XVIIth Gliwice Scientific Meetings 2013

Gliwice, November 15-16, 2013

http://gsn.io.gliwice.pl/

Organizers:

Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch
Silesian University of Technology
Association for the Support of Cancer Research

Patronage and Co-organizers:

Marshal's Office of the Silesian Voivodship
Ministry of Science and Higher Education
Polish Academy of Sciences
Municipal Office in Gliwice

Scientific Committee
of Gliwice Scientific Meetings 2013:

Professor Mieczysław Chorąży *(Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice)*
Professor Marek Kimmel *(Rice University, Houston)*
Professor Marek Los *(Linköping University, Linköping)*
Professor Carmel Mothersill *(McMaster University, Hamilton)*
Professor Joanna Rzeszowska-Wolny *(Silesian University of Technology, Gliwice)* **- President**
Professor Andrzej Świerniak *(Silesian University of Technology, Gliwice)*
Professor Piotr Widłak *(Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice)*

Organizing Committee:

Jacek Rogoliński, Ph. D.
Joanna Łanuszewska, Ph. D.
Aleksander Sochanik, Ph. D.
Elżbieta Zielińska-Król, MSE
Magdalena Skonieczna, Ph. D.
Lucyna Ponge
Iwona Domińczyk

17th Gliwice Scientific Meetings, November 15-16, 2013
Scientific Program

Friday, November 15th, 2013

9.00 - 9.15 Opening Ceremony

9.15 - 11.00 Session I

Regulation of gene expression and replication
Chairman: Ronald Hancock

Anke van den Berg (*University of Groningen, Netherlands*): The role of microRNAs in B cell Hodgkin and non-Hodgkin lymphoma

Joost Kluiver (*University of Groningen, Netherlands*): Long noncoding (lnc)RNAs: novel players in B-cell lymphoma

Małgorzata Czyż (*Medical University of Łódź, Poland*): Heterogeneity of melanospheres: therapeutic implications

Krzysztof Puszyński *(Silesian University of Technology, Poland)*: Dynamics of intracellular processes

11.00 - 11.15 Coffee break

11.15 - 12.30 Session II

Cellular pathways driven by reactive oxygen species
Chairwoman: Joanna Rzeszowska

Sebastian Student (*Silesian University of Technology, Poland*): Reactive oxygen species in the nucleus and cell death

Carmel Mothersill (*McMaster University, Canada*): Radiation-induced non-targeted effects: horizontal and vertical transmission of genetic change mediated by oxidative stress?

Barbara Tudek (*Institute of Biochemistry and Biophysics, Poland*): Lipid peroxidation modulates DNA repair and sensitizes cells to genotoxic factors

Natasha Kopitar (*Josef Stefan Institute, Slovenia*): Deletion of stefin B gene enhances mitochondrial ROS formation and Nlrp3 inflammasome activation

12.30 - 14.00 Poster session and lunch

14.00 - 16.00 Session III

DNA repair in aging and cancerogenesis
Chairwoman: Barbara Tudek

Leon H Mullenders (*Leiden University, Netherlands*): Spot on the DNA damage response: from lesion recognition to human disease and therapy

Wojciech Niedźwiedź (*Oxford University, Great Britain*): BLM collaborates with TOPBP1 in promoting checkpoint activation and replication fork stability

Miroslav Pirsel (*Cancer Research Institute, SAS, Slovakia*): DNA repair helicase – the story with an unexpected end

Grażyna Mosieniak (*Nencki Institute of Experimental Biology, Poland*): DNA damage response in cancer cell senescence

16.00 - 16.15 Coffee break

16.15 -18.15 Session IV

Cancer proteomics and metabolomics *(session co-organized by Polish Society of Proteomics)*
Chairman: Piotr Widłak

Corinna Henkel (*Ruhr University, Germany*): MALDI imaging in clinical research: searching for the Holy Grail

Angels Sierra *(IDIBAPS, Spain*): Proteins and protein-protein interacting motifs to therapeutic target of metastasis

Tone F. Bathen (*Norwegian University of Science and Technology, Norway*): Metabolic profiling of breast cancer

André Goffeau (*Institut des Sciences de la Vie, Belgium*): Targeting selectively the unique energy metabolism of cancer with a small molecule inhibitor

18.15 – 18.30 Company presentation

Andrzej Swinarew *(SHIM-POL, Poland):* The instrumental analysis of novel polymeric materials for bioapplications

20.00 Reception/social event

Saturday, November 16th, 2013

9.00 - 12.30 Session V

Dietary factors in cancer prevention *(session co-organized by European Association for Cancer Research)*
Chairwoman: Wanda Baer-Dubowska

Wanda Baer-Dubowska *(Poznan University of Medical Sciences, Poland):* Diet and cancer: from epidemiological data to mechanism-based cancer prevention

Adriana Albini *(IRCCS MutiMedica, Italy)*: The tumor microenvironment as target for cancer therapy and prevention by dietary components

Karen Brown *(University of Leicester, UK):* Development of resveratrol for cancer chemoprevention.

Albena Dinkova-Kostova (*University of Dundee, UK*): Chemoprevention against cancer by isothiocyanates

10.30 - 11.15 **Meeting of the Polish EACR Group and coffee break**

Jędrzej Antosiewicz (*Gdańsk University of Medical Sciences, Poland*) Chemoprevention of prostate cancer by organosulfur compounds from garlic

Wim Vanden Berghe (*University Antwerp, Belgium*): Promises & challenges in epigenetic remodeling of breast cancer metastasis by natural withanolides from *Withania somnifera (Ashwagandha)*

Agnieszka Bartoszek (*Gdańsk University of Technology, Poland*) Discoveries of experimental chemoprevention - how to exploit them in rational design of health quality and therapeutic foods

12.30 - 12.45 Coffee break

12.45 - 13.30 **Awarded Posters' Presentation**

13.30 - 14.15 Lunch

14.15 - 17.00 Session VI

Cancer killing
Chairman: Marek Los

Sun Xiao-Feng (*Linkoping University, Sweden*): Biomarkers in colorectal cancer

Marek Los (*Linkoping University, Sweden*): Salinomycin – experimental drug with preferred toxicity towards cancer stem cells, the role of autophagy in salinomycin toxicity

Nikolaos Sfakianakis *(University of Mainz, Germany)*: Numerical study of cancer invasion of extracellular matrix

15.30 - 15.45 Coffee break

Katrina Erenpreisa (*University of Riga, Latvia*): Senescence, polyploidy, and stemness – three components of the response of tumour cells to DNA damage

Kourosh Lotfi (*Linkoping University, Sweden*): P-glycoprotein transport of the active imatinib metabolite, CGP74588, in chronic myeloid leukemia cells

Jerzy Pieczykolan *(ADAMED, Poland):* Evaluation of novel, anticancer, targeted fusion biomolecule with high antiangiogenic and cytotoxic activity – summary of preclinical findings

17.00 Closing remarks

Lecture abstracts

Session I:
Regulation of gene expression and replication

THE ROLE OF microRNAs IN B CELL HODGKIN AND NON-HODGKIN LYMPHOMA

Anke van den Berg, Izabella Slezak-Prochazka, Jan Lukas Robertus, Debora de Jong, Gertrud Kortman, Bea Rutgers, Jasper Koerts, Bart-Jan Kroesen and Joost Kluiver

Department of Pathology & Medical Biology, University of Groningen, University Medical Center Groningen, Netherlands.

MicroRNAs (miRNAs) are important regulator of B-cell development and maturation. B cells at the germinal center (GC) stage of differentiation display the most characteristic miRNA signature. These GC B cells are the normal counterparts of various B cell lymphoma subtypes including Burkitt lymphoma and Hodgkin lymphoma. Deregulation of miRNAs is one of the characteristics of B cell lymphoma and the oncogenic transcription factor MYC significantly contributes to the miRNA signature of B-cell lymphoma. To determine the functional relevance of deregulated miRNAs we studied the effect of miRNA overexpression and inhibition on proliferation and defined the miRNA-targetome for a subset of these miRNAs. Overexpression of ten MYC-repressed miRNAs in ST486 BL cells revealed for 6 miRNAs a more than 50% decrease in the percentage of miRNA overexpressing cells in a period of 18 days indicating tumor suppressive activity. One MYC repressed miRNA, i.e. miR-155, induced a more than 50% increase upon overexpression indicating a growth advantage. Inhibition of the miR-17~92 cluster, including the only MYC-induced miRNA, miR-18a, revealed no significant effect on cell growth in Burkitt lymphoma. Inhibition of six miRNAs highly expressed in Hodgkin lymphoma did show a significant effect on proliferation for two miRNAs. Based on the dual effects of miR-155 in Burkitt and Hodgkin lymphoma we performed Ago2-RIP-Chip in miR-155-transduced cells. *In silico* validation of the 54 experimentally identified miR-155 target genes indicated that 32% were predicted as miR-155 targets and 77% contained the 6-mer miR-155-binding motif in the 3'UTR. Using shRNA constructs we showed that inhibition of TBRG1 pheno-copied the effect of miR-155. Our data indicate specific miRNA signatures for different B cell lymphoma subtypes and support crucial roles for individual miRNAs in the pathogenesis of B cell lymphoma.

LONG NON-CODING RNAs: NOVEL PLAYERS IN B CELL LYMPHOMA

J. Kluiver[1], M. Winkle[1], M. Tayari[1], M. Terpstra[2], G. Kortman[1], D. de Jong[1], J. Sietzema[1], B.J. Kroesen[1], K. Kok[2], A van den Berg[1]

[1]Department of Pathology and Medical Biology; [2]Department of Genetics, University of Groningen, University Medical Center Groningen, The Netherlands

B-cell lymphoma is a common malignancy of the B cells, often characterized by translocations involving one of the immunoglobulin loci and an oncogene. A well-known example is MYC, an important oncogenic transcription factor overexpressed in B-cell lymphoma. MYC involved B cell lymphomas are associated with aggressive behavior and poor clinical outcome. A large number of genes are regulated by MYC, several of which are shown to contribute to the MYC induced phenotype. Long non-coding (lnc)RNAs have recently emerged as a novel class of regulatory RNAs acting at the epigenetic, transcriptional or posttranscriptional level. Aberrant expression of several lncRNAs has already been implicated in various aspects of tumorigenesis. It is currently unknown to what extend MYC can regulate lncRNA expression and whether these lncRNAs contribute to the pathogenesis of B-cell lymphoma.

Using an inducible MYC B cell lymphoma model and a custom microarray we investigated the expression >10,000 lncRNA loci and identified 1,820 lncRNA probes that show a MYC regulated expression pattern. Of these, 355 responded already after 4h, indicating direct MYC regulation. To identify transcripts relevant to lymphoma pathogenesis, we determined if these 355 lncRNAs were differentially expressed between primary lymphoma cases with high and low MYC expression and in addition also between MYC-high lymphoma cell lines and normal germinal center B cells. This revealed an overlap of 176 lncRNAs that were MYC regulated, aberrantly expressed in B cell lymphomas and differentially expressed between MYC-high and MYC-low lymphomas. Differential expression patterns were validated by qRT-PCR. As a first indication for lncRNA function, we isolated RNA from nuclear and cytoplasmic fractions of B cell lymphoma cell lines and determined lncRNA enrichment in comparison to RNA isolated from the total cell lysates. Approximately 40% of all lncRNA transcripts showed specific subcellular localization, 80% nuclear and 20% cytoplasmic enriched. 31 of the 176 candidate lncRNAs were enriched in a specific cellular fraction. Furthermore, we analyzed which lncRNAs are enriched in Argonaute 2 containing complexes as an indication for lncRNA-miRNA interaction. For ~5% of all expressed lncRNAs we found evidence for miRNA-lncRNA interactions, including 8 of the 176 differentially expressed MYC-induced lncRNAs.

This study identified 176 MYC responsive lncRNAs that are deregulated in B cell lymphoma. To establish a definitive role in B cell lymphoma pathogenesis a further characterization is warranted.

HETEROGENEITY OF MELANOSPHERES: THERAPEUTIC IMPLICATIONS

Małgorzata Czyż

Department of Molecular Biology of Cancer, Medical University of Lodz, Poland.

Multiple factors have been implicated in heterogeneity of human tumors. Tumor heterogeneity poses a major challenge for effective cancer treatment. In case of melanoma, a high tumor plasticity driven by changes in the microenvironment additionally contributes to complexity of this disease. Testing drug efficacies *in vitro* in two-dimensional, serum-driven monolayer cultures has a poor predictive value. Therefore, more reliable in vitro model is needed. Patient-derived melanospheres grown in anchorage independent manner in stem cell medium were investigated. Transcriptome profiles were generated to explore the molecules governing phenotypes of melanospheres and monolayers. We found that melanospheres better portrayed the original tumor than monolayers. Current results showing activities of selected anticancer drugs against cells from melanospheres will be outlined.

Acknowledgement: This research was supported by grants N° SFI20121205624 from Association pour la Recherche sur le Cancer and 2011/01/B/NZ4/04921 & 2012/06/M/NZ2/00109 from National Science Centre.

DYNAMICS OF INTRACELLULAR PROCESSES

Krzysztof Puszyński

Silesian University of Technology, Gliwice, Poland.

The rapid development of the biological research techniques, we can observe in recent years, provides more detail and complex data. With this technology we are able to not only observe the tissue or cells but also observe what happens inside the single cell. We can detect single molecules and their location change, their phosphorylation status change and we can measure how the amount of the particular molecules changes over time. With this knowledge we can explore the dependences network of the particular peptides and with this exploration it becomes obvious that the intracellular interactions between various molecules are not straightforward but more complex with many mutual dependencies and feedback loops. To fully understand of the complex dependencies network and its function, one has to explore its dynamics. This can be done by construction of mathematical models of observed phenomena and then their analysis. Mathematical models might be deterministic, based on ordinary differential equations or stochastic based on reaction propensities. The network of intracellular species dependences is called signaling pathway. The examples of the signaling pathways models will be shown. Their dynamics will be discussed with focus to the new hypothesis resulting from proper dynamics analysis. This will reveal the advantages of the dynamics of the intracellular processes analysis.

Session II:
Cellular pathways driven by reactive oxygen species

REACTIVE OXYGEN SPECIES IN THE NUCLEUS AND CELL DEATH

Sebastian Student, Magdalena Skonieczna, Joanna Rzeszowska-Wolny

Biosystems Group, Institute of Automatic Control, Silesian University of Technology, 44-100 Gliwice, Poland.

Reactive oxygen species (ROS) are chemically reactive molecules that contain oxygen. ROS such as superoxide, hydrogen peroxide, hydroxyl radical or singlet oxygen are produced permanently in low quantities during cellular metabolism, but their levels can be increased radically by external factors such as ionizing or UV radiation. The main cellular producers of ROS are mitochondria, where oxidants are formed predominantly by complex I or III of the cytochrome chain [rev. in 1]. Another important source of intracellular oxidants are oxidases that catalyze reactions involving molecular oxygen as the electron acceptor, the best studied of which are the seven membrane-bound NADPH-dependent oxidases (Nox1–5 and Duox1–2) which are widely expressed and evolutionarily conserved [2,3]. Cellular ROS are important for redox-signaling pathways which by oxidation of proteins activate transcription factors, kinases, and phosphates [1,4,5] but also can interact with nucleic acids inducing DNA damage and mutations.

We have studied the reactive oxygen species levels in different types of human cells using three fluorescent dyes: 2′,7′-dichlorofluorescein diacetate (DCFH-DA), MitoSOX and CellROX whose fluorescence is proportional to the level of total ROS or to superoxide. Al l cells produced ROS, but they differed in ROS levels. In most of cell types superoxide foci were detected not only in cytoplasm but also in the cell nucleus. Long term observations performed on living HCT116 cells in which the fluorescence was assessed in 100 cells at 15 min intervals for 24 h by CellROX showed that the levels of nuclear ROS fluctuate with different amplitudes. Cells with dark not fluorescent nuclei which did not show nuclear ROS were dying during observation. Our observations suggest that nuclear ROS are important for cell survival.

References:

[1] Finkel T. (2011) Signal transduction by reactive oxygen species. *J. Cell Biol.* **194**, 7-15.
[2] Brown D.I. Griendling K.K. (2009) Nox proteins in signal transduction. *Free Radic. Biol. Med.* 47, 1239-1253.
[3] Aguirre J. Lambeth J.D. (2010) Nox enzymes from fungus to fly to fish and what they tell us about Nox function in mammals. *Free Radic. Biol. Med.* **49**, 1342-1353.
[4] Kensler T.W. Wakabayash N. Biswal S. (2007) Cell survival responses to environmental stresses via the Keap1-Nrf2-ARE pathway. *Annu. Rev. Pharmacol. Toxicol.* **47**, 89-116.
[5] Wright V.P. Reiser P.J. Clanton T.L. (2009) Redox modulation of global phosphatase activity and protein phosphorylation in intact skeletal muscle. *J. Physiol.-London* **587**, 5767-5781.

This work was supported by grants No. DEC-2012/05/B/ST6/03472 from NCN and BKM / 514 /RAU-1/2013.

RADIATION-INDUCED NON-TARGETED EFFECTS: HORIZONTAL AND VERTICAL TRANSMISSION OF GENETIC CHANGE MEDIATED BY OXIDATIVE STRESS?

Carmel Mothersill, Colin Seymour

Department of Medical Physics and Applied Radiation Sciences, McMaster University, Hamilton, Ontario, Canada.
mothers@mcmaster.ca, seymouc@mcmaster.ca

The "non-targeted effects" of ionizing radiation including bystander effects and genomic instability are unique in that no classic mutagenic event occurs in the cell showing the effect. In the case of bystander effects, cells which were not in the field affected by the radiation show high levels of mutations, chromosome aberrations, ROS and membrane signaling changes (horizontal transmission of mutations and information which may be damaging) while in the case of genomic instability, generations of cells derived from an irradiated progenitor appear normal but then lethal and non-lethal mutations appear in distant progeny (vertical transmission). The phenomena are characterized by high yields of mutations and distant occurrence of events both in space and time. This precludes a mutator phenotype or other conventional explanation and appears to indicate a generalized form of ROS mediated stress induced mutatgenesis which is well documented in bacteria. The nature of the signal travelling between irradiated and unirradiated cells and organisms is currently unknown but recent evidence suggests that there may be a physical component such as a vibration wave involved. UV mediated transmission has also been documented. This presentation will discuss the phenomenology of non-targeted effects both in vitro and in vivo, including recent data suggesting that ROS and neurochemicals are important in signal production while the cytokine mediated pathways determine response to the signal. By highlighting some key challenges and controversies, concerning the mechanisms and more importantly, the reason these effects exist, we will discuss current ideas about the wider implications of non-targeted effects in evolution and biology.

LIPID PEROXIDATION MODULATES DNA REPAIR AND SENSITIZES CELLS TO GENOTOXIC FACTORS

Barbara Tudek[1,2], Alicja Winczura[1], Konrad Kosicki[2], Alicja Czubaty[3]

[1]Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawińskiego 5a, 02-106 Warsaw, Poland; [2]Institute of Genetics and Biotechnology, University of Warsaw, Pawińskiego 5a, Poland; [3]Institute of Biochemistry, Faculty of Biology, University of Warsaw, Miecznikowa 1, Poland.

Oxidative stress driven lipid peroxidation (LPO) is involved in the pathogenesis of several human diseases, including cancer. LPO products react with cellular proteins changing their properties, and with DNA bases to form mutagenic exocyclic-DNA adducts, removed from DNA by Base Excision Repair (BER), Nucleotide Excision Repair (NER) and Homologous Recombination (HR) pathways. One of the major reactive aldehydes generated by LPO is *trans-4-hydroxy-2- nonenal* (HNE). We investigated the effect of HNE on BER enzymes *in vitro* and in human wild type cells, as well as in cells lacking several Fanconi Anemia (FANC) proteins, and thus deficient in HR.

The activity of purified ANPG and TDG glycosylases excising from DNA 1,N^6-ethenoadenine (εA) and 3,N^4-ethenocytosine (εC), but not of APE1 endonuclease and 8-oxoGua-DNA glycosylase 1 (OGG1) were inhibited by high concentrations of HNE. K21 cells pretreated with physiological HNE concentrations were more sensitive to oxidative and alkylating agents, H_2O_2 and MMS, when compared to untreated cells. Examination of HNE influence on particular stages of BER in K21 cells revealed that HNE may decrease the rate of εA and εC excision, but not that of 8-oxoguanine. Simultaneously HNE increased the rate of AP sites incision and blocked the re-ligation step after the gap-filling by DNA polymerases. Such imbalance resulted in the increased number of DNA single-strand breaks as revealed by augmentation of the level of poly(ADP-ribose) foci.

The DNA damage response was also affected by HNE. Treatment of HeLa cells with HNE resulted in inhibition of phosphorylation of SMC1, KAP1 and Chk1 proteins engaged in multiple cellular processes like, regulation of cell cycle checkpoints, DNA repair, cell growth an proliferation, apoptosis and maintenance of cellular integrity.

These results suggest that LPO products act not only by forming DNA adducts, but also have the ability to deregulate activities of BER enzymes, and DNA damage response. This may have an impact on cell survival and genome stability, and may be an additional mechanism of pro-carcinogenic effect of inflammations.

GENE DELETION OF STEFIN B ENHANCES MITOCHONDRIAL ROS FORMATION AND NLRP3 INFLAMMASOME ACTIVATION

Katarina Maher[1], Miha Butinar[1], Georgy Mikhaylov[1], Mateja Manček-Keber[2], Barbara Jerič[1] Olga Vasiljeva[1], Boris Turk[1], Nataša Kopitar-Jerala[1]

[1]Department of Biochemistry, Molecular and Structural Biology, Jožef Stefan Institute, Jamova 39, SI-1000 Ljubljana, Slovenia; [2]Department of Biotechnology, National Institute of Chemistry, Hajdrihova 19, Ljubljana SI-1000, Slovenia.

Stefin B (cystatin B) is an endogenous cysteine cathepsin inhibitor localized in the cytosol and nucleus. Its expression is upregulated upon macrophage activation and cellular stress. Mutations in the gene of stefin B are associated with the neurodegenerative disease known as Unverricht-Lundborg disease. The NLRP3 inflammasome is a caspase-1-activating complex involved in the maturation of pro-inflammatory cytokines IL-1β and IL-18. We studied the role of stefin B in the regulation of IL-1β production and activation of pro-inflammatory caspases-1 and -11. Stefin B-deficient (StB KO) mice were significantly more sensitive to the LPS-induced sepsis and secreted higher amounts of pro-inflammatory cytokines IL-1β and IL-18. We further showed that increased caspase-11 gene expression and better pro-inflammatory caspase activation determined in stefin B-deficient bone marrow-derived macrophages (BMDMs) resulted in enchanced IL-1β processing due to extensive destabilization of mitochondria and mitochondrial superoxide generation upon nlrp 3 inflammasome activation. Pretreatment of macrophages with a broad spectrum cathepsin inhibitor E-64d did not affect secretion of IL-1β, suggesting that the increased cathepsin activity determined in stefin B-deficient BMDMs is not essential for the inflammasome activation. Our study demonstrates that the LPS-induced sepsis in stefin B-deficient mice is dependent on caspase-11 and mitochondrial reactive oxygen species (mtROS), but is not associated with the lysosomal destabilization. We propose that the inflammatory signaling pathways determined in stefin B-deficient macrophages could be important in the pathology of the Unverricht-Lundborg disease.

Session III:
DNA repair in aging and cancerogenesis

SPOT ON THE DNA DAMAGE RESPONSE: FROM LESION RECOGNITION TO HUMAN DISEASE AND THERAPY

Leon H.F. Mullenders

Department of Toxicogenetics, Leiden University Medical Center, Leiden, the Netherlands.

A wide variety of genome-caretaking mechanisms (collectively referred to as the DNA damage response or DDR) can counteract the deleterious effects of DNA damage and are essential to prevent toxicity, mutagenesis, genomic instability and disease. The DDR comprises a complex signal transduction cascade that can sense DNA damage, coordinate distinct repair activities and enforce cell cycle arrest ensuring timely and efficient removal of DNA damages from the genome. A corrupted DDR drives mutagenesis and genomic instability with ultimately pathological consequences.

In my presentation I will focus on nucleotide excision repair (carried out by a multiprotein complex) a multistep process involved in removal of structurally diverse DNA lesions including ultraviolet (UV) light-induced photolesions. I will discuss a variety of aspects: the molecular mechanisms of DNA damage recognition, DNA damage signaling via NER-dependent and NER-independent process, NER related human syndromes, the role of NER in controlling mutagenesis. Finally I will address NER related opportunities for cancer treatment.

BLM COLLABORATES WITH TOPBP1 IN PROMOTING CHECKPOINT ACTIVATION AND REPLICATION FORK STABILITY

Andrew N. Blackford[1,2], Jadwiga Nieminuszczy[1], Rebekka A. Schwab[1], Stephen P. Jackson[2], Wojciech Niedzwiedz[1]

[1]WIMM, Oxford University, United Kingdom; [2]Wellcome Trust/Cancer Research, Cambridge, United Kingdom.

Maintenance of DNA replication fork stability is essential for dividing cells to preserve genomic integrity in the face of endogenous and exogenous DNA damage. Critical to these responses is ATR kinase, which promotes checkpoint activation to stabilize stalled forks and to prevent late origin firing. Current models for ATR activation suggest a multistep process that involves initial recruitment of ATR to DNA lesions via its cognate binding partner ATRIP and the subsequent recruitment of topoisomerase 2-binding protein 1 (TopBP1). The mechanisms that regulate TopBP1 recruitment to stalled forks are not yet clear.

Recently, we have identified FANCM as a master regulator of replication stress-response. In line with this, we showed that FANCM-/- cells are impaired in stable association of TopBP1 on chromatin and subsequent activation ATR signaling following replication stress. Here I will provide evidence that a ternary complex of TopBP1, FANCM and BLM is required to maintain fork stability upon replication stress, in part by promoting ATR signaling. Phosphorylation of BLM at a CDK consensus site creates a binding site for tandem BRCT domains of TopBP1, thereby promoting assembly of the ternary complex. Disruption of the TopBP1/BLM interaction in cells leads to ATR signaling defects in response to replication stress, including reduced Chk1 phosphorylation and increased origin firing. Moreover, these cells also display increased sister chromatid exchanges, that we hypothesize arise as a result of unscheduled homologous recombination associated with stalled replication forks.

Taken together, these results provide a mechanistic basis for the fork stability defects previously observed in both FANCM and BLM-deficient cells, and may also help to explain why many Fanconi anemia and Bloom's syndrome patients display symptoms such as short stature and microcephaly that are also found in ATR-Seckel patients.

DNA REPAIR HELICASE – THE STORY WITH AN UNEXPECTED END

Zuzana Šestáková[1], Ján Gurský[1,4], Dana Cholujová[2], Ivan Chalupa[3], Miroslav Piršel[1]

[1]Laboratory of Molecular Genetics; [2]Laboratory of Tumor Immunology; [3]Laboratory of Mutagenesis and Carcinogenesis, Cancer Research Institute, Slovak Academy of Sciences, Bratislava, Slovakia; [4]Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University Olomouc, Olomouc, Czech Republic.

The ERCC3/XPB DNA helicase is the subunit of transcription factor II H (TFIIH) which is involved directly in transcription and nucleotide excision repair and via its Cdk-activating kinase (CAK) complex in cell cycle control. The TFIIH structure is known to be affected by truncation mutations of its subunits. Here, we showed that there is two times and three times lower amount of TFIIH in Chinese hamster UV68 and UV24 *ERCC3* nucleotide excision repair deficient mutants, respectively, comparing with the parental wild type CHO AA8 cells. The degree of this instability is proportionally reflected in the extent of reduction of the cell cycle progression after UV-irradiation measured by pulse- and pulse-chase labeling of the cells by ethynyldeoxiuridine. In fact, the cell cycle profiles of UV68 and UV24 mutants are comparable after 12 Jm^{-2} and 6 Jm^{-2} of UV-irradiation, respectively. The slower S-phase transition in UV24 cells resulted in the higher expression of phosphorylated form of the histone H2AX – the marker of the presence of DNA double-strand breaks. This creates an additional apoptotic signal (resulting in about 20% more apoptotic cells), contributing to the overall UV-sensitivity of the UV24 mutant caused primarily by the nucleotide excision repair deficiency.

The authors acknowledge financial support from Slovak Research and Development Agency (APVV-0208-07) and VEGA Grant Agency of Slovak Republic (2/0051/10, 2/0150/11).

DNA DAMAGE RESPONSE IN CANCER CELL SENESCENCE

Grażyna Mosieniak, Małgorzata Śliwińska, Anna Strzeszewska, Halina Waś, Ewa Sikora

Laboratory of Molecular Bases of Aging, Nencki Institute of Experimental Biology, PAS, 02-093 Warsaw, Pasteura 3 St., Poland.

It is believed that anticancer therapy relies on cytotoxic treatment strategies that would lead to complete destruction of tumor. These approaches, that induce cell death within solid tumor from one hand, can also cause severe side effect in patient due to high doses of drugs or irradiation used during therapy. An alternative strategy to restrict tumor growth and eventually eliminate cancer cells with limited toxicity to patients is therapy-induced senescence. Cellular senescence was primarily described as an effect of gradual exhaustion of replicative potential in normal cells cultured *in vitro* that results in permanent growth arrest. Currently it is recognized as an stress response of both normal and tumor cells that is induced by a verity of exogenous and endogenous factors. It was also show, both *in vitro* and *in vivo,* that senescence sustain an anticancer barrier during carcinogenesis as well as restrict tumor growth upon radio- or chemotherapy. The main cause of senescence is induction of unrepairable double strand DNA damage (DBS) within telomeric regions that persist in senescent cells. We have shown that human colon cancer HCT116 cell undergo cellular senescence upon treatment with low, non toxic, doses of DNA damaging agent – doxorubicin. They ceased proliferation (Ki-67 negative), enlarged their size and increased activity of senescence associated -β-galactosidase, which are hallmarks of senescence. Activation of DNA damage response (DDR) pathway, namely activation of ATM kinase and upregulation of p53 and p21 proteins were observed. The cancer cell senescence was also induced when cells were treated with natural polyphenol – curcumin. In contrary to doxorubicin, curcumin lead to accumulation of cells in mitosis due to improper mitotic spindle formation. Prolong mitotic arrest resulted in DSB, which persisted in cells that exit mitosis, leading to DDR activation and induction of senescence. Interestingly, both doxorubicin and curcumin were able to induced permanent growth arrest in HCT116 cells that do not express p53 (p53-/- cells), which is one of the main component of DDR signaling pathway. Moreover p53-independent induction of p21 protein was observed. Altogether our results prove that DNA damage induced senescence could be obtained in p53 proficient and deficient tumor cells, however different signaling pathways must be activated in order to sustain growth arrest of those cells.

Session IV:
Cancer proteomics and metabolomics
(session co-organized by Polish Society of Proteomics)

MALDI IMAGING IN CLINICAL RESEARCH: SEARCHING FOR THE HOLY GRAIL

Corinna Henkel, Hanna Diehl, Julian Elm, Birte Beine, Helmut E. Meyer

Medizinisches Proteom-Center, Ruhr-University Bochum, Germany.

Mass spectrometry imaging (MSI) has become a powerful and successful tool in the context of biomarker detection especially in recent years. This emerging technique is based on the combination of histological information of a tissue and its corresponding mass spectrometric information. The range of samples to be analyzed is wide and includes besides proteins, peptides, lipids, drugs and their metabolites also glycans and other posttranslational modifications. For data acquisition a tissue section covered with matrix is moved in two dimensions within the mass spectrometer whereas a mass spectrum is recorded for each position. The obtained spatially resolved information of for example protein or peptide abundances can be used to detect differences between healthy and diseased tissue. Hence the method is able to generate peaks specific for histologically defined tissue areas. As an example for a protein MALDI imaging experiment, a bladder cancer study is presented, in which the differentiation of various papillary tumor grades were feasible. In general the identification of such differential peaks is still the methods bottleneck. An alternative approach to get closer to the final goal of biomarker identification, offers peptide imaging. A brief method development of peptide MALDI imaging is also shown.

As conclusion the “holy grail” of biomarker research is not tangible by MALDI imaging yet, but promising attempts in the field seem to gain strength to the technique as will be presented in some examples.

Concluding, MALDI imaging is not yet the “holy grail” of biomarker research, but promising results in this field will be shown to illustrate the great potential of this technique and where it might lead to in the future.

References:

[1] Oezdemir R.F., Gaisa N.T., Lindemann-Docter K., Gostek S., Weiskirchen R., Ahrens M., […] Henkel C. (2012) Proteomic tissue profiling for the improvement of grading of noninvasive papillary urothelial neoplasia. *Clinical Biochem.* **45**, 7–11.

PROTEINS AND PROTEIN-PROTEIN INTERACTING MOTIFS TO THERAPEUTIC TARGET OF METASTASIS

Angels Sierra

Biological Clues of the Invasive and Metastatic Phenotype Group, Bellvitge Biomedical Research Institute (IDIBELL), Barcelona, Spain.

Cancer metastasis is produced by perturbations affecting several genes and pathways. Environmental stimuli trigger uncontrolled cell growth and invasion of other tissues. Understanding cancer progression requires a profound knowledge of the pathways involved in the communication between proteins and genes at a systems level. Consequently, protein-protein interaction networks play an important role in delineating cancer related pathways. Our understanding of cancer has evolved towards the co-operation of groups of genes that constitute pathways. We have described the characteristics of genes involved in breast cancer metastasis and the relationships between them in the context of the protein-protein interaction network. We carried out a systems-level study of the mechanisms underlying organ-specific metastases of breast cancer (Guney et al., 2012).

We followed a network-based approach using microarray expression data from human breast cancer metastases to select organ-specific proteins that exert a range of functions allowing cell survival and growth in the microenvironment of distant organs. MinerProt, a home-made software application, was used to group organ-specific signatures of brain (1191 genes), bone (1623 genes), liver (977 genes) and lung (254 genes) metastases by function and select the most differentially expressed gene in each function. As a result, we obtained 19 functional representative proteins in brain, 23 in bone, 15 in liver and 9 in lung, with which we constructed four organ-specific protein–protein interaction networks (Sanz-Pamplona, 2012). The network taxonomy included seven proteins that interacted in brain metastasis, which were mainly associated with signal transduction. Proteins related to immune response functions were bone specific, while those involved in proteolysis, signal transduction and hepatic glucose metabolism were found in liver metastasis.

In conclusion, the network-based approach is useful to filter information by selecting key protein functions as metastatic markers or therapeutic targets (Stresing et al, 2012; Santana et al., 2013). Towards developing effective network-based therapeutics, we give details of identifying dysregulation patterns using protein-protein interaction networks with an emphasis on the underlying mechanisms of progression in metastatic breast cancer.

References

[1] Sanz-Pamplona, R. et al. (2012) A taxonomy of organ-specific breast cancer metastases based on a protein–protein interaction network. *Mol. BioSyst*. **8**, 2085–2096.

[2] Guney, E. et al. (2012) Springer Book on Systems Biology and Cancer. Part II Understanding Cancer Progression Using Protein Interaction Networks.

[3] Stresing, V. et al. (2012) Peroxiredoxin 2 specifically regulates the oxidative and metabolic-stress response of human breast cancer cells. *Oncogene* 0950-9232/12. DOI: 10.1038/onc.

[4] Santana-Codina N. et al. (2013) Transcriptome-proteome integrated network Identifies endoplasmic reticulum thiol oxidoreductase (ERp57) as a Hub that mediates bone metastasis. *Mol. Cell. Proteom.* 10.1074/mcp.M112.022772, 1–15.

METABOLIC PROFILING OF BREAST CANCER

Tone F. Bathen

Dept. of Circulation and Medical Imaging, NTNU.

Metabolism comprises the integrated network of biochemical reactions that supports life in a living organism, and metabolomics is the systematic study of small-molecular compounds from metabolism. The metabolism of cancer cells is changed compared to normal cells due to high proliferation rates and malignant transformation. Metabolomics, using high resolution magic angle spinning MR spectroscopy (HR MAS MRS), may establish detailed tumor portraits reflecting diagnostic status or therapeutic response, thus potentially leading to the discovery of useful biomarkers in a clinical context. HR MAS MRS enables investigation of tissue samples with minimal sample preparation and keeps the sample intact after analyses. This talk will cover some of the practical issues of HR MAS MRS, and results from published an ongoing studies will be presented.

WEB: http://www.ntnu.edu/isb/mr-cancer

TARGETING SELECTIVELY THE UNIQUE ENERGY METABOLISM OF CANCER WITH A SMALL MOLECULE INHIBITOR

André Goffeau[1], Young H. Ko[2], Peter L. Pedersen[3], Margarida Casal[4], Marek Koziorowski[5], Stanisław Ułaszewski[6]

[1]Institut des Sciences de la Vie, Université Catholique de Louvain-la-Neuve, Place de, l`Université, 1348 Louvain-la-Neuve, Belgium; [2]KoDiscovery, UMBioPark, Innovation Center, 801 West Baltimore Street, Baltimore, MD, USA; [3]Departments of Biological Chemistry and Oncology, Member Sydney Kimmel Comprehensive Cancer Center and Member Center for Obesity Research and Metabolism, John Hopkins University School of Medicine, Baltimore, MD 21205-2185, USA; [4]CBMA, Centre of Molecular and Environmental Biology, University of Minho, Braga, Portugal; [5]Zakład Fizjologii i Rozrodu Zwierząt, Pozawydziałowy Zamiejscowy Instytut Biotechnologii Uniwersytetu, Rzeszowskiego, WERYNIA 502, 36-100 Kolbuszowa, Poland; [6]Institute of Genetics and Microbiology, University of Wroclaw, Przybyszewskiego 63/77, 51-148 Wroclaw, Poland.

The "Warburg Effect" is the most common biochemical phenotype of many cancer types. It is due to elevated tumor glycolysis even in the presence of oxygen. (Under such conditions normal cells convert glucose mainly to carbon dioxide and water and not to lactic acid.) Most cancers show a positive Positron Emission Tomography (PET) scan that monitors the "Warburg Effect". Such cancers export the produced lactic acid *via* specific lactic acid transporters called "monocarboxylate transporters" as the lactic acid inside the cell is accumulating. In contrast, normal cells produce little or no lactic acid under physiological conditions and have very few functioning lactic acid transporters within their cell membrane. Utilizing this knowledge, several agents were screened for their capacity to selectively kill cancer cells. A lactic acid analog, 3-bromopyruvate (3BP) was found to be the best among the agents tested [1-3]. Because 3BP is a structural analog of lactic acid, cancer cells readily take it up, i.e., the 3BP enters these cells on the lactic acid transporters, the same transporters that allowed lactic acid to exit. Once the 3BP enters the cancer cells it targets and destroys their two energy (ATP) production factories, mitochondria and glycolysis. In contrast, little or no 3BP enters normal cells as they have very few lactic acid transporters. This explains why 3BP is highly specific in killing cancer cells. Although there are several other proteins that are targeted by 3BP inside the cancer cells, they are not immediately involved in cell death. "Proof of principle" of 3BP as a potent anticancer agent has been established earlier in a Case Report [4, 5].

In summary, 3BP is a new class of anticancer agent. It is quite different from the currently available chemo-drugs that target one or more of the following: DNA replication, cell cycle/ growth, cellular signal transduction pathways, angiogenesis, and receptors. Significantly, 3BP gains specific entry to cancer cells *via* a monocarboxylate transporter and targets their energy metabolism, thus inhibiting their ATP production and depleting their energy sources (reserves). Therefore, 3BP is a potent "Energy Blocker" of cancer cells and is very effective in killing such cells exhibiting the "Warburg Effect" with little or no effect on normal cells. This unique nature of 3BP as a potent anticancer agent warrants continued development towards clinical treatment of cancer patients. Currently, 3BP is being actively researched *via* global collaborative efforts [5-8].

References

[1] Ko Y.H., Pedersen P.L., Geschwind J.F. (2001) *Cancer Lett.* **173**, 83-91.
[2] Ko Y.H., Smith B.L., Wang Y., Pomper M.G., Rini D.A., Torbenson M.S., Hullihen J., Pedersen P.L. (2004). *Biochem. Biophys. Res. Commun.* **324**, 269-275.
[3] Pedersen P.L. (2007) *J. Bioenerg. Biomemb.* **39**, 211-222.
[4] Pedersen P.L. (2012) *J. Bioenerg. Biomembr.* **44**, 1-6.
[5] Ko Y.H., Verhoeven H.A., Lee M.J., Corbin D.J., Vogl T.J., Pedersen P.L. (2012) *J. Bioenerg. Biomemb.* **44**, 163-170.
[6] Lis P., Zarzycki M., Ko Y.H., Casal M., Pedersen P.L., Goffeau A., Ułaszewski S. (2012) *J. Bioenerg. Biomemb.* **44**, 155-161.
[7] Queirós O., Preto A., Pacheco A., Pinheiro C., Azevedo-Silva J., Moreira R., Pedro M., Ko Y. H., Pedersen P., Baltazar F., Casal M. (2012) *J. Bioenerg. Biomemb.* **44** 141-153.
[8] Dyląg M., Lis P., Niedźwiecka K., Ko Y.H., Pedersen P.L., Goffeau A., Ułaszewski S. (2012) *Biochem. Biophys. Res. Commun.* **434,** 322-327.

Session V:
Dietary factors in cancer prevention

DIET AND CANCER: FROM EPIDEMIOLOGICAL DATA TO MECHANISM-BASED CANCER PREVENTION

Wanda Baer-Dubowska

Department of Pharmaceutical Biochemistry, Poznan University of Medical Sciences, Poznań, Poland.

The ideas that cancer might be preventable, and that food and nutrition might affect the risk of cancer were first made before science emerged in its modern form in the 19th and 20th centuries. Over the years it became evident that cancer of some sites are greatly or mostly affected by food and nutrition. As a consequence authoritative estimates of the preventability of cancer by means of food and nutrition and associated factors have been in broad agreement.

The traditional approach to cancer prevention has consisted of attempts to eliminate carcinogenic agents and to detect and remove precancerous lesions. Currently, efforts are increasingly focused on interrupting, reversing or delaying the neoplastic process. This approach not only complements therapeutic modalities currently in use but may provide alternatives for combating tumors that are unresponsive to treatment. Hence, cancer chemoprevention or reversal of carcinogenesis in the premalignant phase can be defined as the use of natural, or synthetic chemicals to suppress, delay or prevent the process of carcinogenesis. Food is a rich source of potential anticarcinogenic agents. Thus it is reasonable to postulate that dietary components may prevent cancer from reaching its invasive and metastatic stages. Alternatively, components of diet may reduce the risk of second primaries or modify the behavior of established cancer.

Interest in this area of research has markedly increased with the improved understanding of the biology of carcinogenesis and led to mechanism–based cancer prevention approaches.

THE TUMOUR MICROENVIRONMENT AS TARGET FOR CANCER THERAPY AND PREVENTION BY DIETARY COMPONENTS

Adriana Albini

IRCCS "Tecnologie Avanzate e Modelli Assistenziali in Oncologia" - Arcispedale S. Maria Nuova - Reggio Emilia, Italy; Douglas Noonan, Oncology Research, Polo Scientifico e Tecnologico, Fondazione Onlus MultiMedica, Milano, Italy.

The tumour microenvironment (TME) is a "complex society" that involves many "actors" including the malignant cells, but also stromal cells, inflammatory components, the endothelium and the recent discovered cancer stem/initiating cells. All these non-malignant cells of the TME have a dynamic and often tumour-promoting function at all stages of carcinogenesis, including angiogenesis. In this view, targeting the TME represent a valid and necessary strategy in cancer therapy both in intervention and prevention.

The ability some dietary components, like terpenoids (CDDO-Me, CDOO-Im), Resveratrol, Green tea catechins, quercetin, curcumin, to inhibit tumor progression and angiogenesis, both in vitro and in vivo, has been highly documented. Many of these compounds exert anti-oxidant, anti-proliferative, anti-angiogenic and pro-apoptotic effects on a variety of cancers, including leukemia, prostate, breast, colon, brain, melanoma, and pancreatic. In addition these compounds are well tolerated and often found in food products that can be added to diet. Furthermore, most phytochemicals could be taken on a long-term basis to either prevent primary tumor formation or tumor recurrence. We demonstrated the ability of phytochemicals to efficiently target several component of the TME, including the inflammatory component (neutrophils, Natural Killer Cells), the endothelium[1] and interestingly also the "dormant" component represented by the cancer stem cells.

Taken together, the TME complex entity provides many more targets available for cancer therapy than do the tumor cells alone and phytochemicals and dietary components represent a valid tool both for intervention and prevention approaches.

References:

[1] Albini A., Tosetti F., Li V.W., Noonan D.M., Li W.W. (2012) Cancer prevention by targeting angiogenesis. *Nat. Rev. Clin. Oncol.* PMID: 22850752.

DEVELOPMENT OF RESVERATROL FOR CANCER CHEMOPREVENTION

Karen Brown

Department of Cancer Studies and Molecular Medicine, University of Leicester, Leicster, LE2 7LX, UK.

Clinical trials of tamoxifen, finasteride and aspirin have provided proof of principle that cancer chemoprevention in healthy high-risk populations is feasible. However, safety is paramount in this context and the serious side effects of pharmaceutical interventions when taken chronically have hampered their further use. Chemicals derived from the diet, such as resveratrol, are an attractive alternative, with those that are consumed frequently likely to have a favourable safety profile. Such agents are typically multi-targeted and capable of interfering with all stages of carcinogenesis in preclinical models. Unfortunately, despite extremely promising preclinical data, the outcome of clinical chemoprevention trials of isolated micronutrient supplements such as selenium and vitamin E has been disappointing. These failures may be attributed, at least partly, to a lack of sufficient human pharmacokinetic and pharmacodynamic information required to optimise the trial design. This presentation will review our current clinical data relating to the use of resveratrol for cancer chemoprevention, with a focus on potential low dose effects and the role of resveratrol metabolites in mediating efficacy. In addition the major translational challenges in the development of resveratrol for widespread use in human populations will also be addressed.

CHEMOPREVENTION AGAINST CANCER BY ISOTHIOCYANATES

Albena T. Dinkova-Kostova[1,2]

[1]Division of Cancer Research, Medical Research Institute, University of Dundee, Dundee, Scotland, United Kingdom; [2]Department of Pharmacology and Molecular Sciences, Johns Hopkins University School of Medicine, Baltimore, MD, USA.

All aerobic cells are equipped with elaborate networks of highly inducible proteins which protect against the cumulative damaging effects of reactive oxygen species, toxic electrophiles, and misfolded proteins, the major causes of malignancy and chronic degenerative diseases. These cytoprotective proteins are transcriptionally regulated via the Keap1/Nrf2 pathway. Keap1, a substrate adaptor protein for Cullin3/Rbx1 ubiquitin ligase, continuously targets transcription factor Nrf2 for ubiquitination and degradation, but loses this ability in response to various chemical and phytochemical agents (inducers), all of which have sulfhydryl reactivity. We have developed a quantitative Förster Resonance Energy Transfer (FRET)-based system using multiphoton fluorescence lifetime imaging microscopy (FLIM) and found that, under homeostatic conditions, the interaction between Keap1 and Nrf2 follows a cycle whereby the complex sequentially adopts two distinct conformations: "open", in which Nrf2 interacts with one molecule of Keap1, followed by "closed", in which Nrf2 binds to both members of the Keap1 dimer. Inducers disrupt this cycle by causing accumulation of the complex in the "closed" conformation, without release of Nrf2. Consequently, free Keap1 is not regenerated, newly-synthesized Nrf2 is stabilized, and the expression of cytoprotective proteins is upregulated.

The plant isothiocyanates are prominent inducers of the Keap1/Nrf2 pathway. Due to the electrophilic central carbon of the isothiocyanate group, they react with critical cysteine residues of Keap1, disrupting the cycle of Nrf2 ubiquitination, and leading to activation of the transcription factor. The isothiocyanate sulforaphane was first isolated as the principal inducer of the Keap1/Nrf2 pathway from broccoli extracts, and has been shown to be an effective protective agent in numerous preclinical models of gastric, intestinal, prostatic, pulmonary, cutaneous and bladder cancers in animals, and in xenograft models of human tumors. Sulforaphane-rich broccoli preparations have been and currently are in several human studies, ranging from healthy human subjects to populations at high-risk for developing diseases conditions.

CHEMOPREVENTION OF PROSTATE CANCER BY ORGANOSULFUR COMPOUNDS FROM GARLIC

Jędrzej Antosiewicz, Andżelika Borkowska

Department of Bioenergetics and Physiology of Exercise, Medical University of Gdansk, Dębinki 1, 80–211 Gdansk, Poland.

