XVIIIth Gliwice Scientific Meetings 2014

Gliwice, November 21-22, 2014

Organizers of Gliwice Scientific Meetings:

Association for the Support of Cancer Research

Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch

Silesian University of Technology

Institute of Occupational Medicine and Environmental Health, Sosnowiec

Patronage and Co-organizers:

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18th Gliwice Scientific Meetings

Gliwice, 21-22 November 2014

Friday; 21st November, 2014

9.00 – 9.15 ***Opening Ceremony***

9.15 – 12.00 Session I **Transcriptional responses to stress**

Chairman: Piotr Widłak

Allan R. Brasier (Galveston, USA): NF-kB signaling and the innate immune response

Neil D. Perkins (Newcastle, UK): Regulation of cancer cell proliferation and survival by NF-κB

Bożena Kamińska (Warszawa, Poland): Transcriptional and epigenetic mechanisms controlling inflammation

10.45 – 11.00 ***Coffee break***

Marek Kimmel (Houston, USA): Simplicity and complexity in models of cell signaling

Tomasz Lipniacki (Warszawa, Poland): NF-κB and IRF3 crosstalk signaling in MEFs

Mariusz Hartman (Łódź, Poland) – Immediate and long-term molecular response of melanoma cells to changes in microenvironment

12.00 – 13.30 Session II **Biomaterials and nanomedicine**

Chairman: Marek Los

Mehrdad Rafat (Linköping, Sweden): Bioengineered collagen constructs for cell-based regeneration of the cornea

Katerina Chlichlia (Alexandroupolis, Greece): Magnetic and biogenic nanoparticles for cancer therapy

Ramunas Valiokas (Vilnius, Lithuania): Biopatterning techniques in tissue engineering

Łukasz Zarodkiewicz (COMEF): AFM-IR: Combining atomic force microscopy and infrared spectroscopy for multifunctional measurements - nanoscale chemical characterization

13.30 – 14.00 ***Lunch***

14.00 – 15.30 **Poster Session**

15.30 – 18.15 Session III **Bioinformatics and regulatory mechanisms** (session co-organized by EACR)

Chairman: Roman Jaksik

Till Bretschneider (Coventry, UK): Image-based Modelling of Cell Motility

Sascha Ott (Coventry, UK): Analysis of DNase-seq data

Frank Delaunay (Nice, France) Multispectral fluorescent single live cell imaging to analyse the cell cycle and the circadian clock dynamics

16.30 – 16.45 ***Coffee break***

Carmel Mothersill (Hamilton, Canada): Radiation-induced bystander signals: evidence for a role of secondary UVA emission in the generation of oxidative stress and bystander effects by beta irradiation of human keratinocytes

Piotr Formanowicz (Poznań, Poland): Petri net based approach for modeling and analysis of selected aspects of atherosclerosis

Krzysztof Kucharczyk (BioVectis): Minor genetic variants discovery and detection down to 0,1% level at the heterogenic tumor material by the MSSCP method

Michal Braczkowski (ROCHE Diagnostics): Application of ROCHE Diagnostics products for gene expression studies

20.00 – Reception/social event

Saturday; 22nd November, 2014

9.00 – 9.30 **Meeting of the Polish EACR Group**

9.30 – 11.00 Session IV **Glycobiology in medicine**

Chairman: Maciej Ugorski

Anna Lityńska (Kraków, Poland): The role of aberrant glycosylation in human melanoma progression to more malignant phenotype

Marcin Czerwiński (Wrocław, Poland): Glycosyltranferases and blood group antigens

Wojciech Jachymek (Wrocław, Poland): Bacterial glycomics: new approach to vaccine production

Yanusz Wegrowski (Reims, France): Matrix proteoglycans as regulators of tumor progression

11.00 – 11.30 ***Coffee break***

11.30 – 12.30 **Poster Session - Presentation of Awarded Posters**

Satellite Workshop:
Genes – environment interactions

Organizers: Danuta Mielżyńska and Korneliusz Miksch)

12.30 – 17.00 **Genes – environment interactions**

Maria Dusińska (Kjeller, Norway): Challenges with gene-environment interactions: Where we are and where we need to go?

Sofia Pavanello (Padova, Italy): Monitoring of exposures to carcinogens: Does the procedure need a rethink?

13.30 – 14.00 ***Lunch***

Karin Broberg Palmgren (Lund, Sweden): Epigenetics of metals

Natalia Pawlas (Sosnowiec, Poland): Genetic susceptibility to metals

Süreyya Meriç Pagano (Tekirdag, Turkey): Multi-bioassay toxicity evaluation of a mixture of different group of pharmaceuticals

Norbert Kreuzinger (Vienna, Austria): Wastewater treatment plants as a hub between clinical and environmental antibiotic resistance

Aneta Łuczkiewicz (Gdańsk, Poland): Human-associated bacteria and their mobile genetic elements - important minority in wastewater and wastewater impacted ecosystems

Aleksandra Ziembińska-Buczyńska (Gliwice, Poland): Antibiotics and their resistance genes in the environment – bacterial opportunity or threat?

17.00 Closing remarks

Dr Jerzy Jurka (1950- 2014)

Shreds of Memories

Jerzy Jurka, PhD, a biologist, born June 4, 1950 in Ponikiew, Wadowice District of southern Poland, died July 19, 2014 in Los Altos, CA (USA) following a two-year battle with a grave disease. Senior staff from the Department of Tumor Biology, MSC Center of Oncology in Gliwice will surely remember Jerzy, an energetic and ambitious young scientist who, from the beginnings of his career, consequently implemented his plans and academic ideas. Jerzy Jurka attended high school in Wadowice and then studied Chemistry at the Jagiellonian University in Cracow. After graduating in 1973 he worked at the Tumor Biology Department of the Institute in Gliwice until 1982. He was then very much interested in work carried out by Professor Stanislaw Szala and concerning repetitive DNA sequences. However, from the very beginning, he had been showing more interest in theoretical aspects of genetic information and problems of evolution than in experimental benchwork. Having been invited by Professor Manfred Eigen, a Nobel laureate, Jerzy went to the Max Planck Institute of Biophysical Chemistry in Göttingen (Germany). He also visited Jacques Monod Memorial Institute of Molecular Biology in Paris to work with Dr. F. Chapeville and Dr. J. Ninio. He was granted the PhD degree in 1979 from the Biology Department, Uniwersity of Warsaw, majoring in the field of molecular biology. His dissertation concerned "Early Evolution of Genetic Information in the light of the structure of Genetic Code and Proteins".

In 1982 Dr Jerzy Jurka left Gliwice for Stockholm where he was working in Arrhenius Laboratory of the Stockholm University, as well as in Karolinska Institute. Then, in 1983 Dr Jurka emigrated to the United States of America, his new homeland. He had been working at Ann Arbor Laboratory (Michigan), at the University of Houston (Texas), at Dana-Farber Institute/Harvard School of Public Health, Boston (Massachusetts), and Bionet Company in Mountain View (California). Finally, in 1989, he joined the Linus Pauling Institute of Science and Medicine. There, he soon became Assistant Director of Research (Linus Pauling deputy), while being the Head of the Computational Biology Group. Jerzy has had working contacts with such distinguished investigators like Roy Britten and Emile Zuckerkandl.

After the death of L. Pauling in 1994, with the assistance of several of his co-workers, Dr. Jurka set up a new research establishment, the Genetic Information Research Institute (GIRI) and became its research director. He stayed in this capacity until his death in 2014. GIRI has been an excellent although not tremendously large institute, employing a dozen of researchers, mainly computer scientists, biologists and statisticians. GIRI has been financed chiefly through long-term contracts and grants from the Human Genome Project led by the US Department of Energy, as well as from the US National Institutes of Health (NIH). GIRI has set up the world-largest database

featuring DNA sequences from various living organisms and a unique databank of repetitive sequences ("Repbase").

DNA repetitive sequences, transposons or transposable elements, their variety, development, biological significance for genome functioning, genome translocations and horizontal transfers (between species), their contribution to evolutionary processes all were of interest to Dr. Jurka and his collaborators at GIRI. They together identified TE from the Alu family and determined that intergenerational transmission occurs *via* paternal side. Another kind of novel mobile sequences discovered in human genome by Dr. Jurka and his co-workers (mainly Dr. Vladimir Kapitonov) are self-replicating transposons called "Helitron" and "Polinton".

Dr. Jurka was a very active researcher. Every year he attended several important international conferences, symposia and congresses, often as an invited speaker or panel discussion member. As such, he visited Poland on three occasions. Among biologists he was considered a world-class specialist in mobile and repetitive DNA sequences. He authored 117 original papers in leading academic journals. It will suffice to mention his works published by Proc. Natl. Acad. Sci. USA, Science, Nature, Nucleic Acids Res., J. Mol. Evol., Genome Res. And others. He also wrote 11 book chapters and elaborations for scientific encyclopedias. Dr. Jerzy Jurka was a Board Member and then deputy Editor-in-Chief of the Journal of Molecular Evolution, board member of Biology Direct, Gene, Mobile DNA and other journals, as well as numerous research societies.

Dr. Jerzy Jurka worked also as a peer reviewer for top research journals, including Proc. Natl. Acad. Sci USA, Science, Nature, J. Mol. Biol., J. Mol. Biol., Genome Res. and others. He was a scientific advisor to several undergraduate and graduate students, mainly computer scientists and biologists. He organized numerous seminars and conferences including the first international meeting on "The influence of mobile eukaryotic elements on eukaryotic genome" in Asilomar, California, in which I had the pleasure to participate. It was very uplifting to listen there to the words of praise from other conference participants, and they all reflected Dr. Jurka's scientific and organizational skills.

Dr. Jerzy Jurka left wife Elżbieta and three sons; he was lucky enough to attend the wedding of one of them, despite being already gravely ill.

Evoking these shreds of memory and witnessing the vanity of the world around us, I believe it is worthy to remember this fellow Pole who gained authentic merits and recognition in Science. In my opinion, among over twenty researchers who had worked previously at the Department of Tumor Biology in the Gliwice Institute of Oncology, and who later settled abroad, there have been a few very gifted and widely known. I think Dr. Jerzy Jurka was at the front ranks of this eminent group of people who excelled in Science.

Mieczysław Chorąży, Gliwice

Lecture abstracts

Session I:
Transcriptional responses to stress

NF-κB SIGNALING AND THE INNATE IMMUNE RESPONSE

AR Brasier, L Fang, S Choudhary, Y Zhao, MR Kalita, B Tian

Sealy Center for Molecular Medicine, Institute for Translational Sciences, University of Texas Medical Branch, Galveston, TX, USA

The Nuclear Factor-κB (NF-κB) signaling pathway controls gene networks important in the innate immune response. In response to extracellular ligands or pathogen associated molecular patterns activated death domain-containing receptors signal the rate-limiting IκB kinase (IKK) complex leading to proteosomal degradation of the IκB inhibitors, releases the sequestered, inactive RelA transcription factor. We have found that RelA release is necessary, but not sufficient for activation of the innate response. A separate ROS signaling pathway converges on members of the Ribosomal S6 Kinase family is involved in activating phosphorylation of RelA at serine (Ser) residue 276, an event coupled to its acetylation and co-activator recruitment. The fully activated RelA complex mediates phosphorylation of RNA polymerase II leading to transcriptional elongation and expression of immediate-early cytokine genes. We have conducted siRNA screens systematically knocking down the human kinome to identify kinases important in TNF signaling. One hit included the PI3 kinase, ataxia telangiectasia mutated (ATM). In this abstract, I will overview our work linking ROS formation with ATM activation via dsDNA breaks and nuclear export. As a cytoplasmic scaffolding protein, ATM binds and activates the catalytic subunit of protein kinase A (PKAc), a ribosmal S6 kinase that controls RelA serine 276 phosphorylation. In ATM shRNA knockdown cells, TNF-induced RelA Ser 276 phosphorylation and immediate early cytokine gene expression is inhibited. Recent work has extended this pathway to that of the anti-viral response, where ATM is involved the regulation of a distinct Ser 276 kinases. We conclude that ATM is a nuclear damage-response signal modulating NF-κB Ser 276 phosphorylation and transcriptional activation.

REGULATION OF CANCER CELL PROLIFERATION AND SURVIVAL BY NF-κB

Neil Perkins

Institute for Cell and Molecular Biosciences (ICaMB), Medical School, Newcastle University, UK

My laboratory is interested in how NF-κB subunits are regulated by oncogenes, tumour suppressors and stimuli associated with cancer development and therapy. A theme emerging from these studies has been the importance of context for NF-κB activity and how subunits can both repress as well as activate gene targets, leading to alterations in cell fate. We have demonstrated the importance of post-translational modifications in controlling these activities and argued that the activity of parallel signalling pathways have a critical role in determining NF-κB dependent transcriptional output. Based on this work, together with that from other researchers, we propose that the concerted action of tumour suppressors functions to keep the oncogenic activities of NF-κB subunits in check and that loss of tumour suppressor activity during tumour development is required to unleash these anti apoptotic and pro-metastatic activities in malignant cancer cells. In my talk I will present recent and unpublished data from mouse and cell line models, where we have investigated the role of NF-κB subunits in cancer. These studies underline context dependent complexity of NF-κB signalling in cancer.

TRANSCRIPTIONAL AND EPIGENETIC MECHANISMS CONTROLLING INFLAMMATION

Bozena Kaminska

Laboratory of Molecular Neurobiology, Nencki Institute of Experimental Biology, Warsaw, Poland

Microglia (brain resident immune cells) rapidly respond to stress conditions, accumulate and produce a wide variety of cytotoxic and pro-inflammatory molecules. Uncontrolled and prolonged inflammation is associated with virtually all neurological diseases including Alzheimer's and Parkinson's disease, brain trauma and stroke. Signal transducer and activator of transcription (Stats) control gene expression programs that regulate immune and glial cell functions, but Stat targets in inflammatory microglia are unknown. We mapped the genome-wide occupancy of phospho(P)-Stat1,3,5 by hybridization of immunoprecipitation-enriched genomic DNA to promoter microarrays. P-Stat1 and P-Stat3 binding sites were found preferentially within promoters of upregulated genes which encode cytokines/chemokines and transcription regulators. The most interesting hit and newly identified Stat target, was *jmjd3*, encoding a JmjC family histone demethylase and transcription factor. Silencing of Stat1 and Stat3 blocked Jmjd3 and inflammatory gene expression in microglial cells, while overexpression of constitutively active Stat1 and Stat3 was sufficient to induce Jmjd3 and inflammation-related genes. Our studies demonstrate that Stat1 and Stat3, in concert with Jmjd3, activate in microglia a transcriptional network that results in neuroinflammation.

The analysis of common activating and repressive histone modifications revealed acetylation of inflammatory genes and acquisition of repressive histone modifications at the cytoprotective genes that correlated to transcription down-regulation. Microglia activation and inflammatory gene expression were blocked by histone modifying enzyme inhibitors. Our results show that epigenetic modifications are crucial for polarization of microglia into inflammatory phenotype and inhibition of histone modifying enzymes blocks acquisition of a specific phenotype.

SIMPLICITY AND COMPLEXITY IN MODELS OF CELL SIGNALING

Marek Kimmel

Silesian University of Technology; Gliwice, Poland

The talk is a review of simplified "bare-bone" structures of regulatory modules in signaling pathways in cells. Two examples are the NF-kB module in innate immune response and the GCSFR - JAK/STAT/SOCS module in granulopoiesis. These simplest models can be reduced to systems of 4 ordinary differential equation, which makes their study related to the classical algebraic problem of quartics. I will explore some of the dynamical effects possible in such systems and discuss their biological feasibility. On the other end, I will review simple results about stability of systems composed of interconnected simple modules, with the stabilizing and destabilizing effects in view. Here, the main biological example is cooperation and competicion of premalignant cell clones. I will also present recent results in the theory of chemical reaction, which are relevant to discussion.

NF-κB AND IRF3 CROSSTALK SIGNALING IN MEFS

Maciej Czerkies, Zbigniew Korwek, Wiktor Prus, Sławomir Błoński, Joanna Jaruszewicz, Marek Kochańczyk, Bing Tian, Marek Kimmel, Allan Brasier, Tomasz Lipniacki

NF-κB, IRF3 and AP-1 are most potent transcription factors controlling innate immune responses to pathogens. Combining single cell and population techniques with mathematical modeling we analyzed crosstalk of these pathways in mouse embryonic fibroblasts in response to LPS and poly(I:C).

We found that both LPS and poly(I:C) activate mediating kinases IKKα/β and TBK1; interestingly, only poly(I:C) stimulation leads to activation of IRF3 (activated by TBK1) and triggering of transcription of IFNβ, IRF7, and RIG-1 and other interferon regulated genes. LPS stimulation leads to transient or oscillatory (in some cells) responses of NF-κB, in contrast to switch-like responses (preceded by one or two pulses in a fraction of cells) to poly(I:C) stimulation, with fraction of switched-on cells increasing with the stimulation dose. As suggested by the experiment in which cells are costimulated by LPS and IFNβ, the difference in NF-κB responses is caused by IFNβ para- and autocrine regulation that leads to activation of EIF2AK2 (PKR) and OAS1A, which results in suppression of NF-κB inhibitors synthesis. Correspondingly, the blockade of INFβ receptor causes attenuation of response to poly(I:C) at latter time points. Overall, this suggests that autocrine regulation breaks the negative regulation of NF-κB (and IRF3) leading to build up of nuclear NF-κB and IRF3, followed by apoptosis in a fraction of cells (not observed in the case of LPS stimulation). The IRF3 activation is stabilized by positive feedback involving strongly upregulated IRF7 and RIG-1 (which is IRF7 responsive).

We confirmed by mathematical modeling the dynamically divergent responses to LPS (mimicking bacterial infection) and to poly(I:C) (mimicking viral infection). NF-κB is known for exhibiting oscillatory responses to TNFα, which are replaced by switch-like responses in A20-deficients cells. Here, we found that activation of the IRF3 pathway, or INFβ stimulation leads to the similar effect on NF-κB signaling, possibly due to inhibition of translation of NF-κB inhibitors. The switch-like behavior, frequently associated with cell fate decisions, is associated with bistability arising here due to the positive feedbacks in IRF3/IRF7 regulation.

IMMEDIATE AND LONG-TERM MOLECULAR RESPONSE OF MELANOMA CELLS TO CHANGES IN MICROENVIRONMENT

Mariusz L. Hartman, Malgorzata Sztiller-Sikorska, Beata Talar, Anna Gajos-Michniewicz, Malgorzata Czyz

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

Melanoma cell response to even small changes in microenvironment may result in strikingly diverse phenotypes. Patient-derived melanoma populations grown *in vitro* in stem cell medium (SCM) form anchorage-independent structures including highly heterogeneous melanospheres. Cultures expanded in SCM are enriched with cells possessing stem-like characteristics as exhibited by their capability to self-renewal and multi-lineage differentiation. These features are significantly reduced in serum-driven adherent monolayers characterized, however, by higher proliferation rate and invasive potential. Transcriptome profiles generated for melanospheres largely differ from those of monolayers. Thus, melanoma cells respond to changes in microenvironment by altering gene expression leading to phenotype switching. MITF, a transcriptional regulator specific for melanocytic lineage and DKK1, a secreted inhibitor of Wnt/β-catenin pathway, have been identified as coordinators of microenvironment-driven alterations in melanoma phenotypes. Other essential genes related to stem-like features, angiogenesis, invasiveness and cell survival are also differentially expressed between melanospheres and monolayers. Transcriptional reprogramming of melanoma cells clearly observed for long-term serum-driven cultures is preceded by immediate adaptive response involving fluctuations of MITF level, as well as transient changes in proliferative potential and pro-survival machinery. Specific anti-apoptotic Bcl-2-like proteins are involved in this response, however, their contribution varies between different melanoma populations. Unraveling molecular mechanisms both governing melanoma phenotypes obtained during the long-term exposure to defined conditions and underlying immediate adaptive response of melanoma cells to changes in microenvironment can extend our understanding of melanoma biology. The observed molecular diversity should be also carefully considered when *in vitro* melanoma models are generated for drug testing.

This work was financially supported by grants 2012/06/M/NZ2/00109 and 2013/09/N/NZ1/01025 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).

Session II:
Biomaterials and nanomedicine

BIOENGINEERED COLLAGEN CONSTRUCTS FOR CELL-BASED REGENERATION OF THE CORNEA

Mehrdad Rafat[1,6], Neil Lagali[2,3], Marina Koulikovska[2,3], Goran Petrovski[4], Zoltán Veréb[4], Saeed Akhtar[5], Per Fagerholm[2,3]

[1]Department of Biomedical Engineering, [2]Department of Clinical and Experimental Medicine, [3]Department of Ophthalmology, Linköping University, Linköping, Sweden; [4]Department of Ophthalmology, University of Szeged, Szeged, Hungary; [5]Department of Optometry, College of Applied Medicine, King Saud University, Saudi Arabia; [6]LinkoCare Life Sciences AB (Ltd), Sweden

A severe worldwide shortage of donor corneal tissue for transplantation, particularly in developing countries, has prompted the advancement of bioengineered tissue alternatives. Tissue engineered corneas with favorable biological properties, which are additionally amenable to low-cost mass production, however, remain elusive. Using medical-grade collagen as a starting material, a bioengineered construct (BC) was developed for corneal implantation. The BC had robust mechanical, chemical, and optical properties and supported colonization by human corneal epithelial cells and stroma-derived mesenchymal stem cells *in vitro*. In a new type of corneal surgery (femtosecond laser-assisted intra-stromal keratoplasty) developed for the first time in a rabbit model, the BC was tested *in vivo* for therapeutic potential in replacing damaged or diseased native corneal stroma. Cell-free BC implants were characterized in vivo for 8 weeks. Implanted corneas rapidly healed without inflammation or damage to surrounding tissue. Host stromal cells migrated into implants and remained quiescent or initiated regeneration by producing new collagen. Moreover, by controlling the biomaterial swelling properties, final corneal thickness and transparency were modulated *in vivo*. One variant of BC implant resulted in a final corneal thickness and transparency similar to native control corneas. BC implants, transparent when fabricated, maintained transparency *in vivo*, while *ex-vivo* electron microscopy revealed a fine porous microstructure, a proteoglycan network, and a remarkable absence of organized collagen fibrils within implants. This study indicates that a BC extracellular matrix equivalent can incorporate cells passively to promote regeneration of the corneal stroma or be seeded with human stem or organ-specific cells for future therapeutic applications in corneal tectonic surgery or transplantation. The new BC implant has a balanced robustness and elasticity, allows proper fibroblast ingrowth to induce regeneration of new natural collagen in vivo, is amenable to low-cost mass production, and can be widely available for corneal transplantation.

Keywords: *bioengineering, collagen implants, cornea, stem cells, femtosecond laser, corneal transplantation, regeneration*

MAGNETIC AND BIOGENIC NANOPARTICLES FOR CANCER THERAPY

Katerina Chlichlia

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Nanomaterials have revolutionized the field of Biomedicine with remarkable prospects for diagnosis and treatment of human diseases, such as cancer. Many approaches based on nanoparticles have been applied in preclinical cancer models with considerable success, and thus, gained increasing attention as potential novel cancer treatments in recent years. All these approaches hold great promise in battling cancer directly or in combination with other therapies.

Nanomaterials, besides being synthesized in the laboratory, can also be produced from nature through biogenic procedures. The biosynthesis of nanoparticles by microbes (biogenic nanoparticles) is thought to be a clean, inexpensive, non-toxic, and environmentally acceptable ‘green chemistry' procedure, leading to the production of highly biocompatible and mono-dispersed nanostructures. Interestingly, nanostructured selenium derived from bacteria or, alternatively, probiotic strains producing selenium nanoparticles, can have anticancer properties in experimental animal models.

In addition, synthetic superparamagnetic nanoparticles with fluorescent properties have been employed. We have used fluorescent superparamagnetic nanoparticles to facilitate, optimize, visualize and monitor *in vivo* tumour growth in a syngeneic mouse tumor model of colon cancer and optimized this model for preclinical screening of new anti-cancer agents. Moreover, synthetically produced nanostructured iron oxides can generate heat when placed in an alternating magnetic field, providing the basis for magnetic hyperthermia, that holds great promise as a cancer treatment. Noteworthy, preclinical data have demonstrated enhanced efficacy of existing immunotherapeutic anti-cancer regimens when localized hyperthermia is applied. Interestingly, magnetic nanoparticles can be used as delivery vehicles for vaccines and can provide adjuvant activity promoting the induction of protective anti-cancer immune responses. They have great potential as useful platforms and start to revolutionize the field of "DNA vaccination" by facilitating delivery and uptake of plasmid DNA *in vivo* in human cells. Conclusively, successful implementation of the above mentioned approaches will have a tremendous impact in cancer therapy.

BIOPATTERNING TECHNIQUES IN TISSUE ENGINEERING

Ramūnas Valiokas

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The last decade has seen a clear trend in production processes, such as microfabrication, roll-to-roll, 3D printing or robotic manipulation, entering the domain of life sciences and medicine. However, many of these processes originally were developed for electronics, plastics or paper industries. Therefore, they are often not compatible with living cells and tissues, although being very attractive as cost-efficient, scalable and reliable alternatives in development of new implants, bioanalytical devices and therapies. To exploit these opportunities, a possible solution is to combine well-established fabrication platforms with emerging techniques, which can be dubbed as "biopatterning". They are based on nanotechnologies, engineering of molecules and surfaces for precise construction of a biocompatible and bio-interactive interface between cells and man-made materials. Our laboratory has been specializing in this area and this presentation will provide an overview of our recent activities in nanomanipulation of single cells, shaping of cell culture, high-throughput screening, and construction of circuit-like structures, chips and scaffolds for tissue architectonics.

AFM-IR: COMBINING ATOMIC FORCE MICROSCOPY AND INFRARED SPECTROSCOPY FOR MULTIFUNCTIONAL MEASUREMENTS - NANOSCALE CHEMICAL CHARACTERIZATION

Łukasz Zarodkiewicz

COMEF
Anasys Instruments, Santa Barbara , CA 93101 USA

Atomic Force Microscopy (AFM) is an invaluable sub-atomic resolution method for topography and quantitative imagining.
It is a first time when researches can receive the exquisite imagining capacity of atomic force microscopy combined with the precise chemical identification of infrared spectroscopy. With pioneering AFM-IR application , the Anasys Instruments technology is a robust, proven platform witch leverages the advantages of both these applications , delivering the unprecedented ability to chemically identify sample components at sub 100nm lateral resolution.

Despite its widespread applications, infrared micro-spectroscopy has fundamental spatial resolution limits set by both the laws of optics and practical design constraints. Fourier Transform IR spectroscopy is generally limited to a spatial resolution of three times the wavelength of the IR radiation. With Attenuated Total Reflection (ATR) may achieve resolution approaching the wavelength. The table below shows practical resolution limits faced by conventional IR spectroscopy. A new Anasys Instruments technology breaks through resolution limits in conventional IR spectroscopy by using the tip of an atomic force microscope probe to measure infrared absorption. The sample is illuminated by a tunable IR source. When IR radiation is absorbed by a region of the sample, the region heats up. The heat generates a rapid thermal expansion pulse that can be detected by the AFM cantilever tip. This technique beats the “far field” optical diffraction limit because the absorbed radiation is measured by the tip in the extreme near field. The tip of an AFM is used to measure local thermal expansion resulting from absorption of IR light. Even though the focused spot of IR radiation is on the scale of many microns, the thermal expansion can be spatially resolved with the AFM tip on scales well below the optical diffraction limit.

The ability to identify material under the tip of an AFM has been identified as one of the “Holy Grails” of probe microscopy.

Session III:
Bioinformatics and Regulatory Mechanisms
(session co-organized by EACR)

IMAGE-BASED MODELLING OF CELL MOTILITY

Richard Tyson[1], Robert Lockley[1], Graham Ladds[2], Till Bretschneider[1]

[1]*Warwick Systems Biology Centre, Senate House, University of Warwick, Coventry CV4 7AL, UK;* [2]*Division of Biomedical Cell Biology, Warwick Medical School, University of Warwick, Coventry, CV4 7AL, UK*

Extracellular gradients of chemoattractants or mechanical signals can guide cell movement by directing polymerization of actin, the cellular motor, to the leading edge. Various mathematical models have been proposed which describe gradient sensing and cell polarization, mostly in terms of local excitation and global inhibition mechanisms related to original work on biological pattern formation by Turing. I will present approaches how imaging data can be used to put these models to the test. Competing reaction-diffusion models have been parameterized, employing experimental data of actin redistributions in *Dictyostelium* cells, which reorient to alternating gradients of shear flow. I will address in particular problems of parameter identifiability and show that a modified, completely identifiable, version of the very first model in the field, by Meinhardt, reproduces not only averaged cell dynamics, but also noisy single cell data remarkably well.

In another mode of cell motility, namely blebbing, hydrostatic pressure forces the cell membrane to detach from the underlying actin cortex, creating protrusions in form of hemispherical blebs. *Dictyostelium* cells sandwiched between a coverslip and a sheet of agar chemotax by employing a mixed mode of actin protrusions and blebs. A detailed analysis of cell shape dynamics allowed us to study how blebs and actin driven protrusions interact. Results suggest that changes in geometry incurred by actin driven protrusions could direct blebs to the cell front thus underpinning a feedback mechanism that acts in concert with signaling. The examples I give highlight the demand for novel computational and statistical methods for synchronizing single cell dynamics in silico, in time and in space.

ANALYSIS OF DNASE-SEQ DATA

Sascha Ott

Warwick Systems Biology Centre, Senate House, University of Warwick, Coventry CV4 7AL, UK

DNase-seq is a powerful approach to analyse the global pattern of protein-DNA binding in regulatory regions of DNA. In this assay, DNA is digested by DNaseI and high-throughput sequencing is applied to reveal "footprints", regions of DNA where digestion is reduced as a result of protein binding. Binding of a variety of proteins can be detected in one assay. In this talk I will discuss this type of data, a recent method for the detection of footprints, and its application to elucidating aberrant regulatory events in cancer cells.

MULTISPECTRAL FLUORESCENT SINGLE LIVE CELL IMAGING TO ANALYSE THE CELL CYCLE AND THE CIRCADIAN CLOCK DYNAMICS

Franck Delaunay and the C5Sys consortium

Institute of Biology Valrose, CNRS, INSERM, University Nice Sophia Antipolis

Daily synchronous rhythms of cell division at the tissue or organism level are observed in many species and suggest that the circadian clock and cell cycle oscillators are coupled. For mammals, despite known mechanistic interactions, the effect of such coupling on clock and cell cycle progression and hence its biological relevance is not understood. In particular, we do not know how the temporal organisation of cell division at the single cell level produces this daily rhythm at the tissue level. Here we use multispectral imaging of single live cells, computational methods and mathematical modelling to address this question in proliferating mouse fibroblasts. We show that in unsynchronized cells, the cell cycle and circadian clock robustly phase-lock each other in a 1:1 fashion so that in an expanding cell population the two oscillators oscillate in a synchronized way with a common frequency. Dexamethasone-induced synchronization of cellular clocks reveals additional clock states: as well as the low-period phase-locked state there are distinct coexisting states with a significantly higher period clock. Cells transition to these states after Dexamethasone synchronization. The temporal coordination of cell division by phase-locking to the clock at a single cell level has significant implications because disordered circadian function is increasingly being linked to the pathogenesis of many diseases including cancer.

RADIATION-INDUCED BYSTANDER SIGNALS: EVIDENCE FOR A ROLE OF SECONDARY UVA EMISSION IN THE GENERATION OF OXIDATIVE STRESS AND BYSTANDER EFFECTS BY BETA IRRADIATION OF HUMAN KERATINOCYTES

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Recent studies in our laboratory have suggested that both in vivo and in vitro, there is a physical component to the mechanism by which irradiated cells communicate with unirradiated bystanders. Early in vitro work showed that melanin could supress signal production in vitro while in the our vivo fish model, physical separation of the fish using a Perspex inner tank did not stop signals travelling from irradiated to bystander fish. Other work also revealed that irradiation of cells using tritium added to the culture medium resulted in the emission of measurable light in the UVA range and was associated with bystander effects in unirradiated cells placed above the tritium treated dishes. Since UVA exerts biological effects mainly through the production of oxy-radicals, which are known to be involved in the bystander mechanism, experiments have been conducted using a photosensitizer (20 μM lomefloxacin, a fluoroqunilone antibiotic) or a photoprotectant (10 μg/ml melanin) added to the reporter flasks, the directly irradiated petri dishes, or both in order to elucidate the role of photons in the production of an effect in reporter cells. In the positive controls the pattern of clonogenic survival was strongly correlated with the photon emission measured at corresponding doses in the range 0.003-1.2Gy (r=0.977, p<0.01). Reporter flasks treated with lomefloxacin showed greater reductions in clonogenic survival compared with untreated irradiated flasks. Reporter flasks that were treated with melanin while placed above irradiated petri dishes showed increased survival. Treatment of directly irradiated petri dish cultures with lomefloxacin or melanin resulted in expected the increase or reduction of photoemission intensity and corresponding changes in reporter cell survival. We conclude that the observed modifications in clonogenic survival upon treatment with photosensitizing/photoprotecting substrates support a role of UVA emission in the bystander signaling mechanism resulting from irradiation of cells with tritium.

PETRI NET BASED APPROACH FOR MODELING AND ANALYSIS OF SELECTED ASPECTS OF ATHEROSCLEROSIS

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Atherosclerosis has been recognized as a chronic inflammatory process, characterized by the activation of many cells with phagocytotic and antigen-presenting capabilities, accumulation of oxidatively modified lipoproteins, increased turnover of immune-competent cells and tissue damage, as well as hypertrophic degeneration of the arterial intima. The process advances through a series of stages that begin with the initial appearance of the fatty streak lesion, composed largely of the foam cells, which are lipid-engulfed macrophages. The fatty streak evolves toward the complex atherosclerotic plaque. The pathogenesis of the atherosclerotic plaque is a dynamic process that usually occurs over decades although very susceptible individuals may have serious lesions in the first decade of life.

Since atherosclerosis is a very complex biological phenomenon systems approach to the analysis of it seems to be necessary in order to fully understand its nature. The basis of such an approach is a precise mathematical model of the analyzed system. Such a model can be expressed in a language of some mathematical theory. Traditionally, for modeling biological phenomena differential equations are used. They are a powerful mathematical tool but in many cases it may be difficult to build a model of a biological system based on them. The reason is that differential equations require exact values of some parameters which correspond to quantitative properties of the biological system. These properties are usually difficult to determine. Hence, for building formal models of biological systems other mathematical tools are recently used. One of them are Petri nets. Among the advantages of using nets of this type are their intuitive graphical representation and well-known mathematical methods for the analysis of their properties.

Here we present Petri net based models of selected processes being parts of the formation and development of atherosclerosis process. The analysis of these models has been focused on transition invariants which correspond to some subprocesses of the studied biological systems. The relationships among these invariants have been analyzed what led to some interesting conclusions concerning properties of the biological systems.

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MINOR GENETIC VARIANTS DISCOVERY AND DETECTION DOWN TO 0,1% LEVEL AT THE HETEROGENIC TUMOR MATERIAL BY THE MSSCP METHOD

Krzysztof Kucharczyk

BioVectis

Multitemperature Single Strand Conformation Polymorphism genotyping method (MSSCP) can detect already known and discover novel genetic variants within the same assay, even if only a small sample of heterogeneous clinical material is available (e.g., a thin needle biopsy). MSSCP sensitivity was establish at 0,1% level which is particularly useful for detecting or discovering infrequent or underrepresented genetic variants in the clinical sample. MSSCP is fast and cost effective.

During the lecture the methodology principle would be introduce and examples of discovering and detecting genetic variants at heterogeneous clinical samples at genes like EGFR, FLT3, PT53 and KRAS would be presented.

