XIXth Gliwice Scientific Meetings 2015

Gliwice, November 20-21, 2015

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

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19th Gliwice Scientific Meetings

Gliwice, 20-21 November 2015

Friday; 20th November, 2015

9.00 – 9.10 ***Opening Ceremony***

9.10 – 13.10 **Session I**

Intra-tumour heterogeneity and evolution *(session organized by EACR members)*

Chairperson: Bożena Kamińska

Urszula Hibner *(University of Montpellier, Montpellier):* Cancer and evolution

Anna Golebiewska *(Luxembourg Institute of Health, Luxembourg)*: Clonal evolution meets cancer stem cells: in-depth analysis of genetic and phenotypic heterogeneity in glioblastoma

Gareth Wilson *(Cancer Research UK, London Research Institute London, London):* Deciphering cancer genome evolution and intra-tumour heterogeneity

Trevor Graham *(Barts Cancer Institute, Queen Mary University of London):* Intra-tumour heterogeneity as a pan-cancer prognostic biomarker

11.10 – 11.30 ***Coffee Break***

Bozena Kaminska *(Nencki Institute of Experimental Biology, Warszawa):* Cancer stem cells and immune microenvironment

Anke van den Berg *(University of Groningen, Groningen):* Genomic inter- and intra-tumor heterogeneity in primary lung cancer and its metastasis

Kathrina Erenpreisa *(Latvian Biomedical Research Center, Riga):* Sub-cellular heterogeneity and asymmetry in fate choice of irradiated HeLa and etoposide-treated ovarian teratocarcinoma PA1 cells

Marek Los *(Linkoping University, Linkoping):* Generation of limbal epithelial cell progenitors by two methods: by differentiation from iPS cells and by direct trans-differentiation from human dermal fibroblasts

13.10 – 14.15 ***Lunch and Poster Viewing*** **(Poster Session I)**

14.15 – 16.45 **Session II**

Bioinformatics

Chairperson: Joanna Polańska

Jacek Koronacki *(IPI-PAN, Warszawa):* Discovering interdependencies of features in disease-related genomic data

Jan Komorowski *(Uppsala University, Uppsala)* Genes causing many cancers are located near tumor-mutated motifs for the CTCF and other transcription factors

Marek Kimmel *(Rice University, Houston):* Modeling lung cancer drivers based on the cancer genome atlas data

Klaus H. Maier-Hein *(DKFZ, Heidelberg):* Data-driven oncologic image analysis

David P. Kreil *(University of Natural Resources and Life Sciences, Vienna):* Power and limitations of RNA-Seq: findings from the SEQC consortium

16.45 – 17.15 ***Coffee Break and Poster Viewing*** **(Poster Session I)**

17.15 – 19.00 **Session III**

Roles of noncoding RNAs

Chairperson: Joanna Rzeszowska-Wolny

Gunter Meister *(Regensburg University, Regensburg):* RNA-binding proteins as regulators of coding and non-coding gene expression

Joanna Rzeszowska-Wolny *(Silesian University of Technology, Gliwice*): X-ray induced changes in RNA interference suggest independent modulation of mRNA and protein levels

Joost Kluiver *(University of Groningen, Groningen):* Non-coding RNAs as oncogenic components of the MYC regulatory network in Burkitt lymphoma

Gabor Szabo *(University of Debrecen, Debrecen):* DNA/RNA hybrids in the chromatin

20.00 – ***Conference Dinner***

Saturday; 21nd November, 2015

9.00 – 10.00 ***Coffee and Poster Viewing*** **(Poster Session II)**

10.00 – 11.50 Session IV
Radiation Biology
Chairman: Piotr Widlak

Serge Candeias (*CEA, Grenoble*): Effects of low dose radiation on the T lymphocyte repertoire in the mouse: analysis from a TCR point of view

Ingeborg Tinhofer-Keilholz (*Charite University, Berlin*): Mutational profiling using next-generation sequencing reveals distinct molecular mechanisms of radioresistance in HPV+ and HPV- head and neck cancer

Piotr Widłak (*Institute of Oncology, Gliwice*): Signature of serum proteome in patients exposed to ionizing radiation

Karol Jelonek (*Institute of Oncology, Gliwice*): Characterization of exosomes released from irradiated cells

Andrzej Swinarew *(SHIM-POL, Izabelin; Silesian University, Katowice)*: MALDI-ToF and UHPLC in peptide and protein characterization - potential application in radiation research

11.50 – 12.30 ***Coffee Break and Poster Viewing*** **(Poster Session II)**

12.30 – 15.00 Session V
Mitochondrial channels and reactive oxygen species
Chairman: Zbigniew Grzywna

Adam Szewczyk *(Nencki Institute of Experimental Biology, Warszawa*): What we don't know about mitochondrial potassium channels

Krzysztof Dołowy (*Warsaw University of Life Sciences, Warszawa*): Ion channels in mitochondria: the matter of life and death

Piotr Bednarczyk (*Warsaw University of Life Sciences, Warszawa*): Regulation of mitochondrial potassium channels

Przemysław Borys *(Silesian University of Technology, Gliwice)*: Estimation of the maximum flux through ion channel

Magdalena Skonieczna *(Silesian University of Technology, Gliwice*): Inhibition of the voltage-dependent anion channels and their influence on cell functioning

Jakub Hanus *(Tulane University, New Orleans*): Induction of necroptosis by oxidative stress in retinal pigment epithelial cells

Katarzyna Szołtysek (*Institute of Oncology*, *Gliwice*): Role of reactive oxygen species in crosstalk between UV and cytokine activated signaling

Szymon Borek *(ALAB, Warszawa):* Modification in modern tools for cell based assays including mitochondria and reactive oxygen species analysis

15.00 – 16.00 (Poster Session I and II)

Best Poster Presentations

16.00 – ***Concluding Remarks, Lunch and Final Discussions***

Lecture abstracts

Session I:
Intra-tumour heterogeneity and evolution

(session organized by EACR members)

Chairperson: Bożena Kamińska

CANCER AND EVOLUTION

Urszula Hibner

Institut de Génétique Moléculaire de Montpellier, CNRS UMR 5535, 1919 route de Mende, Montpellier, France

Cancers are complex ecosystems that are subject to the laws of Darwinian evolution. The evolutionary framework is useful both for understanding the origins of cancer and for representing the interactions between tumour cells and their environment.

The passage from unicellular to multicellular forms of life involved multiple evolutionary trade-offs that gave rise to highly cooperative cellular behaviour. This creates favourable conditions for appearance of "cheaters" or "free-riders". We will briefly discuss how such cheating behaviour leads to a breakdown of foundations of multicellularity and how it is related to carcinogenesis.

Next we will examine perturbations of tissue homeostasis caused by viral infections that lead to an increase in cancer risk. We will use as example physiopathological alterations caused by hepatitis C viral infection that impact on the features of multicellular organisation, thus favouring tumour development.

CLONAL EVOLUTION MEETS CANCER STEM CELLS: IN DEPTH ANALYSIS OF GENETIC AND PHENOTYPIC HETEROGENEITY IN GLIOBLASTOMA

Anna Golebiewska, Daniel Stieber, Anne Dirkse, Sebastien Bougnaud, Nicolas H.C. Brons, Rolf Bjerkvig, Simone P. Niclou

Norlux Neuro-Oncology laboratory, Luxembourg Institute of Health, Luxembourg

The genetic, molecular and pathophysiological heterogeneity of GBM represents a major obstacle for effective therapies. The concepts of clonal evolution and cancer stem cells (CSC) were proposed to shed light on the strong intra-tumoral heterogeneity and tumor recurrence, by the presence of subpopulations of tumor cells resistant to radio- and chemotherapy.

Here we will present our current understanding on CSCs and clonal evolution in GBM and highlight the influence of tumor heterogeneity at the genetic, phenotypic and microenvironmental level on the precise characterization of tumor cell subpopulations. We will further highlight the importance of appropriate clinically relevant research models to adequately comprehend inter- and intra-tumoral heterogeneity. Over the last years we and others have established patient derived xenograft (PDX) models based on organotypic GBM spheroids that recapitulate genetic and histological characteristics of human GBM. Adapting the PDX model to eGFP expressing mice allowed us to clearly discriminate between tumour and host cells. Using flow cytometry we are able to identify and characterize small subpopulations of cells, according to cell surface and internal markers and according to their DNA content (ploidy level). Our data shows that commonly used cancer stem cell markers are not specific to tumor populations and the analysis of CSCs is biased by the presence of stromal cells carrying stem cell properties. Based on DNA ploidy profiling, we show that putative CSCs are genetically heterogeneous and do not constitute a genetically defined subpopulation of tumour cells. Finally, our data suggests that putative CSCs do not represent a stable entity but a population of cells adapting to a changing microenvironment. We propose that intra-tumoral phenotypic heterogeneity may result from dynamic adaptation of tumor cells rather than from a hierarchical organisation of CSCs and their progeny.

DECIPHERING CANCER GENOME EVOLUTION AND INTRA-TUMOUR HETEROGENEITY

Gareth Wilson

Cancer Research UK, London Research Institute London, London

The importance of intratumour genetic and functional heterogeneity is increasingly recognised as a driver of cancer progression and survival outcome. Our recent study in oesophageal adenocarcinoma explored the mutational processes during disease course and following neoadjuvant chemotherapy using multi-region exome-sequencing. This approach is being taken further in TRACERx (TRAcking non-small cell lung Cancer Evolution through therapy [Rx]), a prospective study of patients with primary non-small cell lung cancer (NSCLC). TRACERx aims to investigate the clonal architecture of NSCLC and its evolution over time through multi-region and longitudinal tumour sampling and genetic analysis. This study links complex genomic data with clinical outcome and aims to identify potential therapeutic targets, which may ultimately guide patient treatment and management, and therefore improve clinical outcome through the delivery of personalised cancer medicine.

INTRA-TUMOUR HETEROGENEITY AS A PAN-CANCER PROGNOSTIC BIOMARKER

Trevor Graham

Evolution and Cancer Laboratory, Centre for Tumour Biology, Barts Cancer Institute, Queen Mary University of London John Vane Science Centre, Charterhouse Square, London EC1M 6BQ

Carcinogenesis is an evolutionary process; establishing the prognosis for a cancer therefore requires predicting the future course of cancer evolution. The same is true in pre-cancerous conditions: the risk of developing cancer is determined by how the pre-cancerous lesion is evolving. However, it is unclear how best to measure tumour evolution, and relatedly, how best to physically sample a neoplasm, in order to determine prognosis.

One way to quantify the evolvability of a population is by the level of within-lesion diversity. If there is no diversity natural selection cannot operate, whereas diverse populations are likely to contain well-adapted individuals that can prosper in changing environments and consequently quantification of within-tumour diversity is likely to be a proxy-measure of the rate of the underlying evolutionary process that drives carcinogenesis. In this talk, I will describe how we have measured within-tumour diversity, both genetically and phenotypically, and used these measures to successfully determine prognosis in both cancers and in premalignant lesions. Diversity is just one way to measure tumour evolution though, and other measures may be more prognostic. In the second part of my talk, I will describe how we have been using computational modelling to search for optimal prognostic biomarkers and tissue sampling strategies.

CANCER STEM CELLS AND IMMUNE MICROENVIRONMENT

Bozena Kaminska

Neurobiology Center, Nencki Institute of Experimental Biology

Malignant gliomas attract immune brain resident microglia and peripheral macrophages, and re-program these cells into pro-invasive, immunosuppressive cells. It results in formation of the tumor supportive microenvironment and evasion of antitumor responses. Gene expression profiling in glioblastoma (GBM) derived microglia/macrophages indicates their switch to the immunosuppressive, M2-like phenotype that is associated with accumulation of regulatory T cells and myeloid derived suppressive cells. Computational analysis of gene expression networks revealed dysfunction of IKKβ-NFkB signaling pathways Downregulation of IKKβ expression in tumor infiltrating immune cells and deficits in NFkB activation were confirmed by biochemical and immunocytochemical studies of GBM tissues and in animal models. Signals responsible for polarization of immune cells into protumorigenic phenotype in GBM are poorly known. Proteomic analysis of glioma secretome combined with a functional assay revealed osteopontin (SPP1) and lactadherin (MGF-E8) as activating factors. Both proteins stimulated primary microglia cultures via integrin signaling that results in activation of PI-3K/Akt and FAK, enhancement of microglial migration, phagocytosis and transcriptional responses. *SPP1* (encoding osteopontin) was overexprressed in glioma stem cells and contributed to their self renewal. Knockdown of *SPP1* in glioma cells strongly reduced growth of intracranial gliomas and blocked pro-invasive polarization of infiltrating immune cells. Accumulation of T regulatory cells was significantly reduced SPP1-depleted gliomas and T cytotoxic cells were switched to Th1 responses which is consistent with partial restoring of antitumor responses. This defines osteopontin/SPP1, both glioma-derived and originated from stromal cells in the perivascular niche as a new biomarker and target for glioma therapy.

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GENOMIC INTER- AND INTRA-TUMOR HETEROGENEITY IN PRIMARY LUNG CANCER AND ITS METASTASIS

Ali Saber[1], Jeroen T. Hiltermann[2], Klaas Kok[3], Diana Spierings[4], M. Martijn Terpstra[3], Aaron Taudt[4], Wim Timens[1], Maria Colomé Tatché[4], Peter Lansdorp[4,5], Harry J.M. Groen[2], Anke van den Berg[1]

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[2]*Department of Pulmonary Diseases,*
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[4]*European Research Institute for the Biology of Ageing, University of Groningen, University Medical Centre Groningen, The Netherlands.*
[5]*Terry Fox Laboratory, BC Cancer Research Centre, Vancouver, Canada*

Introduction: Several studies showed intra-tumor heterogeneity in lung cancer, with multiple clones characterized by their own specific mutational landscape. The extent to which minor clones become dominant in distinct metastasis is not clear. Aim of our study is to investigate inter and intra-heterogeneity in lung cancer in primary and multiple distant metastases.

Materials and methods: Whole exome sequencing (WES) was performed on DNA isolated from total, macro-dissected, or laser micro-dissected tissue sections of 29 samples of 3 non-small cell lung carcinoma (NSCLC) and 2 small cell lung carcinoma (NSCLC) patients using a hybridization-based target enrichment protocol. Genome analysis toolkit (GATK) was used to analyze data and the 1000-Genome database was used to remove single nucleotide polymorphisms. Additional personal variants were excluded using z-score. Mutant read frequencies were corrected based on the estimated normal cell admixture. Validation of a large subset of the somatic mutations was carried out by a PCR-based target enrichment protocol (NuGEN). Single cell whole genome sequencing (WGS) was carried out on the primary tumors and the liver metastasis of one SCLC patient.

Results: Ninety-two to 462 somatic mutations were observed in the five lung cancer patients. Approximately, 50% of all detected mutations were predicted to be damaging at the protein level. For each of the three NSCLC patients, about half of the mutations was shared between all tumor samples, whereas over 95% of mutations were shared in the tumor samples of the two SCLC patients. WES-based copy number variations (CNV) plots showed inter-tumor heterogeneity in all the NSCLC patients and in one of the SCLC patients. Single cell WGS-based CNV plots of the primary and liver metastasis revealed a much higher intra-tumor variation in primary tumor compared to the metastasis. Interestingly, we observed a CNV pattern similar to the liver metastasis cells in two out of 83 single cells of the primary tumor.

Conclusion: Patients with advanced NSCLC have a high percentage of non-ubiquitous mutations. In contrast, SCLC patients showed a limited number of non-ubiquitous mutations, but did reveal a high degree of heterogeneity based on the single cell WGS-based CNV plots. Moreover, single cell derived CNV plots of 2 primary tumor derived single cells closely resembled the CNV plot of the bulk analysis of the liver metastasis.

SUB-CELLULAR HETEROGENEITY AND ASYMMETRY IN FATE CHOICE OF IRRADIATED HELA AND ETOPOSIDE-TREATED OVARIAN TERATOCARCINOMA PA1 CELLS

Je. Erenpreisa, K. Salmina, A. Huna, A. Vazguez-Martin

Latvian Biomedical Research and Study Centre, Riga, Latvia

The whole genome doublings played a prominent role in diversification of genes and evolution of species, while endopolyploidy (multi-nuclearity) allowing diversification of phenotypes originated multi-cellular organisms. The polyploidy of tumour cells induced by genotoxic damage is started with tetraploidy, which may display the autonomy of sub-nuclei in a cell cycle. The next degree of heterogeneity is the emergence of a bi-potential metastable state allowing competition between self-renewal and senescence with alternative fate choice. However, a more pervasive heterogeneity was also observed in about 1-5% of ETO-treated PA1 showing polarity in distribution of stress determinants in tetraploid tumour cells with two partly separated sub-nuclei. Observations showed that this asymmetry can be likely resoluted in the first asymmetric division segregating senescence (or mitotic catastrophe) from stemness, while the next symmetric division returns self-renewing diploid daughters into mitotic cycle.