Epidemiologic data strongly suggests that dietary intake of Allium vegetables, including garlic, may be protective against the risk of prostate cancer. The risk of prostate cancer was shown to be significantly lower in men consuming >10g/d of total Allium vegetables than in men with total Allium vegetable intake of <2.2 g/d in a population-based case-control study.

Anticarcinogenic effect of Allium vegetables is attributed to organosulfur compounds (OSC) such as dially trisulfide (DATS), dially disulfide and many others. Evidence is accumulating to indicate that DATS and other OSC can inhibit prostate cell cycle arrest and induced apoptosis and that this is mediate by reactive oxygen species (ROS). In this study we provide experimental evidence to indicate that in DATS treated prostate cancer cells iron-dependent ROS formation is mediated by JNK1-ITCH-p66shc signaling pathway.

Ferritin is an iron storage protein and its concentration is inversely related to the level of labile iron pool (LIP). Increased ferritin degradation or down regulation of its biosynthesis leads to an increase of LIP which is able to participate in redox reaction and generation of free radicals. Thus signaling pathways which regulate ferritin degradation may have impact on intracellular ROS formation. However, the mechanism of the ferritin degradation has not been fully elucidated so far. In cells stably expressing a dominant negative mutant of p66Shc (p66ShcS36A), DATS did not induce ROS formation. Previously we were able to demonstrate that JNK signaling pathway regulates ferritin ubiquitination and proteasomal degradation. As p66Shc and ubiquitin ligaze Itch are activated by JNK their role in ferritin degradation has been studied. We were able to demonstrate that DATS-induced ferritin degradation in prostate cancer cells expressing inactive form of p66Shc (p66ShcS36A) neither an increase in ferritin H degradation nor an increase in LIP were observed. In addition the cells transfected with inactive form of Itch were more resistant against cytotoxicity of DATS and showed lower DATS-induced ferritin degradation. In conclusion, our results suggest that ferritin degradation is a regulated process where JNK1, p66Shc and Itch play important role. In addition, the data suggest a role of p66Shc in a posttranslational modification of ferritin H, which needs additional work to be elucidated.

PROMISES & CHALLENGES IN EPIGENETIC REMODELLING OF BREAST CANCER METASTASIS BY NATURAL WITHANOLIDES FROM *Withania Somnifera (Ashwagandha)*

Katarzyna Szarc vel Szic[1], David Scherf[2], Ilse M. Beck[3], Marc Bracke[3], Tim De Meyer[4], Clarissa Gerhauser[2], Wim Vanden Berghe[1]

[1]Laboratory of Protein Chemistry, Proteomics and Epigenetic Signalling, Department of Biomedical Sciences, University of Antwerp (UA), Universiteitsplein 1, Campus Drie Eiken, 2610, Wilrijk, Belgium; [2]Workgroup Cancer Chemoprevention and Epigenomics, Division of Epigenomics and Cancer Risk Factors, German Cancer Research Center (DKFZ), Im Neuenheimer Feld 280, 69120, Heidelberg, Germany; [3]Laboratory of Experimental Cancer Research, Department of Radiation Therapy & Experimental Cancer Research, De Pintelaan 185, Building 1P7, Ghent University Hospital, B-9000, Gent, Belgium; [4]Department of Mathematical Modelling, Statistics & Bioinformatics, Ghent University, B-9000, Ghent, Belgium.

A vast majority of cancer patients succumb to metastatic disease. Intriguingly, interfering with metastatic spread remains one of the main challenges in cancer therapy. Metastasis development includes several discrete steps: local invasion, intravasation (or dissemination in lymph nodes or body cavities), circulation and cell survival, extravasation, growth at distinct sites and angiogenesis, all of which occur in a context of tumour-promoting microenvironment. It is now becoming apparent that these cell-microenvironment interactions are highly susceptible to epigenetic regulation, both by internal and external cues.

Here we show that essential components of metastasis development, including urokinase plasminogen activator, ADAM8 metallopeptidase, and tumour promoting cytokine TNFSF12 are regulated epigenetically by DNA methylation in breast cancer as revealed by 450k Illumina BeadChipArray, MCIp and EpiTyper MassArray. Moreover, Withaferin A, a natural compound derived from *Withania somnifera* decreases breast cancer invasion by increasing methylation of these genes leading to lowered gene expression as revealed by RT-qPCR.

DISCOVERIES OF EXPERIMENTAL CHEMOPREVENTION - HOW TO EXPLOIT THEM IN RATIONAL DESIGN OF HEALTH QUALITY AND THERAPEUTIC FOODS

Agnieszka Bartoszek, Barbara Kusznierewicz, Anna Lewandowska, Anna Piekarska, Tadeusz Pilipczuk, Dominik Kołodziejski, Izabela Koss, Piotr Konieczka, Jacek Namieśnik

Chemical Faculty, Gdańsk University of Technology, Gdańsk, Poland.

Translational research is usually associated with the development of new therapeutic treatments as summarized by the popular phrase - from lab bench to bedside. However, in the case of health promoting foods, the transfer of knowledge from research laboratories to industry - from lab bench to shop shelf - may actually occur much more efficiently as evidenced by the plethora of so called functional foods available on the market. These are usually food items or dietary supplements fortified with a single or couple of natural compounds whose health benefits were experimentally demonstrated. Recently, a more scientifically advanced food synergy concept has been proposed by Jacobs et al. (Pub. Hlth. Rev., 2012) in which reductionists research focussed on single food components shifts to natural combinations of nutrients and non-nutrients as they may play a concerted role in influencing health. Such a philosophy of designing new food products with enhanced health quality has been executed by our group over past decade. The examples of commercialized and being currently elaborated anticarcinogenic foods will be presented along with some scientific background behind their development.

Session VI
Cancer killing

BIOMARKERS IN COLORECTAL CANCER

Sun Xiao-Feng

Linkoping University, Linkoping, Sweden.

The presentation includes the three parts: Firstly, there is an introduction of the RESEARCH TEAM. Secondly, the AIMS of this translational study (bedside-bench-bedside): 1) detect biomarkers for making early diagnosis and finding high risk individuals; 2) identify biomarkers associated with therapy response for designing individual therapy; and 3) identify the mechanisms behind the relationships of biomarkers with clinicopathological variables. Thirdly, the RESULTS AND IMPORTANCE of the study: 1) Early diagnosis: The detection of early-stage tumour is a prerequisite for improving therapy response and prognosis. By studying the process of tumour development from normal mucosa to adenoma, primary cancer and metastasis, we may find biomarkers for early diagnosis. The study of polymorphisms will provide information for identifying high risk individuals; 2) Therapy response: Radio-, chemo- and/or antibody therapy have improved patient survival but the patients elicit a large variation in therapy response. Certain molecular mechanisms are involved in therapy response. It is crucial to search for biomarkers used for individual therapy in order to improve prognosis and limit side-effects; and 3) Prognosis: TNM staging is a rather broad classification since cancers in the same stage result in different survival rate. The determination of biomarkers may identify prognosis in the patients within the same TNM stage.

SALINOMYCIN – EXPERIMENTAL DRUG WITH PREFERRED TOXICITY TOWARDS CANCER STEM CELLS, THE ROLE OF AUTOPHAGY IN SALINOMYCIN TOXICITY

Marek J. Łos[1], Jaganmohan Reddy Jangamreddy[1], Saeid Ghavami[2], Artur Cieslar-Pobuda[1,3]

[1]Depart. Clinical and Experimental Medicine (IKE), Division of Cell Biology, Integrative Regenerative Medicine Center (IGEN), Linköping University, Sweden; [2]Department of Human Anatomy and Cell Science, Univ. Manitoba, Canada; [3]Biosystems Group, Institute of Automatic Control, Silesian University of Technology, Gliwice, Poland

Salinomycin is an antibioticum and potassium ionophore. It has recently been identified to preferentially kill breast cancer stem cells. The molecular mechanism of Salinomycin's toxicity is not fully understood, as it cannot be explained solely by its ionophore activity. Various studies reported that Ca^{2+}, cytochrome c, and caspase activation play a role in Salinomycin-induced action. Furthermore, Salinomycin may target Wnt/beta-catenin signaling pathway to promote differentiation and thus elimination of cancer stem cells. In this study, we show a massive autophagic response to Salinomycin (substantially stronger than to commonly used autophagic inducer Rapamycin) in prostrate-, breast cancer cells, and to lesser degree in human normal dermal fibroblasts. Interestingly, autophagy induced by Salinomycin is a cell protective mechanism in all tested cancer cell lines. Furthermore, Salinomycin induces mitophagy, mitoptosis and increased mitochondrial membrane potential in a subpopulation of cells. Salinomycin strongly, and in time-dependent manner decreases cellular ATP level. Contrastingly, human normal dermal fibroblasts treated with Salinomycin show some initial decrease in mitochondrial mass, however they are largely resistant to Salinomycin-triggered ATP-depletion. Our data provide new insight into the molecular mechanism of preferential toxicity of Salinomycin towards cancer cells, and suggest possible clinical application of Salinomycin in combination with autophagy inhibitors (i.e. clinically-used Chloroquine).

NUMERICAL STUDY OF CANCER INVASION OF EXTRACELLULAR MATRIX

Nikolaos Sfakianakis

University of Mainz, Germany

In this work we study high order numerical methods resolving a class of reaction-diffusion-taxis systems modeling the dynamics of cancer tumor invasion on the extracellular matrix. The complication of this type of systems and the dynamics that arise make the analytical study of the system almost impossible and the numerical study extremely difficult. We propose in this work a numerical method to analyse this type of systems to extend of their complexity.

SENESCENCE, POLYPLOIDY, AND STEMNESS – THREE COMPONENTS OF THE RESPONSE OF TUMOUR CELLS TO DNA DAMAGE

Jekaterina Erenpreisa[1], Anda Huna[1], Thomas Jackson[2], Kristine Salmina[1], Paul Townsend[2], Mark Cragg[2]

[1]Latvian Biomedical Research & Study Centre, Riga, Latvia; [2]Cancer Sciences Unit, University of Southampton, Southampton, UK.

All aggressive tumours display signature of embryonal stem cells which is responsible for resistance to genotoxic treatments and cancer progression. Furthermore, recent data from several tumour types shows that stemness is actually induced by genotoxic treatments in differentiated tumour cellls [1,2] and that this response is associated with induced polyploidy and its reverse [3,4]. Intriguingly, genotoxic treatments concurrently induce senescence in the same cells, akin to what happens in induced pluripotent stem cells (iPSC) during somatic cell reprogramming. Therefore, we studied the genotoxic response of the TP53- and telomerase-competent ovarian cancer cell line PA1 [5] and showed a TP53-dependent activation of both stemness promoting (OCT4A) and senescence promoting (p21cip1) regulators. Mechanistic and dynamic studies of individual cells show that high level of two regulators keep each other in balance and the cell in a bi-potential state within the G2 arrest favouring DNA damage repair and vitality, whilst preventing premature exit, which triggers terminal senescence and aberrant cell division. This bi-potential state is leaky for tetraploidy coupled to persistent DNA damage, thus carrying the risk of cancer progression. Our conclusion is that the induction of stemness is inevitably coupled to senescence and DNA damage signalling and that involvement of tetraploidy leads to overcoming the genome-guarding functions of TP53. The same mechanism may be induced during artificial reprogramming for iPSC, raising the possibility of cancer development from these cells.

References:

[1] Lee G.Y. et al.(2011) Stochastic acquisition of a stem cell-like state and drug tolerance in leukemia cells stressed by radiation. *Int. J. Hematol.* **93,** 27-35.
[2] Ghisolfi L. et al. (2012) Ionizing radiation induces stemness in cancer cells. *PloS one* **7**, e43628.
[3] Salmina K. et al. (2010) Up-regulation of the embryonic self-renewal network through reversible polyploidy in irradiated p53-mutant tumour cells. *Exp. Cell Res.* **316**, 2099-2112.
[4] Lagadec C. et al. (2012) Radiation induced reprogramming of breast cancer cells. *Stem Cells* **30**, 833-844.
[5] Jackson T. et al. (2013) DNA damage causes TP53-dependent coupling of self-renewal and senescence pathways in embryonal carcinoma cells. *Cell Cycle* **12**, 430-441.

P-GLYCOPROTEIN TRANSPORT OF THE ACTIVE IMATINIB METABOLITE, CGP74588, IN CHRONIC MYELOID LEUKEMIA CELLS

Kourosh Lotfi[1,2] Karin Skoglund[1], Samuel Boiso Moreno[1], Jan-Ingvar Jönsson[3], Henrik Gréen[1,4]

[1]Division of Drug Research/Clinical Pharmacology, Department of Medical and Health Sciences, Linköping University, Sweden; [2]Department of Hematology, Linköping University Hospital, Sweden; [3]Division of Cell Biology, Department of Clinical and Experimental Medicine, Linköping University, Sweden; [4]Science for Life Laboratory, Division of Gene Technology, Royal Institute of Technology, Solna, Sweden.

The tyrosine kinase inhibitor (TKI) imatinib is, despite the introduction of second generation TKI's, the standard first-line therapy in chronic myeloid leukemia (CML). Early response to therapy is correlated to long-term therapeutic effect in CML. To be able to switch to second generation TKI's in an early stage of CML, it would be advantageous to identify predictive markers for failure on imatinib.

Imatinib is metabolized by hepatic CYP3A4 and CYP3A5, forming approximately 30 metabolites. The main metabolite, CGP74588, is pharmacologically active with similar potency to that of imatinib. CGP74588 is present in ~20% of imatinib plasma concentrations but with a large inter-individual variation. We have previously reported that high CYP3A activity is associated with a better therapeutic outcome of imatinib, indicating a clinical significance of imatinib metabolites (Gréen et al. 2010). Furthermore, imatinib is a substrate for the efflux transporter P-glycoprotein which is the product of the ABCB1 gene. Several ABCB1 polymorphisms have been described and some have been shown to influence the therapeutic response to imatinib in CML patients.

The aim of this study was to investigate the effects of ABCB1 over-expression on the *in vitro* resistance to imatinib and its CYP3A metabolite CGP74588.

ABCB1 wild type human cDNA was infected to the CML cell line K562 using a retroviral system. Co-expression of ABCB1 and the reporter gene for enhanced yellow fluorescent protein (EYFP) was used for the assessment of ABCB1 expression by the analysis of EYFP in FACS. The influence of ABCB1 expression on the cytotoxic effects of imatinib and CGP74588 was assessed using MTT assays.

FACS analysis of EYFP confirmed an over-expression of ABCB1 in infected K562 cells with a mean fluorescence intensity of 23.5 compared to 0.55 in parental K562 cells. ABCB1-expressing cells (K562/ABCB1) were slightly, but not significantly, more resistance to imatinib than K562 cells (IC_{50} K562 = 0.41μM, K562/ABCB1 = 0.50μM). However, ABCB1 expression induced a 12-fold increase in resistance when cells were treated with the imatinib metabolite CGP74588 (IC_{50} K562 = 0.72μM, K562/ABCB1 = 8.53μM, p = <0.000). Recent reports have shown that CGP74588 accumulates in cell lines with acquired multi-drug resistance and high P-gp expression. Our studies on cells with ABCB1 expression as a single resistance mechanism proves that P-gp is indeed confer resistance to CGP74588 in CML cells. Furthermore, CGP74588 cytotoxicity is affected by ABCB1 expression to a greater extent than imatinib itself, indicating that ABCB1 activity could be important for CGP74588 plasma and target cell concentrations *in vivo* and might contribute to the large intra-individual variation seen in patient plasma concentrations. Future studies will be needed to determine the clinical significance of P-gp activity and CGP74588 pharmacokinetics in relation to TKI therapy of CML.

EVALUATION OF NOVEL, ANTICANCER, TARGETED FUSION BIOMOLECULE WITH HIGH ANTIANGIOGENIC AND CYTOTOXIC ACTIVITY – SUMMARY OF PRECLINICAL FINDINGS

Jerzy Pieczykolan[1], Piotr Rózga[1], Anna Pieczykolan[1], Sebastian Pawlak[1], Bartlomiej Zerek[1], Malgorzata Teska-Kamińska[1], Marlena Galazka[1], Katarzyna Bukato[1], Michal Szymanik[1], Albert Jaworski[1], Wojciech Strozek[1], Katarzyna Poleszak[1], Anna Grochot-Przeczek[2], Alicja Józkowicz[2]

[1]Drug Discovery Department, Oncology, Pieńków 149, 05-270 Czosnów, Poland; [2]Department of Medical Biotechnology, Jagiellonian University, Poland.

One of the most important mechanisms of tumorgenesis is formation of new blood vessels and tissue remodeling. Vascular endothelial growth factor (VEGF) is one of the most important and widely studied proangiogenic factor that has an important role in many physiological and pathological processes. Blockade of VEGF pathway has been shown to inhibit both pathological angiogenesis and tumor growth. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL/Apo2L) was considered as a promising anticancer agent due its remarkable ability to induce apoptosis in cancer cells without harming normal cells. However in clinical practice TRAIL efficacy was too low to become the effective single therapy agent.

Here we present the novel fusion protein based on TRAIL/Apo2L, equipped with an antiangiogenic, effector peptide, fused to its N- terminus. Effector, is a 7-amino acid long peptide that binds to natural VEGF receptors competing with their natural ligand. The proposed fusion protein AD-O51.4 consists of extracellular soluble portion of TRAIL linked to two tandemly arranged copies of effector peptide separated by the sequence containing the motif recognized by tumor-specific proteases (MMP's, uPa). TRAIL targeted peptide is able to bind and sequester the VEGF receptors on malignant (cancer) and endothelial cells. As a consequence peptide blocks the binding of the VEGF ligand, blocking new vessels formation. There is also a lot of evidences confirming expression of the VEGF receptors on the surfaces of different cancer cell lines. Due to the presence of VEGF receptors those cell lines can be also targeted by VEGF receptors blocking peptide what makes them susceptible for TRAIL induced apoptosis.

AD-O51.4 showed in vitro specific cytotoxic effect on various cancer cell lines at the level of IC50 below 0.1 ng/mL without toxic effects on normal cells. Cytotoxic activity of TRAIL was significantly lower in comparison to AD-O51.4. We demonstrated that TRAIL/Apo2L-VEGF-antagonist is a strong activator of caspase 3 and Bid processing. Additionally its direct antiangiogenic activity was confirmed with the ring aortic assay and HUVEC spheroid assay.

Strong antitumor activity of novel fusion molecule was also confirmed on xenograft model of multidrug resistant human uterine sarcoma MES-SA/Dx5 where our protein caused complete tumor remission and showed much higher efficacy than TRAIL alone. The similar effect was observed with human colorectal adenocarcinoma Colo205, human pancreatic carcinoma MIA PaCa-2 and orthotropic model of human lung carcinoma NCI-H460-luc2. Therefore we postulate that the use of the fusion proteins can open a new and exciting filed in anticancer compounds development.

Poster abstracts

Abstracts are ordered according to nine main themes.
Number next to the abstract title correlates with poster number.

Cancer (Cell) Biology

1. ANALYSIS OF *CDK1* GENE EXPRESSION IN SQUAMOUS CELL CARCINOMA OF LARYNX

Kinga Bednarek[1], Magdalena Kostrzewska-Poczekaj[1], Marcin Szaumkessel[1], Maciej Giefing[1], Katarzyna Kiwerska[1], Reidar Grenman[2], Krzysztof Szyfter[1,3], Małgorzata Jarmuż-Szymczak[1,4]

[1]Institute of Human Genetics, Polish Academy of Sciences, Poznan, Poland; [2]Department of Otorhinolaryngology, Head and Neck Surgery, University of Turku, Turku, Finland; [3]Department of Phoniatrics and Audiology, University of Medical Sciences, Poznan, Poland; [4]Department of Hematology, University of Medical Sciences, Poznan, Poland.

Laryngeal squamous cell carcinoma (LSCC) is one of the most common head and neck cancers. Because of difficulties in diagnosis and treatment, 5-year survival rate remains low, despite development of chemo- and radiotherapy.

The attention was focused on head and neck cancer genetics – searching for new oncogenes. Aim of the research was the analysis of *CDK1* gene. In a first step we analyzed larynx squamous cell carcinoma cell lines, and next we used material derived from patients from Poznan University of Medical Sciences – primary larynx tumor samples.

The study included both analyses of gene expression and attempt to explain the mechanism, that can be responsible for observed changes. We applied techniques that involved DNA (pyrosequencing, microarray-based DNA-copy number analysis), RNA (microarray-based gene expression analysis, real-time PCR), microRNA (microarray-based microRNA expression analysis), proteins (Western Blot).

We have shown, that expression for *CDK1* - on mRNA and protein level – is increased as compared to non-cancer control derived from larynx (total RNA and larynx tissue whole cell lysate). We found, that these changes are not connected with gene amplification or duplication. We also observed, that DNA methylation level and microRNA expression are on an equal level in analyzed material and non-cancer controls. Further analysis of mechanism for the observed changes explanation is still in progress.

Due to our results indicating different expression profile in LSCC cell lines and larynx tumors, we propose *CDK1* gene as a new potential oncogene in LSCC.

2. ANALYSIS OF THE *MONOCYTE CHEMOTACTIC PROTEIN-1-INDUCED PROTEIN 1 (MCPIP1)* GENE EXPRESSION IN HUMAN NEUROBLASTOMA

Elżbieta Boratyn[1], Irena Horwacik[1], Anna Skalniak[1], Sylwia Tyrkalska[1], Małgorzata Durbas[1], Maria Łastowska[2], Barbara Lipert[3], Jolanta Jura[3], Hanna Rokita[1]

[1]Laboratory of Molecular Genetics and Virology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Gronostajowa 7, 30-387 Kraków, Poland; [2]Department of Pathology, Institute "Pomnik – Centrum Zdrowia Dziecka", Aleja Dzieci Polskich 20, 04-730 Warszawa, Poland; [3]Department of General Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Gronostajowa 7, 30-387 Kraków, Poland.

The recently discovered MCPIP1 (monocyte chemotactic protein-1-induced protein 1) multidomain protein encoded by the *MCPIP1* (*ZC3H12A)* gene, has so far been described as a new transcription [1] and differentiation [2] factor, a ribonuclease [3, 4], and deubiquitinase [5]. However, its role in cancer development is not yet established. Neuroblastoma (NB) is an embryonal malignant neoplasm of the postganglionic sympathetic nervous system, exhibiting considerable clinical and biological heterogeneity. It is the most common extracranial solid tumour in children, representing approximately 10% cancers in infancy and childhood. The main reason of the low cure rate of children with high risk neuroblastoma is resistance of cancer cells to treatment. Attempts to improve the outcome of advanced neuroblastoma have focused so far mainly on intensification of the induction and consolidation phases of chemo-radio therapy, with or without stem cell rescue [6].

We studied *MCPIP1* gene expression in several cell lines like BE(2)-C, IMR-32, HTLA230 and SH-SY5Y. It was observed that the low expression of the MCPIP1 protein measured in human neuroblastoma cell lines might be important for their survival. Enforced *MCPIP1* gene expression in BE(2)-C cells performed through transfection of plasmid constructs bearing either *MCPIPwt* or a mutant form lacking the RNase domain, caused significant decrease in neuroblastoma cell proliferation and viability. Additionally, analysis of microarrays data for *MCPIP1* mRNA expression levels in human primary neuroblastoma tumours showed a lack of expression of the MCPIP1 transcript in all primary tumours. Transcripts of several genes known to be involved in MCPIP1 regulation, like MCP1 (CCL2), MCP-1 receptor (CCR2) and IL-1β receptor – IL1R1, as well as NFκB family members were also evaluated on the same set of primary neuroblastoma tumours.

These results may help to gain more insight into the *MCPIP1* gene expression levels importance in cancer development and indicate the possible □ignalling pathways involved in neuroblastoma cell survival.

References:

[1] Zhou L., Azfer A., Niu J., et al. (2006) Monocyte Chemoattractant Protein-1 induces a novel transcription factor that causes cardiac monocyte apoptosis and ventricular dysfunction *Circ. Res.* **98**, 1177-1158.

[2] Vrotsos E.G., Kalattukudy P.E., Sugaya K. (2009) MCP-1 involvement in glial differentiation of neuroprogenitor cells through APP signaling *Brain Res. Bull.* **79**, 97-103.

[3] Matsushita K., Takeuchi O., Standley D.M., et al. (2009) Zc3k12a is an RNase essential for controlling immune responses by regulating mRNA decay *Nature* **458,** 1185-1190.

[4] Mizgalska D., Węgrzyn P., Murzyn K., et al. (2009) Interleukin-1-inductible MCPIP protein has structural and functional properties of RNase participating in degradation ol IL-1-mRNA *FEBS J.* **276**, 7386-7399.

[5] Liang J., Saad Y., Lei T., et al. (2010) MCP-iduced protein 1 deubiquitinates TRAF proteins and negatively regulates JNK and NF-κB signalin" *J. Exp. Med.* **207**, 2959-2973.

[6] Brodeur G.M. (2003) Neuroblastoma: biological insights into a clinical enigma *Nat. Rev. Cancer* 3, 203-216.

The study was supported by grant no. 2011/03/B/NZ1/00024 from the Polish National Science Center.

3. CELL COMMUNICATION VIA EXOSOMES VISUALIZED BY THE TRANSFER OF FLUORESCENT DYES

Liliana Czernek, Markus Düchler

Department of Bioorganic Chemistry, Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences, 112 Sienkiewicza St., 90-363 Lodz, Poland.

Exosomes are small biological membrane vesicles (30/40-100 nm in diameter), delivered by many cells including tumor cells. These vesicles contain a specific composition of proteins (tetraspanins, receptors for targeting and adhesion), lipids, and various RNA species (mRNA, miRNA). Exosomes are involved in cell-to-cell communication and may serve as vehicles for the transfer of proteins and RNAs to distant locations.

To visualize trafficking of exosomes between cells, we labelled them with two different dyes. One has been described earlier for that purpose - carboxyfluoresceine diacetate succinimidyl-ester (CFSE) which enters the cytoplasm of the cell where it binds to proteins and becomes fluorescent after cleavage of the acetate groups by intracellular esterases. The second one, DSSN (4,4'-bis(4'-(N,N-bis(6''-(N,N,N-trimethylammonium)hexyl)-styryl)stilbene tetra-iodide))), has not been used for exosome labelling so far. DSSN is a water soluble distyrylstilbene oligoelectrolyte which stably intercalates into the cell membranes. In the lipid bilayer it is located perpendicular to the plane of the membrane in an orientation like the hydrophobic long molecular axis. The intercalation is stabilized by terminally charged groups at the ends of DSSN molecules.

Exosomes were harvested by ultracentrifugation from the supernatant of two melanoma cell lines, A375 and 1205Lu, and characterized by flow cytometry. Exosomes were labelled with CFSE or DSSN. Uptake of these vesicles by the same cell line from where they originated (in the case of A375) or by ovarian cancer cells (OvC-16) was monitored by immunofluorescence microscopy and flow cytometry analysis.

The recipient cells acquired strong fluorescence indicating that exosomes were efficiently taken up. There was no significant difference between exosome transfer to the same cells from which they originated and to the cells from a different line. Depending on the dye used, differences in the intracellular localization of the fluorescence were noticed. Usage of dyes with diverse localization preferences might help to clarify the route of exosome uptake by recipient cells. In conclusion, labelling with fluorescent dyes very clearly demonstrated cell-cell communication via exosomes.

4. EFFECT OF PACLITAXEL ON CELL CYCLE IN MCF-7 BREAST CANCER CELLS

Kamil Durka, Krzysztof Kochel, Dominika Szewczyk, Anna Pieniążek, Aneta Koceva-Chyła

Department of Thermobiology, Faculty of Biology and Environmental Protection, University of Lodz, Pomorska 141/143, 90-236 Lodz, Poland.

Paclitaxel belongs to the taxane family, a semisynthetic anticancer drugs that areused in a treatment of advanced ovarian, breast and non-small-cell lung cancers. The drug binds to β-tubulin which prevents microtubul depolymerization and blocks normal cell division.

The aim of the study was analysis of the effect of paclitaxel on cell cycle progression of breast cancer cells (MCF-7). The cells were treated with 0.2μM, 0.4μM and 0.8 μM of paclitaxel for 2 h and after then incubated in fresh medium for 0 and 24 h. Cell cycle distribution was determined by flow cytometry at 0 h time point (in the end of incubation) and after 24 h post-treatment cell growth in a fresh medium. DNA was stained with propidium iodide after permealization of the cell plasma membrane anddigestion of RNA and protein with RN-ase and proteinase K, respectively. Cell cycle distribution was analyzed cytometricallyon the basis of DNA histograms and the significance of observed changes was evaluated statistically using a statistical program STATISTICA (StatSoft, Tulsa, OK, USA).

Obtained results showed that paclitaxel arrested most of the MCF-7 cells at the G2/M checkpoint of the cell cycle in the time-and concentration-dependent manner.

5. SERUM PROTEOME ANALYSIS OF METASTATIC CHANGES IN PATIENTS WITH GASTRIC CANCER BY MALDI-TOF MASS SPECTROMETRY

Agnieszka Gdowicz-Kłosok[1], Agata Chwieduk[1], Agnieszka Namysł-Kaletka[1], Katarzyna Szołtysek[1], Monika Pietrowska[1], Jerzy Wydmański[1], Małgorzata Plechawska-Wójcik[3], Joanna Polańska[2], Piotr Widłak[1]

[1]Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice, Poland; [2]Silesian University of Technology, Gliwice, Poland; [3]Lublin University of Technology, Institute of Computer Science.

Gastric cancer is one of the most common malignant tumor in Poland. Because of the absence of the characteristic symptoms it is usually diagnosed in advanced stadium and only 36-50% of cases are qualified for radical treatment. The biggest problem are numerous therapeutic metastasis and recurrence. Gastric cancer is not only the serious issue from the epidemiological point of view, but also therapeutically and diagnostically. The only reasonable solution of this problem seems to rely on individualized and combined modality treatment.

Few years ago our Centre initiated an original prospective randomized clinical trial to evaluate effectiveness of pre-surgical radio-chemiotherapy treatment. Aim of a research accompanying this trial is to identify possible markers for individualization and optimization of the treatment. Here we aimed to search for serum proteome profiles characteristic for gastric cancer patients with.

Mass spectrometry-based analysis of the blood proteome is an emerging method of clinical proteomics and cancer diagnostics. Although no single peptide is expected to be a reliable bio-marker in such analyses, multi-peptide profiles selected in numerical tests have been already shown in a few studies to have potential values in cancer diagnostics. Here we performed mass spectrometry-based serum proteome pattern analysis aimed at identifying features specific for patients with gastric cancer. Blood samples were collected before the start of therapy from patients with advanced gastric cancer. 148 patients with diagnosed metastasis and 107 patients without metastasis. Serum was isolated after blood clotting and the low-molecular-weight proteome fraction (2-14 kDa) was analyzed using MALDI-ToF mass spectrometry. Registered mass spectra were analyzed using bioinformatics tool created and optimized in our group. The aim of our project was to find protein biomarkers of metastasis. Our pilot study allowed identification of multi-peptide signatures characteristic for serum proteome of patients with gastric cancer.

Key words: gastric cancer, protein biomarkers, multi-peptide profiles, MALDI-ToF mass spectrometry.

The work is supported by Ministry of Science and Higher Education, grant no. NN 403 283 140.

6.TRANSCRIPTOME PROFILES OF MELANOSPHERES AND MONOLAYERS DERIVED FROM NODULAR MELANOMA SPECIMENS

Mariusz L. Hartman[1], Malgorzata Sztiller-Sikorska[1], Beata Talar[1], Salem Chouaib[2], Małgorzata Czyż[1]

[1]Department of Molecular Biology of Cancer, Medical University of Lodz, 92-215 Lodz, ul. Mazowiecka 6/8, Poland; [2]U753, Institute Gustave Roussy, 94-805 Villejuif-Cedex, 114 rue Édouard-Vaillant, France.

Several studies indicated that melanospheres grown in serum-free, EGF/bFGF-supplemented medium are a better tool for study melanoma than monolayers grown in serum-containing medium. Multicellular spheres consist of a heterogeneous population of melanoma cells, including a subpopulation with stem cell-like phenotype characterized e.g. by enhanced self-renewal capacity and ability to differentiate into other cell lineages. However, the molecular characteristics of these two distinct melanoma phenotypes is poorly defined.

In the present study, transcriptome profiles were generated to explore the molecules governing melanospheres and monolayer phenotypes of melanoma cells derived from tumor tissues classified as nodular melanoma, with clinical staging III or IV. The differentially expressed genes were shown as Significance Analysis of Microarray (SAM) and heatmap, and the enriched pathways were collected using KEGG database in Gene Set Enrichment Analysis (GSEA). Selected genes were validated using quantitative Real-Time PCR (qRT-PCR).

The results show that the microenvironment strongly determines the transcriptomes of melanoma cells since gene expression profiles related to melanospheres differed in 1181 transcripts from those of monolayers. When SAM was applied on genes with FC > 8, fifty eight genes appeared to be highly up-regulated (FC from 8.2 to 48.3) in monolayers and seventy nine genes were highly up-regulated (FC from 8.0 to 376.7) in melanospheres. The functional bioinformatics analysis revealed several signaling pathways with potentially modulated activity in melanospheres or monolayers. The results confirm that melanospheres better resemble the original tumor in terms of selected gene expression patterns than monolayer cultures thus, melanospheres can be exploited as an interesting model of melanoma in *in vitro* studies.

This work was financially supported by 2012/06/M/NZ2/00109 from National Science Centre (Poland) and by the ARC (Association pour la Recherche sur le Cancer) 2012-2013: N° SFI20121205624 and Ligue contre le Cancer (France).

7. SALINOMYCIN INDUCED ACTIVATION OF AUTOPHAGY, AUTOPHAGIC FLUX AND MITOPHAGY AMONG PRIMARY, AND CANCER CELLS – ROLE IN CANCER CELL SPECIFIC SENSITIZATION TO APOPTOSIS

Jaganmohan Reddy Jangamreddy, Marek J. Los

Depart. Clinical and Experimental Medicine (IKE), Division of Cell Biology, Linköping Univ., Sweden.

The molecular mechanism of Salinomycin's toxicity is not fully understood. Various studies reported that Ca^{2+}, cytochorme c, and caspase activation play a role in Salinomycin-induced cytotoxicity. Furthermore, Salinomycin may target Wnt/β-catenin signaling pathway to promote differentiation and thus elimination of cancer stem cells. In this study, we show a massive autophagic response to Salinomycin (substantially stronger than to commonly used autophagic inducer rapamycin) in prostrate-, breast cancer cells, and human primary fibroblasts. However, only cancer cells showed increased LC3II flux when treated at lower concentrations of salinomycinbut not primary fibroblasts. Interestingly, autophagy induced by Salinomycin is a cell protective mechanism in all tested cancer cell lines. Furthermore, Salinomycin induces mitochondrial fission, mitophagy, and strongly, and in time-dependent manner decreases cellular ATP level. Contrastingly, primary human fibroblasts treated with Salinomycin are largely resistant to Salinomycin-triggered ATP-depletion. Our data provide new insight into the molecular mechanism of preferential toxicity of Salinomycin towards cancer cells, and suggests possible clinical application of Salinomycin in combination with autophagy inhibitors (like i.e. clinically-used Chloroquine).

8. EXPRESSION OF ANGIOGENIC GENES ACTIVATED BY THE WNT/β-CATENIN PATHWAY IN COLORECTAL CANCER

Tomasz Janikowski, Urszula Mazurek

Medical University of Silesia, Poniatowskiego 15, 40-055 Katowice, Poland.

Introduction: The wnt/β-catenin signaling pathway had been determined as one of the main causes of colorectal cancer development. Destabilization of β-catenin in the cell can lead to uncontrolled expression of genes involved in proliferation, metastasis, inflammation and angiogenesis. From what we know the process of angiogenesis in colorectal cancer has a crucial role in its progression. Despite of antiangiogenic therapy development and improvements in survival prognostics it still is a serious problem. This enables an efficient therapy at the source of uncontrolled angiogenesis whereas two main factor compete in signaling the mentioned β-catenin and hipoksja factor 1.

Aim: The aim of this study was to try to decide which pathway has a greater influence.

Material and methods: To estimate the gene expression profile for both angiogenesis and wnt/B-catenin pathway we performed an microarray analysis with the use of genechip HGU-133a from every microarray we had obtained 22 283 id mRNA . The analyzed samples had been divided into four clinical stage groups accordingly to WHO specifics and the normal control. To create a highly significant and universal group we added samples from the MIAME database (GSE41258).

Results: From the obtained 22 283 id mRNA we peaked using NetAffx database 984 connected to angiogenesis. Because of the lack on β-catenin id mRNA in the angiogenesis group we obtained another 1450 related to the wnt/β-catenin signaling pathway. Next we performed normalization using RMA express and further statistical analysis in GeneSpring 11.5, MS Excel. The next step was to check the biological significance in literature. Afterwards crucial angiogenesis genes had been once more divided into groups based on the main signaling pathways - B-catenin, HIF-α and influenced by both.

Conclusion: In conclusion we could estimate the influence of β-catenin on the expression profile of angiogenic genes.

9. NEGATIVE REGULATION OF THE NFκB SIGNALING PATHWAY BY THE HEAT SHOCK TRANSCRIPTION FACTOR 1

P. Janus[1], M. Kalinowska-Herok[1], K. Szołtysek[1], R. Jaksik[2], L. Handschuh[3], M. Kimmel[2], P. Widlak[1]

[1]Maria Sklodowska-Curie Memorial Center and Institute of Oncology, Gliwice, Poland; [2]Silesian University of Technology, Gliwice, Poland; [3]Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Poland.

NFκB- and HSF1-dependent pathways are the major components of cellular response to stress, which play essential role in pathogenesis and therapy of serious human diseases, including cancer. Both transcription factors regulate numerous genes involved in apoptosis, cell proliferation and inflammatory responses. Here we aimed to identify NFκB-regulated genes, which expression is affected by HSF1.

Expression of NFκB-dependent genes was analyzed in U2-OS human osteosarcoma cells stimulated with TNFα cytokine, which activates the canonical NFκB pathway. Cells were either preconditioned with hyperthermia (HS) to activate endogenous HSF1 or engineered to express a constitutively active form of HSF1 in the absence of heat shock (aHSF1). The expression of TNFα-induced genes was analyzed by the expression microarrays. Bioinformatics analysis was made for prediction of hypothetical κB and HSE motifs in promoter regions of analyzed genes. Actual sites of HSF1 binding to chromatin were detected in cells subjected to HS by chromatin immunoprecipitation assay coupled with DNA sequencing (ChIP-Seq approach).

Stimulation of U2-OS cells with TNFα resulted in up-regulation of 190 genes and down-regulation of 133 genes. Among such 323 TNF-regulated genes 113 genes contained hypothetical κB-binding motifs and could be considered as putatively NFκB-dependent. Actual sites of HSF1 binding were detected in 68 out of 323 TNF-regulated genes, including 35 NFκB-dependent genes. We have analyzed effects of hyperthermia and the presence of constitutively active aHSF1 upon expression of TNF-regulated genes. The same effect of hyperthermia and aHSF1 was observed for 192 TNF-regulated genes, including 64 NFκB-dependent genes, hence we concluded that observed effect was related to hyperthermia-induced HSF1. For the majority of NFκB-dependent genes (54 genes), HSF1 and NFκB revealed antagonistic effect: HSF1 inhibited genes up-regulated by TNF (39 genes) or stimulated genes down-regulated by TNF (15 genes). Among them there were 16 NFκB-dependent genes where actual HSF1 binding in their promoter regions were detected, including 13 genes inhibited by HSF1, which indicated direct effect of HSF1 upon their regulation.

We concluded that HSF1, in addition of canonical activation of *HSP* genes, could be involved in direct regulation of genes participating in the NFκB-dependent pathway. Here we showed evidence that HSF1 is a potential negative regulator of 13 NFκB-dependent genes stimulated by TNFα: *IL8, CCL2, CD83, FOSB, EGR1, ATF3, SLC12A7, ZFP36, PPP1R15A, GADD45B, RRAD, IFNGR2, EPB41L2.*

This work was supported by Polish National Science Center, Grant N401_563740 and Grant DEC-2012/04/A/ST7/00353.

10. 3-BROMOPYRUVATE DECREASES ACTIVITY OF CATHEPSIN B IN RPMI 8226 MYELOMA CELLS, TREATED WITH HYPOXIA-INDUCING AGENT

Mateusz Kędzior[1], Rafał Seredyński[1], Urszula Godzik[1], Dagmara Tomczyk[1], Oskar Uchański[1], Daria Augustyniak[2], Young H. Ko[3], Jan Gutowicz[1]

[1]Department of Physical Chemistry of Microorganisms, Institute of Genetics and Microbiology, University of Wrocław, Poland; [2]Department of Pathogen Biology and Immunology, Institute of Genetics and Microbiology, University of Wrocław, Poland; [3]KoDiscovery LLC, UM BioPark, Baltimore, USA.

Cathepsin B is a lysosomal cysteine protease involved in number of physiological and pathological processes. The enzyme is often overexpressed in late stages of tumorigenesis and its cellular localization may, additionally, be altered. Proteolytic activity of secreted and membrane-bound cathepsin B against extracellular matrix components seems to be an important contributor of cancer cells' motility, including their migration through lymph/blood vessels to secondary sites. Therefore, cathepsin B is considered as prognostic marker and putative target in anti-cancer therapies. Many natural and synthetic inhibitors of cathepsin B have been discovered, and some of them are thoroughly investigated in clinical trials.

3-Bromopyruvate (3-BP) is a potent anti-cancer drug, which affects glycolytic pathway and reduces metabolic efficiency of tumor cells. As an alkylating agent, 3-BP is believed to affect thiol residues of proteins including modifications in cathepsin's active site's structure.

Present work evaluates the influence of 3-BP on cathepsin B activity in RPMI 8226 myeloma cell line. At first, fluorimetric assay was used to check direct inhibitory effect of 3-BP on purified bovine cathepsin B. To determine the specificity of obtained results, experiment was also carried out with papain as a key representative of the cysteine proteases family. Subsequently, the effect of 3-BP on cathepsin B in myeloma cells was tested. In order to increase cellular expression of proteases by activation of the hypoxia response pathway, the cell culture was treated with dimethyloxaloylglycine (DMOG) for 12 hours prior to addition of 3-BP. Subsequently, gelatin zymography was performed to measure the activity of cathepsin B in sonicates of three types of cell samples - (1) untreated, (2) treated with DMOG exclusively and (3) treated with DMOG and 3-BP.

Unexpectedly, fluorimetric assays for purified enzymes showed that neither cathepsin B nor papain activities changed significantly after incubation with 3-BP, which excludes direct alkylation of active site's cystein residues. In contrast, gelatin zymography revealed huge decrease of cathepsin B activity in DMOG-stimulated myeloma cells after treatment with 3-BP.

Our results indicate that 3-BP may interfere with hypoxia-induced upregulation of cathepsin B activity in myeloma cell line. We speculate that it can be related to lower production of cathepsin B in myeloma cells or to the expression of damaged enzyme with limited proteolytic properties.

11. IMPACT OF S1 ON MITOCHONDRIAL RESPIRATORY AND HEXOKINASE II ACTIVITY IN HEPG2 CELLS

Krzysztof Kochel[1], Kamil Durka[1], Janusz Skolimowski[2], Aneta Koceva-Chyła[1]

[1]Department of Thermobiology, Faculty of Biology and Environmental Protection; [2]Department of Organic Chemistry, Faculty of Chemistry, University of Lodz, 141/143 Pomorska St., 90-236, Lodz, Poland.

Introduction/Aim: Hexokinase II (HKII; ATP: D-hexose-6-phosphotransferase, EC 2.7.1.1) is a key hexokinase izoenzyme, which catalyzes the first step of the glycolysis pathway. HKII is overexpressed in the majority of tumor cells and leads to the inhibition of apoptosis. It has been well documented that mitochondria-bound HKII prevents the formation of the mitochondrial permeability pores (MPT phenomena), which is responsible for the triggering of the intrinsic pathway of apoptosis via the suppression of the transport of the pro-apoptotic protein Bax to the outer mitochondrial membrane. Some recent reports also indicated that the succinate dehydrogenase (SDH) – the component of the mitochondrial electron transfer system (ETS) - is the main target for 3-bromopyruvate, a well known inhibitor of HKII.

In the context of this knowledge, the aim of our study was to find a specific inhibitor of the hexokinase II activity, effective enough to induce apoptosis of the cancer HepG2 cells. For this purpose, using computational methods for biomolecular docking, we designed and synthesized a new compound: 2-(3,4-dimethoxyphenyl)-3-oxopropanenitrile (S1) and verified whether it could serve as the inhibitor of HKII and contribute to the apoptosis of the model cancer cells HepG2 by inhibiting of HKII activity.

Materials and methods: All experiments were performed on human hepatoma HepG2 cells. In order to check our hypothesis stating that S1 is able to inhibit the activity of hexokinase II, the following experiments were conducted:

- assessment of the conformational changes in hexokinase II structure with the increasing S1 concentrations - model studies on commercially available hexokinase II (spectrofluorometric measurements)
- study of mitochondrial bioenergetics in permeabilized HepG2 cells (respirometric measurements)
- evaluation of hexokinase II activity in permeabilized HepG2 cells (respirometric measurements)

Results: S1 used at the concentrations of 0.025 μM and 0.05 μM contributed to a significant reduction in the tryptophan fluorescence of hexokinase II. In the bioenergetics studies we have shown that S1 may limit the mitochondrial electron transfer by the inhibition of succinate dehydrogenase (complex II). Nevertheless, the activity of hexokinase II remained unchanged upon 24 h-exposure to S1.

Conclusions: S1 can directly interact with hexokinase II, however, without affecting the HKII activity. On the basis of these results we hypothesize that the main target of the S1 action maight be the complex II (succinate dehydrogenase) of mitochondrial electron transport chain. The evidence that S1 affects the mitochondrial bioenergetics makes reasonable the initiation of further studies in order to reveal the overall effect of S1 on mitochondrial function.

12. ERN1 MEDIATED ENDOPLASMIC RETICULUM STRESS CONTROLS THE EXPRESSION OF THBS1, THBS2, CTGF, UPA AND UPAR GENES IN U87 GLIOMA CELLS

K.I. Kubaichuk, D.O. Minchenko, A.P. Kharkova, O.H. Minchenko

Department of Molecular Biology, Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine, Kyiv, Ukraine; Department of Pediatrics, National O.O. Bogomolets Medical University, Kyiv, Ukraine.

The endoplasmic reticulum stress, mediated by ERN1 enzyme is linked to the processes of neovascularisation and tumor growth. Blockade of ERN1 has anti-tumor effects via suppression of these processes. Ischemia is known to induce ER stress and is responsible for regulation of growth factors involved in tumorogenesis, such as thrombospondin 1 and 2 (THBS1, THBS2), connective tissue growth factor (CTGF), urokinase plasminogen activator (uPA) and uPA receptor (uPAR). Thus, it's important to study the role of these genes in ERN1 signaling in relation to ischemia and tumor growth.

We used human glioma cell line U87 as well its modified variant with suppression of ERN1 enzymatic activities. For glucose/glutamine deprivation cells were grown in the medium without glucose or glutamine. The expression of genes in cells was measured by qPCR.

It was shown that blockade of ERN1 activity in glioma cells significantly increased expression of CTGF, THBS2 and THBS2 (inhibitors of angio- and tumorogenesis) genes as compared with control cells, while the levels of uPA and uPAR (activators of cell migration and angiogenesis) expression were decreased. Moreover, overexpression of CTGF and THBS was observed in control cells under glucose/glutamine deprivation, when the level of uPAR gene expression went down. However, in glioma cells with ERN1 deficiency, more significant effect of glucose deprivation on expression of uPA mRNA was shown. Meanwhile, the expression of uPAR gene was resistant to glutamine deprivation in control cells but was down-regulated in cells with ERN1 loss of function. It's possible that changes in gene expression, can lead to inhibition of tumor growth.

Thus, results of this investigation demonstrate the role of THBS1, THBS2, CTGF, uPA and uPAR in tumorogenesis and are important for developing a new understanding of molecular mechanisms of tumor growth in relation to ERN1 signaling and define new targets for anti-tumor therapies.