Session IV:
Glycobiology in Medicine

THE ROLE OF ABERRANT GLYCOSYLATION IN HUMAN MELANOMA PROGRESSION TO MORE MALIGNANT PHENOTYPE

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Glycosylation is one of the most important posttranslational modification of proteins. In post-genomic era, studies of glycan structure and function become complementary to proteomic research. Cell surface glycoproteins contribute to a variety of interactions between the cell and its surrounding. It is also widely known that aberrant glycosylation has been implicated in many different diseases, due its participation in key biological processes including cell adhesion, receptor activation and signal transduction. Changes in glycosylation occur in essentially all types of human cancers, but the detailed analysis of glycosylation still remains challenging because of the heterogeneity and complexity of oligosaccharides. The good understanding of differences in glycosylation between malignant and healthy tissues can influence biomedicine in many ways. First, the neoglycoforms of cancer cell proteins which are released from the cells can be treated as potential sensitive and specific biomarkers. Secondly, one can study the exact influence of changed glycosylation on cancer cell biology at the molecular and cellular level and finally, manipulating the glycosylation machinery can be considered as a novel therapeutic strategy. A growing body of evidence supports crucial role of glycans at various stages of tumor progression and it is well known that differences in glycan profile are associated with transition from normal to the transformed phenotype. The most frequently observed alterations during tumorogenesis include the extensive synthesis of highly branched and sialylated glycans, premature termination of synthesis and re-expression of foetal-type antigens. Malignant melanoma remains the deadliest form of skin cancer because of its highly aggressive nature. The differences in survival rates between primary and metastatic melanoma potentially reflects the big changes in cancer cell physiology that accompany progression of disease. Human melanoma seems to be a good model of neoplasma because its development occurs through quite well distinguished stages. Therefore, malignant melanoma is one of the most studied cancers with approx. 5000 independent cell line strains established by many laboratories. The multistage nature of melanoma development and the availability of cell lines from different stages of progression provide a chance for quite complex analyses. The studies carried out by our group include analysis of interactions of glycosylated cell surface adhesion proteins (integrins, cadherins) with extracellular matrix proteins, glycosyltrasferases expression profiling and use of lectins to identify characteristic glycan types. Another method include cell culture in the presence of glycosylation inhibitors and specific enzyme treatment of cell glycoptoteins. Finally, MALDI MS/MS and HPLC analysis of N-glycans released from isolated glycoproteins from melanoma cell lines. These studies have shown the changes in sialic acid linkage of integrin glycans and increase in total cell surface abundance and number of proteins bearing tri- and tetra-antennary glycans in the progression from primary to metastatic melanoma. The glycosylation profile of L1CAM as well as nucleolin have been also performed.

GLYCOSYLTRANSFERASES AND BLOOD GROUP ANTIGENS

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Among 35 blood groups systems recognized by the International Society of Blood Transfusion, six consist solely of oligosaccharide antigens and only two contain antigens that are not glycosylated. Thus, glycosyltransferases are important factors in synthesis of blood group antigens. Of all these systems, the P1PK seems to be the most complicated and elusive one. It contains three glycosphingolipid antigens, P^k, P1 and NOR, which are products of the same enzyme, α-1,4-galactosyltransferase ($P1/P^k$ synthase, Gb3/CD77 synthase) encoded by *A4GALT* gene. P^k and P1 antigens contain Galα1>4Gal terminal structures, but P^k belongs to the globo series, while P1 is a member of neolacto series. Conversely, P^k and NOR differ in terminal disaccharide moieties but emerge in the same globo-series pathway. The presence or absence of P1 antigen on erythrocytes gives rise to the P_1 and P_2 blood groups, respectively. The cause of this polymorphism is still not completely solved, but the expression levels of *A4GALT* mRNA were found to be higher in P_1 individuals than in P_2. It may be argued that the upregulated transcript might cause increased production of $P1/P^k$ synthase, and the upregulation may result from single nucleotide polymorphisms in the promoter region of *A4GALT*. On the other hand, the riddle of NOR polyagglutination, caused by the presence of terminal Galα1>4GalNAcβ1 moiety (an unusual structure, never before found in mammals), has been completely solved in our laboratory. We found that all NOR-positive individuals carry C>G mutation at position 631 of the *A4GALT* ORF. The enzyme encoded by the mutated gene contains glutamic acid instead of glutamine at position 211 (substitution Q211E). To determine whether this mutation could change the enzyme specificity so dramatically, we embarked on a multilevel study including stable transfection of a teratocarcinoma cell line (2102Ep) with vectors encoding the consensus or variant α-1,4-galactosyltransferase with Glu at position 211, and flow cytometric analysis of obtained clones, followed by high-performance thin layer chromatography, enzymatic degradation, and MALDI-TOF mass spectrometric analysis of glycosphingolipids isolated from the transfectants. Cells transfected with either vector expressed the P1 blood group antigen, which was absent from untransfected cells. Cells transfected with vector encoding the variant α-1,4-galactosyltransferase expressed both P1 and NOR antigens. To provide further insight into the $P1/P^k$ synthase specificity, we produced recombinant catalytic domains of the consensus and variant enzyme using Sf9 insect cell line and baculovirus expression vector system. We found that the consensus α-1,4-galactosyltransferase can synthesize both P^k and P1 antigens, while α-1,4-galactosyltransferase with Q211E substitution synthesizes P^k, P1 and NOR antigens. These results present the first biochemical evidence that P^k and P1 antigens are indeed products of the same enzyme and that the Q211E substitution renders its ability to additionally synthesize NOR antigen.

MATRIX PROTEOGLYCANS AS REGULATORS OF TUMOR PROGRESSION

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Tumor micro-environment extracellular matrix is a theatre of numerous interactions orchestrating malignant cell for either growth/migration to form metastasis or for tumor growth inhibition by stromal reaction, turning the cells into dormancy, apoptosis or into necrosis. Extracellular matrix is non permissive for normal epithelial cells. Recent two decades of studies pointed to the matrix Small Leucine-Rich Proteoglycans (SLRPs) family as the regulators of tumor cell behavior. Former studies showed that SLRPs control the appropriate collagen network formation in all the tissues. Two SLRPs: decorin and lumican were extensively studied for their engagement in tumor progression. Decorin is an agonist/ligand of several receptors (Met, EGFR, and IGF-1R) and though regulates tumor growth. Recently, decorin engagement in cellular autophagy was discovered. Our studies focus on lumican. This extracellular macromolecule exists in, at least, three forms: as a protein (M_m 37k) as a glycoprotein (M_m 57k) or proteoglycan (M_m 80k - 120k). While proteoglycan form seems to be pro-metastatic, glycoprotein or protein forms inhibit tumor growth, metastasis, and angiogenesis and are pro-apoptotic. This SLRP decreases cell migration acting through different receptors including integrins and tyrosine receptor kinase. Other mechanism of lumican action is its interaction with membrane type 1 matrix metalloproteinase (MT1 MMP or MMP14). While lumican protein is degraded by this enzyme, glycoprotein form is not only resistant to degradation but inhibits enzymatic activity thus protecting the integrity of extracellular matrix and decreasing tumor cell invasion. These SLRPs emerge as matrix factors regulating tumor growth with pharmacologic potential in the future.

Satellite Workshop

Genes-environment interactions

CHALLENGES WITH GENE-ENVIRONMENT INTERACTIONS: WHERE WE ARE AND WHERE WE NEED TO GO

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Environmental diseases are seen as the result of exposure to environmental stressors modulated by individual susceptibility factors. Human population studies typically involve measuring numerous biomarkers of both phenotype and genotype. Biomarkers can reflect exposure, effect (disease risk), or individual susceptibility. For environmentally-induced diseases, molecular biomarkers play a key role in understanding the relationships between environmental stressors and the development of chronic diseases, and in identifying individuals at increased risk.

The molecular epidemiological approach using molecular markers including single nucleotide polymorphisms (SNPs) has been in use for over a quarter of a century in the fields of environmental medicine, genetic epidemiology, and especially in monitoring environmental and occupational exposure, showing that genetic susceptibility to suspected chemical and environmental carcinogens may modify the response to exposure.

More recently, genome-wide association studies (GWAS) have offered various techniques to study DNA variations that are associated with human diseases. However, knowledge of the effect of SNPs on common diseases is still needed in order to understand the underlying genetic factors affecting human diseases – whether gene-gene or gene-environment interactions.

Applying the prevailing 'omics' approaches (transcriptomics, metabolomics, exposomics, whateveromics) to study these interactions produces a superabundance of data, and as a result an urgent need for appropriate statistical and computational methods. Traditional statistical methods are inadequate, owing to the high dimensionality of the data and the occurrence of multiple polymorphisms.

The future clearly lies with omics; but while a large number of genetic variants and gene-environment interactions have been explored for cancer and other environmental diseases, to date replication of studies is lacking and therefore the findings remain to be validated. There is still scope for novel standardized high throughput methodologies, more focused predictive biomarker assays based on specific endpoints, larger samples and cohorts, and better environmental assessment. The new multi-dimensional approaches provide great opportunities for prospective studies to identify and characterize the genes that interact with other genes and environment factors, and to elucidate their causative and modulatory influences on complex multifactorial diseases.

MONITORING OF EXPOSURES TO CARCINOGENS: DOES THE PROCEDURE NEED A RETHINK?

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A procedure known as human biomonitoring to carcinogens is a common practice that measures biomarkers of current exposures, biochemical effects and individual susceptibility. New insight on cancer cell characteristics, increasingly evolved in the last decades, calls for reflecting on the place of biomarkers in the complex gene-environment scenario within cancer development. Biomarkers, commonly used for carcinogen biomonitoring, and highlights their values and limitations, will be considered, restricting the examples of exposures to those chemicals classified in groups 1 and 2A by the International Agency for Research on Cancer.

Current exposures to carcinogens, mainly at work places, are estimated by measuring either the chemical itself or its metabolite(s) in body fluids. Little attention has been paid to the multiplicity and variability of exposures at different critical life stages. Evaluation of carcinogenic exposure may be useful to assess the hygienic conditions and practices at work place and also to the compliance with reference values and limits of exposure, when available.

Several markers of biological effect, including lymphocyte chromosomal aberrations, micronuclei formation, telomere shortening and specific mutations, have been proposed to assess cancer risk. However, only few studies were designed prospectively, among which none was linked to a given exposure. Further consideration should also be given to the necessity of measuring such biomarkers in accessible tissues - usually blood components, especially lymphocyte - and consequently to the uncertain relationship with the corresponding changes in target organs. Most carcinogens require metabolic and subsequent detoxification via conjugation and therefore enzyme polymorphisms could account, at least in part, for cancer susceptibility of exposure to carcinogens. However, most of these studies analyzed the genotype not knowing the degree of accuracy by which it predicts the phenotype. Lastly most, if not all biomonitoring studies, assessed cancer risk on a group basis through snapshots taken at unknown time-points of a postulated chain of events.

New opportunities and suggestions for biomonitoring exposures to carcinogens could derive from the results of comprehensive molecular characterization of environmental cancers and -omic studies. Based on these premises it is possible to envisage new scenarios for biomarker discovery and new challenges as well, that include the need of repeated measurements of global sets of biomarkers to be collected at different critical life stages.

EPIGENETICS OF METALS

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Increasing evidence shows that the environment, particularly early in life, can affect the development of the children and the later risk of chronic diseases and mental dyscapacity: a poor intrauterine environment results in a lower probability of good health later in life. The mechanism for this likely involves disruption of epigenetic processes. Epigenetic processes, inherited changes in phenotype or gene expression caused by mechanisms other than changes in the underlying DNA sequence, include DNA methylation and multiple modifications of DNA-associated proteins, such as histones. Emerging research indicates that epigenetic regulation has major effects on gene expression, and key roles in human disease. Although epigenetics is a rapidly expanding research area, we still know very little about how toxicants in the environment affect the epigenome. Animal studies and a few human studies have indicated that a number of environmental factors can damage the epigenome: metals in food and drinking water, endocrine-disrupting chemicals, plastics, particles, and pesticides; and these act primarily when the epigenome is most sensitive to changes, i.e. during prenatal and neonatal development. I will present evidence from human environmental studies that low-to-moderate exposure to metals, arsenic, cadmium and lead, are linked to epigenetic changes and to toxic effects. On the basis of current knowledge I will propose possible models for the interplay between metals and the human epigenome.

GENETIC SUSCEPTIBILITY TO METALS

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Genes determine all processes in human organism. There is a number of genetic loci in which single mutation leads to disease manifestation. These are monogenic diseases. However, there are much more loci in which mutation or allelic polymorphism increase or decrease chances to develop a disease. In these conditions usually concomitant environmental factors are needed to develop the pathological state. Genetic polymorphism is defined as occurring when the most frequent allele is present less frequently than in 99% of the population. There are several types of polymorphisms depending on the sequence of affected DNA. Different alleles of coding sequences are responsible for differences in many enzymes activity, including those necessary for alcohol, drugs and other xenobiotics metabolism (e.g. metals). There is a growing body of evidence suggesting that genetic polymorphisms play a crucial role in metal accumulation and its toxic effects on human organism.

Susceptibility to metals depends on age, development, concomitant diseases, nutrition, competition with essential metals and vitamins, form of metal (organic or inorganic), lifestyle, occupation, home environment and genetics.

Genetic susceptibility to metals was studied and proved for a number of metals like lead, arsenic, cadmium, berrylium and mercury. Some alleles are related to increased blood concentration and body burden of metals, while others even if they do not affect the metal levels, may influence organism response to metal exposure.

MULTI-BIOASSAY TOXICITY EVALUATION OF A MIXTURE OF DIFFERENT GROUP OF PHARMACEUTICALS

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There are few studies available in the literature on mixture toxicity of pharmaceuticals, which are briefly to be summarized as: the combined effect of ibuprofen, fluoxetine, and ciprofloxacin to *Lemna gibba* and *Myriophyllum* spp for 35 days; mixture of atorvastatin, acetaminophen, caffeine, sulfamethoxazole, carbamazepine, levofloxacin, sertraline, and trimethoprim assessed on a variety of somatic and pigment endpoints in rooted (*M. sibiricum*) and floating (*L. gibba*); acute toxicity of three b1-selective blockers (acebutolol, atenolol, and metoprolol) and three non-b1-selective blockers (nadolol, oxprenolol, and propranolol) in mixture using acute 2 days *Ceriodaphnia dubia* immobility test; a mixture of seven common pharmaceutical agents (acetaminophen, diclofenac, gemfibrozil, ibuprofen, naproxen, salicylic acid, and triclosan) using freshwater amphipod *Hyalella azteca* over three generations. To understand the behavior of each group of pharmaceuticals in complex mixtures two issues would play key roles: testing concentrations and type of model to predict toxicity. General opinion expressed for the concentration is that a) mixtures should be tested both at effective (high) dose levels and at realistic (low) dose levels. A pragmatic approach to whole mixture toxicity is to test toxicity without assessing the types of interactions. The most common one is the concentration addition (*CA*) model which seemed to be appropriate, providing a reasonable worst-case estimation of different mixtures.

In the present study a mixture (M1: Erythromycin+Propranolol+Diclofenac) of different groups of pharmaceuticals was tested to two bioassays of *Daphnia magna and Ceriodaphnia dubia for acute toxicity and C. dubia for chronic toxicity* according to standard testing methods. The organisms were exposed to three doses (0.25, 0.5 and 1 mg/L) of each drug singly or introduced in the mixture. Furthermore, mixture toxicity of Erythromycin with two other antibiotics (M2: Erythromycin+Ciprofloxacin; M3: Erythromycin+Amoxicillin) was examined in order to understand how to assess the interaction pattern of antibiotics in complex mixtures.

The results showed how the toxicity is varying among the groups of the pharmaceuticals and that the relationship '*species-response'* is more significant than the '*dose-response'* one. According to acute test results *C. dubia* displayed same sensibility as the case of *D. magna*. The most toxic antibiotic resulted to be Ciprofloxacin in accordance to the literature findings. Mixture toxicity results showed to be the most sensitive cases compared to chronic test results of the drugs tested individually. Risk scores increased around ten times from singular to mixture tests. It can be evidenced here that to do a better risk analysis it is an urging need to increase model species in wide matrixes in order to obtain accurate data sets for testing appropriate models.

WASTEWATER TREATMENT PLANTS AS A HUB BETWEEN CLINICAL AND ENVIRONMENTAL ANTIBIOTIC RESISTANCE

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Antibiotics (AB) are among the most important pharmaceuticals applied in both, human and veterinarian medicine. As long as their use, concerns about an increase in resistance (ABR) of originally targeted organisms exist. Those concerns are supported by increasing observations of a continuous spreading of even multi-drug resistant organisms that are a serious threat to public health. The reasons for the spreading of antibiotic resistance are agreed to be multifactorial with overuse, unnecessary and careless prescriptions (ECDC 2014) being only some of possible reasons. Within the last few years the significance of wastewater treatment plants and the release of antibiotic resistant bacteria (ARB) or genes (ARG) by discharged effluents of even tertiary treated wastewater got into the focus of corresponding scientific research. The number of peer reviewed papers in that context rose from about 35 in 1994 to 100 in 2004 and already is exceeding 700 published 2014. The common agreement in all of the papers is, that wastewater treatment plants are a significant source for the emission of ARBs and ARGs into the environment either via effluents into the aquatic compartment or via application of biosolids in agriculture.

The treatment processes in wastewater treatment plants are based on biological (mainly bacterial) processes for carbon and nitrogen removal. The "pollution" in the wastewater described e.g. as amount of total organic carbon (TOC) is used to about the same extend for assimilatory and dissimilatory processes. Due to kinetic consideration for bacterial growth (Monod kinetics), the bacteria are not able to utilize substrate completely and residual amounts of the substrate is released to the effluents. Additionally not all substances in wastewater can be utilized as substrate in a direct metabolic reaction. Some substances are used as co-metabolits and others cannot be used at all. Beside that adsorption to the biofilm of activated sludge is a relevant removal pathway for hydrophobic substances. The consequence of all processes occurring are, that e.g. antibiotic are only removed to some extent, depending on their inflow concentration, structure and chemical properties resulting in (usually low) residual concentration in the effluent of conventional plants. A second important aspect in wastewater treatment is that the biocenosis involved in the biological treatment process are a mixture of bacteria originating from human and animal intestine and environmental bacteria, both "meeting" at that specific hub. Within the app. 6 to 24 hours of hydraulic retention time in the plants bacteria from human origin die off under the abiotic conditions of wastewater treatment plants quite fast (Akiyama & Savin 2010), but some of them as enterococci are more persistent and survive the process despite the fact that their number is reduced by some log units (e.g. Merlin et al. 2011). ARBs that die off nevertheless carry ARGs that can be incorporated by different means by other to that point not resistant bacteria.

Summing up within a wastewater treatment plant there are two specific conditions that could induce transfer of ARGs and selection of ARBs:

- Low concentrations of antibiotics that are far below a therapeutically dose and can act as selection parameter for ABR
- Living or dead resistant clinically relevant bacteria that can transfer their ARGs via different means to environmental bacteria that are adapted to usual environmental conditions and therefore can transfer ARGs to the gene pool of the aquatic environment

The importance of both aspect can be demonstrated by applying the concept of the "mutant selection window" (Drlica & Zhao 2007) to wastewater treatment plants that implies

that between a miniumum inhibitory concentration (MIC) and a higher mutant prevention concentration (MPC) there is a concentration range where an antibiotic has an effect on the selection of ARBs. Therapeutical dose as well as the duration of an application should be in a way that there is minimum risk for the selction of mutants during medical application. Due to dilution of AB concentrations and partial degradation the concentrations observed in wastewater treatment plants are far below the therapeutical dose and the MPC. It is assumed, that concentrations are slightly above or below the MIC, but no solid scientific information is available on that asumption. It only is clear that the AB concentrations in the plants do not effect the biological treatment processes.

One of the few studies proving the horizontal transfer of ARGs from clinical to environmental bacteria was published by Yang *et al.* (2014) using a metagenomics approach . In that comprehensive study, 271 ARGs belonging to 18 types of ABR were identified in different compartments (inflow; effluent; activated sludge and anaerobic digested sludge) of a wastewater treatment plants. Even the total removal of ARGs was determined with 99.82%, 78 of the ARGs persisted throughout the treatment processes. The aerobic treatment process was found to be more efficient compared to the anaerobic step that shows environmental conditions very similar to those in human intestines (37°C; anaerobic). Some of the ARGs were related to bacteria species that were not found in the inflow of the plants but are commonly found in biological wastewater treatment plants, so it was concluded, that horizontal gene transfer occurred from clinical to environmental bacteria

Despite the fact, that a lot of scientific papers prove the significance of wastewater treatment plants as a significant source for the release of AB, ARBs and ARGs to the environment and even the transfer from clinical relevant bacteria to environmental bacteria, the pathway of resistance back from the environment to the human is not clear now and hardly investigated. Application of sewage sludge as fertilizer or reuse of treated wastewater for irrigation in agriculture as well as direct uptake in recreational waters may be possibilities for closing the loop. As awareness for the potential impacts of the release of ARBs and ARGs by wastewater treatment plants is there now, engineers are about to adopt existing treatment technologies for decreasing the release and are designing new technologies that even are able to inhibit their release to the aquatic environment.

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HUMAN-ASSOCIATED BACTERIA AND THEIR MOBILE GENETIC ELEMENTS - IMPORTANT MINORITY IN WASTEWATER AND WASTEWATER IMPACTED ECOSYSTEMS

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Since human-associated bacteria have been recognized as vectors in two-way gene transmission between the pathogenic and environmental populations, health hazard connected with wastewater discharge requires reevaluation. *Escherichia coli* and *Enterococcus* spp. are commensal bacteria of intestinal tract of human and warm-blooded animals. Thus in environmental studies both are regarded as indicators of fecal contamination. They are also responsible for increasing number of hospital-acquired infections in Europe. In this paper the particular attention was given to antimicrobial-resistance of *E. coli* and enterococci isolated from wastewater and wastewater impacted environments.

Raw and treated wastewater as well as samples of activated sludge were collected from two wastewater treatment plants (WWTPs): Gdansk-Wschod and Gdynia-Debogorze (northern Poland). Additionally coastal water of Gulf of Gdansk was sampled, about 2.5 km from the shoreline, where both WWTPs discharge the treated wastewater via marine outfalls. The fecal indicators were detected and identified using biochemical methods. The bacterial susceptibility was tested and evaluated according to the CLSI guidelines. Isolates were defined as multidrug-resistant (MDR) when they showed resistance to three or more compounds from different chemical groups. Differences in distributions were evaluated using the $\chi 2$ test. Fecal indicators with resistance patterns of clinical relevance were additionally characterised by molecular methods.

The obtained results showed that during the wastewater treatment processes the fecal indicators with antimicrobial resistance patterns were positively selected. Among enterococci isolates resistant to high-level aminoglycoside (HLAR) were detected, while among *E. coli* - extended spectrum β-lactamases producing isolates were found. Detailed analysis of *E. coli* producing extended-spectrum β-lactamases (ESBL) indicated CTX-M β-lactamase type, which is produced by nosocomial and community strains of *Enterobacteriaceae* in different countries. The recently discovered, plasmid-borne sulfonamide resistance gene - *sul*3 was also detected in the WWTP effluent. Additionally among *E. coli* the positive correlations was found between antimicrobial resistance of clinical relevance and presence of class 1 integrons with occasional occurrence of class 2 integrons. Among *E. faecium* subjected to typing hospital-adapted polyclonal high-risk enterococcal complex (HiRECC) constituted altogether 25% of all isolates and were present at all sampling points. This suggests the ability of such clones to survive the wastewater treatment processes. The HiRECC often carried resistance determinants (located on mobile genetic elements) and pathogenicity factors

The conventional wastewater processes do not prevent the receiver from dissemination of fecal bacteria with clinically relevant resistance patterns. It is suspected that antimicrobial-resistance patterns found in hospital settings could be also beneficial for survival in other compartments. Thus special attention should be given to mobile genetic elements in terms of their role in adaptation of bacterial cells to adverse environmental conditions. With reference to the above, in the environmental risk assessment, the resistant bacteria should be monitored in both, treated wastewater and its receiver.

ANTIBIOTICS AND THEIR RESISTANCE GENES IN THE ENVIRONMENT - BACTERIAL OPPORTUNITY OR THREAT?

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Antibiotics are a wide group of natural, synthetic and semisynthetic compounds able to kill or to inhibit the growth of microorganisms. The first discovery of an antibiotic compound - penicillin, was accidental and took place in 1928. Alexander Fleming, a Scottish biologist, pharmacologist and botanist, described the antimicrobial effect of substances produced by *Penicillum notarum* on a Petri dish with a bacterial culture. Since then the era of antibiotics has begun. The worldwide usage of β-lactam antibiotics (penicillins and cephalosporins) in the 1940s and ‘50s was probably one of the landmark events in human medicine. Interestingly, in 1940 *Escherichia coli* producing penicillinase had been already described in Nature Journal. This information was a proof that the antibiotic resistance genes pre-dated the common antibiotic usage. The bacterial resistance against antibiotics can be gained in several ways. It could be by mutation or selection pressure caused by the antibiotics itself but very often resistance is gained by the acquisition of the novel genes in the horizontal gene transfer (HGT) - conjugation, transformation and transduction. The wastes generated by humans and animals may contain both antibiotics and their intermediates as a potential cause of the resistant bacteria selection or dangerous pathogens being a reservoir of resistance genes possible to be exchanged with other non-pathogenic bacteria. Such an exchange can take place in soil, water and wastewater, but also in agricultural facilities (i. e. animal farms) or hospitals and households. The occurrence and mechanisms of antibiotic resistance among microorganisms is a wide scientific issue which is still not completely understood. The aim of this presentation will be a short description of the resistance mechanisms and a brief outline of the selective pressure causing an antibiotic resistant gene transfer, both in the medical and environmental dimension of the problem.

Poster abstracts

Number next to the abstract title correlates with poster number

1. MALDI-ToF-Based Analyses of the Low-Molecular-Weight Fraction of Serum Proteome of Patients with Gastric Cancer

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Gastric cancer is the fourth most common cancer in the world and the second cause of cancer-related deaths (e.g., there is about 5300 new cases and deaths recorded annually in Poland). This type of cancer, because of nonspecific symptoms or even asymptomatic development in its infancy, is usually diagnosed in an advanced stage with poor prognosis. Identification of tumor biomarker(s) for early detection, assessment of risk of metastasis and/or evaluation of the response to anticancer treatment could improve efficacy of the treatment and increase the 5-year survival rate of this fatal disease. The molecular profiling of the serum proteome, especially its low-molecular-weight component, is a potential method of cancer diagnostics. Here we aimed to use this approach to identify serum proteome component associated with gastric cancer and risk of its metastasis.

Serum samples were collected from the two groups of patients with advanced gastric cancer with diagnosed metastasis (102) or without diagnosed metastasis (77), and from the control group of healthy people matched with respect to age (89). MALDI-ToF mass spectrometry was used to register molecular proteome profiles. Serum samples were ultrafiltered with Amicon Ultra 50K filters to remove components of high molecular weight. The samples were purified by reverse phase chromatography on Zip Tip C18 resins and applied with HCCA matrix saturated in TA30 (30% acetonitrile in 0.1% TFA) solution to AnchorChip target. Mass spectra were registered in the 1800 to 12000 Da range using Ultraflextreme MALDI-ToF spectrometer working in the linear mode. Registered mass spectra were processed using bioinformatic algorithms created and optimized by our group and analyzed with statistical tools of Spectrolyzer software.

In our studies we identified some spectral components that differentiated samples from gastric cancer patients and samples from healthy controls; moreover, we were able to indicate the spectral components, that differentiated patients with locally advanced cancer and patients with metastatic cancer. Our results indicate that MALDI-based analyses of the low-molecular-weight fraction of serum proteome could reveal molecular features with some potential in diagnostics of gastric cancer.

The work was supported by Ministry of Science and Higher Education, Poland; Grant no. N N40 283140.

2. EFFECTS OF PHOTOSTRESS-INDUCED NO ON BYSTANDER CELLS

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Photodynamic therapy (PDT), a unique antitumor modality involving a sensitizing agent, photoexciting light and molecular oxygen, is characterized by local generation of singlet oxygen and other cytotoxic oxidants. When subjected to PDT-induced oxidative stress, many tumors succumb to apoptotic cell death, and much has been learned about how this is affected by factors such as sensitizer localization and efficiency of toxic oxidant generation. However, the influence of metabolic and environmental factors, is still not well understood. Our studies focus largely on the effects of nitric oxide (NO). In this regard nitric oxide synthase (NOS)-generated NO in low doses is known to have pro-survival and growth-promoting effects on various tumors. Using in vitro models of 5-aminolevulinic acid (ALA)-based PDT and chemical NO donors, we show that NO can protect tumor cells against necrotic photokilling by either scavenging lipid-derived radicals or by signaling for heme oxygenase-l and ferritin induction, leading to depletion of prooxidant iron. It was discovered that NO is overproduced by ALA/light-stressed breast tumor cells due to rapid and prolonged upregulation of inducible NOS and that this substantially increases cell resistance to intrinsic apoptotic photokilling. This proposal is based on the following hypothesis: Under PDT stress, many tumors will overexpress NOS and NO as a cytoprotective response, and this can compromise PDT efficacy.

3. FAST AND EFFICIENT TECHNIQUE OF MAGNETIC RESONANCE SPECTRA DECOMPOSITION BASED ON MIXTURE MODEL

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Nuclear Magnetic Resonance Spectroscopy (MRS) is one of the most complex medical tests that are performed for patients with brain tumors. It allows early identification of tumor metabolism trail even if no difference is visible in imaging, and so early detection of possible tumor spread or recurrence. Changes in metabolite amounts are not high thus the estimation of single metabolite concentration must be very precise. We propose to decompose MRS signal into a sum of Gaussian components, which give better estimation of location and spread of signal peaks. As computational time rises with increase of modeling precision, thus there is a need to re-implement an algorithm with use of fast graphic card (GPU).

Proposed methodology was tested on a set of 27 spectra measured on a phantom simulating the human brain. For the proposed methodology a full pre-processing path was designed and applied. Five most popular metabolites: NAA, Creatine, Choline, Myo-inositol and Lactates were identified among all components in decomposed signal. Results for main five metabolites share only very small (less than 5%) dispersion among analyzed spectra. This may be explained by natural dispersion of scanner parameters. We also tested efficiency of different implementations of EM algorithm. GPU-based version drastically speeds up modeling algorithm with comparison to the standard implementation on a single processor.

It was observed that proposed GM spectrum together with complete pre-processing and efficient computational techniques result in fast and precise estimation of metabolite amount. By comparison to other techniques such as LC Model or Tarquin we observed that the proposed method outperforms the two in the analysis of phantom data. The main positive aspects of GPU-based approach is the possibility to increase model precision without the rapid increase of computational time and to quickly analyze big datasets. It is very important since the development of new stronger in vivo scanners results in obtaining much complex signals.

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4. COMPARISON STUDY OF TRANSCRIPTOMIC AND PROTEOMIC ANALYSES OF THREE MOUSE RADIATION-INDUCED AMLS

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Aim: Aim of this study was to show differences of gene and protein expression levels between different ways of induction of acute myeloid leukaemia in mice.
Materials and methods: An experiment was performed for three types of mouse AMLs: RF12, RF26 and MLP3. For MLP3, AML was inducted by implanting leukaemia cells; while for RF12 and RF26 the inducting factor was ionizing radiation. There was also a control group of mice. After transcriptomic and proteomic experiments as well as data preprocessing measured was expression level for 21266 genes (3 technical replicates for AMLs and 6 for control) and level of 320 proteins (1 technical replicate as signal to control ratio). In case of genes, comparison between control group and AMLs for each mouse acute myeloid leukaemia was performed using t-test. As a next step common statistically significant genes (p-value<=0.05) between three AMLs has been found. The same was done for proteins, but because of data complexity and specificity, proteins with statistically changed expression were found using assumed cut off on 2 or ½ fold change level. Using Sørensen-Dice similarity coefficient the pairwise similarity among AMLs mice was analysed.
Results: As a result, Sørensen-Dice index value was found for each pair of mouse AMLs: RF12, RF26 and MLP3. What is more Dice similarity coefficient was obtained for three AMLs together. The analysis was done separately for genes and for proteins. The number of common significant genes and proteins and Sørensen-Dice index values are present in table 1.

Compared groups	Number of common genes	Sorensen-Dice similarity index for genes	Number of common proteins	Sorensen-Dice similarity index for proteins
MLP3 and RF12 and RF26	5521	0.8122	3	0.6458
MLP3 and RF12	6657	0.6376	11	0.1134
MLP3 and RF26	6399	0.6934	4	0.04545
RF12 and RF26	7347	0.7218	85	0.7658

Table 1. Number of common significant genes and Sørensen-Dice index values

Conclusions: There occur many common statistically significant genes and proteins among mouse AMLs. Since RF12 and RF26 are more similar mutually than MLP3 to them, one may conclude that the way of induction of acute myeloid leukaemia has impact on gene changes and protein expression.

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5. CURCUMIN AS ADJUVANT IN GLIOBLASTOMA TREATMENT WITH TYRPHOSTINS

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An increased production of transforming growth factor α (TGFα) or epidermal growth factor (EGF) and its receptor (EGFR), so called autocrine loop, was found in several cancer cell lines [1,2,3], including human brain cancer (*Glioblastoma multiforme*; LN229; CRL-2611). One of the way of inhibition of transduction signal via EGFR is application of low molecular **tyr**osine **pho**sphorylation **in**hibitors (tyrphostins) [4,5,6]. In the present work, we tested two tyrphostins: AG494 and AG1478. Both the investigated compounds are specific ATP-competitive inhibitors of tyrosine kinase, located in the intracellular domain of the EGFR. However, tyrphostin AG1478, unlike AG494, acts in a non-reversible manner. Also AG1478 structurally is very similar to clinically used erlotinib (Tarceva^{TM}) and gefitinib (Iressa^{TM}), which implies it as a potent anti-cancer factor. Supplied with diet polyphenol, curcumin (spice, food coloring E100, dietary supplement) is characterized by anti-cancer and cytoprotective properties [7]. Thus, it was decided to evaluate the effect of curcumin on therapy with EGFR's inhibitors.

In the present study the influence of tyrphostins, curcumin (C) and their mixtures (AG1478+AG494, AG1478+C, AG494+C) on mobility, viability, oxidative stress and cell cycle was evaluated in LN229 cells. Cultures were exposed to a such a concentration of tested compounds as well as their mixtures at which 50% (IC_{50}), 90% (IC_{90}) or theoretically 100% ($2xIC_{50}$) of cells were growth nhibited. The wound healing assay was used to determine the influence of the tyrphostins, polyphenol and their mixtures on the mobility of LN229 cells. The activity of caspases 3 and 7 was measured by intensity of luminescence (Caspase-Glo® 3/7 Promega kit) in order to indicate whether the tyrphostins, curcumin and their mixtures were able to induce apoptosis in glioblastoma cells. Dichlorofluorescein assay was used in the investigation of the effect of EGFR's inhibitors and curcumin on the induction of Reactive Oxidant Species (ROS). Flow cytometer analysis was used to determine the influence of tested compounds on cell cycle.

The results show that curcumin is a stronger inhibitor of mobility of LN229 cells than tyrphostin AG1478 (both compounds in IC_{50}), however their mixture completely stopped cell migration. Furthermore, tested tyrphostins had different apoptotic properties. AG494 in IC_{90} concentration was a moderate apoptotic inductor, while AG1478 had no influence on viability of glioblastoma cells. Surprisingly, their mixture had strongly cytotoxic properties. Curcumin however activated caspases 3 and 7 more strongly than AG494 and tyrphostin's mixture. What is interesting the polyphenol - AG494 mixture had completely opposite properties than each of the compounds alone. That mixture does not induce apoptosis at all, though each of the compounds had strong proapoptotic properties. Curcumin and AG1478 mixture induced the activity of effector caspases, wherein their activity was comparable with the activity of the caspases caused by curcumin alone. Curcumin did not induce the production of ROS within cells (even at $2xIC_{50}$ concentration), while AG1478 and AG1478 with curcumin in $2xIC_{50}$ did. In case of ROS scavenging after hydrogen peroxide treatment, AG494 had strong antioxidative properties, which were directly proportional to concentration used. Higher concentration results in more efficient ability to scavenge ROS. To the contrary, AG1478 and curcumin induced ROS within cells at IC_{50} concentration. While at $2xIC_{50}$ concentration tyrphostin AG1478 lost its oxidative properties, curcumin acted like a strong antioxidative agent. Strong antioxidant properties of AG494 were observed in mixture with AG1489 or polyphenol. It completely abolished oxidative properties of AG1478 (regardless of concentration used) and curcumin at $2xIC_{50}$. The flow cytometer analysis showed that tyrphostins and curcumin at IC_{50} concentration did not influence the cell cycle of glioblastoma cell line. Only mixtures caused the accumulation of cells in different stages of cell cycle.

Our results suggest that curcumin together with tyrphostins is a modulatory agent in glioblastoma treatment. On the one hand polyphenol supports the tyrphostin treatment (complete suppression of migration, antioxidative and cytostatic properties, cell cycle inhibition). But on the other hand it has no influence on EGFR inhibitors (no additive effects on cytostatic properties of tyrphostins).

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6. MICROARRAY ANALYSIS OF THE REGNASE-1 ENFORCED OVEREXPRESSION IN HUMAN NEUROBLASTOMA

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The recently discovered MCPIP1 (monocyte chemotactic protein-1-induced protein 1) also called regnase-1, multidomain protein encoded by the *MCPIP1* (*ZC3H12A)* gene, has so far been described as a new transcription and differentiation factor, a ribonuclease, and a deubiquitinase. However, its role in cancer development is poorly recognized. Our recent analysis of microarray data showed a lack of expression of the MCPIP1 transcript in primary neuroblastoma - the most common extracranial solid tumour in children. Enforced *MCPIP1* gene expression in BE(2)-C cells caused a significant decrease in neuroblastoma proliferation and viability.