GENERATION OF LIMBAL EPITHELIAL CELL PROGENITORS BY TWO METHODS: BY DIFFERENTIATION FORM IPS CELLS AND BY DIRECT TRANS-DIFFERENTIATION FROM HUMAN DERMAL FIBROBLASTS

Artur Cieślar-Pobuda[1,2,3] Viktoria Knoflach[1,3], Saeid Ghavami[4], Marek Los[1,3]

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The corneal epithelium is maintained by a small pool of tissue stem cells located at the limbus. Through certain injuries or diseases this pool of stem cells may get depleted. This leads to visual impairment. Standard treatment options include autologous or allogeneic limbal stem cell transplantation, but graft rejection and chronic inflammation lowers the success rate over long time. Induced pluripotent stem (iPS) cells have opened new possibilities for treating various diseases with patient specific cells, eliminating the risk of immune rejection. In recent years, several protocols have been developed, aimed at the differentiation of iPS cells into the corneal epithelial lineage by mimicking the environmental niche of limbal stem cells. The use of iPS cells increases the risk of teratoma formation. Furthermore, it has been observed that tissues derived form lentiviral-method generated iPS are more prone to cancer development. Both reasons hinder the application of iPS cells in the clinics. Here we show that the differentiation of iPS cells into corneal epithelial cells results in the expression of corneal epithelial markers showing a successful differentiation, but the level of gene expression for the pluripotency markers does not vanish completely and Klf4 is expressed at very high levels, probably due to transgene activation. Therefore, we have tested a direct transdifferntiation approach to circumvent the intermediate state of pluripotency (iPS-stage). The resulting cells exhibited corneal epithelial cell morphology and expressed corneal epithelial markers (please see the attached figure). We shows for the first time a direct transdifferentiation of human dermal fibroblasts into the corneal epithelial lineage. Thus, easily-obtainable autologous human dermal fibroblasts may serve as progenitors for the production of corneal epithelial cell for transplantation approaches.

Session II:
Bioinformatics
Chairperson: Joanna Polańska

DISCOVERING INTERDEPENDENCIES OF FEATURES IN DISEASE-RELATED GENOMIC DATA

Jacek Koronacki

Institute of Computer Science, Polish Academy of Sciences, Poland

More often than not, contemporary computational methods discover single variables associated with phenotypes under the degenerate assumption that biological systems can be characterized by single parameters. This diverges from the principle that a variety of variables (features) are to define the fate of the living organisms, through non-linear complexity.

Our aim has been to apply a new approach established on classification-based feature selection and rule-based modeling. We use classifiers to learn which features contribute best to classifying observations (objects, samples) into distinct classes and what are the interdependencies between the features that describe the observation. The underlying hypothesis is that the interdependent features do not only reflect some syntactical properties of the data and classifiers but also may convey meaningful clues about true interactions in the modeled biological system.

Specifically, we have further developed our method of Monte Carlo Feature Selection and Interdependency Discovery, the goal of which is to discover the most informative features and interdependencies between them. The network of interdependencies is given in the form of a directed graph of interactions between extracted features. We then proceed with modeling interactions on a finer level with rule sets to assign feature values to specific decision classes.

This approach was successfully used in a number of applications. We shall illustrate it with examples from the study of human immune system responses to various stimuli of CD4+ T-cells depending on the racial background. We learnt, e.g., that gene-responses related to bacteria characterize Afro-Americans while those related to viruses characterize Asians.

GENES CAUSING MANY CANCERS ARE LOCATED NEAR TUMOR-MUTATED MOTIFS FOR THE CTCF AND OTHER TRANSCRIPTION FACTORS

Jan Komorowski

Program in Computational Biology and Bioinformatics, Department of Cell and Molecular Biology, Uppsala University, Sweden;
The Computational Biology Laboratory. Institute of Computer Science, Polish Academy of Sciences, Warszawa, Poland.

It has been known that somatic mutations drive cancer. Previous studies concentrated on coding sequences. However, there is growing evidence that mutations in the regulatory regions are over-represented in cancer and thus may be associated with the disease. The availability of public data such as, for instance, the ENCyclopedia of CODing Elements (ENCODE), the Epigenome Roadmap, The Cancer Genome Atlas (TCGA) and the International Cancer Genome Consortium (ICGC) allow for studying the human genome at a base pair level. Interestingly, the mutations in coding regions have been investigated in great detail, while mutations in the regulatory regions have been researched on a more general level using statistical approaches based on transcription factor (TF) motifs or TF binding.

We currently analyze the roles of CTCF, a highly conserved zinc finger protein that is best known as a transcription factor. It has known roles as a transcriptional activator, a repressor/insulator protein and a blocker of the communication between between enhancers and promoters. CTCF can be a recruiter of other transcription factors, too. It has been also known that genomes of many species are covered with many CTCF-binding sites.

Specifically, we developed a computational method for finding regulatory regions using ChIP-seq data from the ENCODE project. Then, we introduced a novel concept of so-called motif-breaking mutations. These are mutations that affect TF- binding disruption in a cell-specific manner and are located at bases with high information content. Only regulatory motifs derived from the corresponding TFs that had an experimental evidence of binding in a matched cancer line are considered. We found 1214 mutations predicted to significantly reduce binding affinity of many TFs. In hepatocellular carcinoma (HCC) there were 905 mutations with most of them at a high affinity position of the CTCF motifs in HCC and in gastric and pancreatic cancers.

Near the mutated motifs for all TFs in HCC and near the mutated motifs for CTCF in the three cancers there was a significant enrichment of 1) genes mutated in cancer, 2) tumor suppressor genes, and a highly significant enrichment of 3) genes in KEGG cancer pathways and 4) sets of genes previously associated with cancer. These findings were supported by experimental validation with electrophoresis mobility shift assays. Mutations located at regulatory motifs but not defined as motif-breaking showed no effect on TF binding.

CTCF is important for insulator function and for establishing the domain structure of the genome. It follows that mutations in its motif are likely to affect expression of many genes. Our strategy can be applied to any cell type with established TF motifs and will aid in identifications of genes regulating the development of cancer.

In this talk I will make a reasonable effort to present the material in a self-contained way without significant assumptions about prior knowledge of cancer etiology or computational background.

MODELING LUNG CANCER DRIVERS BASED ON THE CANCER GENOME ATLAS DATA

Marek Kimmel

Rice University, Houston and Silesian University of Technology, Gliwice.

We analyze the occurrence of passenger and driver mutations in The Cancer Genome Atlas adenocarcinoma of the lung (LUAD) data. We focus on two types of drivers: "curated drivers" which have been demonstrated to be associated with disease in the sense of a biological mechanism, and "inferred drivers" which have been determined by computerized algorithms based primarily on evolutionary and other principles such as PolyPhen-2 and CHASM. As it is known, the number of mutations in different LUAD cases varies widely. When the mutations are categorized into inferred drivers and passengers, the number of passenger mutations is increasing with the number of inferred drivers, as it has been suggested by several theoretical models. In contrast, there is no such dependence between passengers and curated drivers. Moreover, unsupervised association analysis indicates that each curated driver co-occurs with a subset of inferred drivers, the subsets only partly overlapping. Clusters of inferred drivers contain genes implicated in cancer-related processes, with functionalities varying from one cluster to another. These finding seem consistent with a hypothesis by which malignancy may be driven by a very limited number of mutations (such as our "curated drivers") which, aided by natural selection, cause a larger subset of gene variants (our "inferred drivers") to be fixed in the tumor, with a still larger set persisting due to genetic drift or hitchhiking (genetic draft). Discussion includes an outline of a conceptual stochastic model, which might reproduce the findings. [Joint work with Thomas O. Macdonald, Siyi Chen and David Wallace-Bradley at Rice and Roman Jaksik at Silesian Tech].

DATA-DRIVEN ONCOLOGIC IMAGE ANALYSIS

Michael Goetz, Klaus H. Maier-Hein

Juniour Group Medical Imaging Computing, German Cancer Research Center (DKFZ) Heidelberg, Germany

Data-driven, learning-based methods are commonly used to improve the analysis of oncological images. While these methods show usually good performance they require annotated training data to learn a data-specific model. The collection and annotation of necessary data is often very time-consuming and error-prone due to the heterogeneity, ambiguity, and size of the bio-medical data. The collection of appropriate training data is therefore a major obstacle for the use of these methods.

We present a method that allows using only sparsely and unambiguously annotated training data in combination with commonly used learning-based methods. By weighting the annotated training data to match the distribution of all available and unlabeled data a sampling error is avoided. This reduces the sampling error that is introduced by the partial annotation.

A further improvement of the methods is achieved by selecting only training data of high quality and high similarity to the new data. We describe a new method that selects the best training for any new image based on a learned similarity. An optimal classifier is therefore trained for every new and unlabeled image.

The evaluation – based on image segmentation problems – shows that the required annotation time can be significantly reduced by using only sparse and unambiguous annotations. Using our method reduces the introduced sampling bias leading to results that are similar to results that are obtained with full annotation. We show further that the selection of appropriate training data for every training image further improves the quality of the results.

POWER AND LIMITATIONS OF RNA-SEQ: FINDINGS FROM THE SEQC CONSORTIUM

D. P. Kreil, P. P. Łabaj

Boku University Vienna

In a large benchmark study coordinated by the US FDA, we have tested different sequencing platforms at multiple sites using well-established reference RNA samples. Exploiting "built-in truths" in the experimental design, we have assessed the discovery and expression-profiling performances of alternative platforms and analysis pipelines. The results demonstrate that novel exon-exon junctions can still be discovered beyond existing comprehensive annotations and at high sequencing depths. Extensive investigations encompassing diverse performance metrics characterizing reproducibility, accuracy, and information content were combined with comparisons to qPCR and microarray platforms. We have shown that good inter-site and cross-platform concordances for differentially expressed genes are possible with sufficiently effective filters. This is particularly critical in clinical and regulatory settings. In general, performance is application, platform, and pipeline dependent, with transcript-level profiling affected more strongly. Together with data from applications of RNA-Seq from several preclinical and clinical problems, the entire SEQC data sets comprise >100 billion reads (10Tb) and provide a unique resource for testing future developments of RNA-Seq.

Session III:
Roles of noncoding RNAs
Chairperson: Joanna Rzeszowska

RNA BINDING PROTEINS AS REGULATORS OF CODING AND NON-CODING GENE EXPRESSION

Gunter Meister

University of Regensburg, Regensburg, Germany

In animals, microRNAs (miRNAs) are transcribed as capped and polyadenylated primary transcripts. Mature miRNAs are processed from these transcripts by the subsequent action of the two RNase III enzymes Drosha and Dicer. In the cytoplasm, miRNAs directly bind to a member of the Argonaute (Ago) protein family and guide it to partially complementary target sites on mRNAs leading to inhibition of gene expression. Various studies have found that miRNA levels can be regulated post-transcriptionally at almost all steps of maturation and very often RNA binding proteins (RBP) are involved in such regulatory events. To find novel RBPs with functions in miRNA biogenesis, we have performed a proteomics screen and identify more than 100 RNA binding proteins, which specifically interact with miRNA precursors. We find that many of them positively or negatively regulate miRNA processing. It is becoming more and more apparent that a large number of RNA binding proteins form an additional layer of complexity in miRNA biogenesis and function.

Based on our findings, we can also assign RNA-binding activity to proteins that have previously not been involved in RNA binding. We find that the NHL domain of TRIM-NHL proteins is a novel RNA binding domain. We have identified the binding motif of the TRIM-NHL domain protein Brain Tumor (BRAT) from Drosophila and crystallized it in complex with RNA. Brat has a specific function in self-renewal and differentiation processes in neuronal stem cells. Our work assigns a novel regulatory function to this important stem cell factor in Drosophila.

X-RAY-INDUCED CHANGES IN RNA INTERFERENCE SUGGEST INDEPENDENT MODULATION OF MRNA AND PROTEIN LEVELS

Joanna Rzeszowska-Wolny

Systems Engineering Group, Faculty of Automatic Control, Electronics and Informatics, Silesian University of Technology, Gliwice, Poland

Genes are regulated by mechanisms operating at different levels. One of such mechanisms, RNA interference, is based on interactions between RNA-induced silencing complexes (RISCs) and mRNAs. RISC-mRNA interactions may lead to negative regulation of translation or degradation of mRNA. RISCs are addressed to their cognate mRNAs through complementary interactions between microRNAs (miRNAs) and mRNAs, and mutations or RNA modifications may influence the efficiency of RNA interference. Complementary interactions are also important for miRNA biogenesis and their interaction with Argonaute (AGO) proteins. In experiments in which we studied the expression of reporter genes containing sequence motifs targeted by three different miRNAs which differ in their complementarity to the mRNA targets, we observed a correlation between miRNA-target complementarity and protein but not mRNA levels. We observed fluctuations of mRNA and protein levels but high levels of proteins did not correlate with high levels of mRNA, suggesting that mRNA degradation and inhibition of translation could be separate processes. The time course of mRNA level fluctuations are different in X-irradiated cells, and in these cells maximal mRNA and protein levels are observed at the same time points. X-rays are known to induce a wave of reactive oxygen species that can modify the structure of mRNA, miRNA and pre-miRNA, thus potentially changing their mutual interactions and interactions with RNA binding proteins. To test if different types of AGO proteins may differ in pre-miRNA structure recognition, we performed multi-parameter analysis in which the structural features of 127 pre-miRNAs bound to AGO1 or AGO2 were compared. This analysis showed that A-G mismatches in pre-miRNA structure may favor its binding to AGO1 protein. Such binding preferences and their modification in certain conditions may be important elements in switching between RNA degradation and inhibition of translation in RNA interference mechanisms.

NON-CODING RNAS AS ONCOGENIC COMPONENTS OF THE MYC REGULATORY NETWORK IN BURKITT LYMPHOMA

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Burkitt lymphoma (BL) is an aggressive form of B-cell lymphoma characterized by a hallmark translocation resulting in overexpression of the oncogenic transcription factor MYC. A large number of protein-coding genes are known to be regulated by MYC as well as a substantial amount of non-coding RNAs (ncRNAs), including microRNAs (miRNAs) and long ncRNAs (lncRNAs). MiRNAs are short RNA molecules that are known to act at the posttranscriptional level while lncRNAs have more recently emerged as a novel class of regulatory RNAs acting at the epigenetic, transcriptional or posttranscriptional level. Aberrant expression of both types of ncRNAs have been clearly implicated in various aspects of tumorigenesis. In this study we investigated which short and long ncRNAs are regulated by MYC and to what extend these ncRNAs contribute to BL pathogenesis.

MicroRNA expression profiling revealed that MYC significantly contributes to the miRNA signature of BL. To determine the functional relevance of MYC-regulated miRNAs we studied the effect of miRNA overexpression and inhibition on proliferation. Both the inhibition of MYC-induced miRNAs as well as the induction of MYC-repressed miRNAs resulted in several instances in repression of BL cell growth. In particular the overexpression of MYC-repressed miR-150, miR-26a and miR-26b strongly reduced BL cell growth, similar to the effect of MYC inhibition itself. By applying AGO2-RIP-CHIP we identified 12 genes consistently targeted by miR-150 in 2 BL cell lines. The top 3 AGO2-IP-enriched genes included MYB, a validated miR-150 target, ZDHHC11 and ZDHHC11B. Interestingly, the latter 2 transcripts contain high numbers of tandem repeats of the miR-150 binding motif. We are currently investigating the role of these genes in BL pathogenesis.

To identify MYC regulated lncRNAs we generated expression profiles of a B cell lymphoma model with a tetracyclin repressible MYC allele, primary lymphoma cases with high and low MYC expression, BL cell lines and normal B-cells. This revealed that lncRNAs are extensively regulated by MYC. We demonstrate that both Myc-induced mRNAs and lncRNAs are significantly enriched for MYC binding sites. Subcellular localization analysis revealed that compared to mRNAs, lncRNAs more often have a specific subcellular localization. We are currently screening a subset of consistently MYC-induced lncRNAs in a loss-of-function screen. Our first results indicate that next to miRNAs also MYC-regulated lncRNAs contribute to BL pathogenesis. Thus, our data indicate that ncRNAs, including miRNAs and lncRNAs, are a substantial component of the MYC network and contribute to the MYC-dependent growth of BL cells.