13. ITGBL1 OVEREXPRESSION STIMULATES OVARIAN CANCER CELL MIGRATION RATE

Katarzyna Kujawa[1], Agnieszka Macioła[1], Alexander Cortez[1,2], Magdalena Olbryt[1], Tomasz Kujawa[1], Katarzyna Lisowska[1]

[1]Center for Translational Research and Molecular Biology of Cancer; Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch, Wybrzeże Armii Krajowej 15, 44-101 Gliwice, Poland; [2]Polish-Japanese Institute of Information Technology, Koszykowa 86, 02-008 Warsaw, Poland.

Introduction: In our previous microarray study we identified two molecular subtypes of serous ovarian cancers, with distinct gene expression profile. These subtypes are correlated with different survival of the patients. One of the genes that were differentially expressed between two subtypes of ovarian cancer was Integrin, beta-like 1 gene (ITGBL1). It codes for a poorly characterized adhesion protein that contains 10 tandem EGF-like repeats similar to those found in the cysteine-rich structure of integrin subunits. ITGBL1 is supposed to be evolutionarily and functionally cognate with integrin β. On this basis this is speculated that this protein may influence adhesion and motility, thus affecting the ability of cancer cell to spread and metastasize. Our aim was to study whether and how ITGBL1 can influence the biology of ovarian cancer cells.

Methods: ITGBL1 expression in 5 ovarian cancer cell lines (OAW42,SKOV3,OVCAR-3,OVP-10,ES2) was assayed using semi-quantitative RT-PCR. ITGBL1 coding sequence was amplified from cDNA and cloned in pLNCX2 vector. Retroviral system was used to obtain cell lines with ITGBL1 overexpression. Wild type and ITGBL1-expressing isogenics cell lines were then used in scratch assay.

Results: We successfully obtained SKOV3, OAW42 and OVCAR-3 cell lines overexpressing ITGBL1. Than SKOV3/ITGBL1(+) and OAW42/ITGBL1(+) cell lines were assayed for cell proliferation and migration rate. The results were analyzed in comparison to the non-modified SKOV3 and OAW42 cell lines.We found the scratch area was faster covered with the cells overexpressing ITGBL1 than with the cells that do not express ITGBL1.

Conclusions: Our results indicate that ITGBL1 protein may enhance ovarian cancer cell proliferation and motility. This suggests that ITGBL1 may affect the aggressiveness of cancer cells and play an important role in ovarian cancer progression. These results must be further confirmed.

Katarzyna Kujawa was supported by the European Community from the European Social Fund within the DoktoRIS project.
Alexander Cortez was supported by the European Social Fund within the INTERKADRA project (UDA - POKL-04.01.01-00-014/10-00).
This work was partially supported by the grant 2012/04/M/NZ2/00133 to K.L.

14. THE INFLUENCE OF GENISTEIN GLYCOCONJUGATES ON SIGNALING PATHWAYS DEPENDENT ON EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR)

Anna Mucha[1,2], Aleksandra Gruca[1,2], Wiesław Szeja[2], Zdzisław Krawczyk[1,2], Aleksandra Rusin[1]

[1]Center for Translational Research and Molecular Biology of Cancer. Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch, Gliwice, Poland; [2]Department of Organic Chemistry, Bioorganic Chemistry and Biotechnology, Silesian Technical University, Gliwice, Poland.

Background: Association between overactivation of epidermal growth factor receptor EGFR leading to AKT and ERK1/2 activation, and a worsened prognosis motivates many researchers to develop inhibitors of EGFR stimulation. AKT (Protein Kinase B) plays a critical role in controlling survival and apoptosis. Mitogen activated protein kinases (MAPKs) are a family of serine/threonine protein kinase involved in modulation of cell proliferation, differentiation, motility and death. Both AKT and MAPK are activated in EGFR-dependent or independent manner. Identifying the compound able to inhibit EGFR-dependent signaling pathways is the critical point in EGFR inhibitors designing.The alternative to currently tested selective EGFR inhibitors may be non-toxic products of natural origin, such as general tyrosine kinase inhibitor, genistein and/or their derivatives. Genistein is a molecule of great interest as an innovative chemotherapeutic agent and a leading compoundin anticancer drug design.

Aim: The aim of this study was to test the hypothesis that the derivatives of genistein, which cause profound inhibitory effects on EGFR phosphorylation act in one of the following modes: inhibit EGFR-dependent activation of PI3K/AKT signaling pathway and/or inhibit Ras/Raf/MEK/ERK pathway.

Results: We have selected the compound able to inhibit both AKT and ERK phosphorylation in EGFR-dependent manner. The genistein derivative Ram-5 significantly inhibited the activation of AKT and ERK1/2.

Conclusions: The experiment rationalizes the use of Ram-5 for inhibition of certain EGFR dependent signaling pathways in cancer cells.

15. ACTIVATION OF NF-κB SIGNALING PATHWAY DURING THE HEAT SHOCK RESPONSE

Anna Naumowicz[1,2], Patryk Janus[1,2], Wiesława Widlak[1], Marek Kimmel[2]

[1]Center for Translational Research and Molecular Biology of Cancer, M. Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch, Poland; [2]Instituite of Automatic Control, Faculty of Automatic Control, Electronics and Computer Sciences, Silesian University of Technology, Gliwice, Poland.

The NF-κB signaling pathway is an essential component of cellular response to stress as the main ragulator of numerous genes important for inflammation, immune response and cell survival. The family of mammalian NF-κB proteins consists of five NF-κB/Rel and four IκB proteins. NF-κB/Rel are subunits of NF-κB transcription factor acting as dimers, while IκB are their specific inhibitors. The NF-κB heterodimers play a physiological role and their activity remains under the strict control. The most common is a dimer composed of p50/RelA (p50/p65) proteins. The TNFα-stimulated canonical activation of NF-κB pathway leads to phosphorylation and proteasomal degradation of IκB protein, resulting in nuclear translocation of NF-κB and its binding to DNA in the κB motif.

Previous studies have shown that the NF-κB activation pathway is blocked by heat shock. We investigated activation of NF-κB pathway in the human lung adenocarcionoma A549 and osteosarcoma U-2 OS cell lines by Western blotting. TNFα treatment for 15 minutes led to activation of NF-κB signaling which was monitored by following the phosphorylation and degradation of IκB and phosphorylation/activation of RelA. Activation reached the maximum 15 minutes post TNFα treatment and IκB level was restored after 60 minutes in both cell lines. Dynamic live cell imaging studies with A549 cell line stably transfected with a construct coding the EGFP-RelA fusion protein confirmed that RelA was translocated to nucleus following TNFα stimulation.

Cells were subjected to heat shock in 43°C and allowed to recover for 1 hour. Then, they were treated with TNFα for 15 minutes. In U-2 OS cells we observed inhibition of TNFα-induced degradation of IκB, thus NF-κB was not activated at least up to 90 minutes post treatment. Surprisingly in A549 cell, we observed activation of the NF-κB signaling during the heat shock response, although it was weaker and delayed in time (15-30 min) comparing to the normal conditions. The living cell imaging of A549 cells with EGFP-RelA fusion protein has also shown the differences in the kinetics of RelA translocation from cytoplasm to nucleus during normal conditions or in response to heat shock after cytokine stimulation.

16. EXPRESSION OF BNIP3 IN DIFFERENT HUMAN CARCINOMA CELL LINES. RELATIONSHIP BETWEEN THE ONCOGENIC RAS AND BNIP3 EXPRESSION

Alicja Pawlak, Edyta Wysokińska, Wojciech Kałas, Ewa Zioło, Leon Strządała

Department of Experimental Oncology, Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Science, 53-114 Wrocław, Rudolfa Weigla 12, Poland.

BNIP3 (Bcl-2/adenovirus E1B 19 kDa interacting protein 3) belongs to the Bcl-2 family of proteins which are involved in the regulation of programmed cell death. It has been reported that BNIP3 is implicated in the apoptotic, autophagic and necrotic cell death. The aim of our research was to investigate the expression status of BNIP3 in different human carcinoma cell lines. Furthermore we focused on possible relationship between the oncogenic Ras and expression of BNIP3.

Our results obtained by the real-time RT-PCR (comparative C_T method) show that in DLD-1, DKs-8 and HT-29 colorectal adenocarcinoma cell lines there is no BNIP3 expression. This is in accordance with current knowledge and follow from BNIP3 promoter hypermethylation. In order to induce this expression we were using 5-aza-2'-deoxycytidine (5-aza-dC) which inhibits DNA (cytosine-5-)-methyltransferase 1 (DNMT1). As analyzed by the MTS assay addition of 5-aza-dC has no effect on vitality of cells. On the other hand are cancer cell lines which produce BNIP3 constantly and that is the case of A549 (lung) and HCT116 (colorectal) carcinoma cell lines. It is believed that continual expression of BNIP3 may contribute to survival of cells under stress conditions such as hypoxia or reactive oxygen species and this is related to autophagy.

In order to investigate the influence of oncogenic Ras on BNIP3 expression we made use of dominant-negative mutant of Ras (RasN17) and inhibitors of MEK1/2 (PD98059 and U0126). 5-aza-dC was added where there was a need to repress the hypermethylation of BNIP3 promoter. Results were examined by real-time RT-PCR. It has been observed that DLD-1 and HT-29 cells revealed significantly reduced expression of BNIP3 after treatment with either RasN17 or Ras pathway inhibitors compared to negative controls i.e. cells treated with empty vector pcDNA3 or inactive inhibitor U0124, respectively. Additionally, removal of single copy activating mutation K-Ras^{G13D} in DLD-1 cells (resulting in DKs-8 cells), which significantly reduces the activity of Ras protein, makes it impossible to induce the expression of BNIP3 by 5-aza-dC in these cells. By contrast, A549 and HCT116 cells treated with either RasN17 or Ras pathway inhibitors revealed increased expression of BNIP3 mRNA. These results suggest that oncogenic Ras may have different influence on BNIP3 expression depending on the cell type – in cells where BNIP3 is epigenetically silenced there seems to be a positive regulation of this protein expression by oncogenic Ras, whereas in cells expressing BNIP3 constantly this influence is probably negative. What is worth noting, the two of colorectal adenocarcinoma cell lines (HT-29 and HCT-116) exhibit opposite dependency between oncogenic Ras and BNIP3.

17. IDENTIFICATION OF SERUM PROTEOME COMPONENTS ASSOCIATED WITH PROGRESSION OF NON-SMALL CELL LUNG CANCER

Monika Pietrowska[1], Karol Jelonek[1,2], Malwina Michalak[1], Agnieszka Gdowicz-Kłosok[1], Małgorzata Roś[1,2], Paweł Rodziewicz[3], Klaudia Chmielewska[3], Krzysztof Polański[4], Joanna Polańska[4], Monika Giglok[1], Rafał Suwiński[1], Rafał Tarnawski[1], Rafał Dziadziuszko[5], Witold Rzyman[5], Piotr Widłak[1]

[1]Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice, Poland; [2]Polish-Japanese Institute of Information Technology, Bytom, Poland; [3]Polish Academy of Science, Institute of Bioorganic Chemistry, Poznań, Poland; [4]Silesian University of Technology, Gliwice, Poland, [5]Medical University of Gdańsk, Gdańsk, Poland.

Introduction: Implementation of novel molecular prognostic and predictive markers to support the classical TNM-based staging of non-small cell lung cancer (NSCLC) is an emerging approach of personalized treatment. The aim of the present study was to perform comparative analysis of serum of patients with different stages of NSCLC using three complementary proteomic approaches to identify proteome components associated with progression of cancer.

Methods: Serum samples were collected before any treatment from 200 patients with NSCLC, including 103 early stage, 64 locally advanced and 33 metastatic cancer samples, and from 200 donors without malignancy. The low-molecular-weight fraction of serum proteome was MALDI-profiled in all samples. Serum proteins were characterized using 2D-PAGE and LC-MS/MS approaches in a representative group of 30 donors.

Results: Several significant differences were detected between serum samples collected from patients with early stage cancer and patients with locally advanced cancer, as well as between patients with metastatic cancer and patients with local disease. Of note, serum components discriminating samples from early stage cancer and healthy persons were also detected. In general, about 70 differentiating serum proteins were identified, including inflammatory and acute phase proteins already reported to be associated with progression of lung cancer (serum amyloid A or haptoglobin). Several differentiating proteins, including apolipoprotein H or apolipoprotein A1, were not previously associated with NSCLC. No significant differences in patterns of serum proteome components were detected between patients with adenocarcinoma and squamous cell carcinoma.

Conclusions: Several biomarker candidates were identified with potential importance for molecular proteomic staging of NSCLC. Additionally, several serum proteome components revealed their potential applicability in early detection of lung cancer.

The project was supported by the National Science Center, Grant no. N402 3506 38, and National Centre for Research and Development, Project no. POIG.01.01.02-20-080/09.
KJ and MR were supported by the European Social Fund within the INTERKADRA project UDA-POKL-04.01.01-00-014/10-00.

18. PROTEOMIC PATTERNS IN CLASSIFICATION OF THYROID CANCER, A PRELIMINARY REPORT

Monika Pietrowska[1], Magdalena Kalinowska-Herok[1], Corinna Henkel[2], Hanna Diehl[2], Mykola Chekan[3], Dariusz Lange[3], Piotr Widłak[1]

[1]Center for Translational Research and Molecular Biology of Cancer, Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology Gliwice Branch, Gliwice; [2]Medizinisches Proteom-Center Ruhr-Universität Bochum AG Quantitative Proteomics, Bochum, Germany; [3]Tumor Pathology Department, Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology Gliwice Branch, Gliwice.

Thyroid cancer diagnostics are based on histopathological features. Classification of thyroid cancer is crucial for the assessment of prognosis and selection of the treatment. Unfortunately, in some cases an unequivocal classification of thyroid tumors are difficult or even impossible. There are not good molecular markers available to support the classification of thyroid tumors. Earlier genomic studies showed distinct difference in gene expression profile of major thyroid tumors types that have to be reflected in tumors proteomic profile.
Imaging mass spectrometry (IMS) technology has enabled development of high-throughput proteomic approaches in identify and simultaneously localize biomolecules in tissue sections. Also, the ability to conduct mass spectrometry based proteomic analyses on formalin fixed paraffin embedded tissues opens new opportunities for biomarker discovery using hospital biopsy libraries.

The analysis was performed on formalin fixed paraffin embedded tissues resected from patients diagnosed with thyroid follicular carcinoma, papillary carcinoma and medullary carcinoma. All patients involved in the study were treated with surgery at the Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology in Gliwice.
To find and identify specific proteomic signature for different thyroid type cancer we used two techniques: 1) proteins specific for the examined tissues were identified in lysates obtained from certain areas of the material by LC-MS/MS; 2) their spatial distribution in the tissue was analyzed using molecular imaging IMS. Results from both analytical methods, supplemented by the results of traditional morphological and histological studies, will allow the use of appropriate bioinformatics tools to develop molecular models of tissue and molecular characteristics correlate with morphological features.
Such approach allows determining the classification rules set by global molecular profile, to characterize the molecular differences between types of thyroid cancer and to identify new potential candidates for the molecular diagnosis of thyroid cancer.

The work was supported by grant no 011/03/D/NZ4/03507 and 2012/07/B/NZ4/01450 from National Science Center.

19. CELLULAR STRESS INDUCED BY UV-C IRRADIATION CAUSES P53-INDEPENDENT INHIBITION OF NF-kappaB SIGNALLING PATHWAY

K. Szołtysek[1], A. Walaszczyk[1], A. Abramowicz[1], P. Janus[1], M.Kimmel[2], P. Widłak[1]

[1]Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology; Gliwice, Poland; [2]Silesian University of Technology; Gliwice, Poland.

Ultraviolet (UV) radiation is a harmful environmental factor, which causes suppression of the immune system, chronic skin damage and cancer. Exposure to UV induces a variety of cell signalling pathways, including signal transduction pathway regulated by p53, NF-κB and MAPK (ERK, JNK and p38). Actual cellular response to UV-induced stress depends on interactions and crosstalk between different pathways. Here we aimed to analyze kinetics of activation of different signalling pathways in UV-treated cells, and to assess the effect of p53 on the NF-κB-dependent pathway.

Human colon carcinoma HCT116 cell line was used as a model in two congenic variants: either containing or lacking (bi-allelic gene knock-out) transcriptionaly competent p53. Cells were treated with the UV-C radiation (20 J/m^2) and/or incubated with TNFα cytokine (10 ng/ml for 15 minutes) to induce the classical NF-κB pathway; stimulation with the cytokine was performed at different time points after irradiation (from 1 to 24 hours after UV). Expression of genes and proteins representative for different signalling pathways was assessed by QRT-PCR and Western blotting: AKT (AKT/PKB signalling), *JUN* and ERK1/2 (MAPK signalling), *PTEN*, *PPM1D*, p53 and WIP1 (p53 signalling), *IL8, BCL3, NFKB1* and IκBα (NF-κB signalling) and HSPA2, HSPB2 and HSPH2 (the heat shock response).

In both cell variants exposed to UV (p53 positive and p53 negative cells) fast activation of AKT/PKB and WIP1 accumulation (phosphorylation level of AKT declined due to WIP1 induction) was observed, as well as accumulation of HSPs characteristic for cellular response to stress. Strong induction and accumulation of p53 was observed in cells with the wild type *TP53* gene. Activation of MAPK signalling was similar in both cell variants at the protein level (late phosphorylation of ERK1/2), however, higher expression level of *JUN* was observed in cells lacking p53.

Strong effect of UV irradiation was observed on the TNFα-induced activation of the NF-κB pathway. Pre-exposure to UV resulted in suppression of the NF-κB activation (inhibition of the IκBα inhibitor degradation and reduction of nuclear NF-κB) and marked down-regulation of NF-κB-dependent genes (e.g. *IL8, BCL3, NFKB1*). The strongest inhibitory effect was observed when UV irradiation preceded TNFα stimulation for 6 hours. Most notably, similar inhibitory effect of UV was observed in cells with the different p53 status.

We concluded that UV-related stress-response pathways interfered with the cytokine-induced activation of the NF-κB pathways in the p53-independent manner.

This work was supported by the Polish National Science Center, Grant N301-264536 and Grant DEC-2012/04/A/ST7/00353.

20. PHENOTYPIC HETEROGENEITY AND PLASTICITY OF CELLS IN MELANOSPHERES DERIVED FROM NODULAR MELANOMA SPECIMENS

Beata Talar, Małgorzata Sztiller-Sikorska, Anna Gajos-Michniewicz, Kamila Koprowska, Małgorzata Czyż

Department of Molecular Biology of Cancer, Medical University of Lodz, 6/8 Mazowiecka street, 92-215 Lodz, Poland.

Melanoma stem cells are functionally defined but not well characterized at the molecular level. Our study aims at characterization of cells derived from five surgical specimens of nodular melanoma and grown as anchorage-independent melanospheres. Gross populations analyses were performed without any selections approach employing specific markers. Differentiations and changes in morphology were registered under the microscope, self-renewing capacity was assessed by a clonogenic assay and immunophenotype was characterized by flow cytometry. Some cells in melanospheres possessed defining features of cancers stem cells as they were able to self-renew and differentiate. No correlations between proliferation rate and the number of cells with self-renewing capacity was observed. The cellular heterogeneity within melanospheres seems to be an intrinsic characteristics of particular sample and might portray the original tumor heterogeneity. When cells in melanospheres were compared with their adherent counterparts, change in immunophenotype and differentiation status evidenced a high plasticity of those cells. Hypoxic-like conditions enhanced ability of melanoma cells to form spheres. Both, the extent of immunophenotypic variability among melanoma specimens and plasticity of their phenotype, pose a significant challenge to the characterizations of melanoma stem-like cells at the molecular level.

21. ACTIVE HEAT SHOCK TRANSCRIPTION FACTOR 1 SUPPORTS MIGRATION OF MOUSE MELANOMA B16F10 CELLS

Agnieszka Toma*, Natalia Vydra, Tomasz Cichoń, Wiesława Widłak

Center for Translational Research and Molecular Biology of Cancer, Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch, Gliwice, Poland.

Heat Shock Transcription Factor 1 (HSF1) is a main regulator of the heat (stress) response. It activates heat shock genes, which encode heat shock proteins (HSPs). HSPs accumulation enables cells to survive in suboptimal conditions. Beyond the classical induction of HSPs, HSF1 binds to a broad array of non-*HSP* genes. This property of HSF1 seems to be important in processes associated with tumorigenesis.

We aimed to study the role of HSF1 in tumor growth. We have constructed a model of mouse B16F10 melanoma cells with an overexpression of constitutively active, human HSF1, with deletion of the regulatory domain (amino acids 221-315; aHSF1). Simultaneously we have constructed cells with down-regulated HSF1 expression using shRNAs specific for 3'UTR or coding sequences of mouse HSF1. The expression of aHSF1 in stably transfected cells led to activation of several inducible *Hsp* genes (*Hsph1*, *Hspb1*, *Hspa1*), what was confirmed on mRNA and protein level. Transfection with HSF1 shRNA constructs was connected with a decreased level (up to 50-70%) of HSF1 protein and inhibition of the expression of inducible *Hsp* genes. We found that expression of constitutively active HSF1 as well as reduced HSF1 expression did not affect proliferation of B16F10 melanoma cells. However cells with expression of constitutively active HSF1 were able to proliferate more efficiently in the soft agar and gave rise to larger colonies than control cells. They also migrated more effectively in the transwell migration assay. Moreover, the number of metastases in the lungs was increased after the injection of B16F10 cells with expression of constitutively active HSF1 into the tail vein of C57BL6 mice. Down-regulation of HSF1 expression did not change the ability of cells to grow in soft agar and migrate, but its potential to form lung metastasis *in vivo* was reduced. Because of aHSF1 enhanced cell migration, we decided to determine the expression profile of genes associated with cell motility using a specific RT^2PCR array. We found that transcription of several genes involved in focal adhesion (*Vcl*, *Cav1*, *Capn1*) was decreased in cells expressing aHSF1, but was not changed in cells with down-regulated expression of HSF1. At the protein level, down-regulation of VCL expression was confirmed.

We conclude that HSF1 activation might support cell motility and enhance tumor metastasis via influence on the connections between cytoskeleton of a cell and the extracellular matrix.

A.T. was supported by the European Community from the European Social Fund within the INTERKADRA project UDA - POKL-04.01.01-00-014/10-00

22. DETECTION OF TUMOR-RELATED CHANGES IN SERUM PROTEOME OF BREAST CANCER PATIENTS

Anna Walaszczyk, Katarzyna Szołtysek, Patryk Janus, Monika Pietrowska, Piotr Widłak

Maria Sklodowska – Curie Memorial Cancer Center and Institute of Oncology, Gliwice, Poland.

Background: Proteomics is the study of the proteome – a complete protein component of the cell. In contrast to the genome, the proteome is dynamic and its fluctuations depend on combination of numerous internal and external factors. Identifying and understanding changes in the proteome related to a disease development and therapy is a subject of clinical proteomics. Here we aimed to identify in the circulating blood a set of polypeptide biomarkers that could be useful for the early detection, diagnosis, prognosis and management of cancer and to correlate them with known pathological and clinical prognostic and predictive factors.

Methods: Analysis of the low-molecular-weight region of the blood proteome (using either serum or plasma samples) by mass spectrometry (MS) methods is one of the basic approaches of clinical proteomics. Although no single peptide is expected to be a reliable bio-marker in such analyses, multi-peptide sets of markers selected in numerical tests have been already shown in a few studies to have prognostic and predictive value in cancer diagnostics. In our study we have analyzed low-molecular-weight serum polypeptides (<10 kD) using MALDI-TOF mass spectrometry.

Results: Blood samples were collected in the group of 92 operable breast cancer patients before the start of therapy and during a treatment (after the surgical resection of tumors and one year after the end of therapy in a group of 70 patients diagnosed at early stages of the disease. Patients were treated with surgery either independently (26) or in combination with neoadjuvant chemotherapy (5) or adjuvant radio/chemotherapy (39), as well as in the group of 104 healthy controls matched according to age. The clinical data and pathological characteristics are presented. Specific patterns of low-molecular-weight polypeptides (2-10 kD) were identified due to mathematical analyses and cross-correlated between experimental groups. A multi-component set of polypeptides has been selected as a classifier that differentiate control and cancer samples. We identified the classifier built of four spectral components that differentiated healthy persons and breast cancer patients with ~85% specificity and sensitivity. Spectral components (i.e., protein ions) that were the most frequent in such classifier had approximate m/z value of 2866, 3579, and 2303 Da. The classifier intentionally built of above three components showed 80% specificity and 88% sensitivity. We have also observed significantly ($p=0.0003$) increased level of osteopontin in blood of analyzed group of cancer patients. However, the classifier built of osteopontin level showed 28% specificity and 88% sensitivity, and thus was outperformed by the classifier built of the most frequent spectral components identified in serum by MALDI-ToF mass spectrometry.

We found that surgical resection of tumors did not have an immediate effect on the mass profiles of the serum proteome. On the other hand, significant long-term effects were observed in serum proteome patterns one year after the end of basic treatment (we found that about 20 peptides exhibited significant changes in their abundances).

Moreover, the significant differences were found primarily in the subgroup of patients treated with adjuvant therapy, but not in the subgroup subjected only to surgery. This suggests that the observed changes reflect overall responses of the patients to the toxic effects of adjuvant radio/chemotherapy. In line with this hypothesis we detected two serum peptides (registered m/z values 2,184 and 5,403 Da) whose changes correlated significantly with the type of treatment employed (their abundances decreased after adjuvant therapy, but increased in patients treated only with surgery).

Conclusions: Here we have presented report from the project aimed to identify a set of polypeptide biomarkers that could be used for diagnostics and management of breast cancer patients. Preliminary data showed that cancer-specific multi-component polypeptide pattern could be identified in serum of breast cancer patients. However, their importance for cancer diagnostics remains to be validated.

The work was supported by ***UMO-2012/05/N/NZ4/02307.***

23. DIFFERENTIAL EXPRESSION OF microRNA IN DIVERSE PHENOTYPES OF MELANOMA CELLS

Michał Woźniak, Małgorzata Sztiller-Sikorska, Małgorzata Czyż

Department of Molecular Biology of Cancer, Medical University of Lodz, Poland.

Melanoma is a highly heterogeneous tumor strongly dependent on microenvironment. Melanoma cells can grow *in vitro* in anchorage-independent manner in a serum-free medium, however, when serum is introduced to the medium they switch to monolayer phenotype. The mechanism of this phenomenon is not clear, however, the role of microRNAs (miRNAs) is taken into consideration. MiRNAs have been found to control many important molecular processes and signaling pathways including differentiation, epithelial-mesenchymal transition or self-renewal, and even slight changes in their expression or activity can drastically alter cancer cell biology.

We aimed to evaluate the relationships between changes in phenotype triggered by different growth conditions and differentially expressed miRNAs. The miRNA expression analysis was performed with miRNA microarray, and obtained data were validated with qRT-PCR method. Performed analysis revealed that 19 miRNAs were differentially expressed between cells grown as monolayers and anchorage-independent spheres Bioinformatics analysis of differentially expressed miRNAs pointed to their involvement in many signaling pathways including Transcriptional misregulation in cancer, MAPK signaling pathway or Pathways in cancer. Moreover, we found chromosome 19 miRNA cluster (C19MC) upregulated in cells with non-adherent growth. C19MC is frequently upregulated in cells with stem-like properties, which indicates the enrichment of melanoma stem cells in serum-free culture.

This study identifies several microRNAs, which can be responsible for phenotypic changes in melanoma cells, and provides a rationale for subsequent analysis of their role in melanoma development and progression

24. MUTATIONS TRUNCATING THE C-TERMINUS OF WIP1 PHOSPHATASE CAN CODE FOR HYPERACTIVE PROTEIN AND CAN BE FOUND IN DNA FROM BLOOD OF LUNG CANCER PATIENTS

Artur Zajkowicz[1], Dorota Butkiewicz[1], Iwona Matuszczyk[1], Rafał Suwiński[2] Marek Rusin[1]

[1]Center for Translational Research and Molecular Biology of Cancer, [2]II Clinic of Radiotherapy and Chemotherapy, Maria Skłodowska-Curie Memorial Center and Institute of Oncology, Gliwice Branch, 44-101 Gliwice, ul. Wybrzeże Armii Krajowej 15, Poland.

The WIP1 phosphatase coded by the *PPM1D* gene is a negative regulator of proteins activated by double-strand breaks of DNA (e.g. ATM, CHK2, p53). These proteins, coded by tumor suppressers, induce the cell cycle arrest or apoptosis of cells with damaged DNA. Hence, *PPM1D* is considered a proto-oncogene. Recently, we and independently other researchers found a nonsense mutation of *PPM1D* gene in U-2 OS cell line (458:Arg>STOP). The mutant gene codes for protein truncated at the carboxyl terminus what suggested that the mutation may result in formation of a non-functional protein. However, the mechanistic studies performed by others indicated that this mutation codes for WIP1 phosphatase hyperactive towards some of its substrates. Our analyses demonstrated that the expression of the mutant cDNA of WIP1 induces strong dephosphorylation of serine 15 of p53 protein. Unexpectedly, dephosphorylation of p53 does not modulate the expression of p21 – the cell cycle inhibitor protein coded by the p53-inducible gene. Thus, our experiment confirms that the mutant WIP1 from U-2 OS cells is hyperactive, however the biological outcome of its increased activity remains elusive. Interestingly, truncating mutations of *PPM1D* in DNA from lymphocytes of breast or ovarian cancer patients were found by others; these mutations were not common (0,3% of cases) but were more frequently discovered in patients with ovarian (1,1 %) than with breast cancer (0,26%). Interestingly these mutations were not inherited but were formed as the mosaic mutations. In order to find out if similar mutations are present in blood of lung cancer patients, we sequenced exon 6 of *PPM1D* from DNA samples of approximately 300 patients. We found the truncating mutations in about 1% of cases. This is the first report of truncating *PPM1D* mutations in blood from individuals with lung cancer. Interestingly, in one sample the mutant DNA constituted a substantial fraction (approximately 40%) of DNA preparation suggesting that large number of white blood cells have hyperactive WIP1 protein. The clinical implications of this phenomenon are not known but potentially they may be serious.

This work was supported by the Polish Ministry of Science and Higher Education (Grant no. NN-401-597240, to MR) and by "Silesian BIO-FARMA" project POIG.02.01.00-00-166/08.
AZ was supported by the European Community from the European Social Fund within the DoktoRIS project.

25. LOSS OF PROTEIN EXPRESSION AND RECURRENT DNA HYPERMETHYLATION OF THE *GNG7* GENE IN SQUAMOUS CELL CARCINOMA OF THE HEAD AND NECK

Natalia Zemke[1], Sylvia Hartmann[2], Marcin Szaumkessel[1], Itziar Salaverria[3], Ronald Simon[5], Guido Sauter[5], Katarzyna Kiwerska[1], Wojciech Gawecki[4], Magdalena Bodnar[6], Andrzej Marszalek[6], Julia Richter[3], Damian Brauze[1], Malgorzata Jarmuż-Szymczak[1], Martin-Leo Hansmann[2], Reiner Siebert[3], Krzysztof Szyfter[1,4], Maciej Giefing[1]

[1]Institute of Human Genetics, Polish Academy of Sciences, Poznan, Poland; [2]Institute of Pathology, University Hospital, Frankfurt, Germany; [3]Institute of Human Genetics, Univ. Hospital Schleswig-Holstein Campus Kiel, Christian-Albrechts University, Kiel, Germany; [4]Department of Foniatry and Audiology, University of Medical Sciences, Poznan, Poland; [5]Institute of Pathology, University Hospital Hamburg-Eppendorf, Hamburg, Germany; [6]Department of Clinical Pathomorphology, Collegium Medicum, Bydgoszcz, Poland.

The *GNG7* gene (guanine nucleotide binding protein 7) encodes one of three subunits of G protein – a known transmembrane signaling protein. GNG7 (subunit γ) plays a major role in signal transmission and may regulate many signal transducting pathways including "growth arrest induced by cell contact" - a mechanism evolved in multicellular organism to prevent uncontrolled cell proliferation. Recently, several publications reported *GNG7* down-regulation in pancreatic cancer and tumors of the gastrointestinal tract. This together makes *GNG7* an interesting candidate for a new tumor suppressor gene inactivated in cancer.

To test this hypothesis, in the present study 10 laryngeal squamous cell carcinoma cell lines (LSCC) were analyzed by array-CGH to detect copy number alterations of the *GNG7* locus, and coding exons of *GNG7* were sequenced to identify potential mutations. Expression profiling in cell lines was performed by microarray and confirmed by RT-PCR for the analyzed gene (mRNA expression). Moreover, we examined 188 primary squamous cell carcinomas of the head and neck for the GNG7 protein expression, by immunohistochemical staining, and analyzed for *GNG7* promoter methylation in the 10 cell lines as well as 15 primary tumors by bisulfite pyrosequencing.

Expression profiling showed statistically significant, down-regulation of *GNG7* in 6/10 cell lines, out of which two harbored a heterozygous deletion of the *GNG7* locus. Sequencing of the coding exons did not reveal any mutations. In primary cases immunohistochemical stainings demonstrated loss of the GNG7 protein in 68/188 cases (36%). Moreover, by bisulfite pyrosequencing, we have detected hypermethylation of the *GNG7* promoter region in 7/10 (70%) cell lines and 6/15 (40%) primary tumors. Loss of *GNG7* was significantly associated with promoter hypermethylation ($p<0.001$).

Taking together, our results show frequent loss of *GNG7* expression both, in laryngeal cancer cell lines as well as primary head and neck tumors, that may suggest a role in laryngeal cancer pathogenesis. We suggest, moreover, epigenetic silencing as major molecular mechanism responsible for *GNG7* down-regulation.

Cancer Therapy and New Therapeutics

26. THE EFFECT OF BAICALIN ON ANTICANCER ACTIVITY OF DOXORUBICIN IN MCF-7 HUMAN BREAST CANCER CELLS

J. Bernasińska, P. Lewarska, I. Jędrzejewska, A. Koceva-Chyła

Department of Thermobiology, Institute of Biophysics, Faculty of Biology and Environmental Protection, University of Lodz, 90-237 Łódź, Pomorska St. 141/143, Poland.

Doxorubicin (DOX) has a broad spectrum of activity and is one of the most effective chemotherapeutic drug in breast cancer therapy. The clinical utility of this anthracycline is, however, limited by the toxic side effects to healthy tissues and the development of cardiotoxicity. Therefore, elaboration of more effective alternative strategies that increase the therapeutic efficacy and minimize the systemic toxicity of anticancer drugs remains an outgoing challenge. In an effort to develop such effective strategies, most of them are being directed toward the investigation of dietary supplements and phytotherapeutic agents known for their anticancer properties and low toxicity to search for their beneficial therapeutic effects when used in combination with anticancer drugs.

Scutellaria baicalensis is a herb widely used in traditional Chinese medicines, because of its broad spectrum of pharmacological activity. Molecular mechanisms of *Scutellaria baicalensis* activity are diverse and include: antioxidant, anti-inflammatory, cytotoxic, anticancer and proapoptotic activity. The major constituents of *Scutellaria baicalensis* are flavonoids baicalin (BLIN), baicalein (BLEIN), wogonin (W) and wogonoside, which shows various of biological activity.

In this study we have evaluated the effect of baicalin (BLIN) on the anticancer activity of doxorubicin in MCF-7 human estrogen-dependent breast cancer cells. The cells before their incubation with IC_{50} concentration of DOX (2 h) were preincubated for 24 hours with IC_{50} concentration of BLIN. MCF-7 cell viability was evaluated by a reduction of 3-(4,5-dimethyl-2-thiazol)-2,5-diphenyl-2H-tetrazolium bromide (MTT). Mitochondrial membrane potential was estimated with a fluorescent carbocyjanine dye JC-1, reactive oxygen species were measured with dihydroethidium (DHEt) fluorescent probe and the lipid peroxidation with C11-BODIPY$^{581/591}$ fluorescent probe.

On the basis of our results we assume that preincubation with investigated flavonoid enhance cytotoxicity of doxorubicin in cancer cells. Moreover, baicalin showed antioxidant properties and reduced amount of reactive oxygen species and lipid peroxidation generated by doxorubicin in breast cancer calls.

In conclusion, the results of our study suggest that chemotherapy based on a combination of previously well-known and widely used cancer drugs such as doxorubicin with main flavonoids obtained from the root of *Scutellaria baicalensis Georgi,* especially baicalin, might be a new approach for improving doxorubicin therapy through decreasing its effective dose, side effects and patients′ mortality.

27. C-1748 IS HIGHLY ACTIVE IN PANCREATIC CANCER CELL LINE MIAPACA-2 AND INDUCES CELL CYCLE ARREST AND APOPTOSIS

Barbara Borowa-Mazgaj, Ewa Augustin, Jerzy Konopa[1] Zofia Mazerska

Department of Pharmaceutical Technology and Biochemistry, Gdańsk University of Technology, Chemical Faculty, ul. Narutowicza 11/12, 80-233 Gdańsk, Poland.

Pancreatic cancer is the fifth leading cause of cancer death and has the lowest survival rate of any solid cancer in the industrial countries. The poor prognosis of pancreatic cancer results from its tendency for late presentation, aggressive local invasion, early metastasis, and poor response to chemotherapy. Only 1-4% of patients with adenocarcinoma of the pancreas are alive 5 years after diagnosis. Currently, gemcitabine remains the best chemotherapeutic agent available for the treatment of advanced pancreatic cancer. However, gemcitabine treatment results in an objective tumor response rate of <10% and only a marginal survival advantage. Thus there is a strong need for the continual development of novel therapeutic agents to improve pancreatic cancer therapy.

The compound 9-(2′-hydroxyethylamino)-4-methyl-1-nitroacridine, (Capridine ß), C-1748 is the most active derivative of 1-nitroacridine antitumor agents developed in our laboratory. Strong cytotoxic activity against colon cancer cell lines (HCT8 and HT29) and high antitumour activity against xenografts in nude mice of prostate (LnCaP, TSU) and colon carcinoma (HCT8), along with low mutagenic potential and slight myelosuppressive properties allowed the selection of C-1748 for preclinical studies.

The aim of the current study was to investigate and characterize the cellular response of human pancreatic cancer cell line MiaPaca-2 to C-1748 treatment. This cancer cell line was selected due to its high sensitivity to C-1748 (EC_{80} value 0,037 μM). Experiments were performed at EC_{80} concentration of the drug up to 144 h.

Cell cycle analysis revealed that between 24 h and 48 h of C-1748 treatment, MiaPaca-2 cells underwent transient accumulation in the G2/M phase which was followed by prolonged arrest in the G1 phase. Starting from 96 h of drug exposure, decrease in G1 phase population was accompanied by progressive increase in sub-G1 fraction (78% after 192 h), suggesting that initial G1 arrest led to cell death through apoptosis.

Morphological changes of MiaPaca-2 cell line in response to C-1748 treatment were observed using fluorescent microscopy. DAPI staining revealed that cells exposed to C-1748 exhibited features characteristic for apoptosis, like condensed chromatin and apoptosis-body like structures. The drug induced apoptosis in time- and dose-dependent manner. Flow cytometry analysis indicated that caspase-3 activation, typical for apoptosis was detectable already after 24 h of treatment and after 144 h percentage of caspase-3-positive cells increased to 50 % of total population. Preliminary results from acridine orange staining have shown that C-1748 did not induce autophagy even after prolonged drug treatment. Similarly, MiaPaca-2 cells did not undergo accelerated senescence, although such effect was previously observed in colon cancer cells.

To sum up, major cellular response triggered by C1748 in pancreatic MiaPaca-2 cells was the effective induction of apoptosis. These results highlight the therapeutic potential of C-1748 in pancreatic cancer and support rationale for its further investigation towards this type of malignancy.

28. RELATIONSHIP BETWEEN POLYMORPHISMS IN OXIDATIVE STRESS-RELATED GENES AND THERAPY OUTCOME IN NON-SMALL CELL LUNG CANCER

D. Butkiewicz[1], M. Krześniak[1], M. Giglok[2], A. Drosik[2,3], A. Milewska[1], I. Matuszczyk[1], M. Rusin[1], R. Suwiński[2]

[1]Center for Translational Research and Molecular Biology of Cancer, [2]II Clinic of Radiotherapy and Chemotherapy, [3]Department of Clinical Oncology, M. Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch, 44-101 Gliwice, Poland.

Ionizing radiation and platinum analogs generate reactive oxygen species (ROS) that cause DNA strand breaks and oxidative damage in cancer cells. The activity of key antioxidant enzymes, such as e.g. glutathione S- transferases (GSTs), protects cells from this damage. GSTs are a large family of enzymes expressed in many tissues and participating in detoxification of products derived from oxidative stress and exposure to various carcinogens. It has been observed that high levels of GSTs correlated with less favorable response to ROS-inducing anticancer treatments. Thus, one may assume that reduced levels of these antioxidant enzymes, e.g. caused by functional polymorphisms in *GST* genes, might be associated with a greater cytotoxicity and better survival of patients after radio- and/or Pt-based therapy. Some polymorphisms in *GST* genes have been implicated in increased lung cancer risk. Their possible impact on therapy results in lung cancer has been poorly studied, yielding inconsistent results. Thus, the aim of our study was to evaluate an association of the common *GSTP1* Ile105Val, *GSTM1* del and *GSTT1* del polymorphisms with therapy outcome in non-small cell lung cancer (NSCLC).

A group of 344 patients with inoperable NSCLC treated with radiotherapy and Pt-based chemotherapy was investigated using PCR-RFLP. Survival curves were determined with Kaplan-Meier method and compared by log-rank test. HRs and ORs (95% CI) were estimated using uni- and multivariate Cox proportional hazards or logistic regression models.

The *GSTP1* Val allele was associated with lower risk of progressive disease (PD) in response to chemotherapy (P = 0.027) and better overall survival (OS) in univariate model (P = 0.055). In uni- as well as in multivariate models, carriers of at least one *GSTP1* Val allele and *GSTM1* deletion showed longer progression-free survival (PFS) than other patients (P = 0.016). Also, *GSTM1* gene deletion alone was associated with better PFS in multivariate analysis (P = 0.047). Our results demonstrate that selected polymorphisms in *GST* genes may be predictors of response to chemotherapy and serve as prognostic markers in NSCLC patients after radio- and radiochemotherapy.

This work was supported by the Polish National Science Centre (NCN) grant no. 2012/05/B/NZ5/01905 to D.B.

29. HUMAN-GYROVIRUS-APOPTIN TRIGGERS THE MITOCHONDRIAL PATHWAY OF APOPTOSIS FOR ITS CANCER CELL SELECTIVE TOXICITY

Wiem Chaabane

Department of Experimental and Clinical medicine, Linkoping university, IGEN, S-58185, Linkoping,Sweden.

The Human gyrovirus derived protein Apoptin (HGyv-Apoptin) a homolg of the chiken anemia virus apoptin (CAV-Apoptin), a protein with high cancer cells selective toxicity, trigger apoptosis selectively in cancer cells. The mechanism by which Hgyv-Apoptin accomplish its cytotoxic effect is completely unknown. In the present study, we show that HGyv-Apoptin induces apoptosis via the activation of the mitochondrial intrinsic pathway, it induces both mitochondrial inner and outer membrane permebilization, caracterised by the loss of the mitochondrial potential and the release in the cytoplasm of the pro-apoptotic molecules including apoptosis inducing factor and cytochrome c. HGyv-Apoptin acts via the apoptosome, as lack of expression of APAF1 in Mouse embryonic fibroblast strongly protected the cells from HGyv-Apoptin induced apoptosis. This work provides a first insight into the mechanism of Hgyv-Apoptin selective toxicity toward cancer cells.

30. GPR30 – A NOVEL MEMBER OF ESTROGEN SIGNALING PATHWAY AS POTENTIAL NEW THERAPEUTIC TARGET FOR ENDOMETRIAL CANCER

Adam I. Cygankiewicz, Katarzyna Kolanowska, Wanda M. Krajewska

Department of Cytobiochemistry, Faculty of Biology and Environmental Protection, University of Lodz, Pomorska 141/143 90-236 Lodz, Poland.
e-mail: adam.c@biol.uni.lodz.pl

Estrogen receptors play the key role in estrogen signaling. They are divided into classical (nuclear) receptors (i.e. ERα and ERβ) and membrane estrogen receptor GPR30.

GPR30 receptor belongs to the family of seven-transmembrane G protein-coupled receptors (GPCR). In response to 17-β-estradiol GPR30 stimulation, kinases activation, calcium mobilization, cAMP production occurs, which leads to the rapid transcriptional activation of genes. In addition to 17-β-estradiol, some xenoestrogens were characterized as GPR30 ligands. These include phytoestrogens (e.g. genistein), pesticides (e.g. atrazine), bisphenol A, drugs (tamoxifen) and synthetic compounds (e.g. G-1, G36).

The aim of the work was to assess the expression of GPR30 as compared to ERα and ERβ receptors in endometrial cancer of different degree of neoplasia and to identify the molecular mechanisms responsible for the GPR30 expression aberrations.

In endometrial cancer as compared to normal endometrium up-regulation of mRNA expression of not only canonical estrogen receptors ERα and ERβ, but also GPR30 receptors was shown. It was also noticed that the level of GPR30 mRNA increase is associated with tumor grade.

Lower level of methylation of two CpG islands in the promoter region of the GPR30 receptor gene in endometrial cancer comparing to normal material was found. It suggests that one of the mechanisms responsible for the up-regulation of GPR30 receptor gene expression in endometrial cancer may be the decrease of methylation of CpG islands in the GPR30 promoter region.

31. THE EFFECT OF THE NEW ANTHRACYCLINE DERIVATIVE WP 631 ON THE OVARIAN CANCER CELLS

Marta Denel, Arkadiusz Gajek, Agnieszka Marczak

Department of Thermobiology, *University of Łódź, Poland.*

Anthracycline antibiotics are one of the most effective anticancer drugs. They are widely used in many cancer diseases, included solid tumors and haematological disorders. In spite of its high therapeutic effectiveness, the anthracyclines' clinical usage is limited by many series adverse effects. The most dangerous are cardiotoxicity, which can finally lead to the severe cardiomyopathy [1]. There is an urgent need to develop new effective anticancer drugs. WP 631 is a new anthracycline analog, composed of two daunorubicin molecules. Monomers are linked by *p*-xenyl group. WP 631 exhibit higher DNA binding affinity than doxorubicin.

The aim of our studies was to investigate the effect of the WP 631 on the ovarian cancer cell line SKOV-3 (*human ovarian adenocarcinoma)*. Firstly, ability of WP 631 to induce apoptosis and necrosis was examined (double staining with Annexin V and propidium iodide, PARP cleavage). Additionally, DNA damage was studied: comet assay, DNA laddering and TUNEL (terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate biotin nick-labeling) assay. The effects were compared with doxorubicin (DOX), a first generation anthracycline.

Our study provides further information to explain the nuclear events induced by WP 631. According to the data, WP 631 induced both apoptotic and necrotic cell death, but in comparison to DOX, the percentage of apoptotic cells remained at a higher level. The percentage of Annexin V-positive and PI-negative cells reached the highest value at 48 h.
Basing on comet assay outcomes we indicated, that WP 631-induced DNA damages were significantly greater than those induced by DOX. Typical for apoptosis DNA fragmentation (DNA ladder) was obtained after long-lasting (24 and 48 hours) incubation with DOX. In the same experimental conditions this effect was not achieved after using WP 631, but we noted the PARP cleavage. Interestingly, the significantly greater number of TUNEL-positive cells were observed after exposure on DOX than after incubation with new analog.
Our results suggest, that WP 631 seems to be very promising anticancer agent, but still there is a need to further investigate the way of its action.

References:
[1] Hrdina R. et al. (2000) Anthracycline-induced cardiotoxicity. *Acta Medica* **43,** 75-82.

This study was supported by Grant no. N N405 100939 of the Ministry of Science and Higher Education (Poland).

32. CHEMOIMUNOTHERAPEUTICAL APPROACH TO ENHANCE CELL DEATH IN NEUROBLASTOMA CELL LINES

Małgorzata Durbas, Irena Horwacik, Elżbieta Boratyn, Hanna Rokita

Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Gronostajowa 7, 30-387 Kraków, Poland.

One of the most significant target molecules for neuroblastoma (NB) therapy is **GD2 ganglioside** (GD2), an antigen that is highly expressed on NB cells with limited distribution on healthy tissues. GD2 constitutes an ideal target for both active and passive immunotherapy. Our recent studies showed that the GD2-specific 14G2a monoclonal antibody (mAb) exhibits cytotoxic effects on IMR-32 cell line by induction of apoptosis. Another important target molecule is **aurora A kinase** that regulates key stages of mitosis and is overexpressed in various human cancers, including NB. We aimed our efforts at exploring effects of GD2 and aurora A - targeted approach on several NB cell lines because novel therapeutic approaches are needed to improve survival of high-risk NB patients.