The aim of the present study was to investigate the role of regnase-1 in neuroblastoma, using high-throughput analytical methods such as expression DNA microarrays and microRNA microarrays.

Enforced *MCPIP1* gene expression in BE(2)-C cells was obtained through transfection of plasmid constructs bearing *MCPIP1wt* or a mutant form lacking the RNase domain (*MCPIP1ΔPIN*). Changes in the transcriptome and microRNA expression were analyzed using expression DNA microarrays and microRNA microarrays, respectively. The microarrays results were verified using the RT-qPCR system.

Expression microarray analysis, performed on samples isolated from BE(2)-C cells, showed decrease in expression level of a few interesting genes, which encode among others: one of minichromosome maintence proteins, involved in the initiation of eukaryotic genome replication (MCM10), endosialin (CD248) - related to angiogenesis, tumour development and growth, as well as genes encoding neurotransmitter's transporters such as SLC29A4 and SLC44A1. The microRNA profiling, done on RNA samples obtained from BE(2)-C cells, identified a subset of 8 microRNA, which were differentially expressed in MCPIP1wt-transfected and MCPIP1ΔPIN-transfected cells. Bioinformatic analysis showed a large group of possible target genes for Hsa-miR-3613-3p microRNA. More detailed analysis of the genes revealed members of apoptotic machinery (APAF1, DFFB), proteins involved in mTOR pathway (DICER) and also genes which are known as prognostic factors, markers of suppression and differentiation like NF1.

Additionally, expression level of genes indicated using expression arrays and Has-miR-3613-3p target genes was examined in available data from mRNA microarrays performed on human primary neuroblastoma tumours and neuroblastoma cell lines.

These results show that enforced regnase-1 expression can play important role in many pathways and processes involved in carcinogenesis.

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7. THE SYNERGISTIC WAY OF ACTION OF WP 631 AND EPOTHILONE B IN OVARIAN CANCER CELLS

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Ovarian cancer is a leading cause of cancer-related death among women. Most of the cases are diagnosed in advanced stages, when currently used therapies are ineffective. Nowadays, besides surgery, the basic way of treatment is chemotherapy, which mostly has the form of combined therapy. Such a solution has many advantages. Firstly, it increases the therapeutic effect, especially when applied drugs have different mechanisms of action. Secondly, it allows decreasing dosage of each component and thus their toxicity. The third reason is minimizing the development of drug resistance. WP 631, which is a new bisanthracycline, and epothilone B (Epo B) with mechanism of action similar to taxanes, are two new substances, which, given in a combination, synergize in SKOV-3 human ovarian cancer cells. In the present study we investigated if the sequence of WP 631 and Epo B induces apoptosis, influences ROS level and damages DNA in SKOV-3 cell line.

Methods: The morphological changes characteristic of apoptosis and necrosis in drug-treated cells were analyzed by flow cytometry after staining with Annexin V-FITC and PI. The production of reactive oxygen species was studied using specific fluorescence probe - $DCFH_2$-DA and the DNA damages were measured by using TUNEL assay.

Results: The data showed that the compounds added in a combination after 72 hours incubation induced both apoptotic and necrotic cell death. The percentage of apoptotic cells was much more higher after treatment with combination (59.6 ±4.78%) in comparison to drugs applied separately: 44.92 ±5.24% and 5.42 ±1.26% for Epo B and WP 631, respectively. The percentage of necrotic cells reached the highest value also after treatment with drug combination, but in this case changes between sequence and WP 631 or Epo B given alone were not so noticeable. Our further results demonstrated that only WP 631 taken alone, in contrast to Epo B, generates ROS in ovarian cancer cells in the 24 h incubation period (117%). After 72 h of incubation, ROS were not detected either for WP 631 or for Epo B. The maximal increase in the ROS level was reached after 4 h (121 %) when the drugs were given together. The TUNEL assay was performed in order to find an answer if the apoptotic changes induced by drug combination are related to DNA fragmentation or not. It was shown that WP 631 and Epo B given together caused the highest increase (approximately 20%) in the level of apoptotic cells with DNA-strand breaks characteristic of TUNEL-possitive cells. Epo B induced similar alternations, but not as significant as drug combination.

Conclusions: The obtained results provide direct evidence that the drug combination is much more cytotoxic to SKOV-3 human ovarian cancer cells than WP 631 or Epo B given alone.

8. 124-I PRODUCTION FROM TELLURIUM DIOXIDE

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The use of radiopharmaceuticals for molecular imaging has become a significant diagnostic tool in oncology. Positron Emission Tomography (PET) is a very popular diagnostic tool, mainly due to unrivalled high sensitivity and resolution, which allows to diagnose many different physiological processes. The most frequently used radionuclides in PET are 11-C and 18-F, due to its ease of production. These radionuclides have short decay times, measured in hours and minutes. The long half-life time (4.2 days) is suitable for in vivo studies, i.e. in thyroid cancer.

Methods: Target was made by mixing Al_2O_3 with TeO_2 at 5:95 ratio, then mixture was melted using TERIMO module. Next, the target was bombarded by proton beam in 18/9 IBA Cyclotron, according to reaction Te(p,n)I. Using dry distillation method, products of the reaction were separated, and 124-I trapped into NaOH solution.

Results: Successfully produced 4 mCi in 2 ml solution in the first run. Solution was tested in high purity Germanium detector and scintillation counter. Tests show that the level of impurities was insignificant, and sample consists solely of 124-Iodine. Radionuclide was placed in a water phantom and scanned using Siemens mCT Biograph PET/CT scanner. The scanner registered gamma rays from annihilation effect.

Conclusion: Production of 124-Iodine in reaction Te(p,n)I yields good results, and acquisition using PET/CT scanner yields good image quality.

9. BIOLOGICAL INTERACTIONS BETWEEN OMENTAL-DERIVED ADIPOSE STEM CELLS (O-ASC) AND OVARIAN CANCER CELLS; PRELIMINARY MICROARRAY RESULTS

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Epidemiological studies suggest that visceral obesity is a risk factor for development of ovarian cancer. Adipose tissue contains stem cells that can stimulate tumor growth, invasiveness and resistance to therapy. We have previously demonstrated that omental-derived adipose stem cells (O-ASC) significantly promoted *in vitro* proliferation and migration rate of ovarian cancer cell lines, as well as chemo- and radio-resistance.

Our present study was aimed to analyze molecular background of biological interactions between O-ASC and ovarian cancer cells. Three lines of adipose stem cells, derived from omentum of ovarian cancer patients that differed by body mass index (BMI) and presence/lack of omental metastases were used. The O-ASCs were grown in co-culture with ovarian cancer cells, using cell culture inserts with 0.4 μM pores. Then, total RNA was isolated and used for gene expression profiling with Affymetrix Human Gene 1.0 oligonucleotide microarrays. Gene expression was compared between cancer cells grown alone (control) and cancer cells grown in co-culture with O-ASCs. We also compared gene expression profiles of O-ASCs grown alone and in co-culture with cancer cells. Finally, we also compared gene expression in O-ASCs versus ovarian cancer cells.

We found that 301 genes were differentially expressed (FDR<5%) when O-ASCs were grown in co-culture with ovarian cancer cells. Twenty eight genes from this list had over twofold expression change (FC>2). On the contrary, the influence O-ASCs on ovarian cancer cells was more discrete: we found only 20 genes with significant differential expression (FDR<5%). However, all these genes had expression change smaller than two-fold.

Comparison of O-ASC cell lines with ovarian cancer cell lines revealed huge molecular differences between these two cell types: 2121 genes passed criteria of FDR <5% and FC>2, while the greatest differences in expression were reaching 50-fold change level.

Four genes upregulated in O-ASCs grown in co-culture with cancer cells: BIRC3, CCL2, GPRC5B and TNFAIP3, were selected for further validation with quantitative reverse transcription PCR (qRT-PCR). In addition, we chose also IL1A, MFAP4 and POSTN that were among top genes differentially expressed in O-ASCs and cancer cells. All these genes were positively validated with respect to the experimental points for which they were chosen. Interestingly, the differences in expression measured by real-time PCR were higher than measured by microarrays. These preliminary results encourage further studies of molecular processes and signaling pathways engaged in the interactions between O-ASCs and ovarian cancer cells.

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10. EXPRESSION PROFILE OF DNA REPAIR GENES IN ENDOMETRIAL CANCER

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In the recent years endometrial cancer has become one of the most common gynecological malignancies diagnosed in women. Its pathogenesis is linked to proliferation and estrogen receptor signaling, but as every cancer it is induced by mutations in the cell genome. The DNA repair system is probably one of the most important aspects of a healthy cell. The main goal of these systems is to repair deletions, insertions and miss-incorporations that appear during DNA replication. If the system does not work it can be caused by a failure of suppressor genes and/or change of oncogenes' expression. These aberrations can lead directly to cancer transformation and cause a serious problem.

The aim of this study was to evaluate expression profile of genes related to DNA repair systems in endometrial cancer.

Endometrial cancer samples had been collected during hysterectomy at the Department of Gynecology and Obstetrics of the Medical University of Silesia in Katowice. Next, the samples were analyzed histopathologically. On this basis they were divided into three cancer groups: G1, G2, G3. The control samples were taken from normal tissue of patients also after hysterectomy, but performed for other reasons than endometrial cancer. The samples were analyzed using oligonucleotide microarrays HGU-133A with 22 283 ID mRNA; 613 ID mRNA were connected with DNA repair. This group was analyzed statistically with GeneSpring 13.0 (Agilent).

To estimate differences in the gene expression level between cancer and control samples, ANOVA test with post hoc Tukey test were employed. This gave 136 ID mRNA for all cancer grades which were divided in the Venn diagram and specified to different groups. Based on biological significance 6 genes were found to be differentiating: YY1 in the G1, XPA, MGMT and MSH6 in G2, and SSRP1, PRKDC in G3.

Changes in expression of various DNA repair related genes can be a valuable indicator for selecting chemotherapy treatment in patients with endometrial cancer.

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11. EXOSOME UPTAKE IS INFLUENCED BY THE DIFFERENTIATION STATUS OF IMMUNE CELLS

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Exosomes are intraluminal vesicles (30-150 nm in diameter) generated from multivesicular bodies (MVBs) by inward budding. These small membrane vesicles are released to the extracellular space, thus they are present in most biological fluids. Moreover, exosomes can act as intercellular messengers (via proteins, RNAs) to distant locations. Therefore, exsomes are considered to be involved in cell-to-cell communication. Cancers take advantage of this possiblity to influence tumor progression, metastasis or angiogenesis. Cancer-derived vesicles may also exert an immunosuppressive function, as well as trigger an anti-tumor response by transfering tumor antigens to immune cells. We wanted to investigate the efficiency of exosome uptake by different kinds of immune cells.

To vizualize and quantify their uptake into the cells, exosomes were labeled using three different dyes: carboxyfluorescein diacetate succinimidyl-ester (CFSE) - a membrane premeable compound, DSSN+ and DSNN-DEA - water soluble distyrylstilbene oligoelectrolytes which preferentially intercalate into the cell membrane. With the help of cytokines, monocytes were differentated into dendritic cells or macrophages. Exosome uptake was monitored by flow cytometry.

The results show that macrophages and mature dendritic cells take up exosomes more efficiently than monocytes or immature dendritic cells. Macrophages, the 'big eaters' of the immune system, are highly specialized in removal of dying or dead cells and cellular debris. Immature dendritic cells constantly scavenge the surrounding environment for pathogens. Upon maturation (down-regulation of endocytosis) degraded pathogen protein fragments are presented at their cell surface by MHC molecules. Our results indicate that the cell differentiation status has impact on the efficiency of exosome uptake.

12. BIOLOGICAL ACTIVITY OF THIO-SUGAR DERIVATIVES ON HUMAN COLORECTAL ADENOCARCINOMA CELL LINE

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Functionalized carbohydrate derivatives are known to exhibit diverse biological properties like low susceptibility to enzymatic degradation or being a potent enzyme inhibitors. These biological properties are also related to their potential anticancer activity. We synthesized and tested for anticancer properties two sugar derivatives containing sulphur as heteroatom in their sugar ring, i.e. 5-thio-D-glucose and 6-thio-β-D-fructopyranose. Both thio-sugars were cytotoxic to human colorectal adenocarcinoma (LoVo) cell line. As a continuation of this research, we investigated the potential mechanism of their cytotoxicity by evaluating their genotoxicity.

Genotoxicity of thio-sugars to LoVo cell line was investigated by alkaline, neutral and pH 12.1 versions of comet assay. Additionally, we tested the ability of both thio-sugars to induce oxidative DNA damage in human cancer cells by comet assay with DNA repair enzymes.

The comet assay showed dose-dependent genotoxicity of thio-sugars in LoVo cells. Both thio-sugars evoked DNA damage in the alkaline version of comet assay at 0.5 mM and introduced oxidative modifications to DNA bases. It suggests that the potential mechanism of cytotoxic action of investigated 5-thio-D- glucose in LoVo cells may be related to the induction of oxidative stress. We are also planning to determine the exact mechanism of ROS generation in cancer cells by both investigated thio-sugars.

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13. CYTOTOXICITY AND INDUCTION OF APOPTOSIS BY FORMAMIDINODOXORUBICINS IN COMPARISON TO DOXORUBICIN IN HUMAN OVARIAN ADENOCARCINOMA CELLS

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Objectives: The aim of our study was to investigate the antiproliferative activity of five new derivatives of doxorubicin against the SKOV-3 ovarian cancer cells. All these compounds at the position C-3' in daunosamine moiety contain a formamidine group (-N=CH-NRR) at the 3' position with pyrrolidine (DOX-F PYR), piperidine (DOX-F PIP), morpholine (DOX-F MOR), N-methylpiperazine (DOX-F PAZ) and hexamethyleneimine (DOX-F HEX) ring.
Methods: The antiproliferative activity of DOX and tested derivatives was assessed using MTT assay. To evaluate the ability to induce apoptosis the following method was used: double staining using fluorescence probes: Hoechst 33258/propidium iodide (PI) and measurements of phosphatidylserine externalization using Annexin V-FITC (flow cytometry).
Results: All of the investigated derivatives were considerably more cytotoxic to SKOV-3 cell line than DOX. The predominant type of cell death induced by the anthracycline analogues was apoptosis. Necrotic cells represented only a small percentage (<5%) of all cells. The number of apoptotic cells was dependent on the compound and the incubation time.
Conclusions: All new formamidine derivatives of DOX were effective against ovarian cancer cells.The most promising results were obtained for DOX-F MOR and DOX-F PAZ analogues. The least potent was DOX-F HEX.

In summary, we report here synthetic doxorubicin analogs with strong cytotoxicity and which induce high level of apoptosis in human ovarian adenocarcinoma cells in comparison to doxorubicin. Promising results of our study encourage further investigation of their mode of action.

14. EVALUATION OF THE LIPOPHILIC PROPERTIES OF SELECTED BIOLOGICALLY ACTIVE STEROID COMPOUNDS

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Among numerous physicochemical properties the lipophilicity of a molecule has significant impact on biological activity of different organic substances like for example steroids [1,2]. Therefore, the main goal of this work was to determine lipophilic properties of the selected steroid compounds such as androsterone (A), epiandrosterone (EP), dehydroepiandrosterone (DHEA), testosterone (T), estradiol (E) and hydrocortisone (H) which belong to the group of well-known hormones. In order to estimate lipophilic character of all the examined steroids we applied thin-layer chromatography in RP-HPTLC system and also various theoretical methods enabling determination of logP which is the measure of lipophilicity (*e.g.*, AlogPs, IAlogP, ClogP, $logP_{KOWWIN}$, xlogP, milogP) [3]. Chromatographic investigations by means of RP-18WF_{254} silica plates and also the use of two mobile phases consisted of methanol-water and acetonitrile-water allowed to obtain R_M values for investigated compounds. In further study obtained R_M values were converted using Ościk's and also Soczewiński-Wachtmeister's equation into two lipophilicity parameters R_{MW0} and also R_{MWS}, respectively. All chromatographically determined lipophilicity parameters R_{MW0} and R_{MWS} were compared with partition coefficient (logP), determined computationally by means of different software packages and also with *n*-octanol-water partition coefficient ($logP_{exp}$). For all the studied compounds the results of chromatographic lipophilicity parameters such as R_{MW0} determined by Ościk's equation, were relatively higher than R_{MWS} predicted using Soczewiński-Wachtmeister's equation. Of all chromatographically predicted lipophilicity parameters the best agreement with *n*-octanol-water partition coefficient ($logP_{exp}$) was found with R_{MW0} obtained in the methanol-water system in the case of androsterone, epiandrosterone, dehydroepiandrosterone, testosterone and estradiol. For hydrocortisone a strong similarity was observed between experimental logP and R_{MW0} determined by use of mixture acetonitrile-water as mobile phase.

Our study confirms that RP-HPTLC is a reliable, precise and cost-effective analytical tool which allows determining the lipophilic character of biologically active steroids.

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15. A ROLE OF PHLDA1 PROTEIN IN APOPTOSIS AND AUTOPHAGY IN ANTI-GD2 GANGLIOSIDE ANTIBODY-TREATED IMR-32 NEUROBLASTOMA CELLS

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Despite expanding knowledge on neuroblastoma (NB) in recent years, the therapeutic outcome for children at high-risk of NB and patients with relapse of the disease has not significantly improved. Therefore, novel therapeutic approaches are needed to improve survival of the patients. This might be achieved by directing future efforts at recently proposed targets for NB therapy.
PHLDA1/TDAG51 (pleckstrin homology-like domain family A member 1/T cell death-associated gene 51) is ubiquitously expressed in a wide range of normal and cancer tissues. The exact biological function of PHLDA1 in neuroblastoma is unknown, however there is evidence showing that it might act as a mediator of apoptosis and autophagy.

Results obtained from whole-genome microarray assay in the IMR-32 human neuroblastoma cell line led us to select PHLDA1 protein as a key target induced upon the 14G2a mAb treatment. It revealed that the most significant increase in PHLDA1 specific mRNA content occurred in anti-GD2 mAb treated cells. Quantitative RT-PCR on total RNA samples after 14G2a mAb treatment confirmed increased mRNA content in the IMR-32 cells and the highest *PHLDA1* level was found at 48 h. PHLDA1 protein was also significantly upregulated at 6, 16 and 24 h in the IMR-32 cells.

These results prompted us to thoroughly investigate the role of PHLDA1 in the anti-GD2 mAb-treated neuroblastoma cells. We used a lentivirus vector-based RNAi approach using shRNA expression to silence *PHLDA1* gene in IMR-32 cells to observe its effect on the 14G2a mAb-induced cellular cytotoxicity. We observed that downregulation of *PHLDA1* expression promotes proliferation of IMR-32 cells. The rate of proliferation in *PHLDA1*-silenced cells is significantly higher than in mock (*i.e.* non interfering control shRNA) treated cells and wild type cells as assessed by measurement of cellular ATP content. The viability of the 14G2a mAb-treated silenced cells is increased as compared to mock and wild type cells. This inhibition of cytotoxic effect of the 14G2a mAb observed in PHLDA1-silenced cells indicates that cytotoxic effect of the 14G2a mAb on neuroblastoma may be PHLDA1-dependent.

Our following studies focused on describing the effect of PHLDA1 silencing on expression of molecules involved in apoptosis e.g. caspase 3, 7 and PARP and autophagy process e.g. LC3B, Atg proteins. We showed that *PHLDA1* silencing slightly inhibits caspase-3 cleavage in silenced clones when compared to mock and wild type IMR-32 cells. The results of these studies demonstrate that down-regulation of *PHLDA1* in IMR-32 may contribute to apoptosis resistance suggesting proapoptotic role of PHLDA1. To investigate whether PHLDA1 protein plays a part in autophagy process in 14G2a mAb-treated cells, PHLDA1-silenced IMR-32 cells were analyzed in terms of proteolysis process of a microtubule associated protein 1 light chain 3 (MAP-LC3). The higher levels of MAP-LC3B converted form were detected by immunoblotting in PHLDA1-silenced clones than in mock and wild type cells providing an evidence of autophagosomes formation in *PHLDA1* gene-silenced setting.

Further studies are warranted to investigate the mechanism responsible for PHLDA1 induction in 14G2a mAb-treated neuroblastoma cells and its role in apoptosis and autophagy.

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16. EFFECT OF HYBRID NANOSPHERES PA66/POM ON CELL CYCLE OF MCF-7 BREAST CANCER CELLS

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Cancer is the main cause of death worldwide. Each year, cancer is diagnosed in nearly 12 million people. The most effective cancer therapy is still based on the use of cytostatics. However, such treatments have different side effects like cardiotoxicity, hepatotoxicity and others. Thus, a key direction of current research in this field is to improve cancer therapy through a design of new compounds with anticancer activity and side effects lower than those of currently used drugs. Elaboration of new effective drug carriers for targeted therapy is also of great interest. Over the past few years, interest in the use of nanomaterials and nanotechnology in medicine has grown rapidly. Several nanoparticles displaying anticancer properties have been engineered and investigated in different laboratories as new promising compounds for application in cancer therapy.

In this study, we have evaluated the effect of hybrid nanospheres PA66/POM on cell cycle progression of MCF-7 breast cancer cells. PA66/POM hybrid nanospheres are classified as polyoxymethalates and considered as compounds with potential antitumor properties. Analyzed nanoparticles contained two components: polymer PA66 and varying content of tungsten trioxide (WO3) - 3%, 10% and 30%. MCF-7 cells were incubated for 24 h with a nanohybrid. Afterwards, nanoparticles were removed and cells were incubated in fresh medium for 0, 24 or 48 h. DNA was stained with propidium iodide and after permeabilization of the cell plasma membrane and digestion of RNA and protein with RNase and proteinase K. Cell cycle distribution was analyzed cytometrically on the basis of DNA histograms (FlowJo) and the significance of observed changes was evaluated statistically using STATISTICA. In some of the experiments the cells were preincubated for 1 h with antioxidants N-acetylcysteine (NAC) or vitamin C (vit. C) before the treatment with PA66/POM hybrid nanospheres.

We have found that PA66/POM displayed anticancer properties and inhibited proliferation of MCF-7 breast cancer cells arresting most of them at the G2/M checkpoint of the cell cycle. The largest changes were observed in cells treated with tungsten trioxide (WO3) alone. Preincubation with antioxidants did not significantly affect changes generated by the hybrid nanospheres investigated, which implies that oxidative stress is not directly involved in inhibition of proliferation of MCF-7 breast cancer cells by these compounds.

17. RIGID IMAGE REGISTRATION ALGORITHM AS A TEMPLATE MATCHING TECHNIQUE IN DERMOSCOPIC IMAGES

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Dermatoscopy is a major method used in diagnosing skin cancer. One of the problems that arise with this diagnostic technique, is the storage and searching of the database of previously acquired skin lesions. During a new diagnostic procedure skin lesions are acquired and the basic task for the clinicians is to find the previous record of the currently studied selected lesion. Information about the dynamics of this lesions would be very precious for the diagnostic process. Lesions with dramatic changes in their size or shape should be marked as suspicious and marked as a potential malignant melanoma, likewise lesions that were not present during previous diagnostic procedure.

In this study we used simple preprocessing step to select the shape of region of interest in the dermoscopic image. Next task of the algorithm is to search the database using affine transformation of the object and rigid image registration algorithm. Having two images, one from the current measurement and the other selected from a database we are looking for the best match and store the value of similarity measure used. Images that are suspicious to be similar will have the lowest value of this measure. Some studies of a few popular similarity measures were performed, including: Mutual Information [1], Normalized Mutual Information, Normalized Cross-Correlation and Phase-Correlation, to check the accuracy of those measures and performance. The algorithm was tested on dermatoscopic images supplied from PH^2 image database [2].

The test performed on a selected subset of images allowed us to check the accuracy of this method to the given transformation (known translations, rotations). The next step in our work is to search for similarity in the real database from the real diagnostic measurements.

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18. GENOTOXICITY OF GRAPHENE OXIDE AND GRAPHENE OXIDE WITH MANGANASE IONS IN HUMAN LUNG CANCER CELL LINE

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Graphene is an allotrope of carbon in the form of a one-atom-thick sheet. Other carbon allotropes are also made out of graphene, for example graphite and nanotubes. It is very strong compared to its weight, and it is about 100 times stronger than steel. Graphene oxide drug delivery system for anti-cancer therapy is based on direct immobilization of anti-cancer drug molecules on graphene and graphene oxide (GO) surfaces. GO nanocarriers are formed by loading drugs, antibodies for cell targeting and a probe for tracking the carrier inside the cell. GO can be chemically functionalized with polyethylenimine and polyethylene glycol (GO-PEI-PEG). Potential usability of graphene oxide in therapy is rooted in its low toxicity. There have been data suggesting that the relative cell viability was reduced to 69% when the GO concentration was as high as 320 mg/ml. GO-PEI-PEG complexes at concentrations from 0 to 320 mg/ml were not cytotoxic. We know that GO can be contaminated with manganese ions during the preparation process. Different laboratories remove the manganese ions with varying effectiveness while preparing the material. This is why in our study we decided to check the genotoxicity of two different GO samples, which were cleared of manganese ions with varying effectiveness.

In the experiment we used the A549 cell line (the human lung adenocarcinoma cell line) to analyze DNA damage after exposition to graphene oxide (GO) and graphene oxide contaminated with manganese ions (GO+Mn). For both samples we used two concentrations of graphene oxide: 1 mg/L and 10 mg/L. After 3 and 24 hours of incubation of the A549 cell line with graphene oxide the DNA damage was measured with the use of comet assay.

DNA damage level in cells from the A549 line was always higher after 24 hours of incubation, irrespectively of the type of graphene oxide probe (GO or GO+Mn) as well as the concentration (1 or 10 mg/L). After 3 hours of incubation with graphene oxide in concentration of 1 mg/L we observed a higher level of DNA damage in the manganese contaminated probe (TDNA - % DNA in the comet tail was as high as 7%), however after 24 hours of incubation the damage level was similar in both probes, oscillating around 12% TDNA. Incubation with graphene oxide in concentration of 10 mg/L didn't influence the DNA damage level in cells from the A549 line in a major way. After 3 hours of incubation the damage level was oscillating around 3%, and after 24 hours of incubation it was slightly higher for manganese contaminated probes and reached a level of 12 %.

In conclusion we can state that the exposition time of cells to graphene oxide can have a significant influence on the level of DNA damage on A549 line cells. The level of manganese contamination in graphene probes has a smaller influence on the DNA damage, although in lower GO concentrations and after short exposition times the toxicity level of the manganese contaminated probes was significantly higher. Further research is planned.

19. DNA METHYLATION OF GENE PROMOTER REGION OF ENDOTHELIN RECEPTOR TYPE A IN ENDOMETRIAL CANCER

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All three isoforms of the endothelin show higher or lower affinity to endothelin receptor type A (ETA), which participates in the regulation of cell proliferation, angiogenesis and contributes to tumor invasion. The decrease of transcriptional activity of endothelin receptor type A gene was observed in endometrial cancer but mechanisms responsible for silencing are still unknown.

The purpose of the work was to investigate if decreased expression level is caused by epigenetic silencing by promoter methylation.

The research included samples of the endometrium: 6 histopathologically confirmed as normal and 17 as endometrial cancer, further divided according to the tumor grade: G1-3, G2-13 and G3-1. Expression profile of endothelin receptor type A gene was determined by Affymetrix HGU-133 A microarrays. Promoter methylation profile was performed using the method of Wang et al. (2013).

The study revealed absence of endothelin receptor type A promoter methylation in normal endometrium samples and confirmed the presence of ETA DNA methylation in endometrial cancer: G1 - 2/3 samples, G2 - 7/13 samples and 1/1 in G3. The obtained results show that the mechanism responsible for transcriptional activity silencing of endothelin receptor type A gene is associated with promoter methylation of this gene.

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20. THE CAUSES OF GAPS CREATION IN SHOTGUN SEQUENCING

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The shotgun sequencing used on a large scale is a technique which links automatic sequencing devices and computer analysis. This method[1, 4] is based on random cutting of DNA fragments into smaller pieces and then computational fold by overlapping these fragments into contiguous sequence of DNA (called contigs). Thanks to this approach, researchers are able to sequence huge genomes such as human one which contains about $3*10^9$ bp[4]. This method can lead to gaps between contigs, caused by errors[5] associated with observation of false bases, that reduce the quality of the sequencing process.

The aim of the research was to create a model [1, 2] according to shotgun sequencing assumptions. The main purpose of model creation was to check influence of the basic process parameters, such as reads length, number of reads, number of genome and threshold of overlap, into number of gaps. Furthermore, we modified a model to take account the amount of reads that contain errors, which show us the influence of incorrect reads into quality of this sequencing technique.

In the present study the effects of gap creation [3] was tested for dependence of the tandem repeating sequences number. We check some organisms chromosome sequences (according to tandem repeats) and their shotgun sequencing statistics and plot the results. In the next step we check tandem repeating sequences included on the beginnings and ends of the contigs relative to whole contigs sequences.

The results show that the model is a good way to predict the number of gaps creating during the shotgun sequencing method, according to the basic parameter of process and percentage of errors. Further, the number of tandem repeats analysis demonstrated impact of tandem repeating sequences into gaps formation. Discussion about the causes of gaps creation may be helpful in understanding and improving the quality of shotgun sequencing process.

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21. THE *SIPA1* GENE POLYMORPHISM AS POTENTIAL PROGNOSTIC MARKER IN NON-SMALL CELL LUNG CANCER - PRELIMINARY RESULTS

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Lung cancer has one of the lowest survival outcomes of all neoplasms worldwide. Metastases are responsible for majority of lung cancer mortality. SIPA1 (signal-induced proliferation-associated 1) is a mitogen-induced GTPase activation protein that participates in cell cycle inhibition, decreased cell adhesion, increased motility and in modulating metastatic efficiency of cancer cells. Germline polymorphisms in *SIPA1* gene may contribute to the development of metastasis in human cancers. In this preliminary report, we aimed to examine the association of the *SIPA1* -313A>G (rs931127) promoter polymorphism with overall survival (OS) and progression-free survival (PFS) in non-small cell lung cancer (NSCLC). So far, its role as potential prognostic marker has been studied only in breast cancer.

A group of 351 patients with inoperable NSCLC treated with radiotherapy or radiochemotherapy was genotyped using PCR-RFLP. Survival curves were determined with Kaplan-Meier method and compared by log-rank test. Uni- and multivariate Cox proportional hazards regression was also applied.

The statistically significant association between the SNP and survival was observed in multivariate analysis. Under co-dominant and recessive models, the GG homozygotes had significantly shorter PFS in all cohort and in advanced stage group. The GG genotype was also associated with reduced OS and PFS in radiotherapy alone subgroup. Moreover, the *SIPA1* -313 GG was identified as an independent adverse prognostic factor for PFS. Our preliminary findings suggest that rs931127 variant may be a potential predictor of poor survival in NSCLC due to an increased risk of progression, however, considering the relatively small sample size of the group and its clinical heterogeneity, further verification of the results is required.

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22. EFFECT OF OXIDATIVE MODIFICATION OF CYSTEINE RESIDUES OF GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE ON FUNCTION AND LOCATION IN CELL

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Glyceraldehyde 3-phosphate dehydrogenase (GAPDH, EC 1.2.1.12) is an enzyme which belongs to the class of oxidoreductases. It is one of the most common proteins: GAPDH concentration is between 10-20% of the total cellular proteins. This enzyme belongs to the so-called housekeeping enzymes, i.e. those which are involved in processes essential for cell survival and expressed at relatively constant unchanging levels in most tissues of the body. Numerous studies showed that GAPDH is involved in glycolysis and participates in a number of processes such as plasma and nuclear membrane fusion, formation of the cytoskeleton, transport of secretory vesicles, translational and transcriptional control of gene expression, maintaining the integrity of DNA and apoptosis.

GAPDH is particularly susceptible to oxidative modification of reactive cysteine residues present in the active site (Cys -152). These modifications occur as a result of S-thiolation, S-nitrosylation or disulfide bonds that lead to aggregate formation. The consequence of modifications include changes the properties of the glycolytic enzyme, the translocation of the enzyme from the cytoplasm to the nucleus and initiation of apoptosis. Reversible modifications of Cys-152 may also advantageously affect on the cell by restoring redox balance in the cytosol. This state is due to the forwarding the glucose-6-phosphate from glycolytic pathway to the pentose-phosphate pathway, a major source of production of NADPH in the cytosol .

Redox state of Cys-152 present in the active site of GAPDH determines both the location and function of the enzyme in the cell. Particularly interesting issue is the role of GAPDH in neurodegenerative processes associated with age. In fact many studies have shown that oxidatively modified glyceraldehyde 3-phosphate dehydrogenase is involved in the pathogenesis of neurodegenerative diseases such as Alzheimer's, Parkinson's and Huntington's.

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23. PROLIFERATION, APOPTOSIS, INTRACELLULAR CALCIUM AND EXPRESSION OF VASOACTIVE FACTORS IN THE HUMAN ENDOTHELIAL MODEL OF MUCOPOLYSACCHARIDOSIS TYPE VI

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Mucopolysaccharidosis type VI (MPS VI, Maroteaux-Lamy syndrome) is an autosomal recessive lysosomal disorder determined by mutations in the arylosulfatase B (ARSB) gene. This leads to incomplete degradation and cellular accumulation of the glycosaminoglycans - dermatan sulfate (DS), predominantly in skeletomuscular and cardiovascular system. Endothelial cells are the key regulatory cells of the cardiovascular system and maintain vascular tonus through interactions with vascular smooth muscle cells. Pulmonary hypertension occurs in patients with MPS VI and is a marker of bad prognosis.

The aim of the study was to establish a human MPS VI cellular model of pulmonary artery endothelial cells (HPAECs) and evaluate how it effects proliferation, apoptosis, intracellular calcium levels and expression endothelial nitric oxide synthase (eNOS), natriuretic peptide type C (NPPC) and vascular endothelial growth factor A (VEGFA) - factors, that may trigger PH.

Results: Raising DS concentrations reduces viability of the cells both in case of ARSB deficiency and controls, however it hardly influences apoptosis. The intracellular calcium is reduced by 26.88% in case of ARSB deficiency and increases with elastin receptor stimulation. The MPS VI HPAECs show, in contrast to the controls, up to a 53% increase in intracellular calcium with increased DS levels. The expression of eNOS in HPAECs is reduced down by two thirds in the presence of DS. NPPC show a biphasic expression reaction with increase at 50 μg/mL DS and reduction at 0 and 100 μg/mL DS. The expression of VEGFA is reduced with rising DS concentrations and absence of elastin and goes up with rising DS in the presence of elastin.

Our data suggest that MPS VI endothelium is functionally abnormal and shows a modified expression of vasoactive factors.

24. METFORMIN AND RESVERATROL DO NOT SHOW CYTOPROTECTIVE ACTIVITY IN CARDIOMYOCYTES EXPOSED TO IONIZNG RADIATION AND/OR EPIRUBICIN

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With the development of cancer therapies the efficiency of treatment and survival time of patients after treatment improve. At the same time the frequency of long term adverse side effects increases, including toxicity to cardiovascular system (cardiotoxicity). Since almost every cancer therapy is associated with some risk of developing cardiotoxicity (risk ranges between 2% to even 30%) there is a need for a method of limiting or completely preventing such side effects. Use of cardioprotectants (compounds with protective activity on cardiovascular system) that will protect cardiovascular system from damage is an emerging concept in anticancer treatment. Available literature describes a number of compounds, the use of which would have a beneficial effect. In this study we analyzed the potential of two of them - resveratrol and metformin - to minimize cytotoxic effect of ionizing radiation and/or epirubicin on human cardiomyocytes.

Cultured *in vitro* human cardiomyocytes were treated with epirubicin and/or ionizing radiation. Simultaneously, the cells were treated with metformin or resveratrol. Survival of cells after different doses of both toxic and protective agents was assessed by MTT and clonogenic assay. Changes in cell cycle phases distribution, apoptosis (TUNEL assay, CD95 expression), and autophagy (LC3-II expression) intensity were measured as well.