RNA/DNA-HYBRIDS IN THE CHROMATIN

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R-loops form when an RNA strand displaces one of the strands of a DNA duplex, yielding an RNA/DNA-hybrid and single-stranded (ss) DNA, encompassing an interval that may be as long as in the order of thousand bp. Long R-loops may have physiological function in class-switch recombination, regulation of transcriptional termination, and have been also linked to genomic instability. The RNA/DNA-hybrids can be detected by a hybrid specific monoclonal antibody (S9.6), while the ss DNA can be visualized using labeled single-stranded DNA binding protein (SSBP). Fluorescence resonance energy transfer suggestive of molecular distance between the two is taken as evidence of the R-loop nature of the structures seen. The S9.6 antibody can be used for chip purposes, making chip chip or chipseq mapping studies possible. We have also elaborated a reverse South-Western (rSW) blot procedure to detect the hybrids within nitrocellulose blotted restriction fragments, and used S9.6 on combed DNA molecules as well. Using these tools, we have been studying the localization of these molecular entities in the yeast genomic DNA, in the repetitive rDNA locus of S. cerevisia, and in human genomic DNA. Having developed a laser scanning cytometry (LSC) based method to analyze DNA loops present in the halos of salt-extracted nuclei preparations, we could show that the nuclear domains harbouring R-loops are localized to the nuclear matrix containing relaxed DNA, confined within the space delimited by the nuclear lamina in these samples, sparing the superhelical loops that protrude out. We speculate that R-loops are part of the structures involved in the higher-order organization of chromatin in eukaryotic cells.

Session IV:
Radiation Biology
Chairman: Piotr Widlak

EFFECTS OF LOW DOSE RADIATION ON THE T LYMPHOCYTE REPERTOIRE IN THE MOUSE: ANALYSIS FROM A TCR POINT OF VIEW

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Genetic instability is one of the main predicted consequences of low dose radiation exposure. Although considerable efforts are focused on developing much needed biomarkers of radiation-exposure and long term effects, it remains a challenge to identify a marker sensitive enough for detection of low doses exposure and effects, yet suitable for use in large scale epidemiological studies.

T lymphocytes play an essential role in the defense of the organism against pathogens and cancer. This role is mediated by the clonally distributed antigenic T cell receptor (TCR) expressed on their surface. The genes coding for the two TCR subunits exist in the germline a discrete V, D and J gene segments that need to be assembled during T lymphocyte development in the thymus by a site-directed somatic rearrangement process known as V(D)J recombination. Several molecular aspects of V(D)J recombination were recently shown to be altered in a murine model of genetic instability. In addition, illegitimate TCR gene recombination events have been shown to be increased in situations of genetic instability due to exposure to genotoxic chemicals in man, or gene inactivation in mice. As a result, the repertoire of functional T lymphocytes in the periphery might be altered. We postulated that similar events might take place during radiation-induced genetic instability and that TCR alterations could be used as biomarker of exposure to - and long term effects of - low dose radiation. Therefore, we performed an in-depth molecular profiling of the TCR repertoire expressed in peripheral blood lymphocytes in mice exposed to a single low or intermediate dose of ionizing radiation at several time points post irradiation, and specifically investigated the onset of illegitimate V(D)J recombination events in mice after acute or chronic exposure to low dose radiation.

Finally, the large amount of molecular data generated in these studies led to the identification of new aspects of the TCR gene recombination in mice and men, some of which may have consequences for the onset of T cell leukemia.

The work was financially supported by the European Commissions (DoReMi, European Atomic Energy Community's Seventh Framework Programme (FP7/2007-2011) under grant agreement n°249689) and NCN grant HARMONIA 4 register number 2013/08/M/ST6/00924 (JP, CB).Calculations were carried out using infrastructure of GeCONiI (POIG.02.03.01-24-099/13).

MUTATIONAL PROFILING USING NEXT-GENERATION SEQUENCING REVEALS DISTINCT MOLECULAR MECHANISMS OF RADIORESISTANCE IN HPV+ AND HPV- HEAD AND NECK CANCER

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German Cancer Research Center (DKFZ), Heidelberg, Germany and German Cancer Consortium (DKTK) partner sites: [1]Berlin, [2]Dresden, [3]Essen, [4]Frankfurt, [5]Freiburg, [6]Heidelberg, [7]München, [8]Tübingen, Germany; [9]Charité University Hospital, Dept. of Radiooncology and Radiotherapy, Berlin, Germany.

Background: The genetic landscape of SCCHN is currently being unravelled by an increasing number of studies using next-generation sequencing (NGS). The role of distinct mutational patterns for treatment outcome remains largely unknown. We here analyzed mutational patterns of HPV+ and HPV- tumors and compared results with outcome after uniform chemoradiation.

Methods: Archival tumor specimens from 208 patients with locally advanced SCCHN of the hypopharynx, oropharynx or oral cavity, all uniformly treated with surgery and adjuvant cisplatin-based radiochemotherapy at one of the eight partner sites of the German Cancer Consortium were included in this study. An in-house gene panel for semiconductor-based sequencing, covering 211 exons from 45 genes frequently altered in SCCHN was used for mutational analysis. Genetic alterations were correlated with HPV status, clinical risk parameters and patient outcome.

Findings: Mutational profiles were successfully established for 185 SCCHN cases. Interestingly, HPV+ carcinomas were significantly enriched for activating mutations in driver genes (*PIK3CA* 27%, *KRAS* 8%, *NRAS* 4%, *HRAS* 2%) compared to HPV- cases (P=0.002). Conversely, HPV- tumors showed an increased frequency of loss-of-function alterations in tumor suppressor genes (*TP53* 67%, *CDKN2A* 30%, *PTEN* 4%, *SMAD4* 3%) compared to HPV+ cases (P<0.001). After a median follow-up of 55 months, detection of alterations in tumor suppressor genes significantly increased the risk of death (HR 2.9, 95% CI 1.5-5.8, P=0.001), locoregional recurrence (HR 5.4, 95% CI 1.6-18.1, P=0.006) and distant metastasis (HR 2.3, 95% CI 1.0-5.1, P=0.04). The occurrence of activating driver gene mutations did not significantly influence outcome in the total cohort of patients, however, they were associated per trend with increased risks of locoregional recurrence and death (HR 3.7, 95% CI 0.7-20.6, P=0.12) in the subgroup of HPV+p16+ oropharyngeal carcinomas.

Conclusion: Activating mutations in driver genes occuring in one third of HPV-driven SCCHN seem to negatively interfere with efficacy of adjuvant cisplatin-based chemoradiation. These genes or their associated signaling pathways might represent therapeutic targets for improving cure rates of HPV+ disease.

SIGNATURE OF SERUM PROTEOME IN PATIENTS EXPOSED TO IONIZING RADIATION

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Purpose: Ionizing radiation effects the proteome of irradiated cells and tissue, yet data concerning changes induced during radiotherapy (RT) in human blood are fragmentary and inconclusive. We aimed to identify features of serum proteome and associated processes involved in response to partial body irradiation during cancer treatment.

Methods and Materials: Twenty patients with head and neck squamous cell cancer (HNSCC) and 20 patients with prostate cancer received definitive intensity-modulated RT. Blood samples were collected before RT, just after, and one month after the end of RT. Complete serum proteome was analyzed in individual samples using a shotgun LC-MS/MS approach, which allowed identification of about 450 proteins. About 100 unique proteins were quantified in all samples after exclusion of immunoglobulins, and statistical significance of differences between consecutive samples was assessed. Processes associated with quantified proteins and their functional interaction were predicted using gene ontology tools.

Results: RT-induced changes were marked in HNSCC patient group: 22 upregulated and 33 downregulated proteins were detected in post-RT sera. The majority of changes reversed during follow-up, yet levels of some proteins remained affected one month after the end of RT. RT-upregulated proteins were associated with acute phase, inflammatory response and complement activation. RT-downregulated proteins were associated with transport and metabolism of lipids (plasma apolipoproteins), and blood coagulation. RT-induced changes were much weaker in group of prostate cancer patients, which corresponded to differences in acute radiation toxicity observed in both groups. Nevertheless, general pattern of RT-induced serum proteome changes was similar in both groups of cancer patients.

Conclusions: In this pilot study we proposed molecular signature of radiation response, which is based on specific features of serum proteome. The signature includes upregulation of factors involved in acute/inflammatory response, but also downregulation of plasma apolipoproteins and factors involved in blood coagulation.

CHARACTERIZATION OF EXOSOMES RELEASED FROM IRRADIATED CELLS

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Exosomes are membrane vesicles of endocytic origin that participate in intercellular 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 non-irradiated cells. Exosomes secreted from FaDu cells originating from human squamous head and neck cell carcinoma were analyzed using LC-MS/MS approach.

We found that exposure to ionizing radiation resulted in gross changes in exosomal cargo. There were 217 proteins identified in exosomes from control cells and 384 proteins identified in exosomes from irradiated cells, including 148 “common” proteins, 236 proteins detected specifically after irradiation and 69 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. This 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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MALDI-TOF AND UHPLC IN PEPTIDE AND PROTEIN CHARACTERIZATION - POTENTIAL APPLICATION IN RADIATION RESEARCH

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Rapid development of mass spectrometry in recent years in medical application (fig.1) is associated with search for new techniques andbio compatible materials that can be used to produce a novel type of highly selective analytical sensors for medicine, oras a new technique for medical dosimetry applications.

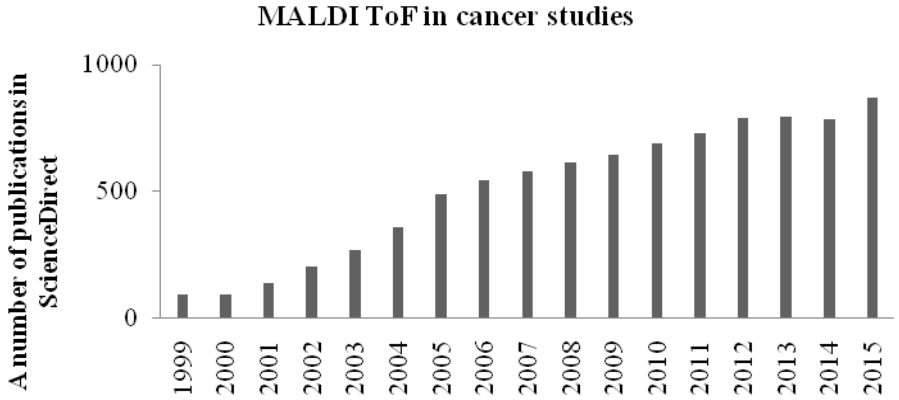

Figure 1. Number of publications according to MALDI – ToF in cancer studies between 1999 and 2015 presented on sciencedirect.com

A very large group of compounds used in this study are macrocyclic compounds, among others synthetic macrocyclic polyethers, discovered in 1967. That discovery involves the development of coordination chemistry of metal ions, which are strongly and selectively complexed by selected criptands or criptand's groups.

The aim of research in this area is the selection of cancer fingerprints by the use of highly sensitive and selective technique for applications in oncology and determination of its interaction with ionization beam.

Keywords: mass spectrometry, cancer fingerprints, solid phase micro extraction, gas chromatography,

This study presents also the preliminary results on the gel response on irradiation. Gel samples were irradiated in a MedTec water phantom using 6MV X-rays generated by a linear accelerator Varian Clinac 2300Ex and were analyzed by the MALDI-ToF.

Session V:
Mitochondrial channels and reactive oxygen species

Chairman: Zbigniew Grzywna

WHAT WE DON'T KNOW ABOUT MITOCHONDRIAL POTASSIUM CHANNELS?

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In this lecture we summarize our knowledge about mitochondrial potassium channels with special focus on unanswered questions of this field. The following potassium channels have been described in the inner mitochondrial membrane: the ATP-regulated potassium channels, Ca^{2+}-activated potassium channels, the voltage-gated Kv1.3 potassium channels, and the two-pore domain TASK-3 potassium channels. The primary functional role of these channels includes changes in membrane potential and mitochondrial respiration. Additionally, they modulate the mitochondrial matrix volume and synthesis of reactive oxygen species by mitochondrial. Mitochondrial potassium channels are believed to contribute to cytoprotection and cell death. In this paper, we discuss observations on fundamental issues concerning mitochondrial potassium channels: their molecular identity, channels pharmacology and their functional properties.

ION CHANNELS IN MITOCHONDRIA: THE MATTER OF LIFE AND DEATH

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Lack of oxygen (ischemia) kills heart and brain cells after oxygen supply is restored. The repeated episodes of limited oxygen supply to heart and brain cells make them less sensitive to apoptosis (ischemic preconditioning). A similar phenomenon was discovered with hearts preconditioned by changing the calcium concentration (called calcium preconditioning) and by the use of K_{ATP} or BK_{Ca} ion channels openers. In both ischemic and calcium preconditioned cells ATP concentration is higher than in non-preconditioned cells. Mitochondria are the organelles that are likely involved in preconditioning because they use oxygen, produce ATP, store calcium, have K_{ATP} or BK_{Ca} ion channels and are involved in programmed cell death (apoptosis). The hypothetical mechanism of the preconditioning is presented. pH of mitochondrial matrix is buffered by apatite-hydroxyapatite equilibrium. In the case of sufficient nutrient and oxygen supply, ATP is produced and its concentration rises. The increased mitochondrial membrane potential $\Delta\psi$ sucks available calcium ions from the cytoplasm. Calcium ions loaded to the matrix are bound to apatite forming hydroxyapatite. During the period of ischemia ATP can still be produced at the expense of ΔpH and calcium ion efflux via the mito-Ca channel maintains the electroneutrality of the process. Thus, the higher the ΔpH (the higher proportion of hydroxyapatite in the matrix) the longer mitochondria will produce ATP during ischemia and thereby prevent damage to heart muscle cells.

REGULATION OF MITOCHONDRIAL POTASSIUM CHANNELS

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In recent years, a number of potassium channels present in the inner membrane of mitochondria have been discovered. Their physiological roles are still unclear. However, it has been observed that potassium channels affect mitochondrial matrix swelling, regulate the concentration of reactive oxygen species, change the mitochondrial membrane potential, and transport calcium into mitochondria. Additionally, it has been shown that activation of mitochondrial potassium channels protects against necrotic and apoptotic cell death during myocardial infarction or cerebral hypoxia. These findings stimulated an intensive study of the pharmacology of mitochondrial ion channels and contributed to the development of many hypotheses concerning the role of mitochondrial ion channels in cell death.

One example of mitochondrial channels is the large-conductance calcium-regulated potassium channel (mitoBKCa channel). We described pharmacological and electrophysiological properties of this channel using patch-clamping mitoplasts isolated from the astrocytoma U-87 MG cell line. The channel was activated by calcium at micromolar concentrations and by potassium channel opener NS1619. On the other hand, this channel was inhibited by paxilline and iberiotoxin, both known inhibitors of BKCa channels. Also, we showed that substrates of the respiratory chain (e.g. succinate) decrease activity of the channel at positive voltages. This effect was abolished by inhibitors of the respiratory chain (e.g. antimycin). Our findings indicate possible structural and functional coupling of the mitoBKCa channel with the mitochondrial respiratory chain in human astrocytoma U-87 MG cells.

This work was supported partially by the Nencki Institute of Experimental Biology, financial resources of WULS-SGGW (505-10-060200-M00390-99) and Polish Mitochondrial Network MitoNet.pl.

ESTIMATION OF THE MAXIMUM FLUX THROUGH ION CHANNEL

Przemysław Borys

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In this work I investigated the quality of the analytical pore size relation to the maximum flux of ions through the channel in the half-spherical approximation of the channel absorption on the channel's mouth. This approximation was used for example as a model to determine the diameter of the BK channel pore. I compared these predictions with the results for a more realistic ion absorption on the selectivity filter in a real electric potential of the channel. I also compared it to the results obtained for two other absorption models. The results differ to a high extent suggesting caution in the use of the considered analytical approximation.

INHIBITION OF VOLTAGE-DEPENDENT ANION CHANNELS AND THEIR INFLUENCE ON CELL FUNCTIONING

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Exposure of cells to ionizing radiation (IR) causes damage to various biomolecules, and may disrupt cellular metabolism. The reduced viability of cancer cells after irradiation is mainly associated with the induction of double-stranded breaks in DNA, but it also depends on mitochondrial status which influences the energy balance of cells. The condition of mitochondria depends on their membrane permeability, its potential, and the status of membrane channels.

In this work we studied the effects of inhibiting mitochondrial membrane voltage-dependent anion channels (VDACs) by 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid (DIDS) on cellular processes and radiosensitivity of K562 cancer cells. We compared cell cycle, viability, induction of apoptosis and clonogenic potential in the presence or absence of DIDS, and after exposure to 4 or 12 Gy of X-rays.

Control unirradiated cells, treated with DIDS, did not show changes in the cell cycle but they showed a significant decrease in clonogenicity. Twelve hours after exposure to 4 or 12 Gy of X-rays, inhibition of the cell cycle and accumulation in G2 phase was observed but irradiation together with DIDS treatment caused accumulation in the S phase, an effect observed for different DIDS concentrations.

Irradiated and unirradiated cells showed an increased apoptotic fraction a short time (1h) after DIDS application, but 24h later irradiated and DIDS-treated cells showed no difference or even lower levels of apoptosis than cells which were only irradiated. At the same time we observed changes in the cytochrome C concentration in the cytoplasm.

DIDS-treated cells showed changes in global reactive oxygen species (ROS) and a lowering of cellular ROS level was most pronounced 12h after treatment.

Altogether, our results show that inhibition of VDACs influences many cellular processes and systemic circuits regulating cell cycle check points, clonogenicity, and apoptosis and may influence the cellular response to ionizing radiation.