We used immunoblotting method to evaluate the response of several human neuroblastoma cell lines to the anti-GD2 14G2a mAb. We showed that the mAb decreases all three aurora kinases expression and phosphorylation in IMR-32, LA-N-1 and CHP-134 cells.

Additionally, we studied expression levels of several substrates of Aurora A kinase such as MYCN, P53 and PHLDA1. It was shown that downregulation of aurora A kinase by the therapeutic antibody is associated with decreased levels of MYCN protein in cytoplasm, and induced expression of PHLDA1 and P53 proteins.

Our further studies focused on the possibility of combining the GD2 specific 14G2a mAb with a new Aurora A inhibitor (MK-5108). It was shown that MK-5108 specific aurora A kinase inhibitor decreases neuroblastoma cell survival, and when used in combination with the mAb, significantly potentiates cytotoxicity against IMR-32, CHP-134, and LA-N-5 neuroblastoma cells *in vitro* as determined by measurements of cellular ATP content.

We performed additional experiments with 13-*cis* retinoic (RA) used alone or in combination with the mAb. We observed that heterogeneous effects were obtained with RA alone and in combinations with the mAb on the six neuroblastoma cell lines tested. The combination of the antibody and the RA further enhances the antitumor activity compared to both agents applied alone, as shown for four cell lines out of six tested.

The results of these studies confirmed the advantage of combining targeted immunotherapy and chemotherapy in cell death enhancement in cultures of neuroblastoma cell lines. Therefore, such approach should be acknowledged in broadening of current treatment strategies to improve in future the outcome of NB patients.

The study was supported by grant no. 2012/07/B/NZ1/02808 from the Polish National Science Center.

33. TOXICITY OF EPIRUBICIN AND TRASTUZUMAB TO HUMAN CARDIOMYOCYTES AND BREAST CANCER CELLS *IN VITRO*

Michalina Gramatyka

Foundation of Cardiac Surgery Development, Zabrze, Poland.

Several factors used in anticancer therapy (e.g. ionizing radiation and anthracyclines) reveal cadiotoxic activity and oncological treatment is frequently followed by malfunction of cardiovascular system. Combining potentially cardiotoxic therapeutic agents, commonly used in combination therapy, may result in escalation of toxic side effects. At presence monitoring damage to cardiac muscle is practiced during anticancer treatment – it allows optimal dose management but does not prevent damage to the myocardium. Use of cardioprotectants that will protect cardiovascular system from damage is an emerging concept in anticancer treatment. Here we analyzed and compared the toxic effect of epirubicin and trastuzumab on cardiomyocytes and breast cancer cells.

Human cardiac cells and BT-474 cells (with HER2 overexpression) were incubated with epirubicin (concentration range 0,5 – 100 μg/ml), trastuzumab (concentration range 1 - 150 μg/ml) or combination of both. Cell viability was assessed by XTT test.

Incubation of cells with epirubicin and trastuzumab had different effect on cardiomyocytes and BT-474 cells. Concentration of epirubicin equal to 5 μg/ml reduced viability of cardiomyocytes by 40% and had no effect on BT-474, while concentration 10 μg/ml reduced viability of cardiomyocytes by 60% and of BT-474 by only 15%. This shows that BT-474 are more resistant to epirubicin treatment than cardiomyocytes. Interestingly incubation of both cells with trastuzumab did not result in reduction in cell viability in tested concentration range. Incubation of cells with combination of epirubicin and trastuzumab slightly reduced cell viability, but the difference was not statistically significant.

This work was supported by the National Science Centre, Grant UMO-2012/05/N/NZ7/01936.

34. GENISTEIN DERIVATIVE RAM-5 ACTS AS RADIOSENSITISER IN CANCER CELL LINES THROUGH EGFR AND TOPOISOMERASE II ACTIVATION INHIBITION

Aleksandra Gruca[1,2], Ania Mucha[2], Anita Miczka[2], Wiesław Szeja[2], Zdzisław Krawczyk[1,2], Aleksandra Rusin[1]

[1]Center for Translational Research and Molecular Biology of Cancer. Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch, Gliwice, Poland; [2]Department of Organic Chemistry, Bioorganic Chemistry and Biotechnology, Silesian Technical University, Gliwice, Poland.

Background: Isoflavonoids, non-toxic products of natural origin, were reported to enhance the effects of radiotherapy in cell culture and animal models. One of the early observations relevant to possible anticancer application of genistein was its inhibitory activity on topoisomerase II and tyrosine kinases, including epidermal growth factor receptor: EGFR. Topoisomerase inhibition leads to cell division blockage. EGFR modulates critical molecular prosurvival programs, including: proliferation, migration, adhesion and inhibition of apoptosis. EGFR has been shown to be highly overexpressed in many types of cancers including lung and colon. Furthermore, radiotherapy increases EGFR phosphorylation level resulting in worsened treatement outcome. There are different EGFR-dependent mechanisms of radioresistanse: (i) EGFR nuclear translocation and interaction with DNA-PK, enzyme involved in DNA repair; (ii) activation of PI3K/AKT signaling pathway leading to suppression of DNA damage–induced apoptosis; (iii) activation of reaction cascade downstream of MAPK or STAT5 leading to rapid repopulation. Inhibition of EGFR activity may improve effectiveness of radiotherapy which motivates many researchers to develop specific inhibitors of its kinase activity. The coexisting influence on topoisomerase II make genistein a promising agent for combined chemoradiotherapy treatement. The relative simplicity of genistein molecule structure and many possibilities of its derivatization offers ample space for obtaining derivatives with improved activity or affecting new molecular targets.

Aim: The aim of the project was to get deeper understanding of the mechanism of radiosensitization of cells by genistein and its derivatives associated with EGFR inhibition and coexisting topoisomerase II inhibition. Important aspect of this project was the selection of most potent radiosensitizer.

Results: Our studies revealed that certain novel derivatives of genistein inhibit cancer cell growth of HCT-116 and DU-145 lines much stronger than the parent drug. We have selected a compound able to inhibit both: topoisomerase II and EGFR activation and moreover to lead to EGFR downstream signaling pathways including pAKT and pMAPK level decrease after ionizing irradiation and thus synergistically potentiate clonogenic cell death after irradiation.

Conclusion: Our experiment delivered rationale for use of RAM-5 genistein sugar derivative to enhance the effect of cancer irradiation by targeting EGFR and topoisomerase II activation.

35. EVALUATION OF EFFICACY OF RKI-123, NOVEL POTENT GENISTEIN DERIVATIVE IN THREE-DIMENSIONAL MODEL OF COLORECTAL CANCER

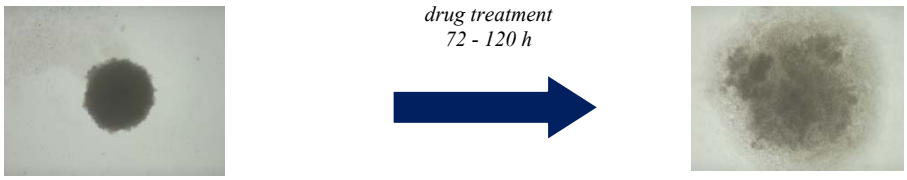
Radosław Kitel[1,2], Łukasz Filipczyk[2], Aleksandra Rusin[2], Wiesław Szeja[1]

[1]Organic, Bioorganic Chemistry and Biotechnology Department, Faculty of Chemistry, Silesian University of Technology, Krzywoustego 8 Str, 44-100 Gliwice; [2]Center for Translational Research and Molecular Biology of Cancer, Maris Skłodowska-Curie Memorial Institute of Oncology, Branch Gliwice, Wybrzeze AK 15, 44-101 Gliwice, Poland.

Two-dimensional cell cultures are nowadays gold standard in anticancer drug evaluation however they do not reflect the complexity of tumor environment *in vivo*. This often leads to failure of some drug discovery programs. Therefore modern drug discovery process requires novel screening tools and models that mimics tumor biology better than monolayer cultures. In order to minimize cost and improve overall efficacy of entire drug discovery process novel simple and high-throughput assays are required.

In our research program on development of novel isoflavone derivatives with high proapoptotic activity we used multicellular tumor spheroid (MCTS) model in order to determine activity of most potent molecules in conditions similar to those *in vivo*. Uniform in size MCTS were obtained from suspension of HCT-116 and HT-29 cells using ultra-low attachment 96-well plates. After 4 days from establishment, MCTS were exposed to different concentrations of selected compounds for indicated period of time. The efficacy of compounds was assessed in terms of ability to decreasing spheroid volume and cell viability, as indicated by light microscopy and viability assays. We compared the results with several drugs used in clinical practice, for example erlotinib (Tarceva), an EGFR tyrosine kinase inhibitor and antimetabolite 5-fluorouracil. Additionally we performed analysis for parent compound, genistein. Novel compounds promotes cell death both in monolayer and MTCS cultures, although to achieve comparable effects higher concentrations were needed in MTCS.

Our results clearly shows that introducing of MCTS model in high-throughput manner in drug discovery pipelines is very convenient method for selecting of drug candidates prior *in vivo* assays. MCTS are an appropriate model for studying tumor response to novel therapeutic agents and they bridges the gap between monolayer and *in vivo* models.



The project is realized within the VENTURES programme (VENTURES/2012-9/6) of Foundation for Polish Science, cofinanced from European Union, Regional Development Fund.

36. RKI-123 – A NOVEL GENISTEIN DERIVATIVE PROMOTES APOPTOSIS IN HUMAN COLORECTAL CANCER CELLS RESISTANT TO ERLOTINIB

Radosław Kitel[1,2], Łukasz Filipczyk[2], Aleksandra Rusin[2], Wiesław Szeja[1]

[1]Organic, Bioorganic Chemistry and Biotechnology Department, Faculty of Chemistry, Silesian University of Technology, Krzywoustego 8 Str, 44-100 Gliwice; [2]Center for Translational Research and Molecular Biology of Cancer, Maria Skłodowska-Curie Memorial Institute of Oncology, Branch Gliwice, Wybrzeze AK 15, 44-101 Gliwice, Poland.

Natural products and their derivatives still predominate as lead structures for design antitumor drugs. Among thousands compounds screened isoflavone genistein is one of the most investigated molecules. However introduction of genistein into clinic practice is rather doubtful due to low bioavailability. Nevertheless, it is well known that proper chemical derivatization may change pharmacokinetic profile of molecule. Indeed two genistein derivatives, KBU2046 and AXP-107-11 have entered clinical trials, first for treatment prostate cancer, the latter for pancreatic ductal adenocarcinoma.

Our research program on developing novel genistein derivatives lead us to discovery of novel potent compound with ability to inducing cell death. Among several compounds the most prominent effects were obtained for RKI-123. This novel derivative reduced cell viability in HCT-116 and HT-29 cell lines. Analysis of subG1 population in cell cycle, annexinV-FITC/PI double staining and dissipation of mitochondrial potential ($\Delta\psi$) done by flow cytometry revealed that this compound induced cell death in HCT-116 cell line. Since RKI-123 was designed as inhibitor of EGFR tyrosine kinase we compared results with FDA-approved tyrosine kinase inhibitor – erlotinib (Tarceva). In contrast to RKI-123, erlotinib did not trigger cell death in HCT-116 cell line.

We conclude that chemical derivatization of genistein supported by computer-assisted drug design methods lead to novel chemical entities with ability to induce cell death in submicromolar concentration several times lower than parent compound.

The project is realized within the VENTURES programme (VENTURES/2012-9/6) of Foundation for Polish Science, cofinanced from European Union, Regional Development Fund.

37. CASPASE-3/CASPASE-8-DEPENDENT DEATH OF LEUKAEMIA SENSITIVE HL60 AND MULTIDRUG RESISTANT HL60/MX-2 CELLS INDUCED BY SELECTED PYRIDINIUM SALTS

Dorota Kostrzewa-Nowak, Jolanta Tarasiuk

Department of Biochemistry, Faculty of Biology, University of Szczecin, 3c Felczaka St, 71-412 Szczecin, Poland.

An important goal of experimental chemotherapy is the development of novel anticancer drugs retaining a high activity against tumours resistant to classic chemotherapy. Current data show an important role of reactive oxygen species (ROS) in triggering programmed cancer by apoptosis or lysosome membrane permeabilization-dependent mechanisms.

In our previous study, we have demonstrated that pyridinium salts: 1-methyl-3-nitropyridinium salt (MNP) and 3,3,6,6,10-pentamethyl-3,4,6,7-tetrahydro-[1,8(2H,5H)-dion]acridine salt (MDION) exhibit a comparable activity against human promyelocytic sensitive leukaemia HL60 cell line and its multidrug resistant subline HL60/MX-2 characterized by the presence of mutated α isoform of topoisomerase II and absence of the β isoform of this enzyme. Moreover, it was evidenced that studied pyridinium salts were able to shift NAD(P)H/NAD(P)$^+$ equilibrium in cancer cells and highly increase the cellular level of ROS. It was also observed that MDION caused lysomal membrane disruption in HL60 and HL60/MX-2, in contrast to MNP that influenced only the lysomal membrane integrity of HL60/MX-2 cells.

The aim of this study was to examine the effect of pyridinium salts (MNP, MDION) on inducing caspase-3/caspase-8-dependent apoptosis of human promyelocytic leukaemia sensitive HL60 cell line and its multidrug resistant HL60/MX-2 subline. Cell cycle distribution analysis was performed with the use of propidium iodide. The activity of caspase-3 and caspase-8 was measured using membrane-permeant, fluorescent inhibitor-based FLICA caspase probes. All measurements were made with the aid of BD FACSCalibur flow cytometer.

It was showed that MNP used at IC_{90} caused significant increase in the percentage of subdiploid (sub-G1) cells as well as caspase-3 and caspase-8 activity in both sensitive HL60 and resistant HL60/MX-2 cells. In the case of MDION used at IC_{90}, a slight G2/M arrest was firstly observed followed by the appearance of sub-G1 subpopulation of both studied sensitive and resistant cells. MDION used at this concentration caused an important increase in caspase-3 and caspase-8 activity in HL60 and HL60/MX-2 cells.

The obtained results indicate that studied pyridinium salts, MNP and MDION, are able to induce apoptotic cell death of leukaemia sensitive HL60 and multidrug resistant HL60/MX-2 cells by inducing the intrinsic and extrinsic pathways occurring with the caspase-3 and caspase-8 involvement.

This study was supported by the Faculty of Biology, University of Szczecin, Poland (Grant no. 504-1000-240-847).

38. IMPACT OF BIOREDUCTIVE ACTIVATION OF ANTITUMOUR ANTHRACYCLINE DRUGS, DOXORUBICIN AND PIRARUBICIN, BY NADPH CYTOCHROME P450 REDUCTASE ON THEIR APOPTOTIC STIMULI PROPERTIES IN REGARD TO SENSITIVE AND MULTIDRUG RESISTANCE LEUKAEMIA HL60 CELLS

Dorota Kostrzewa-Nowak, Jolanta Tarasiuk

Department of Biochemistry, Faculty of Biology, University of Szczecin, 3c Felczaka St, 71-412 Szczecin, Poland.

The anthracycline antitumor agents are among the most effective drugs, currently available for the treatment of various human neoplastic diseases including leukaemias, lymphomas and solid tumours. However, the clinical usefulness of these drugs is limited by the occurrence of multidrug resistance (MDR) associated with the presence of drug efflux pumps (e.g. P-glycoprotein, MRP1), belonging to the ATP-binding cassette protein family.

In our previous study we have evidenced the important role of bioreductive activation of anthracycline compounds having non-modified quinone structure (among them doxorubicin, DOX and pirarubicin, PIRA) by exogenously added NADPH cytochrome P450 reductase (CPR) from human liver and NADPH in cytotoxic activity against human promyelocytic sensitive leukaemia HL60 cell line as well as its MDR sublines exhibiting two different phenotypes of MDR related to the overexpression of P-glycoprotein (HL60/VINC) or MRP1 (HL60/DOX).

The objective of this study was to examine whether the bioreductive activation of DOX and PIRA by CPR changes their apoptotic stimuli properties in regard to sensitive HL60 and multidrug resistant (HL60/VINC and HL60/DOX) leukaemia cells.

It was found that non-activated as well as CPR-activated forms of DOX and PIRA used at IC90 were able to cause significant alterations in cell cycle distribution of sensitive HL60 as well as resistant HL60/VINC and HL60/DOX cells and induce apoptosis. Interestingly, it was evidenced that HL60/VINC cells were more susceptible to undergo caspase-3/caspase-8-dependent apoptosis induced by both studied forms of anthracycline drugs compared to HL60 and HL60/DOX cells. However, the examined agents did not change the expression of Fas receptors on the surface of HL60 sensitive as well as resistant cells regardless of its form used in the study. Obtained results suggest that CPR-dependent reductive activation of examined anthracycline drugs (DOX and PIRA) does not change their apoptotic stimuli properties in regard to sensitive HL60 and multidrug resistant (HL60/VINC and HL60/DOX) leukaemia cells. Nevertheless, taking into account that side toxic effects observed in course of patient treatment with antitumour drugs are dose-dependent, it seems that the reported increase in antiproliferative activity and ability to induce apoptosis of DOX and PIRA after their reductive activation by exogenous CPR against the MDR cells overexpressing both P-glycoprotein and MRP1 at much more lower concentrations of these drugs could be of clinical importance for the treatment of tumours resistant to classical chemotherapy. However, the clinical significance of the presented results obtained in the model system remains to be proven. Thus, further studies are needed to prove the potential importance of the proposed approach in leukaemia therapy.

These studies were supported by the Faculty of Biology, University of Szczecin, Poland and Ministry of Science and Higher Education, Poland (Grant no. N401 138 31/2952).

39. PROAPOPTOTIC ACTIVITIES OF FERROCENYL COMPOUNDS AND THEIR CONTRIBUTION TO DISSIPATION OF MITOCHONDRIAL TRANSMEMBRANE POTENTIAL IN HEPG2 HUMAN HEPATOMA CANCER CELLS

Paulina Lewarska[1], Paweł Hikisz[1], Konrad Kowalski[2], Aneta Koceva-Chyła[1]

[1]Department of Thermobiology, Institute of Biophysics, Faculty of Biology and Environmental Protection, University of Lodz, Pomorska 141/143, 90-236 Lodz, Poland; [2]Department of Organic Chemistry, Faculty of Chemistry, University of Lodz, Tamka 12, 90-403 Lodz, Poland.

Attractive biological properties of ferrocenes bring about the fact that they captured the attention of many scientists. The scope of their potential application can be extremely broad. Several reports have indicated that some of ferrocenes exhibit a highly promising activity *in vitro* and *in vivo* against certain diseases such as bacterial and fungal infections, HIV, malaria. It has been also shown that ferrocene exhibit wide range of antitumor properties. It is believed that the basis of their biological activity is the induction of apoptosis, and cytotoxic properties against various cancer cell types. A distinctive feature of the early stages of apoptosis is the disruption of active mitochondria. This includes changes in the mitochondria transmembrane potential and alterations of the redox potential.

This study aimed at evaluating the proapoptotic activity of 2 acryloylo ferrocenyl derivatives (15, 15Cl) and their impact on changes in mitochondrial transmembrane potential of HepG2 cancer cells. The membrane-permeant JC-1 dye was used to monitor mitochondrial activity. The cells were treated with investigated compounds for 3 and 6 hours with IC_{50} or IC_{90} concentration of ferrocenes and after then fluorescence of JC-1 monomers and dimers was measured.

The investigation showed that ferrocene derivatives exhibited proapototic properties. They cause depolarization of mitochondrial membrane of HepG2 cells which proves the induction of programmed cell death of this type of cancer cells by ferrocenyl compounds and suggests that apoptosis of HepG2 cells can proceed via mitochondrial pathway.

40. INHIBITION OF MULTIPLE MYELOMA CANCER CELLS (RPMI 8226) BY THE SMALL MOLECULE 3-BROMOPYRUVATE

Grażyna Majkowska-Skrobek[1], Daria Augustyniak[1], Paweł Lis[1], Anna Bartkowiak[1], Mykhailo Gonchar[2] ,Young H. Ko[3], Peter L. Pedersen[4], Andre Goffeau[5], Stanisław Ułaszewski[1]

[1]Institute of Genetics and Microbiology, University of Wroclaw, Przybyszewskiego 63/77,51-148 Wroclaw, Poland; [2]Institute of Biotechnology, University of Rzeszów, ul. Sokołowska 26, 36-100 Kolbuszowa, Poland; [3]KoDiscovery, UMBioPark, Innovation Center, Suites 502 E and F, 801 Baltimore Street, Baltimore, Maryland, 21201, USA; [4]Department of Biological Chemistry, John Hopkins University School of Medicine,Baltimore, MD 21205-2185, USA; [5]Institut des Sciences de la Vie , Université Catholique de Louvain-la-Neuve, Place de l`Université, 1348 Louvain-la-Neuve, Belgium.

The phenomenon of multiple resistance of eukaryotic cells to cytotoxic drugs, antibiotics and fungicides is a major medical problem in terms of both cancer chemotherapy and infectious diseases. Therefore, there is a strong need for novel inexpensive and effective anti-cancer drugs. Our basic research is focused on the potential of the cytotoxic drug 3-bromopyruvate (3-BP) which is a synthetic derivative of lactate, interacting with SH groups of methionine and cysteine residues. 3-BP is a strong alkylating agent that inhibits both cellular ATP production sources of cancer cells., i.e., glycolysis and mitochondria. 3-BP is a potent anticancer drug that has been used successfully in animals and humans by Dr Young Ko with no apparent side effects.

Why 3-BP effect is so specific to cancer cells? We hypothesize that this behavior is related to the properties of the cell membrane MCT transporters which are induced in all cancer cell. To investigate this issue we have compared the anticancer activity of 3-BP and the well known anti-leukemia compound imatinib methanesulfonate (Glivec) on Human Multiple Myeloma (MM) cells (RPMI 8226) as well as control Peripheral Blood Mononuclear Cells (PBMC). The IC50 values for MM and PBM cells were of 24 and 58 μM of 3-BP, respectively. Similarily, Glivec inhibited preferencially the growth of MM cells (IC50 = 33.2 μM), compared to the control PBMC (IC50 = 46 μM). The cytotoxicity of 3-BP is potentiated by the glutathione inhibitor buthionine sulfoximine (BSO). Moreover, the level of intracellular ATP of MM cells decreases by over 90% in 1h after addition of 100 μmolar 3-BP. The rate of uptake of radiolabelled 3-BP by MM cells was higher than in PBMC. The Km for intracellular accumulation of 3-BP in MM cells (0.30mM 3-BP) was 25 times lower than that of control cells (7.2mM 3-BP). This uptake was accompanied by massive overexpression of the MCT1 gene encoding monocarboxylate plasma membrane lactate transporter which is probably responsible for 3-BP uptake. 3-BP is a polar compound and is thus not a substrate for the Multiple Drug Resistance network (MDR) which inactivates many anticancer drugs in mammals. This was confirmed by our study on the Pleiotropic Drug Resistance (PDR) network in the yeast *Saccharomyces cerevisiae*.

Based on these results and literature data, a mechanism for the anticancer properties of 3-BP in MM cancer cells is proposed. This mechanism has implications for clinical therapy of cancer cells by 3-BP.

This work was supported by the Ministry of Science and Higher Education (Poland) within of "Statutory Research 1016/S/IGM/13". PLP acknowledges support from NIH grant CA10951.

41. MIXED LINEAGE KINASES ACTIVATE MEK INDEPENDENTLY OF RAF TO MEDIATE RESISTANCE TO RAF INHIBITORS

Anna A. Marusiak[1], Zoe C. Edwards[1], Eleanor W. Trotter[1], Natalie L. Stephenson[1], Willy Hugo[2], Xiangju Kong[2], Michael G. Gartside[3], Shameem Fawdar[1], Andrew Hudson[1],Wolfgang Breitwieser[4], Nicholas K. Hayward[3], Roger S. Lo[2], John Brognard[1]

Cancer Research UK, [1]Signalling Networks in Cancer Group and [4]Cell Regulation Group, Paterson Institute for Cancer Research, The University of Manchester, Manchester, UK; [2]Division of Dermatology, Department of Medicine, University of California, Los Angeles, California, USA; [3]Oncogenomics Research Group, Queensland Institute of Medical Research, Herston, Brisbane, Australia.

Vemurafenib (PLX4032) is a first-line therapeutic for approximately 50% of metastatic melanoma patients that carry an activating $BRAF^{V600E}$ mutation. However, complete responses in patients are rare (6%) and resistance occurs within 2-18 months. Here we demonstrate that the mixed lineage kinases (MLK1-4) are MEK kinases that can reactivate the MEK/ERK pathway in the presence of RAF inhibitors. Furthermore, expression of MLKs mediates resistance to vemurafenib in V600E-positive melanoma cell lines and endogenous MLK2 contributes to acquired resistance to vemurafenib in a cell line model. In vemurafenib-treated patients, we observe increased expression of MLK3 in melanomas with acquired vemurafenib resistance, consistent with MLK3 expression mediating resistance in a mouse model. Lastly we observe MLK1 mutations identified in patients are gain-of-function mutations. In summary, our data sheds light on a novel function of the MLKs as direct activators of the MEK/ERK pathway with implications for melanomagenesis and resistance to RAF inhibitors.

42. PRELIMINARY STUDIES ON THE BIOLOGICAL EVALUATION OF STAR COPOLYMERS WITH D-GLUCOPYRANOSIDE CORES

Anna Mielańczyk[1], Magdalena Skonieczna[2], Sebastian Grządka[1], Dorota Neugebauer[1]

[1]Department of Physical Chemistry and Technology of Polymers, Faculty of Chemistry, Silesian University of Technology, M. Strzody 9, 44-100 Gliwice, Poland; [2]Institute of Automatic Control, Faculty of Automatic Control, Electronics and Computer Science, Silesian University of Technology, Akademicka 16, 44-100 Gliwice, Poland.

Drug delivery carriers (DDC) are designed in order to lower toxicity of the biologically active compound by means of prolonged circulation in the blood stream as much as targeted release. Polymers are a popular choice for the DDC since they offer a wide range of architectures with engineered characteristics such as tunable sizes with narrow distribution, defined solubility and stability, protection of drugs during circulation, and transportation to targeted organ or tissue [1].

Our research focuses on the preparation of polymers in the controlled manner and optimization of their properties towards the use as DDC. To the purpose acetal derivatives of D-glucopyranosides are utilized as initiators for Atom Transfer Radical Polymerization (ATRP) of methyl methacrylate (MMA) with the selected comonomer i.e. *tert*-butyl methacrylate (*t*BMA) or glycidyl methacrylate (GMA). We have also managed to obtain an star miktopolymers by combination of Ring Opening Polymerization (ROP) of ε-caprolactone and ATRP of *t*BMA. The biodegradable and biocompatible polyester arms degrade into product being capable of absorption by the body with minimal tissue reaction.

In the present study the effects of 4- and 6-arm star copolymers on both HCT-116 and NHDF cells were examined. The preparation of polymeric carriers included: synthesis of acetal-based initiators, copolymerization, post-polymerization modifications of GMA and *t*BMA units. The last step based on chemical conjugation between the amino groups present in the modified with ethylenediamine (ED) units of glycerol methacrylate (GOHMA) or the carboxylate groups in the methacrylic acid (MAA) units, and fluorescein isothiocyanate (FITC) or fluoresceinamine (FA), respectively.

Our studies revealed that P(MMA-*co*-MAA)$_6$ stars are polyanions and possess negative surface charge whereas P(MMA-*co*-GOHMA^{ED})$_4$ are polycations with positive surface charge. Surface charge is an extremely important factor which affects drug carriers in terms of their cytotoxicity. The effect of selected star copolymers on NHDF and HCT-116 cell viability was estimated using MTS assay and disclosed toxicity only in the case of P(MMA-*co*-GOHMA^{ED}) toward HCT-116. Furthermore, in agreement with the assay of MTS, Annexin-V assay results also showed that 100 μg mL-1 of P(MMA-*co*-GOHMA^{ED})$_4$ after 24h induced more apoptotic (A+) and necrotic (PI+) cells A+/PI+=35% than P(MMA-*co*-MAA)$_6$ where A+/PI+=5%. The confocal microscopy images of fluorescein-modified copolymers proved successful cell internalization.

References:
[1] De Jong W.H., Borm P.J. (2008) Drug delivery and nanoparticles: Applications and hazards, *Int. J. Nanomedicine* **3**, 133-149.

This work was financially supported by the National Science Center (NCN, Poland) according to Decision No DEC-2012/07/N/ST5/01875(A.M.& D.N.).
Biological experiments were supported by grant No. BKM/514/RAU-1/2013 t.26 from Silesian University of Technology (M.S.) and were performed in the Biotechnology Center of the Silesian University of Technology using equipment financed by the ''Silesian Biofarma'' program.

43. EFFECT OF REDUCTIVE ACTIVATION OF IDARUBICIN BY NADPH CYTOCHROME P450 REDUCTASE ON ITS INTRACELLULAR ACCUMULATION IN SENSITIVE AND MULTIDRUG RESISTANT MCF7 BREAST CANCER CELLS

Robert Nowak[1,2], Dorota Kostrzewa-Nowak[1,2], Jolanta Tarasiuk[1,2]

[1]Department of Biochemistry, Faculty of Biology, University of Szczecin, 3c Felczaka St, 71-412 Szczecin, Poland; [2]Molecular Biology and Biotechnology Center, Faculty of Biology, University of Szczecin, Wąska 13 St, 71-415 Szczecin, Poland.

Idarubicin (IDA) is one of clinically important anticancer drugs belonging to anthracycline antibiotic family. Their clinical usefulness is limited by the development of multidrug resistance (MDR) by cancer cells associated with the overexpression of genes encoding ATP-binding cassette (ABC) membrane transporters (e.g. P-glycoprotein, P-gp and MRP1) responsible for active efflux of drugs out of resistant cells resulting in the decreased intracellular accumulation insufficient to inhibit resistant cell proliferation.

In our previous works we have demonstrated that some anthracycline drugs (among them IDA) and synthetic anthraquinone derivatives having non-modified quinone structure could undergo reductive activation by NADPH cytochrome P450 reductase (CPR) and that this activation has a high impact on increasing their activity not only in regard to sensitive cancer cell lines but also against MDR sublines overexpressing P-gp and MRP1. It was postulated that CPR-activation of these compounds could generate reactive intermediates able to irreversibly bind to cellular constituents before being removed from resistant cells by MDR exporting pumps.

The aim of the study was to examine the intracellular accumulation of CPR-activated IDA in human sensitive MCF7 and multidrug resistant MCF7/DOX (overexpressing P-gp) breast adenocarcinoma cells. The study was performed with the aid of BD FACSCalibur flow cytometer and real-time living cell imaging system BD Pathway Bioimager 855 by utilizing fluorescence properties of IDA.

The results of flow cytometry analyses showed much more important increase in intracellular accumulation of CPR-activated form of IDA in resistant MCF7/DOX cells than in MCF7 cells in comparison to the level observed for the drug alone (non-activated). Additional analyses performed with the use of the bioimaging system demonstrated irregular zones of intracellular distribution of both activated and non-activated form of IDA. The identification of IDA accumulation sites in sensitive MCF7 and resistant MCF7/DOX cells with the use of specific markers of intracellular organelles as well as the determination of an availability of this drug for the MDR exporting pumps need further studies.

This study was supported by National Science Center (NCN), Poland (Grant no. 2012/05/B/NZ3/01985).

44. EFFECT OF SELECTED POLYPHENOLS, GALLIC ACID AND EPIGALLOCATECHIN GALLATE, ON MATRIX METALLOPROTEINASES ACTIVITY IN SENSITIVE AND MULTIDRUG RESISTANT MCF7 BREAST CANCER CELLS

Anna Nowakowska[1,2], Jolanta Tarasiuk[1,2]

[1]Department of Biochemistry, Faculty of Biology, University of Szczecin, 3c Felczaka St, 71-412 Szczecin, Poland; [2]Molecular Biology and Biotechnology Center, Faculty of Biology, University of Szczecin, Wąska 13 St, 71-415 Szczecin, Poland.

Invasion and metastasis are the major causes of morbidity and mortality in breast cancer patients. The degradation of the extracellular matrix by cancer cells involves several proteolytic enzymes (e.g. metalloproteinases, MMPs; serine proteases and cathepsins). Among the members of the MMPs family, gelatinase A (72 kDa type IV collagenase, MMP-2) and gelatinase B (92 kDa type IV collagenase, MMP-9) play a critical role in extracellular matrix degradation and tumour cell invasion in breast cancer. Recently, increasing interest of many investigators is focused on the use of dietary polyphenols in cancer prevention and chemotherapy of tumours with high metastatic potential.

The aim of this study was to investigate the effect of selected polyphenols: gallic acid (GA) and epigallocatechin gallate (EGCG) on matrix metalloproteinase (MMP-9 and MMP-2) activity in sensitive MCF7 human breast adenocarcinoma line and its multidrug resistant (MDR) sublines obtained by doxorubicin selection: MCF7/DOX and MCF7/DOX_{500}.

The activity of MMP-2 and MMP-9 and the effect of studied polyphenols on these matrix proteases were examined by gelatin zymography assays. Interestingly, we have found that the activity of MMP-2 and MMP-9 significantly increased in resistant MCF7/DOX and MCF7/DOX_{500} cells whereas they were not detected in sensitive MCF7 cells. It was also observed that GA (30, 60, 100 and 120 μM) and EGCG (5, 10 and 20 μM) caused a concentration-dependent inhibition of MMP-2 and MMP-9 activity in MCF7/DOX and MCF7/DOX_{500} cells. Control experiments performed by counting viable cells in the presence of trypan blue using a Burker hemocytometer confirmed that examined compounds in these ranges of concentration did not affect the cell growth of MCF7/DOX and MCF7/DOX_{500} sublines.

In summary – obtained results show that studied polyphenols – gallic acid and epigallocatechin gallate could exert antimetastatic effect on multidrug resistant breast cancer cells by inhibiting matrix metalloproteinases involved in metastasis phenomenon.

This study was supported by the Faculty of Biology, University of Szczecin, Poland (Grant no. 504-1000-240-852).

45. DETERMINATION OF CYTOXICITY OF GENISTEIN DERIVATIVES IN CACO-2 CELLS

Katarzyna Papaj[1], Aleksandra Rusin[2], Wiesław Szeja[1]

[1]Department of Organic Chemistry, Bioorganic Chemistry and Biotechnology, Silesian University of Technology, Gliwice, Poland; [2]Center for Translational Research and Molecular Biology of Cancer, Maria Skłodowska-Curie Memorial Center and Institute of Oncology, Gliwice Branch, Poland.

Genistein analogues designed and synthesized in Department of Organic Chemistry, Bioorganic Chemistry and Biotechnology of Silesian Technical University in Gliwice inhibit proliferation of cancer cells *in vitro* at the concentration several-fold lower than genistein. They block the cell cycle, influence the mitotic spindle and induce apoptosis [1-2]. In the next step of our drug development program we want to determine bioavailability and metabolism of these semi-synthetic genistein derivatives.

In bioavailability study we used the Caco-2 cell monolayer growing on porous membranes, which is an accredited *in vitro* model suitable for prediction of a drug transport through the human intestine wall. The experiments *in vitro* will allow us to find out the derivatives of sufficient bioavailability suitable for oral administration. One of the prerequisites of this model is the lack of toxicity of the drugs transported through the cell monolayer. The cytotoxicity assay will allow us to determine highest nontoxic concentrations of compounds. The highest non-toxic concentration of the drug will be used in experiments aiming evaluation of transport through cell monolayer measured with HPLC-MS/MS.

We determined toxicity of the tested compounds in Caco-2 cell line with use of MTT assay. MTT test measures the activity of mitochondrial enzyme, which catalyses the reduction of colorless 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl tetrazolium bromide into purple formazan. This process requires active mitochondria, and even freshly dead cells do not cleave significant amounts of MTT. Amount of formazan is proportional to the number of living cells in a culture dish and it is measured spectrophotometrically with a microplate reader at 570nm wavelength.

Caco-2 cells were plated in 96-well plate at the density $2*10^4$ cells per well and grown for 72h to simulate long-term culture in plates used for trans-well transport measurements. Then, cells were treated by the tested compounds for 24h with a series of concentrations: 5.0, 10.0, 25.0 and 50.0 μM. After 3h incubation with MTT substrate the absorbance of samples was measured.

In our study we showed that 50.0μM concentration reduced viability of Caco-2 cell line to 60-80% of untreated control. 25 μM solutions of the tested drugs were safe for Caco-2 cell monolayer and this concentration was selected for HPLC-MS/MS studies.

References:

[1] Rusin A., Zawisza-Puchałka J., Kujawa K., et al. (2011) *Bioorg. Medic. Chem.* **19**, 295-305.
[2] Rusin A., Gogler A., Glowala-Kosińska M., et al. (2009) *Bioorg. Medic. Chem. Lett.* **19**, 4939-4943.

Research studies part-financed by the National Science Centre (2011/01/N/NZ4/01141).

46. AD-O54.9 - NOVEL FUSION PROTEIN WITH DUALISTIC PROAPOPTOTIC AND ANTIANGIOGENIC ACTIVITY AS A NEW PRECLINICAL STRATEGY IN CANCER TREATMENT

P.K. Rózga[1]; B. Żerek[1]; A. Pieczykolan[1]; M. Gałązka[1]; K. Bukato[1]; S.D. Pawlak[1]; M. Szymanik[1]; A. Jaworski[1]; M. Teska-Kamińska[1]; A. Grochot-Przęczek[2], J.S. Pieczykolan[1]

[1]Drug Discovery Department, Adamed, 05-152 Czosnów, Pieńków 149, Poland; [2]Department of Medical Biotechnology, Jagiellonian University, 30-387 Krakow, Gronostajowa 7, Poland.

Background: For almost two decades tumor necrosis factor-related apoptosis-inducing ligand (TRAIL/Apo2L) has been under extensive development as a potential therapeutic due to its ability to induce apoptosis in cancer cells while remaining neutral to normal cells.

Tumor growth is tightly related to new blood vessel formation and tissue remodeling. Platelet derived growth factor (PDGF) is important for vascular development in physiological and pathological processes. Blockade of PDGF pathway has been shown to inhibit pathological angiogenesis and tumor growth.

According to this knowledge, we proposed a novel fusion protein (AD-O54.9) with dualistic proapoptotic and antiangiogenic activity.

Our new molecule consists of a recombinant TRAIL/Apo2L variant linked with an effector peptide sequence and an activation motif recognized by tumor-specific proteases (MMPs, uPa) between. The effector peptide is formed by 19-amino acid fragment of human PDGF which binds the PDGF receptors competitively to the natural ligand while being itself devoid of activity. As a consequence, angiogenic activity of PDGF is blocked, stimulation of new blood vessels formation does not occur and finally tumor growth is inhibited.

Materials and methods: AD-O54.9 protein was expressed in *E. coli*, using pET expression system, and purified by IEC chromatography. Cytotoxic activity was examined using a propidium iodide assay. Its antiangiogenic activity was evaluated by HUVEC tube formation and ring aortic assays. Safety was tested on human primary hepatocytes. The proapoptotic and antiproliferative activity was tested using flow cytometry methods and propidium iodide staining. In vivo potential was examined on mice xenograft models of human colorectal adenocarcinoma (Colo205), human lung carcinoma (NCI-H460-luc2), human pancreatic carcinoma (MIA PaCa-2) and human oesophageal adenocarcinoma (OE19) cell lines where molecules showed anticancer activity.

Results: The molecule showed in vitro specific cytotoxic effect on various primary cancer cell lines at IC50 below 0.01 ng/ml. We demonstrated that AD-O54.9 is a very potent apoptosis inducer and inhibitor of angiogenesis. Fusion protein showed superior effect displaying significant tumor volume regression compared with TRAIL/Apo2L and standard chemotherapeutic agents.

Conclusions: The obtained results confirm that we developed a very promising molecule with a high potential of anticancer activity that could be considered as a novel therapeutic agent.

47. MODULATION OF EGFR AND STAT3 ACTIVATION BY RESVERATROL AND ITS METHYLTHIO-DERIVATIVES IN HUMAN HACAT AND A431 CELLS

Hanna Szaefer[1], Michał Cichocki[1], Violetta Krajka-Kuźniak[1], Tomasz Stefański[2], Stanisław Sobiak[2], Wanda Baer-Dubowska[1]

[1]Poznan University of Medical Sciences, Department of Pharmaceutical Biochemistry, Poznań, Poland; [2]Poznan University of Medical Sciences, Department of Chemical Technology of Drugs, Poznań, Poland.

Epidermal growth factor receptor (EGFR) located on the cellular membrane of keratinocytes is widely recognized as a key regulator of numerous essential processes underlying skin development, homeostasis and repair. EGFR interacting with signal transducers and activators of transcription (Stat) is considered an attractive therapeutic target. In the current study we investigated the effect of resveratrol and its two 4'-methylthio-*trans*-stilbene derivatives (3-M-4'-MTS; S2) (3,5-DM-4"-MTS; S5) on EGFR and Stat3 activation in human immortalized HaCaT keratinocytes and epidermoid carcinoma A431 cells.

Cells were treated with resveratrol and its derivatives for 24 hours and cytosolic, nuclear fractions and whole cell lysates were isolated. The level of EGFR and Stat3 was determined by Western blot analysis. Activation of Stat-3 was evaluated in the nuclear fraction using Transcription Factor TransAM ELISA kits, with consensus site oligonucleotides specifically binding activated Stat-3.

In HaCaT cells both derivatives, similarly as the parent compound, decreased only the total level of EGFR receptor. In A431 cells resveratrol reduced the Y1173 and Y1068 EGFR residues phosphorylation while S2 affected only the phosphorylation of Y1068 residue. In this cell line resveratrol and S2 derivative diminished Stat3 binding capacity to the DNA consensus site. The effect of tested compounds on Stat3 activation in HaCaT cells was only slightly affected.

These results indicate that methylthiostilbenes are not more potent modulators of EGFR/Stat3 complex than resveratrol and introducing additional methoxy group make them less effective.

This work was supported by the Ministry of Science and Higher Education of Poland, grant N N 405 209737.

48. BEETROOT AND CHOKEBERRY JUICES PROTECT AGAINST CARCINOGENS-INDUCED LIVER AND MAMMARY GLAND INJURIES IN RATS

Hanna Szaefer[1], Violetta Krajka-Kuźniak[1], Ewa Ignatowicz[1], Małgorzata Kujawska[2], Wanda Baer-Dubowska[1], Jadwiga Jodynis-Liebert[2]

[1]Department of Pharmaceutical Biochemistry, University of Medical Sciences, Święcickiego 4, 60-781 Poznań, Poland; [2]Department of Toxicology, University of Medical Sciences, Dojazd 30, 60-631 Poznań, Poland.

Red beetroot, a common ingredient of diet, is a rich source of a specific class of non-phenolic, water soluble antioxidants, betalains, which have been shown to have anticarcinogenic and anti-inflammatory potential. Chokeberry fruits contain phenolic antioxidants, namely anthocyanins, polymeric proanthocyanins and phenolic acids.
The aim of this study was to examine the effect of 28 days feeding (8 ml/kg b. w.) with beetroot juice or chokeberry juice on phase I and phase II enzymes in male Wistar rats treated with hepatocarcinogenic N-nitrosodiethylamine (NDEA) and on the hepatic and mammary gland phase I and phase II enzymes in the Sprague-Dawley females treated with 7,12-dimethylbenz[a]anthracene (DMBA).

When compared to controls, in Wistar males long term feeding with beetroot or chokeberry juice decreased activities of CYP1A1/1A2 with no effect on CYP2E1 and CYP2B. NQO1 activity was markedly increased after treatment with both juices, however beetroot juice increased GST mu and chokeberry juice increased GST alpha. In the liver of Sprague-Dawley females similar effects were observed. In Wistar male rats a single dose of NDEA (150 mg/kg b. w.) caused decrease in activities of cytochrome P450 markers, CYP1A1/1A2 and CYP2E1 in the liver, whereas activity of CYP2B was significantly elevated and phase II enzymes (GST and NQO1) were not affected. Combined treatment with juices and NDEA caused decrease in CYP1A1/1A2 and CYP2E1 activities and raised CYP2B. GST was not particularly affected, however NQO1 was diminished in comparison to NDEA-treated animals.

In the Sprague-Dawley females which obtained DMBA twice (on the 27^{th} and 28^{th} days of treatment; 10 mg/kg b. w.) the carcinogen caused increase in CYP1A1, CYP1A2 and CYP2B activities in the liver when compared to controls, with the most pronounced effect measured in the case of CYP1A1. Activities of GST and NQO1 were elevated. Combined treatment with juices and DMBA increased the activities of tested phase I and phase II enzymes. Changes in enzyme activities were accompanied with similar alterations of the relevant proteins concentrations estimated in the Western blot technique.

In the mammary gland DMBA treatment increased CYP1A1 protein level with no effect on CYP2B. Beetroot juice alone increased the level of GST pi, enzyme involved in active metabolites of DMBA detoxification. This effect was moderately expressed in the case of chokeberry juice. Joint treatment with beetroot or chokeberry juices and carcinogen elevated CYP1A1/1A2, GST mu and pi protein levels.

Conclusions: metabolic alterations induced by beetroot or chokeberry active ingredients which may change metabolic activation of xenobiotics are tissue specific and depend on the class of carcinogen.

49. THE INFLUENCE OF NANOPARTICLES ON HUMAN BREAST ADENOCARCINOMA CELLS

Marzena Szwed[1], Aneta Rogalska[1], Ralph Santos-Oliveira[2], Sotiris Missailidis[2], Agnieszka Marczak[1]

[1]Department of Thermobiology, Faculty of Biology and Environmental Protection, University of Lodz, Pomorska 141/143 st., 90-236 Lodz, Poland; *[2]Laboratory of Nanoradiopharmaceuticals, Zona Oeste Estadual University, 14 Brazil.*

Chemotherapy is a primary treatment for cancer, but its efficacy is often limited by the adverse effects of cytotoxic agents. Targeted drug delivery may reduce the non-specific toxicity of chemotherapy by selectively directing anticancer drugs to tumor cells. Especially, breast cancer has a poor prognosis and advanced breast cancer lacks effective therapy. In this study, we are established targeting therapy for cancer therapy through tumor tissue-specific delivery of nanoparticles. We compared the effect of two different types of nanoparticles: EDTMP and MUC-1 protein aptamer on estrogen receptor positive (MCF-7) and negative (MDA-MB-231) breast cancer cell cultures. We analyzed the viability of cells, their morphological changes, and influence of nanoparticles on the cell proliferation. The *in vitro* growth-inhibition assay MTT, indicated that MUC-1 protein aptamer is more cytotoxic for both analyzed cancer cell lines. Besides, MCF-7 cells are more sensitive to nanoparticle than MDA-MB-231. During the assessment of cellular morphology we confirmed that MCF-7 cells and MDA-MB-231 cell lines were able to transform their morphology. Comparison of kinetic growth parameters showed that the rate of cells proliferation was dramatically decreased after tested nanoparticles application. The results presented here should contribute to the understanding of the differences in antitumor activities of the analyzed nanoparticles.

50. PROGESTERONE RECEPTOR, COPPER TRANSPORTER ATP7B AND GLUTATHIONE TRANSFERASE T1 GENES POLYMORPHISMS- DETERMINANTS OF THE GASTRO-INTESTINAL TOXICITY OF PACLITAXEL/CISPLATIN CHEMOTHERAPY

Karolina Tęcza, Jolanta Pamuła-Piłat, Ewa Grzybowska

Center for Translational Research and Molecular Biology of Cancer, Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch, Poland.

Cytotoxic drugs used in cancer chemotherapy aim at the intensively proliferating cancer cells. Unfortunately these drugs also destroy normal cells and tissues with high proliferation rates, such as epithelia in gastro-intestinal track or cells in the bone marrow and skin, leading to chemotherapy-related toxicities. Gastro-intestinal (GI) toxicity is one of the most common adverse events in chemotherapy. Particularly early and severe symptoms considerably decrease the chances of chemotherapy success, as they require immediate treatment discontinuance. Cisplatin has high emetogenic properties- more than 90% of patients after monotherapy without appropriate premedication experience certain level of GI toxicity. It is believed, that patients' susceptibility to treatment toxicity could be in part caused by the genetic variation in genes encoding the key proteins of drugs' metabolic pathways and transport systems. Also, there are evidences, that the variation in genes necessary for organism's homeostasis, i.e. hormonal signaling, may be linked to adverse events during cytotoxic treatment, but the extent of such dependence is still mostly unknown.
Relationships between genetic polymorphisms and chemotherapy-induced toxicity were analyzed in group of 129 ovarian cancer patients treated with paclitaxel and cisplatin in first-line chemotherapy. 14 genetic modifications in 9 genes were selected for this study, including functional variants in copper and cisplatin transporter ATP7B, component of cisplatin detoxification pathway GSTT1 and progesterone receptor (PGR) genes.