Exposure of cardiomyocytes to epirubicin, either alone or in combination with ionizing radiation, had a negative effect on cell survival. Resveratrol and metformin alone had no significant effect on cardiac cells. When combined with epirubicin and/or ionizing radiation, resveratrol had no impact on cell cycle phases distribution, but it slightly reduced the number of TUNEL positive (apoptotic) cells. Metformin enhanced radiation and epirubicin toxicity, which was manifested primarily by a disruption in the distribution of cell cycle phases (accumulation of cells in G2/M and S phase). In conclusion, this study did not confirm unambiguously the potential of resveratrol or metformin to decrease the toxicity of ionizing radiation and epirubicin on cardiomyocytes cultured *in vitro.*

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25. A VALIDATED GENE REGULATORY NETWORK AND GWAS TO IDENTIFY EARLY TRANSCRIPTION FACTORS IN T-CELL ASSOCIATED DISEASES

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The identification of early regulators of disease is important for understanding disease mechanisms, as well as finding candidates for early diagnosis and treatment. Such regulators are difficult to identify because patients generally present when they are symptomatic, after early disease processes. Here, we present an analytical strategy to systematically identify early regulators by combining gene regulatory networks (GRNs) with GWAS. We hypothesized that early regulators of T-cell associated diseases could be found by defining upstream transcription factors (TFs) in T-cell differentiation. Time-series expression profiling identified upstream TFs of T-cell differentiation into Th1/Th2 subsets to be enriched for disease associated SNPs identified by GWAS. We constructed a Th1/Th2 GRN based on time-series expression, DNA methylation profiling, and sequence-based predictions, using the LASSO algorithm. The GRN was validated by ChIP-seq and siRNA knockdowns. GATA3, MAF and MYB were prioritized based on GWAS and the number of GRN predicted targets. The disease relevance was supported by differential mRNA expression of the three TFs and their targets in six T-cell associated diseases. We tested if the TFs or their splice variants changed early in disease by exon profiling of two relapsing diseases, namely multiple sclerosis and seasonal allergic rhinitis. This showed differential expression of splice variants of the TFs during relapse-free, asymptomatic stages. Potential targets of the splice variants were validated based on expression profiling and siRNA knockdowns. Those targets changed during symptomatic stages. Our results show that combining construction of GRNs with GWAS can be used to infer early regulators of disease.

26. 5-METHYLCYTOSINE, 5-HYDROXYMETHYLCYTOSINE AND 5 HYDROXYMETHYLURACIL IN HUMAN LEUKOCYTES AND SPERM AS EPIGENETIC MARKS - COMPARISON OF THE ABSOLUTE LEVEL

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5-Methylcytosine is one of the most important epigenetic modifications which has a profound impact on embryonic development. After gametes fusion there is a widespread and rapid active demethylation process of sperm DNA, which in turn suggests that the paternal epigenome plays an important role during embryonic development.

To better understand the epigenome of sperm DNA and its possible involvement in a developing embryo we determined epigenetic marks in human sperm DNA and in surrogate, somatic tissue - leukocytes; the analysed epigenetic modifications included 5-methyl-2'-deoxycytidine, 5-hydroxymethyl-2'-deoxycytidine, 5-hydroxymethyl-2'-deoxyuridine. For absolute determination of the modification we used LC/UV/MS/MS techniques with isotopically labeled internal standards.

Our analyses demonstrated, for the first time, that absolute global values of 5-methyl-2'-deoxycytidine, 5-hydroxymethyl-2'-deoxycytidine and 5-hydroxymethyl-2'-deoxyuridine in sperm are highly statistically different from those observed for leukocytes DNA with respective mean values 3.815 vs. 4.307%; 0.797 vs. 2.945 per 10^4 deoxynucleosides; 5.209 vs. 0.492 per 10^6 deoxynucleosides. We hypothesize that an exceptionally high value of 5-hydroxymethyluracil in sperm (more than 10-fold higher than in leukocytes) may play a not-yet-recognized regulatory role in the paternal genome.

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27. DUAL ROLE OF HIF-1 IN REGULATION OF THE *HSPA2* GENE IN NORMAL AND CANCER CELLS

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The *HSPA2* gene, is a member of the HSPA family of the so-called heat shock or stress genes. Based on the results of initial studies, it was thought that this gene is expressed specifically in spermatogenic cells. At present it is known that the *HSPA2* gene is also active in various cancer cells, as well as in some populations of somatic cells, mainly in epithelial ones. Neither the function of the HSPA2 protein nor the mechanism that regulate the corresponding gene expression in cancer and somatic cells are known.

The *in silico* analysis revealed that a regulatory region of the *HSPA2* gene contains Hypoxia Responsive Element (HRE), a *"cis"* regulatory element which might potentially bind the transcription factor HIF-1 (*Hypoxia Inducible Factor 1*). Consequently, our goal was to investigate the potential role of HIF-1 in regulation of the *HSPA2* gene expression in normal somatic and cancer cells.

By means of transient transfection assay, chromatin immunoprecipitation and analysis of the *HSPA2* gene expression under conditions facilitating stabilization of HIF-1 or inhibiting its transactivatory action, we found out that in normal keratinocytes the HIF-1 acts as a negative regulator of the *HSPA2* gene. However, our results also demonstrated that in cancer cells the HSPA2 gene expression can be induced along with increased HIF-1 activity. Overexpression of constitutively active mutant of HIF-1α in normal keratinocytes and cancer cells confirmed the opposite role of HIF-1 in regulation of the HSPA2 gene expression. While in normal cells HSPA2 is negatively regulated by HIF-1, in cancer cells this transcription factor acts as a positive regulator of this gene. To conclude, our results show for the first time the dual role of HIF-1 in regulation of the same gene in normal and corresponding cancer cells.

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28. STUDIES ON MEDIUM COMPOSITION IN ENZYMATIC GLYCOSYLATION REACTIONS

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Carbohydrates play a key role in various cellular recognition systems involving cell differentiation [1] inflammation [2], bacterial/viral infections, immune response [3], disease states such as cancer [4] and many other intercellular communication [5]. Sugar part modification of glycoconjugates on the surface of cells can have a significant impact on their biological properties and functions.

Non-conventional biocatalysis involves enzymatic reactions carried out in a medium other than buffer systems. By changing composition of the reaction mixture and influencing water activity, it is possible to conduct the same reaction obtaining a much higher yield.

This communication describes the use of ionic liquids as cosolvent in the glycosylation reaction catalysed by β-glucosidase from *Aspergillus niger K0440*. During the investigation we examined the influence of eight different ionic liquids on the final reaction efficiency, as measured by an HPLC system. We received rather unexpected results (increased reaction efficiency) three times.

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29. NEW FERROCENYL AND DICOBALT HEXACARBONYL CHROMONES - PROAPOPTOTIC PROPERTIES AND ARRESTING HUMAN CANCER CELLS (MCF-7 AND CCRF-CEM) IN G2/M PHASE

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Modern oncology has seen new trends for therapeutic use of metal complexes with anticancer properties. Ferrocene compounds (Fc) belong to the most commonly used organometallic derivatives. The interest in them is mainly due to their properties in biological media as well as strong redox properties.

The aim of the study was to evaluate antiproliferative and anticancer properties of new ferrocenyl and dicobalt hexacarbonyl chromones. Estrogen-dependent breast cancer (MCF-7) and acute lymphoblastic leukemia(CCRF-CEM) cell lines were used in the experiments. Proapoptotic properties of compounds were analyzed by fluorescence microscopy on the basis of morphology changes in the treated cells simultaneously stained with fluorescent dyes Hoechst 33258 and propidium iodide. The percentages of live, early-apoptotic, late-apoptotic and necrotic cells were estimated. Ferrocene impact on cell cycle was examined by flow cytometry and analysis of DNA histogram. DNA was stained with propidium iodide after permeabilization of the cell plasma membrane and digestion of RNA and protein with RNA-se and proteinase K, respectively.

Tested ferrocenyl derivatives induced both apoptosis and necrosis of tumor cells with prevalence of apoptosis. The percentage of apoptotic cells was dependent on the concentration of ferrocenes. Moreover, ferrocenyl compounds inhibited proliferation of cancer cells and caused G2/M block in theircell cycle.

30. THE ROLE OF ARE AND MRE BASED GENE REGULATION IN CELLULAR RESPONSE TO IONIZING RADIATION

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Regulation of gene expression in response to stress conditions allows activating a variety of mechanisms that either protect the cell or activate apoptosis in case of unrecoverable damages. The regulation on mRNA level can involve either changes in the yield of transcript production or its stability, triggering a very fast response to the current intracellular conditions. Transcript level stability involves two basic mechanisms which utilize either ARE or MRE classes of sequence motifs. ARE (AU-rich regions) are responsible for the recruitment of specific proteins which by attaching to the mRNA decrease or increase its stability, depending on the protein involved. MRE motifs are recognized by miRNA particles that either lead to mRNA degradation or inhibition of translation.

In order to assess the influence of both mechanisms on radiation induced cellular response we measured the transcript level changes in Me45, K562 and HCT116 cells, using microarrays, in various time intervals after 4Gy irradiation. Our results indicate that 12h after irradiation up-regulated transcripts show a significant increase in the occurrence of both MRE and ARE motifs, comparing to those down-regulated. An additional experiment in which we measured the expression levels of AUF1 and DICER1 genes, which serve a crucial role in ARE and MRE-based regulation respectively, lead to a hypothesis that radiation influences at least one of those mechanisms.

In order to verify this hypothesis we performed additional experiments using plasmids that code for two luciferase genes in one of which we modified the 3'-UTR by adding specific ARE or MRE regulatory motifs. This experimental setup allows to reduce the influence of other factors by controlling the structure of the regulatory region and allowing to focus only on a specific mechanism. By using four distinct plasmids with various regulatory elements we have shown that in all cases 12h after irradiation the protein level of genes with additional regulatory elements increases, compering to the unmodified control. This observation is consistent with our previous results, indicating that those regulatory mechanisms can be impaired, which leads to a reduced degradation level. In order to assess how radiation influences the regulatory mechanisms we focused on both changes of ARE-recognizing proteins and processes that affect the miRNA levels in the cell. This allowed us to show that one of the crucial steps in cellular response to radiation may be the inhibition of pre-miRNA processing and ARE based mRNA stability regulation.

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31. DOWN-REGULATION OF GENE EXPRESSION OF WNT/PCP SIGNALING TRANSMITTERS IN ENDOMETRIAL CANCER CLINICAL SAMPLES

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Yearly morbidity due to endometrial cancer is approximately 142 000 worldwide. The disease is fatal for 42 000 patients. Pathogenesis of endometrial cancer has been related to estrogen up-regulation, which probably is caused by disruption of the canonical Wnt/B-catenin signaling pathway. However, Wnt molecules have not only the potential to activate the canonical pathway. Non-canonical Wnt signaling has even wider influence on the cells signaling pathways. Signal transduction via Wnt/PCP activates the MAPK pathway. Specifically it has influence on c-jun and c-fos transcription factors. These have the ability to stimulate apoptosis in normal cells. The down regulation of MAPK signaling has its outcome in cancer survival and progression.

The aim of this study was to estimate gene expression of all critical for MAPK and Wnt/PCP transmitters.

Endometrial cancer and normal endometrial samples were obtained from patients after total hysterectomy. The control samples were from patients which surgery was performed for other reasons than endometrial cancer. All samples were histopathologically analyzed and grouped according to Figo. Total mRNA was extracted from the samples and purified. Afterwards Microarray HGU-133A (affymetix) has been performed. The obtained results were statistically evaluated with the use of GeneSpring 13.0 and ANOVA with post hoc Tukey was employed.

From the microarray analysis 22 283 probe signals were obtained. Based on the Affymetrix database 2792 had been chosen which latter on were analyzed with ANOVA with post hoc Tukey. There were only 232 ID mRNA statistically significant at $p<0.05$. From those ID mRNA had been obtained that is biologically significant in the Wnt/PCP and MAPK signaling pathways. Amongst the respective genes were JUN, MAPK8, MAPK10 Wnt5a and Wnt4.

The Wnt/PCP and MAPK signaling pathway main transmitters are downregulated in endometrial cancer. This can suggest that the pathway in endometrial cancer has been inhibited by cancer changes.

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32. CROSSTALK BETWEEN HYPERTHERMIA-ACTIVATED HSF1 AND EXPRESSION OF NF-κB-REGULATED GENES

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As the result of stress conditions, different signal transduction pathways are activated in cell that determine the mechanisms responsible for adaptation and survival. Among them are two major pathways, regulated and executed by NFκB and HSF1 transcription factors, which are critical for growth, development and response to the treatment of cancer and other human diseases. Both signaling pathways can interfere with each other, but mechanism of this regulation is not clear yet. Here we aimed to identify NFκB-regulated genes, the expression of which is affected by HSF1, in a positive or negative way.

Expression of NFκB-dependent genes was analyzed in U2OS human osteosarcoma. These cells, either control and preconditioned with hyperthermia (HS) to activate endogenous HSF1, were stimulated with TNFα cytokine and the expression of TNFα-induced genes was analyzed by the expression microarrays. 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). Bioinformatics analysis was also made for prediction of hypothetical κB and HSE motifs in promoter regions of the analyzed genes.

We observed that 324 genes changed their expression upon stimulation with TNFα; 191 genes were up-regulated while 133 genes were down-regulated compared to untreated control. Hypothetical κB motifs were found in proximal promoters of 114 of these genes (this group putatively represents a set of genes regulated by NFκB). We found that expression of 187 of TNFα-modulated genes was affected by the hyperthermia pre-treatment. Two modes of co-effects were observed: synergistic/additive (strengthen of TNFα effect) and antagonistic (reduction of TNFα effect). In general, among genes co-affected by TNFα and HS, the κB motif was present in proximal promoters of 74 genes, while the HSE motif and/or actual HSF1 binding was observed in regulatory regions of 52 genes. We identified 27 co-affected genes with binding sites (either hypothetical or actual) for both transcription factors. Among them there were 10 genes, for which observed effect of HS on their TNFα-induced expression was synergistic/additive (eg *EGR1, FOSB, RRAD*) and 17 genes with antagonistic effect (eg. *CCL2, CCL20, CD83, IL8, TNF*).

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33. IONIZING RADIATION AFFECTS PROTEIN COMPOSITION OF EXOSOMES SECRETED IN VITRO FROM HEAD AND NECK SQUAMOUS CELL CARCINOMA

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Exosomes are membrane vesicles of endocytic origin that participate in inter-cellular communication. Environmental and physiological conditions affect composition of secreted exosomes, their abundance and potential influence on recipient cells.

Here we analyzed protein component of exosomes released *in vitro* from cells exposed to ionizing radiation (2Gy dose) and compared their content with composition of exosomes released from control not irradiated cells. Exosomes secreted from FaDu cells originating from human squamous head and neck cell carcinoma were analyzed using LC-MS/MS approach.

We have found that exposure to ionizing radiation resulted in gross changes in exosomal cargo. There were 217 proteins identified in exosomes from control cells and 383 proteins identified in exosomes from irradiated cells, including 147 "common" proteins, 236 proteins detected specifically after irradiation and 70 proteins not detected after irradiation. Among proteins specifically overrepresented in exosomes from irradiated cells were those involved in transcription, translation, protein turnover, cell division and cell signaling.

Our results indicated that exosomal cargo reflected radiation-induced changes in cellular processes like transient suppression of transcription and translation or stress-induced signaling.

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34. PROTEOMIC PATTERNS OF SQUAMOUS CARCINOMA

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Most head and neck cancers are squamous cell carcinoma (SCC). The anatomical features of SCC have been histochemically evaluated with hematoxylin and eosin. However, the border between the cancer and stromal regions is unclear and large portions of the cancer and stromal regions are resected in surgery. To reduce the resected area new methods of diagnosis are needed.

In our study we made an attempt to clearly distinguish the border on the basis of proteins distribution visualised by IMS (Imaging Mass Spectrometry). Eight cases of SCC resected with margin and normal epithelium were analyzed in mass range: 800-4000 m/z for tryptic peptides with 100 μm raster. The results of MALDI-IMS data were compared with HE staining, followed by selection of peptides which were specific for normal and cancerous tissues. In the IMS dataset signals were significantly different in intensity ($p<0.05$) between cancer and tumour surrounded tissues for proteins. We found signals characteristic for tumour tissue (e.g. 1167 m/z, 2050 m/z, 2191 m/z, 2165 m/z), and epithelial cells (e.g. 1392.86 m/z, 965.41 m/z, 879.61 m/z).

In conclusion, the cancer and tumour surrounded tissues of SCC were clearly distinguished with the use of these features and IMS analysis, and this molecular identification can provide important information to elucidate the mechanism of cancer invasion.

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35. INTERACTIONS BETWEEN HSF AND NF-κB PATHWAYS

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Inhibition of NF-κB pathway is known to promote apoptosis and therefore may constitute one of the goals in anticancer therapies. Experimental results show that heat shock induces such inhibition in cancer cells. However, the mechanisms of interactions between heat shock and NF-κB pathways are not fully understood yet. Development of a combined mathematical model of these pathways and its subsequent computational analysis should help to uncover these mechanisms and determine the time window in which heat shock treatment preceding chemotherapy would be the most efficient.

The model proposed in our work was built on the previously published ones, which described either NF-κB (Lipniacki *et al*., 2004) or HSF (Szymanska & Zylicz, 2009) pathways separately. In order to incorporate crosstalk beetween HSF and NF-κB pathways, they had to be modified to accommodate the following assumptions: (1) nuclear and cytoplasmic levels of proteins and complexes had to be separated; (2) constitutive and inducible HSPs were described by separate variables; (3) additional interactions between these two pathways have been added in order to connect both models.

The interactions between the HSF and NF-κB pathways take into account creation HSP:IKK complexes and temperature-dependent inactivation of proteins located upstream of IKK activation. Both of these interactions lead to inhibition of IKK activity and prevent the p65 phosphorylation.

Activation of HSR and HSF1-dependent signaling after hyperthermia as well as activation of classical NF-κB-dependent signaling after TNFα cytokine stimulation was analyzed experimentally in different cell lines (A549, U2-OS, GMO7492). As expected, we found that heat shock resulted in a general blockade in the p65 phosphorylation and nuclear translocation of p65 and expression of NF-κB-dependent target genes. The kinetics of activation of NF-κB was studied comprehensively using human fibroblasts cells. Interestingly, hyperthermia blocked activation of NF-κB when cells were stimulated with TNFα directly after hyperthermia or after 1 hour recovery.

The influence of hyperthermia on activation of NF-κB pathway was analyzed also at the single cell level using live-cell microscopy. The inhibition of nuclear translocation of activated NF-κB (p65/RelA-EGFP fusion protein) in A549 and U2OS cells was also observed, yet heterogeneity in kinetics of corresponding response could be observed.

Based on the simulations we showed that the proposed model reflects the dynamics of NF-κB response suppression for some time after the heat shock, which we saw also in the experimental data.

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36. EFFECT OF CLOFARABINE ON DNMT1 METHYLATION AND EXPRESSION IN K562 CELLS

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The DNMT methyltransferase 1 (DNMT1) plays a significant role in maintaining DNA methylation status and regulating expression of tumor suppressor genes. Inhibition of DNMT1 activity could reduce hypermethylation of silenced genes and promote the re-expression. Thus inhibition of transcription and translation of DNMT1 is one of possible strategies for cancer therapy.

The aim of presented research was to investigate the relationship between DNMT1 and abnormal methylation of tumor suppressor genes in human leukaemic cell line K562 (representing chronic myeloid leukaemia) - also after cells' treatment with clofarabine (2'-deoxyadenosine analogue). In all experiments K562 cells were treated for 96 hours with clofarabine at 8 to 20 nM concentrations. The *DNMT1* promoter methylation was estimated with PCR-based methylation sensitive restriction analysis. The level of *DNMT1* mRNA was evaluated with real-time qPCR assays, and the DNMT1 protein expression was measured with ELISA-like immunoassay (EpiQuik™ DNMT1 Assay Kit, Epigentek). Additionally, the total DNMTs activity was measured with ELISA-like immunoassay kit (EpiQuik™ DNMT Activity/Inhibition Assay Ultra Kit, Epigentek).

After clofarabine treatment the methylation status of *DNMT1* promoter decreased slightly (less than 10 %). The expression level of DNMT1 was decreased significantly compared with control cells, and we observed almost 30 % reduction of DNMT1 mRNA and about 20 % reduction of DNMT1 protein level in K562 cells treated with the highest drug concentration (20 nM). However, we did not notice significant changes in the total DNMTs activity.

In our previous work we demonstrated that decreased expression of DNMT1 mRNA caused promoter demethylation of a few suppressor genes (*PTEN*, *APC*, *RARβ2*) and increase in mRNA expression of these genes. A methylation status of *DNMT1* promoter can influence transcriptional activity of some tumour suppressor genes, but it is only one of possible ways of regulation.

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37. EFFECT OF RANIBIZUMAB ON EXPRESSION PROFILE OF GENES ASSOCIATED WITH PROLIFERATION IN HUMAN RETINAL PIGMENT EPITHELIAL CELLS

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Retinal pigment epithelial cells (RPE) play an important role in many normal eye biological processes. RPE cells can often undergo reactive hyperplasia due to trauma, inflammation, and other ocular injuries. In turn, RPE damage or loss invariably results in retinal degeneration and diseases such as age-related macular degeneration (AMD). Furthermore, vascular endothelial growth factor (VEGF) is a key factor in the pathogenesis of AMD. Currently, the most widely used treatments for the neovascular exudative form of AMD include intravitreal injections of anti-VEGF agents, such as ranibizumab (Lucentis). However, the influence of ranibizumab on RPE cells at molecular level remains still poorly understood. Therefore, the present study aims at the identification of differences in expression pattern of genes associated with proliferation in human retinal pigment epithelial cells in response to ranibizumab treatment compared to the control cells.
RPE cells were exposed to ranibizumab (0.125mg/ml) for 6 hours. Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA). The analysis of the expression profile of genes involved in proliferation was performed using oligonucleotide microarrays of HG-U133A 2.0 (Affymetrix, Santa Clara, CA). Appointment of differentiating genes was performed with the use of GeneSpring 12.0 and Pl-Grid platforms.
Typing of differentially expressed genes was performed in a panel of 2734 transcripts of genes encoding proteins involved in cell proliferation. The changed expression of 399 transcripts after ranibizumab treatment of RPE cells was identified (unpaired t-test, $p < 0.05$) by a cutoff of at least 1.1-fold change. Among these transcripts, 159 were up-regulated, 240 were down-regulated and four were found to be regulated by more than 1.5-fold.
The expression changes in genes associated with cell proliferation may lead to further insights into the molecular mechanism of ranibizumab action on RPE cells.

This study was supported by the grant KNW-1-007/K/4/0

38. RKI-123, NOVEL GENISTEIN DERIVATIVE, EXHIBITS ITS PROAPOPTOTIC ACTIVITY THROUGH ACTIVATION OF C-JUN AND P-38 KINASES

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Natural products and their derivatives play important roles in cancer treatment. Several novel experimental anticancer therapeutics are derived from small molecules found in nature. Among hundreds of natural-derived scaffolds, isoflavones are of particular interest. Genistein, a member of isoflavone family, possess broad spectrum of anticancer activity, although due to unfavourable properties its clinical application is rather doubtful. Therefore several groups including ours, made attempts to obtain derivatives of genistein with enhanced potency against cancer cells.

In our drug discovery programme we have obtained several novel genistein glycoconjugates with enhanced potency in decreasing viablilty of HCT-116 cancer cells. Among them, RKI-123 showed the strongest cytostatic and cytotoxic activity . This derivative was able to arrest HCT-116 cells in G1 phase at 10 uM and caused apoptosis at 20 uM which was confirmed by FACS analysis. At this concentrations parent compound had no effect on HCT-116 cells. To get some insights into molecular mechanism of action of RKI-123 we used comercially avaliable Proteome Profiler Human Phosphokinase array kit. Results clealry show that apoptotic activity of RKI-123 is connected with activation of c-Jun and p38 kinases.

In conclusion, we proved that proper modification of genistein scaffold led to derivatives with increased potency and novel molecular mechanisms of action compared to 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

39. RKI-123, NOVEL GENISTEIN DERIVATIVE, SENSITIZES HT-29 CELL LINE TO TRAIL

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Tumor necrosis factor (TNF)-related apoptosis inducing ligand (TRAIL), due to its ability to trigger apoptotic cell death of tumor cells in a selective manner, is an attractive candidate for anticancer drug. However, many tumors, including colon cancers, were found to show resistance to TRAIL-induced apoptosis.

In our drug discovery programme we have identified a small molecule compound, shortly named RKI-123, that was previously found to induce apoptosis in HCT-116 cell line. Evaluation of its activity in HT-29 showed that up to 20 uM concentrations RKI-123 had growth inhibitory activity but no death-inducing effects. By contrast, pretreatment of HT-29 cells with RKI-123 for 48 h and then rhTRAIL for 24 h, substantially increased TRAIL-induced cell death. Increased content of sub-G1 population was detected in FACS analysis. Interestingly, pretraetment of HT-29 cells with genistein, a compound from which RKI-123 is derived, did not show sensitizing effects to TRAIL.

Taken together, we have shown that RKI-123 could represent a novel strategy for overcoming TRAIL resistance and combined treatment with RKI-123 and rhTRAIL ma be an effective strategy for human colon cancer therapy.

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40. THE EXPRESSION AND FUNCTION OF HSPA2 IN HUMAN EPIDERMAL KERATINOCYTES

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HSPA2 was originally described as testis-specific member of the HSPA (HSP70) heat shock protein family, which is crucial for spermatogenesis. Recently we have shown that HSPA2 is also synthesized in human normal somatic tissues in cell- and tissue-type specific manner. High level of HSPA2 was found in epidermis and other stratified epithelia.

This study was designed to characterize the expression and function of HSPA2 in human epidermis. In order to identify the type of epidermal keratinocytes subpopulation in which HSPA2 is highly expressed we performed immunofluorescence analysis of human skin and immunophenotypization of primary normal human epidermal keratinocytes (NHEK), followed by fluorescence-activated cell sorting and analysis of HSPA2 as well as differentiation markers expression levels in selected subpopulation of cells. For functional analysis of HSPA2 we selected HaCaT cells, an immortal human epidermal keratinocyte line. In these cells HSPA2 expression was stably silenced by virally delivered shRNAs. The potential impact of HSPA2 on HaCaT cells phenotype was evaluated by proliferation, clonogenic, migration and adhesion assays.

We found that undifferentiated keratinocytes (expressing cytokeratines CK5 and CK14), present in basal layer of epidermis, are highly positive for HSPA2 expression. Immunophenotypisation of NHEK cells (performed directly after isolation from the skin), revealed that HSPA2 is not expressed in subpopulation enriched for stem cells. These results suggest that HSPA2 expression is activated along with initiation of keratinocytes' differentiation, thus potential functions of HSPA2 could be exerted in epidermal primitive basal keratinocytes but not in epidermal stem cells.

In order to uncover the function of HSPA2 in keratinocytes we established stable HaCaT cell lines characterized by decreased expression of the corresponding gene. We found that HSPA2 silencing did not affect neither the proliferation nor migration of HaCaT cells. However, differences in cells clonogenic as well as adhesion potential, which correlated with degree of the *HSPA2* gene silencing level, were detected. In summary, we hypothesize that HSPA2 may participate in control of keratinocytes differentiation by regulation of keratinocytes release from junction with basal layer, setting them on the path of terminal differentiation.

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41. MRI IMAGES DISTORTION STUDIES BASED ON RIGID REGISTRATION OF MULTI-MODAL IMAGES

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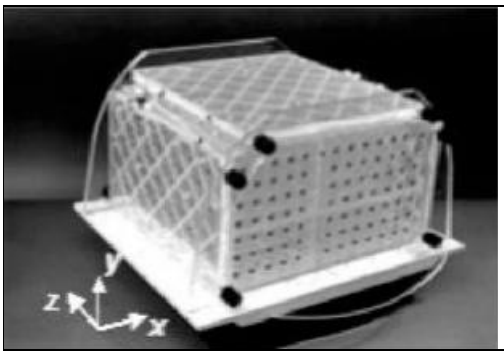
Medical imaging techniques are commonly used to recognize structural anomalies, tumors or as a device for i.e. planning radiotherapy treatment. One of the basic techniques is magnetic resonance imaging (MRI). However, MRI images may not be accurate geometrically in the presence of susceptibility gradients (i.e. metal implant or air tissue boundaries). This is especially problematic in the context of radiotherapy planning, where superior soft tissue contrast might be used for target delineation.
The purpose of the study was to implement an algorithm for a rigid registration, which would be able to find the correct match of two multimodal images: undistorted CT and MRI with possible local distortion. Affine transformations were implemented to account for translations and rotations between MRI and CT data sets and Mutual Information [1] was used as a quality measure.
The method was tested in two different phantom objects with well defined geometrical structures (LEGO phantom and Linear Test Object - LTO [2,3], with dimensions 440x270x360 mm, Figures 1 and 2). Images were acquired with Philips 3T MRI scanner and GE CT.



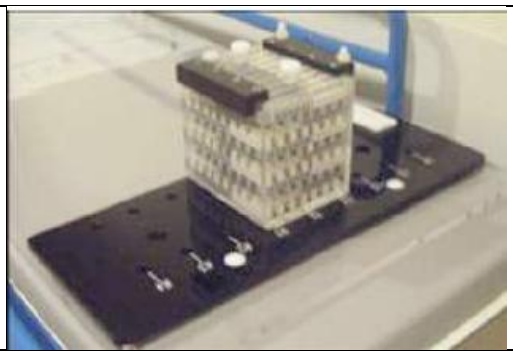


Fig.1 Linearity test object (LTO) developed at the Fig.2 LEGO test object developed at the Institute for Cancer Research.

As a result we have obtained rigid fusion of MRI and CT phantom images that allows us to visualize the level of distortions in each image region. Performed tests shows that some distortions are present especially in LTO object in distant to center locations. This results will be the basis for the method of distortion measurement and correction in 3D MRI image sets. In our future work we are planning to use non-rigid registration algorithms to obtain mentioned effects.

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42. SYNTHESIS AND BIOLOGICAL ACTIVITY OF URIDINE AND ARYL 1-THIOGLYCOSIDE CONJUGATES

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Glycosyltransferases (GTs) constitute a large group of enzymes that are involved in the biosynthesis of oligosaccharides, polysaccharides, glycolipids and glycoproteins. These molecules of fascinating diversity mediate a wide range of functions, from structure and storage to specific signaling. The GTs transfer sugar residues from an activated donor substrate, usually a nucleotide sugar, to an acceptor that may be a lipid, a protein or a growing saccharide [1]. In eukaryotes, most of GTs are resident membrane proteins of the endoplasmic reticulum and the Golgi apparatus. These enzymes received the most attention because they are responsible for the synthesis of complex glycans that play major role in recognition or siganalling events, cell adhesion, cell differentiation, glycoprotein folding, targeting organelles and bacterial/viral infections [2]. Selective inhibition and controlling the activity of the enzymes responsible for the synthesis of cell-surface oligosaccharides is thus of interest as it may lead to the development of new therapeutic agents. Analogues of NDP-sugar (sugar donor analogues) can have an important role as new drugs. Designing of these compounds is generally based on the modification of one of three structural part: carbohydrate part, the diphosphate linkage or the nucleoside moiety.

Herein, we present a synthesis of a wide range of uridine glycoconjugates in which 1-thioglycosides derivatives of D-glucose and D-galactose are connected to uridine through the amide bond. Synthesized compounds have been subjected to the biological evaluations of their inhibitory activity against commercially available β-1,4-galactosyltransferase from bovine milk. This method involves observation of product formation by transfer D-galactose unit from UDP-galactose to an acceptor – esculine, and compared to reactions carried out under the same conditions with the addition of glycoconjugates as competitive inhibitors to UDP-galactose [3]. To accurately determine the changes in concentrations of substrate and product, RP-HPLC method was used. Additionally, the anticancer activity of selected glycoconjugates was determined using MTT assay. Cytotoxicity tests were performed using two cell lines: HCT 116 and DU 145.

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43. POSITIONING OF CELLS WITH RESPECT TO BEAM FIELD AFFECTS THEIR BIOLOGICAL RESPONSE

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Purpose: RT treatment sessions are planned based on the assumption that the effects of radiation on cells are target-oriented and occur only in cells lying in the beam field. Recently it has been shown that there are other non-targeted mechanisms which may affect cells that are not directly exposed to radiation. These non-targeted effects, such as genomic instability, bystander effect, hyperradiosensitivity and adaptive response are more complex than those assumed by the LNT model and can be important in case of cells lying outside the radiation field and which were exposed to low dose of radiation.

In the present study we tested cytotoxic and genotoxic effects of photon radiation upon cells placed inside a water phantom in the beam axis or outside the radiation field during exposure. We also carried out cell cycle analysis of such cells.

Materials/Methods: Measurements were performed on 5.4 cm depth in a water phantom. As the irradiation source, a linear accelerator was used (Clinac 2300 CD) and 6 MV photon radiation was applied at 100 MU/min accelerator mode, field size 20x20 cm. On this depth, the dose of 5 Gy is in the beam axis, and ca. 0.2 Gy is outside of irradiated field.

Human lung cancer cells A549 were exposed to radiation, both in the beam field or outside of it. The cytotoxic effect of irradiation was evaluated as cell viability (MTS assay); genotoxic effects was determined as induction of apoptosis measured by microscopic observation and annexin V-FITC flow-cytometry analysis; cell cycle was also analyzed by flow-cytometry.

Results: Our measurements revealed that cells placed within the beam field show decreased viability and increased frequency of apoptosis and micronuclei as compared to non-irradiated control cells. Surprisingly, compared to non-irradiated cells, the cells placed during exposure outside the beam field show decreased viability and higher level of apoptosis. Cells irradiated in the beam field showed inhibition of cell cycle in G2 phase, whereas in outlying cells no cell cycle-related changes were observed in comparison with control.

Conclusions: This finding suggests that healthy cells lying outside the beam field may be killed as a result of irradiation during RT. This relationship observed for therapeutic radiation ought be taken into consideration in both treatment planning and in clinical practice.

44 PRO-DEATH SIGNALLING DURING HYPERTHERMIA: UP-REGULATION OF THE PROAPOPTOTIC MEDIATOR PMAIP1/NOXA BY HEAT SHOCK FACTOR 1

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HSF1 is the primary transcription factor activated by different forms of cellular stress. In somatic cells activation of HSF1 leads to accumulation of heat shock proteins (HSPs) and cytoprotection. On the contrary, in heat shocked spermatocytes, which are extremely heat-sensitive, activation of HSF1 induces caspase-3-dependent apoptosis. We assume that these two opposed processes can be directly regulated by HSF1.

To elucidate mechanism of such diverse HSF1 activity we carried out genome-wide transcriptional analysis combined with global mapping of HSF1 binding sites by ChIP-chip or ChIP-Seq. We searched for HSF1-dependent genes differentially regulated after temperature elevation in heat-sensitive (spermatocytes) and heat-resistant (hepatocytes) cells. Results of our study show that HSF1 is activated and binds to promoters of *Hsp* genes in both types of cells, but *Hsps* expression is blocked in heat shocked spermatocytes. However, in spermatocytes we found binding of HSF1 to the introns of the proapoptotic, p53-dependent *Pmaip1/Noxa* gene, what was next confirmed by ChIP-PCR. Moreover, we detected up-regulation of the *Pmaip1* transcription at physiological temperature in testes of transgenic mice with constitutively active form of HSF1. Evident up-regulation of the *Pmaip1* transcription was also found in isolated spermatocytes and whole testes from wild type animals subjected to hyperthermia. Additionally, we analyzed expression of *Pmaip1* after temperature elevation in several mouse organs (stomach, small intestine, kidney, colon, liver) characterized by different heat sensitivity, and in a panel of mouse and human somatic cell lines i.e. mouse HECa10 (from lymph nodes), Renca (renal carcinoma), and C26 (colon adenocarcinoma), and human 1205Lu (melanoma) and MCF7 (breast cancer). Looking for HSF1 binding (by ChIP-PCR) we found it only in cell lines activating the *Pmaip1*/*PMAIP1* transcription after hyperthermia. We noticed also that hyperthermia-induced HSF1-mediated activation of *Pmaip1*/*PMAIP1* is associated with higher sensitivity of cells to elevated temperature.

To the best of our knowledge we present here the first evidence that the binding of HSF1 is associated with increased expression of a strictly proapoptotic gene. Thus, HSF1 may play a dual role in response to heat shock, either cytoprotective or lethal. We hypothesize that increased transcription mediated by binding of HSF1 to the *PMAIP1* gene contributes to differential heat/stress sensitivity of cells. HSF1-mediated activation of *PMAIP1* in combination with differential expression of cytoprotective HSPs, could be the main molecular mechanism involved in switching from pro-survival to pro-death signaling in cells subjected to elevated temperatures.

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45. ALTERNATIVE SPLICING OF THE MOUSE *MDM1* GENE INSIDE EXONS

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The mouse *Mdm1* (transformed mouse 3T3 cell double minute 1), as well as *Mdm2* (transformed mouse 3T3 cell double minute 2), were first described as genes amplified 25-30-fold in transformed mouse 3T3 cells. Amplified sequences originated from mouse chromosome 10 and formed numerous, small, acentromeric chromatin bodies. Multiple transcripts are generated from the *Mdm1* gene through the mechanisms of alternative splicing and/or polyadenylation signal choice. By Northern blotting three different length transcripts were previously detected: 1.4, 2.4 and 3.1 kb. The shortest *Mdm1* transcript consists of four exons, the longest - 15 exons (it differs only by short, 30bp, additional exon located between previously identified exons 7 and 8). There is also a variant of *Mdm1* transcript which lacks the exon 4. Our results suggest that some new *Mdm1* splicing variants could exist.