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INDUCTION OF NECROPTOSIS BY OXIDATIVE STRESS IN RETINAL PIGMENT EPITHELIAL CELLS

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Age-related macular degeneration (AMD) is the leading cause of severe vision loss in people aged over 50. It is a multi-factorial disease with unclear etiology. Age is the most consistent risk factor associated with AMD. Genetic factors, oxidative stress, and inflammation ethnicity are considered to be contributors to the pathogenesis of AMD. Currently, the mechanism of oxidative stress-induced RPE cell death in AMD is still controversial, most pointing to a dominant role for apoptosis in the process.

The purpose of this study was to systematically dissect the mechanism of RPE cell death induced by oxidative stress. ARPE-19 cell line, human RPE cells, and mouse model of sodium iodate-induced RPE degeneration were used to analyze oxidative stress-induced RPE cell death. Features like membrane permeability, TUNEL assay, caspase 3 cleavage, release of HMGB1, and activation of RIP3 kinase were analyzed.

Oxidative stress-induced RPE cell death was prevented by RIP1 kinase inhibitor necrostatin-1 but not Caspase inhibitor z-VAD, suggesting necrotic feature of RPE cell death. Moreover, key features of necrosis like RIPK3 aggregation, release of HMGB1 from nucleus to cytoplasm, and plasma membrane leakage indicated by PI staining, were observed in RPE cells upon oxidative stress. Silencing of RIPK3 and PGAM5, key proteins in necrosis, largely prevented oxidative stress-induced RPE death. Sodium iodate ($NaIO_3$)-induced RPE degeneration in mice revealed swollen RPE cells breaking off from the monolayer characterized by nuclear pyknosis and vacuolization. RIPK3 aggregation indicating formation of necrosome and PI membrane permeability were observed in RPE cell layer. TUNEL positive RPE cells and photoreceptors were detected after $NaIO_3$ treatment, but active caspase 3 was detected only in the photoreceptors layer.

ROLE OF REACTIVE OXYGEN SPECIES IN CROSSTALK BETWEEN UV AND CYTOKINE ACTIVATED SIGNALING

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Cellular response to UV induces reactive oxygen species (ROS) accumulation and activation of variety of cell signalling pathways. Among them, signal transduction pathways regulated by p53, NF-κB and AP-1 transcription factors play major role. Here we aimed to assess the effect of cellular stress induced by UV irradiation on the classical NF-κB-dependent pathway.

Colon carcinoma HCT116 cell line was used, in two congenic variants either containing or lacking transcriptionally competent p53. Stress response was induced by treatment with the UV-C radiation (20J/m^2); level of proteins or mRNA representing different signalling pathways was assessed by Western blot or QRT-PCR at a few time points after exposure. To analyse potential crosstalk between cellular stress- and cytokine-activated signalling, cells were also subjected to the stimulation with TNFα alone (10ng/ml) or in combination with UV-C irradiation; stimulation with TNFα was performed 1-6 hours after irradiation. Activation of NF-κB was monitored by Western blot (IκBα degradation) and EMSA (DNA-binding by NF-κB forms); NF-κB-dependent transcriptional response was assessed by QRT-PCR. To determine if signal transduction pathways activated by the performed treatment are linked with cellular redox imbalance and oxidative stress response, total ROS and superoxide levels were also analysed.

Upon UV exposure, strong induction of p53 was observed in cells with the wild type *TP53* gene. In both cell variants activation of AP-1 signalling (stronger in cells lacking p53), NF-κB-dependent genes (e.g. *IL8, MnSOD/SOD2*) and accumulation of ROS was observed. ROS accumulation was also observed in both cell variants stimulated with TNFα alone. Strong effect of UV irradiation was observed on the TNFα-induced activation of the NF-κB pathway. Pre-exposure to UV resulted in suppression of the NF-κB activation (inhibition of the IκBα degradation and reduction of nuclear NF-κB) and marked down-regulation of NF-κB-dependent genes (e.g. *IL8, NFKB1*). The strongest inhibitory effect was observed when UV irradiation preceded TNFα stimulation for 6 hours. Moreover, we observed a decreased level of ROS induced by TNFα in cells pre-treated with UV-C irradiation. Most notably, similar inhibitory effect of UV was observed in cells with the different p53 status.

We conclude that UV-related stress-response pathways interfere with the cytokine-induced activation of the NF-κB pathways in the p53-independent manner. Observed interference is based on the homeostasis and redox regulation in cellular signalling.

This work was supported by Polish National Science Center, Grant UMO-2013/08/M/NZ1/00935 and Grant DEC-2012/04/A/ST7/00353.

MODIFICATION IN MODERN TOOLS FOR CELL BASED ASSAYS INCLUDING MITOCHONDRIA AND REACTIVE OXYGEN SPECIES ANALYSIS

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ALAB, Warszawa, Poland.

High interest in cell-based assays encourages development of new tools and methods of analysis or modification of currently used ones. There are many directions of such progress with direct or indirect means of sample data collection. Some of them lead to truly revolutionary solutions.

Advanced cellular / subcellular motion analysis or hyperspectral cytometry are examples of tools which redefine cell-based assay and put collection of quantitative data on a completely different level. They open new areas allowing development of label free assays and improvements in currently used methods.

Our presentation will cover some details and applications which hopefully unveil great potential hidden in new instruments.

Poster abstracts

Genome and cell heterogeneity (F1 – F13)
Bioinformatics; Modeling biological processes (F14 – F26)
Mechanisms regulating gene expression (F27 – F45)
New methods and new approaches (F46-F58)
Searching for markers of disease (S1-S16)
New molecules and new therapies (S17 – S45)
Modeling of molecules and structures (S46 – S54)

Numbers in parantheses before the abstract title show the poster viewing time and place (F- Friday, S – Saturday)

(S3) EXOSOMAL CARGO: EFFECT OF ALBUMIN AND IGG REMOVAL ON MASS SPECTROMETRY ANALYSIS

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Exosomes are nanovesicles (30 – 100 nm in diameter) released by many cell types and identified in the body fluids such as blood, urine, saliva or cerebrospinal fluid. They are formed in endosomal compartments and secreted upon fusion of multivesicular bodies with plasma membrane. Their diverse cargo (proteins, lipids and different types of nucleic acids) is a subject of intensive study, especially focused on characterizing biological function of exosomes, for example their role in intracellular communication. Nowadays, several different protocols of exosome isolation are available, but it is a challenge to obtain high-quality exosomal samples, free from other extracellular vesicles or non-specific proteins derived from culture media supplemented with bovine serum.

In our study we characterize the proteomic profile of exosomes present in supernatants from established HNSCC (head and neck squamous cell carcinoma) cell lines exposed to ionizing radiation. Two cell lines with similar radiosensitivity, but different status of *TP53,* were selected for further analysis: HN30 (p53-wild type) and OSC-19 (p53-null), both HPV-negative. The supernatant was collected 24 and 48 hours after irradiation with 2, 4 or 8 Gy dose and exosomes were isolated for mass spectrometry analysis using two methods based on ultracentrifugation: (i) traditional differential centrifugation and (ii) differential centrifugation supplemented with size exclusion chromatography. The Sepharose 2B®-based columns allowed removal of high-abundance proteins like albumin co-isolated with exosomes, which significantly increased number of identified proteins and veracity of the results.

Summing up, the presence of co-isolated proteins such as albumin and IgG affects the qualitative and quantitative evaluation of exosomal cargo performed by mass spectrometry techniques. The phenomenon of ion suppression occurring during the analysis causes that tryptic ions originating from proteins present in greater amounts are measured more frequently while lowering the intensity of measurement of other ions. A reflection of this phenomenon is statistically significant higher number of identified proteins and their higher sequence coverage in the samples purified from the high-abundant proteins.

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(F18) LUMPED PARAMETER MODEL OF HUMAN CIRCULATORY SYSTEM BASED ON ELECTRICAL ANALOGY

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Advanced computer technology, tools, like computational fluid dynamics (CFD) and knowledge about the functioning of the human blood circulatory system, structure of blood, behaviour of vessels - allow improving understanding of blood flow in human body. Complex simulation has to assume multiphase approach, wall elasticity and pulsating blood flow conditions, based on work of the heart [1].

On the inlet of vessel, periodical cardiac pulsatile flow will be implemented using User Define Function capability of ANSYS Fluent [2].

In the systemic peripheral vascular beds the characteristic impedance, peripheral resistance, capacitance will be taken into the consideration in electrical analogues as a lumped parameter (LPM) model of circulatory system [3].

The resistance in a vessel can be modeled by electronic component – resistor. The blood flow is not stationary, blood is stored (eg. in the vessels and kidneys) just like energy in the capacitors. Coil is an analog to the inert tendency of blood, which mass resist to move due to the pressure difference. Furthermore it can be assumed that flow has only one direction – so vessels act like diodes in electronic circuits. Additionally the conservation of mass can be applied converted into the Kirchhoff's law [4].

The realistic, time-course, lumped parameter (0D) model will represent the cardiovascular system and serve as boundary conditions in further CFD analyses.

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(S11) EXPRESSION PROFILE OF LAMP3 GENE INVOLVED OF AUTOPHAGY IN COLON ADENOCARCINOMA

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Autophagy is a biological process of controlled distribution of molecules, fragments of cells and cell organelles to obtain an additional source of energy. There are three types of autophagy: microautophagy, macroautophagy and chaperone-mediated one.

LAMP3 (Lysosomal-associated membrane protein 3) belongs to a family of LAMP proteins.It is a heavily glycosylated integral membrane protein located mainly in the lysosomal membrane. It is a newly identified tumor specific protein. Recent studies have shown that it participates in tumor metastasis and drug resistance. Its main role is to contribute to cell proliferation, induce migration and invasion of tumor cells.

The aim of this study was to compare the expression profile of LAMP3 in colon cancer specimens from different clinical stages of cancer.

The analysis of the expression profile of LAMP3 gene was performed using HG-U133A oligonucleotide microarrays (Affymetrix, Santa Clara, CA). Appointment of differentiating genes was performed with the use the PL-Grid Infrastructure (http://www.plgrid.pl/).

The results showed that the gene encoding LAMP3 is overexpressed in colon adenocarcinoma, regardless of the clinical stage of this cancer ($p = 6.866942E-4$). The value of FC, which shows log2 fluorescence signal difference between the treatment groups was K2 vs K = -1.0977846 and LCS vs K = 1.784912, and HCS vs. K = 1.7770386.

Conclusions: Molecular changes precede phenotypic changes. Overexpression of the transcriptional activity gene LAMP3 does not depend on the clinical stage of adenocarcinoma.

(S36) APPLICATION OF ISOTHERMAL TITRATION CALORIMETRY (ITC) TECHNIQUE TO STUDY INTERACTIONS BETWEEN DRUGS AND THEIR CARRIERS

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Isothermal titration calorimetry (ITC) is one of the best techniques to obtain complete thermodynamic profile of phenomena occurring in the measuring cell. Heat is adsorbed or released during both chemical and physical processes. During a titration process the heat of changes in the composition of a system at constant pressure and temperature is measured. A single ITC experiment allows for direct determination of the reaction stoichiometry (n), the enthalpy change (ΔH) and the equilibrium constant (K), from which the entropy of binding (ΔS) and Gibbs energy (ΔG) can be calculated. ITC technique has found many applications, including biological ones. The following types of studies can be distinguished: protein interactions, macromolecular assembly, study of kinetics, interaction of surface with nanoparticles, drug design and characterization, testing crystal chirality in chiral silicate zeolites, and others.

Our aim was to verify application of ITC technique in the description of reaction thermodynamics between biologically active model substances and their carriers. Novel star-shaped copolymers (potential carriers) with sugar core were studied in terms of their the reaction with fluorescein isothiocyanate (FITC), doxorubicin (DOX) and methotrexate (MTX). Obtained thermodynamic parameters contributed to a better understanding of such phenomena and selection of the carrier with the best thermodynamic properties.

Studies carried out by ITC showed that all reactions examined occured in the desired direction. The study allowed to describe both chemical reactions between drugs and their carriers, as well as physical phenomena, such as self-association of doxorubicin. The obtained results showed that, in the future, ITC may enable determination of reactivity between active sites present in carriers with biologically active compounds.

(F32) THE EXAMINATION OF PROMOTER METHYLATION AND EXPRESSION OF *LGALS1* AND *LGALS3* GENES IN THE A2780 OVARIAN CANCER CELL LINE

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Ovarian cancer constitutes barely 3% of all female cancers, however it still remains the most lethal gynecological cancer due to difficulties in early detection. Only 30% of cases are diagnosed in non-advanced stage. Epigenetic alternations, such as DNA methylation, play an important role in molecular pathogenesis of ovarian cancer. Aberrant methylation of gene promoters underlies the changes in their expression and can be potentially useful as a biomarker or a target for cancer therapy. Galectin-1 and 3 are β-galactoside-binding proteins involved in many cellular processes, both physiological (e.g. proliferation, apoptosis, immune response) and pathological (e.g. tumor progression, angiogenesis and metastasis). Increasing evidence points to the abnormal expression of *LGALS1* and *LGALS3* genes (coding respectively galectin-1 and galectin-3) in ovarian cancer, although there are still insufficient data regarding the contribution of DNA methylation in these alterations.

The aim of this study was to seek the link between expression of *LGALS1* and *LGALS3* genes and methylation pattern of their promoter regions in A2780 ovarian cancer cell line. The cells were cultured under standard conditions (37˚C; 5% CO_2, RPMI-1640 supplemented with 10% FBS). Spin column technology was used for DNA and RNA extraction. The methylation patterns of *LGALS1* and *LGALS3* gene promoters were examined by methylation specific PCR (MS-PCR). The expression level of analyzed genes was investigated by reverse transcription PCR (RT-PCR). Specific primers were designed using MethPrimer (for MS-PCR) and Primer-BLAST (for RT-PCR) tools. *TBP* (TATA-binding protein) gene was used as a positive control for both PCR reactions. The amplicons were separated by gel electrophoresis.

The results show that in A2780 cancer cell line, the lack of *LGALS1* gene methylation is accompanied by the expression of galectin-1. At the same time, the methylation of *LGALS3* gene promoter correlates with the lack of galectin-3 expression. Obtained data may suggest that examination of methylation pattern of *LGALS1* and *LGALS3* gene promoters will very likely provide information on the expression of galectin-1 and 3.

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(F27) INFLUENCE OF PRE-MIRNA STRUCTURE ON MIRNA BINDING TO ARGONAUTE PROTEINS

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MicroRNAs are short 22-24nt-long RNA molecules responsible for the regulation of gene expression at the post-transcriptional level. These molecules are the elements of the RNA-induced silencing complex (RISC) whose main components are miRNAs and Argonaute (AGO) proteins. RISC may interfere with gene expression by degradation of mRNA or blockade of translation. Literature data suggest that mRNA degradation by RISC may depend on miRNA-mRNA complementarity (mRNAs with target motifs fully complementary to miRNA are degraded) and the type of AGO protein present in RISC (AGO 2 is more efficient in degradation).

In our experiments we studied the expression of reporter genes containing sequence motifs targeted by three different miRNAs which differ in their complementarity to the mRNA targets. HCT116 human colon cancer and Me45 human melanoma cells were transfected with Psi-CHECK2 plasmid containing the Renilla luciferase gene with motifs recognized by let-7, miR-21 and miR-24 in its 3'UTR. Renilla luciferase mRNA levels were assessed by RT-qPCR and protein levels were estimated on the basis of luciferase activity. The results showed a correlation between miRNA-target complementarity and protein but not mRNA levels. We also did not observe a correlation between mRNA and protein levels in transfected cells, which suggested that mRNA degradation and inhibition of translation could be separate processes. One of the possible hypotheses could be a different engagement of different AGO proteins in these two processes and differences in affinity to different miRNAs. In recent studies Suzuki and coworkers have shown that Argonaute proteins are capable of differentiating between nucleotides at the 5' or 3' end of mature double stranded pre-miRNA, and on this basis to decide which strand will become the guide. To better understand if different types of AGO proteins may differ in pre-miRNA structure recognition and show some selectivity in binding to different miRNAs, we performed multi-parameter analysis in which we compared structural features of 127 pre-miRNAs bound to AGO1 or AGO2. Analysis based on immunoprecipitation data (described by Turchinovich and Burvinkel, 2012) showed some differences in pre-miRNA structure of AGO1- and AGO2-bound miRNA. Our results suggest that pre-miRNA affinities to different AGO proteins can be important elements in switching between RNA degradation or inhibition of translation in RNA interference mechanisms.

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(F53) TUNING OF CSF FILTRATION TECHNIQUES AS AN IMPROVEMENT FOR AUTOMATED TUMOUR DETECTION IN ANALYSIS OF MAGNETIC RESONANCE DIFFUSION WEIGHTED IMAGING

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Aim: Aim of this work is to propose a tuning procedure for T2 FLAIR based method for cerebrospinal fluid filtration on magnetic resonance imaging.