Three polymorphic variants of progesterone receptor together with one *ATP7B* variant and GSTT1 gene deletion were independently responsible for high risk of GI toxicity, including early and severe symptoms. More importantly, for the carriers of one and more unfavorable genotypes we observed strong accumulation of the GI toxicity risks. At the same time none of the clinical factors, like tumor FIGO stage, its histotype, patients' age and presence of *BRCA1* mutation, were connected to any toxic symptoms.

The results suggest, that impaired function of ATP7B-mediated cisplatin efflux system or GSTT1-driven detoxification route combined with modified organism response to progesterone due to change function of its receptor, are the strong determinants of GI toxicity of paclitaxel/cisplatin chemotherapy. We believe, that genetic variants responsible for above listed changed protein functions could make a promising potential predictive markers of paclitaxel/cisplatin-induced GI toxicity for the ovarian cancer patients.

This work was financially supported by grant no. N N401 329939

51. FUSION OF TRAIL/Apo2L WITH A MEMBRANE DISRUPTING PEPTIDE – AD-O56.9 AS A NOVEL ANTICANCER THERAPEUTIC

B. Żerek, M. Szymanik, P.K. Rózga, S.D. Pawlak, A. Pieczykolan, M. Gałązka, K. Bukato, A. Jaworski, K. Poleszak, J.S. Pieczykolan

Drug Discovery Department, Adamed, 05-152 Czosnów, Pieńków 149, Poland.

Background: The tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL/Apo2L) is a member of the TNF superfamily that initiate apoptosis of tumor cells through the activation of their death receptors. The ability of TRAIL/Apo2L to selectively induce apoptosis of tumor cells but not normal cells makes it an attractive agent for cancer therapy. However, many cancer types have developed resistance mechanisms, such as dysfunctions of proapoptotic proteins.
Here, we report a novel molecule – AD-O56.9, which is composed of the soluble domain of TRAIL/Apo2L (carrier and in some cases also an effector) fused with a cationic, alpha-helical (KLAKLAK)2 antimicrobial peptide (the effector). (KLAKLAK)2 peptide fused to protein transduction domain can induce cancer cell death by triggering mitochondrial membrane permeabilization and swelling, resulting in the release of cytochrome c and induction of apoptosis. It creates also the capacity to cause aggregation of mitochondria, what is also mechanism of cytotoxic action. We added poly-arginine cell penetrating domain to (KLAKLAK)2 peptide, to increase efficiency of its internalization. To allow separation of TRAIL/Apo2L domain and the RRRRRRRR(KLAKLAK)2 peptide after reaching the tumor, we linked these two domains with a sequence motif recognized by MMPs and uPa proteases, present in tumor cells membranes or their proximity.

Methods: AD-O56.9 protein was produced in E. coli, using pET expression system, and purified by IEX chromatography. The obtained molecule was characterized biochemically and biophysically. MTT (3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide) cell viability assay was used to assess AD-O56.9-mediated as well as sole RRRRRRRR(KLAKLAK)2-mediated killing of carcinoma cells. Flow cytometric analysis were used to evaluate influence of the AD-O56.9 on plasma membrane and mitochondrial membrane integrity, caspase 3 activation, PARP cleavage, as well as influence on cell cycle of cancer cells. The tumoricidal activity of AD-O56.9 was evaluated in NOD/SCID mice bearing different types of cancer xenografts.

Results: AD-O56.9 exhibited cytotoxic effect on various cancer cell lines, both TRAIL-sensitive and TRAIL-resistant (IC50 about 10 ng/ml), but showed no toxic effect on normal cells. This protein was also highly cytotoxic against primary cancer cells. The element that overcomes resistance to TRAIL/Apo2L is RRRRRRRR(KLAKLAK)2 peptide, but only being a part of AD-O56.9. Analyzing cell cycle and plasma membrane integrity in relatively sensitive cell line (NCI-H460) and TRAIL-resistant cell line (A549) we showed that AD-O56.9 induced apoptosis in these cells. This protein led to activation of caspase 3, cleavage of PARP [poly (ADP-ribose) polymerase] as well as caused strong depolarization of mitochondrial membrane. Importantly, AD-O56.9 administration caused significant regression of TRAIL sensitive human pancreatic carcinoma MIA PaCa-2, human oesophageal adenocarcinoma OE19, human colorectal adenocarcinoma Colo205 and TRAIL-resistant human hepatocellular carcinoma HepG2 grown as xenografts in NOD SCID mice.

Conclusions: Our novel fusion protein AD-O56.9 is able to induce apoptosis in many cancer cell lines, even TRAIL resistant and causes tumor regression in mice bearing human cancer cells. Obtained results make this very promising molecule worth further preclinical development.

Non-Cancerous Pathologies

52. PULMONARY ARTERY ENDOTHELIAL CELLS ARE MODIFIED IN HUMAN MODEL OF MUCOPOLYSACCHARIDOSIS TYPE VI.

Adam Golda[1], Anna Lalik[2], Agnieszka Jurecka[3,4], Anna Tylki-Szymańska[5]

[1]Department of Cardiology, Gliwice Medical Center, ul. Kościuszki 29, 44-100 Gliwice, Poland; [2]Systems Engineering Group, Faculty of Automatic Control, Electronics and Informatics, Silesian University of Technology, Gliwice; [3]Department of Medical Genetics, The Children's Memorial Health Institute, Dzieci Polskich 20, 04-730 Warsaw, Poland; [4]Department of Genetics, University of Gdańsk, W. Stwosza 59, 80-308, Gdańsk, Poland; [5]Department of Pediatrics, Nutrition and Metabolic Diseases, The Children's Memorial Health Institute, Dzieci Polskich 20, 04-730 Warsaw, Poland.

Mucopolysaccharidosis VI (MPS VI) is a rare genetic disorder caused by deficient activity of arylsulfatase B (ARSB), an enzyme involved in the degradation of glycosaminoglycans (GAGs), namely dermatan sulfate and chondroitin sulfate. In the absence of this enzyme activity, the stepwise degradation of the glycosaminoglycans is blocked, resulting in intracellular accumulation of the substrates into the lysosomes and leading to a progressive disorder with multiple tissue and organ involvement. The negative role of dermatan sulfate on cardiovascular system has been widely documented; the excess of dermatan sulfate in the tissue of heart valves leads to the valvular heart disease with all consequences. Endothelium plays a key regulatory function in the vascular system. It regulates vascular tone, platelet activity, leukocyte adhesion and angiogenesis. Increased occurrence of pulmonary hypertension in MPS patients contributes to higher mortality, which at least partially can be contributed to endothelial dysfunction.

The aim of this study was to investigate the impact of ARBS deficiency in human pulmonary artery endothelial cells on differentiation, exocrine function, viability and apoptosis. The human MPS VI model of pulmonary artery endothelial cells (HPAECs) was achieved using siRNA ARSB transfection (35.5% reduction of ARSB level). Gene expression study using Real-Time showed statistically significant ($p<0.05$) reduction of expression of NNPC (34.9%), Ve-cadherin (38.8%) and ICAM 2 (26.8%), and no expression differences of vWF in siRNA-transfected versus control cells. Underexpression of ARSB was associated with HAPEC cells diminished viability. No differences in HAPEC apoptosis levels were seen between control and siRNA ARSB transfected cells; both - alone and upon stimulation of the elastin receptor with various concentrations of dermatan sulfate.

Our results show that mucopolysaccharidosis type VI causes not only morphologic changes in the cardiovascular system, but also many functional and structural alternations of key regulatory cells - endothelial cells.

This work was supported by "Grant Servier 2012" from Polish Cardiac Society.
The experiments were performed in the Biotechnology Center of the Silesian University of Technology using equipment financed by the "Silesian Biofarma".

53. OCCURRENCE OF OSTEOPROTEGERIN IN THE ARTERIES AND CALCIUM DEPOSITS

Aleksandra Kuzan[1,2], Agnieszka Chwiłkowska[2], Magdalena Kobielarz[1,3]

[1]Regional Specialist Hospital in Wroclaw, Research and Development Centre; [2]Department of Medical Biochemistry, Medical University in Wroclaw; [3]University of Technology, Department of Biomedical Engineering and Experimental Mechanics.

Osteoprotegerin (OPG) is one of the key bone matrix regulatory proteins, but also is found in the blood vessels walls and enters the blood serum. It has been suggested to be involved in the formation of calcium deposits in the arteries.

The purpose of study was to analyze the relationship between the content of OPG in the arteries and the severity of atherosclerosis. The research material consist of 30 fragments of arteries from people who died a sudden death, classified by macroscopic evaluation to the five stages of atherosclerosis. Among the tested samples there were nine with large (more than 0.5 cm^2) calcium deposits that were isolated from the tissue and analyzed independently. ELISA method was used to determine the OPG level.

The average content of OPG in tested arterial fragments was 0,004 μg / mg tissue, and in calcium deposits was four times lower- 0.001 μg / mg tissue. It was noticed that in the tissue surrounding the deposits does not occur more of the protein than in tissues without signs of calcification. There was observed differences in the OPG content but no correlation between the degree of atherosclerosis. Understanding the role played by the OPG in the blood system, both blood and vessel wall, is important in the determination of the function of this protein.

This publication is part of project „Wrovasc – Integrated Cardiovascular Centre", co-financed by the European Regional Development Fund, within Innovative Economy Operational Program, 2007-2013 realized in Provincial Specialized Hospital, Research and Development Center in Wroclaw.

54. RELATION OF VITAMIN D_3 AVAILABILITY TO RANKL/RANK/OPG SIGNALING PATHWAY IN REMODELING OF BONE TISSUE IN EXPERIMENTAL TYPE 1 DIABETES

D. Labudzynskyi, I. Shymanskyy, O. Lisakovskaya, M. Veliky

Laboratory of Medical Biochemistry, Palladin Institute of Biochemistry, Kyiv, Ukraine.

Background and aims: Vitamin D_3 (D_3) deficiency is known to be correlated with the development of bone resorption in the primary osteoporosis and other disorders, including diabetes. More recently it has been also shown that signaling pathway of the receptor activator of nuclear factor κB (RANK), RANK ligand (RANKL), and its decoy receptor osteoprotegerin (OPG) plays an important role in the maintaining of functional and structural state of bone tissue. In particular, it determines osteoblasto- and osteoclastogenesis, being a key element in the bone remodeling. Notably, the expression of receptor activator of RANKL is regulated by bone-seeking hormones such as parathyroid hormone and 1,25-dihydroxyvitamin D_3. The present study was design to determine the relationship between mouse vitamin D_3 status and RANKL/OPG signaling pathway in impairment of bone tissue remodeling associated with type 1 diabetes.

Materials and methods: Type 1 diabetes was induced in male C57BL/J6 mice (weighing 25.0 ± 1.5g) by i.p. injection of multiple low dose streptozotocin (40 mg/kg b.w.). Control and STZ-diabetic mice were maintained with or without treatment with D_3, at 15 IU/mouse per os, for 8 weeks (a prevention paradigm). Serum 25-hydroxyvitamin D_3 ($25OHD_3$), RANKL and OPG were assessed by ELISA. The levels of cytochrome P 450 27A1 (CYP 27A1), CYP 2R1 (mitochondrial and microsomal isoenzymes respectively) were assayed by Western-blot analysis. Blood serum contents of calcium (Ca^{2+}), phosphorus (Pi) and bone alkaline phosphatase (ALP) activity were measured spectrophotometrically.

Results: Serum level of $25OHD_3$, the main circulating metabolite of D_3, was shown to be reduced to 23.8±1.9 in diabetes vs. 39.7±2.9 nmol/l in control, that reflects reliably vitamin D_3 deficiency ($p<0.05$). These changes were accompanied by altered expression of hepatic vitamin D_3 25-hydroxylases (CYP 450) involved in D_3 metabolism. Indeed, diabetes was associated with 2.1-fold decrease in CYP 27A1, which is known to be a constitutive isoenzyme with the low affinity to D_3, and 1.7-fold elevation of high affinity 25-hydroxylase isoform CYP 2R1. Upregulated expression of the inducible microsomal isoform of CYP 450 probably provides compensatory mechanism to overcome the lack of D_3. Vitamin D_3 deficiency was found to be related to hypocalcemia, hypophosphatemia and elevated activity of alkaline phosphatase, indicative of increased bone resorption associated with diabetes. Assessment of bone-related cytokine concentrations demonstrated that both OPG and RANKL levels were higher in diabetic mice (by 20% and 31% respectively, $p<0.05$) then in control animals. It is worth noting that RANKL/OPG ratio showed only a slight difference between these two experimental groups. Increased OPG level in diabetic mice most likely occurred to compensate elevated RANKL level that is why the ratio remained comparable to that of nondiabetic control animals. Full restoration of circulatory $25OHD_3$ content was achieved due to D_3 treatment. Better vitamin D_3 availability was resulted from the normalization of hepatic D_3 25-hydroxylases expression and counteracted diabetes-induced abnormalities of mineral metabolism in bone tissue. Administration of vitamin D_3 strongly correlated with a significant decrease in serum levels of RANKL and OPG as compared with diabetic mice.

Conclusion: The findings suggest that diabetes-induced alterations of bone tissue remodeling and bone mineralization are most likely caused by a decline in D_3 availability and can be linked to disturbances in RANKL/OPG signaling pathway. It was demonstrated that diabetes-related impairments may efficiently be prevented or corrected by vitamin D_3 treatment.

55. INFLUENCE OF 25OHD$_3$ LEVEL ON EXPRESSION OF CYTOCHROME P450 (27A1 AND 2R1) AND IMMUNOREGULATORY INDEX VALUE IN EXPERIMENTAL TYPE 1 DIABETES

D. Labudzynskyi, I. Shymanskyy, A. Mazanova, O. Lisakovskaya, M. Veliky

Laboratory of Medical Biochemistry, Palladin Institute of Biochemistry, Kyiv, Ukraine.

Background and aims: Vitamin D_3 (D_3) is currently recognized as a potent immunomodulator affecting the activities of immune cells in various autoimmune diseases. However, the precise mechanisms of D_3 influence on immune homeostasis in diabetes has not been clearly defined. The present study was design to determine the relationship between mouse vitamin D_3 status, expression of D_3 25-hydroxylases (CYP450 27A1 and 2R1, mitochondrial and microsomal isoforms respectively) and CD4/CD8 T-lymphocyte ratio in blood and spleen in diabetes and after chronic administration of D_3.

Materials and methods: Type 1 diabetes was induced in male C57BL/J6 mice (weighing 25.0 ± 1.5g) by i.p. injection of multiple low dose streptozotocin (40 mg/kg b.w.). Control and STZ-diabetic mice were maintained with or without treatment with D_3, at 15 IU/mouse per os, for 8 weeks (a prevention paradigm). Serum 25-hydroxyvitamin D_3 (25OHD$_3$) was measured by ELISA. The levels of cytochrome P450 27A1 and 2R1 isoenzymes were assessed by electrophoresis and immunoblotting. Blood lymphocytes were phenotyped using immunofluorescence antibodies against CD4+ and CD8+ cell markers by flow cytometry.

Results: Serum level of 25OHD$_3$, the main circulating metabolite of vitamin D_3, was shown to be reduced to 23.8±1.9 in diabetes vs. 56.6 ± 4.1 nmol/l in control, that reflects reliably vitamin D3 deficiency ($p<0.05$). These changes were accompanied by altered expression of hepatic vitamin D_3 25-hydroxylases (CYP 450) involved in D_3 metabolism. Indeed, diabetes was associated with 2.1-fold decrease in CYP 27A1, which is known to be a constitutive isoenzyme with the low affinity to D_3, and 1.7-fold elevation of high affinity isoform CYP 2R1 isoform. Rise immunoregulatory index indicating alteration of CD4/CD8 (helper/suppressor) lymphocyte ratio in blood was observed in diabetic group as compared to control (1.84±0.11 vs. 1.55±0.12 respectively, $p<0.05$) whereas spleen immunoregulatory index reciprocally reduced (1.43±0.12 vs. 1.80±0.15 respectively, $p<0.05$). Full restoration of circulatory 25OHD$_3$ content was achieved due to D_3 treatment and it caused the normalizing reciprocal action on D_3 25-hydroxylases that led to augmentation of vitamin bioaviability. Furthermore, it notably attenuated the immune imbalance observed in diabetes by up-regulating CD4/CD8 splenic and blood lymphocyte ratio.

Conclusion: The study confirmed that diabetes was associated with the alterations of vitamin D_3 25-hydroxylases expression and infringement of blood immunoregulatory index that correlated with vitamin D_3 deficits. It was demonstrated potential role of cholecalciferol in the regulation of autoimmune processes and vitamin D3 25-hydroxylase expression in diabetes.

Chemical Synthesis and Analysis

56. ANALYSIS OF DOLICHOLS CONTENT IN *ERCC1*$^{-/-}$ MICE AS A MODEL OF ACCELERATED AGING

Dorota Dziuban[1], Joanna Komaszyło (Siedlecka)[1], Laura Niedernhofer[2], Ewa Swiezewska[1], Barbara Tudek[1,3]

[1]Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland; [2]The Scripps Research Institute, Jupiter, USA; [3]University of Warsaw, Poland.

XPF-ERCC1 endonuclease mutant mice show accelerated aging and increased incidence of cancer. *Ercc1*$^{-/-}$ mice live only 4 weeks and show onset of multiple premature aging-related degenerative diseases.

Cellular lipids and the products of their peroxidation have been implicated in aging. Polyisoprenoids like dolichols, are structural components of the cellular membranes. In mice, dolichols are composed of 16- 22 isoprene units are accumulated. Due to the increase of the dolichols content during the life span these compounds have been proposed as biomarkers of aging. Recently, a new putative function of dolichols as protectors of cellular membranes against peroxidation has been postulated.

In this study we analyzed dolichol level using HPLC/UV method. Brain, liver and kidney tissues were applied for analysis both from ERCC1 proficient and deficient mice. Tissue-specific differences in the amount of dolichol were observed. The highest level was observed in kidneys, while the lowest in brains.

Interestingly, the composition of dolichol mixture was changed in *Ercc1*$^{-/-}$ mice in comparison to wt animals. We observed a significant increase of dolichol composed of 18 isoprene units (D-18), with simultaneous decrease of D-19 and D-20 in kidneys of *Ercc1*$^{-/-}$ mice.

These results suggest that metabolism of polyisoprenoid lipids is affected in *Ercc1*$^{-/-}$ mice. Further investigations are still in progress.

57. DESIGN AND SYNTHESIS OF NEW URIDINE CONJUGATES WITH AMINO ACIDS AS POTENTIAL INHIBITORS OF RIBONUCLEASE A

Andrzej Gondela[1], Marcin Pacholczyk[2], Mateusz Tomczyk[1], Krzysztof Walczak[1]

[1]Silesian University of Technology, Department of Organic Chemistry, Bioorganic Chemistry and Biotechnology, B. Krzywoustego 4, 44-100 Gliwice, Poland; [2]Silesian University of Technology, Institute of Automatic Control, Faculty Of Automatic Control, Electronics and Computer Science, Akademicka 16, 44-100 Gliwice, Poland.

Ribonucleases are type of cellular enzymes responsible for the degradation of RNA into smaller fragments. There are considered as most important enzymes involved in removing of cellular RNA no longer required after protein synthesis. The hydrolytic properties are also used as cell's first line defence against RNA-viruses infections. RNase A catalyses the cleavage of the P–O5' bond of RNA on the 3'-side of cytidine and uridine residues. 2',3'-cCMP is a typical nucleotide inhibitor of RNase formed during breakdown of RNA.

Ribonucleases can be cytotoxic because cleaving RNA renders indecipherable its encoded information. RNase A was shown then to be toxic to tumor cells, both *in vitro* and *in vivo*. The mechanism of cytotoxicity probably involve binding to cell-surface glycolipids, retrograde transport to the specialised cellular organelles (Golgi apparatus, endoplasmic reticulum), translocation into the cytosol, and degradation of cellular RNA.

The design of selective inhibitors for ribonucleases is one of ways for the control of cell's functions. The derivatives of nucleosides can be used as the restricting molecules for the RNase activity. 3'-Estrified by amino acids or alkane dicarboxylic acids derivatives of thymidine are effective inhibitors of Ribonuclease A.

We focused on designing and synthesis of new class of compounds able to bind effectively to active site of RNase and inhibit their activity. Designed molecular models constitute 2'-amino-2'deoxyuridines substituted on amino group with methyl alkanoate moieties. The calculations of docking into active site of Bovine Pancreatic Ribonuclease A (PDP: 1FS3) were performed using Autodock Viena 4 programme. The analysis of obtained results clearly indicated the high affinity of chosen compounds toward RNase A.

The elaborated strategy of synthesis involve preparation of 5'-substituted 2,2'-ahnydrouridine, its transformation into appropriate 2',3'-dideoxy-oxazolidine-2-thione derivative in reaction with methyl omega-isothiocyanatoalkanoates followed by the ring cleavage under basic conditions.

References:

[1] Raines R.T. (1998) *Chem. Rev*. **98**, 1045-1065.
[2] Samanta A., Dasgupta S., Pathak T. (2011) *Bioorg. Med. Chem*. **19**, 2478-2484.
[3] Debnath J. Dasgupta S., Pathak T. (2009) *Bioorg. Med. Chem.* **17**, 4921-4927.
[4] Debnath J., Dasgupta S., Pathak T. (2009) *Bioorg. Med. Chem*. **17**, 6491-6495.
[5] Marshall G.R., Feng J.A., Kuster D.J. (2007) *Peptide Sci.* **90**, 259-277.

58. SCREENING OF β-GALACTOSIDASES SOURCE FOR BIOSYNTHESIS OF 2-DEOXY-β-GALACTOSIDES

Przemysław Hahn, Anna Kasprzycka, Wiesław Szeja

Silesian University of Technology, Department of Organic Chemistry, Bioorganic Chemistry and Biotechnology, Krzywoustego 8, 44-100 Gliwice, Poland.

Glycosylated compound have numerous applications in the food and pharmaceutical industries. Their synthesis is, however, far from trivial Because chemical glycosylation reactions suffer from low yields and lack of selectivity, biocatalytic routes have received increasing attention as efficient alternatives.

Glycosylation is usually carried out by glycosyltransferases which transfer glycosidic residues from NDP-sugar to the respective aglycon. In addition to the glycosyltransferases, the synthesis reaction is also carried out by glycosidases during reverese hydrolysis or transglycosylation.

Although glycosidases typically degrade their substrate in quantitative yields, they can also be used for synthetic purposes. Hydrolases are very attractive in that respect because they are readily available, have wide range of donor specificities and often display activity towards a variety of carbohydrate and non-carbohydrate acceptors.

The focus of the study is on the culture of different strains of microorganism with high production of β-galactosidases and their application in synthesis reaction.

According to these results it is possible to obtain β-galactosidase from different strains of yeasts during a simple and brief methodology. In addition to that, it is readily applied in the synthesis reactions of β-D-2-deoxyglucosides.

Research studies part-financed by the European Union within the European Regional Development Fund (POIG. 01.01.02-14-102/09).

References:

[1] Abbott D.W., Macauley M.S., Vocadlo D.J., Boraston A.B. (2009) *J. Biol. Chem.* **17**, 11676.
[2] Vic G., Hastings J., Crout D.H.G. (1996) *Tetrahedron Asymm.* **7**, 1973.
[4] Hou D., Lowary T.L. (2009) *Carbohydrate Res.* **344**, 1911-1940.
[5] Trincone A., Pagnotta E., Rossi M., Mazzone M. (2001) *Tetrahedron Asymm.* **12**, 2783–2787.
[6] Petit J.M., Paquet F., Beau J.M. (1991) *Tetrahedron Lett.* **32,** 6125–6128.

INNOVATIVE ECONOMY
NATIONAL COHESION STRATEGY

EUROPEAN UNION
EUROPEAN REGIONAL
DEVELOPMENT FUND

59. SYNTHESIS AND SELECTIVE FUNCTIONALIZATION OF A NOVEL DI-UNSATURATED DISACCHARIDES

Katarzyna Komor[1], Wiesław Szeja[1], Joachim Thiem[2]

[1]*Department of Organic Chemistry, Bioorganic Chemistry and Biotechnology, Silesian University of Technology, 44-100 Gliwice, ul. Krzywoustego 4, Poland;* [2]*Department of Chemistry, Faculty of Sciences, University of Hamburg, D-20146 Hamburg, Martin-Luther-King-Platz 6, Germany.*

Selective functionalization of glycals has been widely used for the preparation of 2-deoxy glycosides, which are common structural units in many biologically active natural products such as antibiotics or cardiac glycosides, as well as versatile synthetic intermediates. The development of improved methods for carbohydrate synthesis and particularly glycosidic bond formation is therefore critical. Therefore, utilization of glycals as building blocks for the total synthesis of various natural products is of great interest in bioorganic and medicinal chemistry.

In connection with our own studies in this area we became interest in the prospect of developing a method that would lead to simple as well relative glycoside derivatives of biologically active compounds. We optimized an effective method for synthesis of new disaccharide building blocks containing 1,2- and 2,3-unsaturated 6-deoxyhexoses from glycals. Further transformation of 1,2-unsaturated sugar unit leads to the 2,6-dideoxy glycosides and opens the possibility to synthesize different unusual sugars and glycoconjugates.

References:

[1] Borovika A., Nagorny P. (2012) *J. Carbohydr. Chem.* **31**, 255-283.
[2] Krol E., Wandzik I., Szeja W. et al. (2010) *Antiviral Res.* **86**, 154-162.
[3] Wandzik I., Bieg T. (2006) *Carbohydr. Res*. **341**, 2702-2707.
[5] Marzabadi C.H., Franck R.W. (2000) *Tetrahedron* **56**, 8385–8417.
[6] Danishefsky S.J., Bilodeau M.T. (1996) *Angew. Chem. Int. Ed. Engl.* **35**, 1380–1419.
[7] Thiem J., Klaffke W. (1990) *Top. Curr. Chem.* **154**, 285–332.
[8] Wohlert S.E., Kunzel E., Machinek R. et al. (1990) *J. Nat. Prod.* **62**, 119.

Katarzyna Komor received a scholarship under the project "DoktoRIS - Scholarship Program for Innovative Silesia".

60. PREPARATION OF 1-AMINO SUGAR DERIVATIVES AND THEIR EMPLOYMENT IN SYNTHESIS OF GLYCOCONJUGATES WITH POTENTIAL BIOLOGICAL ACTIVITY TOWARD FAMILY OF GLYCOSYLTRANSFERASES

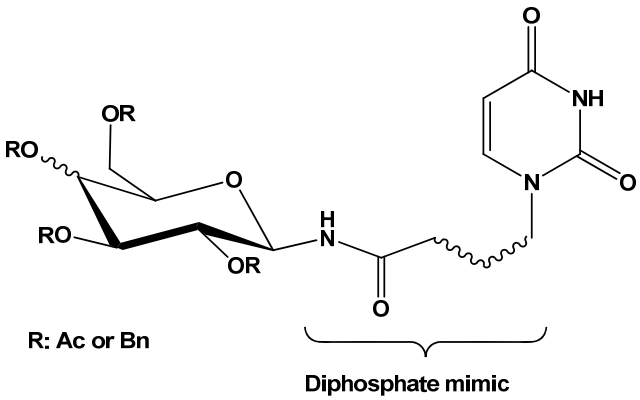
Roman Komor, Gabriela Pastuch, Mateusz Pleśniak

Silesian University of Technology, Department of Organic Chemistry, Bioorganic Chemistry and Biotechnology, 44-100 Gliwice, ul. Krzywoustego 4, Poland.

`Glycosyltransferases (GTs) are family of enzymes which catalyze glycosylation between sugar donor, activated with nucleoside phosphate or lipid phosphate group and acceptor which is usually other sugar but nucleic acids, proteins, lipids, antibiotics and other small molecules are also encountered [1]. In recent years increased attention toward GTs inhibitors has emerged due to key role of this enzymes in biological synthesis of glycoconjugates and polysaccharides. Development of new selective inhibitors is of great importance in dealing with bacterial [2] and fungal diseases [3].

Unfortunately design of active compounds remain still a difficult task because of complex GTs reaction mechanism with protein conformational changes during catalytic cycle and upon substrate binding [1].

Our research concerned synthesis of β-1-amino tetra-*O*-acetyl- and tetra-*O*-benzyl-protected derivatives of D-glucose and D-galactose from corresponding 1-azides. Subsequent amide condensation with uridine derivative (cyclic or acyclic) yielded glycoconjugates which after protecting groups cleavage can serve as potential glycosyltransferases inhibitors.



References:

[1] Lairson L.L., Henrissat B., Davies G.J., Withers S.G. (2008) *Annu. Rev. Biochem*. **77**, 521–555.
[2] Berg S., Kaur D., Jackson M., Brennan P.J. (2007) *Glycobiology* **17**, 35–56.
[3] Behr J.B., Gainvors-Claisse A., Belarbi A. (2007) *Nat. Prod. Res*. **21**, 76–82.

Roman Komor received a scholarship under the project DoktoRIS - Scholarship Program for Innovative Silesia.

61. PREPARATION OF ACYCLIC ANALOGUES OF URIDINE AND THEIR APPLICATION IN SYNTHESIS OF POTENTIAL GLYCOSYLTRANSFERASE INHIBITORS

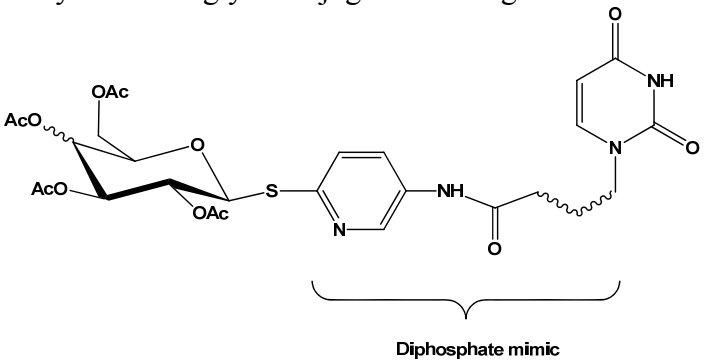
Roman Komor, Gabriela Pastuch, Wiesław Szeja

Silesian University of Technology, Department of Organic Chemistry, Bioorganic Chemistry and Biotechnology, 44-100 Gliwice, ul. Krzywoustego 4, Poland.

Oligosaccharides and glycoconjugates that are found on the cell surface participate in many intracellular and extracellular events such as viral or bacterial infection, tumor metastasis, immune response or inflammation. The development of glycosyltranferases inhibitors may lead to discovery of novel therapeutic agents for the treatment of certain diseases in which carbohydrates-protein interactions are involved. To find suitable and selective inhibitors for this class of enzymes is still challenging. Here, we describe a novel concept that allows the design of inhibitors based on the structure of the donor substrate binding pocket.

The aim of the research is to synthesize potential glycosyltransferase inhibitors, acyclic derivatives of uridine connected to thioglycosides via amide bond, that are analogues of natural donor substrate. Structures that we propose, were carefully designed, concerning requirements for the acyclic part of the nucleoside: (1) chain no longer than six carbon atoms, (2) substituents containing heteroatoms with lone pair of electrons (hydroxyl groups are preffered), and (3) presence of the carboxyl group which is essential to create amide bond with the amino group of glycoside.

Synthesis of such models will complete the already existing structures library and subsequent biological evaluations will be enriched with information concerning the biochemical relevance of size and structure of ribose mimicking motif of obtained glycoconjugates. Modulation or inhibition of glycosyltransferase activity provides an opportunity for intervention of the composition of carbohydrates in oligosaccharides which may clarify the structure-function relationship. Moreover compounds that can modulate the biosynthesis of glycoconjugates are of great interest as novel therapeutic agents.



References:
[1] Zhu X., Stolz F., Schmidt R.R. (2004) *J. Org. Chem.* **69**, 7367-7370.
[2] Vaghefi M.M., Bernacki R.J., Dalley N.K. et al. (1987) *J. Med. Chem*. **30,** 1383-1391.
[3] Szewczyk B., Tyborowska J., Krol E., Szeja,W. (2005) *J. Biotechnol*. **118S1**, S84-S85.
[4] Komor R., Pastuch-Gawołek G., Sobania A., Jadwiński M. **(**2012) *Acta Polon. Pharmaceut. - Drug Res.* **69**, 1259-1269.

Roman Komor received a scholarship under the project DoktoRIS - Scholarship Program for Innovative Silesia.

62. SYNTHESIS OF DIMERIC URIDINE DERIVATIVE – POTENTIAL INHIBITOR OF CHITIN SYNTHASE

Katarzyna Kral, Jadwiga Paszkowska, Tadeusz Bieg, Ilona Wandzik

Department of Organic Chemistry, Bioorganic Chemistry and Biotechnology, Silesian University of Technology, 44 –100 Gliwice, ul. Krzywoustego 4, Poland.

Chitin synthase (CS) is an enzyme responsible for fungal cell wall biosynthesis which is essential for fungal growth and reproduction. In the active site CS polymerizes uridine diphosphoryl-N-acetylglucosamine (UDP-GlcNAc) into polymeric chains of β-(1-4)-linked N-acetylglucosamine (GlcNAc) – chitin. The fact that chitin is absent in human cells makes CS an important antifungal target.

The structure of the active site in chitin synthase is still unknown therefore potential inhibitors are designed based on proposed mechanism. It is called "The two active site mechanism" and it suggests the presence of two active sites in the enzyme with the distance about 14 – 22 Å between them. According to this hypothesis CS simultaneously catalyses polymerization of chitin in this two places.

Several dimeric inhibitors of CS were synthesized and their biological activity showed advantage of dimeric compounds compared to monomer analogues.[1]

Following this theory we designed and synthesized dimeric inhibitor (Figure1.). It contains two molecules of uridine with proper linker between them. The synthetic procedure of this compound is described below.

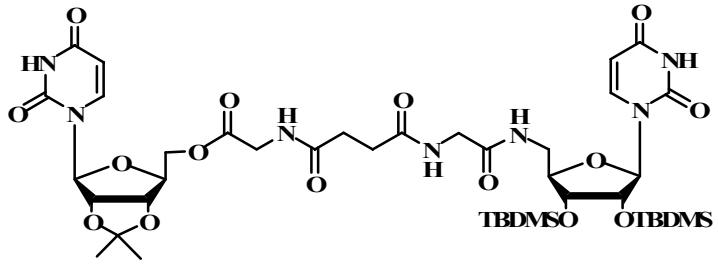


Fig 1. Structure of synthesized dimeric derivative of uridine

The multistep synthesis of dimeric derivative utilizes 2',3'-O-isopropylideneuridine and 5'-amino-2',3'-di-O-*tert*-butyldimethylsilyluridine as main substrates. Both of them were first coupled with N-benzyloxycarbonylglycine, than protective groups were cleaved by hydrogenolysis to obtain derivatives with free amino group. Next step was reaction with succinic anhydride and finally both fragments were coupled with the use of PyBOP which is commonly used as a coupling reagent[2] in peptide bond formation. All compounds were purified by flash chromatography and characterized by NMR spectroscopy. Synthetic details will be presented.

References:

[1] Yeager A.R., Finney N.S. (2004) *J. Org. Chem*. **69**, 613-618.
[2] Coste J., LeNguyen D., Castro B. (1990) *Tetrahedron Lett.* **31**, 205-208.

Research studies part-financed by European Union within the European Regional Development Fund (POIG 01.01.02-14-102/09).

63. SYNTHESIS AND FUNGICIDAL ACTIVITY OF THIOCARBAMATES DERIVATIVES ALCOHOLS

Agata Ptaszek-Budniok[1], Anna Kasprzycka[1], Przemysław Hahn[1], Wiesław Szeja[1], Wioletta Przystaś[2], Ewa Zabłocka-Godlewska[2], Mirosława Sioła[3]

[1]Silesian University of Technology, Faculty of Chemistry, Krzywoustego 4Str., 44-100 Gliwice, Poland; [2]Silesian University of Technology, Faculty of Power And Environmental Engineering, Konarskiego 18 Str., 44-100 Gliwice, Poland; [3]Laboratory of Microbiology Silesian Center for Heart Diseases, M. Curie-Skłodowskiej 9 Str., 41-800 Zabrze.
E-mail: agata.ptaszek-budniok@polsl.pl

N-Allyl and N-aryl Tiocarbamates as reported by literature possess very interesting biological properties.[1-3] Thiocarbamates as fungicides have been extensively studied since 1960.[4] However, little attention had been given to N-allyl and N-aryl substituted thionocarbamates derivatives alcohols until Thorne[5] studied a wide variety of derivetives of thiocarbamic acid and carbamic acids. He found that phenyl carbamates, alkyl-phenyl carbamates, and alkyl thiophenyl carbamates have low fungitoxicity and that some thionocarbamates showed activity. To study the possibility of improving the fungicidal activity of the known thiocarbamates, in the present work a series of N-alkil, N-phenyl and N-benzyl thiocarbamates derivatives alcohols were synthesized, and their fungitoxic activity was studied for test isolates: *Phoma linganum*, *Fusarium solani*, *Fusarium culmorum, Trichoderma viride* and *Botrytis cinerea* which were obtained from the Bank of Plant Pathogens (Institute of Plant Protection National Research Institute in Poznań). In addition, thiocarbamates synthesized were tested for yeasts (*Candida albicans, Candida dubliniensis, Candida tropicalis, Candida glabrata, Candida krusei, Candida parapsilosis*) isolated from clinical materials in the Laboratory of Microbiology, Silesian Center for Heart Diseases in Zabrze.

Results presented in this paper reveal good fungicidal activity of certain N-allil, N-phenyl and N-benzyl thiocarbamates.

References:

[1] Cesarini S., Spalarossa A., Ranise A., et al. (2008) *Bioorg. Medic. Chem.* **16**, 6353-6363.
[2] Albores-Velasco M., Thorne J., Wain R.L. (1995) *J. Agric. Food Chem.* **43**, 2260-2261.
[3] Hart B.W., Faiman M.D. (1995) *Biochem. Pharmacol.,***16**,157-163.
[4] Ludwig R.A., Thorn, G.D. (1960) *Adv.Pest Control Res.* Interscience: New York, Vol.3.
[5] Thorne J. (1967) Chemical structure in relation to biological activity of certain carbamic acid derivatives. Ph.D Dissertation, University of London.

Research studies part-financed by the European Union within the European Regional Development Fund (POIG. 01.01.02-14-102/09).

64. SYNTHESIS OF NOVEL AND ACTIVE SUGAR-BASED DRUG DELIVERY SYSTEMS

Sylwia Waśkiewicz[1], Małgorzata Burek[1], Agata Grzybek[1], Zenon P. Czuba[2], Wojciech Król[2]

[1]Department of Physical Chemistry and Technology of Polymers, Faculty of Chemistry, Silesian University of Technology, ul. M. Strzody 9, 44-100 Gliwice, Poland; [2]Department of Microbiology and Immunology, Medical University of Silesia, 41-808 Zabrze, ul. Jordana 19, Poland.

"Intelligent" hydrogels, which can change their swelling behavior and other properties in response to environmental stimuli such as temperature, pH, solvent composition and electric fields are interesting in view of their unique properties and potential application in the technological and biomedical fields. Temperature and pH are two important parameters that are normally studied in details for drug delivery purposes. It is necessary for them to show a response at body temperature and under acidic pH in order to function as desired at the target area. Among the family of temperature sensitive hydrogels, poly(N-isopropylacrylamide) (PNIPAAm) hydrogel is one of the most widely studied. The hydrogels based on PNIPAAm exhibit the volume phase transition temperature (VPTT) around 32 °C, which mean that above this temperature their nature changes from swollen to skrinked. In other words, hydrogels show an on-off drug release, allowing the desired drug release above VPTT. However their disadvantages are that they mostly show a slow response to external stimuli and are usually non-degradable, which may restrict their applications as biomaterials. One way to improve the response rate and simultaneously biocompability of PNIPAAm based hydrogels is through the introduction of sugar moieties. Moreover sugar units can modify VPTT of PNIPAAm hydrogel to the body temperature.

In the present study we report the synthesis of a series of novel sugar based thermoresponsive hydrogels based on trehalose or salicin. Those hydrogels are proposed to be used as a drug delivery carriers, releasing drug above VPTT with predetermined pharmacokinetics. In the first case acid-degradable trehalose-based diacetals were used as a cross-linker. Hydrogels structure destroys in an acidic environment as a result of deprotection reaction and is accompanied by a release of trehalose molecules. Trehalose is a storage carbohydrate and transport sugar and plays an important role in stress protection, especially during heat stress and dehydratation. As a non-reducing sugar, this saccharide does not undergo Maillard reaction with amino compounds such as amino acids or proteins. It is used in a protection of corneal epithelial cells in culture from death by desiccation and suppression of tissue denaturalization, for effective preservation of organs, in the treatment of osteroporosis and in protein based drugs including Advate (Baxter), Avastin (Genentech), Lucentis (Genentech), and Herceptin (Genentech).

A similar hydrogels were obtained using salicin-monoacetal compounds, where a salicin molecule is released in an acidic environment without hydrogel structure degradation. Salicin, is an active precursor for salicic acids, which is used as an anti-inflammatory drug and could be employed instead of aspirinin.

The preliminary cytotoxicity studies by employing MTT assay have shown that sugar acetals, their hydrogels and copolymers with NIPAAm are not cytotoxic for the human fibroblasts cell line.

The work is financed by the POMOST Programme 2012-5/11 of the Foundation for Polish Science and co-financed by the European Regional Development Fund.

Cellular Stress, Oxidation and DNA Repair

65. THE PARTICIPATION OF WIP1 PHOSPHATASE IN THE RESPONSE OF U-2 OSTEOSARCOMA CELLS TO RADIATION

Agata Abramowicz[1], Katarzyna Szołtysek[1], Patryk Janus[1], Krzysztof Puszyński[2], Piotr Widłak[1]

[1]Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice, Poland; [2]Silesian University of Technology, Gliwice, Poland.

WIP1 is a multi-target serine/threonine protein phosphatase encoded by *PPM1D* gene. Its major targets include MDM2 (Ser395), ATM (Ser1981), p53 (Ser15), CHK1 (Ser345), CHK2 (Thr68), γH2AX (Ser139) and NF-κB (Ser536). These proteins are inactivated by a WIP1-mediated dephosphorylation, hence WIP1 putatively participates in regulation of recovery of cell after induction of DNA damage response (DDR). Overexpression of *PPM1D* gene has been observed in some types of cancer and usually has been associated with poorer prognosis. Here we aimed to characterize the significance of WIP1 in response to radiation in human osteosarcoma U-2 OS cell line.

U-2 OS cells have been characterized by high expression of *PPM1D* gene. Two functional gene products are detected in these cells: full-length *wild-type* protein (66.7 kDa) and C-terminus truncated form (about 50 kDa). The expression of WIP1 in U-2 OS cells was inhibited by lentivirus-delivered shRNA (control cells contained an shRNA construct encoding a scrambled sequence). The WIP1 knock-down was verified by Western-blot – amounts of both protein forms were reduced to approximately 25% of their initial levels. The DDR was induced upon exposure to ionizing radiation (IR, 4 or 10 Gy) or UV-C radiation (10 J/m^2). The levels of DDR-related proteins were analyzed by Western-blot at different time points after irradiation. Expression of selected p53-dependent genes (*NOXA*, *CDKN1A*) was assessed by QRT-PCR. The influence of the WIP1 inhibition on the cell cycle profiles was assessed by flow cytometry. The long-term effects of the WIP1 deficiency on radiation sensitivity of cells were analyzed by the clonogenic test.

Both IR and UV-C induced accumulation of WIP1 in control cells, which was the most prominent after irradiation with 10 Gy, also in WIP1-depleted cells some accumulation of WIP1 could be detected after exposure to IR.

The WIP1 deficiency in U-2 OS cells resulted in prolonged phosphorylation of Chk1 (Ser345) and Chk2 (Thr68) in UV and IR exposed cells, respectively. The effect was visible 24 hours after irradiation, which corresponded to maximal accumulation of WIP1 in WIP1-proficient cells. It suggests that WIP1 could be involved in long-term "switch-off" of cellular response to radiation. Furthermore late activation/phosphorylation of Chk2 (Thr68) could be detected in UV-irradiated WIP1-deficient cells, hence WIP1 could be involved in regulation of late/secondary response to UV-induced damage. However similar distribution of the cell cycle and expression of p53-dependent genes in WIP1-proficient and -deficient cells upon irradiation indicates existence of mechanisms capable of general compensation of WIP1 deficiency.

This work was supported by Polish National Science Center, Grant N N518 287540.

66. CHARACTERIZATION OF ERCC1 DEFICIENT MOUSE EMBRYONIC FIBROBLASTS IN RESPONSE TO 4-HYDROXY-2-NONENAL, A MAJOR LIPID PEROXIDATION PRODUCT

Jolanta Czerwińska[1], Patrycja Berentowicz[2], Małgorzata Nowak[2], Konrad Kosicki[2], Laura Niedernhofer[3], Elżbieta Speina[1], Barbara Tudek[1,2]

[1]Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland; [2]Institute of Genetics and Biotechnology, Warsaw University, Warsaw, Poland; [3]The Scripps Research Institute - Florida, Jupiter, USA.

The ERCC1-XPF protein complex is an endonuclease involved in nucleotide excision repair (NER) and processing of DNA interstrand crosslinks. *Ercc1* knockout mice display retarded postnatal growth and premature aging. Cells derived from *Ercc1*$^{-/-}$ specimens reveal an increased level of reactive oxygen species and are hypersensitive to factors introducing oxidative stress. It was shown that during oxidative stress secondary products of reactive oxygen species – lipid peroxidation (LPO) products are intensively generated. The aim of our study was to determine the role of LPO products in etiology of the *Ercc1*$^{-/-}$ mice phenotype. For this purpose immortalized mouse embryonic fibroblasts (MEFs) derived from *Ercc1*$^{-/-}$ and wild type (wt) mice were used. We investigated the sensitivity of *Ercc1*$^{-/-}$ and wt MEFs to hydrogen peroxide (H_2O_2) and four aldehydes – end products of LPO: 4-hydroxy-2-nonenal (HNE), crotonaldehyde (CRO), malondialdehyde (MDA) and acrolein (ACR). These compounds are very reactive and can form adducts to DNA and proteins as well as DNA-DNA and DNA-protein crosslinks. Our results demonstrated hypersensitivity of *Ercc1*$^{-/-}$ fibroblasts to H_2O_2, as well as to LPO products: HNE, CRO and MDA, but not to ACR. The biggest difference in viability between *Ercc1*$^{-/-}$ and wt cells was in the case of HNE treatment. Since HNE is also one of the major LPO products generated in cells, we chose this compound for further study aimed at characterization of cell death induced in *Ercc1*$^{-/-}$ MEFs by LPO products. Three types of cell death: apoptosis, necrosis and autophagy were investigated by luminescence assays and/or by Western blotting. Necrosis and apoptosis were the preferred types of HNE-induced cell death for *Ercc1*$^{-/-}$ and wt, respectively. There was no activation of autophagy. We also measured the cell cycle of *Ercc1*$^{-/-}$ MEFs by flow cytometry and found in response to HNE a marked accumulation of fibroblasts in phase G2.

67. THE COMET ASSAY AS BIOMARKER OF DNA DAMAGE IN PERIPHERAL BLOOD LEUKOCYTES OF NUCLEAR MEDICINE STAFF

Małgorzata M. Dobrzyńska[1], Krzysztof A. Pachocki[1], Aneta Gajowik[1] Joanna Radzikowska[1], Agata Sackiewicz-Słaby[2]

[1]Department of Radiation Protection and Radiobiology, National Institute of Public Health – National Institute of Hygiene, 00-791 Warsaw, Chocimska 24, Poland; [2]Maria Sklodowska Curie Memorial Center and Institute of Oncology ,02-781 Warsaw, Roentgena 5, Poland.

Ionizing radiation not only at high, but also at low chronic doses are known as mutagenic and carcinogenic in human. Medical staff using radiation for diagnostic and therapeutic purposes are potentially at risk of overexposure.