We amplified the *Mdm1* coding sequence by PCR on cDNA template prepared from mouse testes. PCR products were purified and cloned in vector from PCR Cloning Kit. After restriction analysis, selected clones were sequenced. Beside the full length transcript variants we found eight variants with deletions ranging from 111 bp to 1586 bp. Interestingly, deletions occurred in exons (between exons: 3 and 12; 6 and 14; 8 and 9, 10, or 12; 9 and 12). In most cases an open reading frame (ORF) was shifted after deletion. In one case (containing deletion of the last 96 bp of exon 8 and first 15 bp of exon 9, and the whole exon 12 deleted) ORF was conserved. Additionally, among EST sequences representing *Mdm1* gene we found the variant lacking 33 nucleotides from the beginning of the exon 5, the variant lacking exon 2, and two clones containing intronic sequences (http://www.ncbi.nlm.nih.gov/UniGene/clust.cgi?ORG=Mm&CID=101191&MAXEST=120, GenBank entry: CA318831.1).

The obtained results suggest that unusual alternative splicing inside exons of the *Mdm1* gene could exist. Alternatively, deletions in the transcripts could be generated at the reverse transcription step. This possibility has to be checked in further studies.

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46. ANTIBIOTIC RESISTANCE GENES IN MULTIDRUG-RESISTANT *ESCHERICHIA COLI* ISOLATED FROM WASTEWATERS AND MARINE COASTAL WATERS

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A safe economical and reliable way of wastewater disposal is a principal problem for ecosystems which are highly anthropogenically-impacted, like the Gulf of Gdansk and its shallow western branch - Puck Bay. Due to the limited water exchange between these water bodies and open Baltic Sea, the introduced pollutants remain there for a long time. Nowadays, antibiotic resistant bacteria and antibiotic resistance genes are considered as novel emerging pollutants in marine environments.

Thus, the aim of this study was to investigate antibiotic resistance profiles and antibiotic resistance genes in *Escherichia coli* isolated from wastewater treatment plant (WWTP) effluents and marine waters (Gulf of Gdańsk, Baltic Sea). Bacteria were isolated according to the procedure dedicated for fecal coliforms, from two local wastewater treatment plants and their receiving waters: Gulf of Gdańsk (Baltic Sea). All isolates were biochemically identified and their drug susceptibility was determined. Among 445 tested isolates 36% (n=161) were resistant to at least one antibiotic. Those isolates were tested for prevalence of integrons and antibiotic resistance genes using PCR. Among resistant strains 26% were multidrug-resistant (MDR) (resistant to 3 classes of antibiotics). In 55 isolates (31 %) we detected integrons (class 1 in 49 isolates, class 2 in 6 isolates, class 2 integrons were detected only in multidrug-resistant isolates). Isolates with resistance to β-lactams were the most frequent (n=134, 83% of all resistant isolates). Among them, the bla_{TEM} gene was dominant (found in 78% of isolates resistant to β-lactams). Other genes coding β-lactamases were found only occasionally (bla_{OXA} in 7 isolates, bla_{SHV} in 5 isolates and $bla_{\mathrm{CTX\text{-}M}}$ in 2 isolates). Among isolates resistant to sulphonamides (n=50) *sul*2 gene was predominant (found in 42 isolates), followed by *sul*1 gene (in 29 isolates). *sul*3 gene was found in two isolates from wastewater samples. In tetracycline-resistant isolates (n=87) we found mostly *tet*A and *tet*B genes (in 51 and 35 isolates, respectively), *tet*D and *tet*G genes were found only in two isolates originated from marine outfall of WWTP Gdynia-Debogorze. In isolates resistant to quinolones (ciprofloxacin and levofloxacin) we tested only the presence of plasmid-mediated quinolone resistance genes (PMQR genes). We detected *qnr*S, *qnr*A, *qnr*B, *qep*A and *aac*(6')-Ib genes. In aminoglicoside-resistant strains (n=10) *str*AB and *aac*(3)II genes were the most frequent.

The data obtained in this study indicate that, in general, applied wastewater treatment level together with effective dilution of treated wastewater by marine outfalls were sufficient to protect coastal water quality from sanitary degradation (low number of *E. coli* detected in marine waters). Detailed analyses showed, however, that bacteria carrying integrons and antibiotic resistance genes of clinical significance can survive in wastewater and marine water conditions. These findings highlight the need for further studies to understand the dissemination, stability and transmission of resistance genes in water ecosystems.

47. HAIR REMOVING TECHNIQUE AS A INITIAL STEP IN DERMATOSCOPY IMAGES ANALYSIS

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Background: Image processing is an important part of dermatoscopic diagnosis of malignant melanoma. It allows to quickly diagnose the changes in naevus, is non-invasive and allows to determine the probability of melanoma occurrence. Dermatoscopy images are acquired using a dermatoscope and contain, besides the region of interest, also hairs. Preprocessing is the initial step toward more advanced analysis for example to discretize images of malignant skin lesions and benign changes based on features calculated from region of interest, for example texture parameters that will indicate porosity or directionality. To accurately calculate those features hairs registered in the image have to be removed and then region of interest can be found.
Methods: The proposed algorithm includes a transformation from color image to gray scale, sharpening the image by stretching the histogram, finding the edge of dark and light hair (using Canny edge detector), using mathematical morphology methods to filter inappropriate objects and finally hair removal. After processing the image, the next step is to analyze the structure of the image. The algorithm was tested on dermatoscopic images supplied from PH^2 image database [1].
Results: In most cases, the algorithm removes hairs from the image without disrupting the structure of the lesion and allows us to create contour of the lesion without unnecessary (hair) objects in region of interest. Analysis of the texture will be performed by means of entropy, standard deviation, gray scale image, the average value of brightness, skewness of the histogram and energy level and will be the subject of further analysis.

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48. FRACTAL DESCRIPTION OF THE FRACTURE SURFACE OF POLYMERIC MATERIALS

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Since Mandelbrot's publications [1-2], fractal geometry has been extensively applied to characterize roughness of fracture surfaces and correlate it with mechanical properties [3].

The concept of fractals was used in the analysis of the fracture surfaces of two different polymeric materials. Polymer networks obtained from three popular dental dimethacrylate monomers: Bis-GMA, TEGDMA and UDMA as well as two copolymers of these monomers were analysed [4]. Dense polymer membranes with dispersed magnetic powder (magnetic membranes) for air separation were also investigated [5]. In both cases, profiles of fractures were described by a modified fractal dimension. It is based on scaling the length of a profile with the size of a measuring step. The modified fractal dimension D_β is considered a diagnostic tool for structure-morphology analysis of fracture path and roughness parameter.

The investigated polymer networks were found to possess the fractal characteristics. Hardness of these materials had a correlation with D_β and increased with the increase of D_β value. This can be attributed to the change in the fracture mode depending on the mass distribution. Moreover, it can be observed even in one dimensional analysis. However, the role of fracture morphology and fractal parameters and their relations with the material properties are still not well understood and this issue is worth further research [6].

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49. MOLECULAR SIGNALS OF BYSTANDER EFFECT INDUCED BY UVB RADIATION IN HUMAN MALIGNANT MELANOMA CELLS

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Bystander effect is a phenomenon in which unirradiated cells change their behavior due to operation of molecular signals released by directly irradiated cells. Neighboring stressed cells show decreased apoptosis, reduced survival, elevation of frequency of mutation, chromosomal aberrations, micronuclei formation and single and double-strand breaks. Many publications focused on the bystander effect in the case of ionizing radiation, while little is published about bystander effect after ultraviolet irradiation. UV radiation is divided into three ranges: UVA (320-400nm), UVB (280-320 nm) and UVC (200-280). Whereas UVC is blocked by the stratospheric ozone layer, UVA and UVB radiation reaches the earth's surface, thus it is reasonable to study the bystander effect at these UV spectrum ranges.

The aim of this project was to study the response of malignant melanoma cells (Me45) and normal human dermal fibroblast (NHDF) to direct UVB action and to putative bystander signals. The transwell co-incubation system was used, where cells to be irradiated (10 kJ/m^2 UVB) were seeded in wells, and bystander cells were seeded in inserts. Three experimental sets were used: irradiated Me45 incubated alone, irradiated Me45 incubated with non-irradiated NHDF and irradiated Me45 incubated with non- irradiated cells the same line. The reactive oxygen species, nitric oxide and superoxide radicals were measured by flow cytometry as a potential mediators of bystander effect. Viability was measured by MTS assay and micronucleus and apoptosis assays were evaluated using cytokinesis block by cytochalasin B.

The results show that bystander effect can be induced by irradiated melanoma cells (e.g., after 72 h post irradiation we have ~80 % of living cells in non-irradiated Me45 or NHDF), wherein viability of directly irradiated cells (all three cases) dropped to ~10 % of control cells. These results are consistent with percentage of apoptotic cells and micronuclei. The level of intracellular reactive oxygen species (ROS) increased significantly at 12h and persists elevated up to 24 h in directly irradiated Me45 cells incubated alone. Co-incubation of Me45 with the cells of the same line caused an increase of ROS shortly post irradiation. Simultaneously, bystander melanoma cells show increase of ROS at the same time. Non-hit fibroblasts incubated with irradiated Me45 exhibited smaller elevation of ROS also in the first 6 hours. Bystander fibroblasts seem to reduced superoxide level in irradiated melanoma cells in parallel they present considerable increase of superoxide. We observed also elevation of superoxide in bystander Me45. In the case of cellular nitric oxide, we observed some increase in all irradiated cells at different times and shortly after exposure in bystander cells.

All data indicate that UVB can induce bystander effect in malignant and normal cells and reactive oxygen and nitrogen species seem to be the first molecular signals engaged in this phenomenon.

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50. MATHEMATICAL MODEL OF BYSTANDER EFFECT INDUCED BY UVA RADIATION

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The aim of this study was to propose a mathematical model of bystander effect described by ordinary differential equations and parameter estimation based on biological experiments. The model describes ways in which cell survival is influenced by neighbouring cells. The model also explains two different effects described in the literature: classical bystander effect, where cell survival is reduced when communication takes place with irradiated cells, and reciprocal bystander effect where increase in cell survival of irradiated cells close to unirradiated bystander cells is observed. The *in vitro* experiments were performed using human dermal fibroblasts exposed to UVA[1].

The model reports the dynamics of two cells populations and is described by five ordinary differential equations. The model assumes that normal cells (N) are characterized by unlimited population growth. Subpopulation of damaged and non proliferating cells (D) appears after irradiation and due to native DNA breaks. Molecular signals transmitted via factors (β) released into the medium are generated by irradiated cells. They are inhibited by normal cells. β factors influence the rate of normal cells damage and the repair rate. Numerical estimation of mathematical model parameters is not a trivial task, because there is no universal method of performing this process. Furthermore, standard numerical methods of searching minimum of the objective functions are strongly dependent on the starting point. Therefore, the process must be repeated multiple times using different initial values of the parameters. This considerably increases the time of calculations. The parameters of the model were initially estimated using ADFIT program - a tool for numerical parameter estimation [2]. For further parameters estimation we used sensitivity analysis. Sensitivity analysis allows to determine what level of accuracy is necessary for a parameter to make the model sufficiently useful and valid. As a result we obtained the model, which is well fitted to the experimental data. Moreover, the model can be used to explain both classical and reciprocal effects.

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51. THE NOVEL MATHEMATICAL MODEL OF ATR-P53-WIP1 SIGNALING PATHWAY: STUDIES ON PREDICTION OF CELLULAR RESPONSE TO DNA DAMAGES

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Daily, thousands of DNA lesions are formed in each cell of the human body in response to stress agents, like the UV radiation or some chemicals. The existence of such large number of abnormalities in many cells may cause death of the whole organism after a very short time. During evolution many cellular mechanisms evolved to repair the DNA damages and prevent its transfer to the daughter cells. ATR (ataxia telangiectasia mutated and Rad3-related) module is activated by presence of single stranded DNA areas in the cell, which are caused by resection of various types of lesions or by stalled replication forks.

For a better understanding and prediction of the cell behaviour, the mathematical model of ATR pathway was developed. Model was created on the basis of experimental data and biological assumptions. Numerical implementation was based on Haseltine-Rawlings postulate, which combines deterministic and stochastic approach.

In this study detection and apoptosis thresholds were examined. In addition, it has been proven that the apoptotic threshold shifts when the specified proteins involved in the pathway (especially Wip1 and ATR) are blocked or reduced. Results show the essential role of the phosphatase Wip1 in the DNA damage response of regulatory pathways.

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52. THE NMR STUDY ON STRUCTURE OF *N*-ARYL-*C*-NITROAZOLES

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C-nitroazoles are subunits of wide range of compounds with biological acitivity. Many of them serve as drugs with antiprotozoal or antibacterial activity. This mainly results from reduction of nitro group, present in the structure and is strongly dependent on its position in the heterocyclic ring.

Our research are focused on *N*-aryl-*C*-nitroazoles. Recent studies confirmed the activity of *N*-aryl-4-nitroimidazoles in curing patients with Human African Trypanosomiasis (sleeping sickness) [1] For this reason the interest on *N*-aryl derivatives of *C*-nitroazole ring, seems to be rationalized towards finding new, biologically active compounds.

Due to the fact that azoles can exist in tautomeric forms and therefore give isomeric products or because of regioselectivity of reactions, unequivocal determination of compound structure seems to be crucial for investigating azole derivatives. Analysis of a series of *N*-arylazoles obtained by us was performed. The scope of heterocyclic rings include: 3-nitropyrazole [2], 4-nitropyrazole, 4-nitroimidazole, 2-nitroimidazole, 3-nitro-1,2,4-triazole, 4-nitro-1,2,3-triazole. Standard ^{1}HNMR and ^{13}CNMR spectra did not allow full and definite assignment of signal to elements of product structure. Therefore, HMBC and HSQC experiments were applied. For a full characterization and confirmation of structure of azole ring NOESY spectra analysis was necessary. This experiment allowed observation and determination of coupling between protons through space.

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53. THE STUDY ON THE RELATIONSHIP BETWEEN *N*-ARYL-*C*-NITROAZOLES STRUCTURE AND THE VALUES OF THEIR PARTION COEFFICIENTS

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During design of new biologically active compounds, many properties of structure are considered. The value of partition coefficient is among the most commonly used for QSAR (Quantitative Structure-Activity Relationship) analysis. The value of this parameter is very important for molecules, the biological activity of which results from passing through many biological membranes. The most restricting are conditions at blood-brain barrier.

In our recent studies we performed a series of experiments aiming at measuring the values of partition coefficient of a series of *N*-aryl-*C*-nitroazoles [1]. The experimental values of logP were obtained by use of "shake-flask method" or chromatographic techniques (RP-TLC).

Here, we present results of the analysis of relationship between these experimental results, values calculated by many *on-line* calculators and the structure of compounds for series of *N*-aryl-*C*-nitro- pyrazoles, imidazoles and triazoles. The strong dependences between Hammet constant and the substitution pattern on both experimental and calculated values were found. The position of the substituents and the structure of azole ring can also be correlated with obtained results. Such a relationship may be useful during assessing biological activity of compounds and can allow very rough estimation of value of partition coefficient with the change of substituent.

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54. INCREASE THE EXPLANATORY POWER OF GENE SET ENRICHMENT ANALYSIS BY FUSION OF EXPRESSION AND ONTOLOGY INFORMATION

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Results of high-throughput DNA microarray experiments are summarized by gene signatures - lists of genes exhibiting certain patterns of expression across experiments, very often differentiating expression between cases and controls. Such signature is a starting point in the process of functional analysis which should provide understanding of biological mechanisms involved in the studied experiments. One of the most commonly approach is gene set enrichment analysis of Gene Ontology (GO).

In a classic approach, enrichment analysis is performed individually for each GO term and does not take into account any dependencies resulting from the topology of the GO graph. It results in inheritance problem, as annotations are inherited from more specific descendant terms, and what can lead to false-positive results in biological interpretation. To overcome this problem, and as a side effect to reduce the size of gene signatures, some approaches include additional steps of decorrelation with respect to the GO graph.

Here we are presenting another approach, where in addition significance of the differences in gene expressions is fused into the gene set enrichment analysis. Such an approach allows us to filter out GO terms of low Information Content, what is crucial for many applications, e.g. tumor classification. To show advantages of our new approach we compare its preliminary results with several already existing and commonly used methods on a pilot data set.

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55. PA66/POM HYBRID NANOSPHERES AS POTENTIAL TARGETED NANOCARRIERS IN ANTICANCER THERAPY

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Currently, scientific research in the field of anticancer therapy is focused on design and synthesis of new effective compounds, which could act as potential cytostatic drugs. It is particularly important to develop chemotherapeutic agents with reduced systemic toxicity. These new compounds should also allow for reduction of multidrug resistance of tumor cells, which now is the sturdiest limitation to successful treatment of cancer diseases. In addition, selective therapeutic agents enable to minimize side effects, which currently are an integral part of the standard chemotherapy treatment.

The solution to all these problems seems to be the introduction of new nanoparticles as drug nanocarriers. Application of a suitable nanocarriers gives many advantages. First of all, the nanometric size of nanoparticles allow them to freely cross the biological barriers. Very important is also the protective effect of the nanocarriers to normal cells, by limiting their exposure to the toxic effects of anticancer drugs.

Hybrid nanospheres PA66/POM, which belong to polyoxometalate group of compounds, act as inorganic inhibitors of casein kinase (CK2). Therefore they play a key role in tumor cell proliferation and thus can exhibit potential anticancer properties.

The aim of the study was to evaluate the proapoptotic properties of hybryd nanospheres PA66/POM against MCF-7 human breast cancer cells. The studied material consisted of hybrid nanospheres PA66/POM which differ in the percentage of tungsten trioxide in their structure. Pure polymer 66 and pure tungsten trioxide were also studied for comparison.

Proapoptotic properties of hybrid nanomaterials were estimated on the basis of changes in the asymmetry of membrane lipids and membrane integrity estimated by cytometric analysis of phosphatidylserine externalization using Annexin V FITC Apoptosis Detection Kit (Santa Cruz Biotechnology, USA). MCF-7 breast cancer cells were incubated with tested compounds for 24 hours, then the medium was replaced by fresh one and the apoptosis process was evaluated over 0 - 72 h of post-incubation period.

Directly after the incubation with the tested compounds (0 h time point) a slight decrease in the onset of live cells and similar small fractions (5-10%) of apoptotic and necrotic cells were observed. Treatment of cells with pure polymer 66 and PA66 containing 3 wt% WO_3 resulted in a significant decrease in the fraction of live cells (55-60 %) and in a increase in necrotic cell fraction (>30%) at 24 h time point of postincubation period. During the further 48 h and 72 h a progressive reduction of the fraction of live cells(65-80%) and a simultaneous increase in the percentage of necrotic cells were found in cells incubated with pure PA66 and PA66 containing 3, 10 and 30 wt% WO_3. This implies proapoptotic properties of the investigated hybrid materials in human breast cancer cells.

56. ARONIA MELANOCARPA PEELS AS UNCONVENTIONAL ADDITIVE WITH STRONG ANTIOXIDANT PROPERIETES USED IN LAMBS' FATTERING

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Antioxidants appear in food in low concentrations and are capable of preventing and stopping oxidation. The substances with the strongest antioxidant effect are phenolic compounds. Plant-derived phenols are obtained in reactions of metabolic processes. Unfortunately, the animal feed additives do not include by-products of the fruit and vegetable industry. As mentioned above, it is in fruits that the largest number of substances reducing oxidative stress is found. However, the policy for recycling by-products of the Polish fruit processing industry has led to a steadily increasing emission of biological waste into the environment. In the recent years there have been attempts of enriching animal products with plant-derived, highly antioxidant compounds. This may significantly decrease some of the disadvantageous features of meat related to the metabolic transformations of organic compounds in tissues. The use of by-products of the fruit processing industry in the fodder for slaughter animals may increase the content of biologically active components in meat, giving the products and materials the prosperities of functional food.

Studies provided an analysis of lipids peroxidation indicators, which included malondialdehyde (MDA). Reactive forms of oxygen species could degrade lipids, especially polyunsaturated ones. It may cause cascade of reactions which induce formation of malondialdehyde. Level of MDA is used as a bio-indicator to determine the level of oxidative stress in an organism.

Research was conducted on the 48 male lambs of Polish Merino and Wrzosówka - dual type of typical genotype. Animals were assigned in three different nutrition groups - control and two experimental. All lambs were fed with use of well-balanced, completed fodder. Furthermore, experimental groups received addition of dried *Aronia melanocarpa* peels with different amount - 150 g and 300 g per 1kg of basic fodder. MDA was analyzed in muscle tissue (comber, haunch, entrecote) and liver.

Obtained results present positives changes in biological samples, particularly in all experimental groups. Significantly lower level of MDA ($P \leq 0.01$) was observed in tissues collected from lambs which addition of *Aronia melanocarpa* was given. Concentration of MDA in samples was calculated by using Gen5 programme, dedicated to ELISA technique.
Addition of dried *Aronia melanocarpa* peels is a very useful solution in animal feeding, which protects the organism from oxidative stress and pathological disorders. Moreover, dried *Aronia melanocarpa* peels are better digestible and cheaper than synthetic or chemical additives containing antioxidants.

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57. CLASP1 GENE EXPRESSION HAS PROGNOSTIC SIGNIFICANCE IN OVARIAN CANCER

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Ovarian cancer is usually diagnosed at an advanced stage and thus the results of treatment are poor. Scientific efforts are aimed on better understanding of biology of this cancer and on search for diagnostic, prognostic and predictive biomarkers.

We examined gene expression in 97 ovarian cancer samples and performed a number of systematic analyses of expression patterns in relation to several defined clinical and molecular features. Analysis of survival resulted in 93 probesets related with overall survival (OS) and 18 probesets related with disease free survival (DFS). Fifteen genes related with OS, two of them (ATRX and CLASP1) showing also correlation with DFS, were chosen for validation with quantitative RT-PCR. Only four genes were confirmed to be significantly correlated with OS. This were CLASP1 ($p = 0.005$), MBNL1 ($p = 0.038$), SPPL2B ($p = 0.027$) and VAV2 oncogene ($p = 0.013$). None gene was validated according to DFS. We also did other comparisons based on the above RT-PCR measurements. Interestingly, CLASP1 showed also strong correlation with CHT response ($p = 0.0005$) as well as with BRCA1 mutation status ($p = 0.035$). We performed also an external validation in the independent set of 33 ovarian cancer samples. CLASP1 appeared again to be significantly related both with OS (p=0,049) and DFS (p=0,004). CLASP1 (cytoplasmic linker associated protein 1) is relatively poorly characterized protein that is thought to play a role in regulation of tubule dynamics in interphase and during cell division. Thus, this protein may be important in tumor cell response to taxanes. Possibly, it may also be engaged in differential response to CHT in patients with hereditary, BRCA1 mutation-linked ovarian cancer. We think that CLASP1 may be worth further investigation as a potential prognostic and predictive marker.

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58. OPTIMIZATION OF SPATIOTEMPORAL PROTOCOL FOR RADIOTHERAPY BY USING ADJOINT SENSITIVITY ANALYSIS

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Radiotherapy is a common treatment method for cancer disease. It is based on irradiation of cancer cells by high-energy radiation that cause destroys their molecular organization. In fact the radiation destroys not only the cancer cells but also the healthy cells. An important thing in radiotherapy is to maximize the effect of destroying only cancer cells. In this work we present a method for optimization the spatiotemporal signal of irradiation which affects the example tumor growth model.

In the present study we analyze avascular tumor growth model from work [1]. The model is one dimensional in space and is described by system of three partial differential equations:

$$\begin{cases} \frac{\partial p}{\partial t} = \frac{\partial}{\partial x}\left(\frac{p}{p+q}\frac{\partial (p+q)}{\partial x}\right) + g(c)\cdot p\cdot(1-p-q-n) - f(c)\cdot p - k_{pIR}\cdot IR\cdot p \\ \frac{\partial q}{\partial t} = \frac{\partial}{\partial x}\left(\frac{q}{p+q}\frac{\partial (p+q)}{\partial x}\right) + f(c)\cdot p - h(c)\cdot q + k_{pIR}\cdot IR\cdot p - k_{qIR}\cdot IR\cdot q \\ \frac{\partial n}{\partial t} = h(c)\cdot q + k_{qIR}\cdot IR\cdot q \end{cases}$$

where: *p, q, n* are relative amounts of: proliferation, quiescent and necrotic cells, *g*(*c*), *f*(*c*), *h*(*c*) are growth functions (which values depending on nutrient concentration *c*) for particular type of cells. *IR* is variable that represent the radiation dose and variables *kpIR* and *kqIR* are radiosensitivity for proliferation and quiescent cells.

We used adjoint sensitivity analysis to calculate the spatiotemporal gradient of objective function with respect to the irradiation signal. The objective function is defined as total number of quiescent and proliferation cells in final time of simulation. The gradient with appropriate constraints can be used to optimize signal of irradiation. The optimized signal can be useful in developing new radiotherapy protocols.

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59. THE MANUFACTURE AND PROPERTIES OF ELECTROSPUN PLA NANOFIBRES FOR CARTILAGE TISSUE ENGINEERING

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Current clinical methods to repair defective cartilage caused by trauma or cancer injuries are limited in their ability to regenerate its functional properties both in terms of composition and mechanics. Recent methods have focused on the use of tissue engineering approaches to repair cartilage tissue. Particular attention has been paid to fibrous polymer scaffolds at the nanoscale obtained by electrospinning technique. The method has gained interest due to its ability to form a fibrous structure similar to the natural extracellular matrix. While several studies have been carried out to develop porous, isotropic polymer-based scaffolds for tissue engineering, as well as to manufacture bulk scaffolds, nanofibre-based materials have not yet been extensively studied. Considering the similarities between the electrospun nanofibres and the extracellular matrix, materials formed by the electrospinnig, such structures seem to be suitable for regenerating cartilaginous and neural tissues.

The work presents preliminary results on the manufacture and biomechanical evaluation of nanofibres in the form of membranes. The fibrous materials were obtained from poly(lactid acid) (PLA) nanofibres modified with carbon nanotubes (CNTs) and gelatin (GEL) .

The polymer nanofibres were additionally modified to obtain three different types membranes, i.e., the hybrid structure of PLA and gelatine nanofibres, carbon nanotube (CNT)-modified PLA nanofibres and pure PLA-based nanofibres. Gelatin was used as a moderator of resorption time of PLA. CNTs were added to polymer matrix to promote cell formation and to enhance electrical and mechanical properties of PLA nanofibres. Selected physical and mechanical properties of materials were assessed. The samples were also studied *in vitro* using cell culture of human chondrocytes collected from patients. The influence of the nanofibrous scaffolds upon chondrocytes was determined by cytotoxicity and genotoxicity assays.

60. ANGIOGENESIS-LINKED GENES EXPRESSION IN LARYNX CANCER

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Every year 600 000 new cases of head and neck cancers are diagnosed in the world, nearly one fourth of them is larynx cancer. The average five-year survival especially for larynx cancer is approximately 60%. The progression and metastasis of all head and neck cancers depends on the rate of angiogenesis. The process of angiogenesis is complex and regulated by a variety of signaling pathways, where the distinct influence have Wnt and hypoxia. Their main role in this process is a regulation of gene expression of various growth factor and extracellular matrix modulators. Vascular endothelial growth factor (VEGF) is probably the crucial and the best know one. Five isoforms: VEGFA, VEGFB, VEGFC, VDGFD and PIGF have been identified.

Each of them has a different impact on various processes, however they are similar in the role of crucial angiogenesis stimulators.

The aim of this study was to evaluate the potential specific angiogenesis-linked gene markers for larynx cancer.

Larynx cancer and normal larynx samples were obtained during surgery from patients of ENT Department of Medical University of Silesia. The samples underwent routine histopathological examination and were subsequently divided into three cancer groups G1, G2 and G3, and one control group. Total RNA was extracted with Trizol® reagent and purified. Obtained mRNA was hybridized with HGU-133A 2.0 Plus microarrays (Affymetrix). Based on microarray results expression of selected genes was estimated with QRT-PCR. The microarray results were analyzed with the use of Gene Spring 13.0. The ANOVA and Tukey *post hoc* tests with Benjamini-Hochberg correction were used in statistical analysis. The results from QRT-PCR were analyzed using Statistica 11.0 software.

The microarray analysis gave more than 58,000 fluorescence signals of mRNAs, from which 992 were selected as related to angiogenesis according to the Affymetrix database. After the statistical analysis, only 179 mRNAs were considered as statistically significant at $p<0.05$. Based on biological significance of obtained genes and fold change higher than 2.0 VEGFA, SERPINE1 and FGF9 were distinguished between others. Afterwards, gene expression of all VEGF isoforms was evaluated with QRT-PCR giving a different expression pattern.

The trend of VEGFA gene expression was different in the microarray experiment than QRT-PCR results. Our microarray research confirmed SERPINE1 gene to be a potential biomarker in the larynx cancer.

61. MICROARRAY EVALUATION OF LARYNX CANCER PROLIFERATION RELATED GENE EXPRESSION AND SIGNALING PATHWAYS

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Larynx cancer is usually researched as a part of a larger group of malignancies classified as head and neck cancer and is responsible for approximately 25-30% of cases in this group. Ongoing signaling pathways in various histopathological grades hardly have been studied. Like in all know malignancies, also in larynx cancer cells proliferation has a crucial role for patient's survival chances. A variety of signaling pathways directly influence cell proliferation and differentiation. Among them, TGF-β, Wnt canonical and non-canonical signaling are among the most important ones. They also participate in the regulation of other processes like inflammation or apoptosis.

The aim of this study was to evaluate using microarray analysis the overall expression trend of signaling pathways taking direct and indirect part in cell proliferation in larynx cancer.

Tissue samples were obtained from patients with larynx cancer. The control group was formed from samples taken from the margin, which had no visual marks of malignancy. The obtained tissue samples underwent routine histopathological examination and were classified according to FIGO. Total RNA was extracted with Trizol® and purified. The mRNA was used for the microarray experiment with chipset HGU 133A (Affymetrix) according to the manufacturer protocol. The last step was the statistical analysis of the obtained results which was made using GeneSpring 13.0 software. The results showed a normal distribution, which was the basis to implement ANOVA with Tukey post hoc test and Benjamini-Hochberg correction for the analysis of proliferation related genes taken from the list of the Affymetrix database. The last step was to check the significance of signaling pathways in the ANOVA results with Panther gene onthology program.

From all microarray HGU-133A 2.0 plus probe sets only 4017 ID mRNA were distinguished as relevant in the process of proliferation according to Affymetrix database. After the statistical analysis there were 798 probe sets statistically significant. The next step was to evaluate their biological significance and participation in different signaling pathways that could influence proliferation. The obtained entity has designated TGF-β, Wnt inflammatory response signaling pathways as statistically significant. By correlating the results from Panther and Gene Spring of the significant genes they were evaluated as up or down regulated. Based on this analysis the TGF-β related genes were most over-expressed, while Wnt signaling was downregulated in the cancer samples.

The TGF-β signaling pathway was probably the spearhead of larynx cancer proliferation because of down-regulation of other significant proliferation pathways like Wnt.

62. EFFECT OF SELECTED PLANT POLYPHENOLS ON INTRACELLULAR ACCUMULATION OF ANTITUMOUR ANTHRACYCLINE DRUGS IN MULTIDRUG RESISTANT LEUKAEMIA HL60/VINC CELLS OVEREXPRESSING P-GLYCOPROTEIN

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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, P-gp; MRP1 and BCRP), belonging to the ATP-binding cassette protein family responsible for active efflux of drugs out of resistant cells resulting in the decreased intracellular accumulation insufficient to inhibit resistant cell proliferation. Therefore, many efforts are focused on the search of agents reversing MDR of tumour cells.

Because the food of plant origin (especially fruits and vegetables) is rich in biologically active compounds, it is extensively explored as the potential source of new antitumour drugs active against tumours resistant to classical chemotherapy as well as effective modulators of MDR. It is postulated that plant polyphenols could inhibit the efflux of chemotherapeutics out of resistant tumour cells by P-gp.

The aim of this study was to examine the effect of selected polyphenols, quercetin (Q), gallic acid (GA) and ellagic acid (EA), on intracellular accumulation of three anthracycline drugs: doxorubicin (DOX), daunorubicin (DR) and pirarubicin (PIRA) in human promyelocytic leukaemia HL60/VINC cells exhibiting a MDR phenotype related to the overexpression of P-gp. The study was performed with the aid of flow cytometry by utilizing fluorescence properties of anthracycline drugs.

It was found that Q, GA and EA did not increase the intracellular accumulation of DOX, DR and PIRA in resistant HL60/VINC cells. Obtained results suggest that studied polyphenols are not able to interact with P-gp in HL60/VINC cells or that they bind to this MDR pump in an independent manner to anthracycline compounds having in mind that P-gp is a multi-site transporter.

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63. SYNTHESIS AND PRELIMINARY EVALUATION OF BIOLOGICAL ACTIVITY OF SELECTED QUINOLINE GLYCOCONJUGATES

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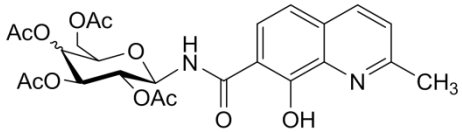
Quinoline scaffold can be found in many classes of biologically active compounds used as antifungals, antibacterials, anticancer, as well as antituberculotic agents [1-4]. Nevertheless structures designed on the core of quinoline moiety still suffers from poor bioavailability/membrane transport. This prompted us to elaborate sugar derivatives of quinoline, which can be a potentially effective strategy for targeting quinoline derivatives toward tumor cells [5,6].

2-Methyl-8-hydroxy-7-carboxyquinoline was selected as quinoline derivative used for connection with a sugar part. Sugar part could be connected to this quinoline derivative by amide, ester, thioester or glycosidic bond. Based on the earlier results and literature reports, it was concluded that important for biological activity is the presence in the glycoconjugate structure aromatic fragment or an amide bond.



The example of synthesized compounds

In the present study a method of synthesis of quinoline glycoconjugates is presented. Structure of synthetized compounds contain sugar moiety linked directly with 2-methyl-8-hydroxy-7-carboxyquinoline by an amide bond. Compounds were synthetized by simple and commonly used condensation methods using different coupling agents. Furthermore, the results of the initial assessment of the biological activity of these compounds against a selected tumor line will be presented.

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64. GENOME-WIDE DNA METHYLATION PROFILING OF GASTRIC CANCER-ASSOCIATED MYOFIBROBLASTS IDENTIFIES CANCER-INDUCED DNA METHYLATION CHANGES

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The tumor microenvironment plays an important role in cancer development and progression. In particular, Cancer Associated Myofibroblasts (CAMs) promote the growth, proliferation and migration of cancer cells through a complex mechanism of reciprocal communication. While it is well established that DNA methylation plays a key role in cancer progression, little is known about DNA methylation in non-malignant neighboring stromal cells. Unpublished data from our lab shows that gastric CAMs exhibit distinct gene expression profiles compared to adjacent tissue myofibroblasts or normal tissue myofibroblasts therefore we hypothesize that CAMs are epigenetically programmed to promote tumor growth and migration. Our data shows that changes in global DNA methylation status alter CAM induced changes in cancer cell proliferation and migration. To further investigate the mechanism of epigenetic reprograming of gastric myofibroblasts, we performed DNA methylation and RNA expression arrays on a collection of primary human gastric myofibroblasts to identify CAM-specific genome-wide DNA methylation signatures and their role in regulating gene expression. Data from this study provide strong evidence for CAM specific methylation signatures and show that tumor-promoting properties of CAMs are dependent on epigenetic programming.

65. THE OVEREXPRESSION OF LOC66598 PROTEIN AFTER TRANSIENT TRANSFECTION OF 3110001I22RIK-EGFP CONSTRUCT INDUCES APOPTOSIS IN NIH3T3 AND MCF7 CELLS

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The mouse *3110001I22Rik* gene is localized in the first intron of Bfar (bifunctional apoptosis regulator) gene. It codes for uncharacterized protein LOC66598 (312 aa). A conditional knockout mouse were generated as part of the International Knockout Mouse Consortium program (https://www.mousephenotype.org/). No significant abnormalities were observed in twenty two tests carried out on *3110001I22Rik* mutant mice. Thus, the function of a hypothetical protein encoded by *3110001I22Rik* gene is still not known. According to BioGPS database, the highest expression of the gene was noticed in oocytes and fertilized eggs, in embryonic stem lines, and in hematopoietic cells (http://biogps.org/#goto=genereport&id=66598). In macrophages *3110001I22Rik* expression is induced after LPS treatment.