Introduction: Due to the fact that cerebrospinal fluid (CSF) signal is characterized by a similar diffusion as tumour it should be removed before a magnetic resonance image analysis. The standard technique is to use FLAIR sequence on which CSF is visible as dark pixels (almost fully attenuated signal). Taking into account difference in resolution between FLAIR and ADC image, a partial volume effect must be considered that results in bigger CSV areas on ADC image. To deal with the problem a simple FLAIR-based technique may be extended by addition of scaling parameter that shifts the CSF cut-off value.

Material and methods: For this study 6 patients diagnosed with Astrocytoma fibrillare II were chosen. All images from training set were filtered from CSF, with the use of a defined range for scaling parameter values according to the scheme: CSF identification on FLAIR, then translation to the co-registered ADC map. For each value of scaling parameter the CSF filtered ADC maps were segmented with the use of MiMSeg (Binczyk, Polanska et al.) threshold value of: 547.63 10-3 mm^2/s. The quality of detection was compared to the manual expert detection and measured in Dice similarity coefficient (DSC). The scaling parameter value characterized by highest mean DSC was chosen as optimal. It was used and compared to the standard FLAIR-based method on a 3-patient independent validation set.

Results: The examined range for scaling parameter was spanned from 0.5 to 1.5. The optimal value was found to be 1.18 with the mean DSC equal to 87.63 % and 95%, CI (84.56 – 88.70). The obtained value of scaling parameter has been fixed and verified on a 3-patient validation set obtaining the following result: 86.71% of DSC with 95 % CI (85.30-88.12). For comparison, the standard non-scaling parameter algorithm was used and in automated tumour detection of DSC it equals to 84.54% with 95 % CI (83.39-85.69). In the last step the modified CSF filtration scheme was compared to BRATUMIA method.

Conclusions: It was proven that addition of scaling factor to the standard FLAIR-based CSF filtration technique results in improved automated tumour detection measured in mean DSC. The main improvement is visible as lower number of CSF residuals on filtered image that are detected as false positives. The comparison to the BRATUMIA confirmed that the examined technique is promising and authors plan to further develop it.

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(F7) DETECTION OF DIFFERENTIALLY METHYLADED REGIONS OF GENOME IN HUMAN LAUKAEMIAS

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Introduction: DNA methylation is one of epigenetic mechanisms which is very important in cell differentiation. The hypermethylation of gene regulatory sequence can inactivate its transcription. Methylation is a signal for proteins regulating chromatin structure and it initiates the process of chromatin condensation what makes DNA methylation one of gene expression control processes. Differantially methylated genome regions are specific for various types of cancer. Hypermethylation can occur in cancer disease at gene promoters, and this inactivates tumour suppressor genes, as well as global hypomethylation of the cancer genome, which leads to chromosomal instability. In this study we concentrated on genome methylation profile in human leukaemias.

Materials and methods: an experiment was performed for two kinds of human leukaemia due to different causes. One was *de novo* leukaemia (5 patients) while the other a side effect of chemotherapy (4 patients). For these cases, DNA methylation profile was checked, using Infinium 450k microarrays. 485512 sites of whole genome (all chromosomes) were examined for methylation level. For each site we obtained the results in M-value, which is methylated to unmethylated (level) logarithmic ratio. The aim of this study was to discern differentially methylated regions in genome between *de novo* and *after chemotherapy* leukaemias. In order to find these regions, peak detection method was used. Before that, signal smoothing methods were applied, due to high diversity of methylation level in contiguous sites of the genome.

Result: The analysis shows that there are significant differences of methylation level in several regions of genome. Rough analysis revealed that, generally, methylation level in *de novo* leukaemia is higher than in *after chemotherapy* leukaemia. However, detailed analysis showed also genome regions where methylation in *after chemotherapy* leukaemia is higher than in *de novo* leukaemia. Information about probes in Infinium 450k microarray enable identification of differentially methylated genes which, consequently, characterize differential expression.

Conclusion: DNA methylation is a gene expression control process. It can change the functionality of cells and influence molecular processes in them. Detection of differentially methylated genome regions provide an information about deregulated genes. Thanks to this information we can learn about differential functions and processes in cancer cells.

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(F29) MICRORNAS- MEDIATED REGULATION OF CELL ADHESION IN RENAL CELL CANCER

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Background: Renal cell carcinoma (RCC) is the most common, highly metastatic and therapy-resistant subtype of renal cancer. We have recently found that RCC tumours are characterized by severely disturbed expression of 19 genes involved in adhesion and remodelling of extracellular matrix (ECM). In this study we hypothesized that: a) disturbed expression of these genes is caused by microRNAs (short, non-coding RNAs that bind to transcripts of target genes and inhibit their expression); b) the microRNAs targeting genes related to adhesion and ECM remodelling affect adhesion of RCC cells.

Material and methods: The material for the study consisted of: a) 74 matched-pairs of RCC tumours and non-tumorous control samples; b) RCC-derived Caki2- cell line. The prediction of microRNAs potentially targeting 19 genes involved in adhesion/ECM remodelling was performed using miRsystem. Only microRNAs predicted by at least two of the seven independent algorithms were selected for further analysis. The expression of 34 predicted microRNAs was analysed using Pick-&-Mix microRNA PCR Panels (Exiqon). The expression was normalised to miR-28 and miR-103 whose stable combination of expression was indicated by NormFinder. microRNA-mediated regulation of target genes was evaluated using transfections of microRNA mimics or inhibitors and real-time PCR analyssi. The influence of microRNAs on adhesion was analysed using transfections of Caki-2 cells with microRNA mimics or inhibitors and ECM Cell Adhesion Array Kit (Millipore).

Results: The expression of 28 microRNAs potentially targeting the expression of adhesion and ECM-related genes was statistically significantly disturbed in RCC tumour samples when compared with paired matched controls. The expression of 22 microRNAs (let-7p, miR-107, miR-10b-5p, miR-130b-3p, miR-148-3p, miR-182-5p, miR-192-5p, miR-196-5p, miR-200c-3p, miR-204-5p, miR-223-3p, miR-25-3p, miR-26a-3p, miR-26b-5p, miR-29-3p, miR-30a-5p, miR-32-5p, miR-328, miR-363-3p, miR-429, miR-9-5p, miR-98-5p) was decreased, while expression of six microRNAs (miR-142-5p, miR-144-3p, miR-23a-3p, miR-382-5p, miR-590-5p, miR-93-5p) was increased in RCC tumours when compared with paired matched controls. Four microRNAs (miR-26-5p, miR-30a-5p, miR-328, and miR-363-3p) were selected for functional studies. Bioinformatic predictions revealed that all these microRNAs are potential regulators of genes involved in cellular adhesion. Functional assays showed that: a) miR-26a-3p mimic downregulated the expression of ITGA5, miR-363-3p downregulated the expression of COL5A1 and ITGA5, while miR-328 downregulated the expression of MMP16; b) miR-26a-3p enhanced adhesion od RCC cells to collagen II and IV, while miR-363-3p decreased cellular adhesion to collagen I.

Conclusion: The expression of microRNAs targeting genes involved in cellular adhesion and ECM remodelling is disturbed in RCC tumours. Re-expression of microRNAs miR-26a-3p and miR-363-3p changes adhesive properties of RCC cells.

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(F12) ENFORCED MCPIP1 EXPRESSION DOWNREGULATES MYCN ONCOGENE EXPRESSION AND MTOR PATHWAY SIGNAL TRANSSMITION AND MIGHT CAUSE DIFFERENTIATION AND DEATH OF NEUROBLASTOMA CELLS

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Neuroblastoma is the most common extracranial solid tumour occurring in childchood. One of the most important neuroblastoma markers is MYCN oncogene, which plays an important role in many aspects of the discussed neoplasm. Additionally, high MYCN expression causes poor patient outcome. mTOR complex is another vital player in neuroblastoma, which is responsible for crucial cell processes, commonly deregulated in cancers. Changes in mTOR signalling pathway may affect: apoptosis, autophagy, differentiation, changes in transcription, translation and cell cycle.

Our recent studies showed an inverse correlation between MYCN and MCPIP1 expression levels in neuroblastoma cell lines and tumours. In the present study we investigated the impact of the enforced wild type or mutant MCPIP1 expression in BE(2)-C neuroblastoma cell line. We found significant decrease in MYCN expression on gene and protein levels, which might suggest possible induction of differentiation. mTOR pathway analysis showed a decrease of expression or phosphorylation in majority of the proteins belonging to this pathway. Further we performed protein microarray analysis which excluded apoptosis. Next we examined autophagy by analyzing expression of microtubule associated protein 1 light chain 3B (LC3B) and showed that converted LC3B form is increased in cells overexpressing MCPIP1 protein.

Our results indicate that both MYCN oncogene expression and signal transmission via mTOR pathway are inhibited in neuroblastoma cells with enforced expression of MCPIP1 and such conditions can lead to differentiation and death of tested cancer cells.

(F1) COLORECTAL ADENOCARCINOMA STEM CELLS SHOW DISTINCT GROWTH PATTERNS DEPENDING ON THE CULTURE ENVIRONMENT

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Colorectal cancer is one of the most common malignancies in human population. The high mortality and morbidity rates are driving forces of extensive studies utilizing cutting-edge techniques. According to current knowledge, failure of chemotherapy can be attributed in large part to the presence of cancer stem cells (CSC) in the tumor, that exhibit a high degree of heterogeneity and plasticity.

Up to date basic research in this field was mostly based on two disease models: established cell lines and animal studies. Identification of Lgr5, an R-spondin receptor, as a marker of stem cells of the gastrointestinal tract, provided researchers with a possibility to selectively culture them *in vitro* and to observe all steps of differentiation and self-organization from stem cells to “mini-guts”. Organoid (or 'colonoid' for colon tissue) cell culture is a method of growing cells in the laminin-rich extracellular matrix that allows and supports cell growth into three-dimensional structures, which better resemble *in vivo* conditions than traditional monolayer. As such, the three-dimensional matrix, in which the specific growth factors are dispersed, is considered a necessity.

Recently, several groups have succeeded to sustain organoids from various tissue types for prolonged time. In the gastrointestinal tract research, organoid cultures are currently the state-of-the-art model for biological studies of colon, esophagus and small intestine.

LoVo cell line (ATCC® CCL-229™) was established from Dukes' type C, grade IV, colorectal adenocarcinoma. Cells were derived from metastatic site. LoVo cell line was described as containing a significant fraction of CSC.

In the present study, we were trying to assess whether established cell line could be a source of cancer stem cells and if those cells could generate 'colonoidal spheroids'. For that purpose, colon cancer stem cells were isolated and cultured in different conditions. Lgr5 positive stem cells were sorted from cells propagated in standard culture of adherent cells. Magnetic activated cell sorting (MACS) with bead-conjugated antibodies was utilized for that matter. 2D and 3D growth environments were used to analyze phenotype of growing structures. Cultures were also exposed to cytotoxic agents with different mechanism of action.

The results show that established LoVo cell line contains small but relevant stem cell content (1.5-3%). It is possible to isolate those cells and to culture them. The type of environment has a major impact on the growth pattern and cell morphology. Cells kept in 2D conditions form cell aggregates very similar to general LoVo population with elongated cells extending toward the inter-cellular space. Cells cultured in 3D matrix grow as compact 'spheroids' with almost invisible cell junctions and very smooth edges.

The support provided by extracellular matrix gives the cells better conditions to grow and proliferate than traditional 2D, even if in both conditions medium is supplemented with key growth factors. Also, in 3D environment less dead cell are present than in 2D one, as indicated by the cytotoxicity assay.

(S44) THE COMBINATION OF WP 631 AND EPO B DISTURBS CELL CYCLE IN OVARIAN CANCER CELLS

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Background: Ovarian cancer is one of the main reasons of death among women. In spite of rapid medical developments, the curability is still on the very low level. Nowadays, besides surgery, the basic way of treatment is chemotherapy, mostly combined therapy. We have already shown that the combination of bisanthracycline and epothilone B (Epo B) induces apoptosis, leads to DNA damage and provokes oxidative stress more intensively than when these compounds are used separately. The exact mechanism of action of this drug combination remains undiscovered. As cell cycle dysregulation is a hallmark of cancer cells, fully understanding the influence of anticancer drugs on cell cycle distribution allows to develop novel chemotherapeutic strategies and thus to improve clinical outcomes. For this reason we investigated the effect of drug combination on the cell cycle of SKOV-3 cells. In order to find out at which point of cell cycle the combination of WP 631 and Epo B is the most active, we chose, based on literature search, three inhibitors: alsterpaullone, DAPT and metformin, which inhibit CDK1/cyclin B complex, EpCAM and HMGB1, respectively.

Methods: The effects of the tested substances on the cell cycle were determined based on the nuclear DNA contents in the cells by using flow cytometry.

Results: After 24 h incubation Epo B induced the highest G2/M arrest and also rise in sub-G1 population to about 13%. At this point of time the combination also stopped the cell cycle at the G2/M phase, but the percentage of cells was lower in comparison to Epo B. The combination in turn caused stronger apoptosis, observed as a more significant grow of sub-G1 cells (about 21%). WP 631 at this concentration did not influence the SKOV-3 cell cycle. The inhibition of EpCAM and HMGB1 by DAPT and metformin, respectively, did not change the activity of WP 631, Epo B and their combination. In turn, preincubation with CDK1/cyclin B inhibitor, alsterpaullone, caused the significant drop in the level of cells arrested at G2/M phase after treatment with Epo B. Such an observation was not confirmed for the combination, what points to the fact that Epo B in lower concentration used in a combination (5 nM) exhibits its activity through distinct mechanisms than Epo B at the concentration of 10 nM.

Conclusions: Epo B induce cell cycle stoppage in SKOV-3 cells observed as a G2/M arrest. Inhibition of cyclin B/CDK1 decreased activity of a drug, meaning that Epo B acts through the cyclin B/CDK1 complex. We suppose, that after combination treatment the cells escaped from mitotic arrest what is known as a mitotic slippage. While mitotic arrest in Epo B-treated cells could be overcame after DNA damage repair, apoptosis which occurs after mitotic slippage in combination-treated cell is irreversible. It clearly explains higher activity of drug combination in comparison to single Epo B and allows to state that the combination of WP 631 and Epo B is a better therapeutic option than single drugs.

(F26) HEAT TRANSFER IN HUMAN TISSUE UNDER MILD COOLING – NUMERICAL MODEL VALIDATION

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Measurements of heat transfer and temporal temperature distribution can be used as input in diagnostic tools and methods of skin lesions, with special interest in malignant melanoma identification. Such approach requires use of skin temperature and heat flux measurements combined with numerical simulation. Mild skin cooling process by copper compress is considered. Temperature distribution on the skin and heat flux between metal and tissues are measured. They are used in the course of validation studies of the proposed numerical model.

Numerical model of heat transfer in living tissues is described by Pennes' bioheat equation [1] augmented with additional models of thermoregulation [2]. The information regarding material properties of all involved in simulation bodies (ie. tissues & cooling compress) is essential to accurately solve this problem. Properties of human tissues, hard to measure *in vivo*, are based on well-established literature sources. Sensitivity analysis based on results obtained in previous simulations, shows that thermal properties of cooling compress (namely: specific heat capacity, heat diffusivity and density) are of high importance as well. Therefore, the main purpose of this work was to obtain accurate material property information by means of laboratory experiments.

Laser flash analysis (LFA) was employed to determine heat diffusivity of cooling compress material [3]. Heat capacity was examined using differential scanning calorimetry (DSC) [4].

The measured material properties of cooling compress were then used in numerical simulation carried out using ANSYS Fluent commercial CFD package. Simulation results were validated against experimental data.

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(S25) SILYL DERIVATIVES OF 3,4-DIBROMO-5-HYDROXY-2(5*H*)-FURANONE AS COMPOUNDS WITH POTENTIAL ANTI-CANCER ACTIVITY

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Introduction of the silyl group to a biologicaly active compound may cause increasing the properties desired in medicinal chemistry, like lipophilicity. The lipophilic compounds have better ability to penetrate cell membranes. One of the first examples of compounds with confirmed biological activity which contained silicon in their structure was 5'-*O*-*tert*-butyl-dimethylsilyluridine. This uridine derivative shows anti-cancer activity against HT-1080 lung cancer cell line. Moreover, this effect couldn't be observed with the starting compound.

The aim of our research was a preliminary evaluation of anti-cancer activity of 3,4-dibromo-5-hydroxy-2(5*H*)-furanone derivatives containing silyl groups within their structure.

In the first stage of investigation, the cytotoxicity of compounds was ascertained by MTT assay. Influence of 2(5*H*)-furanone derivatives on cell cycles was confirmed using flow cytometry. In the end, by using comet assay, we determined the induction of DNA damage by our compounds.

On the basis of the research, it has been concluded that the synthesized 3,4-dibromo-5-hydroxy-2(5*H*)-furanone derivatives exhibit more pro-apoptotic properties against the cancer cells than unmodified mucobromic acid.