The purpose of the study was the examination of DNA damage in peripheral blood leukocytes of staff the Department of Nuclear Medicine of two medical centers in Warsaw, where several kind of radionuclides are used for radiodiagnosis and radiotherapy. Control were chosen among unexposed staff. Both exposed and control individuals were asked to fill up a questionnaire to get necessary information about sex, age, smoking habits, exact description of work, use of therapeutic drugs, previous diagnostic exposure to X-rays, nuclear medicine examination. The blood was taken by venopuncture. We used the alkaline Comet assay to assess the DNA damage. Percentage of DNA in the tail and Comet tail moment were used for further analysis.

The results show the variability between individuals were from both control and exposed groups. The significantly higher mean of DNA damage in lymphocytes of investigated group compared to unexposed control was observed. Among exposed staff there were differences dependent on the kind of work. Mean percent of DNA in Comet tail and Comet tail moment of technicians involved in scintigraphy and positron emission tomography markedly exceeded the mean values of other group. The level DNA damages of lymphocytes of nurses, doctors, pharmacists and administrative workers (contact only with treated patients) were much lower. The level of DNA damage of peripheral blood leukocytes was independent on the period and category of work of exposed people. Cigarette smoking was not related to increases in DNA damage in white blood cells of nuclear medicine personnel in contrary to DNA damage of control individuals. There were no differences between females and males among control and exposed subjects.

In conclusion, monitoring and improvement of health safety of nuclear medicine personnel should be taken under consideration to prevent induction of DNA strand breaks.

This work was funded by Ministry of Science and Higher Education/National Centre for Research and Development, Project "Improvement of Safety and Working Conditions" coordinated by Central Institute for Labour Protection-National Research Institute (2011-2013).

68. 8-OXO-7,8-DIHYDROGUANINE (8OXOGUA) AND URIC ACID AS AN EFFICIENT PREDICTORS OF SURVIVAL IN COLON CANCER (CRC) PATIENTS. RELATIONSHIP BETWEEN THE MAIN ENZYMES (PARP AND OGG) RESPONSIBLE FOR 8OXOGUA REPAIR IN CRC

Tomasz Dziaman[1], Marek Foksiński[1], Daniel Gackowski[1], Rafał Różalski[1], Ewelina Zarakowska[1], Anna Szpila[1], Jolanta Guz[1], Beata Sikorska[1], Agnieszka Siomek[1], Hubert Ludwiczak[2], Jarosław M. Cieśla[2], Mateusz Chmielarczyk[2], Ewa Wiśniewska[3], Andrzej Marszałek[3,4], Barbara Tudek[2,5], Ryszard Oliński[1]

[1]Department of Clinical Biochemistry, Collegium Medicum, Nicolaus Copernicus University, Karlowicza 24, Bydgoszcz, Poland; [2]Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawinskiego 5a, Warsaw, Poland; [3]Department of Clinical Pathomorphology, Collegium Medicum, Nicolaus Copernicus University, Sklodowskiej-Curie 9, Bydgoszcz, Poland; [4]Department of Oncologic Pathology, University of Medical Sciences and Wielkopolskie Oncology Center, Garbary 15, 61-868 Poznan, Poland; [5]Institute of Genetics and Biotechnology, Faculty of Biology, University of Warsaw, Pawinskiego 5a, Warsaw, Poland.

The aim of this work was to answer the question whether the broad range of parameters which describe oxidative stress and oxidatively damaged DNA and repair are appropriate prognosis factors of colon cancer (CRC) patients survival? The following parameters were analyzed for 89 CRC patients: concentration of uric acid and vitamins A, E, C in plasma; levels of 8-oxodG in DNA of leukocytes and colon tissues; urinary excretion rates of 8-oxodG and 8-oxoGua; the activity and mRNA or protein level of repair enzymes OGG1, APE1, ANPG, TDG and PARP1.

All DNA modifications and plasma antioxidants were analyzed using high performance liquid chromatography (HPLC) or HPLC/gas chromatography-mass spectrometry techniques. Expression of repair proteins was analyzed by QPCR, Western or immunohistochemistry methods.

Longer survival coincided with low levels of 8-oxodG/8oxoGua in urine and 8-oxodG in DNA as well as with high concentration of uric acid plasma level. In contrast to expectations, longer survival coincided with lower mRNA level in normal colon tissue of the main 8-oxoGua DNA glycosylase, OGG1, but no association was found for PARP-1 expression.

When analyzing simultaneously two parameters the discriminating power increased significantly. Survival prognosis for patients with low level of urinary 8-oxoGua together with low level of 8-oxodG in leukocytes (both below median value) or high concentration of plasma uric acid (above median value) have the best prediction power. Since prediction value of these parameters seems to be comparable to conventional staging procedure, they could possibly be used as markers to predict clinical success in CRC treatment.

In our work we have also observed good, highly statistically significant correlation between mRNA expression of OGG1and PARP-1 in all investigated tissues/settings; leukocytes of all groups (control, patients with adenoma and CRC) and in marginal/healthy tissues as well as in cancerous one. The good correlation may indicate that the both genes, which are main players in repair of oxidatively damaged DNA, are commonly expressed as an answer to the same stimuli – oxidative stress, which is a feature of CRC.

Interestingly, we observed significant correlation between protein expression of PARP-1 and OGG1only in diseased tissues – polyps and cancerous one. This in turn may be explained by the finding that direct interaction of the proteins is significantly enhanced by oxidative stress.

69. ANTICANCER PROPERTIES OF FERROCENYL DERIVATIVES: DNA DAMAGE IN HUMAN HEPATOMA CANCER CELLS HEPG2 TREATED WITH ACRYLOYLO FERROCENES

Paweł Hikisz[1], Paulina Lewarska[1], Łukasz Szczupak[2], Konrad Kowalski[2], Aneta Koceva-Chyła[1]

[1]Department of Thermobiology, Institute of Biophysics, Faculty of Biology and Environmental Protection, University of Lodz, Pomorska 141/143, 90-236 Lodz, Poland; [2]Department of Organic Chemistry, Institute of Chemistry, Faculty of Chemistry, University of Lodz, Tamka 12, 91-403 Lodz, Poland.

In today's modern oncology, there are new trends for metal complexes with anticancer properties. High hopes in the treatment of cancer is reposed in ferrocene derivatives. Their chemical stability, hydrophobicity and lipophilicity, easy chemical modification and functionalization, as well as, strong redox properties make them suitable candidates as potential chemotherapeutic agents. Cytotoxic properties of ferrocene corresponds to their ability to generate free radicals and DNA cleavage activity thus induce oxidative damage to DNA and leads to strand breaks in deoxyribonucleic acid.

The aim of this study was to investigate the effect of 3 ferrocene derivatives (15, 15Cl and 12a) on the generation of DNA damage in HepG2 human liver cancer cells. The single cell electrophoresis/comet assay, performed under alkaline conditions, was used to measure DNA damage. The cells were incubated for 24 hours with IC_{50} concentration of ferrocenes cells and after then cultured in fresh medium for 0 and 24 hours.

The results indicated that investigated ferrocenes derivatives cause DNA damage in HepG2 cells. Ferrocenes containing chlorine atom in their structure showed greater genotoxic properties. After 24 hours of cell culture in drug free medium, a decrease in the amount of damaged DNA was observed compared with a time of 0 hours. This may suggest that at lest a part of DNA damage caused by investigated ferrocene derivatives are easily repaired in a short time after the treatment by the efficient DNA repair systems in this cancer cell line.

70. MAJOR LIPID PEROXIDATION PRODUCT 4-HYDROKSYNONENAL AFFECTS DNA DAMAGE RESPONSE IN HeLa CELLS

Konrad Kosicki[1], Jolanta Czerwińska[2], Jakub Kucharczyk[1], Elżbieta Speina[2], Wojciech Niedźwiedź[3], Barbara Tudek[1,2]

[1]Institute of Genetics and Biotechnology, University of Warsaw, Pawińskiego 5a, Poland.; [2]Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawińskiego 5a, 02-106 Warsaw, Poland; [3]The Weatherall Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, Headington, Oxford OX3 9DS, UK.

Lipid peroxidation (LPO) is involved in the pathogenesis of several human diseases, including cancer. LPO products react with cellular proteins changing their properties, and with DNA bases to form mutagenic exocyclic-DNA adducts, DNA-DNA and DNA-protein crosslinks. We have shown that HNE induced single- (SSB) and double-strand DNA breaks (DSB) in HeLa cells, which number increased with time up to 24 h after cells treatment with HNE. SSB and DSB caused replication stress should induce the Fanconi system. However, FANCD2 ubiquitination was not induced following HNE treatment. Activation of Fanconi pathway is performed by phosphorylation of FANC core proteins by ATM/ATR kinases. We observed that HNE inhibits phosphorylation of several DNA Damage Response (DDR) proteins, namely Chk1, SMC1, KAP1, RPA, and histone H2AX. This inhibition was visible up to 12 h following cells exposition to HNE. However, phosphorylation of Chk1 was inhibited for the whole time of conducting the experiment, that is 72 h. Only very faint bands of P-Chk1 were seen 24-72 h after HNE treatment of HeLa cells. This could suggest that ATR kinase phosphorylating Chk1 is inhibited by HNE. Since Chk1 is the only kinase, which phosphorylates one of FANC core proteins FANCE, and this phosphorylation is indispensable for FANC core activation and FANCD2 ubiquitination, Chk1 inhibition could explain lack of FANC system activation following HNE treatment in spite of the presence of DNA damage. Interestingly, when HeLa cells were pre-treated with HNE, DDR was inhibited, when cells were subsequently exposed to another DNA damaging agent, camptothecin. DDR inhibition by HNE resulted in cell cycle arrest in G2 phase, and increase of DNA fragmentation. However, 6 h following HNE treatment phosphorylation of DNA-PK_{cs} was induced. This could suggest that in response to HNE high fidelity homologous recombination is substituted by low fidelity non-homologous end joining, thus decreasing genome integrity. Thus, genotoxic activity of HNE is complex and involves both DNA damage and modulation of DNA damage response.

71. QUERCETIN TRIGGERS REACTIVE OXYGEN SPECIES-DEPENDENT INDUCTION OF APOPTOSIS AND LYSOSOMAL DEATH OF SENSITIVE AND MULTIDRUG RESISTANT LEUKAEMIA HL60 CELLS

Agnieszka Maruszewska , Jolanta Tarasiuk

Department of Biochemistry, Faculty of Biology, University of Szczecin, 3c Felczaka St, 71-412 Szczecin, Poland.

Multidrug resistance (MDR) is one of the major causes of the failure of anticancer therapy. One of the most important mechanisms leading to the occurrence of MDR is related to the modulation of cellular death pathways. Reactive oxygen species (ROS) are among the most crucial factors triggering programmed cell death by apoptosis or lysosome membrane permeabilization-dependent mechanisms.

Polyphenols are compounds widely distributed in common diet (e.g. in fruits, vegetables, nuts, wine and tea). Quercetin belongs to the flavonoids – one of the subclasses of polyphenols and there is an increasing body of evidence showing that it is able to modulate cellular death pathways of tumour cells.

The aim of this study was to determine the effect of quercetin on triggering the programmed death of human promyelocytic leukaemia sensitive cells HL60 and multidrug resistant HL60/VINC cells overexpressing P-glycoprotein.

It was found that quercetin exerts comparable cytotoxic activity towards sensitive HL60 and resistant HL60/VINC cells. It has modulated the cellular level of ROS acting as prooxidant or antioxidant agent depending on the concentration used and time of incubation. The results of cell cycle distribution analysis showed significant increase (to about 40-50 %) in the percentage of sub-G1 subpopulation of cells exposed to quercetin used at IC_{90} in the case of both HL60 and HL60/VINC cells. It was also demonstrated that this compound caused oligonucleosomal DNA fragmentation characteristic for apoptosis. It caused significant activation of caspase-3 (1,5-fold in sensitive HL60 and 3,6-fold in resistant HL60/VINC cells) as well as caspase-8 (2,6-fold in sensitive HL60 and 5,6-fold in resistant HL60/VINC cells). Quercetin induced also lysosome membrane permeability of both HL60 and HL60/VINC cells in a concentration-dependent and time-dependent manner.

In summary, obtained results show that quercetin is able to trigger ROS-dependent induction of apoptosis and lysosomal death of sensitive and multidrug resistant leukaemia HL60 cells.

This study was supported by the Faculty of Biology, University of Szczecin, Poland (Grant no. 504-1000-240-850).

72. EFFECT OF FLAVONOID GLYCOSIDES ON REDUCTION OF SUPEROXIDE LEVEL AND DNA DAMAGES AFTER X-RAY RADIATION OF HUMAN LYMPHOCYTES *IN VITRO*

Małgorzata Materska[1], Irena Perucka[1], Maria Konopacka[2], Jacek Rogoliński[2], Krzysztof Ślosarek[3], Barbara Chilczuk[1]

[1]Research Group of Phytochemistry, Department of Chemistry, Agricultural University, Akademicka 15, 20-950 Lublin, Poland; [2]Center of Translational Research and Molecular Biology of Cancer; [3]Radiotherapy and Brachytherapy Treatment Planning, Institute of Oncology, ul. Wybrzeże Armii Krajowej 15, 44-100 Gliwice, Poland.

The antioxidant and radioprotective effect of four main glycosides of phenolic compounds of *Capsicum annuum* L. were studied. Sinapoyl-*E*-glucoside; quercetin-3-*O*-rhamnoside-7-*O*-glucoside, quercetin-3-*O*-rhamnoside and luteolin-7-*O*-(2-apiosyl)-glucoside were isolated and purified by preparative liquid chromatography and their structure was confirmed by spectroscopic methods (UV, ESI-MS). For the first time compounds were assayed for their radioprotective effect on human cell lymphocytes from oxidative damage induced by X radiation. Simultaneously antioxidant activity according to superoxide radical generated by enzymatic and nonenzymatic methods was determined. Glycosides of quercetin, luteolin and sinapic acid showed radioprotective activity against the X radiation higher, than their aglycones, even though they showed weaker antiradical activity. The highest radioprotective potential was noticed for quercetin-3-*O*-rhamnoside. Furthermore, quercetin and luteolin derivatives in contrast to the free aglycones, were not cytotoxic against human lymphocytes in the whole range of concentrations used. The obtained results indicated that derivatives of flavonoid are safer and more efficient in protecting of cellular components from the harmful effects of X-rays in comparison to their aglycones. These compounds can potentially be used as a cell protective substances.

73. MODIFICATION OF WRN PROTEIN WITH 4-HYDROXYNONENAL CHANGES ITS ENZYMATIC ACTIVITIES AND FUNCTIONAL DYNAMICS

Elżbieta Speina[1], Jolanta Czerwińska[1], Jarosław Poznański[1], Janusz Dębski[1], Vilhelm A. Bohr[2], Barbara Tudek[1,3]

[1]Department of Molecular Biology, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, 02-106 Warsaw, ul. Pawińskiego 5a, Poland; [2]Laboratory of Molecular Gerontology, National Institute on Aging, National Institutes of Health, 21224 MD, Baltimore, 251 Bayview Blvd, USA; [3]Institute of Genetics and Biotechnology, Warsaw University, 02-106 Warsaw, ul. Pawińskiego 5a, Poland.

4-Hydroxy-2-nonenal (HNE) is a reactive a α,β-unsaturated aldehyde produced during oxidative stress and subsequent lipid peroxidation of polyunsaturated fatty acids. The reactivity of 4-HNE towards DNA has been well established. Here, Werner (WRN) protein is identified as a target for modification by 4-HNE. Werner syndrome arises through mutations in both copies of the *WRN* gene that encode RecQ 3'-5' DNA helicase and exonuclease essential for genomic stability. This hereditary disease is associated with chromosomal instability, premature aging and cancer predisposition. WRN appears to participate in the cellular response to oxidative stress and cells devoid of WRN display elevated levels of oxidative DNA damage.

Western blot, immunoprecipitation and mass spectrometry were used to identify and characterize the *in vitro* and *in vivo* covalent modifications of WRN by HNE. Sites of 4-HNE adduction on human recombinant WRN were mapped as Lys577 (within ATP binding domain), Cys727, His1290, Cys1367, Lys1371 (within nuclear localization signal) and Lys1389. HNE adduction of WRN protein was shown to appear also *in vivo*, in cells pretreated with HNE or carbon tertrachloride (the agent known to cause lipid peroxidation), suggesting that this modification could influence WRN catalytic activities. We demonstrated that that both helicase and exonuclease activities of HNE modified WRN protein were inhibited both *in vitro* and in immunoprecipitates from cell extracts. However, WRN binding to DNA was unaffected. We applied molecular modeling analysis of HNE adducted to Lys577 and Cys727 residues to provide a potential mechanism of deregulation of WRN enzymatic activity.

74. INVOLVEMENT OF REACTIVE OXYGEN SPECIES AND microRNAs IN CELLULAR RESPONSE TO OXIDATIVE STRESS

Izabella Ślęzak-Prochazka[1,2], Karolina Gajda[1], Roman Jaksik[1], Joanna Rzeszowska-Wolny[1]

[1]Institute of Automatic Control, Silesian University of Technology, 44- 100 Gliwice, Akademicka 16, Poland; [2]Department of Public Health, Czestochowa University of Technology, 42-200 Częstochowa, Armii Krajowej 36b, Poland.

MicroRNAs (miRNAs) are small (~22nt) noncoding RNA molecules that negatively regulate gene expression by binding to the 3'untranslated region (3'UTR) of their target mRNAs. MiRNAs play an important role in cellular processes like apoptosis, proliferation and stress response. It is evident that miRNAs are involved in regulation of the biological pathways associated with ionizing radiation-induced stress responses. However, the exact mechanisms causing altered miRNA levels and the direct targets of many stress-induced miRNAs are largely unknown. The aim of this project is to determine miRNA contribution to cellular response to ionizing radiation.

Leukemic K562 and HL60 and melanoma Me45 cell lines were exposed to ionizing radiation. We determined levels of reactive oxygen species (ROS) using 2′,7′-dichlorofluorescein diacetate (DCFH) and analyzed cell cycle distribution using propidium iodide 1, 4, 8, 12 and 24h after irradiation. In both K562 and HL60 cells, the highest increase in ROS levels was observed 12h after irradiation compared to control non-irradiated cells, whereas we did not observe this increase in Me45 cells. K562 and HL60, but not Me45, cells showed cell cycle arrest in G2/M phase 12h after irradiation. Next, to investigate whether ROS increase is dependent on cell cycle phase, we arrested K562 cells by nocodazole or thymidine block and we observed a similar G2/M-specific increase of ROS detected by DCFH. To determine the involvement of miRNAs, we compared number of predicted miRNA target genes in transcripts up- or downregulated 1, 12 and 24h after irradiation. We observed changes in number of miRNA target genes 12h after irradiation in K562 cells but not in Me45 cells.

These results suggest that miRNAs may cooperate with ROS to induce cellular response to ionizing radiation and regulate cell cycle.

This work was financially supported by Grant No. DEC-2012/04/A/ST7/00353, National Science Centre (NCN) and BKM-514/Rau1/2013/t26, Silesian University of Technology.

Normal Cell Biology

75. PROINFLAMMATORY CONDITIONS STIMULATE CD274 EXPRESSION ON THE HSCS

Agnieszka Ciomber[1,2], Iwona Mitrus[1], Magdalena Głowala-Kosińska[1], Wojciech Fidyk[1], Andrzej Smagur[1], Sebastian Giebel[1]

[1]Department of Bone Marrow Transplantation, Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch, 44-101 Gliwice, Wybrzeże Armii Krajowej 15, Poland; [2]Department of Animal Physiology and Ecotoxicology, Faculty of Biology and Environmental Protection, University of Silesia, 40- 007 Katowice, ul. Bankowa 9, Poland.

Hematopoetic stem cells (HSCs) are responsible for the production of all the lineages of blood cells. HSCs reside inside the bone marrow's microevironment, where several groups of cells and extracellular matrix elements interact with HSCs to regulate their self renewal, differentiation and migration. HSCs may directly interact with the immune system. They for instance possess the ability to regulate expression of CD274 proteins, which is believed to have immunosupressive function. Cell-surface CD274 inhibits T- cell immunity by induction of apoptosis in T lymphocytes. Expression of this protein on HSCs can be induced by stress or immune signals but this process is still poorly described.

The aim of the study was to analyze the expression of CD274 on the surface of HSCs. Firstly we compared the percentage of $CD274^+$ HSCs in bone marrow samples obtained from patients before allogeneic HSCs transplantation and 28 days after the procedure. Besides, in the *in vitro* culture we investigate the impact of proinflammatory and immmunosupressive cytokines on the level of CD274 on the HSCs obtained from peripheral blood stem cells (PBSCs). We examined the effect of IL-2, IL-4, IL-10, TGF-□ and IFN-□ after 24, 48 and 72 hours incubation. The analysis were performed using flow cytometry and appropriate antibodies.

Our results demonstrate increased level of CD274 protein on HSCs after HSCs allotransplantation. The percent of CD274 positive HSCs is 2- fold higher than the one before transplantation. *In vitro* studies show that proinflammatory cytokines stimulate CD274 expression on HSCs. The percent of $CD274^+$ HSCs increases after 24 hours of incubation from 3 to 25% under the influence of IL-2 and to 75% after stimulation with IFN- □ the effect maintains or increases over time. There are no changes in the level of this protein after stimulation with IL-4, IL-10 and TGF-□which have immunosuppressive properties. We assume that the increased expression of CD274 on HSCs is associated with inflammatory conditions in bone marrow microenvironment after HSCs transplantation. The higher expression of this protein probably allows them to survive in recipient's microenvironment.

The study was supported by Grant No. 2011/03/B/NZ6/04917 KTS.

76. INFLUENCE OF TX-100, SDS AND TRYPSIN ON VIABILITY OF FIBROBLASTS *IN VITRO*

Michalina Gramatyka, Piotr Wilczek

Foundation of Cardiac Surgery Development, 41-800 Zabrze, Wolności 345a.

Heart valve diseases are an important mortality cause in patients suffering from cardiovascular diseases. The mechanical and biological valve prostheses, which are currently used, show many limitations associated with their fast degradation (in case of biological prostheses) or ability to cause thromboembolic complications (in case of mechanical prostheses). Removal of the donor cells (decellularization) from tissue and replacing them with recipient cells is the promising method to overcome these limitations. Such approach presents a number of potential advantages including: reduction of the immune response, reduction of the valve calcification intensity and reduction of the risk of transmitting diseases. Most common methods of tissue decellularization include detergent and/or enzymatic treatment. Because all compounds used in the decellularization process show some degree of cytotoxicity, the question arises if their remains in the tissue can negatively affect condition of reseeded cells.

Here we aimed to assess toxicity of trypsin, SDS and Triton X-100 at concentrations corresponding to that used for decuellularization of heart valaves (for trypsin 1, 2 and 3 μg/ml; for SDS 10, 20 and 30 μg/ml; for Triton X-100 2, 4 and 6 μg/ml). We tested influence of these reagents on viability (TUNEL, JC-1 and AnnexinV tests), proliferation (Ki-67 test), adhesion (vinculin, ICAM-1, PECAM-1, VCAM-1 and E-cadherin proteins) and morphology (actin, β-tubulin and vimentin proteins) of fibroblasts cultured *in vitro*, .

We observed that in all concentrations and incubation times neither trypsin nor SDS nor Triton X-100 affected significantly viability of fibroblasts. Similarly, no effect on expression of cytoskeleton and adhesion proteins was observed. There was a slight decrease in proliferation capacity when incubating the cells with the highest concentration of Triton X-100. Further studies with different cell types could allow to confirm (or deny) if compounds remaining in the tissue after the decellularization process may have adverse effect on the condition of the recipient cells.

77. AN INTRIGUING ROLE OF HIF1α IN REGULATION OF HSPA2 IN KERATINOCYTES

Anna Habryka[1,2], Agnieszka Gogler-Pigłowska[1], Mariusz Kryj[3], Zdzisław Krawczyk[1,2], Katarzyna Klarzyńska[1,4], Dorota Ścieglińska[1]

[1]Center for Translational Research and Molecular Biology of Cancer, [3]Oncologic Surgery Clinic, Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch, Poland; [2]Department of Organic Chemistry, Bioorganic Chemistry and Biotechnology, Faculty of Chemistry, Silesian University of Technology, Poland; [4]Department of Biochemistry and Medical Genetics, School of Health Sciences, Medical University of Silesia, Katowice, Poland.

Epidermis, the most superficial layer of the skin, is composed mostly of keratinocytes. It is widely known that keratinocytes environment is hypoxic due to a lack of vasculature system in the epidermis. This phenomenon is manifested by stabilization of HIF1α in the basal layer, the most hypoxic part of the epidermis. Interestingly, as demonstrated by our previous results, HSPA2 is highly expressed in the epidermal basal layer.

We have performed an *in silico* analysis of the potential transcription factors binding sites in the *HSPA2* gene promoter identifying the presence of Hypoxia Responsive Element (HRE), HIF1α binding regulatory sequence, as a result. Consequently, our goal was to investigate the potential role of HIF1α in regulation of the *HSPA2* gene expression in human keratinocytes.

We have determined that during hypoxia in normal human epidermal keratinocytes (NHEK), as well as in immortalized human keratinocytes HaCaT (human adult low calcium high temperature) and in squamous cell carcinoma (A431) cells expression of the *HSPA2* gene is downregulated. Functional analysis of *HSPA2* promoter confirmed these results, showing decreased promoter activity under hypoxic conditions. Furthermore, deletion of HRE from the *HSPA2* promoter significantly reduced activity of reporter Luc gene both under hypoxia and normoxia. This result suggests that HRE is required to maintain the expression of *HSPA2* gene at normoxia. In Chromatin Immunoprecipitation assay (CHIP) we confirmed that HIF1α binds to *HSPA2* promoter in keratinocytes cultured at low oxygen tension. In order to confirm whether HIF1 binding reduces HSPA2 promoter activity at hypoxia, we are currently performing chemical inhibition of HIF1α activity using N-acetylocystein (NAC), which reduces HIF1α stability and echinomycin, which specifically blocks interaction of HIF1α with DNA.

Our results enabled us to draw an overall conclusion that HIF1α acts as a negative regulator of the *HSPA2* promoter activity in keratinocytes under hypoxia.

Research was financed from MNiSW grant No. N401 683740 and NCN grant No. N/NZ1/05/2012/00022.

78. HSPA2 IS A MARKER OF EPIDERMAL UNDIFFERENTIATED KERATINOCYTES

Katarzyna Klarzyńska[1,3], Anna Habryka[1,2], Agnieszka Gogler-Pigłowska[1], Mariusz Kryj[3], Zdzisław Krawczyk[1,4], Damian Sojka[1], Dorota Ścieglińska[1]

[1]Center for Translational Research and Molecular Biology of Cancer; [3]Oncologic Surgery Clinic, Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch, Poland; [2]Department of Organic Chemistry, Bioorganic Chemistry and Biotechnology, Faculty of Chemistry, Silesian University of Technology, Poland; [4]Department of Biochemistry and Medical Genetics, School of Health Sciences, Medical University of Silesia, Katowice, Poland.

This study was designed to characterize the expression of HSPA2 in human epidermis and during process of keratinocytes differentiation. HSPA2 was originally described as testis-specific member of the HSPA (HSP70) heat shock protein family, which is crucial for spermatogenesis. However, recently we have shown that HSPA2 protein is synthesized in human normal somatic tissues in cell- and tissue-type specific manner. High level of HSPA2 protein was found in basal layer of epidermis and other stratified epithelia. Nevertheless, possible function of HSPA2 and mechanisms regulating its expression in epidermis remain to be elucidated.

In the present study, by means of immunofluorescence analysis, we have demonstrated that in human epidermis HSPA2 positive cells colocalize with cells expressing markers specific for undifferentiated keratinocytes (CK5 and CK14). Therefore, we assumed that HSPA2 expression can be regulated during keratinocytes' differentiation. We applied the model of calcium-induced differentiation of HaCaT cells to search for relation between the level of HSPA2 protein and keratinocytes differentiation. HaCaT cells grown in low calcium medium (LCM) showed marker signature specific for undifferentiated (basal) keratinocytes, reduced proliferation rate and significant upregulation of HSPA2 expression. On the contrary, HaCaT cells cultured in high-calcium medium (HCM) showed upregulation of differentiation markers, elevated proliferation rate and stable HSPA2 transcript level. Our results also suggested that the final level of HSPA2 protein can be determined by so far unidentified posttranslational mechanism. We have observed accumulation of HSPA2 protein in high density cell culture, which surprisingly was not accompanied by increase in the level of transcription.

In summary, our results strongly suggest that *HSPA2* gene is highly active in undifferentiated keratinocytes (possibly epidermal progenitor and/or stem cells). In order to confirm this hypothesis, we aim to determine the differences in HSPA2 level between two keratinocytes populations, namely differentiated and enriched with stem cells undifferentiated keratinocytes, isolated by flow cytometry based sorting of primary normal human epidermal keratinocytes (NHEK).

Research was financed from MNiSW grant No. N401 683740.

79. INTERPLAY BETWEEN HSF1 AND HSF2 DURING THE HEAT SHOCK RESPONSE IN MOUSE TESTES

Joanna Korfanty[1], Tomasz Stokowy[1], Natalia Vydra[1], Agnieszka Gogler-Pigłowska[1], Anna Naumowicz[1], Luiza Handschuh[2], Jan Podkowiński[2], Wiesława Widłak[1]

[1]Center for Translational Research and Molecular Biology of Cancer, Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch, 44-101 Gliwice, Wybrzeże Armii Krajowej 15, Poland; [2]Institute of Bioorganic Chemistry Polish Academy of Sciences, 61-704 Poznań, Noskowskiego 12/14, Poland

Heat Shock Factors (HSFs) are transcriptional activators of heat shock genes. Once activated they form trimers and bind specifically to Heat Shock sequence Elements (HSEs) throughout the genome. HSF1 is the primary factor responsible for the response to different forms of cellular stress (e.g. heat shock), while HSF2 becomes activated during development and differentiation (e.g. during spermatogenesis). Both HSF1 and HSF2 cooperate either during stress or under physiological conditions.

Despite the high degree of conservation of the heat shock response, different cells vary in their ability to induce Heat Shock Proteins (HSPs) synthesis and consequently in sensitivity to damaging agents. Interestingly, some types of cells (for example spermatocytes) lack the typical heat shock response and are hypersensitive to hyperthermia. What's more, activation of HSF1 in spermatocytes initiates apoptosis leading to infertility of males. To elucidate mechanisms assisting heat induced apoptosis we studied how HSF1 and HSF2 cooperate with each other in response to heat shock in mouse spermatocytes.

For studies of HSF1 and HSF2 binding to DNA in control and heat shocked (for 5-20 minutes at 38ºC or 43ºC) spermatocytes we applied ChIP-seq (chromatin immunoprecipitation combined with massive parallel sequencing). Analyses revealed that both transcription factors are able to bind to promoter, intragenic and intergenic regions. Increased temperatures induced remodeling of the binding. Following heat shock we stated enhanced HSF1 binding to many *Hsp* and non-*Hsp* promoters. HSF2 was only bound to these promoters at physiological temperature and/or at 38ºC, then it was completely released at 43ºC. Additionally, we stated that binding of HSF1 to the *Hsps* promoters did not correlate with their transcriptional activation following heat shock.

Additionally, we studied direct interactions (heterotrimers are also possible) between HSF1 and HSF2 in control and heat shocked mouse testes using the Duolink In Situ kit. At physiological temperature we observed up to five HSF1/HSF2 complexes per spermatogenic cell. Many of complexes were also located outside of chromatin (stained by DAPI). Significant reduction of HSF1/HSF2 interactions was observed following heat shock, especially after 15 min treatment at 43ºC.

Obtained results suggest that HSF1 and HSF2 could cooperate in regulation of the transcription of some genes only at physiological temperatures and in some cases at 38ºC, since they bind to the same region of the promoter in these conditions. Although HSF1 and HSF2 interactions exist in spermatogenic cells following heat shock, they are markedly reduced and do not stimulate activation of *Hsp* genes expression.

J.K. was supported by the European Community from the European Social Fund within the INTERKADRA project UDA-POKL-04.01.01-00-014/10-00.

80. MODULATION THE NRF2-ARE PATHWAYS BY RESVERATROL AND ITS METHYLTHIO-DERIVATIVES IN MOUSE EPIDERMIS AND HaCaT KERATINOCYTES

Violetta Krajka-Kuźniak[1], Hanna Szaefer[1], Tomasz Stefański[2], Stanisław Sobiak[2], Michał Cichocki[1], Wanda Baer-Dubowska[1]

[1]Department of Pharmaceutical Biochemistry, Poznań University of Medical Sciences, Poznań, Poland; [2]Department of Chemical Technology of Drugs, Poznań University of Medical Sciences, Poznań, Poland.

Resveratrol is the most extensively studied stilbene derivative. We previously showed that resveratrol, beside inhibiting CYP1A1 and 1B1 activities, induced phase II enzymes in mouse skin treated with initiating dose of benzo[a]pyrene or 7,12-dimethylbenz[a]anthracene. Transcription factor Nrf2 plays a key role in regulation of the inducible expression of phase II enzymes such as GST and NQO catalyzing the detoxication of reactive electrophiles and oxidants that contribute to the formation of mutations and ultimately cancers.

In this study, we investigated whether resveratrol and its three 4'-methylthio-*trans*-stilbene derivatives possessing one (3-M-4'-MTS; S2,), two (3,5-DM-4'-MTS; S5) and three (3,4,5-TM-4'-MTS; S7) additional methoxy groups could activate Nrf2 signaling in mouse epidermis *in vivo* and human keratinocytes in culture.

Female CD-1 mice were treated with resveratrol and methylthio-derivatives (S2, S5 and S7) in the doses of 16 μmol in 0.2 ml of acetone per mouse. HaCaT cells were treated with either 20 or 60 μM resveratrol, 20 or 60 μM S2 derivative, 5 or 20 μM S5 compound, or 0.1% DMSO.

Western blot analysis showed translocation of Nrf2 from cytosol to nucleus in both models. Phosphorylation at Ser40 of Nrf2 was detected as a result of treatment with resveratrol and its derivatives, however its level did not differ significantly from that found in the control group of animals or HaCaT cells. The level of Keap1 protein was not significantly changed by methylthio-stilbenes nor resveratrol in both tested model. All tested stilbenes increased GST activity, but resveratrol was the most effective inducer. Moreover, only resveratrol increased protein level of GSTP in mouse epidermis. The cytosolic content of GSTM was enhanced in HaCaT cells after the treatment with derivatives S2 and S5. The same effect was observed for GSTP in the case of compound S2. Resveratrol and its methylthio-derivatives reduced the NQO2 protein level in HaCaT cells. Thus it is possible that increased expression of GSTP or GSTM and GST activity was linked with NQO2 inhibition in these cells.

The results of this study indicate that resveratrol as well as its methylthio-derivatives activate Nrf2 not only in mouse epidermis but also in human keratinocytes. Upregulating the GST isozymes might be particularly important for deactivation of chemical carcinogens such as PAH.

This work was supported by the Ministry of Science and Higher Education of Poland, grant N N 405 209737.

Data Analysis and Computer Modelling

81. A STOCHASTIC MODEL OF THE P53 UBIQUITINATION SYSTEM

Wojciech Bensz, Krzysztof Puszyński

Silesian University of Technology, Gliwice, Poland.

The p53 protein is one of the most widely investigated proteins known to science as it is transcription factor responsible for induction of processes crucial for cell fate such as DNA repair, cell cycle arrest and apoptosis. Mdm2 protein is the main negative regulator of p53, acting via mechanism of ubiquitination - a post-translational protein modification usually designating proteins for efficient, proteasome dependent degradation. Constant fast degradation of p53 protein maintains its low level in normal cells.

New stochastic model of p53 protein ubiquitination process was proposed, basing on p53|Mdm2 feedback loop model by Puszynski et al.[1] and more recent experimental discoveries. One step protein degradation reactions of the original model were replaced by ubiquitination and autoubiquitination reactions catalysed by Mdm2, ultimately leading to degradation of polyubiquitinated proteins. Deubiquitination reactions catalysed by HAUSP protein were additionally incorporated. Stochastic nature of DNA breaks generation, gene copy activation and deactivation included in the model lets for single cell response simulations and estimation of apoptotic and surviving fractions of cells.

Simulation analyses of cells irradiated with different doses of ionizing radiation and expressing different levels of HAUSP deubiquitinase showed that control of the latter is possibly useful strategy to increase apoptotic fraction of irradiated cells. In general, obtained results were in agreement with available knowledge derived from wet laboratory experiments and also suggested some new possibilities which are yet to be verified *in vitro* or *in vivo*.

References:

[1] Puszyński K., Hat B., Lipniacki T. (2008) Oscillations and bistability in the stochastic model of p53 regulation. *J. Theor. Biol.* **254**, 452– 465.

This work was supported by National Science Centre – decision no. DEC-2012/05/D/ST7/02072.

82. SIMULATION ANALYSIS OF THE ATR MODULE AS A DNA DAMAGE DETECTOR UNIT

Monika Kurpas, Krzysztof Puszyński

Silesian University of Technology, Faculty of Automatic Control, Electronics and Computer Science, 44-100 Gliwice, Akademicka 16, Poland

In the human body thousands of DNA lesions occurred every day. Quick detection, amplification and transduction of the damage signal to the effector module are necessary for maintaining the integrity of the DNA in the cell. Damage response results with cell cycle arrest, DNA repair or apoptosis. In eukaryotic cells (especially human), modules: ATM, which responds to the formation of double DNA strand breaks and ATR, which is responsible for detecting single-strand damage, performs a detector function.

Simulation analysis of a constructed mathematical model of ATR pathway is the subject of this study. According to Haseltine-Rawlings postulate, deterministic (for quick reactions; Runge-Kutta fourth-order method) and stochastic (for slow reactions; Gillespie method) simulation algorithm was used. Model parameters were based on informations from literature.

Obtained results agree with the biological data and show that ATR is an effective system for damage detection and strong amplification of the signal. Module can detect even a single lesion. Response of the ATR pathway is very fast - detection takes place within a few seconds after the occurrence of the damage. Lock of the some interactions (in example performed by Chk1, Chk2 and ATR phosphorylation of p53) can lead to cancer or another genetic diseases. Additionally, the created mathematical model explains that the base level of production and activation of p53 protein signaling pathway observed in cells may be caused by persistent cellular stress.

The project was financed by the National Science Centre, in the grant number N N518 287540.

83. MIR-MASK AS A TOOL FOR THE REGULATION OF P53-MDM2 INTRACELLULAR PATHWAYS

Anna Lalik, Roman Jaksik, Krzysztof Puszyński

Systems Engineering Group, Faculty of Automatic Control, Electronics and Informatics, Silesian University of Technology, 44-100 Gliwice, Akademicka 16, Poland.

Disruption of the p53-Mdm2 interaction might have important biological consequences like cell cycle arrest, senesces or apoptosis, therefore the p53-Mdm2 control loop is an attractive therapeutic target.

The aim of this work was to assess the regulatory possibilities of miRNA-masking antisense oligonucleotides in the p53-Mdm2 pathway, by blocking a specific miRNA binding motif in the 3'UTR of the target mRNA [Wang Z (2011) *Methods Mol Biol*.; 676:43-9].

In this study we transfected HCT116 cells with oligonucleotides complementary to specific miRNA binding sites in the Mdm2 mRNA. Cells were collected 24h after transfection and both cell viability as well as apoptosis were evaluated using MTS assay and flow cytometry, respectively. The level of MDM2 was determined by Real-Time PCR and Western blotting.

Our results indicate that the size of apoptotic cells fraction in control and transfected cells varies substantially suggesting a significant impact of the miRNA-masking oligonucleotides on the functioning of the p53 pathway. We also showed that the cells differ in the level of Mdm2 depending on the kind of oligonucleotides used, demonstrating their possibilities as a regulatory agents.

This project was funded by the Polish National Center for Science granted by decision number DEC-2012/05/D/ST7/02072. The experiments were performed in the Biotechnology Center of the Silesian University of Technology using equipment financed by the "Silesian Biofarma".

84. MULTIPLICATIVE NOISE REMOVAL IN MEDICAL ULTRASOUND IMAGES USING TRIMMED NON-LOCAL MEANS TECHNIQUE

Krystian Radlak, Bogdan Smolka, Natalia Radlak

Institute of Automatic Control, Silesian University of Technology, 44-100 Gliwice, Akademicka 16, Poland.

Synovital joint disorders may cause several limitations in joint function and body movement. Progression of structural bone damage in a single joint can be assessed by quantitative Power Doppler Sonography (PDS). However, the images obtained by PDS are contaminated by multiplicative noise and consequently a proper interpretation of the results and a correct diagnosis can be difficult. Therefore the image denoising and enhancement is strongly needed.

Multiplicative noise, also known as speckle noise is a signal distortion appearing due to signal multiplication by a noise process, and is quite difficult to remove. The main aim of the noise removal techniques is to recover the true signal from the corrupted image. The traditional methods of restoration of PDS images are based on Fourier transform and a strategy aiming at converting the multiplicative noise into additive one and to suppress it in the frequency domain.

The main aim of this research is to apply the Trimmed Non-Local Means technique (TNLM) to PDS images to suppress multiplicative noise without the transformation to frequency domain. The TNLM method is a modification of the Non-Local Means algorithm, in which the image pixels are restored by a weighted average of pixels, whose local neighborhood is similar to the local neighborhood of the pixel which is currently being processed. The results show, that this algorithm significantly improves image quality, so that the denoised image is visually more pleasing and easier for interpretation.

The research leading to these results has received funding from the Norwegian Financial Mechanism 2009-2014 under Project Contract No. Pol-Nor/204256/16/2013.

85. MODELING AND TESTING OF TRANSCRIPTION FACTOR BINDING SITES

Karolina Smolińska, Marcin Pacholczyk

Institute of Automatic Control, Silesian University of Technology, Akademicka 16, 44-100 Gliwice, Poland.

Complexes of transcription factors - DNA binding sites play a major role in regulation of gene transcription. Transcription factors have specific structure which allows to bind to transcription factors binding sites (TFBS) on DNA. Nowadays exist many different methods of detecting TFBS which use experimentals or computational tools.

The aim of project was to create methodology that allows to compute models of transcription factors binding sites. The algorithm is based on computional methods and uses crystalographic data and is a modification of Alamanova et al. approach [1]. The result are Position Weigth Matrices (PWMs), that represent models of TFBS. In this work we construct PWMs using two different statistical potentials for estimation of free energy of binding, i.e. a volume-fraction corrected DFIRE- based energy function [2] and statistical potential developed by Robertson and Varani [3]. The main code was written in Python. To test the proposed approach we used NF-κB family of transcription factors (p50p50, p50p65 and p50RelB) and heat shock factor HSF1. PWMs, calculated for different statistical potentials and PWMs based on experimental data fom TRANSFAC database has show significant simillarity and comparable performance.

The present study has shown that computional method of modeling TFBS based on statistical potential can be a good alternative for experimental techniques.

References:

[1] Alamanova D., Steigmaier P., Kel A. (2010) Creating PWMs of transcription factors using 3D structure-based computation of protein-DNA free binding energies. *BMC Bioinformatics* **11**, 225.

[2] Zhao H., Yang Y., Zhou Y. (2010) Structure-based prediction of DNA-binding proteins by structural alignment and a volume-fraction corrected DFIRE-based energy function. *Bioinformatics* **11**, 1857-1863.

[3] Robertson T.A., Varani G. (2007) An all-atom, distance-dependent scoring function for the prediction of protein-DNA interactions from structure. *PROTEINS Struct. Funct. .Bioinform.* **66**, 359-374.

86. LANGTEVIN DYNAMICS IN LOGARITHMIC POTENTIAL APPLIED TO MODEL ACTIVITY OF AN ION CHANNEL GATE

Agata Wawrzkiewicz, Zbigniew Grzywna

Department of Physical Chemistry and Technology of Polymers, Faculty of Chemistry, Silesian University of Technology, Strzody 9, 44-100 Gliwice, Poland.

We consider activity of ion channel gate as a stochastic process which can be modelled by the Langevin dynamics in logarithmic potential. Thanks to this approach, it is possible to describe such features of considered system as: power-law tailed dwell-time distributions, the long-term correlations between the lengths of subsequent channel states, or fractal scaling of some the statistical characteristics with time.

Biological interpretation of the model is provided. We indicate biological counterparts for every model element and parameter, associate the origin of the recognised long-term memory with thickness fluctuations of the cell membrane in vicinity of the channel and describe the indications that lead us to choose logarithmic form of potential function.

Our model exhibits a great flexibility, because of the general view of the problem based on the motion of a reaction coordinate in an asymptotically logarithmic potential in the high friction regime. Depending on the chosen parameter, model can exhibit a very rich behaviour of channel gate dynamics.

The proposed theoretical model was applied for large conductance voltage and Ca^{2+}-activated potassium channel (BK_{Ca}). Obtained results are in good agreement with experimental data.

Methodology

87. FORMATION OF BIOACTIVE COATINGS ON Ti-15Mo ALLOY VIA PLASMA ELECTROLYTIC OXIDATION

Dorota Babilas[1], Joanna Michalska[2], Katarzyna Służalska[3], Anna M. Osyczka[3], Wojciech Simka[1]

[1]Silesian University of Technology, Faculty of Chemistry, Gliwice, Poland; [2]Silesian University of Technology, Faculty of Materials Engineering and Metallurgy, Katowice, Poland; [3]Jagiellonian University, Faculty of Biology and Earth Science, Krakow, Poland.

In recent years a rapid development of surface engineering of metal implants have been noted. The metal implants should have high corrosion resistance, minimal reactivity in the tissue environment, good biotolerance and bioactivity [1]. In the group of metallic materials widely used in biomedical applications titanium and titanium alloys take a special place [2]. In comparison with other metallic materials used for implants, titanium and its alloys have very good properties such as high local corrosion resistance, low density and low modulus of elasticity [3]. Moreover, among the Ti-based alloys used for orthopedic implants β-phase titanium alloys occupy a special place. Ti-15Mo alloy is an example of β-phase titanium alloys and it is particularly characterized by very good electrochemical stability with biocompatibility [4].

In order to improve the integration of the implant with the bone, a surface of the implant should be modified [5]. One of electrochemical methods of surface modification of metal implants is the plasma electrolytic oxidation (PEO). The PEO technique allows to obtain high quality coatings, which are characterized by high surface roughness, high microhardness, good adhesion strength and abrasion resistance. Plasma electrolytic oxidation process is also used for the preparation of bioactive coatings on titanium and its alloys [6].

The aim of this study was to present influence of the chemical composition of the bath and the applied voltage on the morphology, chemical composition and bioactivity of oxide coatings produced on Ti-15Mo alloy by plasma electrolytic oxidation. The PEO process was conducted at voltages in the range of 100 to 400 V. The samples were modified in the 0.5 and 1.0 molar solution of K_2SiO_3 and 5 g/dm^3 KOH. After anodic oxidation the samples of Ti-15Mo alloy were submitted for biocompatibility examinations with human bone marrow-derived mesenchymal stem cells. The results show that voltage passivation has the biggest influence on the morphology and chemical composition of the coatings produced on Ti-15Mo alloy by plasma electrolytic oxidation. As a result of the passivation voltage above 200 V developed and rough oxide layer with characteristic "craters" was obtained. Bioactivity of the Ti-15Mo alloy depends on the applied voltage and solutions in which the passivation process was done. Modification of Ti-15Mo alloy by plasma electrolytic oxidation in bath containing potassium silicate leads to the increase of a bioactivity of the studied alloy.

References:

[1] Wierzchoń T., Czarnowska E., Krupa D. (2004) Inżynieria powierzchni w wytwarzaniu biomateriałów tytanowych, Oficyna Wydawnicza Politechniki Warszawskiej, Warszawa.
[2] Huang C.F. et al. (2009) *J. All. Compounds* **476**, 683.
[3] Hanawa T. (2010) *Japan. Dent. Sci. Rev.* **46**, 93.
[4] Oliveira N.T.C. et al. (2009) *Acta Biomater.* **5**, 399-405.
[5] Liu X. et al. (2004) *Mater. Sci. Eng.* **R 47**, 49-121.
[6] Cheng S. et al. (2011) *Ceramics Intern.* **37**, 1761-1768.

88. THERMODYNAMIC DESCRIPTION OF DIVALENT MANGANESE BINDING TO URIDINE AND ITS SELECTED DERIVATIVES

Katarzyna Bernaczek, Monika Krasowska

Department of Physical Chemistry and Technology of Polymers; Faculty of Chemistry, Silesian University of Technology; Strzody 9, 44-100 Gliwice, Poland.