We constructed vectors coding for the 2HA-LOC66598 and LOC66598-EGFP fusion proteins. Since we failed to establish the stably transfected cell lines with these constructs, we focused on analyses of cells transiently transfected with the *3110001I22Rik-EGFP* construct. Life imaging microscopy analysis of mouse NIH3T3 cells revealed that transfected cells die by apoptosis within 18-42h after transfection. Similar effect was observed in transfected human MCF7 cells, although some cells efficiently removed the fusion protein and survived. Control cells (transfected with EGFP only) of both cell lines were not affected.

To conclude, we found that ectopic expression of *3110001I22Rik* gene could result in apoptosis. It takes a few/a few dozen hours from protein production to cell death (the effect is not immediate), and the protein is localized predominantly in the nucleus.

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66. HYBRID MODEL OF SOLID TUMOUR GROWTH AND SPROUTING ANGIOGENESIS

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Solid tumour growth is inseparably connected with a delivery of oxygen and nutrition factors from surrounding vascular network. However, due to a fast metabolism of the tumour cells, hypoxic regions may occur, causing a creation of tumour necrosis sites. The phenomenon of hypoxia is important because it typically leads to the process of angiogenesis and as an addition it reduces efficiency of many therapies. Angiogenesis is the physiological process of growth of new blood vessels from existing vasculature. It is a fundamental step in the transition of tumors from benign to the malignant state. There are many inhibitors of angiogenesis that are used in the treatment. They are often connected with inhibition of vascular endothelial growth factor (VEGF) or its receptor.

In the present study, the authors have connected continuous and discrete model into one hybrid model describing the behavior of tumour growth and process of angiogenesis. Continuous part of the model is based on the multiphase model, and it is represented as a set of advection equations for different types of the cells. It is also complemented by reaction-diffusion equations for oxygen, VEGF and (chemotherapeutic and anti-angiogenic) drugs. The discrete part of the model is used for modeling the angiogenesis. It enables modeling sprouting of the new vascular branches, connecting them into loops and thus also flow of the blood through it. The branching process is regulated by the delta-notch signaling pathway. In order to speed up the simulations of the model, it was calculated in parallel.

A number of simulations were performed with different discrete model parameters in order to generate vascular networks with different vessel tortuosity and density of branches. Two-dimensional simulations of the tumour growing in healthy tissue (with and without angiogenesis) were consistent with the biological picture of this pathological process. Result of simulations of the tumour growth in hexagonal-vascular structure with process of angiogenesis and two types of therapies was quantitative comparison of applied treatment protocols. Developed algorithm is ready for implementation in a three-dimensional environment.

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67. TDSCOPE PROJECT - TOWARDS AN INNOVATIVE METHOD AND DEVICE FOR EARLY DIAGNOSIS OF SKIN LESIONS

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Malignant melanoma is one of the world's fastest-growing types of cancer. However, if discovered early, the tumor may be removed surgically [1]. Success of such treatment depends on the properties of the tumor and the stage at which the cancer is detected. The 10-year survival ranged from 93% for stage IA to 39% for stage IIC melanoma [2].

The aim of *TDscope* project [3] is to develop method and device for early stage skin cancer detection. The project arose from an innovative idea of combining two diagnostic non-invasive techniques for in-vivo skin lesion diagnostics:

- *optical* –dermatoscopy (dermoscopy), which allows the clinician to examine cutaneous micro-features not visible under macroscopic examination,
- *thermal* – thermodermatoscopy, which allows the clinician to examine thermal state of lesion, by means of analysis of skin temperature map (IR images).

The project is executed by a consortium of medical, technical as well as commercial partners, namely: Center and Institute of Oncology, Gliwice Branch (medical partner), Silesian University of Technology (technical partner) and Juwena (consortium leader). The project execution is scheduled for 2013-2016, with ongoing research phase – medical experiment – ending in 2015. Following the research phase, the prototype and further clinical tests are anticipated.

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68. DOUBLE ROLE OF THE P53 IN THE INDUCTION OF CELL RESPONSE TO DNA DAMAGES

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Protein p53 is one of the most important tumor suppressor, because of its function in cell responses e.g. for DNA damages such as its repair, cell cycle arrest, senescence and apoptosis. Abnormality in p53 regulation or its total inactivity is resulting in abnormal cell growth with the potential to promote malignant progression. p53 plays its role by transcriptional and mitochondrial activity. We wanted to explore, by means of simulation experiment, which of this activity is crucial for apoptosis induction.

In our work we presented an expanded model of the p53 protein role in cell response to DNA damages. The model consists on autoregulatory positive and negative feedback loops, spontaneous and p53 induced activation, mono- and poly-ubiquitynation of p53 and mitochondrial fraction containing mitochondrial p53, apoptotic proteins and its complexes. We took into consideration well known anti- and proapoptotic mitochondrial proteins, which are responsible for mitochondrial membrane permeabilization, Cytochrome-C release, caspases cascade activation and finally apoptosis. We consider presence the p53 protein in three compartments: the nucleus, the cytoplasm and the mitochondria, which enables evidencing the double role of p53 in cell.

In our work we examined cell response after different dose of irradiation. Cell response is dependent on nuclear p53 level, mitochondrial p53 level and free Bax level. Low IR dose (2 Gy) induce mainly increase of nuclear p53 but not mitochondrial p53 and free Bax, which result rather in cell cycle arrest (21% of the cell population) then apoptosis (30.6%). The 4 Gy irradiation induce higher amount of DSBs and in result increase levels of both p53 and free Bax over the proposed apoptosis threshold. In this case apoptosis is induced in 65.4% of the cell population and cell cycle arrest in 31.2%. Moreover, we examined response to irradiation in cell with repression of the p53 nuclear import or the p53 mitochondrial export. The blockade of the p53 transport to nucleus significantly reduce cell ability to induce apoptosis, however accumulation of the mitochondrial p53 and following release of the Bax from Bcl-2/XL -Bax complexes cause cell death in part of cell population. In this case the apoptotic fraction in response to 2 Gy of irradiation is equal 24.4% while to 4 Gy – 29.8%. In irradiated cells repression of the p53 translocation to mitochondria results in Bcl-2/XL - Bax heterodimers formation from newly produced Bax and the increase free Bax level over introduced threshold is observed in smaller fraction of the cell population – 13.2% with 2 Gy and 52.6% with 4 Gy.

The results show that cell response after DSB formation depends on p53 level: low p53 level induce cell cycle arrest and high level induce apoptosis. The results underline that cell response depends not only on total p53 level, but also p53 levels in separate parts: nucleus, mitochondria and cytoplasm. Repression of the p53 transport to nucleus or to mitochondria resulted in smaller apoptotic fraction, which demonstrate importance of both p53 activity, as a transcription factor and by direct activity on the mitochondrial membrane. Increase of mitochondrial p53 is sufficient to induce apoptosis because of its ability to liberation Bax from complexes. After mitochondrial export blockade accumulation of nuclear p53 cause total Bax level increase by induction of its transcription, so in the part of cell population free Bax level is high enough to induce apoptosis.

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69. ANTI-MELANOMA ACTIVITY OF WP760 IN VARIOUS IN VITRO CULTURING CONDITIONS

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Background: Efficient treatment for metastatic melanoma remains an urgent need. Potential anti-melanoma drugs include WP760, a novel bis-anthracycline demonstrated to be active specifically towards melanoma cells [Zheng et al, 2007]. However, there have been no data concerning activity of this pharmaceutical against melanoma cells under differing tumor microenvironment conditions.
Aim: Examination of WP760 activity against melanoma cells maintained in two-dimensional (conventional, 2D) or three-dimensional (spheroids, 3D) cell culture systems, as well as in cultures exposed to experimental underoxygenation.
Materials and Methods: Six human melanoma cell lines. Parameters monitored: cell viability (MTS assay), apoptosis (TUNEL test), clonogenicity (clonogenic test), cell cycle (flow cytometry), and spheroid viability (growth kinetics).
Results: Strong anti-melanoma activity of WP760 was found in a 2D culture models, with average IC_{50} approx. 30nM. Such activity was dose- and exposure time-dependent, with the IC_{50} effect at 30nM concentration observed after 1-24 hours of exposure to drug, depending on cell line. Under hypoxic conditions, WP760 was less active towards those cell lines for which proliferation rate declined under hypoxia. In 3D models lower activity of WP760 was observed. Inhibiting growth of melanoma spheroid cultures required WP760 concentrations higher than those needed in 2D systems. WP760 reduced clonogenicity of melanoma cells, triggered their apoptosis and influenced cell cycle in 2D models.
Conclusions: Our results have confirmed significant anti-melanoma activity of WP760 in *in vitro* 2D culturing systems. The results revealed attenuated anticancer activity against spheroids as well as, to some extent, under hypoxic conditions. Further investigation is required to better explore the potency of WP760. Due to the drug hydrophobicity, liposomal formulations of this drug are likely to contribute relevant information and allow testing this drug action *in vivo.*

70. NUMERICAL MODEL OF HEAT TRANSFER IN SKIN LESIONS – MODEL VALIDATION BASED ON EXPERIMENTAL DATA

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The accurate numerical models of living tissue are of high importance among numerous modern biomedical engineering challenges. Such models not only allow to understand processes involved, but they can help to develop new treatments and/or equipment used to assist medical staff during diagnosis and controlled treatment process.

The work presented here is a part of wider research project targeted in verifying the possibility of early diagnosis of skin lesions, with special interest in malignant melanoma identification.

Numerical model of skin undergoing thermal stimulation (mild cooling) in a human forearm is presented. The skin temperature recovery process was then analysed. The heat transfer in the living tissues is modelled using Pennes' bioheat equation [1] augmented with additional models of thermoregulation [2]. The numerical model of human forearm, cooling compress and surrounding air was developed. A numerical model of heat transfer in tissues and CFD model of surrounding air (natural convection) was proposed. The simulations were carried out using ANSYS Fluent 14 commercial CFD package. The additional source terms of heat conduction equation arising in Pennes' bioheat transfer equation were introduced by means of UDF (user-defined function) mechanism.

Heat flux (during cooling phase) and skin temperature history (during recovery phase) samples were recorded in group of male adults. Simulation results are validated against experimental data.

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71. IMPAIRMENT OF REVERSE CHOLESTEROL TRANSPORT BY STAR-DEPENDENT CHOLESTEROL HYDROPEROXIDE TRAFFICKING: IMPLICATIONS FOR ATHEROGENESIS UNDER OXIDDATIVE STRESS

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Low density lipoprotein (LDL) is rich in unsaturated lipids, including free cholesterol and cholesteryl esters. All of them can be exposed to action of reactive oxygen species, which may result in numerous modifications. Main product of free radical oxidation is a large variety of oxidized lipids, including cholesterol ring oxides such as 7-hydroxyperoxide (7-OOH), 7- hydroxide (7-OH), and ketone (7=O). All of these products can be cytotoxic depending on the concentration, but only ChOOHs are redox-active , i.e. capable of inducing propagative oxidative damage via 1-electron reductive turnover. 7=O and 7-OH are more abundand in vascular lesions have garnered more interest than the latter vis-à-vis involvement in atherogenesis. However, small amounts of 7α/β-OOH intermediates detected in arterial lesions should not signify minor importance.

Macrophages may accumulate large amounts of cholesterol esters, non-estrified cholesterol, and other lipids from oxidized LDL, potentially leading to arhterogenic "foam cell" formation. Reverse cholesterol transport RCT is a key pathway by which macrophage attempt to maintain cholesterol homeostasis by exporting the sterol when is reaches excessive levels. Of our special interest are proteins of the steroidogenic acute regulatory (StAR) family, which bind and transport cholesterol and deliver it into/to mitochondria. On the inner membrane of mitochondria cholesterol is converted to 27-hydroxycholesterol (27-OH) by 27-hydroxylase (CYP27A1). 27-OH is a key agonist of transcription factor liver-X-receptor (LXR), which upon forming a heterodimer with retinoid-X-receptor (RXR) in the nucleus, induces expression of the plasma membrane ATP-binding cassette transporters ABCA1 and ABCG1.

72. DETERMINATION OF RAT ORAL BIOAVAILABILITY OF GENISTEIN DERIVATIVES

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Genistein derivatives synthesized in our laboratory are compounds that inhibit cancer cell proliferation at the concentration several fold lower than genistein through various mechanisms, such as blocking the cell cycle or interacting with mitotic spindle (1-4). In our previous studies we determined *in vitro* bioavailability of these drugs using Caco-2 cell line. In the next stage, we decided to determine their bioavailability and metabolism *in vivo*.

This work presents preliminary data on bioavailability and metabolism of genistein derivatives in rats after oral administration of the drugs.

Genistein derivatives and genistein, which served as a reference compound, were administered by oral gavage to the animals divided at random into six groups. Next, the animals (3 animals with each group) were placed in metabolic cages for urine collection, and 9 animals were placed in livig cage for blood collection. Blood was collected from animals at different timepoints after drug administration (0.5; 1; 1.5; 2; 2.5; 3; 3.5 and 4 hours). Urine was collected during 0-12 hours and 12-24 hours after administration. The urine and blood serum samples were purified using Solid Plase Extration and analyzed using HPLC-ESI-MS/MS.

Genistein and its derivatives were present in urine samples in the unchanged form. Compounds were rapidly eliminated from animals body with urine; in the samples collected between 12-24 h after drug administration, in comparison to samples collected between 0-12h, the concentration of detected compounds was 3-7 lower. Moreover, we showed that all compounds were metabolized *in vivo* by connecting with glucurinic or sulfuric acid. In plasma of experimental animals administered with genistein derivatives the drugs were not detected, while in genistein-treated group, the isoflavonoid was reliably determined.

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73. IMPACT OF THE SELECTION OF STATISTICAL TEST ON THE QUALITY OF GENE SIGNATURES IN AN INTEGRATIVE ANALYSIS APPROACH

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The development of high-throughput experimental methods in molecular biology has enabled the connection of data and results from independent studies. This integrative concept addresses various issues related with the analysis of high-dimensional data. It allows for increasing the power of statistical testing by raising the number of samples as well as gives the opportunity to use a wide range of procedures for the validation of a single experiment.

This research focused on the problem of selecting the appropriate type of statistical test for the purpose of identifying differentially expressed genes. The data sets consisted of two independent expression sets obtained in the course of microarray experiments on radiosensitivity. The experiments were designed with the objective of identifying genes differentiating radioresistant and radiosensitive people in a group of breast cancer patients undergoing radiotherapy.

The data from both studies was analyzed separately and the resulting gene signatures were validated using a restrictive approach which involved comparing the genes from each experiment and investigating their intersection. The quality of these genes as markers of radiosensitivity was assessed using a logistic regression classifier. The separability of the samples was measured by applying the common differentially expressed genes as features for the classifier.

The first setting of this study implied the use of two-sided statistical tests, chosen accordingly to the normality of the feature distribution and homogeneity of variance. This, however, proved to be misleading, as in such a manner the situation where in one experiment a gene is up-regulated and in the other down-regulated was not taken into account. Therefore, another approach was used in which one-sided tests were performed for the corresponding genes. This difference had a strong impact on the quality of classification which may be illustrated by the ROC charts.

This research revealed the significance of careful choice of procedures for statistical testing when analyzing high-dimensional 'omics' data. A profound understanding of the experimental design and possible issues allows for the improvement of genetic signatures in terms of quality of classification and meaningfulness of biological interpretation of the results.

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74. GAME-THEORETIC APPROACH TO IMAGE SEGMENTATION

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Segmentation is one of the many techniques for dealing with image analysis of high importance. Scientists are working on improving existing solutions, combining algorithms, finding new more precise. This process consists in dividing the image into regions called segments. Pixels belong to the region have a common feature, which does not occur outside the nearest neighborhood.

In this work, we have implemented and tested image segmentation algorithm based on evolutionary games theory introduced by M. Pellilo and the group [1-3]. An image is represented as edge-weighted graph where the vertices correspond to individual pixels and their weight reflects on the similarity between them. The clustering algorithm used in this work is based on finding similarity between pixels using replicator dynamics, assigns it to the cluster and removes it from the graph. The process will be repeated until all pixels are assigned to corresponding clusters. We applied the method to segmentation of intensity (gray scale) images. The research was carried out on artificially generated, syntetic images. Implementation have been done in Matlab.

Some preliminary results of syntetic images segmentation have been obtained. For simple structured images results are correct. However, those images were relatively small (about 100x100 pixels) that was caused by inefficient graph representation in computer memory in Matlab. Further steps in our work will concentrate on improving the efficiency of the algorithm to be able to process medical images in high resolution (at least 512x512 pixels) and on testing this algorithm on real medical data (PET/CT/MRI) with the presence of noise.

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75. BLENDGATE - A USEFUL TOOL TO SUPPORT GEOMETRY MODELING PROCESS FOR MONTE CARLO SIMULATIONS IN MEDICAL PHYSICS

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Modeling the geometry of the simulation environment is a task that requires a large amount of time and precision. Immediate visualization of input elements of the environment can be very useful especially while creating a complex environment. Unfortunately, this problem is not solved by any popular and frequently used tools for simulations, which is GEANT4 [1]. GEANT4 is a set of object-oriented libraries, that allows to build geometry directly in C++ code for Monte Carlo simulations. However, the visualization of whole created environment is possible only by running compiled code. This way the user needs to have a vision of the whole environment and the position of each object must be described by the abstract objects and its parameters.

The proposed solution is based on the WYSIWYG (what you see is what you get) idea. An open-source software called Blender [2] was used in order to visualize objects. Blender has been extended by the possibility to save the modeled environment to file that can be read by GEANT4. Modeling process using proposed module consist of following stages. First, we need to create an environment simulation in Blender. It is possible to take advantage of the introduced additional elements which are for example duplications of objects or assign materials. Next stage, is to save the model to a file in a format GDML [3] (Geometry Description Markup Language - an XML-based language for Describing the Geometries).

Prepared program allows to speed up work in modeling, as well as facilitates applying changes to existing models. Thanks to drag and drop method, it is not required to consider the location of each object in numerical form. Parameters, such as the size and location will be saved in a file by the program. In conclusion, the user's work is focused primarily on the visualization and the geometry description is produced as an output from the module.

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76. ISOLATION AND CHARACTERIZATION OF MESENCHYMAL STROMAL CELLS DERIVED FROM BONE MARROW AND ADIPOSE TISSUE OF MICE

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Mesenchymal stromal cells (MSCs) are nonhematopoietic multipotent cells. The best sources of MSCs are mesodermal tissues such as the bone marrow or adipose tissue. Murine MSCs have the adhesive ability to plastic dishes and express the antigens such as CD29, CD44, CD90 or Sca-1. MSCs differentiate into three cell lines: adipocytes, chondrocytes and osteoblasts. Moreover, these cells possess immunomodulatory properties, produce a number of growth factors and cytokines, as well as exhibit tropism for areas covered by the inflammatory reaction.

Cells were isolated using a collagenase solution and then grown in IMDM medium containing 10% Fetal Bovine Serum. Phenotype of the cells was verified by flow cytometry. For confirmation of tropism to GL261 cells the Boyden chamber migration assay was used. MTT assay was performed to investigate the influence of MSCs on mouse melanoma tumor growth.

In this study the isolation of MSCs from murine bone marrow and adipose tissue method was developed. Isolated cells have the phenotype of murine mesenchymal stromal cells, show morphological similarity to fibroblasts and differentiate into adipocytes and osteoblasts. Obtained cells produce the factors that influence the growth and proliferation of murine melanoma cells *in vitro* and also show the ability to form vascular-like structures in matrigel. Isolated cells exhibit tropism for glioma tumor cells and accelerate murine melanoma tumor growth *in vivo*.

77. INFLUENCE OF DERMATAN SULFATE ON NORMAL HUMAN CELLS

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Dermatan sulfate (DS) is a glycosoaminoglycan (GAG) present in skin, blood vessels, heart valve, bones and cartilages. It is involved in infection, tumorigenesis, growth, development and wound repair - it regulates the coagulation cascade by binding to heparin cofactor II, thrombin and activated protein C [1, 2]. Several publication presented DS interaction with keratinocyte growth factor and fibroblast growth factor [3, 4]. Delehedde et al. showed that viability and proliferation of immortalized aneuploidy human keratinocyte are increased in the presence of dermatan sulfate [5]. Similar effect was observed for human fibroblasts [4].

Dermatan sulfate is composed of repeating disaccharide units built from L-iduronic acid and N-acetylgalactosaminidase and it is accumulated in several of the mucopolysaccharidosis disorders (MPS), especially in MPS type VI. MPS VI is caused by deficiency of arylfulfatase B, one of key enzymes involved in DS degradation.

The aim of this study was to investigate the influence of different, empirically-chosen dermatan sulfate concentrations on human pulmonary artery smooth muscle (hPASM) cells with functional and silenced arylsulfatase B gene.

In this work depletion of endogenous *ARSB* was achieved by siRNA transfection. Cell viability and intracellular calcium level were determined by MTS assay and fluorescent calcium indicator Fluo-4, respectively.

The obtained results suggest that high levels of dermatan sulfate may slightly increase hPASM cells viability. It has also showed that dermatan sulfate or ARSB level did not influence on intracellular calcium concentrations.

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78. NEW POTENTIAL CONTRAST AGENTS FOR MRI

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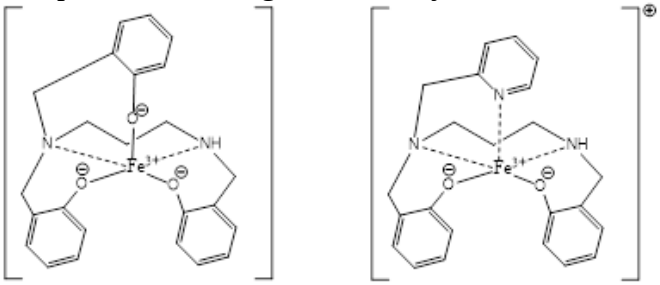
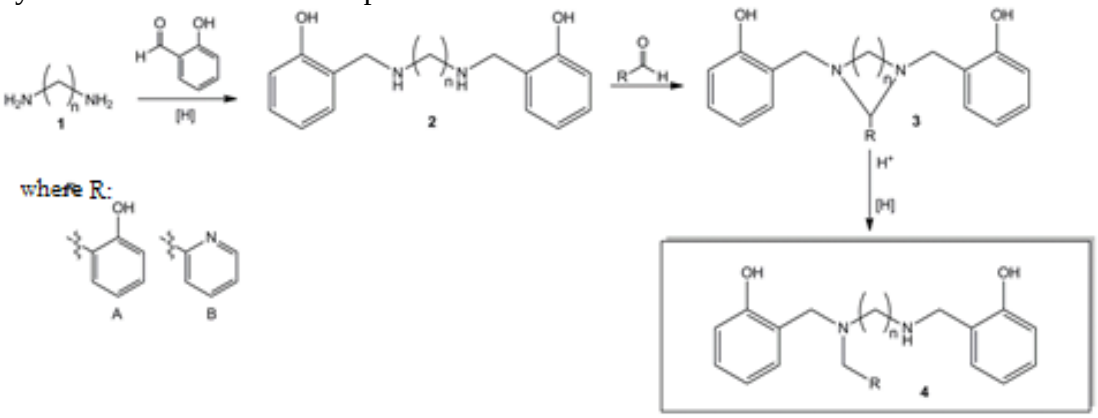
Modern diagnostic methods should allow early detection of cancer by means of non-invasive diagnostic methods. Magnetic resonance imaging (MRI) is currently one of the most important diagnostic methods. Clinical application requires the use of effective and safe contrast agents. Currently used gadolinium contrast agents have limited safety for human health. The solution to this problem requires the use of new contrast agents based on endogenous metal ions. We investigated the application of iron(III) complexes as new contrast agents for MRI [1].

For this purpose, a series of pentadentate asymmetric multiamino-phenolic ligands were synthetized. Based on two models (Scheme 1), in which the increased number of carbon atoms in the alkyl chain we examined the relationship between the stability constant of the complex and the length of the alkyl chain.



Scheme 1

The main element of the project was the synthesis of unsymmetrical ligands (4). Several synthetic pathways of target ligands, one of which is based on reductive amination of simple symmetrical diamines (1) 1,3-diazacycle form intermediate (3). Which in the last step is subjected to selective opening and a reduction (Scheme 2) [2]. The intermediates have been isolated and their structures confirmed by X-ray studies. An alternative approach of the synthesis involves selective protectition of the diamine.



Scheme 2

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79. THIONOCARBAMATES – FUNGICIDAL ACTIVITY

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In recent years, increased interest in combatting fungal infections can be noticed. The incidence of fungal infections continues to increase in hospitalized patients, HIV- positive individuals/AIDS patient, immunocompromised and immunocompetent patients, organ transplant recipients [1-2]. Increasingly, fungal infections is observed that are caused by different pathogens. They cause increased incidence of morbidity and mortality in infected patients. Most often these infections are caused by *Candida* spp., *Aspergillus fumigatus* and *Cryptococcus neoformans*, rarely by: *Zygomycetes*, *Fusarium* spp, *Trichosporon beigelii*, *Blastoschizomyces*, *Scedosporium*, *Acremonium* [3]. Further is a visible increase the development of antifungal agents, due to the increase of resistance emerging pathogenic fungi [5] Successful treatment of fungal infections depends on the proper selection of appropriate antifungal preparations. In recent years, several dozen antifungal drugs were introduced but many of them were later withdrawn, because they caused serious side effects. [4-5].

N-Allyl and N-aryl thionocarbamates are an important class of compounds of interest due to their numerous biological effects including anesthetic, fungicidal, bactericidal, pesticidal and antiviral [1-5]. Thionocarbamates as fungicides have been extensively studied since 1960 [5]. However, little attention had been given to N-allyl and N-aryl thionocarbamates derivatives alcohols until Thorne studied a wide variety of derivatives of thiocarbamic and carbamic acids. Tolciclate and Tolnaflate are compounds belonging to the class thionocarbamates and commercially used as antifungal agents. Tolnaftate and Tolciclate blocked sterol biosynthesis in fungal cells and cell extracts, with accumulation of squalene.

To study the possibility of improving the fungicidal activity of the known thionocarbamates, in the present work a series of N-alkyl, N-phenyl and N-benzyl thionocarbamates derivatives alcohols were synthesized, and their fungitoxic activity was 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

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80. ROLE OF MECHANICAL AND MAGNETIC PROPERTIES OF MMM MEMBRANES IN THE AIR SEPARATION PROCESS

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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. One of them are polymer composites, called mixed matrix membranes (MMMs), which combine the selectivity of fillers and the simplicity of polymer membrane processing. The dispersion of impermeable particles in polymers could change and improve the electrical, magnetic, mechanical and gas separation properties of membranes [1, 2].

Over the last few years, our research had concentrated on magnetic membranes used for the air enrichment in oxygen. This work is the continuation of our earlier research [3-5], where we have found that incorporation of magnetic micropowders into the polymer matrix improved the gas transport properties of membranes. We have examined the influence of mechanical (conventional yield plasticity, clear boundary plasticity, tensile strength and Young modulus) and magnetic parameters (coercivity, remanence and saturation magnetization) on gas separation properties of various types of magnetic membranes (EC and PPO with dispersed magnetic powders, like MQP-14-12, MQP-16-7 and MQP-B with a particle size 5, 25 μm, below 20 μm and 20-50 μm). The results showed that membrane's coercivity depended on composition and microstructure of the magnetic powder. On the other hand, remanence and saturation magnetization increased with the increase of the powder addition in the membrane. It was found that the magnetic membrane's gas transport properties were improved with the increase of membrane's remanence, saturation magnetization, magnetic neodymium particle filling and decrease in powder particle size. It was observed that the magnetic EC and PPO membranes had higher gas permeability and diffusivity, while their permselectivity and solubility were rather maintained or slightly increased. It was also analyzed the dependence of the drift coefficient *w* on the magnetic parameters of investigated membranes. The increase of the magnetic powder addition and decrease of its granulation improved mechanical properties of the tested membranes. This also had a positive effect on their gas separation properties.

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81. THE APPLICATION OF ELECTROPORATION DUE TO EFFECTIVE BLEOMYCIN DELIVERY IN "DIFFICULT TO TREAT" HUMAN OVARIAN CELL LINES

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The electroporation method is currently applied to enhance the transport of various molecules such as genes, ions or drugs. During EP process permeabilization of the membrane is induced and more intense drug transport is possible. The combination of electroporation (EP) and chemotherapy is one of the new technique called electrochemotherapy (ECT).

Ovarian cancer is one of the most lethal gynecological tumors. The prognosis remains poor due to ovarian cancers are diagnosed in advanced stages. Thus there is a need for new therapeutic methods.

The aim of our investigation was the evaluation of the electrochemotherapy effectiveness in contrast to standard chemotherapy with bleomycin in two varian cancer cell lines. Two human ovarian cells lines were used: OvBH-1- human clear ovarian carcinoma with silent mutation of P53 gene and resistant to chemo- and radiotherapy and SKOV-3 line - human ovarian carcinoma cells resistant to diphtheria toxin, cisplatin and Adriamycin. In EP and ECT experiments were used different voltage values: from 0 to 1200 V/cm, 8 pulses with duration of 100μs and intervals between pulses 1s. Cells were treated with bleomycin in various concentrations (from 1 to 300 nM). The cells viability after applied treatments was evaluated by MTT assay. The expression of heat shock proteins - HSP27 was examined by immunocytochemical ABC method.

The cytotoxicity with different concentrations of bleomycin was not significant decreased in both cell lines. The highest decrease was observed after EP with bleomycin after 48h of incubation for 1000 (V/cm). The intensity of expression of small heat shock proteins HSP27 slightly increased after ECT in both treated cell lines in particular in OvBH-1. The results indicated that electroporation effectively supports chemotherapy with bleomycin on human ovarian cells in vitro.

Keywords: ovarian cancer, bleomycin, electroporation, HSP27

82. THE EVALUATION OF THIO-DISACCHARIDES GENOTOXICITY IN HUMAN CERVIX ADENOCARCINOMA (HELA) CANCER CELL LINE

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Thio-sugars possess important biological properties including resistance to carbohydrate degrading enzymes and enzyme inhibitory activity due to presence of sulfur atom, which distinguish them from their oxygen analogs. Recent evidence suggests that these compounds may have the therapeutic potential in the treatment of cancer and infectious diseases. In our research we turned our attention to novel synthetized thio-disaccharides containing biologically important sugar moieties such as glucose and galactose linked with sulfur bridge. These thio-disaccharides were cytotoxic to human cervix adenocarcinoma (HeLa) in micromolar concentrations, but the exact mechanism of their action is unknown. Our previous data suggests that anticancer properties of thio-sugars may be linked with their DNA damaging potential.

The aim of our project was to determine genotoxic potential of thio-sugars on HeLa cell line. We have investigated three thio-disaccharides: 1,6-anhydro-3-deoxy-4-*S*-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-4-thio-β-D-*erythro*-hexopyranos-2-ulose (denoted as TVI);1,6-anhydro-3-deoxy-4-*S*-(β-D-glucopyranosyl)-4-thio-β-D-*erythro*-hexopyranos-2 ulose (denoted as TVII); 1,6-anhydro-3-deoxy-4-*S*-(β-D-galactopyranosyl)-4-thio-β-D-*erythro*-hexopyranos-2-ulose (denoted as TIX). HeLa cell line was cultured in DMEM (BioWhitaker) medium supplemented with penicillin-streptomycin mix (Sigma) and 10% fetal bovine serum (BII). Cells were incubated in humidified incubator at 37°C, 5% CO_2 and passaged at 80% confluence. Genotoxicity of the selected thio-sugars was analyzed after one hour incubation -with HeLa cells. All experiments were repeated at least three times. We used three types of comet assay to evaluate the level and type of DNA lesions induced by thio-disaccharides. We also used a neutral version of comet assay to detect DNA double strand breaks (DSB), single strand breaks (SSB) was detected by unwinding and electrophoresis at pH 12.1 and alkaline version of comet assay were used to detected alkali labile sites (ALS).

All the tested thio-disaccharides are genotoxic at designated concentrations in dose dependent manner. We observed only ALS and SSB, nor DSB's. There was no significant difference between tested compounds containing glucose or galactose as one sugar moiety and between compounds with four hydroxyl group (VII) in the sugar ring functionalized by the acetyl group (VI). ALS and SSB effect could be the results of oxidative stress induced by thio-disaccharides. Further detailed analysis is under intense investigation in our laboratory.

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83. DIFFERENCES IN EXPRESSION OF SIGNIFICANT METALLOPROTEINASES IN ECM OF LARYNX CANCER

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Metalloproteinases (MMPs) are proteases degrading extracellular matrix (ECM) proteins such as collagens, elastin, proteoglycans and laminins. They can be functionally broadly divided into collagenases, gelatinasis, stromelysins and membrane-type MMPs. The high expression of MMPs in cancer can alter the cancer cells environment favoring tumor progression and the occurrence of metastasis. In many neoplasms their expression is correlated with tumor invasiveness. Their expression is thought to be a negative prognostic factor.

The aim of this study was to analyze the expression pattern of metalloproteinase family members in larynx cancer.

Larynx cancer and normal larynx samples were obtained from patients during surgery. The samples underwent routine histopathological examination and were subsequently classified as G1, G2 and G3 grade or control. Total cell RNA was extracted with Trizol® reagent and purified. After quality and quantity control the samples were selected for further analysis. The obtained mRNA was hybridized with HGU-133A 2.0 Plus microarrays (Affymetrix). QRT-PCR was performed for metalloproteinases selected after microarray analysis. The statistical evaluation of the microarray experiment was made in Gene Spring 13.0 with ANOVA and Tukey *post hoc* tests with Benjamini-Hochberg correction. The results from QRT-PCR were analyzed using Statistica 11.0 software. The first step was to select ECM related genes from the Affymetrix database. The ANOVA results gave 252 ID mRNAs statistically significant and MMP1, MMP9, MMP13 were selected for QRT-PCR because of their high expression level in cancer tissue. The MMP1, MMP9 and MMP13 were highly over-expressed and probably influenced the cancer microenvironment in larynx cancer thus contributing to its progression.

84. INFLUENCE OF AMPHOTERICIN B AND ITS MODIFIED FORMS ON EXPRESSION PROFILE OF MELATONIN RELATED GENES IN RPTEC CELLS

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The presence of membrane melatonin receptors in kidney cells may indicate repair mechanisms induced by the nephrotoxic effect of amphotericin. Melatonin is a hormone involved in many physiological and pathological process. Melatonin acts through receptor dependent and independent mechanism. Receptors belong to the G protein-coupled receptor superfamily and are divided into two groups: MT1 and MT2. Amphotericin B is a polyene antifungal drug. However, it also exhibits strong toxicity, including DNA damage. Numbers of experimental and clinical studies have been aimed at the prevention of AmB nephrotoxicity. Last studies have shown that complex of AmB with copper ions (II) (AmB-Cu2+) reveals weaker toxicity comparing to amphotericin B. The aim of this study was to evaluate influence of amphotericin B on melatonin related genes expression in human RPTEC (Human Renal Proximal Tubule Cells) cells.

Based on cytotoxicity test results RPTEC cells were treated with 0.5μg amphotericin B and AmB-Cu2+ complex per ml of medium. The extraction of total RNA was performed with the use of phenol-chlorophorm method. The expression profile of 66 genes related to activity of melatonin receptors was appointed using oligonucleotide microarrays HG-U133A 2.0 (Affymetrix). Differentiating genes were selected with the use of GeneSpring 12.0 and PL-Grid platforms ($p<0.05$ and $FC>1$).

Analysis showed one differentially expressed gene in group of cells treated with AmB, comparing to control. RGS4 gene, which expression was decreased, is responsible for regulation of GTP-ase activity of G protein alpha subunit. It was proven that protein product of gene RGS4 can modulate melatonin receptors (MT2) activity. Five genes were specific for cells treated with AmB-Cu2+ complex. There was also one gene specific for group of cells treated with Fungizon, amphotericin deoxycholate.

Effect of amphotericin B on kidney cells could be related to regulatory pathway induced by melatonin. We observed different expression profile of genes in individual groups.

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85. EXPRESSION PROFILE OF CASPASE ACTIVITY-RELATED GENES IN RPTEC CELLS TREATED WITH AMPHOTERICIN B-COPPER(II) COMPLEX AND OXIDIZED FORMS OF AMPHOTERICIN B

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From year to year the number of very difficult to treat, fungal infections increases. One of the most commonly used antifungal drug is amphotericin B - polyene antibiotic. Unfortunately, the mechanism of amphotericin B action is not selective and leads to a number of adverse effects, including nephrotoxicity. It is possible that amphotericin induces apoptosis by influence on the expression of genes encoding caspases. Caspases are intracellular enzymes from the group of cysteine proteases that play a central role in apoptosis. There are numerous studies being conducted over the modification in the structure of amphotericin in order to reduce its toxicity.