(F28) IMPORTANCE OF THE HEAT SHOCK ELEMENT (HSE) LOCATED IN THE INTRON OF THE *PMAIP1* GENE FOR ITS ACTIVATION DURING STRESS

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Cellular stress can engage two fundamental responses: apoptosis, that eliminates irreparably damaged cells, and the heat shock response, that functions to sustain survival by limiting cellular damage and accelerating recovery. This paradoxical activation of both pro- and anti-apoptotic events in response to the same stimulus ensures that neither aberrant cellular survival nor inappropriate cell death arises and, in doing so, averts the onset and persistence of the pathological state. We assume that these two opposed processes can be directly regulated by Heat Shock transcription Factor 1 (HSF1). It activates both the expression of genes encoding the cytoprotective HSPs (Heat Shock Proteins, which prevents apoptosis) and the expression of proapoptotic, p53-dependent gene, *Pmaip1*/*Noxa* (what we have found in our earlier genomic studies).

HSF1 binds specifically to Heat Shock Elements (HSEs) throughout the genome. Typically, several HSEs can be found in the promoters of *HSPs* genes which guarantee their strong activation during stress. Using ChIP-Seq we detected HSF1 binding not in the promoter, but in the second intron of the *Pmaip1* gene which is known as the major p53-responsive proapoptotic gene. To prove that the HSE sequence in the *Pmaip1* intron is indispensable for the gene activation during stress we cloned this part of the intron and inserted it as an enhancer into pGL3 vector (containing firefly luciferase reporter gene). We obtained a series of pGL3 constructs with or without enhancer and/or with different promoters (*Pmaip1* or *SV40*). Additionally, we constructed a vector with mutated HSE sequence. Vectors were transiently transfected to different types of cells and their activity was tested following heat shock. Moreover, to show that PMAIP1 can induce apoptosis, we cloned *Pmaip1* coding sequence in-frame with EGFP. This enabled us to monitor PMAIP1/EGFP fusion protein by live imaging microscopy. Preliminary results indicate that HSE from *Pmaip1* intron in combination with *Pmaip1* promoter can activate firefly luciferase in response to heat shock, but only in cells with correct p53 protein present.

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(S10) OVEREXPRESSION OF SFRP2 AND MFAP5 PROTEINS AFFECTS OVARIAN CANCER CELL PROLIFERATION AND MIGRATION RATE

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Introduction: In our previous microarray study we identified two molecular subtypes of serous ovarian cancers, with distinct gene expression profile. These subtypes are correlated with different patient survival. Among the genes that were differentially expressed between two subtypes of ovarian cancer were, e.g., Secreted Frizzled-Related Protein 2 (SFRP2) and Microfibrillar Associated Protein 5 (MFAP5). Our aim was to study whether and how SFRP2 and MFAP5 can influence proliferation rate and motility of ovarian cancer cells, the features related with cancer malignancy and metastatic potential.

Material and methods: SFRP2 and MFAP5 coding sequences were amplified from cDNA and cloned in pLNCX2 vector. Retroviral system was used to obtain ovarian cancer cell lines with overexpression of each particular gene. Wild type OAW42 and OAW42/SFRP2(+) iso-genic cell lines were compared using MTS and crystal violet proliferation assays, as well as in scratch assay for migration rate. The same tests were used to compare wild type ES2 and ES2/MFAP5(+) cells.

Results and discussion: We found that the scratch area was faster covered with cells overexpressing each gene studied, in comparison to the wild type cells that were not express-ing SFRP2 and MFAP5. Proliferation tests showed that cells overexpressing MFAP5 exhibited trend toward slightly faster proliferation than non-modified cells, although it was not al-ways statistically significant. On the contrary, SFRP2 doesn't influence proliferation rate.

Conclusions: Our results indicate that MFAP5 may increase ovarian cancer cell proliferation rate and it stimulates these cells' migration rate. SFRP2 protein does not affect proliferation but it enhances ovarian cancer cell motility. This suggests that SFRP2 and MFAP5 are members of a complex regulatory network affecting ovarian cancer biology. Better understanding of these regulatory associations requires further studies.

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(S27) BIOLOGICAL PROPERTIES OF OXAZOLINOANTHRACYCLINES

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Objectives: The purpose of our study was the evaluation of biological properties of oxazolinoderivatives of daunorubicin and doxorubicin, such as measurements of cytotoxicity and ability to induce apoptosis in three human cancer lines: human ovarian adenocarcinoma (SKOV-3), human lung adenocarcinoma (A549) and human hepatocellular cell line (Hep-G2**)**. Oxazolinoanthracyclines are obtained from formamidinoderivatives of daunorubicin or doxorubicin in the reaction of cleavage of the bond between carbon and nitrogen atoms in formamidine group, elimination of secondary amine molecule and formation of 5-membered ring corresponding oxazolinoanthracyclines.

Methods: The antiproliferative activity was assessed using MTT assay. To evaluate the ability of the compounds to induce apoptosis, double staining using fluorescence probes: Hoechst 33258/propidium iodide (PI) were used.

Results: Our results confirmed beneficial biological properties of oxazoline derivatives of doxorubicin and daunorubicin. It has been proven that the antiproliferative activities of O-DOX in tested tumor cell lines were higher in comparison to the parental DOX. In Hep-G2 cell line we obtained a high activity of O-DAU also. It has been demonstrated, that oxazoline derivatives of DOX and DAU are able to induce apoptosis. Typical morphological features of apoptosis, cell shrinkage, chromatin condensation and apoptotic bodies formation were observed. The most potent apoptosis inducer in tested cell lines was O-DOX. The percentage of necrotic cells persisted at low level.

Conclusion: The higher antiproliferative potential as well as the ability to induce apoptosis cause the oxazoline derivatives of DOX and DAU seems to be good candidates as effective anticancer drugs. Promising results of our study encourage to further investigate the way of their action.

(F4) VARIABILITY IN CELL CYCLE REGULATION BETWEEN CELLS FROM ONE LINEAGE

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Cellular heterogeneity (variability among cells) is always observed in cell populations. Frequently, this fact is disregarded, and only the population features of cell behavior are considered. However, for various reasons [1] it is important and challenging to learn how individual cell behave and to understand this mechanism.

Several types of biological experiments can be used to generate single-cell expression data [2]. In this communication we consider differences among cells due to the stochastic nature of cell cycle regulation, which is one of the most important mechanisms of cell. To observe individuals we analyze data from system FUCCI - the fluorescence ubiquitination cell cycle indicator. In this system two markers were used to indicate the G0/G1and S/G2/M phase of the cell cycle, respectively. Cells can be observed using a confocal microscope. We re-analyzed data first published in ref. [3], where the authors analyzed relations between cell cycle and mammalian clock.

In our work we tracked the individual cells using the LineageTracker software. It detects fluorescently marked cell nuclei and quantifies their fluorescence levels. Additional plugins allow detecting divisions and identify parent and progeny cells [4]. We obtained a few hundred trajectories of individual cells labeled for the 2 FUCCI proteins. Resulting data varied with respect to noise levels and record lengths (cells were born during the experiment).

During tracking we observed similarities in the behavior of cells from the same lineage. To confirm this theory a quantitative analysis was carried out. The most important element of the analysis is the method for automatic detection of cell phases and cell cycle endpoints. The two crucial steps of algorithm are: (i) to identify the level of noise and apply the appropriate parameters of the smoothing algorithm and (ii) to analyze dynamics of proteins by numerical differentiation. The algorithm is computationally efficient; We calculated among other the correlations of the cell cycle lengths between cells from the same lineages. This result and other characteristics confirm the conclusions regarding cell cycle regulations obtained by us in a previous study using a different cell system [5].

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(S40) PLANT POLYPHENOLS AS NATURAL IMMUNOMODULATORS

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Plant polyphenols possess one or more aromatic rings in a molecule, which contain anywhere from one to several tens of hydroxyl groups of acidic character. These are secondary metabolites of which the largest group is made up of flavonoids, phenolic acids, phenolic alcohols, stilbenes and lignans. Flavonoids are made up of two benzene rings connected with a heterocyclic pyran or pyron ring. They are divided into subclasses: flavonols, flavones, isoflavones, flavanones, anthocyanins, flavanoles, catechins, chalcones, aurones and others.

Polyphenol compounds are widespread in the plant world. They are present in edible parts of plant products and in medicinal plants. Phenolic acids are present in fruits of raspberry, black currant, wild strawberries, in red onions and in tea, lignans – in linseed, grains, coffee, stilbenes (resveratrol) – in fruits, grape seeds, red wine, catechins – in apricots, cherries, red wine, cocoa, green tea, flavones – in celery, parsley, grains, isoflavones – in soy grains, flavanones – in white parts of citruses, peach seeds [1].

The presence of a large number of hydroxyl groups in a particle gives these compounds antioxidant properties. Theaction mechanism of polyphenol compounds is based on inhibiting the activity of enzymes, which are responsible for the formation of xanthine RFT-oxidase, NADPH oxidase and myeloperoxidase. Polyphenols chelate copper iron ions, which catalyze the reactions of RFT creation (reactive oxygen forms). The simplest to bind are hydroxyl radicals, anion radical superoxide, singlet oxygen and lipid radicals.

Plant polyphenols inhibit, or modulate pro-inflammatory cytokine expression, as well as the expression of chemokines – TNF- α, IL-1β, IL-6, IL-8 and protein of chemoktatic monocytes MCP-1 [1,2,3].

Polyphenols also exhibit anticoagulant activity, anti-fungal, antiviral, and blood vessel sealing. Using polyphenols in a diet is an important element in prevention of many civilizational diseases and a diet rich in polyphenol compounds is beneficial to the health, lowering the risk of circulatory diseases, diabetes, cancer, and osteoporosis.

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(S32) RAMAN SPECTROSCOPY IN RESEARCHING VARIOUS DRUG FORMS

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In 1928 an Indian physicist C.V Raman experimentally proved the existence of non-flexible photon dispersion by substance particles. This phenomenon was named the Raman effect.

Using lasers as a source of stimulating radiation gave rise to the development of Raman spectroscopy. Laser radiation beam is characterized by high monochromatism and intensity, while radiation polarization allows for a definition of the degree of depolarizations of Raman bands. Thanks to using laser light to stimulate particles and using very sensitive signal detection systems (CCD detectors – Coupled Charge Device), we can register Raman dispersion spectrum at the same time, with a good spectral resolution and with bands of high intensity [1,2].

The pharmaceutical industry uses Raman spectroscopy more and more often, indentifying active substances and excipients in researching quality control, polymorphism of substances and processes connected with changes of polymorphic forms. Raman spectroscopy enables the monitoring of solvation and hydration processes, testing final drug form in order to define placement of the active substance, excipients and the creation of agglomerates. It allows for testing the repetitiveness of the process of drug coating and for the definition of drug stability.

The benefit of Raman spectroscopy is using a small samples for the reserach, which do not require special preparation, powdering or mixing with dispersing factors. Direct analysis through a glass or polymer container is possible. The reserach can be conducted using a dispersing Raman spectroscope or a spectroscope using Fourier transform (FT) [1,2,3].
Raman spectroscopy can successfully be used to detect counterfeit medicinal products, which do not fulfill the quality requirements and constitute a danger to the health and life of the patient.

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(F54) HIERARCHICAL AGGLOMERATIVE CLUSTER ANALYSIS FOR ASSIGNING HERBS TO THE SUITABLE PLANT FAMILY

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Hierarchical agglomerative cluster analysis was performed using two methods: Euclidean distance as similarity measurement and the Ward's method as amalgamation rule. Selected herbs (Artemisia dracunculus, Taraxacum officinale, Plantago lanceolata, Hypericum perforatum, Ocimum basilicum, Satureja hortensis, Origanum vulgare, Origanum majorana, Melissa officinalis, Mentha piperita, Rosmarinus officinalis, Salvia officinalis, Thymus vulgaris, Eruca vesicaria, Urtica dioica, Coriandrum sativum, Anethum graveolens, Levisticum officinale, Petroselinum crispum, Allium schoenoprasum) were tested in order to determine the content of chlorophyll pigments, carotenoids and flavonoids. The pigments were identified by the spectrophotometric method. The dendrograms were constructed using chlorophylls and carotenoids variables and hyperoside and quercetin variables. The results showed that the hierarchical agglomerative cluster analysis, based on chlorophyll and carotenoids pigments, gave three clusters with a few misclassified samples. The dendrogram based on data for flavonoids gave two separated clusters. The first one contained herbs of Lamiaceae family, and the second - other samples. The study showed that the dendrogram obtained using hyperoside and quercetin variables, is useful for classification herbs to their corresponding variety plants family.

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(F33) *PHLDA1* GENE SILENCING IN IMR-32 NEUROBLASTOMA CELLS CAUSES ELEVATION OF AURORA A KINASE EXPRESSION AND AFFECTS APOPTOSIS AND AUTOPHAGY-RELATED MOLECULES

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Despite the expansion of knowledge on neuroblastoma (NB) in recent years, therapeutic outcome for children with a high-risk 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 aiming 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 yet unknown, however there is evidence showing that it might act as a mediator of apoptosis and autophagy. In our study PHLDA1 gene is upregulated in 14G2a monoclonal antibody-treated IMR-32 cells as assessed by qRT-PCR.

We analyzed expression levels of PHLDA1 and two oncogenic drivers i.e. aurora A and MYCN in neuroblastoma cell lines: Kelly, CHP-134, LA-N-1, LA-N-5, HTLA230, IMR-32 and *MYCN*-non amplified SK-N-SH neuroblastoma cell line. The highest expression levels of *PHLDA1* gene and proteins in IMR-32 and SK-N-SH correlated with the lowest expression levels of *AURKA* gene and protein and the lowest *MYCN* gene and protein content in IMR-32 cells.

These results prompted us to thoroughly investigate the role of PHLDA1 in IMR-32 neuroblastoma cells. We used a lentivirus vector-based RNAi approach using shRNA expression to silence *PHLDA1* gene in order to observe its effect on metabolic activity, apoptosis and autophagy process in IMR-32 cells.

We compared metabolic activity of *PHLDA1*-silenced clones to control and wild type cells based on measurements of cellular ATP contents. From the experiments, we concluded that from day 4 of culture, a clearly visible increase in metabolic activity can be measured for all three clones with decreased expression of *PHLDA1* gene.

Our further studies focused on describing the effect of *PHLDA1* silencing on expression of molecules involved in apoptosis and autophagy. We report that the levels of PARP and caspase 3 proteins were smaller in *PHLDA1*-silenced clones when compared to respective controls of both the 14G2a-treated and untreated groups. Finally, we measured a rise in levels of converted microtubule associated protein 1 light chain 3 (MAP-LC3) form in *PHLDA1*-clones when compared to respective controls of both the 14G2a-treated and untreated groups. The results of these studies demonstrate that down-regulation of *PHLDA1* in IMR-32 may contribute to apoptosis resistance suggesting proapoptotic role of PHLDA1.

Based on the experiments, we can conclude that there is an inverse correlation between protein levels of aurora A and PHLDA1 in IMR-32 cells. Furthermore, downregulation of *PHLDA1* is linked to a higher metabolic activity of selected IMR-32 clones. More thorough studies are warranted to characterize involvement of PHLDA1 in biology of neuroblastoma cancer cells, including its function in apoptosis and autophagy.

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(F56) TEMPLATE MATCHING TECHNIQUE USING FEATURES FINDING FOR DERMATOSCOPIC IMAGES DATABASE

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Melanoma, the most frequent malignant skin cancer, can be characterised by aggressive growth with early and numerous metastases. Early diagnosis, likewise in many other tumours, followed by immediate surgery procedure, allows to save most patients, even those with a poor diagnosis. Dermatoscopy is a major method used in a diagnosis of skin cancer. However, to distinguish suspicious pigment changes and to detect new lesions, it is crucial to observe changes of skin structure in time. Multiple examinations are the basic source of information, but one of the problems that arise is to store and efficiently search the database of previously acquired skin lesions. During each examination, full set of a visible lesion is acquired and can be analysed to detect new and changed lesions. This situation can be solved by using template matching techniques on previously stored images database.

In previous work [1] we have demonstrated rigid registration algorithm to be a reliable technique for template matching in dermatoscopic images database. This approach was very precise in finding an appropriate image from a dataset. However, registration algorithms use an optimisation algorithm to find optimal parameters of affine transformation, which can be very time-consuming. Here, we present the other approach to template matching problem that uses feature detection algorithm. The idea is to find invariant features in each images pair - current lesion image and stored in database lesions. For this purpose, we have used well known in literature algorithm SIFT [2] which finds corresponding features between two images. The number of those common features can be an index of similarity between the images. The algorithm was tested on dermatoscopic images supplied from PH^2 image database [3].

Tests performed on available database showed that the number of features for the appropriate image was significantly higher (>100) than for other images (<15). This fact can lead to threshold definition that will distinguish similar images from others. Presented approach has an additional advantage, the execution time is significantly (about 20 times) lower comparing to the technique based on registration algorithm and takes ~30 seconds for one image pair.