A fundamental principle of all biological processes is molecular organization and recognition. Biological macromolecules are able to interact with various small and large molecules, with a high degree of specificity and high affinity. Calorimetry is one of technique enabling to study directly the basic physical forces between and within a macromolecule In sufficient detail by measuring heat quantities or heat effects. Modern isothermal titration calorimeters (ITC) are sensitive enough to probe even weak biological interactions in molecular scale. Uridine and its derivatives bounded with divalent manganese ions are presumed to be inhibitors of glycosyltransferases, while being able to link to the enzyme molecule by Mn2+ ions [1,2]. Bond energy inhibitor - manganese was measured by using calorimetric methods (ITC). Thermodynamic parameters were determined to eliminate those substances which have no possibility of binding with enzyme.

References:

[1] Wang R., Steensma D.H., Takaoka Y., Yun J.W., Kajimoto T., Wong C.-H. (1997) *Bioorg. Med. Chem.* **5**, 661.
[2] Wandzik I. (2008) *Acta Pol. Pharm.* **65**, 735

89. MAGNETIC RESONANCE IMAGING AND SPECTROSCOPY OF MOUSE GLIOMA

Łukasz Boguszewicz[1], Agnieszka Skorupa[1], Tomasz Cichoń[2], Ryszard Smolarczyk[2], Magdalena Jarosz[2]

[1]Department of Medical Physics; [2]Molecular Biology Department - Experimental Therapy Laboratory, Maria Skłodowska-Curie Memorial Center and Institute of Oncology, Gliwice Branch, 44-101 Gliwice, Wybrzeże Armii Krajowej 15, Poland.

Magnetic resonance imaging (MRI) and spectroscopy (MRS) are a powerful, non-invasive tools for *in vivo* tumor studies: diagnosis, response to therapy, recurrence and metastases. Small animals are used to run preclinical trials for evaluation and modification of treatment schemes and some similarities between mouse and human glioma models have been reported. We present an equipment setup and simple MRI/MRS acquisition protocol for *in vivo* evaluation of mouse glioma.

All procedures involving animals were performed with the consent of the Local Ethics Committee, Medical Academy, Katowice, Poland. Therapy was performed using 6 to 8-week-old C57BL/6NCrl mice from our own animal facility. Each animal was injected intracranially with 1×10^5 murine glioma GL261 cells /1μL. The cell inoculum was infused at depth of 2,5 mm from the surface of the brain after creating 0,5 mm pocket using stereotactic headframe. Mice were anesthetized by intraperitoneal injection of 2,5% Avertine. The MRI/MRS examinations were performed after 3 weeks of inoculation.

The MRI/MRS examinations were performed on BRUKER Avance III 400 MHz spectrometer with 30 mm microimaging probe and dedicated small animal monitoring/gating system. Standard T1 and T2 weighted as well as high resolution T2 weighted sequences were used. 1H MR single voxel spectra were acquired using PRESS sequence. Total examination time including the animal setup in the NMR probe was about 30 minutes per mouse.

High resolution mouse brain images with large, distinct tumor mass were obtained. MR spectroscopy revealed high mobile lipids levels in the tumor tissue comparing to normal appearing brain tissue.

90. MODELING OF PERVAPORATION IN COMPLEX SYSTEMS

Gabriela Dudek, Anna Strzelewicz, Monika Krasowska, Roman Turczyn, Aleksandra Rybak

Department of Physical Chemistry and Technology of Polymers, Faculty of Chemistry, Silesian University of Technology, Strzody 9, 44-100 Gliwice, Poland.

Pervaporation is considered as a clean and energetically efficient process which has a wide range of applications. It can work separately or it can be integrated into a hybrid process. The modeling of the mass transport in pervaporation seems to be one of the fundamental aspects to understand and therefore can improve the process performance. This paper discuss problem of ethanol/water separation through the complex systems i.e. iron oxide magnetite chitosan composite membranes. This model is based on two steps in pervaporation: (1) sorption into the membrane, and (2) diffusion in the membrane. With the help of this model, key design parameters of the process (diffusion coefficient, permability coefficient, flux, solubility coefficient) were determined.

Finally, the comments on the application of this model with regard to two main research fields in pervaporation: development of membranes and design of processes and modules, were presented.

The authors would like to thank The Silesian University of Technology for providing financial support under the project GG 104/CZP2/2012

References:

[1] Dudek G., Turczyn R., Strzelewicz A. et al. (2013) Studies of separation of vapours and gases through composite membranes with ferroferric oxide mag-netic nanoparticles. *Sep. Purif. Technol.* **109**, 55-63.
[2] Dudek G., Turczyn R., Strzelewicz A. et al. (2012) Preparation and characterization of iron oxides-polymer composite membranes. *Sep. Sci. Technol.* **47**, 1390–1394.
[3] Rybak A., Krasowska M., Strzelewicz A., Grzywna Z.J. (2009) "Smoluchowski type" equations for modelling of air separation by membranes with various structure. *Acta Phys. Polon. B* **40**, 1001-1008.
[4] Strzelewicz A. Krasowska M., Dudek G. et al. (2013) Anomalous diffusion on fractal structure of magnetic membranes. *Acta Phys. Polon. B* **44**, 955-965.
[5] Zielińska K., Kujawski W., Chostenko A.G. (2011) Chitosan hydrogel membranes for pervaporative dehydration of alcohols. *Sep. Purif. Technol.* **83**, 114-120.
[6] Kopeć R., Meller M., Kujawski W., Kujawa J. (2013) Polyamide-6 based pervaporation membranes for organic–organic separation. *Sep. Purif. Technol.* **110**, 63-73.

91. PERCOLATION PHENOMENA IN POLYMERIC MEMBRANES USED FOR GAS SEPARATION

Monika Krasowska, Anna Strzelewicz, Gabriela Dudek, Aleksandra Rybak, Roman Turczyn

Department of Physical Chemistry and Technology of Polymers, Faculty of Chemistry, Silesian University of Technology, Strzody 9, 44-100 Gliwice, Poland.

The cluster properties in macroscopically uniform porous media with a random distribution of elements are the subject of "percolation" theory. The percolation idea was introduced in 1957 by Broadbent and Hammersley [1].

The main problem in percolation [2] is the determination of the percolation threshold (PT), which is the critical concentration of a certain type of elements (sites or bonds, if the porous medium is simulated by relevant lattices), at which a cluster consisting of such elements of an infinite (macroscopic) length - the so-called "percolation cluster" (PC) - emerges in the system for the first time. Until the moment when a percolation cluster is reached, only clusters of finite limited size exist in the system. Upon the emergence of a percolation cluster, the system's properties qualitatively change. Each randomly selected element may now belong to a finite-size cluster or to an infinite percolation cluster. A calculation of the probability of the last realization, having become known as the percolation probability (p), and makes the second most important problem of a percolation theory.

Structure and morphology of dense polymer membranes with dispersed magnetic powder (magnetic membranes) for air separation were investigated [3]. This transport process can be considered as percolation process. The physico-chemical structure of polymer affects the percolation threshold and the percolation concentration. Membranes with various amount and granulation of magnetic powder have different topological structures and may lead to different behaviours of the penetrant.

References:

[1]. Broadbent S., Hammersley J. (1957) Percolation processes I. Crystals and mazes. *Proc. Cambr. Philos. Soc.* **53**, 629–641.

[2] Bunde A., Havlin S. (1991) Fractals and disordered systems. Springer

[3] Krasowska M., Rybak A., Dudek G. et al. (2012) Structure morphology problems in the air separation by magnetic membranes. *J. Membr. Sci.* **415–416**, 864–870.

92. *BRCA1* AND *BRCA2* MUTATION SCANNING USING HIGH RESOLUTION MELTING ANALYSIS (HRM)

Jolanta Pamuła-Piłat, Karolina Tęcza, Joanna Łanuszewska, Ewa Grzybowska

Center for Translational Research and Molecular Biology of Cancer, Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch, Gliwice, Poland.

Hereditary mutations in *BRCA1* and *BRCA2* genes are one of the known causes of the breast and ovarian cancer. The most of them are detected by DNA sequencing, RFPL analysis and ASA-PCR reactions. High resolution melting analysis is an alternative valuable method for screening alternations in genes.

In our study HRM method was used to detect the most frequent founder mutations in Polish population c.68_69delAG, c.181T>G, c.4034delA, c.5266dupC in *BRCA1* and c.5946delT, c.9403delC in *BRCA2* gene. We tested 50 breast cancer patients with strong familial history of breast/ovarian cancer. All reactions were performed in two replicates. This methodology is not specific for these mutations. Differences in plot shapes were compared to the positive and wild type controls. Founder mutations detected by HRM method were confirmed by RFLP-PCR and ASA-PCR. In our studies we have found a few rare genetic alterations.

High resolution melting analysis method is an accurate method for rapid screening of the most frequent founder mutations in *BRCA* genes. HRM offers the possibility of detecting unexpected genetic variants in the PCR products.

93. POLYIMIDE MAGNETIC MIXED MATRIX MEMBRANES FOR THE AIR SEPARATION

Aleksandra Rybak[1], Monika Krasowska[1], Gabriela Dudek[1], Anna Strzelewicz[1], Zbigniew J. Grzywna[1], Petr Sysel[2]

[1]Department of Physical Chemistry and Technology of Polymers, Faculty of Chemistry, Silesian University of Technology, Strzody 9, 44-100 Gliwice, Poland; [2]Institute of Chemical Technology, Faculty of Chemical Technology, Department of Polymers, Technicka 5, CZ-166 28 Prague 6, Czech Republic.

Although oxygen is normally present in the air, higher concentrations are required to treat many disease processes. Over the past few decades, membrane separation process was found to be promising for various medical and industrial applications (air separation, hydrogen recovery and CO_2 removal). In order to combat the limitations of polymer and inorganic membranes research is underway for alternative membrane materials. A very promising strategy for improving the mass transport through polymer films is the incorporation of inorganic materials (zeolites, carbon molecular sieves and silica nanoparticles) into a polymer matrix. They are called mixed matrix membranes (MMMs), a new class of membrane materials, that offers the significant potential in membrane separation technology [1-3].

In this paper, we continue the work on polymer membranes filled with various magnetic powders (magnetic membranes) used for an air enrichment. The idea of magnetic membranes is based on the observation that oxygen and nitrogen have quite different magnetic properties i.e. oxygen is paramagnetic whereas nitrogen diamagnetic, what gives a real chance for their separation [4, 5]. Magnetic membranes with LPI and HBPI matrices were made by casting of HBPAA or LPAA in NMP with a dispersed magnetic powder MQP-14-12 (of the appropriate amount: 0.5 – 1.7g) in the external field of a magnet and keeping at gradually increased temperatures, finally at 230^{o}C for 1 h. For final magnetization, a strong field magnet of about 2,5 T, was used. All these membranes were examined for nitrogen, oxygen and air permeability in the experimental setup with a gas chromatograph HP 5890A. Data analysis was carried out using Time Lag method. The effect of magnetic powder particles on the gas transport properties of heterogeneous membranes was studied. Permeability and diffusion coefficients of O_2, N_2 and synthetic air components were estimated for homogeneous and heterogeneous membranes. The results showed that the membrane permeation properties were improved with the magnetic neodymium particle filling. It was observed that the magnetic polyimide membranes showed higher gas permeability. While their permselectivity was rather maintained or slightly increased. The results also showed that the magnetic powder addition enhanced significantly gas diffusivity in polyimide membranes.

References:

[1] Li N.N., Fane A.G., Ho W.S.W. Matsuura T. (2008) Advanced membrane technology and applications, Hoboken, John Wiley & Sons Ltd.
[2] Polotskaya G.A. et al. (2007) *Sep. Sci. Technol.* **42**, 333- 347.
[3] Goh P.S. et al. (2011) *Sep. Purif. Technol.* **81**, 243-264.
[4] Rybak A., Grzywna Z.J., Kaszuwara W. (2009) *J. Membr. Sci.* **336**, 79- 85.
[5] Rybak A., Dudek G., Krasowska M. et al. (2012) *Sep. Sci. Technol.* **47**, 1395.

94. APPLICATION OF DIFFUSION EDITED ^{1}H NMR SPECTROSCOPY TO QUANTIFICATION OF SERUM LIPOPROTEINS

Agnieszka Skorupa, Łukasz Boguszewicz, Marek Kijonka, Maria Sokół

Department of Medical Physics, Maria Skłodowska-Curie Memorial Center and Institute of Oncology, Gliwice Branch,44-101 Gliwice, ul. Wybrzeże Armii Krajowej 15, Poland.

The pulse-acquire ^{1}H NMR spectra of blood serum consist of sharp resonances from low-molecular weight metabolites and broad signals arising from proteins and lipids. Strong signals in the 0.8-1.3 ppm region have been assigned to CH_3 and CH_2 groups of 'mobile' lipid components of lipoproteins. Lipoproteins are divided into five main classes: chylomicrons, very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL), low density lipoproteins (LDL) and high density lipoproteins (HDL). Moreover, these classes can be further divided into subclasses to obtain a more detailed characterization of the pathological states. Since the beginning of the 1990s Otvos et al. and Ala-Korpela et al. contributed significantly to application of ^{1}H NMR techniques to lipoprotein quantification avoiding tedious physical isolation of these particles from serum [1]. The first group created the LipoProfile test which is commercially available. Interestingly, NMR techniques provide information on lipoprotein particle concentrations. Several studies have shown that the number of LDL particles is a stronger predictor of pathological conditions than LDL-cholesterol which is routinely evaluated. However, accuracy of lipoprotein quantification has been shown to be subclass-dependent due to high signals overlap. Recently information about diffusion coefficients of various lipoprotein subclasses measured by diffusion edited ^{1}H NMR spectroscopy has been exploited. Although results are promising the usefulness of this method should be carefully evaluated by various research groups.

The purpose of this work was to apply diffusion weighted ^{1}H NMR spectroscopy to characterization of serum lipoproteins. The samples were prepared by mixing 250 µl of serum with 250 µl of saline. ^{1}H NMR spectra were acquired at 310 K on Bruker Avance III 400 MHz. One-dimensional ^{1}H NMR spectra were measured using noesypr1d pulse sequence (90º flip angle, 6.2 s acquisition time, relaxation delay of 100 ms). Diffusion edited spectra were measured using double stimulated echo pulse program including bipolar gradient pulses and a longitudinal eddy current delay. The gradient pulse strength was increased from 5 to 95% of the maximum strength in 32 steps. The diffusion time was equal to 120 ms, length of the gradient pulse was equal to 6 ms and relaxation delay was equal to 2 s.

The results of our work indicate usefulness diffusion-weighted ^{1}H NMR spectroscopy in lipoprotein characterization.

References:

[1] Mallol R., Rodriguez M.A., Brezmes J. et al. (2013) Human serum/plasma lipoprotein analysis by NMR: application to the study of diabetic dyslipidemia. *Prog. Nucl. Magn. Reson. Spectrosc.* **70**, 1-24.

95. FRACTAL GEOMETRY CHARACTERIZATION OF MAGNETIC MEMBRANES

Anna Strzelewicz[1], Monika Krasowska[1], Gabriela Dudek[1], Aleksandra Rybak[1], Roman Turczyn[1], Michał Cieśla[2]

[1]Faculty of Chemistry, Silesian University of Technology, Strzody 9, 44-100 Gliwice, Poland; [2]M. Smoluchowski Institute of Physics, Jagellonian University, Reymonta 4, 30-059 Kraków, Poland.

Diffusion in disordered systems does not follow the classical laws and that's why great number of studies have been carried out in order to gain a better understanding of transport phenomenon in membranes with disordered structure. Recently [1-3], we have discussed structure-morphology problems of ethylcelullose membranes with magnetic powder used to the air separation. Fractals are a very good model for the geometrical structure of most disordered materials, and thus in the case of our magnetic membranes. In this paper, we go one step beyond the previous research by analysing membranes with different type and granulation of magnetic powders. The concept of diffusion on fractal structure of polymer membrane with dispersed magnetic powder is discussed. Magnetic membrane, is a medium with penetrant-scale gaps whose size and position are changing randomly. It exhibits distinctive fractal characteristics and can be described by using the fractal geometry i.e. anomalous diffusion exponent α, static fractal dimension d_f and fractal dimension of the trajectory of the random walk d_w.

References:

[1] Rybak A., Krasowska M., Strzelewicz A., Grzywna Z.J. (2009) "Smoluchowski type" equations for modelling of air separation by membranes with various structure. *Acta Phys. Polon. B* **40**, 1001-1008.
[2] Krasowska M., Rybak A., Dudek G. et al. (2012) Structure morphology problems in the air separation by magnetic membranes. *J. Membr. Sci.* **415–416**, 864–870.
[3] Strzelewicz A. Krasowska M., Dudek G. et al. (2013) Anomalous diffusion on fractal structure of magnetic membranes. *Acta Phys. Polon. B* **44**, 955-965.

ADDENDUM

96. A MATHEMATICAL MODEL FOR PREDICTION OF CHANGES IN mRNA-miRNA INTERFERENCE AFTER IRRADIATION

Danuta Gaweł, Roman Jaksik, Joanna Rzeszowska-Wolny, Krzysztof Fujarewicz

Silesian University of Technology, Gliwice, Poland.

To answer the question how irradiation affects mRNA-miRNA interference we have built the prediction model of changes in mRNA expression levels before and after irradiation induced by the miRNA. To do that we have assumed that in short period of time (up to 1 hour after irradiation) the change in mRNA expression levels depends on changes in mRNA-miRNA interference.

Our analysis are based on microarray expression data sets of Me45 cells, K562 and HCT116 with normal p53 gene and with knocked out gene p53. Expression levels of mRNA(microRNA) were measured in irradiated and non-irradiated cells in 1 hour after with Affymetrix(Agilent) microarrays.

In the model described below we have focused on seeds (since the interaction between microRNA and mRNA is possible when the sequence of microRNAs seed region is fully complementary to the mRNA sequence):

$$FCmRNA_i = \sum_{j=1}^{N_s} \left(k_j c_{ij}^{ms} \sum_{l=1}^{N_\mu} c_{jl}^{s\mu} \, \mu RNA_l \right)$$

where $FCmRNA_i$ is the fold change of gene i and N_s is the number of unique seeds. k_j is the coefficient of change in expression of i-th mRNA caused by the presence of j-th seed. C_{ij}^{ms} is the matrix of the size N_m x N_s, where N_m is the number of genes. In i-th row (for each gene) and j-th column (for each seed) is the number of potential binding sides for j-th seed in the 3'UTR region of i-th mRNA. Therefore, assuming that there are 4 potential target sides for j-th seed in the 3'UTR region of i-th mRNA C_{ij}^{ms} would be equal to 4. N_μ is the number of microRNAs, μRNA_l is the expression of l-th microRNA, and $c_{jl}^{s\mu}$ is the matrix of the size $N_s x N_\mu$ which contains the information if l-th microRNA has j-th seed ($c_{jl}^{s\mu} = 1$) or not ($c_{jl}^{s\mu} = 0$).

Results show significant correlation between real and predicted fold change in Me45, HCT116m and HCT116p cells.

This work was funded by Polish National Science Centre, grant DEC-2012/05/B/ST6/03472.

97. GC-CONTENT BIAS IN SHORT OLIGONUCLEOTIDE MICROARRAY STUDIES

Roman Jaksik

Institute of Automatic Control, Silesian University of Technology, Akademicka 16, 44-100 Gliwice, Poland.

High throughput microarray studies can provide invaluable information concerning gene expression changes although the reproducibility of results was many times shown to be very low, suffering from various technical aspects of the experiment.

In this study we asses various sources of technical signal variability focusing on the differences in the GC content of individual probes which can lead to an over 100-fold variations in the measured signal intensity for a specific transcript. The extent of the differences, which result most likely from variations in the reaction efficiency of the main microarray preparation steps (RNA isolation, amplification and hybridization), varies between individual microarrays introducing a strong bias in the data. Based on our study which involves over 160 thousand microarrays from 7 thousand experiments conducted on ten distinct microarray platforms, we show that GC-content related bias has a huge impact on the identification of differentially expressed genes, especially those which show large or low GC percentages of their transcript sequence.

In order to reduce the bias we propose a new pre-processing method csGC-RMA, based on a sample specific background intensity estimation and LOESS regression, which significantly reduces the between sample, signal differences, increasing the dataset integrity. The method was tested on two spike-in microarray datasets increasing the sensitivity and specificity of methods used to identify differentially expressed genes, proving its usefulness as a data preprocessing method.

Or work describes the extent to which probe intensities are affected by GC-content bias, its possible sources and a novel correction method, providing guidelines for the interpretation of microarray data, originating from experiments conducted through the last decade on various microarray platforms.

This work was supported by the Polish National Science Centre grant number 2011/01/N/NZ2/05358. Calculations were carried out using the computer cluster Ziemowit (http://www.ziemowit.hpc.polsl.pl) funded by the Silesian BIO-FARMA project No. POIG.02.01.00-00-166/08 in the Computational Biology and Bioinformatics Laboratory of the Biotechnology Centre in the Silesian University of Technology.

NIE PRZYJEDZIE, BRAK PLAKATU

98. THE EVALUATION OF ULTRASTRUCTURAL CHANGES IN MOUSE'S HEPATOCYTES AND CARDIOMIOCYTES AFTER EXPOSITION TO IONIZING RADIATION

Małgorzata Łysek-Gładysińska[1], Anna Wieczorek[1], Teodora Król[1], Anna Walaszczyk[2], Dorota Gabryś[3], Monika Pietrowska[2]

[1]Department of Cell Biology and Electron Microscope, Institute of Biology, University of Jan Kochanowski, Swiętokrzyska 15, 25-406, Kielce, Poland; [2]Center for Translational Research and Molecular Biology of Cancer, Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology Gliwice Branch Wybrzeże Armii Krajowej 15, 44-101 Gliwice, Poland; [3]Department of Radiology, Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology Gliwice Branch Wybrzeże Armii Krajowej 15, 44-101 Gliwice, Poland.
e-mail:mglad@ujk.kielce.pl

The ionizing radiation is used mainly in oncological radiotherapy to treat breast and genital organs cancer. It may cause inconvertible cell changes which sometimes induce apoptosis and necrosis. Ionizing radiation also induces modifications of lysosomal compartment which is responsible for the intracellular homeostasis. Numerous experimental data demonstrate that ionizing radiation causes the number and volume fraction of lysosomes to increase. At the same time lysosomal enzyme activity in many cell types raises. The ionizing radiation administered at high doses induces significant changes in cells which lead to the intensive lysosomal degradation, apoptosis and necrosis (Ahmed, 2005;Telbisz et al; 2002).

The aim of this study was to monitor the morphological changes at the ultrastructural level in the hepatocytes and cardiomiocytes after ionizing radiation.

The experiment were conducted on male mice (strain C57BL/6J) aged 8-, 48- and 68-weeks. The animals were kept in room temperature (21º) in naturally controlled ratio of light and dark 12 : 12, they were fed a standard food ad libitum. Mice were divided into three control groups and four experimental groups. The experimental animals aged 8 -weeks were treated with single exposition of ionizing radiation at dose 8Gy and 16Gy. Those animals were irradiated with the help of therapeutic linear accelerator Clinac 2300. After 40- and 60 weeks since the irradiation the animals were killed by cervical dislocation and animal tissues were immediately taken for morphological research studies (Marzella and Glaumann, 1980). Thin sections (70-80 nm) were cut on an ultramicrotome Reichert-Jung and double stained with uranyl acetate and lead citrate. The evaluation of the morphological changes of the liver and heart cells was performed using transmission electron microscope (TEM).

The results of morphologic examination have shown changes in the ultrastructure of mouse hepatocytes and cardiomiocytes 40- and 60 –weeks after radiation exposure (at dose 8Gy and 16Gy) in regard to mitochondrion, rough endoplasmic reticulum, lysosomes, Golgi apparatus. A significant increase of lipofuscine accumulated in the cardiomiocytes of mouse have been observed of mice aged 48- and 68- weeks as well as in the group of mice after radiation.

References:

[1] Ahmed R.G. (2005) Damage pattern as function of various types of radiations. *Med. J. Islam. World Acad. Sci.* **15**, 135-147.
[2] Marzella L., Glaumann H. (1980) Increased degradation in rat liver induced by vinblastine II. Morphological characterization. *J. Lab. Invest.* **42**, 18-27.
[3] Telbisz A., Kovács A., Somosy Z. (2002) Influence of X-ray on the autophagic-lysosomal system in rat pancreatic acini. *Micron* **33**, 143-151.

This project was supported by the 7. FP EURATOM 211403 CARDIORISC.

99. FUCCI REPORTER SYSTEM OF CELL CYCLE PROGRESSION IN CANCER CELLS STRESS RESPONSE STUDIES

M. Skonieczna[1], K. Gajda[1], C.A. Feillet[2], F. Delaunay[2], P. Martin[2], M. Kimmel[1,3]

[1]Biosystems Group, Institute of Automatic Control, Silesian University of Technology, Akademicka 16, 44-100 Gliwice, Poland; [2]Institut de Biologie Valrose, Université Nice-Sophia Antipolis, CNRS UMR7277/INSERM U1091 Parc Valrose, 06108 Nice cedex 2, France; [3]Department of Statistics, Rice University, Houston, Texas.

High level of reactive oxygen species (ROS) appear in diverse cellular processes and may cause cellular damage, oxidative stress, and nucleic acid modifications, also in cells exposed to genotoxic factors such as ionizing radiation. Low endogenous ROS levels play a role in redox signaling pathways in cell biology and are generated synchronously with the normal cell cycle. To better understand the role of free radicals in cancer cells a relation between their production and cell cycle progression should be studied.

Progression through each cell cycle phase and transition from one phase to the next are monitored by sensor mechanisms, called checkpoints, which are driven by cyclin-dependent kinase (CDK) family of serine/threonine kinases and their regulatory partners the cyclins [1]. The cell cycle is regulated not only by intracellular signals, but also by extracellular signals, with differentiation, morphogenesis, and cell death in response to stress conditions. To visualize that, combined monitoring system of spatiotemporal dynamics of cell-cycle progression were originally developed and named "fluorescent ubiquitination-based cell cycle indicator" (FUCCI) [2]. Red- and green-emitting fluorescent proteins were fused to CDT1 and GEMININ proteins, which oscillate reciprocally during the cell cycle. CDT1 level is highest in the G1 phase and falls down when the cell enters the S phase, whereas GEMININ level is highest in the S, G2 and M phases and falls when the cell enters the G1 phase. CDT1 and GEMININ are degraded due to the process of ubiquitination, what is referred to (\U") in the name of the reporter method [3].

In our studies we used mouse hepatoma Hepa1-6 FUCCI cells, cultured for 48h in DMEM supplemented with 5% or 10% of FBS to get the distributions of the duration of cell-cycle phases within the population in response to different stress conditions. Cells were observed *in vivo* and images were captured every 15 minutes, by timelapse fluorescence microscope at 37ºC and 5% CO_2.

The Hepa1-6 FUCCI cells were tracked using "LINEAGE TRACKER 2.0", software developed by Warwick Systems Biology at the University of Warwick, UK [3]. As expected, the nucleus of a cancer cell fluoresces in red when this cell is in the G1 phase of the cell cycle, and in green when it is in S, G2 or M phases. Duration of each cell-cycle phase changes in response to different concentration of FBS in growth medium. Limiting access to stimulating factors (5% of FBS) caused cellular stress, and as a consequence, slowing down of the cell cycle and proliferation of cancer cells. That could also explain well known ROS over-production during cell cycle inhibition.

References:

[1] Williams G.H. . Stoeber K. (2012) The cell cycle and cancer. *J. Pathol.* **226**, 352-364.

[2] Sakaue-Sawano A., Kurokawa H., Morimura T. et al. (2008) Visualizing spatiotemporal synamics of multicellular cell-cycle progression. *Cell* **132**, 487–498.

[3] Billy F., Clairambault J., Delaunay F. et al. (2013) Age-structured cell population model to study the influence of growth factors on cell cycle dynamics. *Math. Biosci. Eng.* **10**, 1-17.

This work was financially supported by the National Science Center (NCN, Poland) under Decision No DEC-2012/05/NZ2/01618; DEC-2012/05/B/ST6/03472 and by grant BKM / 514 /RAU-1/2013 t.26 from Silesian University of Technology (M.S. and K.G.), and also ERASysBio+ project C5Sys.

Funding was through ANR (Grant No. ANR-2009-SYSB-002-02) and through the "Investments for the Future" LABEX SIGNALIFE program ANR-11-LABX-0028-01 (FD).

100. CONSEQUENCES OF P53 LEVEL FOR CELL CYCLE PROGRESSION AND DEATH

Magdalena Ochab, Krzysztof Puszyński

Institute of Automatic Control, Faculty of Automatic Control, Electronics and Computer Science, Silesian University of Technology, 44-100 Gliwice, ul. Akademicka 2A, Poland.

P53 regulatory pathway is responsible for cell response like cell cycle arrest, DNA repair or apoptosis in the case of DNA damages. In healthy cells low level of p53 is maintained by rapid ubiquitilation forced by Mdm2, while DNA damage results in p53 stabilization and activation. Phosphorylated form of p53 is capable of activate expression of proteins, which regulate cell cycle (p21) and mediate apoptosis (Bax and BH3-only proteins), inhibit expression of antyapoptotic proteins (Bcl-2 and Bcl-Xl) and inducing synthesis of its own regulators (PTEN and Mdm2). Decision of cell fate is depended of phosphorylated p53 levels and proportion between proapoptotic and antiapoptotic proteins.

Proposed stochastic model include negative feedback loop with Mdm2 and positive feedback loop which include PTEN, PIP3 and AKT, mono- and multiubiquitiation of p53 caused by nuclear MDM2, p53 phosphorylation – spontaneous and DSB-mediated and apoptotic proteins. The balance between proapoptotic and antiapoptotic members of the BCl-2 family can be destroyed by increase level of Bax, which is caused by Bax transcription and P53 forming an complex with the antiapoptotic protein like BCL-2 or BCL-XL. Bax homodimers are require to make the mitochondrial membrane permeable for release of Cytochrome-C, caspase activator.

The results show that cells stressed by irradiation by dose 2 Gy after 36 hours can make different decision like DNA repair, cell cycle arrest or apoptosis. P53 mutation, which make it immpossible to interact in mitochondrial membrane decreases cell ability to induce apoptosis. Moreover, p53 direct interaction with Bcl-2 is sufficient for apoptosis. Cell fate decision between G1 arrest and apoptosis is determined by its ability to increase p53 level and its functions as transcription factor and direct activator.

101. ESTIMATION PARAMETERS OF JAK-STAT PATHWAY MODEL BY USING ADJOINT SENSITIVITY ANALYSIS

Krzysztof Łakomiec, Krzysztof Fujarewicz

Silesian University of Technology, Gliwice, Poland.

The JAK-STAT signaling pathway transfer the information from cellular membrane to nucleus by using the janus kinase (JAK) and signal transducer and activation transcription protein (STAT5). The conformation changes of the membrane erythropoietin receptor causes janus kinase to phosphorylation the cytoplasmic STAT5 proteins. Next STAT5 binds into dimer and travel to nucleus where activate the transcription of target gene. After transcription is done the dimer of STAT5 are decomposed and recycled to cytoplasm.

Experimental data and model are acquired from work [1]. Model contains system of four differential equations with one delay:

$$\begin{cases} \frac{dx_1}{dt} = -k_1 \cdot x_1(t) \cdot EpoRa(t) + 2 \cdot k_4 \cdot x_3(t - tau) \\ \frac{dx_2}{dt} = k_1 \cdot x_1(t) \cdot EpoRa(t) - k_2 \cdot x_2(t)^2 \\ \frac{dx_3}{dt} = -k_3 \cdot x_3(t) + 0.5 \cdot k_2 \cdot x_2(t)^2 \\ \frac{dx_4}{dt} = k_3 \cdot x_3(t) - k_4 \cdot x_3(t - tau) \end{cases} \quad (1)$$

The variables x_1, x_2, x_3, x_4 are quantities of: non-phosphorylated STAT5 protein, the phosphorylated STAT5, dimer of phosphorylated STAT5 and dimer of STAT5 that are in the nucleus. $EpoRa$ is a variable that is correspond to activation at particular time of the erythropoietin receptor. The time tau correspond to time which particular STAT5 molecule remains in the nucleus.

Work presents the utilization of adjoint sensitivity analysis [2] to parameter estimation of this model. Additionally sensitivity analysis indicate that dephosphorylation of single STAT5 protein in cytoplasm can impact this pathway.

References:

[1] Swameye I., Müller T.G., Timmer J. et. al. (2003) Identification of nucleocytoplasmic cycling as a remote sensor in cellular signaling by databased modeling. *PNAS* **100**, 1028-1033.

[2] Fujarewicz K. (2012) Sprzężona analiza wrażliwości układów z opóźnieniami, *X Jubileuszowe seminarium naukowe* Wybrane Zagadnienia Elektrotechniki i Elektroniki, Ustroń 27-29 września 2012

This work was supported by the Polish National Science Center under grant DEC-2012/05/B/ST6/03472.

102. A PARALLEL IMPLEMENTATION OF MALDI-ToF SPECTRA MODELING ON CUDA-ENABLED GRAPHICS HARDWARE

Michal Marczyk[1], Joanna Polanska[1], Andrzej Polanski[2]

[1]Data Mining Group, Institute of Automatic Control; [2]Institute of Informatics, Silesian University of Technology, 44-100 Gliwice, ul. Akademicka 16, Poland.

MALDI-ToF mass spectrometry allows characterization of human proteome giving the possibility to identify protein signatures that can distinguish between profiles of two various biological conditions. We model mass spectrum by a mixture of Gaussian distributions, where components represent signal peaks which defines composition of the sample. We propose to divide spectrum into segments and modeling separate segments using EM algorithm. Obtained model parameters are used for modeling of the whole spectrum. Due to risk of modeling noise we introduced post-processing of spectral components by merging and filtering.

GMM modeling of MALDI-ToF spectra is a complex iterative and nonlinear process which makes it very time consuming, but gives an opportunity to parallelize existing algorithm. Different solutions are available by implementation on graphical processor units or multicore central processor units. The best strategy is to combine these two solutions. To check the efficiency of parallel implementation we used two different platforms with multiple CPUs and different GPUs.

CUDA environment enables efficient and intuitive parallelism of algorithm for decomposition of mass spectrum. Parallelization of the code drastically speed-ups modeling algorithm comparison to the standard implementation on a single CPU. Obtained results depend on the type of hardware used. The most efficient implementation was based on mixed strategy, maximizing usage of available CPUs and GPUs.

This work was funded by the National Science Centre grant (2011/01/N/NZ2/04813).

103. BYSTANDER EFFECT INDUCED BY UVA AND UVB RADIATION IN HUMAN MALIGNANT MELANOMA CELLS; IMPLICATION OF REACTIVE OXYGEN SPECIES

Aleksandra Krzywon, Maria Widel, Joanna Rzeszowska-Wolny

Institute of Automatic Control, Silesian University of Technology, Akademicka 16 Street, 44 – 100 Gliwice, Poland.

Bystander effect is the phenomenon where molecular signals produced by directly irradiated cells induce changes in unirradiated neighbors which manifest as decreased survival, cytogenetic damage, increase in the level of apoptosis, some biochemical and genetic changes. Bystander effect in the case of UV radiation is as yet poorly understood. UV radiation consists of UVA, UVB and UVC waves. UVA (320-400 nm) is predominant part of sunlight reaching the Earth (~95%), whereas UVB (280-320 nm) reaches ~5% and UVC (200-290 nm) is almost completely absorbed by protective ozone layer. UVA and UVB are responsible for skin cancer, however participation of bystander effect in UV induced carcinogenesis is completely unknown.

The aim of presented experiments was to compare the response of malignant melanoma cells (Me45) to direct action of UVA and UVB radiation and response of co-incubated with them non-exposed neighbors of the same line. Using a transwell co-culture system, melamomas growing in 6- well plates were irradiated with UVA (20 kJ/m^2) or UVB (10 kJ/m^2) and co-cultured with non-irradiated cells growing in inserts. Reactive oxygen species, nitric oxide and superoxide radicals were measured by flow cytometry as suspected mediators of bystander effect(s).

The results indicate that bystander effect appeared as diminution of survival measured by MTS assay. Both bands of UV caused an increase of superoxide level which reached the highest values at 24 h in directly irradiated cells and bystander cells (~300% after UVA and ~500% after UVB in comparison with control). In contrast, the level of intracellular reactive oxygen species (ROS) significantly increased after UVA and UVB radiation at 3h of co-incubation and then decreased to control level. In the case of cellular nitric oxide we did not observe any changes after UV light in comparison with control. UVA showed statistically significant increase in mitochondrial membrane potential between 6 and 24 h in directly irradiated and bystander cells. UVB caused increase of mitochondrial membrane potential at 12 and 24 h in directly exposed and bystander cells. Our data indicate that reactive oxygen species but not nitrogen species are potential signaling molecules mediating bystander effect after UVA and UVB radiation.

Supported by the grants No N N518 497 639 and DEC2012/05/B/ST6/03472 from the Polish Ministry of Science and Higher Education and grant BKM 514/RAU1/2013 from the Silesian University of Technology.

104. GENE - CELL-CYCLE CORRELATION IN RESPONSE FOR IONIZING RADIATION (PRELMINARY ANALYSIS)

Joanna Zyla[1], Ghazi Alsbeih[2], Christophe Badie[3], Joanna Polanska[1]

[1]Data Mining Group, Faculty Of Automatic Control, Electronics and Computer Science, Silesian University of Technology, 44-100 Gliwice, Akademicka 16 Poland; [2]Faisal Specialist Hospital & Research Centre, Riyadh 11211, Kingdom of Saudi Arabia; [3]Public Health England, Centre for Radiation, Chemical & Environmental Hazards, Chilton, Didcot, Oxfordshire OX11 ORQ United Kingdom.

Aim: The aim of the study was to show interactions between level of cells in different cell cycles phases and linkage their amount to genes expression level. As a stimulus in research ionizing radiation was used.

Materials and methods: As a population under investigation fraternal twin was used (56 individuals - divided to 28 pairs). Percentage contribution of cells at each cell cycle phase were measured in two checkpoints 0Gy and 2Gy dose (24 hours after irradiation) accompanied by apoptosis fraction (0Gy and 5Gy). The second group of data include measurements of DNA material amount (gene expression) to 10 characteristic genes for radiation sensitivity (0Gy and 2Gy). To all group of data standard fold change were calculated. The Spearman correlation (rho) between each cell cycle phase and each gene (at both checkpoints) was calculated. To randomize the population, twins pairs were divided to two independent groups with using the Bernoulli principle (5000 repeat). What is more the difference and ratio between groups of random twin pair was investigated and correlation was calculated. Additionally the T test for each rho was performed.

Results: As a results, to each check gene-cell cycle interaction the correlation with p-value was obtained. Mean value was calculated and related to p-value<0.05 and compared to correspondence rho. The statistic significance was obtained only for difference and ratio form twin group signal, where various genes were marked at different cell-cycle phase. The number of significant genes for cell cycle phase are present at table 1.

	Differnece | Ratio			
	G1	S	G2	Apoptosis
No. of sig. Genes at 0Gy	3 | 3	1 | 1	2 | 2	0 | 1
No. of sig. Genes at FCH	0 | 0	0 | 0	0 | 0	1 | 1

Table 1. Number of significant genes in integrations with cell cycle to difference and ratio of the signal

Conclusions: There is significant linkage between cells and genes was obtained only for difference and ratio. The tested genes have mostly influence at 0Gy to cell cycle phases. The apoptosis interaction occurred in different gene between 0Gy and FCH. Proposed methodology need to be improved and subjected to further analysis.

The work was financially supported by NCN grant HARMONIA 4 2013/08/M/ST6/00924 (JP, CB), BKM/514/RAU-1/2013t.6 (JZ).
Additionally, Joanna Zyla is holder of scholarship DoktoRis – Scholarship program for Innovative Silesia.

105. THE DIFFERENTIATION OF HEAD/NECK TUMOURS WITH USE OF INFORMATION ABOUT THE DIFFUSION OF WATER MOLECULES OBTAINED FROM NUCLEAR MAGNETIC RESONANCE DATA

Franciszek Binczyk[1], Barbara Bobek-Billewicz[2], Anna Hebda[2], Beata Hejduk[2], Joanna Polańska[1]

[1]Silesian University of Technology in Gliwice, Institute of Automatic Control, Data Mining Group; [2]Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology Gliwice Branch, Radiodiagnostics Department.

Aim of the project: Head/neck tumours are more and more common tumours in the population. They exists number of types of tumours that differs in malignancy, location and expected survival time. The most common approach while identifying the tumour is to look at the cells after biopsy. Hoverer it was noticed that another non invasive technique may be used that requires only Nuclear Magnetic Resonance test. Aim of this work is to analyse information about diffusion of water molecules around and inside tumour tissue to detect characteristic values that describes tumour according to its type.

Materials and methods: Magnetic Resonance Diffusion Weighted Imaging (DWI) is a commonly used sequence that is applied to oncological patients during NMR scan. Obtained information about diffusion of water molecules in the tissue is in most cases useful to answer the question about type of tissue that is examined. It was already proven that in high malignant tumours the capacity of proliferating cells is large and as a result the water movement/diffusion is not limited. On the other hand benign tumours are build of slow proliferating tissue and diffusion of water molecules is limited. The information in DWI is high dimensional and rather complex thus in standard routine a few factors are evaluated and analysed. Such a factors are Relative and Fractional Anisotropy and Apparent Diffusion Coefficient (ADC). In their previous work authors proven that analysis of ADC distribution is useful for analysis of brain tumours. The different head/neck tumour types are different in their structures and more similar (by means of water diffusion) to each other what results in slightly different analysis. The proposed methodology is based on analysis of distribution of factors, obtained by DWI, with emphasis on their characteristic values for different types of tumour tissue. The characteristic values are obtained by analysis of histograms obtained from segmented tumour tissues.

Results: During experiments large data sets of DWI obtained from Institute of Oncology in Gliwice was used. All tumour tissue was manually segmented by experienced physician in order to look for a features of diffusion information that may differentiate/ be characteristic for certain types of tumours. In this work authors present the results of such a analysis with emphasis on the features obtained from DWI that seems to be promising. While the result may be useful directly in clinic research authors will continue their work in the nearest future with emphasis on other NMR modalities to enhance quality of already obtained results.

This project was supported by SUT grant BK/RAU1/2013/10 and UMO-2013/08/M/ST/6/00924 Harmonia 4 NCN Grant.

106. THE DETECTION OF NEURAL PROGENITOR CELL SIGNAL IN NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY DATA

Franciszek Binczyk[1], Michał Staniszewski[2], Agnieszka Polnik[3], Łukasz Boguszewicz[3], Maria Sokół[3], Andrzej Polański[2], Joanna Polańska[1]

[1]Silesian University of Technology in Gliwice, Institute of Automatic Control, Data mining group; [2]Silesian University of Technology in Gliwice, Institute of Informatics; [3]Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology Gliwice Branch, Department of Medical Physics.

Aim of the project: Neural Progenitor cells are the cells that are origin's for new type of cells in human brain. Their existence its important especially in the process of brain repair after stroke or more severe brain injuries. The region of high concentration of NPC in a human brain is said to be a Hipocampus. The NPC's like all other cells in brain may be differentiated by its specific chemical structure. The aim of this work is to verify hypothesis that NPC signal may be found in Nuclear magnetic Resonance Spectroscopy data, with use of two type of data analysis: signal decomposition into Gaussian Mixture Model and signal analysis by SVD techniques.

Materials and methods: Expected NPC signal is of low amplitude since the number of NPC in a brain is low. That implies usage of efficient pre-processing protocol for the used NMR data that cons is of: noise filtering, eddy current correction, phase correction and baseline correction. Pre processed signal is then analysed with use of two techniques in order to switch from large raw data ion set of features that describes peaks that are present in NMR spectrum. The first method is based on frequency signal decomposition into GMM. In such a model peak or group of peaks are represented by a Gaussian component of the model that is described by parameters that key inform about peak height, position and width. Such an approach is promising for a detection of peaks that are overlap with others or are hidden in the signal as expected small peak of NPC.

The second examined method is based on signal decomposition wit SVD approach. The second method differs from first by the input signal. While GMM operates on spectrum in frequency domain, SVD takes free induction decay signal that is still in time domain. The method then decompose the signal into harmonics that are later represented in spectrum as peaks. The method seems to be promising for a detection of small amplitude NPC signal because even if signal is small the frequency may be still detected in induction decay signal as well as parameters of the peak in frequency spectrum.

Results: Large data set consist of single voxel spectroscopy studies was pre-processed and examined with use of two mentioned techniques. The data was obtained in Institute of Oncology in Gliwice for which the presence of NPC signal was expected. The obtained result is promising because at expected chemical shift the peak was found. Authors present a comprehensive analysis that consist of: discussion about strong and weak points of assumed methodology, detected signal and analysis of NPC peak presence with respect to factor that may influence a number of actual cells in hipocampus such as for example age.

This project was supported by SUT grant BKM-514/RAu-1/2013/7 and UMO-2013/08/M/ST/6/00924 Harmonia 4 NCN Grant.

107. THE ASSOCIATION OF GSTP1 GENOTYPE WITH TREATMENT OUTCOME OF CISPLATIN - COMPARISON FUNCTIONS OF THE ENZYMES ON A MOLECULAR LEVEL

Natalia Radlak, Marcin Pacholczyk

Institute of Automatic Control, Silesian University of Technology Gliwice, Poland.

Ovarian cancer is one of the most common cancer among women. Glutathione-S-transferases family enzymes (GSTs) metabolize exogenous and endogenous substances that may have a role in ovarian cancer carcinogenesis. The main aim of this research is to determine if functions of the enzymes associated with environmental exposures are modulated by genetic polymorphism in the GSTP1 gene.

Molecular Docking (MD) - computational simulation of a candidate ligand binding to a receptor, abbreviated as docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules using for example scoring functions. The main goal of this molecular modelling method was to studied the binding mode especially around the active site in order to see how accessible this region is for ligand binding.

GSTP1, can substantially limit the amount of free platinum drugs available for interaction with DNA, by catalyzing their binding to tripeptide glutathione, one of the most abundant molecules in cells. The change of amino acid in the protein structure, one containing an isoleucine [Ile105]GSTP1 and the other a valine residue [105Val]GSTP1 in position 105 has been observed in the hydrophobic substrate-binding site (H-site). Therefore, the presence of valine versus isoleucine in position 105 could change substrate binding, and consequently, enzyme activity.

The work of Natalia Radlak has been supported under "DoktoRIS Scholarship program for innovative Silesia" part-financed by the European Union under the European Social Fund and grant BKM 1514/ RAU1/ 2013 from Silesian University of Technology.

108. METHODS FOR META-ANALYSIS OF EXPRESSION DATA FROM DIFFERENT MICROARRAY PLATFORMS

Anna Papież[1], Paul Finnon[2], Simon Bouffler[2], Christophe Badie[2], Joanna Polańska[1]

[1]Institute of Automatic Control, Silesian University of Technology, Akademicka 16, 44-100 Gliwice, Poland; [2]Public Health England, Centre for Radiation, Chemical & Environmental Hazards, Chilton, Didcot, Oxfordshire OX11 ORQ United Kingdom.

The goal of the study was to examine the impact of combining data from independent microarray experiments on the potential of finding a genetic signature for radiosensitivity among breast cancer patients. Meta-analysis may allow for the overcoming of numerous issues related with the high dimensionality of microarray data sets and thus, gives the opportunity of obtaining an improved estimate of the true effect.

The data consisted of results from two sets of microarray experiments performed after 24h on material from lymphocytes of radiosensitive and radioresistant breast cancer patients subjected to high dose radiation (2 Gy and 4Gy). The experiments were performed on HuGene 1.0 ST oligonucletide chips and Breakthrough 20K cDNA chips.

The genes common for both microarray platforms were chosen for further research. Data processing required batch effect filtration in order to limit the influence of systematic bias due to distinct chip types. After data preprocessing and normalization the differentialy expressed genes were investigated for Gene Ontology annotations along with related signaling pathways. This comparative analysis showed that the most common annotations for genes presenting differential expression in the two experiments occurred repeatedly in both cases. Moreover, these annotations proved to be linked to cancer-associated processes.

Comparative analysis of two microarray experiments resulted in the selection of candidates for radiosensitivity biomarkers. Further study involving the merging of data sets as well as knowledge of the biological processes annotated to the signature genes would enable more accurate prediction of radiosensitivity.

This work was supported by HARMONIA 4 grant register number 2013/08/M/ST6/00924.