The aim of the study was to evaluate the effect of pure amphotericin B and its modified forms on the expression of genes associated with caspase pathway.

RPTEC cells (Human Renal Proximal Tubule Cells) were treated with 0,5μg amphotericin B (A), fungizon (F), amphotericin B-copper(II) complex (AC) and oxidized forms of amphotericin B by Fe3+ iron(III) (AF) per ml of medium. Total RNA was extracted using phenol-chlorophorm method. The expression profiles of genes related to caspase activity were determined using oligonucleotide microarrays (HG-U133A 2.0, Affymetrix).

Analysis of 67 ID associated with the caspase pathway indicated only 4 differentiating ID for CASP5 and NOL3 genes. Expression of CASP5 was higher in all samples treated with amphotericin in comparison to control cells, but significant increase was observed only in cells treated with A and AC. NOL3 expression was decreased in all treated cells.

Amphotericin can induce apoptosis by increasing the expression of caspase 5 and NOL 3 gene silencing, which is an inhibitor of this process. However, the clear identification of the mechanism of toxicity of amphotericin requires further study .

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86. DISTRIBUTION OF REACTIVE OXYGEN SPECIES IN THE CELL

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Reactive oxygen species (ROS) are chemically reactive molecules that contain oxygen and are important players in the regulation of cellular signaling and energy production processes. The main producers of ROS in living cells are mitochondria, complex I and III of the cytochrome chain, where superoxide anions may appear as byproducts (rev. in [1]). Another important source of intracellular oxidants are oxidases such as membrane-bound NADPH-dependent oxidases (Nox1–5 and Duox1–2), which are widely expressed and evolutionarily conserved [2,3] and whose only known function is the regulated generation of ROS. The levels of cellular ROS influence the oxidized or reduced states of cellular proteins which create redox-signaling pathways, activating some transcription factors, kinases, and phosphatases ([4,5] and reviewed in [1]). ROS levels are significantly increased by some external factors such as ionizing and UV-A radiation and chemical compounds, and these increased levels cause oxidative damage to nucleic acids [6].

We observed that cells exposed to ionizing radiation start to produce increased levels of ROS 12-24 h after treatment [7]. In the present work, using confocal fluorescent microscopy and a specific probe detecting superoxide anions (Mitosox), we studied the distribution of superoxide between the cytoplasm and the nucleus in control and irradiated cells. In nuclei of unirradiated cells of different types we observed fluorescent foci suggesting the existence of nuclear superoxide sources.

Analysis of the global levels of cellular, nuclear, and cytoplasmic superoxides in irradiated and control cells showed increased levels of nuclear superoxide in comparison to cytoplasm which were not observed in control cells, suggesting a possible role of ROS in regulation of cellular responses to radiation.

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87. INFLUENCE OF VARIOUS DRUG FORMS OF AMPHOTERICIN B ON HISTAMINE RELATED GENES IN RPTEC CELLS

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Amphotericin B is a golden standard in treating systematic fungal diseases. However therapy with amphotericin B is associated with infusion-related reactions (IRRs) and side effects of nephrotoxicity. Little is known about the molecular mechanism underlying observed renal tubular toxicity. One of the possible mechanism of amphotericin B-induced nephrotoxicity is related to various pro-inflammatory effects resulting from stimulation of Toll-like receptors (TLR), e.g. raised cytokine levels. Amphotericin B is dosed in various drug forms to reduce risk of side effects and their severity. Biogenic amine - histamine - exerts many biological effects. It acts through foursubtypes of G-protein-coupled receptors (H1-4), expressed in various cell types and tissues. Histamine is synthesised by mast cells and other cell types, including kidney (proximal tubule).

The aim of this study was to assess influence of various drug forms of amphotericin B on histamine related genes expression in human RPTEC (Human Renal Proximal Tubule Epithelial Cells) cells.

RPTEC cells were treated with four different forms of amphotericin B: copper complex, AmB oxidized by iron, fungizone and pure amphotericin B (0,5μg AmB/ml of medium). The concentration of drug was selected based on cytotoxicity test. Total RNA was extracted with the use of phenol-chlorophorm method. The expression profile of histamine related genes was appointed using oligonucleotide microarrays HG-U133A 2.0 (Affymetrix). Comparative analysis of transcriptomes was performed with the use of GeneSpring 12.0 and PL-Gridplatforms. Differentiating genes were considered when $p<0,05$ and $FC>1$.

Statistical analysis revealed that 15 genes were differentiating every Amphotericin B form with control samples. However only 8 genes' expression was changed differently between each tested drug form.

Amphotericin B influence on histamine related genes in RPTEC cells differs between examined forms of drug. Nevertheless pure Amphotericin B has the weakest influence on histamine related genes. Molecular mechanism of renal tubular toxicity caused by amphotericin B needs further studies.

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88. EXPRESSION PROFILE OF SUPPRESSOR GENES IN CELLS TREATED WITH AMPHOTERICIN B AND ITS MODIFIED FORMS

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Amfotericin B is a polyene antifungal drug which exhibits strong toxicity, including DNA damage. Suppressor genes participate in regulation of cell damage and apoptosis. Increase in their expression results in growth inhibition and cell division arrest. Amphotericin B exert strong nephrotoxicity which could be caused by DNA damage in renal tubule cells. During the last few years, some randomized trials have tested different strategies to reduce AmB-induced renal toxicity. During the last few years some experiments have been tested different strategies to reduce AmB-induced renal toxicity e.g. complex AmB with copper.

The aim of the study was to determine whether in RPTEC treated by modified forms of amphotericin B expression profile of suppressor genes has changed.

RPTEC cells were treated with 0.5μg amphotericin B, AmB-Cu2+ complex and oxidized AmB per ml of medium. Total RNA was extracted with the use of TRIZOL, according to manufacturer protocol. Gene expression profile was evaluated by oligonucleotide microarray HG-U133A 2 (Affymetrix). Comparative analysis included 2744 ID of suppressor genes mRNA.

Statistical analysis ANOVA showed 131 genes with $p<0.05$. Post hoc analysis showed 56 differentially expressed genes. Among them 17 genes are specific for cells treated with AmB, 11 for cells treated with oxidized AmB and 21 for cells treated with AmB in complex with Cu2+ ion. These genes are involved in regulation of apoptosis, cell survival and MAPK activity.

Amphotericin B modified forms change expression profile of suppressor genes. These results suggest participation of suppressor genes in amphotericin-induced nephrotoxicity, depending on modified form of this drug.

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89. COMPUTATIONAL METHOD FOR MODELING OF TRANSCRIPTION FACTORS BINDING SITES

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Transcription factor binding sites are involved in many intracellular processes. This is the reason why new methods of detecting TFBSs structures in DNA are sought. These approaches can be divided into experimental techniques and computational tools. Transcription factor binding sites are commonly represented by Position Weight Matrices (PWMs).

In this study we presented a computational approach, which allowed us to create PWMs based on the statistical potential. The focus of this work is on the members of NF-κB family: p50p50, p50p65 and p50RelB. This method is a modification of Alamanova et al.[1] approach. First of all, we used different statistical potential to estimate energies in TF-DNA complexes. We based our computation on a volume-fraction corrected DFIRE - based energy function [2], while Alamanova et al. used potential developed by Robertson and Varani [3]. Moreover, we created an algorithm of improving the PWMs quality, which allowed us to increase the chance of detecting experimentally verified transcription factor binding sites by models. The promoter sequences were scanned with *NucleoSeq* 2.0[4] to detect TFBSs. We discovered connection between values of statistical potential parameters and the number of detecting binding sites. We prepared positive set - 58 experimentally verified TFBSs in 41 human promoter sequences and four negative sets. Proposed approach is based on the receiver operator characteristic (ROC). Additionally, we analyzed values of the area under the curve (AUC). We also compared PWMs calculated by our method, Alamanova et al. approach and experimental models from TRANSFAC database.

Logos (graphical representation of PWMs) created for analyzed models show significant similarity. What is more, matrices detected comparable number of TFBSs. The obtained results suggest that computational method could replace laboratory techniques in the future.

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90. THE MECHANICAL PROPERTIES OF THE KERATINOCYTES TREATED WITH UVB RADIATION

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The top layer of the human skin consists of four cell types, where the majority are keratinocytes. Therefore, these cells are exposed to many external factors including ultraviolet radiation. It is well known that the UVB radiation is the main cause of non-melanoma skin cancer, which originates from keratinocytes [1]. If the UVB photons are absorbed by the keratinocytes cells, chromophors like DNA, RNA and proteins convert into chemically modified products. Next, different signal transduction networks are initiated and the cell is directed to one of the two paths: repairment or death through apoptosis. The escape of a mutated cell from the mentioned paths results in increased skin carcinogenesis [2].

Early diagnosis of cancer is a very important subject considering the mortality rate of patients suffering from these diseases. As many as 10 % of all malignant tumors are the non-melanoma carcinomas of the skin. Moreover, the detection of cancer on a single-cell level may enhance the number of survivors and facilitate the treatment of patients. Changes in the cells morphology, elasticity and DNA may also suggest the occurrence of malignant transformation [3]. Such investigations can also help in the understanding of the many physiological processes like differentiation, proliferation and adhesion of cells [4].
For the studies the atomic force microscope (AFM) and fluorescence microscopy are used to analyze the effect of UVB radiation on the morphology and changes in the mechanical properties of keratinocytes. These combined studies revealed dose-dependent changes in the elasticity of the cells. The highest dose of UVB radiation (100 mJ/cm^2) significantly decreased the viability and the stiffness of keratinocytes cells. Also the fluorescence microscopy revealed changes in the organization of cytoskeleton in the radiated cells. Considering all the collected data, this approach is appropriate for tracking the UVB-induced changes in the cell elastic properties under physiological conditions.

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91. HSPA2 ROLE IN NON-SMALL CELL LUNG CARCINOMA CELLS

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Human HSPA2 was considered, for a long time, as a testis-specific and not heat-inducible member of the heat shock protein HSPA (HSP70) family. Recently, we and others have shown that HSPA2 can be expressed in various types of human malignancies as well as in certain types of normal somatic tissues. In our previous studies we found that HSPA2 is frequently expressed in non-small cell lung carcinoma (NSCLC) cell lines. Also, we have shown that high expression of HSPA2 in primary tumors is related to poor prognosis in NSCLC patients. However, the function of HSPA2 in normal and cancer cells is still very poorly characterized.

This study was designed to characterize the role of HSPA2 in NSCLC. NCI H358 cells (adenocarcinoma cell line) were infected with retroviral vectors encoding shRNA to down-regulate the *HSPA2* gene expression. We found that silencing of the *HSPA2* gene did not affect cells proliferation, viability and clonogenic potential. In order to evaluate if decreased HSPA2 expression affects sensitivity of NSCLC cells to anticancer drugs we performed short- and long-time survival assays. We observed positive correlation between the HSPA2 level and resistance of NCI H358 cells to cisplatin. HSPA2-depleted cells were more sensitive to cisplatin-induced cell death than control cells expressing non-targeting shRNA. We also found by flow cytometry-based apoptotic assays that number of apoptotic cells is significantly higher in cells with reduced HSPA2 expression.

To sum up, our preliminary study shows that HSPA2 may play an important role in NSCLC cells response to anticancer drugs, namely to cisplatin. However, our findings should be further verified by extended analysis of several other NSCLC cell lines.

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92. FRACTIONAL DIFFUSION EQUATIONS IN THE DESCRIPTION OF ANOMALOUS TRANSPORT THROUGH MAGNETIC MEMBRANES

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One of the most significant applications of fractional-order derivatives is modelling of diffusion in a specific type of medium (i.e. non-crystalline, disordered materials). The order of the resulting equation is related to the fractal dimension d_f and the fractal dimension of the trajectory of the random walk d_w. Several authors suggested partial differential equations of fractional order for the description of transport processes in fractals [1].

Recently [2-4], 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. Examined membranes are inhomogeneous i.e. there are some molecular clusters of different size which definitely can influence on the membrane properties. The transport properties of membranes depends on many parameters such as: used polymeric matrix, type of powder, its amount and granulation. Description of our complex system bases on the phenomenological, and molecular (random walk on a fractal lattice) approaches. Determined parameters like: anomalous diffusion exponent α static fractal dimension d_f and fractal dimension of the trajectory of the random walk d_w, are implemented to the generalized diffusion equation proposed by Metzler et al.[5].

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93. SCREENING AND IDENTIFICATION OF BIOSURFACTANT AND/OR BIOEMULSIFIER PRODUCING BACTERIA ISOLATED FROM HYDROCARBON-CONTAMINATED MARINE SEDIMENTS. CHARACTERIZATION OF THE MOLECULES

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Biosurfactants are amphiphilic compounds defined as structurally heterogeneous groups of surface-active molecules synthesized by microorganisms, which can be divided in low- and high-molecular weight compounds. The first ones, biosurfactants (BSs), are mostly glycolipids and lipopeptides, which showed decrease surface tension capability, while the second, called bioemulsifiers (BEs), are lipopolysaccharides and lipoproteins and are less effective on lowering the surface tension, but are highly effective on emulsion production. In the last years with increasing environmental and modern industrial awareness and emphasis on a sustainable society in harmony with the global environment, surfactants produced by living cells are getting huge attention because of their improved properties compared to their chemical counterparts as biosurfactants have low toxicity, high biodegradability, ecological acceptability, specific activity at extreme temperatures, pH and salinity and can be produced from renewable substrates. The study of new biosurfactant, with higher activities and/or yields, and their microbial producers is therefore needed to overcome these bottlenecks. An important source of new microorganisms is marine environment. Marine microorganisms develop novel metabolites or produce external substances which are rarely present in earthy origin and allow them to live in such extreme conditions.

The aim of this work was screening of bacteria, isolated from marine environment contaminated by hydrocarbons, for their capability to produce biosurfactants and/or bioemulsifiers. In order to obtain information about presence of BS/BE Drop collapse test and Emulsification Index Assay were determined. The emulsion stability was measured for up to two months and then BE from the best producers were characterized for the influence of extreme conditions, like high concentration of salt, high temperature and pressure during autoclaving and crude oil used instead of other solvents, in the emulsion capabilities. In the end, a preliminary investigation of the genetic diversity of the best BS/BE producers strains was performed through the comparison of the length of the intergenic transcribed spacer (ITS) region between the prokaryotic 16S-23S rDNA genes.

The results show that out of 79 isolates 34 morphologically different ones were able to efficiently growth in liquid medium supplemented with $1\%_{w/v}$ of glucose and were preliminary screened for their capability to produce biosurfactants/bioemulsifiers. In this work, it has been used an experimental approach which reduce time and costs for screening of novel BS/BE-producing bacteria. Out of 34 strains 32 (94%) showed positive results in at least one screening test. 18 isolates (52.9%) showed both biosurfactants and bioemulsifier abilities, 5 strain exhibited biosurfactant capabilities and 9 produced biosurfactants with only emulsion ability, with a stability over 2 months. Moreover, the extracellular biosurfactants produced by the best 17 marine isolated strains showed good stability at high temperature and pressure and also at high salt concentrations. Finally, 7 isolates could emulsify crude oil. From the best 17 biosurfactant and/or bioemulsifier producers thanks to 16S ITS-PCR it was obtained 12 different taxa.

94. NF-κB-DEPENDENT RESPONSE TO IONIZING RADIATION

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Signalling pathways that depend on p53 or NF-κB transcription factors are essential components of cellular response to stress. Both proteins, which participate in regulation of the expression of numerous genes involved in cell cycle arrest, DNA repair, apoptosis, immune response and inflammation, can be activated by the same stimuli and the final cellular outcome is determined by the crosstalk between them. Here we aimed to characterize the effect of ionizing radiation (IR) on regulation of expression of genes controlled by these transcription factors.

U2-OS human osteosarcoma cell line was used as an experimental model. The clonogenic assay was performed to characterize its radiosensitivity, and then two doses: 4 Gy and 10 Gy (LD_{50} and LD_{100}, respectively) were used for further analyses. Activation of the NF-κB and p53 pathways was monitored by Western-blotting. Expression of selected target genes dependent on NF-κB and/or p53 was assessed by qRT-PCR at different time points after irradiation (from 30 minutes to 24 hours). Analysis of RelA (NF-κB subunit) and p53 binding to promoter regions of selected genes was performed using ChIP-qPCR.

Moreover, analysis of the influence of RelA and/or p53 on expression level of genes induced by ionizing radiation was performed. With application of siRNA approach, two additional cellular variants (with down-regulated RelA or p53) were obtained. In order to compare transcriptional response induced by different stimulating factors, all cells were either irradiated or treated with TNFα cytokine and expression of selected target genes was assessed.

Exposure of the U2-OS cells to ionizing radiation resulted in strong activation of both NF-κB and p53 pathways, which was observed as IκBα phosphorylation (Ser32) and subsequent NF-κB nuclear translocation, and by p53 accumulation (irradiation with 10 Gy resulted in faster and stronger activation of NF-κB). As a consequence, both transcription factors were recruited to promoter regions of analyzed target genes (e.g. *IL8*, *CDKN1A*) and expression level of dependent genes was increased.

Moreover, down-regulation of RelA or p53 affects expression levels of (selected) target genes in the way depending on stimulating factor (either genotoxic stress or inflamatory stimuli).

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95. PHARMACOGENETIC MODELS OF BREAST CANCER PATIENTS ADVERSE REACTIONS TO FAC CHEMOTHERAPY

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FAC is the most common breast cancer chemotherapy regime. It combines 5-fluorouracil, doxorubicin and cyclophosphamide, which on the cellular level are responsible for genetic material damage leading to cell cycle checkpoints activation and cell death. Cytotoxic drugs aim the intensively proliferating cancer cells, but 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. Furthermore, these adverse reactions are also observed in relatively slow proliferating tissues in organs involved in systemic detoxification and clearance, i.e. in liver and kidneys. Because of the complexity of cellular pathways of each FAC drug, it is clear that molecular mechanisms of adverse reactions to these three drugs are extremely complicated.
Relationships between genetic polymorphisms and chemotherapy-induced toxicity were analyzed in group of 200 breast cancer patients treated with FAC regime in first-line chemotherapy. 13 genetic modifications were selected for this study, including functional variants in genes encoding proteins involved in FAC drugs transport (ABCB1, ABCC2, ABCG2), metabolism (CYP2C19, GSTT1, GSTM1, GSTP1, TYMS, MTHFR) and drug-induced damage repair (ERCC1, ERCC2, XRCC1).

Our results show that genetic variants responsible for high risk of treatment toxicities belong to transport and metabolic pathways of all three FAC drugs, which confirm the complex causative network of factors underlining systemic adverse reaction to treatment.

Multifactoral pharmacogenetic models were possible to establish for treatment-related overall and early anemia (low hemoglobin as well as anisocytosis), hepatotoxicity and gastro-intestinal symptoms (nausea). Furthermore, accumulation of the unfavorable genotypes was responsible for drastic increase of risk of overall and early anemia, anisocytosis, overall hepatotoxicity and early nausea. Potentially life-threatening severe neutropenia correlated with only one genetic polymorphism, but nonetheless we observed over twofold increase in toxicity risk for common allele of VNTR variant in TYMS gene.

The results suggest, that accumulated modifications in transport, metabolic and damage repair FAC drugs pathways are strongly responsible for systemic unfavorable reaction to treatment. It is believed, that comprehensive model consisting of many genetic alterations could make a promising potential predictive tool of treatment-related toxicity for the 5-fluorouracil, doxorubicin and cyclophosphamide-treated breast cancer patients.

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96. CISPLATIN-BASED CHEMOTHERAPY AND COPPER TRANSPORT SYSTEMS

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Copper is the trace element essential for the proper functioning of the cells because of its role as cofactor of many crucial enzymes, such as cytochrome c oxidase, superoxide dismutase and lysyl oxidase. Cellular transport system ensures the exact distribution of copper throughout the body and consequently its malfunction could lead to serious medical conditions, such as Menkes and Wilson disease. Apart from copper transport this system is used to move platinum and its derivates through the cell and body- including the widely used chemotherapeutic drug cisplatin. It is therefore believed that polymorphic variants in genes encoding the importer (CTR1) and intracellular exporters via the TNG network (ATP7A and ATP7B) could alter the drug availability and its therapeutic concentration. As the result of such alterations cisplatin resistance or oversensitivity could be developed leading to cancer therapy failure and/or serious deterioration of patients' condition. Similar consequences could also be the result of modifications in genes encoding multidrug and toxin extrusion proteins (MATE family). These efflux transporters are not the part of the main copper transport system, but are crucial for efficient elimination of toxins, including copper and platinum drugs, in liver and kidneys.

Impact of genetic polymorphisms in copper transport systems on the response to cancer treatment was analysed in the group of 129 women diagnosed with epithelial ovarian cancer receiving cisplatin-based first-line chemotherapy. For this study we selected 11 functional variants in CTR1, ATP7A, ATP7B, MATE1 and MATE2-K genes.

The results show that decrease of platinum importer CTR1 expression, as the consequence of intronic rs12686377 variant, leads to platinum-resistant phenotype and significantly rises the risk of death (HR 2,62; p=0,005). On the other hand three functional variants in ATP7B transporter gene are responsible for treatment-related, both overall and early, neutropenia. Furthermore the risk of bone marrow damage increases with the accumulation of unfavourable genotypes, reaching OR 7,89 for overall and OR 27,33 for early neutropenia. Chemotherapy-induced liver damage seems to correlate with rs2289669 variant in hepatocytes guardian MATE1, which in combination with polymorphism p.Arg72Pro in TP53 gene is responsible for over 17-fold increase of hepatotoxicity risk.

The results indicate that efficient platinum influx to the cells is crucial for positive reaction to treatment and patients' longer overall survival. Cisplatin-induced toxicity on the other hand seems to be dependent on the management of the drug's concentration- by both intracellular transport (ATP7B) and extrusion (MATE1) systems.

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97. HSF1-DEPENDENT DOWN-REGULATIONOF VINCULIN IS RESPONSIBLE FOR ENHANCED MIGRATION OF THE MELANOMA CELLS

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Heat Shock transcription Factor 1 (HSF1), the major regulator of stress response is frequently activated in cancer and has an apparent role in malignant transformation. Here we analyzed the influence of the over-expression of a constitutively active transcriptionally-competent HSF1 mutant form (with deletion of the regulatory domain: amino acids 221-315; aHSF1), or HSF1 silencing (by shRNA), on phenotypes of mouse B16F10 melanoma cells. The expression of aHSF1 as well as reduced HSF1 expression did not affect the proliferation of cells. However, cells with aHSF1were able to grow more efficiently in the soft agar. Moreover, they migrated more effectively in the transwell migration assay, and the number of metastases in the lungs was increased after the injection of cells 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 to migrate, but their potential to form lung metastases *in vivo* was reduced. Furthermore, aHSF1 reduced the adherence of cells to a fibronectin-coated surface, and affected the actin cytoskeleton.

To search for possible migration-related factors targeted by HSF1, we determined the expression profile of genes associated with cell motility using a specific RT^2PCR array. The expression of several genes, namely *Vcl*, *Ptk2b*, *Cav1*, *Capn1*, *Mmp2*, was significantly down-regulated in B16F10 cells expressing aHSF1. Human 1205Lu and WM793B cells over-expressing aHSF1 also showed thelower level of *VCL* gene transcripts compared to control cells. To directly confirm involvement of HSF1 in regulation of *Vcl/VCL* gene, an actual binding of HSF1 was assessed using ChIP assay. In the human *VCL* gene HSF1 binding was detected in the second intron, both in 1205Lu and WM793B cells (in both heat shocked and aHSF1-expressing cells). In the case of B16F10 cells binding of HSF1 was detected in the first *Vcl* exon, yet binding in the second intron was not detected (in any of six checked HSE-like motifs). At the protein level, HSF1-mediated down-regulation of VCL expression was found in B16F10 cells and in 1205Lu cells, but not in WM793B cells (which suggests additional post-transcriptional mechanism of regulation in this cells). Most importantly, down-regulation of vinculin corresponded to cell migration ability analyzed *in vitro* using the Boyden chamber - in the same cells over-expression of aHSF1 resulted in the down-regulation of vinculin and increased motility.

We concluded that the activation of HSF1 apparently enhanced the motility and metastatic potential of cancer cells, exemplified here by melanoma. Among potential HSF1-mediated mechanisms promoting cell motility and invasiveness we found transcriptional down-regulation of vinculin. Vinculin is a motility-related protein participating in the dynamic reorganization of the cytoskeleton and playing important roles in the regulation of cell migration. Hence, HSF1-dependent down-regulation of vinculin offers a mechanistic explanation for HSF1-mediated enhancement of motility and metastatic potential of cancer cells.

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98. L- AND D-LACTATE ENHANCE CELLULAR DNA REPAIR CAPACITY AND MODULATE RESISTANCE TO ANTICANCER CHEMOTHERAPEUTICS THROUGH INHIBITION OF HISTONE DEACETYLASES IN CERVICAL CARCINOMA CELLS

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Lactate, the end product of glycolysis, except of being an alternative fuel for cells, is also regarded as an active metabolite. Although inhibition of histone deacetylases (HDACs) by L- and D-lactate in the context of gene transcription regulation has recently been reported the role of lactate-dependent chromatin remodeling in facilitating the effectiveness of the DNA-damage response has not been characterized so far.

We demonstrated that L- and D-lactate inhibit HDACs in living cervical cancer cells, hyperacetylate H3 and H4 histones and increase chromatin accessibility. Furthermore, inhibition of HDAC activity corresponded with increased nuclear accumulation of DNA-PKcs and its activated form after chemotherapeutics treatment resulting in improved DNA repair rate as evaluated by γ-H2AX and comet assays. Finally, the results of the clonogenic cell survive assay showed that lactate mediates resistance to clinically used chemotherapeutics and this effect could be suppressed by pharmacological inhibition of monocarboxylate transporters.

Our data indicate that L- and D-lactate present in uterine cervix could participate in modulation of cellular DNA damage repair processes and resistance of cervical carcinoma cells to anticancer chemotherapeutics.

99. MACROPHAGE MITOCHONDRIAL DAMAGE FROM StAR TRANSPORT OF 7-HYDROXYPEROXYCHOLESTEROL: IMPLICATIONS FOR OXIDATIVE STRESS-IMPAIRED REVERSE CHOLESTEROL TRANSPORT

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Disorders of the inner macrophage cholesterol transport homeostasis are the heart formed atherosclerotic lesions. Macrophages cells may potentially become overloaded with cholesterol and other lipids when oxidized low density lipoprotein (oxLDL) is taken up. To limit adverse cholesterol accumulation, macrophages can program its export to acceptors such as apolipoprotein -A1 (apo-A1) or high density lipoprotein (HDL). This is an early step of reverse cholesterol transport (RCT), which is delivers cholesterol to the liver for disposal. RCT is required in the maintenance of cholesterol homeostasis.

Macrophage express a number of proteins, which participate in RCT. These include steroidogenic acute regulatory (StAR) family proteins which bind and transport preexisting or incoming cholesterol and deliver it to mitochondria. In effect of stimulation with dibutyryl-cAMP, RAW 264.7 macrophages exhibited a time-dependent induction of StarD1and StarD4. Upon uptake by Mito, 7α-OOH starts lipid peroxidation and membrane depolarization.

In this work is shown, that under oxidative stress, StARs will transport not only cholesterol to macrophage mitochondria, but also pro-oxidant cholesterol hydroperoxides (7-OOHs), thereby impairing early-stage RCT.

100. EXPRESSION PROFILE OF GENES RELATED TO TGF-BETA SIGNALING PATHWAY IN HUMAN RENAL PROXIMAL TUBULE CELLS IN RESPONSE TO AMPHOTERICIN B AND AMPHOTERICIN B-COPPER(II) COMPLEX

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Fungal infections are one of the most common cause of death in patients with cancer and in transplant patients. Amphotericin B (AmB) belongs to polyene antibiotics, which are mainly used in antifungal therapy. Despite its advantages, AmB causes many side effects, including nephrotoxicity. It has cholesterol-binding capacity to human cells which consequently leads to cell death. It may be due to activation of signaling pathways associated with TGF-beta protein. To reduce the toxicity of AmB against human cells, the chemical modifications of the molecule are carried out, including forming a complex of amphotericin B with copper ions.

The aim of this study was to compare the influence of amphotericin B-copper(II) complex and pure form of amphotericin B on expression of genes related to TGF-beta signaling pathway.

Human Renal Proximal Tubule Cells were treated with amphotericin B and amphotericin B-copper(II) complex (AmB-Cu2+). Total RNA was extracted with the use of TRIZOL, according to manufacturer protocol. Expression profile was evaluated by oligonucleotide microarray HG-U133A 2 (Affymetrix).

In this study 449 ID of genes related to TGF-beta signaling pathway was analyzed. Statistical analysis indicated that expression of 13 ID was significant changed (THBS1, DAB2, JUN, SERPINE1, BMP2, SMURF2, MAP3K7, SIK1, FOS, CD24, NEDD4L, TSC22D1, GDF15). Both, in the sample of pure form of AmB and the sample of amphotericin B-copper(II) complex, two genes were down-regulated (BMP2 and FOS) and one gene was up-regulated (GDF15), comparing to control. Three genes (SMURF2, MAP3K7, CD24) changed their expression in the sample of modified form of AmB comparing to control and pure form of AmB.

There was no effects of AmB and AmB-Cu2+ on gene expression of TGF-beta and its receptors. However, they influenced on the expression of genes associated with the signaling pathways regulated by this cytokine. Direction of changes in profile expression was similar after application both forms of amphotericin B.

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101. A NOVEL METHOD OF METABOLITE EXTRACTION FROM FORMALIN FIXED PARAFFIN EMBEDDED TISSUE SECTIONS FOR GC/MS ANALYSIS

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Archival formalin-fixed paraffin-embedded (FFPE) tissue specimens constitute valuable source of clinical material for retrospective molecular studies. Because of their long term stability and widespread availability, FFPE tissue sections might be reliably used for biomarker discovery, as alternative for fresh frozen tissues. Recent studies showed the applications of mass spectrometry techniques for proteomic analysis of FFPE samples. However, metabolomic assessment of archival tissue sections remains still in infancy. In this respect, there is a urgent need for efficient methods enabling extraction and profiling of metabolites present in FFPE tissue samples.

In this study, we demonstrate the novel methodology allows for isolation of primary metabolites obtained from archival tissues of mouse kidney. Moreover, we compared metabolomic data obtained from fresh frozen, formalin fixed and formalin-fixed paraffin embedded tissue specimens. Using gas chromatography followed by mass spectrometry (GC/MS) technique, we identified 82 metabolites (e.g. amino acids, saccharides, carboxylic acids, fatty acids) present in all studied tissue types. 67% of identified compounds overlapped in three different types of samples. Quantity of all metabolites were generally less for the FFPE samples in comparison to fresh frozen and formalin fixed. There were also observed influence of fixation time on the number of identified compounds.

These data demonstrate the possibility of conducting metabolomic study on retrospective FFPE tissues for clinical research. Moreover, formalin fixed tissue could be an alternative clinical material suitable for metabolomic analysis.

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102. CANCER CELL LINES WITH WILD-TYPE TP53 DIFFERENTLY RESPOND TO THE ACTIVATION OF P53 BY ACTINOMYCIN D AND NUTLIN-3A CO-TREATMENT

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In majority of human cancers the p53 tumor suppressor signaling pathway is inhibited in one way or another. The point mutations of p53 are found in approximately 50% of human cancers, although in some cancer types these mutations are much less frequent. The p53 protein can induce apoptosis when a cell is exposed to stress factors, e.g. extensive DNA damage resulting from by cancer therapy. The ability to undergo stress-induced apoptosis may be a favorable predictive factor. We observed that in A549 lung cancer cell line the p53 can be synergistically activated by treatment with two agents used in cancer therapy - actinomycin D and nutlin-3a. The synergy is evident in the phosphorylation of serine 46, which is considered as the marker of the highest activation level of p53 associated with the induction of apoptosis. However, in spite of strong serine 46 phosphorylation, the A549 cells did not undergo apoptosis following actinomycin D and nutlin-3a co-treatment. The serine 46 phosphorylation and apoptosis were visible in A549 cells following exposure to camptothecin - and inhibitor of topoisomerase I and an anti-cancer drug. In order to find out what is the predominant pattern of p53 activation in cancers, we selected 13 cancer cell lines of various origin (lung, colon, stomach, kidney, breast, ovary and skin) as well as normal human fibroblasts. Co-exposure of cells to actinomycin D and nutlin-3a revealed that in majority of cell lines the synergistic activation of p53 takes place. However, the apoptosis, measured as the presence of caspase-3 activity appears only in minority of cell lines. Cells with extensive apoptosis show synergistic activation of p53 however many cells with synergistic activation do not undergo apoptosis. We conclude that contrary to the common belief, the phosphorylation of serine 46 of p53 is not a good indicator of proapoptotic activation of p53.

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103. VITAMIN E – FACTOR AFFECTING OXIDATIVE PROCESSES

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Vitamin E as one of the crucial antioxidants play an important role in lipid and protein protection. The lack or deficiency of antioxidants result in enhanced oxidation process and, consequently, may have an adverse effect on health. It is known that low concentration of antioxidants in diet could reduce immunological activity and may increase the risk of cancer.

Four hundred twenty male meat type chickens of the Polish experimental line were randomly divided into two feeding groups (control (C) with 44 mg of vitamin E and experimental (E) with 244 mg of vitamin E), and reared until 63 days of age. On day 63 birds were slaughtered and received carcasses were chilled in 4^{o}C for 12 h. After this time muscle samples were collected. The content of α-tocopherol was determined by liquid chromatography using method from CSN EN 12822 norm.To determine the lipid oxidation, the malondialdehyde (MDA) value was defined with the extraction method according to Shahidi (1990).

Supplementation with 244 mg of vitamin E result in increase content of vitamin E in tissues. Contents of vitamin E was over three-fold increase ($P \leq 0.01$) of chickens from group E compared to group C (group C – 6.48 mg/kg, group E – 23.03 mg/kg of vitamin E). Increase the content of vitamin E in animals diet cause higher level of vitamin E in meat. Therefore meat became a good source of vitamin E in common diet.

Vitamin E has a significant ($P \leq 0.01$) effect on a decreased content of malondialdehyde (MDA). In meat of chickens from group E, significantly ($P \leq 0.01$) lower oxidative changes were observed 48 h after slaughter (group C – 0.49 mg/g group E - 0.11 mg/g). Four times lower MDA value suggested that vitamin E may effectively inhibit oxidative processes in meat.

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104. ANTICANCER POTENTIAL OF CALCIUM IONS IN FIBROSARCOMA CELLS (WEHI 164)

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Electroporation (EP) is an innovative and effective method and in combination with chemotherapy (CT) can be effectively applied due to cancer cells damage. The application of high-voltage pulses cause temporary permeabilization of cell membrane, allowing the drugs, ions or chemical substances to entry into the cells. The enhanced drug transport increases its intracellular concentration and induces oxidative stress and chain of biochemical reactions which cause death of cells of either apoptosis or necrosis type.

Calcium ions are necessary in many physiological process like: genes and enzymes transcription, regulation of cell cycle or proliferation. Furthermore, the overload of calcium ions play crucial role in initiations of cell death.

In the present study the cytotoxicity of calcium ions combined with electroporation was evaluated in cultured mouse fibrosarcoma cells (WEHI 164). The cells were exposed to external electrical field of strength: 500V/cm and 700V/cm (100μs at 8 pulses for each case). The calcium ions were diluted in EP buffer in the following concentrations: 0.5mM, 1mM and 5mM. Cytotoxicity of therapy was evaluated by MTT assay after 24h and 48h incubation.

The results showed that EP supported by calcium affects the viability of WEHI 164 cells negatively. The mortality of cells is at the highest level when treatment was applied with the following parameters: 5mM and both values of EP. Our research proved that cells viability is inversely proportional to calcium concentrations and time of incubation. Moreover, EP is crucial in terms of cells death rate when enforced with low-level Ca^{2+} .

105. EXPRESSION PROFILE OF ENDOTHELIN-3 GENE IN ENDOMETRIAL CANCER

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Endothelins are the group of proteins, among which there are three isoforms with a similar structure. The level of certain genes expression decides about the character of the changes in a cell and its interaction with the microenvironment. In cells, expression of endothelin-3 gene is responsible for the modulation of apoptosis, proliferation and cell survival, and also angiogenesis. Changes in endothelin-3 gene expression level may participate in cancer initiation and progression.

The purpose of this study was to investigate the level of endothelin-3 gene expression in endometrial cancer, comparing to normal endometrium.

The research included samples of the endometrium: 6 histopathologically confirmed as normal and 17 as endometrial cancer, further divided according to the tumor grade: G1-3, G2-13 and G3-1. Expression profile of endothelin-3 gene was performed using method by Wang et al. (2013).