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(F45) A PETRI NET BASED APPROACH TO THE ANALYSIS OF THE ROLE OF IRON IONS IN THE PROCESS OF ATHEROSCLEROSIS

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Based on the results of numerous studies, influence of iron, which is an essential element for the growth and well-being of almost all living organisms, becomes a noticeable factor in the development and progression of atherosclerosis. The key role played here is the Fenton reaction, a source of highly toxic hydroxyl radicals involved in the lipid peroxidation that is catalyzed by iron ions. Lipid peroxidation increases cell-mediated oxidation of low-density lipoprotein (oxLDL), which plays a critical role in atherosclerotic plaque formation. This process is triggered by the persistence of lipid-laden macrophages as well as interfering endothelial cell motility in arterial wall.

In our work a systems approach to the study of this complex phenomenon is presented. The constructed Petri net model of the participation of iron in the development of atherosclerosis process allows a formal mathematical analysis. As a first step the transition invariants (t-invariants) have been computed and based on them the MCT-sets and t-clusters have been calculated. For both the MCT-sets and t-clusters the biological meanings have been assigned, allowing, especially on the basis of the latter ones, to draw some interesting facts about the model. Another type of the analysis, the so called *knockout* approach, is based on disabling one or more elements of the model. Knockout analysis can be done on the basis of t-invariants or simulation of the net to gather data about the behavior of the model. Both knockout methods have been used in our study, revealing very high influence of the inflammation process on the atherosclerosis progression, much more significant than e.g. influence of the iron ions coming from the Fenton reaction.

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(F15) MODELING AND ANALYSIS OF IMMUNE AND INFLAMMATORY MECHANISMS IN ESSENTIAL HYPERTENSION AND CARDIOVASCULAR DISEASE - A PETRI NETS BASED APPROACH

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In spite of the huge amount of research recently performed, the pathogenesis of human hypertension remains elusive. Hence, for the majority of patients with high blood pressure, hypertension has to be defined as "essential". This issue is crucial considering the fact that its clinical consequences are one of the major sources of morbidity and mortality in the western world. Recently, low-grade inflammation together with the innate and adaptive immune responses have been proposed as key influencing factors in the pathogenesis of this type of hypertension.

In our study, to investigate the importance of the various factors of the development of this type of hypertension, a systems approach has been used. For this purpose a formal model, based on Petri nets theory, of the crucial interactions between immune and inflammatory regulatory processes in essential hypertension has been created and then analyzed.

The analysis was based on the generation of MCT-sets and t-clusters calculated using specifically selected clustering method. We have also simulated single and multiple knockout experiments supported by the state simulator of the net to gain an insight into the system behavior.

As a result we have identified the main pathways responsible for regulating the body's blood pressure. We have revealed that oxidative stress and inflammation appear to play a key role for emergence of the essential hypertension in the human body. As it was expected, the angiotensinogen-angiotensin axis is a very important factor that leads to vasoconstriction.

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(F14) SYSTEMS APPROACH TO MODELING AND ANALYSIS OF MONOCYTES-MACROPHAGES AXIS DISTURBANCES IN ATHEROSCLEROSIS

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The role of monocytes and macrophages is of fundamental interest in our understanding atherosclerotic lesions' development. These cells are mononuclear phagocytes having distinct roles in tissue homeostasis and immunity. In steady state, the patrolling anti-inflammatory monocytes monitor the vasculature and become tissue resident macrophages (M2). However, during defective cholesterol homeostasis they are recruited to the atherosclerotic plaque and differentiate into anti-inflammatory macrophages (M1). M1 via scavenger receptors sense modified low-density lipoproteins and along with the local cytokines induces proliferation of the macrophages and enhances local inflammatory status that is crucial for atherosclerotic plaque instability.

In this work a systems approach to the study of this complex issue is presented. For this purpose a Petri net based model involving monocytes differentiation and M1 and M2 interactions leading to atherosclerotic plaque instability has been built and analyzed.

The analysis of the model has been mainly based on t-invariants. The relationship between such invariants and the structure of MCT-sets have been examined. Moreover, the behavior of the net has been checked on the basis of its simulation. These studies have allowed to identify some important structures and functional dependencies in the model what has lead to formulation of interesting biological conclusions.

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(S29) BIOPOLYMERS AS MEMBRANES FOR THE PERVAPORATIVE DEHYDRATION

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Membrane separation technology, due to economic efficiency, has been widely used in purification, concentration and separation of liquid and gaseous mixtures. One of the most active area in membrane research is pervaporation - a technique aimed at the separation of liquid mixtures and allows to break the technological barriers, enabling the effective and "green" separation of organic mixtures. In recent times pervaporation is widely used for the dehydration of organic solvents, especially for ethanol. There are many studies on developing high performance and selective homogeneous or heterogeneous membranes for dehydration application, for example, on the basic of biopolymer: chitosan and alginate.
The aim of this work was to compare separation properties of membranes based on different biopolymers in the pervaporative dehydration of ethanol. For this purpose membranes based on chitosan and/or alginate crosslinked in the similar manner by phosphoric (V) acid or glutaraldehyde were used.
Pervaporation experiments were caried out at room temperature using feed solution containing 93 wt.% of ethyl alcohol. Then, based on the total flux and GC analysis results several transport characteristic of investigated membranes was evaluated. It was showed that the applied crosslinking agents have differently affected the membrane separation properties. In the case of alginate membranes crosslinking agent can influence the separation factor and selectivity coefficient, whereas for chitosan membranes it influences the obtained fluxes. On the other hand, better results were achieved when phosphoric (V) acid was used as the crosslinking agent i.e. the highest pervaporation separation index and separation factor were obtained for phosphoric (V) acid crosslinked alginate membranes but the greatest total flux obtained for phosphoric acid crosslinked chitosan membranes.

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(F40) DOES HSPA2 CONTRIBUTE TO EPIDERMAL KERATINOCYTES DIFFERENTIATION?

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The epidermis is highly specialized stratified epithelium that has evolved to perform multiple protective functions. Skin epithelium is a dynamic tissue; keratinocytes proliferate in the basal layer and undergo a tightly controlled differentiation program upon movement into the suprabasal layers. The epidermal differentiation program engages a variety of genes that contribute to formation of the multilayered epithelium with stratum corneum barrier.

HSPA2 was originally described as testis-specific member of the HSPA (HSP70) heat shock protein family. We have recently shown that HSPA2 is also expressed in human somatic tissues in cell- and tissue-type specific manner. High level of HSPA2 was found in epidermis and in other stratified epithelia, however the physiological role of HSPA2 in these tissues is not recognized at present.

This study was aimed at characterizing expression and function of the HSPA2 gene in human epidermis. By immunofluorescence analysis we found that undifferentiated keratinocytes residing in the basal layer of epidermis are highly positive for HSPA2. Immunophenotypisation of freshly isolated primary human epidermal keratinocytes followed by fluorescence-activated cell sorting revealed that the HSPA2 gene is not expressed in subpopulation of epidermal cells enriched for stem cells. These results suggest that expression of the HSPA2 gene in basal keratinocytes can be activated immediately before or along with initiation of the terminal differentiation process. The functional significance of HSPA2 expression in epidermis we studied using HaCaT cells (immortalized human epidermal keratinocyte line). We suppressed the HSPA2 gene expression by lentiviral transduction of cells with vectors encoding shRNA sequences targeting HSPA2 mRNA. We found that silencing the *HSPA2* gene had no effect on proliferative potential (tested in short term assay) but significantly attenuated clonogenic ability of HaCaT cells grown in standard 2D culture. This result allowed to presume that HSPA2 deficiency in HaCaT cells could accelerate terminal differentiation. To study HSPA2 role in epidermal morphogenesis and differentiation we growth HaCaT cells in organotypic 3D cultures. We observed that inhibition of HSPA2 expression was associated with elevated expression of terminal differentiation markers in epidermal equivalents, whereas no differences in expression of proliferation markers, as well as in pluristratified architecture were found. These results suggest that in epidermal keratinocytes HSPA2 may participate in controlling the process of terminal differentiation. Thus, it is possible that aberrant HSPA2 expression may contribute to development of skin diseases manifested by defective terminal differentiation of epidermal keratinocytes.

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(S50) IDENTIFICATION OF GATES IN HUMAN SOLUBLE EPOXIDE HYDROLASE

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Gates can reversibly control the access of various ligands and solvent to and from the active site. They can even consist of single amino acid which can be identified in open or closed conformation. Therefore, they contribute in essential enzyme properties like selectivity or specificity.

The aim of this study was identification of gates in human soluble epoxide hydrolase. Epoxide hydrolases are enzymes, which belong to the superfamily of α/β-hydrolases and catalyze the conversion of epoxides to their corresponding diols. These metabolites have a large role in maintaining cardiac, renal, and pulmonary homeostasis.

In presented work the *in silico* study of human soluble epoxide hydrolase were performed. The Amber14 package was used to run and analyse 10 ns molecular dynamics simulation. Caver 3.0.2 was used for tunnel identification. Gates exploration was performed based on analysis of amino acids conformations changes.

Preliminary results of the analysis show that Phe261 significantly modifies the opening and throughput of two tunnels in enzyme.

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(F41) THE IMPACT OF TUMOR NECROSIS FACTOR A (TNF-A) ON THE TRANSCRIPTIONAL ACTIVITY OF THE GENES ENCODING ENZYMES OF THE STEROIDOGENESIS PATHWAY IN H295R CELL LINE

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The cancerogenesis process can occur in endocrine organs, such as cortex of adrenal gland resulting in adenoma formation. Functioning adenomas are associated with the cells' ability to secrete hormones, mainly: glucocorticoid (cortisol) and mineralocorticoid (aldosterone). A large number of reports shows that both inflammatory process and cytokines play an extremely important role in cancerogenesis. Tumor necrosis factor α (TNF-α) is one of the proinflammatory cytokines, produced mainly by monocytes, macrophages, T lymphocytes and mast cells. This cytokine is also known as modulator of adrenal steroidogenesis. Unfortunately, the role of this agent in cancerogenesis is still not clearly understood due to its pro- and antitumorigenic properties.

The aim of the present study was to investigate the differences in expression pattern of genes associated with the steroidogenesis pathway in H295R cell line in response to increasing concentration of TNF-α and exposure time.

H295R cells were exposed to TNF-α (0,001 nM – 10 nM) for 3, 12, 24 and 72 hours. Total RNA was extracted with TRIzol reagent (Invitrogen) according to manufacturer's protocol. Expression profile of selected genes was determined using RT-qPCR.

The results indicate that TNF-α plays an important role in adrenal steroidogenesis modulation. It was observed that the increase of the transcriptional activity of the examined genes was associated with the higher concentration of TNF-α and the longer exposure to this cytokine. In conclusion, the intensification of inflammatory process may cause overexpression of the genes encoding enzymes of the steroidogenesis pathway in adrenal gland cells.

(S54) MULTIFLUID EULER-EULER MODEL OF THE BLOOD FLOW WITHIN THE BLOOD VESSEL WITH RIGID WALLS

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In recent years Computational Fluid Dynamics (CFD) methods [1], widely used in various engineering applications, have become a tool and proved their applicability in the biomedical flows modeling. The scope of the present study shows an Eulerian multiphase approach [2] in model of blood flow in human blood vessel (artery) section. Proposed numerical model assumes blood flow in rigid vessels and blood properties as a non-Newtonian, nonhomogeneous fluid which consists of blood plasma, red blood cells (erythrocytes) and white blood cells (leukocytes). *multifluid Euler-Euler* [3] technique allows to model the flow behavior for each of blood components and treats each phase as a separate interpenetrating continua with defined density, volume fraction and other physical properties.

Detailed numerical simulation of such multiphase model requires implementation of pulsatory boundary conditions for the flow equations. This can be done using User Defined Functions (UDFs) capability of ANSYS Fluent [4], coupling CFD model with an electrical analog (lumped model) of human circulatory system. This approach covers the influence of the human arterial system and allows to reproduce periodical cardiac cycles that determinates the pulsating (transient) blood flow conditions.

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(F3) SINGLE-CELL APPROACH REVEALS NON-GENETIC HETEROGENEITY FOR SENESCENCE AND STEMNESS REGULATORS IN EMBRYONAL CARCINOMA PA1 CELLS TREATED WITH ETOPOSIDE

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Tumour cellular senescence, induced by genotoxic treatments, was typically considered a terminal cell fate. However, it was recently shown to be reversible in certain conditions. At present the mechanisms of reversion are unclear, particularly in cancer stem cells. Using the embryonal teratocarcinoma PA1 cell line as a model of cancer stem cells we have shown that senescence induced by etoposide (ETO) treatment was intimately associated with self-renewal. Surprisingly, we found that these programs are not antagonizing at a single cell level – most cells simultaneously accumulate two opposite regulators in their nuclei – the self-renewal transcription factor OCT4A and mediator of senescence P21CIP1. The upregulation of both regulators is dependent on activation of TP53 [1]. PA-1 cells treated with ETO display highly heterogenous increase of OCT4A and P21CIP1 indicative of dis-adaptation catastrophe and bipotentiality. SOX2 and NANOG expression did not change post ETO treatment, indicating to dissociation of OCT4A from its core pluripotency function. Nevertheless, OCT4A was found to restrict entrance to senescence by suppressing P21CIP1 [2]. Moreover, ETO-induced OCT4 was found to be colocalised and correlating with activated AMPK, a key component of metabolic stress response and regulator of autophagy indicating to previously unknown stress function of TP53-dependant OCT4 response. Removal of OCT4 by RNA silencing ablated the clonogenic survival of the ETO-treated PA1 cells.

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(F30) THE EFFECT OF HISTONE MODIFICATIONS AND DNA SUPERHELICITY ON NUCLEOSOME STABILITY

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The effect of various posttranslational histone tail modifications (PTMs) on nucleosome stability was compared by exposing agarose embedded nuclei to treatments with salt or intercalator dyes, determining the remaining fraction of histones using PTM specific antibodies and laser scanning cytometry. Steep elution profiles could be measured in nuclei of all phases of the cell cycle by both salt and intercalator treatment in the case of H3K4me3 and H3K27ac marks, while the nucleosomes carrying a number of different other marks were relatively resistant, similarly to bulk histone-GFP. The difference is not due to the nucleosome-free neighboring regions in the case of the promoter/enhancer-proximal H3K4me3 and H3K27ac nucleosomes, since eluting by salt ~50% of the nucleosomes prior to intercalator elution did not change the apparent H3-GFP stability. Destabilization of the H3K4me3 marked TSS proximal nucleosomes was uniform along the genome, as revealed by chip sequencing, when doxorubicin was used as the intercalator. Nicking treatments of the nuclei did not affect the stability of nucleosomes carrying H3K4me3 or H3K27ac, while those of the second group, were all destabilized. Nick-induced destabilization was apparent also in the case of the H2A-H2B dimers, revealed at low intercalator concentrations. These data support the notion that superhelicity may assume a gene regulatory role. We also suggest that the H3K4me3 and H3K27ac active marks specify dynamic nucleosomes accomodating already relaxed DNA sequences, while most other nucleosomes hold the DNA in constrained superhelices.

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(S14) ULCERATIVE COLITIS AND CROHN'S DISEASE - ARE ESTROGENS IMPORTANT?

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Ulcerative colitis (UC) and Crohn's disease (CD) are two of the most common types of chronic non-specific inflammatory bowel diseases (IBD). The pathogenesis of UC and CD involves numerous mechanisms, which makes considerable difficulties in the effective treatment of these disease entities. Chronic inflammation in the intestine leads to damage to intestinal epithelial barrier, peristalsis disturbances, changes in microflora, deregulation of the immune response, and may also contribute to an increased risk of cancer. Estrogens were found to be one of the factor responsible for these processes e.g., 17β-estradiol is able to modulate the permeability of the intestinal wall by regulating potassium and calcium channels. In addition, estrogen receptors α and β (ERs), as well as estrogen receptor interacting with G proteins (GPER) are recognized regulators of the immune response by synergistic or antagonistic cross-talk with multiple signaling pathways.

The aim of the study was to assess the expression of canonical estrogen receptor ERα and ERβ and estrogen receptor interacting with G proteins (GPER) in biopsy samples from patients with UC and CD as compared to normal cells of the intestine. Our preliminary studies focused on evaluation of the expression of interleukin 6 and 10 (IL) and tumor necrosis factor α type (TNF α).

The conducted research has revealed different expression profile of the estrogen receptors in UC and CD as compared to normal cells of the intestine. There was also a change in the expression of pro-inflammatory cytokines in examined types of IBD. The results suggest that estrogens through estrogen receptors may influence the pathophysiology of UC and CD, regulating an immune response of the intestine in IBD.