109. THE INCREASED SENSITIVITY OF HUMAN BREAST ADENOCARCINOMA CELL LINES ON ELECTROCHEMOTHERAPY *IN VITRO*

Nina Rembiałkowska[1], Aleksandra Kuzan[1], Julita Kulbacka[1], Małgorzata Kotulska[2], Anna Chromańska[1], Jolanta Saczko[1]

[1]Department of Medical Biochemistry, Medical University, Chalubinskiego 10 St., 50-368 Wroclaw; [2]Institute of Biomedical Engineering and Instrumentation, Wroclaw University of Technology, Wybrzeze Wyspianskiego 27, 50-370 Wroclaw.

Electroporation (EP, Electropearmeabilization) is a biophysical method, performed by the application of high voltage electrical pulses to cells in vitro or tissues in vivo. The method is used to increase the cells uptake of different molecules by permeabilization of the membrane. The combination of electroporation and application of drugs with inhibited transport is known as electrochemotherapy (ECT).

The effect of electroporation with and without drug (bleomycin, doxorubicin) was performed by analysis of cloning efficacy test. We investigated human breast adenocarcinoma (MCF-7/WT) and its doxorubicin resistant subline (MCF-7/DOX). Bleomycin was used at 30nM and 300nM concentration; for doxorubicin was selected concentration 0.17nM. The electroporation parameters were: 100, 500, 1000, 1500, 2000V/cm, 50μs and 5 impulses. As electrodes were used thin stainless-steel parallel plates (4 mm gap). Cloning efficacy test was performed as the cell survival assay based on the ability of a single cell to grow into a colony (24h, 72h and 120h after electroporation).

Cloning efficiency test showed that applied electrical pulses alone have not induced cell death below 1500V/cm for MCF-7/WT and below 1000V/cm electric field intensity for MCF-7/DOX. However the application of 2000V/cm electric field intensity was sufficient to decrease cloning efficacy to 50% of control cells. The addition of bleomycin or doxorubicin in the electroporation buffer enabled reduction of the electric field intensity required to cancer cells elimination.

The project is co-financed by the European Union as part of the European Social Fund.

110. SIMULATION ANALYSIS OF ATM AS AN ACTIVATOR OF P53 SIGNALING PATHWAY INCLUDING PPM1D AS A MAJOR DEACTIVATION AGENT

Katarzyna Jonak, Krzysztof Puszyński

Systems Engineering Group, Institute of Automatic Control, Silesian University of Technology, Gliwice, Poland.

Eukaryotic cells are exposed continuously to the genotoxic stresses caused by various sources, such as ionizing radiation (IR). IR generates DNA double-strand breaks (DSBs) that are known to be one of the most cytotoxic lesions. In order to maintain genomic integrity, the DNA damage response (DDR) is activated upon DSB induction. This biological signaling pathway is a cascade of the signals from different types of macromolecules: detectors that recognize DSBs, proteins mediating signal transduction, and effectors responsible for activation of the damage response. DSBs are detected indirectly by ataxia telangiectasia mutated (ATM) that stabilizes and activates the p53 tumor suppressor protein. Various p53 target genes are involved in cell cycle checkpoint arrest, DNA repair, apoptosis or senescence.

The proper functioning of DDR is essential to enhance the cellular survival. Therefore, it is important to study interactions between different components of DDR and to model cellular response to the signals from the specific elements of the DDR pathway.

We propose a mathematical model that explains p53 regulation based on the ATM dependent detector system. Major DNA damage response regulators play an essential role in ATM-p53 pathway. Mdm2 facilitates p53 degradation, checkpoint kinase 2 (Chk2) inhibits p53 ubiquitination and degradation, and cellular transcription factor CREB transcriptionally activates ATM. Moreover, recent works shown that the critical component of the ATM dependent signaling pathway is played by the protein phosphatase PPM1D (Wip1) that regulates dephosphorylation events. Additionally, in this model we linked ATM-p53 pathway components with PPM1D, which is transcriptionally activated by p53 and CREB.

We simulated the cellular response to the damage combining all of the described elements. The obtained results show that ATM pathway is an effective system for DSBs detection with strong amplification signal and quick response. Furthermore, we observed the strong dependence of the cellular response to the DNA damage on PPM1D, what leads to the conclusion that it plays a role as a gatekeeper in the ATM-Mdm2-p53 regulatory loops, essential in the process of DNA damage repair.

Acknowledgement: This work was financially supported by the Polish National Science Center (NCN) grant no. N N518 287540.

111. NUMERICAL SIMULATIONS OF TUMOUR GROWTH INCLUDING ANGIOGENESIS FOR THERAPY PROTOCOL OPTIMISATION

Damian Borys,Krzysztof Psiuk-Maksymowicz, Sebastian Student, Andrzej Świerniak

Institute of Automatic Control, Silesian University of Technology, 44-100 Gliwice, Akademicka 16, Poland.

Presented mathematical model of tumour growth consist of five nonlinear partial differential equations that describe cancer cells dynamics, surrounding healthy tissue cells dynamics, oxygen concentration and concentration of chemotherapeutic and antiangiogenic agent. The continuous model is supplemented with the discrete description of changes of the vascular network with accompanying angiogenesis phenomenon.

Numerical simulations of our model was executed toward finding a suboptimal therapy protocol including two therapies: chemotherapy and antiangiogenic therapy. Protocols has been selected by means of meta-heuristic algorithms. Among the optimisation algorithms simulated annealing, genetic algorithm and ant colony algorithm were implemented. For those algorithms convergence to suboptimal solution was examined and compared, as well as average tumour size, average iteration count and average execution time.

Presented results show that best results has been obtained for ant colony algorithm, however regarding the protocol solution we couldn't find any general pattern for the treatment.

Calculations were carried out using the computer cluster Ziemowit (http://www.ziemowit.hpc.polsl.pl) funded by the Silesian BIO-FARMA project No. POIG.02.01.00-00-166/08 in the Computational Biology and Bioinformatics Laboratory of the Biotechnology Centre in the Silesian University of Technology.
This work was supported by the National Science Centre (NCN) in Poland under Grant No. N-N519-647840.

112. SYNTHESIS AND PROPERTIES OF ALUMINIUM OCTACARBOXYPHTHALOCYANINE - A POTENTIAL PHOTOSENSITIZER FOR PHOTODYNAMIC THERAPY

Joanna Nackiewicz, Marta Kliber, Małgorzata Broda

Faculty of Chemistry - University of Opole, 45-052 Opole ul. Oleska 48, Poland.

Phthalocyanine (Pc) derivatives which are synthetic analogs of porphyrin belong to an important group – aromatic macrocycles that have a conjugated system containing 18 delocalized π electrons [1–3]. Phthalocyanines found many practical applications due to their specific physicochemical properties [4]. In the last decades, Pcs have been used as blue-green dyes and pigments, catalysts, chemical and gas sensors, photoelements in solar cells, photonic devices, semiconductors, liquid crystals and in nonlinear optics [4, 5–8]. Phthalocyanines are also considered to be one of the most promising compounds in nanotechnology [3, 9]. Recently, much attention has been focused on the possibility of using these compounds as second generation photosensitizers in photodynamic therapy (PDT) [10, 11]. The complexes of phthalocyanines with metal ions like Zn^{2+}, Al^{3+}, Ga^{3+} and Si^{4+} are especially interesting in this respect because of a long triplet lifetime and high triplet quantum yield. These features are essential for high singlet oxygen quantum yields and high cytotoxicity against neoplasmic cells [12–14].

Aluminum 2,3,9,10,16,17,23,24-octacarboxyphthalocyanine, (AlPcOC) has been synthesized from pyromellitic dianhydride, aluminium chloride, DBU, and urea (step1), according to the procedure described in [15].

The carboxylic groups in AlPcOC molecules make the compound well soluble in water. AlPcOC has tendency to associate in pH < 7, in water, whereas alkaline solutions, alcohols, DMSO reduced the aggregation of aluminum octacarboxyphthalocyanines.

The obtained complex will be characterized using UV-Vis, IR, MS, NMR and tested in vitro on living cells.

References:

[1] Karaoğlan G.K. Gümrükçü G. Koca A. et al. (2011) *Dyes Pigments* **90**, 11–20.
[2] Acar I. Bıyıklıoğlu Z, Durmuş M. Kantekin H.J. (2012) *Organomet. Chem.* **708–709**,1–45.
[3] Goślinski T. Osmalek T. Konopka K. et al. (2011) *Polyhedron* **30**, 1538–1546.
[4] Liu M.O. Tai C. Sain M. et al. (2004) *J. Photochem. Photobiol. A: Chem.* **165**, 131–136.
[5] Gao L. Qian X. (2001) *Dyes Pigments* **51**, 51–55.
[6] Erdoğmuş A. Nyokong T. (2010) *Dyes Pigments* **86**, 174–181.
[7] Karaoglan G.K. Gümrükçü G. Koca A. (2011) *Dyes Pigments* **88**, 247–256.
[8] Canlıca M. Nyokong T. (2012) *Polyhedron* **31**, 704–709.
[9] Fernandes E.G. Vieira N.C. de Queiroz A.A. et al. (2010) *J. Phys. Chem. C* **114**, 6478–6483.
[10] Klusona P. Drobek M. Zsigmond A. (2009) *Appl. Catal. B: Environm.* **91**, 605–609.
[11] Ke M.R. Huang J.D. Weng S.M. (2009) *J. Photochem. Photobiol. A: Chem.* 201, 23–31.
[12] Lopez T. Ortiz E. Alvarez M. et al. (2010) *Nanomedicine: NBM* **6**, 777– 785.
[13] Durmus M. Yaman H. Göl C. et al. (2011) *Dyes Pigments* **91**, 153–163.
[14] DeRosa M.C. Crutchley R.J. (2002) *Coord. Chem. Rev.* **233-234**, 351-371.
[15] Sakamoto K., Ohno E. (1997) *Prog. Org. Coat.* **31**, 139–145.

Marta Kliber is a recipient of a Ph.D. scholarship under a project funded by the European Social Fund.

113. MODEL OF CEREBRAL VASCULAR NETWORK BASED ON NON-CONTRAST ENHANCED MAGNETIC RESONANCE ANGIOGRAMS

Krzysztof Psiuk-Maksymowicz, Małgorzata Prejs, Damian Borys, Jarosław Śmieja

Institute of Automatic Control, Silesian University of Technology, 44-100 Gliwice, ul. Akademicka 16, Poland

Two types of non-contrast enhanced magnetic resonance angiograms were the input data of the developed method. First of them, Time of Flight (TOF) Angiography – enabled for the visualization of the arterial tree whereas the next one, Phase Contrast (PC) Angiography – enabled for the visualization of both arterial and venous trees.

Medical images stored in DICOM file format required preprocessing. This process included resizing of the images, segmentation of the area of the brain and morphological white top-hat transformation in case of TOF images for noise reduction and gamma transformation necessary for enhancement of the vessels.

The main core of the algorithm consist of segmentation of the vessel sections, selection of the vessels centroids and constriction of objects, connected with each other, of branch and node type. The crucial step for the quality of the results was the segmentation and quality of the source images. Three type of segmentation algorithms were analyzed: automatic and manual threshold segmentation with threshold based on histogram or binarization with hysteresis. The vascular trees obtained from two types of images were compared.

Developed algorithm allows the creation of three-dimensional model of cerebral vascular network and is potentially useful for the diagnosis of various vascular system diseases of the brain as well as for the scientific simulations for blood flow or prediction of the drug distribution in the brain.

This work was supported by the National Science Centre (NCN) under Grant No. 2011/03/B/ST6/04384.

114. EFFICIENT PARALLEL IMPLEMENTATIONS OF NUMERICAL METHODS FOR SIMULATION OF THE VASCULAR SOLID TUMOUR GROWTH MODEL AND RESPONSE TO THERAPY

Sebastian Student, Damian Borys, Krzysztof Psiuk-Maksymowicz, Andrzej Świerniak

Institute of Automatic Control, Silesian University of Technology, 44-100 Gliwice, Akademicka 16, Poland.

Solid tumour growth and the influence of different types of therapies has attracted the attention of theoreticians from many different fields, becoming important areas of active research in the theoretical biology community. Independently of the type of mathematical model, calculation of its solution is always time and resources demanding.

The main interest of the authors was to develop efficient numerical methods for simulations of the vascularised tumour growth under the influence of different types of therapies. A system of partial differential equations was introduced in order to simulate growth of tumour and normal cells as well as the dynamics of the diffusing nutrient and anti-angiogenic or chemotherapeutic factors within the tissue. We have implemented FDTD (finite difference time-domain) method which was already shown to produce numerical stable solutions.

In order to make calculations in larger space which include complex three-dimensional structure of capillaries a single processor computers are not sufficient. Hence there is need to use more computing power to obtain the results in a reasonable time. We are comparing the implementation of the numerical method for multi-computer system (cluster) with the message passing programming paradigm (MPI) with massively parallel computing implementation using graphic computing accelerators. The code was written in C++ and compared with Matlab implementation with appropriate toolboxes (Parallel Computing Toolbox and Distributed Computing Server). Calculations were carried out using the computer cluster Ziemowit (http://www.ziemowit.hpc.polsl.pl) funded by the Silesian BIO-FARMA project No. POIG.02.01.00-00-166/08 in the Computational Biology and Bioinformatics Laboratory of the Biotechnology Centre in the Silesian University of Technology.

In all cases the use of parallel implementation speedups the simulation time in the comparison to the standard implementation on a single processor computer. Our results showed, that we can significantly reduce the simulation time, when we use parallel computing written in C++. The speedup depends on the size of the computation domain, available memory size and type of used processors and realization accuracy.

This work was supported by the National Science Centre (NCN) in Poland under Grant No. N-N519-647840.

115. EFFECT OF EXPOSURE CONDITIONS (DOSE RATE, MEDIUM DEPTH) ON RADIOTHERAPY EFFICACY

Maria Konopacka[1], Jacek Rogoliński[1], Krzysztof Ślosarek[2]

[1]Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch, Center for Translational Research and Molecular Biology of Cancer, Poland; [2] Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch, Department of Radiotherapy and Brachytherapy Treatment Planning, Poland.

Introduction: Cellular response depends not only on the magnitude of adsorbed dose but also on dose rate, its fractionation, positioning of cells with respect to irradiation field, etc. Cancer radiotherapy regimens use radiation of varying dose rates (100 – 600 MU/min).

We tested whether irradiation of cell cultures with the same dose but different dose rate, at different medium depths, in the beam axis or off the irradiation field will generate the same extent of damaged cells.

Materials and Methods: The study was carried out using two cancer cell lines (A549 and HCT116-/-) and one normal line (BEAS-2B). As a radiation source Cliniac 2300 accelerator was used, delivering photon radiation (6 MV). 5 Gy dose was used (at 100 and 600 MU/min dose rate); cells were placed in a water phantom at two depths (3 or 15 cm), either within or outside of the irradiation field.

Biological effects was assessed as: cell survival and cytogenetical changes (micronuclei frequency, apoptosis induction).

Cytogenetic damage was estimated as frequency of micronuclei and condensation of chromatin characteristic for apoptosis process. Cytokinesis-block micronucleus test was used. We counted also the ratio the extent of cytogenetic damage in cancer cells irradiated in beam axis to the extent of damage to normal cells exposed outside the radiation field.

Results*:* Cytogenetic damage:

1. Lower dose rates (100 Gy/min) induce more cytogenetic damage (formation of micronucleated cells and apoptotic cells) than higher ones (600 Gy/min) in both normal and cancer cells;
2. More micronucleated and apoptotic cells form at greater medium depth (15 cm) as compared to lesser depth (3 cm);

These (1,2) observations holds true for both normal and cancer cell lines studied.

These relationships are observed only within the beam field. Cells placed outside of the irradiation field are damaged to the same extent irrespective of depth and dose rate.

Survival:

In cell survival we observed the same relationships but not markedly as these above.

Conclusion: It was found that cytogenetic effect in irradiated cells depends for the same dose on various radiotherapy exposure conditions (dose rate, depth of medium, positioning with respect to axis).

When using smaller dose rates and greater medium depth a therapeutically more beneficial relationship (QE) is observed.

The observations presented herein can be used for future radiotherapy planning.

116. INDUCTION OF DNA LESIONS IN CANCER CELLS BY THIOSUGAR 1,5-ANHYDRO-6-DEOXY-6-METHANE-SULFAMIDO-D-GLUCITOL AND ITS PRECURSOR 1,6-ANHYDRO-5-C-HYDROXYMETHYL-*A*-L-*ALTRO*-PYRANOSE

Anna Czubatka[1], Joanna Sarnik[1], Paweł Tokarz[2], Zbigniew J Witczak[3], Tomasz Poplawski[1]

[1]Department of Molecular Genetics, University of Lodz, Lodz, 90-236, Poland; [2]Department of Organic Chemistry, University of Lodz, Lodz, 91-403, Poland; [3]Department of Pharmaceutical Sciences, Nesbitt School of Pharmacy, Wilkes University, Wilkes-Barre, PA 18766, USA.

Sugars with sulfur atom (thiosugars) gained great interest in recent times. These chemicals have a number of interesting biological properties that can be successfully used in the treatment of many diseases like cancer or diabetes. Anticancer activity of carbohydrates containing sulfur, like thiodisaccharides or thioglycolipids, has also been reported. However, there is no information about the mechanisms of their action. On the basis of earlier reports we decided to investigate thiosugars and their precursors as a potential anticancer agents.

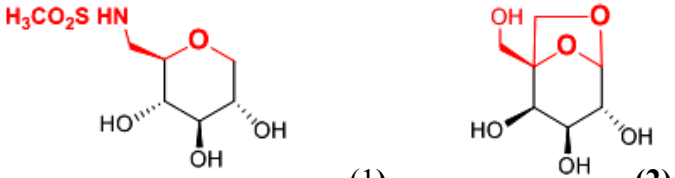


Fig. 1. Thiosugar - 1,5-anhydro-6-deoxy-6-methane-sulfamido-D-glucitol (1) and chemical precursor 1,6-anhydro-5-C-hydroxymethyl-α-L-altro-pyranose(2).

In this work we compared cytotoxic and genotoxic effects induced by studied chemicals in human colon adenocarcinoma cell line (LoVo), human lung adenocarcinoma epithelial cell line (A549) and chronic myelogenous leukemia (K562).A12h exposure of compounds hasn't induced a significant decrease in the viability of LoVo and K562 cell. Only thiosugar increased the viability of A549 cells by 50% in 4 mM concentration. Both compounds evoked DNA damage in all studied cancer cells as evaluated by the alkaline comet assay. Results from the neutral comet assay showed that both chemicals induced DNA double strand breaks (DSBs) and this was confirmed by the plasmid relaxation assay. DNA damage induced by thiosugar and its precursor was persistent and wasn't removed during a 120 min repair incubation.

The results showed that thiosugar and its precursor induced DNA lesions in cancer cells. This compounds may be use in anticancer strategy in combination with the DNA repair inhibitors.

This work was supported by the The National Science Centre, decision nr DEC-2011/01/B/NZ4/03391.

117. BIOCOMPATIBILITY OF NANOPARTICLES LOADED WITH PHOTOSENSIBILIZERS FOR PRACTICAL USE IN SUPPORT OF ANTICANCER THERAPY

Jadwiga Pietkiewicz[1], Urszula Bazylińska[2], Joanna Rossowska[3], Anna Choromańska[1], Andrzej Gamian[1,4], Kazimiera Anna Wilk[2]

[1]Department of Medical Biochemistry, Medical University of Wroclaw, Chalubinskiego 10, 50-368 Wroclaw; [2]Faculty of Chemistry, Wroclaw University of Technology, Wybrzeze Wyspianskiego 27, 50-370 Wroclaw; [3]Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Weigla 12, 53-114 Wroclaw; [4]Wroclaw Research Center EIT+, Stablowicka 147, 50-066 Wroclaw, Poland

In the recent years much effort has been made in the nanomedicine research to develop new effective and biocompatible drug delivery systems having a colloidal phase as a key prerequisite for their effectiveness. Among these systems an important role is played by the template-mediated nanocarriers that form high kinetically stable oil-core nanodispersions of enhanced therapeutic efficiency. The attractiveness of polymeric nanocarriers lies in their ability to incorporate many hydrophobic drugs into the oil phase thereby enhancing their solubility and permeation [1]. The previous our works has been carried out to explore the biological potential of the polymeric nanocapsules loaded with hydrophobic cyanines as effective delivery system of photosensitizer in photodynamic therapy of cancer cells [2,3]. In this report we described physicochemical properties and biocompatibility of multilayer nanocapsules prepared by subsequent adsorption of opposite charged polyelectrolyte layers (dextran and chitosan sulfate sodium salt) on the nanoemulsion liquid core (layer-by-layer (LbL) approach) [1,3]. The L-b-L nanocapsules based on o/w nanoemulsion droplets stabilized by new generation of biocompatible saccharide-derived surfactants, i.e. linear 2-(dodecyldimethylammonio)-ethylgluco-heptonamide bromide (D_2GHA-12) or dicephalic N,N-bis[3,3'(trimethylammonio)propyl] dodecanamide dimethylsulfate (C_{12}(TAPAMS)$_2$) and loaded with hydrophobic cyanine-type photosensibilizers: zinc phtalocyanine ZnPc or cyanine IR-780 were characterized for size, shape and morphology (by DLS, SEM and AFM) and colloidal stability. Additionally, were fabricated analogs functionalized with polyoxyethylene glycol. The obtained nanocarriers offer simultaneously the ease of manufacture with little energy input, a high solubilization capacity and long time-stability. These nanocapsules were subjected to *in vitro* biological analysis for evaluate their biocompatibility and suitability for use in support of anticancer therapy. After parenteral administration, the nanocarriers may interact with plasma proteins and various cells present in the blood stream and for these reason therapeutic efficiency of delivered cargo may be diminished. We characterized biocompatibility of obtained nanocapsules by determination of cytotoxic effect on target cancer cells as well as by estimation of hemolytic activity against human erythrocytes. Additionally, we determined the cytotoxicity of nanocapsules against control normal cells – macrophages and endhotelial cells. Moreover, the degree of macrophage uptake of nanocapsules was examined and the serum albumin influence on this process has been establish for preliminary assessment of biodistribution. The low hemolytic potential and insignificant cytotoxicity against target cancer cells and macrophages as well as endothelial cells indicate good biocompatibility of obtained nanocapsules. Serum albumin at physiological concentration decreased of macrophage uptake of nanocarriers, which suggest the prolongation of their half-life in the circulation. Nanocapsules functionalized with polyoxyethylene glycol were more safe for normal and cancer cells and do not undergo internalization by macrophages.

References:

[1] Bazylińska U., Skrzela R., Szczepanowicz K., Warszyński P., Wilk K.A. (2011) *Soft Matter* **7**, 6113-6124.
[2] Wilk K.A., Zielińska K., Pietkiewicz J., Skołucka N., Choromańska A., Rossowska J., Garbiec A., Saczko J. (2012) *Int. J. Oncol.* **41**, 105-116.
[3] Bazylinska U., Pietkiewicz J., Saczko J., Nattich-Rak M., Rossowska J., Garbiec A., Wilk K.A. (2012) *Eur. J. Pharm. Sci.* **47**, 406–420.

Additional Lecture Abstract:

THE INSTRUMENTAL ANALYSIS OF NOVEL POLYMERIC MATERIALS FOR BIO APPLICATIONS

Andrzej Swinarew[1,2], Sylwia Golba[1], Beata Swinarew[2,3], Jadwiga Gabor[1], Beata Kopek[1], Sylwia Burczyk[1]

[1]Institute of Materials Science, University of Silesia, 40-007 Katowice, Poland; [2]SHIM-POL A.M. Borzymowski E.Borzymowska-Reszka A. Reszka Spółka Jawna; [3]Institute of Engineering, Polymer Materials and Dyes, 87-100 Torun, ul. Maria Sklodowska-Curie 55

Some star-shaped polymers as well as molecularly imprinted polymers (MIPs) are polymeric materials with specific recognition sites complementary in shape, size and functional groups to the template molecule, involving an interaction mechanism based on molecular recognition. These recognition sites mimic the binding sites of biological entities such as antibodies and enzymes. Their stability, ease of preparation and low cost for most of the target analytes make them attractive for numerous applications. The use of star-shaped polymers as stationary phases for HPLC is one of the best studied application of novel polymeric materials, largely because it provides a convenient method for quantitative assessment of the quality of imprints produced by a particular strategy. A wide range of chemical compounds have been separated successfully, ranging from small molecules, such as drugs, to large proteins and cells. The best results have been obtained for molecules with molecular weights in the range of 200–1200Da. The resulting polymers are robust, inexpensive and, in many cases, possess affinity and specificity that is suitable for industrial applications.

In this announcement we present an investigation of new polymeric stationery phase for pathogens detection using the Nexera UHPLC equipped with a Pinnacle DB PAH 1.9 μm, 50 x 2.1 mm column and the multiple DAD and FLD detectors supported with GCMS HS techniques enabling detection of trace-level components. The described method allows for selective absorption of pathogen molecules.

Participant affiliation and e-mail addresses

Name	Participation[1]	Affiliation	City	e-mail address
Abramowicz Agata	P	*Maria Skłodowska-Curie Memorial Center and Institute of Oncology*	*Gliwice*	abramowicz_agata@wp.pl
Agnieszka Bartoszek	L	*Gdańsk University of Technology*	*Gdańsk*	agnieszka.bartoszek@pg.gda.pl
Albini Adriana	L	*IRCCS MutiMedica*	*Milan*	adriana.albini@multimedica.it
Antosiewicz Jędrzej	L	*Gdańsk University of Medical Sciences*	*Gdańsk*	jant@gumed.edu.pl
Baer-Dubowska Wanda	L	*Medical University of Poznan*	*Poznań*	baerw@ump.edu.pl
Bartkowiak Anna	P	*Unversity of Wroclaw*	*Wrocław*	anna.bartkowiak@microb.uni.wroc.pl
Bathen Tone F.	L	*Norwegian University of Science and Technology*	*Trondheim*	tone.f.bathen@ntnu.no
Bednarek Kinga	P	*Institute of Human Genetics PAS*	*Poznań*	pelinska@man.poznan.pl
Bensz Wojciech	P	*Silesian University of Technology*	*Gliwice*	wojciech.bensz@gmail.com
Bernaczek Katarzyna	P	*Silesian University of Technology*	*Gliwice*	Katarzyna.Bernaczek@polsl.pl
Bernasińska Joanna	P	*University of Lodz*	*Łódź*	joannab@biol.uni.lodz.pl
Binns Andrew	EACR	*EACR*	*Nottingham*	Andrew.Binns@nottingham.ac.uk
Błaszczyk Ewa		*Institute of Occupational Medicine and Environmental Health*	*Sosnowiec*	blaszczyk@ietu.katowice.pl
Błażusiak Henryk		*PWiK*	*Gliwice*	pwik@pwik.gliwice.pl
Boratyn Elżbieta	P	*Jagiellonian University*	*Kraków*	elzbieta.boratyn@gmail.com
Borowa-Mazgaj Barbara	P	*Gdańsk University of Technology*	*Gdańsk*	basia.borowa@wp.pl
Borys Damian	P	*Silesian University of Technology*	*Gliwice*	damian.borys@polsl.pl
Brown Karen	L	*University of Leicester*	*Leicester*	kb20@leicester.ac.uk
Budkiewicz Dorota	P	*Maria Skłodowska-Curie Memorial Center and Institute of Oncology*	*Gliwice*	dorotab@rocketmail.com
Burek Małgorzata	P	*Silesian University of Technology*	*Gliwice*	m.burek@op.pl
Byczek Anna		*Silesian University of Technology*	*Gliwice*	anna.byczek@polsl.pl
Chorąży Mieczysław		*Maria Skłodowska-Curie Memorial Center and Institute of Oncology*	*Gliwice*	chorazy@io.gliwice.pl
Cieślar-Pobuda Artur		*Silesian University of Technology*	*Gliwice*	artur.cieslar-pobuda@polsl.pl
Coyle John		*ANIMALAB*	*Poznań*	sw@animalab.pl
Cygankiewicz Adam	P	*University of Lodz*	*Łódź*	adam.c@biol.uni.lodz.pl
Czernek Lilianna	P	*The Centre of Molecular and Macromolecular Studies PAS*	*Łódź*	lczernek@cbmm.lodz.pl
Czerwińska Jolanta	P	*Institute of Biochemistry and Biophysics PAS*	*Warszawa*	jczerwinska@ibb.waw.pl
Czubatka Anna	P	*Unversity of Lodz*	*Łódź*	acz@biol.uni.lodz.pl
Czyż Małgorzata	L	*Medical University of Lodz*	*Łódź*	mczyz@csk.umed.lodz.pl
Denel Marta	P	*Unversity of Lodz*	*Łódź*	martadenel@gmail.com
Dinkova-Kostova Albena	L	*University of Dundee*	*Dundee*	A.DinkovaKostova@dundee.ac.uk
Domińczyk Iwona		*Maria Skłodowska-Curie Memorial Center and Institute of Oncology*	*Gliwice*	
Dudek Gabriela	P	*Silesian University of Technology*	*Gliwice*	gmdudek@polsl.pl
Duechler Marcus	P	*The Centre of Molecular and Macromolecular Studies PAS*	*Łódź*	mduchler@cbmm.lodz.pl
Durbas Małgorzata	P	Jagiellonian University	Kraków	malgorzata.durbas@uj.edu.pl
Dziuban Dorota	P	*Institute of Biochemistry and Biophysics PAS*	*Warszawa*	dziuban@ibb.waw.pl
Erenpreisa Katrina	L	*University of Riga*	*Riga*	katrina@biomed.lu.lv
Fil Dawid	P	*Medical University of Bialystok*	*Białystok*	daveblue7@wp.pl
Filipczyk Anna		*Silesian University of Technology*	*Gliwice*	ania.filipczyk@gmail.com
Filipczyk Łukasz		*Maria Skłodowska-Curie Memorial Center and Institute of Oncology*	*Gliwice*	lukasz.filipczyk@wp.pl
Foksiński Marek	P	*Nicolaus Copernicus University*	*Toruń*	marekf@cm.umk.pl
Frańczak Robert		*Artmedic Sp. z.o.o*	*Jędrzejów*	mglad@ujk.kielce.pl
Gajda Karolina	P	*Silesian University of Technology*	*Gliwice*	Karolina.gajda@polsl.pl
Gdowicz-Kłosok Agnieszka		*Maria Skłodowska-Curie Memorial Center and Institute of Oncology*	*Gliwice*	agagdowicz@wp.pl
Goffeau Andre	L	*Université Catholique*	*Louvain-la-Neuve*	agoffeau@hotmail.com
Golda Adam	P	*Gliwice Medical Center*	*Gliwice*	adamgolda@interia.eu
Gramatyka Michalina	P	*Maria Skłodowska-Curie Memorial Center and Institute of Oncology*	*Gliwice*	mgramatyka@frk.pl
Grochowina Kinga		*Silesian University of Technology*	*Gliwice*	grochowina.kinga@gmail.com
Gruca Aleksandra	P	*Silesian University of Technology*	*Gliwice*	gruca.aleksandra@gmail.com
Grynkiewicz Grzegorz		*Pharmaceutical Research Institute*	*Warszawa*	g.grynkiewicz@ifarm.eu
Grzybek Agata	P	*Silesian University of Technology*	*Gliwice*	agagrzybek@poczta.fm
Grzybowska Ewa	P	*Maria Skłodowska-Curie Memorial Center and Institute of Oncology*	*Gliwice*	ewagrzybowska@yahoo.com
Guz Jolanta	P	*Nicolaus Copernicus University*	*Toruń*	jolaguz@cm.umk.pl
Habryka Hanna	P	*Maria Skłodowska-Curie Memorial Center and Institute of Oncology*	*Gliwice*	habrykaanna@gmail.com
Hahn Przemyslaw	P	*Silesian University of Technology*	*Gliwice*	przemyslaw.hahn@polsl.pl
Hancock Ronald		*Laval University Cancer Research Centre*	*Québec*	ronald.hancock@crhdq.ulaval.ca
Hartman Mariusz	P	*Medical University of Lodz*	*Łódź*	mariuszhartman@gmail.com
Henkel Corinna	L	*Medical Proteome Center, Ruhr University*	*Bochum*	corinna.henkel@rub.de
Herok Robert		*EURx Sp. z o. o.*	*Gdańsk*	robert@eurx.com.pl
Hikisz Paweł	P	*University of Lodz*	*Łódź*	pawelhikisz@gmail.com
Hudy Dorota	P	*Silesian University of Technology*	*Gliwice*	dorota.hudy@dy.pl
Ignatowicz Ewa	P	*Poznan University of Medical Sciences*	*Poznań*	eignato@ump.edu.pl
Jaganmohan Reddy		*Linkoping University*	*Linkoping*	jjmreddy@gmail.com
Janikowski Tomasz	P	*Medical University of Silesia*	*Katowice*	biolmolfarm@sum.edu.pl
Jonak Katarzyna	P	*Silesian University of Technology*	*Gliwice*	jonak.katarzyna@gmail.com
Kalas Wojciech	P	*Institute of Immunology and Experimental Therapy PAS*	*Wrocław*	kalas@iitd.pan.wroc.pl
Karbownik Krystyna		*PWiK*	*Gliwice*	pwik@pwik.gliwice.pl

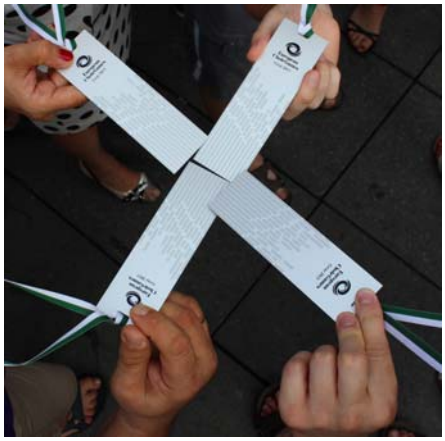
Name	Partici-pation[1]	Affiliation	City	e-mail address
Kardyńska Małgorzata		*Silesian University of Technology*	*Gliwice*	malgorzata.kardynska@polsl.pl
Kędzior Mateusz	P	*University of Wroclaw*	*Wrocław*	mateusz.kedzior@microb.uni.wroc.pl
Kiśluk Joanna		*Medical University of Bialystok*	*Białystok*	jk18@interia.pl
Klarzyńska Katarzyna	P	*Maria Skłodowska-Curie Memorial Center and Institute of Oncology*	*Gliwice*	katarzyna.klarzynska@gmail.com
Kliber Marta	P	*Opole University*	*Opole*	martaemkliber@o2.pl
Kochel Krzysztof	P	*University of Lodz*	*Łódź*	kochel.krzysztof@gmail.com
Komor Katarzyna	P	*Silesian University of Technology*	*Gliwice*	katarzyna.komor@polsl.pl
Komor Roman	P	*Silesian University of Technology*	*Gliwice*	rkomor@polsl.pl
Kopitar Natasha	L	*Josef Stefan Institute*	*Ljubljana*	natasa.kopitar@ijs.si
Korfanty Joanna	P	*Maria Skłodowska-Curie Memorial Center and Institute of Oncology*	*Gliwice*	joanna1540@op.pl
Kosicki Konrad	P	*Warsaw University*	*Warszawa*	konrado@biol.uw.edu.pl
Kostrzewa-Nowak Teresa	P	*Szczecin University*	*Szczecin*	kostrzewa@univ.szczecin.pl
Krajka-Kuźniak Violetta	P	*Poznan University of Medical Sciences*	*Poznan*	vkrajka@ump.edu.pl
Kral Katarzyna	P	*Silesian University of Technology*	*Gliwice*	Katarzyna.Kral@polsl.pl
Krasowska Monika	P	*Silesian University of Technology*	*Gliwice*	Monika.Krasowska@polsl.pl
Krol Ela		*Silesian University of Technology*	*Gliwice*	ela.krol@polsl.pl
Krześniak Małgorzata	P	*Maria Skłodowska-Curie Memorial Center and Institute of Oncology*	*Gliwice*	gosik1@poczta.fm
Kubaichuk Kateryna	P	*Palladin Institute of Biochemistry of NAS*	*Kyiv*	katrissa@ukr.net
Kuzan Aleksandra	P	*Wroclaw Medical University*	*Wrocław*	aleksandra.kuzan@gmail.com
Labudinskyi Dmytro	P	*Palladin Institute of Biochemistry of NAS*	*Kyiv*	konsument3@gmail.com
Lewarska Paulina	P	*University of Lodz*	*Łódź*	paulinalewarska@tlen.pl
Los Marek	L	*Linkoping University*	*Linkoping*	marek.los@liu.se
Lotfi Kourosh	L	*Linkoping University*	*Linkoping*	kourosh.lotfi@liu.se
Łakomiec Krzysztof	P	*Silesian University of Technology*	*Gliwice*	krzysztof.lakomiec@polsl.pl
Łanuszewska Joanna		*Maria Skłodowska-Curie Memorial Center and Institute of Oncology*	*Gliwice*	jlanuszewska@io.gliwice.pl
Łysek-Gładysińska Małgorzata	P	*Jan Kochanowski University in Kielce*	*Kielce*	mglad@ujk.kielce.pl
Marczyk Michał	P	*Silesian University of Technology*	*Gliwice*	Michal.Marczyk@polsl.pl
Marusiak Anna	P	*University of Manchester*	*Manchester*	amarusiak@picr.man.ac.uk
Maruszewska Agnieszka	P	*Szczecin University*	*Szczecin*	maruszewska.a@wp.pl
Materska Małgorzata	P	*University of Life Sciences in Lublin*	*Lublin*	malgorzata.materska@up.lublin.pl
Mielańczyk Anna	P	*Silesian University of Technology*	*Gliwice*	anna.mielanczyk@polsl.pl
Mielżyńska-Svach Danuta		*Institute of Occupational Medicine and Environmental Health*	*Sosnowiec*	d.mielzynska@imp.sosnowiec.pl
Mosieniak Grażyna	L	*Nencki Institute of Experimental Biology*	*Warszawa*	g.mosieniak@nencki.gov.pl
Mothersill Carmel	L	*McMaster University*	*Hamilton*	mothers@MCMASTER.CA
Mucha Anna	P	*Maria Skłodowska-Curie Memorial Center and Institute of Oncology*	*Gliwice*	aniamuz7890@interia.pl
Mullenders Leon	L	*Leiden University Medical Center*	*Leiden*	L.Mullenders@lumc.nl
Nackiewicz Joanna	P	*Opole University*	*Opole*	Joanna.Nackiewicz@uni.opole.pl
Niedźwiedź Wojciech	L	*Oxford University*	*Oxford*	wojciech.niedzwiedz@imm.ox.ac.uk
Nowak Robert	P	*Szczecin University*	*Szczecin*	robnowak@univ.szczecin.pl
Nowakowska Anna	P	*Szczecin University*	*Szczecin*	a.nowakowska76@gmail.com
Ochab Magdalena	P	*Silesian University of Technology*	*Gliwice*	magdaochab@gmail.com
Olbryt Magdalena		*Maria Skłodowska-Curie Memorial Center and Institute of Oncology*	*Gliwice*	molbryt@io.gliwice.pl
Pacholczyk Marcin		*Silesian University of Technology*	*Gliwice*	marcin.pacholczyk@polsl.pl
Pamuła-Piłat Jolanta	P	*Maria Skłodowska-Curie Memorial Center and Institute of Oncology*	*Gliwice*	jpamula@io.gliwice.pl
Papaj Katarzyna	P	*Silesian University of Technology*	*Gliwice*	katarzyna-papaj@wp.pl
Papież Anna	P	*Silesian University of Technology*	*Gliwice*	anna.papiez@polsl.
Paszkowska Jadwiga	P	*Silesian University of Technology*	*Gliwice*	jadwiga.paszkowska@polsl.pl
Pawlak Alicja	P	*Institute of Immunology and Experimental Therapy PAS*	*Wrocław*	alicja8pawlak@gmail.com
Perucka Irena	P	*University of Life Sciences in Lublin*	*Lublin*	irena.perucka@up.lublin.pl
Pieczykolan Jerzy	P	*ADAMED*	*Pieńków*	Jerzy.Pieczykolan@adamed.com.pl
Pirsel Miroslav	L	*Cancer Research Institute, SAS*	*Bratislava*	miroslav.pirsel@savba.sk
Pleśniak Mateusz	P	*Silesian University of Technology*	*Gliwice*	mateusz.plesniak@gmail.com
Ponge Lucyna		*Maria Skłodowska-Curie Memorial Center and Institute of Oncology*	*Gliwice*	lponge@io.gliwice.pl
Psiuk-Maksymowicz Krzysztof	P	*Silesian University of Technology*	*Gliwice*	krzysztof.psiuk-maksymowicz@polsl.pl
Ptaszek-Budniok Agata	P	*Silesian University of Technology*	*Gliwice*	agata.ptaszek-budniok@polsl.pl
Puszyński Krzysztof	L	*Silesian University of Technology*	*Gliwice*	krzysztof.puszynski@polsl.pl
Radlak Krystian	P	*Silesian University of Technology*	*Gliwice*	krystian.radlak@polsl.pl
Radlak Natalia	P	*Silesian University of Technology*	*Gliwice*	natalia.radlak@gmail.com
Rogolinski Jacek		*Maria Skłodowska-Curie Memorial Center and Institute of Oncology*	*Gliwice*	rogolinski@io.gliwice.pl
Rózga Piotr	P	*ADAMED*	*Pieńków*	Piotr.Rozga@adamed.com.pl
Rusin Marek	P	*Maria Skłodowska-Curie Memorial Center and Institute of Oncology*	*Gliwice*	rusinm@rocketmail.com
Rybak Aleksandra	P	*Silesian University of Technology*	*Gliwice*	Aleksandra.Rybak@polsl.pl
Rzeszowska-Wolny Joanna		*Silesian University of Technology*	*Gliwice*	Joanna.Rzeszowska@polsl.pl
Sfakianakis Nikolaos	L	*University of Mainz*	*Mainz*	sfakiana@uni-mainz.de
Sierra Angels	L	*Bellvitge Biomedical Research Institute, IDIBELL*	*Barcelona*	asierra@idibell.cat
Sikorska Beata	P	*Nicolaus Copernicus University*	*Toruń*	sikorska.beata@wp.pl
Skalniak Anna		*Jagiellonian University*	*Kraków*	anna.skalniak@uj.edu.pl
Skonieczna Magdalena	P	*Silesian University of Technology*	*Gliwice*	magdalena.skonieczna@polsl.pl
Skwarska Anna		*Gdańsk University of Technology*	*Gdańsk*	anna.skwarska@pg.gda.pl
Smolińska Karolina	P	*Silesian University of Technology*	*Gliwice*	karolina.smolinska@o2.pl

Name	Partici-pation[1]	Affiliation	City	e-mail address
Sochanik Aleksander		*Maria Sklodowska-Curie Memorial Center and Institute of Oncology*	*Gliwice*	asochanik@io.gliwice.pl
Speina Elżbieta	P	*Institute of Biochemistry and Biophysics PAS*	*Warszawa*	elasp@ibb.waw.pl
Staszewska Anna		*Laborant*	*Łódź*	anna.staszewska@laborant.pl
Stręk Malgorzata		*Medical University of Lodz*	*Łódź*	malgorzata.strek@umed.lodz.pl
Strzelewicz Anna	P	*Silesian University of Technology*	*Gliwice*	Anna.Strzelewicz@polsl.pl
Student Sebastian	L	*Silesian University of Technology*	*Gliwice*	sebastian.student@polsl.pl
Sulewska Anetta		*Medical University of Bialystok*	*Białystok*	zkbm@umb.edu.pl
Sun Xiao-Feng	L	*Linkoping University*	*Linkoping*	xiao-feng.sun@liu.se
Swinarew Andrzej	L	*SHIM-POL A.M.*	*Katowice*	
Szaefer Hanna	P	*Poznan University of Medical Sciences*	*Poznań*	hszaefer@ump.edu.pl
Szczerbik Ola		*Maria Sklodowska-Curie Memorial Center and Institute of Oncology*	*Gliwice*	ola.szczerbik@polsl.pl
Szmajda Ewelina		*Institute of Biochemistry and Biophysics PAS*	*Warszawa*	e.szmajda@ibb.waw.pl
Szmigiero Leszek		*Medical University of Lodz*	*Łódź*	leszek.szmigiero@umed.lodz.pl
Szpila Anna	P	*Nicolaus Copernicus University*	*Toruń*	szpila@cm.umk.pl
Szwed Marzena	P	*University of Lodz*	*Łódź*	szwedmesia@o2.pl
Talar Beata	P	*Medical University of Lodz*	*Łódź*	talar.beata@gmail.com
Tarasiuk Jolanta	P	*Szczecin University*	*Szczecin*	tarasiuk@univ.szczecin.pl
Tęcza Karolina	P	*Maria Sklodowska-Curie Memorial Center and Institute of Oncology*	*Gliwice*	ktecza@io.gliwice.pl
Toma Agnieszka	P	*Maria Sklodowska-Curie Memorial Center and Institute of Oncology*	*Gliwice*	agatoma5@wp.pl
Tudek Barbara	L	*Institute of Biochemistry and Biophysics PAS*	*Warszawa*	tudek@ibb.waw.pl
Ułaszewski Stanisław	P	*University of Wrocław*	*Wrocław*	stanislaw.ulaszewski@microb.uni.wroc.pl
van den Berg Anke	L	*University of Groningen*	*Groningen*	a.van.den.berg01@umcg.nl
Van den Berghe Wim	L	*University Antwerp*	*Wilrijk*	wim.vandenberghe@uantwerpen.be
Vydra Natalia		*Maria Sklodowska-Curie Memorial Center and Institute of Oncology*	*Gliwice*	nvydra@yahoo.co.uk
Waśkiewicz Sylwia	P	*Silesian University of Technology*	*Gliwice*	sylwia.waskiewicz@polsl.pl
Wawrzkiewicz Agata	P	*Silesian University of Technology*	*Gliwice*	Agata.Wawrzkiewicz@polsl.pl
Widłak Wiesława	P	*Maria Sklodowska-Curie Memorial Center and Institute of Oncology*	*Gliwice*	wwidlak@io.gliwice.pl
Wiem Chaabane	P	*Linkoping University*	*Linkoping*	wiemchaabane27@hotmail.fr
Wawrzkiewicz Agata	P	*Silesian University of Technology*	*Gliwice*	Agata.Wawrzkiewicz@polsl.pl
Woźniak Michał	P	*Medical University of Lodz*	*Łódź*	michal.wozniak@umed.lodz.pl
Wyrwicki Szymon		*ANIMALAB*	*Poznań*	sw@animalab.pl
Wysokińska Edyta	P	*Institute of Immunology and Experimental Therapy PAS*	*Wrocław*	edyta.wysokinska@iitd.pan.wroc.pl
Zajkowicz Artur	P	*Maria Sklodowska-Curie Memorial Center and Institute of Oncology*	*Gliwice*	zajkowicza@gmail.pl
Zarakowska Ewelina	P	*Nicolaus Copernicus University*	*Toruń*	ewelinaz@cm.umk.pl
Zemke Natalia	P	*Institute of Human Genetics PAS*	*Poznań*	zemke@man.poznan.pl
Żerek Bartłomiej	P	*ADAMED*	*Pieńków*	Bartlomiej.Zerek@adamed.com.pl
Żyła Joanna	P	*Silesian University of Technology*	*Gliwice*	joanna.zyla@polsl.pl

[1]L-lecture; P-poster

Authors' index

The Academic Music Ensemble (AZM) of Silesian University of Technology was founded in 1996.
Its co-founder and conductor at the same time is Krystyna Łoboda Krzyżanowska – professor at the Music Academy in Katowice.
From the very beginning AZM broke a lot of stereotypes, it includes not only a choir, but also an instrumental ensemble. In its repertoire you can find songs ranging from Gregorian chant to Rock'n'Roll. Every music enthusiast will find something for himself.



The main task of AZM are the musical settings during ceremonies at its alma mater. Another important area of activity of AZM is a large-scale, international cooperation, which highlight is a cyclic event called "International Workshop Musica Pro Europa", which attracts young musicians from around the world and gives them the possibility to learn foreign cultures through work and play.
AZM participated and was awarded at several competitions and festivals in Poland, Czech Republic, Portugal, Spain, Germany, Estonia, Finland, Italy, Malta, Hungary and Ukraine.
This summer AZM won four siver medals in all four disciplines it took part in at an European Choir Games in Austrian Graz.
The ensemble is currently organizing "International Workshop Musica Pro Europa 2013". Final concert contains Bhram's "Ein Deutches Requiem" performed with Hungarian orchestra and Ukrainian choir.

Silesia.

Positive energy

Contents