The results revealed 60% decrease in the transcriptional activity of endothelin-3 gene in G1 endometrial cancer comparing to control. In subsequent stages of endometrial cancer a gradual increase of transcriptional activity of EDN-3 was observed and reached 560% of the control level in G3. Due to obtained results, EDN-3 can contribute to initiation as well as progression of endometrial cancer.

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106. TARGETING THE PD1 - PDL1 AXIS WITH SMALL MOLECULE INHIBITORS

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Almost 120 years ago William Coley introduced the idea of immunotherapy demonstrating the possibility of using host's immune system to fight cancer. Since then, studies on tumor genesis revealed a number of factors that may potentially serve as molecular targets for immunotherapies. One of such promising targets are PD1 and PDL1 proteins. PD1 (Programmed cell death protein 1) is expressed on the surface of activated T cells and plays a critical role in modulation of the host's immune response. Its ligand, PDL1 (Programmed cell death 1 ligand 1), is overexpressed in cancer cells and binds to PD1, thus silencing the immune response against the tumor. This consequently facilitates propagation of the disease. The notion of the mechanisms used by cancer cells to block the immune system response was utilized in the development of therapies blocking PD1-PDL1 interaction. Up to date, human PD1-PDL1 complex has not been crystallized and structure of the mouse - human complex does not provide a complete view of the molecular basis of PD1-PDL1 interactions. The purpose of this study is to obtain crystal structure of the human PD1-PDL1 complex and characterize the interaction interface. This shall allow rational design of small molecule inhibitors of the interaction. In addition, the study presents the results of fragment docking towards PD1 protein which will facilitate the design of small - molecule inhibitors of PD1-PDL1 interaction.

107. EFFECT OF REDUCTIVE ACTIVATION OF DOXORUBICIN BY NADPH CYTOCHROME P450 REDUCTASE ON CELL CYCLE OF SENSITIVE AND MULTIDRUG RESISTANT MCF7 BREAST CANCER CELLS

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Doxorubicin (DOX), belonging to anthracycline antibiotic family, is one of 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 this drug is limited by the occurrence of multidrug resistance (MDR) associated with the presence of efflux pumps (e.g. P-glycoprotein, P-gp; MRP1 and BCRP), belonging to the ATP-binding cassette protein family responsible for active efflux of drugs out of resistant cells resulting in their decreased intracellular accumulation insufficient to inhibit resistant cell proliferation.

In our previous studies we have evidenced that anthracycline compounds having non-modified quinone structure (among them DOX) underwent bioreductive activation by exogenously added NADPH cytochrome P450 reductase (CPR) from human liver and NADPH and that this activation had a high impact on increasing their activity against leukaemia MDR sublines overexpressing P-gp and MRP1.

The aim of the present study was to examine the effect of CPR-activated DOX on the cell cycle of cells originating from solid tumours: human sensitive MCF7 and multidrug resistant MCF7/DOX (overexpressing P-gp) breast adenocarcinoma cells.

The effect of CPR-activated DOX on cell cycle distribution was examined up to 72 h of incubation. At the indicated incubation time points (6h, 24 h, 48 h, 72 h), the cells were stained with fluorescent probe - propidium iodide to measure the cellular DNA content with the aid of flow cytometry. It was found that CPR-activated DOX used at IC_{90} caused a significant changes in the cell cycle distribution of sensitive MCF7 and multidrug resistant MCF7/DOX cells as well as a marked increase in the percentage of the apoptotic subpopulation (sub-G1).

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108. HEREDITY OF THE G1 AND G2 CELL CYCLE PHASE BASED ON GENES IN P53 PATHWAY

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Aim: The aim of the study was to show interactions between level of cells in different phases and linkage their amount to genes expression level and eventual heredity which could explain investigated process. As a trait in this research radiosensitivity was investigated.
Materials and methods: As a population under investigation dizygotic (DZ) and monozygotic (MZ) twins was used (28 DZ pairs and 15 MZ pairs). Percentage contribution of cells at G1 and G2 phases were measured in two checkpoints 0Gy and 3Gy dose (24 hours after irradiation). The second group of data include measurements of p21 (G1 gene) and GADD45A/CycB1 (G2 genes) expression from qPCR at 0Gy and 2Gy. To all group of data standard fold change were calculated. The Spearman correlation (rho) between twins in each cell cycle phase and gene (at both checkpoints) was calculated. To randomize the population, twins pairs were divided to two independent groups with using the Bernoulli principle (10000 repeat). Additionally the T test for each rho was performed. Finally between signal form
DZ twins and MZ twins the narrow-sense heredity was calculated according to Jacquard formula $h^2=(r_{mz}-r_{dz})/(1-r_{dz})$.
Results: As a results, to each gene and cell cycle interaction the correlation with p-value was obtained. Mean value for correlation was calculated with confidential intervals(95%).
We observed high heredity (70%) for G2 cell cycle phase at FCH and 44% for G2 phase 0Gy, and G1 at FCH. The only one investigated gene for G1 step show high heredity for both signals (h^2_0Gy=68%, h^2_FCH=64%). For G2 phase only CycB1 show high h^2 (79%) also we observe disruption and randomization of expression for FCH.
Conclusions: We show that disruption of G2 phase are related to activity of CycB1 which is well known however, we show that the genetic factor play the biggest role. The same situation is observed G1 and p21. The GADD45 do not show and disruption and correlation with G2 phase despite his role in process of G2 cell cycle arrest. This finding need further investigation and validation on independent data sample.

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Addendum

109. THE REGULATION OF *NEPRILYSIN* (*NEP*) EXPRESSION IN OVARIAN CANCER CELL LINES

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The neprilysin (encoded by MME gene - Membrane Metallo-Endopeptidase Variant 2) is a 85514 Da single-pass type II membrane protein built with 750 amino acids, which is an important cell surface marker in the diagnostic of human acute lymphocytic leukemia. It is known that neprylisin is involved in carcinogenesis, Alzheimer desease, hypertension, asthma or motor system diseases. The most interesting seems to be the role of neprylisin in the infiltration and the malignant invasion. There are evidences that neprylisin could be the beneficial and important factor which inhibit the proliferation of pathological cells. There is a theoretical possibility of inhibiting the undergoing of malignant transformation.

The main aim of the study was to analyse the influence of a glucocorticosteroid - Dexaven (Dexamethasoni natrii phosphas) and anti-cancer chemotherapy drug - Etoposide (Etoposide phosphate) on the *Neprilysin* (*NEP*) expression. The research were done on two different ovarian cancer cell lines.

Material and methods: The A2780 human ovarian cancer cell line established from tumour tissue from an untreated patient (European Collection of Cell Cultures – ECACC, 93112519) and OVP-10 ovarian cancer cell line (The Maria Skłodowska-Curie Institute of Oncology in Warsaw) were grown under standard conditions in RPMI 1640 culture medium until the metabolism was stabilised, after that the cells were seeded on a 24-well dish and a serial dilution of examined drugs: Dexaven (10^{-5}M, 10^{-6}M, 10^{-7}M, 10^{-8}M, 10^{-9}M) and Etoposide (50μM, 25μM, 12,5μM, 6,25μM, 3,12μM) were prepared. All different concentrations of drugs were added to culture medium for 24 hours and for 48 hours. After incubation time total RNA was isolated and the expression level of *NEP* was established by Q RT-PCR with MultiScribe™ Reverse Transcriptase and AmpliTaq Gold DNA Polymerase and with SYBR Green Dye. As the reference gene for research the *β-actin* was chosen.

Results show significant influence of dexamethason on the *NEP* expression level what confirmed results obtained in lung cancer by other researcher. In A2780 cells tested drug stimulated the increase of copies of *NEP* mRNA, the highest with the 10^{-7}M concentration (after 24 hours: 2555%, after 48 hours 196% increase in comparison with control). In OVP-10 cells the highest increase of *NEP* expression was observed with 10^{-8}M (after 24 hours: 243%, and after 48 hours 390% increase in comparison with control). Result obtained after etoposide treatment did not confirm our conjecture. The increase of *NEP* expression and after some time the mplecular activity of these gene was lower in comparison with control one.

Dexamethason, as well as, etoposide changed the expression level of *NEP* in A2780 and OVP-10 cells, but the effect of changes depend on concentration of the drug and the time of exposure. Higher ability of modification of *NEP* transcription activity demonstrated dexamethason.

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110. INFLUENCE OF XANTHONES ON THE EXPRESSION OF ANTIOXIDANT GENES: *CAT, GPX, SOD1*

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Natural xanthones, as well as their synthetic derivatives, have been proved to possess interesting biological properties that make them an interesting subject in cancer therapy. However, molecular pathways of their anticancer features remain not fully understood. Reactive oxygen species (ROS) lead to deterioration of pivotal cellular components which is why they have been termed *Pandora's box*. On the other hand, ROS mediate numerous intracellular metabolic pathways and are important component of biological systems, therefore the use of antioxidants during cancer therapy is currently a debated topic due to some contradicted findings (e.g. *SOD paradox*). Intracellular ROS concentration is a result of the balance between their generation and the dynamics of their elimination by antioxidants and antioxidant enzymes.

Our purpose was to investigate expression of the most significant antioxidant genes: catalase (CAT), glutathione peroxidase (GPX), and Zn,Cu-dependent superoxide dismutase (SOD1) in the *in vitro* cultures of bladder cancer and lung cancer (T24 and A549 cell lines). mRNA levels of the studied genes were determined by Real-Time™ RT-PCR. Intracellular ROS level was measured by the detection of fluorescent product (DCF) yielded from the oxidation of carboxy-H_2DCFDA (Molecular Probes), and the total antioxidant level was measured by a commercial kit (Sigma-Aldrich) utilizing ABTS oxidation assessment.

Four xanthone derivatives (HN-17, HN-27, HN-28, HN-30), synthesized at the Department of Bioorganic Chemistry (CMUJ, Kraków, Poland) were included in the study due to their previously designated significant antitumor activity. Additionally, natural xanthones (gambogic acid and α-mangostin) served as standards. Obtained results demonstrate that synthetic xanthones displayed different degree of ROS induction. In general, T24 cells were more prone to ROS generation than A549. On the other hand, T24 cell line was also more resistant to cytotoxic effects of xanthones. HN-28 was found to be the most potent xanthone derivative to induce ROS generation in the A549 cell cultures. Similar strength was displayed by HN-27. Both xanthones increased ROS levels when higher concentrations were used which suggests dose-dependent manner of ROS stimulation. Treatment of A549 cells with these xanthones also led to increase in expression of antioxidant enzymes at 25 μM concentrations. However, the most potent ROS-inducer was gambogic acid while α-mangostin performed moderate antioxidant properties. None of the analyzed xanthones was as strong ROS-inducer as control chemicals (doxorubicin and bleomycin). Altogether, these results suggest that pro- or antioxidant properties of xanthones strictly depend on their chemical structure. Synthetic xanthones analyzed in this study basically induced expression of antioxidant enzymes but that effect was not always accompanied by significant influence on intracellular ROS or antioxidant levels.

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111. EXPRESSION PROFILE OF TOLL-LIKE RECEPTORS IN COLORECTAL CANCER

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Toll-like receptor (TLR) signalling pathways are activated by the binding of various TLR ligands which leads to the synthesis of many proteins, such as pro-inflammatory cytokines and adhesion molecules, involved in the maintenance of tissues homeostasis and immune response.

The aim of this study was to compare the expression profile of 580 mRNAs for genes associated with TLR signalling pathways in colon cancer specimens in the low (LCS) and high (HCS) clinical stages of cancer.

The analysis of the expression profile of genes associated with TLR signalling pathways was performed using oligonucleotide microarrays of HG-U133A (Affymetrix, Santa Clara, CA). Appointment of differentiating genes was performed with the use of GeneSpring 12.0.

The results indicated that 59 mRNAs for genes associated with the biological activity of TLRs were differentially expressed (one-way ANOVA, $p < 0.05$) in colorectal cancer. Among these transcripts, 11 mRNAs were differentially expressed in LCS and 21 mRNAs were differentially expressed in HCS in comparison to control specimens (Tukey post hoc test, $p < 0.05$).

112. MASS PROFILING OF CANCER SERUM LIPIDOME - AN OPTIMIZATION OF THE MALDI-TOF ANALYSIS METHOD

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It is generally accepted that ongoing cancer processes significantly alter the metabolism of cells, and analysis of metabolites allows identifying mechanisms characteristic for cancer. Metabolomics is a field of molecular biology that focuses on global composition and dynamic changes of metabolites and other small molecules. Lipidomics is one of the metabolomic subset that deals with characterization of lipids.

The most commonly used methods in lipidomics are mass spectrometry (MS) and proton nuclear magnetic resonance (1H-NMR). The advantage of mass spectrometry methods over NMR is their higher sensitivity. The mass spectrometry allows identification of particular lipids and their quantification in complex mixtures (like body fluids). The MS methods are based on precise measurement of a mass to charge ratio of ionized molecules of the analyte. For a long time mass spectrometry had not played an important role in lipid research because "traditional" methods of ionization introduces fragmentation of lipid chains. However, the situation changed with invention of the "soft ionization" methods like MALDI and ESI, and MS became a most powerful technique of lipid analysis. Additionally, high throughput of MALDI profiling makes it perfect for large cohort studies, where hundred of samples need to be analyzed. This approach allows identifying many different groups of lipids depending on samples preparation and spectra registration methods. The selection of either positive or negative ion modes allows recording nearly all biologically relevant classes of lipids.

It is obvious that quality of the measurement depends on the choice of appropriate techniques. There are many different methods that can be used for lipids analysis but none of them is recommended for total serum lipids profiling. It is therefore appropriate to carefully select suitable parameters of lipid analysis by MALDI TOF. In the literature, there are few matrices recommended for lipids analysis with MALDI-TOF mass spectrometry. In the study, we used three different matrices (DHB, DHAP and ATT) in order to find the one, which crystallizes homogeneously, is stable in the vacuum and allows observing many classes of lipids in a positive and a negative ion mode. We also tested the solvents in which the matrix can be dissolved, the order of application of sample and matrix on the target plate and we chose suitable temperature of storing samples during the preparation of the analysis.

All the tests were performed for 36 samples. After statistical analysis we decided that for the profiling of cancer serum lipidome, the most suitable are two matrices: ATT (dissolved in 90% ethanol) and DHB (dissolved in 30 % ethanol) in both positive and negative ion modes. The samples should be kept on dry ice during application on the target plate, and due to obtain homogenous crystallization, matrix should be applied before sample. All of these methods allow to register mass spectra in the automatic and repeatable way.

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113. ROLE OF DNA REPAIR GENES IN GENERATING HUMAN CANCER CELL LINES RESISTANT TO PHOTODYNAMIC THERAPY

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Photodynamic therapy (PDT) is relatively non-invasive cancer treatment that involves two individually nontoxic components, light and photosensitizer. PDT leads to generation of cytotoxic oxygen species that damage cell components and cause cell death. There are several cellular defense mechanisms against PDT including, drug resistance pump, activity of antioxidant detoxifying enzymes, heat shock proteins and possibly DNA repair.
In this study we aimed to investigate role of DNA repair especially base excision repair system in PDT resistance. To this end, human cancer cell lines resistant to PDT were isolated by applying 5-ALA and appropriate light dose after several cycles of PDT. After isolation of PDT resistant cells, some of their characteristics were studied. Oxidative stress and activity of selected base excision repair enzymes were measured and expression of ABCG2, HO-1 and DNA repair genes were studied by both qRT-PCR and Western blotting.

From all ALA-PDT treated cell lines, Hela, human glioblastoma (U-87) and cervical carcinoma (SKG-IIIa) cell lines showed stable resistant features. Longer cell doubling time was observed in U87 and HeLa ALA-PDT resistant cell lines compare to parental cells. mRNA level of APE1, OGG1, TDG, UDG and MutT were induced in U87 resistant cell line and the level of BER enzymes in PDT resistant cells was either stable or induced following PDT. We also observed higher activity of APE1 and OGG1 enzymes in U-87 resistant cell line compare to parental line. Data demonstrate the probable role of BER in generating U-87 resistant cell line following PDT.

114. NUCLEAR LOCALIZED AKT ENHANCES BREAST CANCER STEM-LIKE CELLS THROUGH COUNTER-REGULATION OF P21$^{WAF1/CIP1}$ AND P27^{KIP1}

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Cancer stem like cells (CSC) are a rare subpopulation of cancer cells capable of propagating the cancer and causing cancer recurrence. In this study, we found that the cellular localization of Akt kinase affects the maintenance of CSC. When Akt tagged with nuclear localization signal (Akt-NLS) was overexpressed in SKBR3 and MDAMB468 breast cancer cells, these cells showed a 10-15% increase in the number of cells with CSC-like enhanced ALDH-1 activity and showing $CD44^{+High}/CD24^{-Low}$ phenotype. This effect was completely reversed in the presence of Akt-specific inhibitor, Triciribine. Furthermore, cells overexpressing Akt or Akt-NLS were less likely to be in G0/G1 phase of the cell cycle by inactivating $p21^{Waf1/Cip1}$, and exhibited increased clonogenicity and proliferation as assayed by colony-forming assay (mammosphere formation). Thus, our data emphasize the importance the intracellular localization of Akt has on stemness in human breast cancer cells. It also indicates a new robust way for improving the enrichment and culture of CSC for experimental purposes. Furthermore, it allows for the development of simpler protocols to study stemness, clonogenic potency, and screening of new chemotherapeutic agents that preferentially target cancer stem cells.

115. ANTIBODIES AGAINST *ESCHERICHIA COLI* O24 AND O56 O-SPECIFIC POLYSACCHARIDES RECOGNISE EPITOPES ON HUMAN TISSUES REVEALED BY IMMUNOHISTOCHEMICAL INVESTIGATION

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The serotypes O24 and O56 of *Escherichia coli* are characterized by the presence of sialic acid in their lipopolysaccharides (LPS). Lipopolysaccharide is an endotoxic molecule localized on the cell surface of Gram-negative bacteria [1]. The presence of sialic acid in LPS might contribute to the pathogenicity of bacteria by a molecular mimicry mechanism of sharing of a common epitope with host structures. The presence of bacterial epitopes structurally similar to host antigens, due to a molecular mimicry may obviate a host immune response [2]. In *E. coli* O24 and O56 LPS the sialic acid residue is glycosylated at 7-position, thus important was to establish whether structural homology occurs with tissue antigens or other type of the mimicry concerns broader extent of serological similarity [3]. In previous study the specific rabbit sera could be obtained against these bacteria. The aim of the present work was to purify the specific antibody with an affinity chromatography on immobilized LPS and to examine histochemically if these antibodies recognise the specific epitopes on human tissues. Here, we report the isolation of specific antibodies with an affinity chromatography using immobilized lipopolysaccharides. Next, we histologically evaluated the reactivity of anti-O24 and anti-O56 antibody on human tissues. The study was conducted under assumption that the sialic acid based molecular identity of bacterial and tissue structures is not only an understanding the mimicry based bacterial pathogenicity. Cross-reacting antibodies could be used to recognize specific human tissues depending on their histogenesis and differentiation which might be useful for diagnostic purposes. The results indicate that variety of human tissues is recognized by anti-O24 and anti-O56 antibodies. Interestingly, only a single specific reactivity could be found in anti-O56 antibody preparation. Several tissues studied were not reactive with any of both antibody, thus proving that the presence of cross-reactive antigens was tissue specific.

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116. COMPARISON OF UBIQUITIN-DEPENDENT PROTEIN DEGRADATION DYNAMICS OBTAINED WITH VARYING NUMBER OF UBIQUITINATION STEPS IN A MODEL

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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. Concurrently to Mdm2 mediated ubiquitination, deubiquitination reactions mediated by HAUSP protein are affecting p53 degradation rate in the opposite direction. Constant fast degradation of p53 protein maintains its low level in normal unstressed cells.

Ubiquitination designating a protein for proteasome-dependent degradation is an iterative process. The process consists of attachment of first ubiquitin residue to the protein molecule and elongation of ubiquitin chain by consequent addition of ubiquitin molecules to free lysine residue of the terminal ubiquitin of the chain already attached to the protein molecule. First step is known as monoubiquitination whereas addition of the second and next ubiquitins to the chain is called polyubiqutination. Chains consisting of at least 4 ubiquitin molecules are recognized by proteasomes, but models of p53 signalling pathway usually incorporate only one or two consecutive steps of ubiquitination resulting in significant increase of degradation rate. Simulation analyses of ubiquitination models with differing number of ubiquitination steps preceding fast degradation were performed. Obtained dynamics were compared in order to verify if the simplification of the ubiquitination process in present models could be justified.

117. OXIDATIVE MODIFICATIONS IN RNA AND DNA OF HUMAN CANCER CELLS EXPOSED TO X-RAYS

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Ionizing radiation (IR) is widely used in medicine in diagnostics and cancer therapy. The best studied target for IR is DNA, which can suffer variable types of damage such as single- and double-strand breaks, oxidative base damage leading to cell cycle disturbance and apoptosis, or other types of cell death. However, increasing data indicate that ionizing radiation can also induce damage in RNA. Reactive oxygen species (ROS) originating from the radiolysis of water induced by IR have extremely short lifetimes (nanoseconds) and cannot be observed by methods which require longer manipulations but their existence can be deduced only on the basis of oxidative damage to cellular macromolecules. In this study we assessed the levels of oxidative damage in DNA and RNA after irradiation of cultured Me45 and HCT116 cells originating from human melanoma and colon cancer, respectively.

Oxidized nucleotides in nucleic acids, 8-oxo-7,8-dihydroguanosine (8-oxoG) in RNA and 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxo-dG) were measured simultaneously by high pressure liquid chromatography with electrochemical detection (HPLC-EC).

RNA and DNA isolated from irradiated ME45 cells showed increased levels of 8-oxoG and 8-oxodG during the first six hours after irradiation. Control, unirradiated cells showed also fluctuations of the levels of oxidized nucleotides in their nucleic acids during culture. In melanoma cells the level of oxidative modifications of nucleotides in DNA of irradiated cells was significantly higher ($p<0.005$) than that in RNA of control cells. Nucleic acids isolated from HCT116 cells showed levels of oxidative damage one order of magnitude lower than melanoma cells. Our results show that different cell types cope differently with oxidative changes in their nucleic acids and suggest that these differences may be an important element of the cellular response to ionizing radiation.

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118. SYNTHESIS AND PROPERTIES OF ZINC OCTACARBOXYPHTHALOCYANINE - A POTENTIAL PHOTOSENSITIZER FOR PHOTODYNAMIC THERAPY

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Phthalocyanines, as synthetic structural analogues of porphyrin, are investigated as the model compounds of naturally occurring metalloporphyrins. They have many interesting and applicable properties *e.g.* optical, catalytic, electrocatalytic in various processes, photochemical and photobiological (photodynamic therapy PDT) [1]. The complexes of phthalocyanine with metal ions, such as Zn^{2+}, Al^{3+}, Ga^{3+} and Si^{4+} are especially interesting in PDT because they have long triplet lifetime and high triplet quantum yield [2].

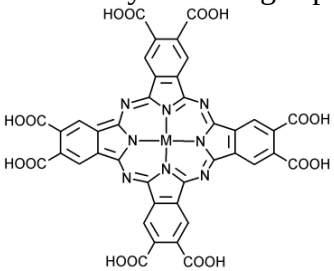

Fig. 1. Structure of octacarboxyphthalocyanine, M - Zn

The aim of our research is zinc phthalocyanine, that has eight carboxylic groups attached to the benzene rings (Fig. 1). The carboxylic groups in ZnPcOC molecules make the compound well soluble in water. Zinc 2,3,9,10,16,17,23,24-octacarboxyphthalocyanine was obtained according to the general procedure described by Wőhrle, et al. [3].

In our preliminary biological studies a biological cytotoxicity of ZnPcOC was evaluated in MTS viability assay, according to the producer's protocol (PROMEGA). The adherent melanoma cancer cells (Me45) were spread in a sterile 96-wells plate, in amount of 10^4 cells per well. After 24 hours, the medium was replaced by a fresh DMEM-F12 solution, supplemented with 12% Fetal Bovine Serum (FBS, PAA). ZnPcOC was tested at different micromolar concentrations: 2, 1, 0.5, 0.25, 0.125 μM, in 24 hours incubation under standard conditions (37°C, 5% CO_2 and 60% humidity). The absorbance for cell viability was measured at 490 nm on an EPOCH apparatus. Additionally, the cells with ZnPcOC addition were exposed to the UV A, UV B and UV C radiation, at dose 100 J/m^2. We expected that UV radiation will improve toxicity of ZnPcOC. For wider biological activity tests, the cell cycle assay was performed, within the melanoma cell cycle, replication inhibition or apoptosis was assayed. After 24 hour incubation of Me45 cells (10^5 cells per well, in 24-wells plate) with ZnPcOC at dose-range: 2, 1, 0.5, 0.25, and 0.125 μM, cells were fixed in 70% EtOH and evaluated using a PI/RNase cell cycle protocol for flow cytometry, using Aria III sorter (BD).

Although the MTS assay didn't show significant decreasing of melanoma cells viability and proliferation, we still believe that compound impact will be observed in flow cytometry cell cycle assay. If ZnPcOC could penetrate the cells, probably the replication process will be blocked, and S phase fraction will be increased. After preliminary biological tests we are not able to confirm or exclude yet a potentially cytotoxicity activity of ZnPcOC, against cancer melanoma cells. In next step we will increase dose concentrations of ZnPcOC with parallel increasing of UV exposition.

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119. EFFECTS OF HYDROPHOBIC FUNCTIONALIZED SILVER NANOPARTICLES ON THE FLUORESCENCE OF COMMON FLUORESCENT MARKERS

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Noble metal nanoparticles are considered for anticancer and antibacterial applications. It has been proved that silver nanoparticles are able to penetrate into selected tissues and bacterial cells, leading to their apoptosis. There is evidence of more efficient incorporation into cancer and bacterial cells with respect to unchanged tissues. This can be used for destroying cancer cells as well as for drug encapsulation and targeted delivery to selected cells. The understanding of the mechanism of incorporation and binding with selected parts of cells is essential for the prospective use of systems based on these nanoparticles for diagnostic and therapeutic purposes. Thus, it is necessary to develop simple monitoring systems that will allow to estimate the kinetics and results of the processes induced by these nanoparticles when incorporated into cells.

In the present study we investigate the effects of introducing silver nanoparticles in model membrane-like systems on the spectral properties of selected fluorescence markers. A micellar suspension of surfactant molecules is used as a model system. Our previous results show that hydrophobic functionalized Ag nanoparticles are encapsulated in neutral Triton X-100 micelles with a diameter of 10 nm without significant change in the size of the micelles. On this basis we assume that one micelle encapsulates one Ag nanoparticle of a diameter of 3 nm to 7 nm.

We investigated the changes in the spectral properties of the fluorescent markers DPH and ANS resulting from the incorporation of silver nanoparticles into micelles. The micelles were first stained with the markers at different concentrations and stabilized for 15 minutes under ambient conditions. Hexane suspensions of colloidal Ag nanoparticles were injected rapidly into the stained surfactant solutions. The spectral characteristics of the obtained systems were measured after full evaporation of hexane, and the measurements were repeated after 3 and 7 days. The results indicate that the interaction between the two elements (nanoparticles and dye molecules) in the vesicles reduce the native fluorescence intensity and quantum yield of dye molecules. The quenching efficiency is much higher in systems with a large number of nanoparticles; however, the decrease in fluorescence after the addition of a small number of nanoparticles is still significant compared to stained micelles. Moreover, the degree of fluorescence quenching and its dependence on the nanoparticle concentration indicate that the observed changes do not result from simple mechanical effects (the displacement of dye molecules by nanoparticles). More complex mechanisms, such as Foerster resonant energy transfer (FRET), must be involved. Our results indicate that silver nanoparticles are capable of penetrating into stained micelles, but the presence of marker molecules can reduce the incorporation efficiency of the nanoparticles.

Besides affecting the global fluorescence of the dye molecules, metallic surfaces in close proximity to DPH molecules can also alter the electron distribution within the molecules. This is indicated by different emission ratios in subbands within the range 400 nm to 500 nm. Comparison of the results obtained with DPH and ANS indicates that ANS-based systems are much more stable, and therefore much better applicable for monitoring the processes in the investigated systems.

120. PROLIFERATION AND APOPTOSIS IN MELANOMA CELLS EXPOSED TO UV RADIATION

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Ultraviolet (UV) light is electromagnetic radiation with a wavelength from 400 nm to 10 nm. The spectrum of UV radiation can be subdivided into three main ranges: UVA (400 – 315 nm), UVB (315 – 280 nm) and UVC (280 – 100 nm). The Sun emits UV at all wavelengths but the atmosphere blocks more than 70% of this UV and the Earth's surface is reached only by UVA, and by a small UVB fraction as UVB passes through the atmosphere depending on cloud cover and atmospheric conditions (reviewed in [1]). UV originating from sunlight is an environmental carcinogen for humans, and exposure causes DNA damage such as cyclobutane pyrimidine dimers and (6-4) photoproducts, which can be repaired by the nucleotide excision repair (NER) system. Chronic exposure to UV leads to photoaging, immunosuppression, and carcinogenesis and the most dangerous UV-induced cancer is melanoma. In the present work we studied the effects of UV radiation on Me45 human melanoma cells alone or co-cultivated with un-irradiated NHDF fibroblasts. We tested the induction of apoptosis and changes in proliferation after exposure of the melanoma cells to different doses of UVA, UVB or UVC and recuperation during 72 hours without or with un-irradiated neighbouring Me45 or fibroblasts. We used the following ranges of radiation doses: 5-20kJ/m2 for UVA, 2-10kJ/m2 for UVB, and 50-200kJ/m2 for UVC. UVB and UVC were very efficient in induction of melanoma cell apoptosis at all doses, but higher doses (5 and 10kJ/m2) of UVB were less efficient than lower doses (2kJ/m2) whereas for UVC the induction of apoptosis was correlated positively with the dose. Co-cultivation with unirradiated NHDF cells protected melanoma cells exposed to low doses of UVB, but showed an opposite effect after exposure to high doses. Un-irradiated Me45 or NHDF cells also showed a protective effect on cells exposed to low UVC doses but no effect in the case of high dose-exposed Me45 cells. Seventy two hours after exposure, using the cytochalasin block, we observed cells that did not divide, cells that divided once and cells that divided asymmetrically giving three nuclei. UVA did not significantly change the distribution of cellular population between these fractions. Neighbouring un-irradiated NHDF cells present during 72 h of recovery slightly stimulated proliferation and inhibited this asymmetric division in UVA-irradiated Me45 cells. Exposure to UVB or UVC completely inhibited the asymmetric division. Our results suggest that neighbours of UV irradiated cells can influence the responses to irradiation such as apoptosis and proliferation.

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121. ELECTRODIALYTIC SEPARATION OF METALLIC CONTAMINANTS FROM ZINC ELECTROPLATING BATH

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Effluents containing heavy metals can be generated by several industries. One of the most hazardous industrial process is an electroplating. Spent electroplating bath and wastewaters are very toxic, carcinogen and cause damaging impact on the environment. Electroplating baths become contaminated, during regular electroplating operation. Some metallic contaminants, such as iron, also cause a deactivation of electroplating baths. The conventional treatment techniques such as precipitation, ion exchange and electrochemical removal can be used for heavy metal removal from these effluents. One of the processes, which has been applied in several studies for the treatment of technological solutions in an electroplating industry is an electrodialysis. The electrodialysis (ED) is a membrane technique for the separation of ions across membrane. Studied process uses two different types of the membranes: cation-exchange membrane (CEM) and anion-exchange membrane (AEM) which allows for selective passage of cations and anions respectively. ED is a promising method for the treatment of effluents contaminated with heavy metals to recover precious substances for later reuse.

In the presented study, the possibility of ED separation of metallic contaminants (Fe^{2+}, Fe^{3+} and Cu^{2+}) from model zinc sulphate electroplating bath was examined. The effects of types of separated ions and presence of chelating ligands (EDTA, sodium citrate) were investigated.

The results show that Fe^{2+} cannot be removed selectively by ED. However, in the case of Fe^{3+} and Cu^{2+} contaminations, some evidence of selective transport of zinc was observed. It was demonstrated that selectivity of ED in Zn^{2+} separation from Fe^{3+} and Cu^{2+} can be enhanced by the addition of chelating ligands, such as EDTA or sodium citrate. The electrodialysis completed with complexation appears to be a viable method for removal of heavy metals from industrial effluents such as waste of zinc electroplating baths. Analysis of results proves that the electrodialysis with complex formation makes possible removal of iron and copper from studied solutions.

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122. OXYGEN CONSUMPTION ASSAY AS A NEW TOOL FOR ANALYSIS OF CELLULAR RESPIRATION AND MITOCHONDRIAL FUNCTION

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Oxygen Consumption Assay [HS Method] is a highly flexible 96 or 384-well fluorescence plate reader-based approach, for the direct, real-time analysis of cellular respiration and mitochondrial function. The easy-to-use MitoXpress® Xtra assay allows measurement of extracellular oxygen consumption rates (OCR) with whole cell populations (both adherent and suspension cells), isolated mitochondria, permeabilised cells and a wide range of 3D cultures including: tissues, small organisms, spheroids, scaffolds and matrixes. The assay is also suitable for measurement of isolated enzymes, bacteria, yeasts and moulds. In this assay, MitoXpress® Xtra is quenched by O2 through molecular collision, and thus the amount of fluorescence signal is inversely proportional to the amount of extracellular O_2 in the sample. Rates of oxygen consumption are calculated from the changes in fluorescence signal over time. The reaction is non destructive and fully reversible (neither MitoXpress® Xtra nor O_2 are consumed), facilitating measurement of time courses and drug treatments. Luxcel's flexible plate reader format, allows multiparametric or multiplex combination with Luxcel's other products, as well as combining with commonly available reagents to measure glycolysis, LDH, JC-1, MMP (Ψ), ROS, and cellular ATP. For example, MitoXpress® Xtra in combination with Luxcel's pH-Xtra® – Glycolysis Assay allows the simultaneous real-time measurement of mitochondrial respiration and glycolysis and analysis of the metabolic phenotype of cells and the shift (flux) between the two pathways under pathological states**. Plate Preparation** 3D RAFT cultures were prepared with either A549 or HepG2 cells at the indicated density in 240ul DMEM / Collagen solution on a 96-well plate. RAFT cultures were formed as per manufacturer's protocol. **Oxygen Consumption Measurements.** For oxygen consumption measurements MitoXpress®-Xtra stock was prepared in 16ml of pre-warmed DMEM and culture media was replaced in each well with 150µl of this solution. Where applicable, 1µl of compound stock (150X) was added to each well. Wells were then sealed by overlaying with 100µl pre-warmed HS mineral oil to inhibit oxygen back diffusion into the sample. This is best done using a repeater pipette. The plate was then measured kinetically on a FLUOstar Omega (BMG Labtech) for 90-120mins with ~2 minute interval exciting the probe at 380nm and measuring emission at 650nm. Ratiometric measurements were performed using the following delay and gate settings. Delay 1: 30s, Gate 1: 30s, Delay 2: 70s, Gate 2: 30s. **Extracellular Acidification Measurements.** Three hours prior to measurement the RAFT culture plate was placed in a CO2 FREE incubator at 37°C, 95% humidity, in order to remove CO_2 from the plate material. Spent media was removed and 2 wash steps were performed using the Respiration Buffer (0.5 mM KH2PO4, 0.5 mM K2HPO4, 20 mM Glucose, 4.5 g/L NaCl, 4.0 g/L KCl, 0.097 g/L MgSO4, 0.265 g/L CaCl2), finally 150µl of Respiration Buffer containing pH-Xtra probe at the recommended concentration was added to each well. The plate was then measured kinetically on a FLUOstar Omega (BMG Labtech) for 90-120mins with ~2 minute interval exciting the probe at 380nm and measuring emission at 615nm. Ratiometric measurements were performed using the following delay and gate settings. Delay 1: 100s, Gate 1: 30s, Delay 2: 300s, Gate 2: 30s.Oxygen consumption provides detailed information on mitochondrial function, specifically on the activity of the electron transport chain (ETC),while extracellular acidification (ECA) informs on glycolytic flux. Measurements are conducted on standard 96-well microtitre plates, on a fluorescence plate reader, and facilitate a deep insight into the metabolic behaviour of the 3D culture and into how metabolism is perturbed by a particular compound or environmental condition. 3D cell culture facilitates the development of complex intra-cellular interactions thereby helping to narrow the gap between in vitro and in vivo biological systems. Adoption of 3D technologies has however been limited, in part due to difficulties associated with producing reproducible 3D cultures. Difficulties can also arise due to an incompatibility with certain in vitro assay technologies.

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[1]L-lecture; P-poster

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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.

Now AZM is preparing for an European Choir Games in German Magdeburg!

Silesia.

Positive energy

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