(F10) MUTATOR PHENOTYPE AND THE ORIGINS OF REPLICATION

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Mutator phenotype is the result of mutations in genes that function in the maintenance of genomic stability, playing a significant role in the DNA detection and damage repair mechanisms. The phenotype is manifested by a significantly increased mutation rate which is often associated with tumor progression. One of the best described mutator phenotypes results from the mutation in the exonuclease domain of polymerase epsilon (POLE-exo). Mutations between aminoacids 268 and 471 of the protein encoded by the POLE gene can impair its exonuclease activity leading to an increased number of context dependent mutations that occur during DNA replication, most of which are either TCT -> TAT or TCG -> TTG. Since polymerase epsilon is responsible only for the leading strand synthesis, this results in a very specific mutation pattern that can be observed only in specific regions of the DNA, in the vicinity of DNA replication origins (ORI). POLE-exo specific mutations can be found predominantly upstream from the ORI while the reverse-complementary mutations are observed mainly downstream. This phenomenon provides the possibility to specifically map origins of replication in the DNA, which are difficult to identify using experimental approaches.

The goal of our study is to identify origins of replication based on a distinct mutation pattern associated with damaged polymerase epsilon, and to determine structural properties of the DNA in their vicinity. For this purpose we use whole genome sequencing data from The Cancer Genome Atlas project, which provides the possibility to assess mutation patterns across the entire DNA sequence. We extracted 17 patients in whom we have found various POLE-exo mutations, and further selected 11 who showed a high number of mutations among which TCT -> TAT and TCG -> TTG were overrepresented. Using our custom algorithm we identified over 2800 potential replication origins, conserved across all samples used in the study. Based on this analysis we have determined that replication origins are located predominantly in the vicinity of genes, in DNA regions of a specific chromatin conformation. We have also determined that the ORI sites share a 26 nucleotide long sequence motif which can be found in the retrotransposons from the Alu family.

Our findings provide insight into the poorly described mechanism which affects the location of DNA replication origins. A comprehensive understanding of this process can be used to determine abnormalities in the DNA that might affect the replication time of various chromosome regions, changes in which are often associated with cancer progression.

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(F23) APPLICATION OF ADJOINT SENSITIVITY ANALYSIS TO PARAMETER ESTIMATION OF AGE-STRUCTURED MODEL OF CELL CYCLE

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Age-structured models describe heterogeneous populations of individuals. Life of the individual consist of a finite number of phases, which differ in properties such as the ability to reproduce. There is the age-structure of a subpopulation in each phase, i.e. individuals in the subpopulation differ in the dwell time in the phase. In every moment of time the individual can go to the beginning of a next phase, become older in a current phase or die - these events depends on a transition functions, which determines their probability. This approach allows more realistic modelling of the population growth than using models assuming homogeneity of the population, which is especially important for complex organisms with long reproduction time.

The aim of this study was to develop software to estimate parameters of the age-structured model of cell cycle using adjoint sensitivity analysis. The model consists of three phases: G_0/G_1, S and G_2/M. Parameters in this case were transition functions and initial cells distributions in each phase. Parametric and nonparametric estimation approaches were used.

The software was designed to use data from the flow cytometry. Results were obtained for artificially generated input data and actual measurement of the cell number of HCT-116 cell line. Our results show that using a combined estimation approach gives the best adjustment of the model to the actual data. Real average durations of the cell cycle phases were achieved.

(F35) CROSSTALK BETWEEN CYTOKINE AND HYPERTHERMIA INDUCED PATHWAYS: IDENTIFICATION OF DIFFERENT SUBSETS OF NF-κB-DEPENDENT GENES REGULATED BY TNF-α AND HEAT SHOCK

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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. Our results revealed predictable inhibitory effects of heat shock on the expression of classical NF-κB-target genes, but also novel patterns of activation (or co-activation) and repression (or co-repression) for some gene subsets.
We established a global picture for heat shock-mediated impact on expression of genes regulated by TNFα cytokine. 193 genes changed expression in human U-2 osteosarcoma cells stimulated with cytokine, including 77 genes with the κB motif in the proximal promoters. Large overlap between sets of genes modulated by cytokine or heat shock was revealed, including 47 genes upregulated and 39 genes downregulated by both treatments. Binding sites for heat shock-induced HSF1 were detected in regulatory regions of 1/3 of genes modulated by both treatments. Furthermore, pre-treatment with heat shock affected expression of 2/3 of cytokine-modulated genes. In the largest subset of co-affected genes (83 genes), heat shock suppressed the cytokine-mediated activation (antagonistic effect), yet subsets of co-activated and co-repressed genes were also revealed. In most cases, the antagonistic effect was observed in genes associated with the canonical functions of NF-κB signaling. On the other hand, genes involved in transcription regulation were over-represented in the subset of genes upregulated by both stimuli, which might have contributed to the robust response of cells to both stress conditions. Pre-treatment with the heat shock resulted in suppression of NF-κB binding in the promoters of the cytokine-upregulated genes, either antagonized or co-activated by both stimuli, which suggested replacement of NF-κB-mediated regulation by heat shock-mediated regulation in the latter subset of genes.

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(S1) LIPIDOME/METABOLOME ANALYSES OF EXOSOMES USING DIRECT AND CHROMATOGRAPHY-AIDED MASS SPECTROMETRY

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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. Compared to the central role of MS methods in exosome proteomics, at present MS-based workflows are less commonly used for lipidomic/metabolomic investigations.

Here we analyzed lipid/metabolite component of exosomes released *in vitro* from human squamous head and neck cell carcinoma using direct and chromatography-aided mass spectrometry methods.

Selection of the optimum method for lipid/metabolite extraction allowed on MS analysis of ionizing radiation influence on lipid/metabolite composition detected in exosomes secreted from human head and neck cancer cells.

Exosome lipidomics/metabolomics has not yet reached its full potential, but holds great promise to discover more about exosome biogenesis and function as well as lipid biomarkers useful in clinical diagnostics.

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(F43) UVA STIMULATES THE PROLIFERATION OF HUMAN TUMOR CELLS

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UVA is a fraction of electromagnetic UV radiation with wavelength from 315 to 400nm. UVA is emitted naturally by the Sun and is invisible to human sight. It provides about 95% of UV radiation which reaches the Earth, it is present throughout the year, and it affects all living organisms. Sun-emitted UVA does not have enough energy to ionize atoms, but it can induce reactive oxygen species in cells. It reaches the dermis and hypodermis and is potentially carcinogenic and directly involved in photoaging.

Here we present the results of studies concerning the effects of low doses of UVA radiation on normal and cancer human cells (NHDF, normal human dermal fibroblasts; Me45, melanoma cells; HCT116, human colorectal carcinoma cells). Using fluorescence microscopy and flow cytometry we tested the cell cycle and proliferation of cells exposed to UVA doses of 5, 10, or 20 kJ/m^2. Cells were treated with cytochalasin B which inhibits cytoplasmic division, and the proliferation rate was estimated on the basis of the fraction of binucleated cells in the population. Exposure of Me45 and HCT116 cells to low doses of UVA radiation stimulated their proliferation activity. We observed a significantly increased fraction of binucleated cells in Me45 and HCT116 cell lines; HCT116 cells also showed a significant increase in polyploidy. A normal cell line (NHDF) did not proliferate more actively after irradiation with the same UVA doses but, on the contrary, it showed a significant increase in apoptosis.

Analysis of numbers of cells that divided more than once during 24 h of observations suggested that exposure to UVA radiation accelerated the passage through the G2 phase.

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(F50) WEB BASED QPCR DATA ANALYSIS SOFTWARE

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Real-time PCR (qPCR) is an advanced technique, which uses fluorescent dyes to monitor the gene amplification process during the PCR reaction. Changes of fluorescent intensity corresponding to the amount of desirable reaction product can be measured and used to count the quantification cycle (Ct) in each reaction. The big advantage of this method is speed and accuracy over conventional PCR. The high sensitivity of qPCR makes it desirable in relative and absolute gene expression quantification. A crucial and very important step in an experiment is the proper analysis, because even the smallest mistake will have a big influence on the analysis results. It's sensitivity is the reason why this method is used for large scale studies, especially in microbiology and medicine, regardless of the costs.

However, Ct values calculated based on the fluorescent intensity curves are not always very precise and require special normalization and statistic calculations. The simplest method of analysis is ΔΔCt [1], where we have control and treated probes with corresponding reference probes. This mathematical formula is quite popular in both small and big experiments.

The problem is when we have a bigger and more complicated experimental scheme, because in many cases probes are divided in many multiwell plates. In such an experiment we often use more than one reference gene where we use statistical techniques to select stable reference genes. In bigger experiments we use real efficiency and calculate firstly the quantity of a gene using ΔΔ Ct method, and then normalize and calibrate the result values [2]. This process requires computer- accurate calculations, and that is why we suggest a new tool for qPCR data analyses.

Biologists often need plots, tables and ability to look into every gene separately to analyze the experimental data correctly. Our software is fully automated and uses as an input the raw files, obtained from a qPCR instrument and allows to analyze them or calculate in different ways. We have implemented the *BioRad* instruments file format, which is one of the most popular qPCR equipment manufacturers, but depending on the needs it can be simply expanded on other file formats. All analysis steps are controlled by the user from a web interface. The user can choose if he is interested in appointing the Ct value manually or using some model-based methodology. The software include all necessary preprocessing steps and also the control plots, tables and statistics. Everything depends on what data is needed and what is our target.

The biggest advantage is that all complicated calculations are computed on the server computer in a quite short time. All results can be exported to EXEL file format or PDF files so we have access to them all the time. This software does not need local computer installation and special mathematical skills, because the configuration and parameter settings are minimal.

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(F20) ANALYSIS OF THE HSF/NF-κB PATHWAY MODEL SENSITIVITY TO CHANGES IN INPUT SIGNALS

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NF-κB is a family of transcription factors that regulate the transcription of hundreds of genes, including genes that determine cell fate. It has been proved that NF-κB can play an antiapoptotic role in cancer cells, e.g. *via* activation of anti-apoptotic genes (Cataldi *et al*., 2003). Therefore, inhibition of NF-kB pathway may constitute one of the goals in anticancer therapies. Experimental results show that heat shock induces such inhibition in cancer cells (Janus *et al*., 2011). However, the precise mechanisms of interactions between heat shock and NF-kB pathways are not fully understood. 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 chemo- or radiotherapy would be the most efficient.

So far, numerous models of NF-κB pathway have been developed, whereas much fewer models of HSF pathway were published. The model proposed in our study 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 between HSF and NF-κB pathways, they had to be modified: nuclear and cytoplasmic levels of proteins and complexes had to be separated and constitutive as well as inducible HSPs were described by separate variables. The interactions between the HSF and NF-κB pathways take into account creation HSP:IKK complexes, temperature-dependent inactivation of proteins located upstream of IKK activation and inhibition of NF-κB import to the nucleus under heat shock condition.

The system input is described by heat shock temperature, the length of heat shock treatment and the recovery time, i.e. time delay between the end of heat shock and beginning of TNF treatment. Their values are also the environmental parameters controlled in the experiment. In order to examine the sensitivity of the system to changes in parameters characterizing the input signal, we performed sensitivity analysis in the frequency domain, using Discrete Fourier Transform. Results of this analysis show that the efficiency of NF-κB response suppression strongly depends on heat shock temperature and recovery time, whereas the length of heat shock treatment is much less important, which is consistent with experimental data.

The simulation analysis show that the proposed model reflects the dynamics of NF-κB response suppression for some time after the heat shock. Moreover, based on the sensitivity analysis and simulations, we found that in order to obtain the most efficient therapeutic procedure we must take into account both the heat shock temperature and time delay between the end of heat shock and the beginning of TNF treatment.

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(F16) IDENTIFICATION AND MODELLING OF BLOOD FLOW PROCESSES IN SECTION OF LARGE BLOOD VESSEL USING HYBRID EULER-LAGRANGE MULTIPHASE APPROACH

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The main objective of current project is to develop and test novel approach of accurate modelling of human blood flow in arteries. Currently available research reports do not cover the spatial interaction of individual blood phases and walls of blood vessels. Such approach could significantly reduce accuracy of such models.

Usage of Computational Fluid Dynamics (CFD) [1] solver allows to assemble all essential information. A hybrid multifluid Euler-Lagrange approach is proposed in current study, basing a part of wider research project targeted at modelling of blood flow in human artery section.

In proposed model the multifluid [2] approach is used to model blood plasma and Red blood Cells (RBCs) - the interpenetrating phases. In addition, White Blood Cells (WBCs), (which volume fraction is about 1% of the overall blood volume) are represented as dispersed phase. Using proposed methodology, where collisions of WBCs can be modelled, is possible using advantages of hybrid Euler Lagrange [3] model.

The interaction between phases and within phases are calculated in Eulerian grid, where the calculated interaction stress tensor is used by dispersed phase to take into account collision effect. As a result, the 3D structure of mutually interacting particles will be modelled.

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(F42) RADIOBIOLOGICAL RESPONSE OF THE GLIOBLASTOMA T98G CELL LINE TO PHOTON RADIATION OF DIFFERENT QUALITIES

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The efficiency of radiation-induced effects vary not only with the adsorbed dose but also with the energy of radiation. It has been well established that photon energy spread increases with penetration depth of the irradiated medium. At greater medium depth, interaction with the fraction of scattered radiation with lower energy becomes more frequent and a larger portion of scattered radiation with energy lower than that of the incidental beam can change the biological effect of irradiated cells in a manner different from that predicted by dosimetric calculation.

The aim of this study was to compare the biological effect of the photon radiation dose rate commonly used under radiation therapy conditions, for two values of photon energy, in human T98G glioblastoma cell line.

Cells were exposed to 6 MV or 20 MV photon energy radiation delivered at either 100 or 600 MU/min, at two different medium depths (3 or 15 cm) in a water phantom. Dose applied was 5 Gy. The radiation induced genetic changes were estimated as frequency of micronucleated and apoptotic-like cells, using cytogenesis-block micronucleus test.

Low dose-rate induced more micronucleated and apoptotic cells than high dose-rate. The lower photon energy induced more genetic damage in T98G cells, as compared to higher photon energy (6 MV versus 20 MV).

At a larger medium depth of water phantom higher cell radiosensitivity was observed, as compared to smaller depth.

Dose-rate and photon energy have a significant influence on micronuclei formation and induction of apoptosis of T98G cell irradiated *in vitro*. Our experiment suggests that application of conventional radiotherapy delivered at low dose rate and low photon energy can be more efficient in inducting DNA damage in T98G cells. Evaluation of this effect in other types of human glioblastoma cells should be useful for planning of radiotherapy treatment because malignant gliomas are mostly treated by radiation.

(F39) SPEN EXPRESSION IN MOUSE SPERMATOGENIC CELLS

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SPEN protein, known also as MINT (MSX2-Interacting Nuclear Target) or SHARP (SMRT/HDAC1-associated repressor), is a large nuclear protein of 402 kDa characterized by four N-terminal RNA-recognition motifs and a highly conserved C-terminal SPOC (Spen Paralog and Ortholog C-terminal) domain. It is known that in osteoblasts SPEN is proteolytically cleaved to mature 110 kDa (N-terminal) and 250 kDa (C-terminal) fragments that both accumulate in nuclear matrix fractions. A 300 kDa protein detected in the cytoplasm probably represents an incompletely processed precursor. SPEN may bind both to DNA and RNA. It was suggested that it may serve as a binding platform for other transcription factors and co-repressors. We hypothesize that this protein may be involved in the general repression of transcription induced by heat shock in spermatocytes, which leads to massive apoptosis.

We detected up-regulation of the *Spen* transcription in isolated spermatocytes and whole testes from wild type mice subjected to hyperthermia. *Spen* transcription was also enhanced in the testes of transgenic mice overexpressing the mutated, active form of HSF1 (*Heat Shock Transcription Factor 1*), the main regulator of the heat shock response. However, this activation was probably not directly dependent on HSF1, since we did not state the binding of HSF1 to the *Spen* promoter by chromatin immunoprecipitation (ChIP). We determined the intracellular distribution of SPEN protein in mouse spermatogenic cells by immunohistochemistry in control and heat shocked testes. SPEN protein preferentially accumulates in the nuclei of spermatogenic cells, especially in spermatocytes, and revealed a significant increase of expression after heat shock. Western blot analysis of fractionated testes extracts showed the presence of the full-length protein (which potentially represents a nascent polypeptide precursor) in the cytoplasm, and a shorter, mature protein formed after proteolytical digestion detected only in the nuclear fractions. Moreover, Western blot analysis of whole testes and nuclear and cytoplasmic fractions revealed an evident increase of SPEN protein level already 15 min after heat shock, both in testes shocked at 38°C and at 43°C.

Currently we work on the identification of direct molecular targets (DNA and proteins) of SPEN. The results of interactions between SPEN and its targets will be combined with already available data on gene expression profiles to draw a general picture of the role of SPEN in the heat shock response in spermatogenic cells.

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(F5) DO THE DOSE AND THE TIME POST IRRADIATION AFFECT FUNCTIONAL V GENES FREQUENCIES IN MURINE TCR?

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The V(D)J recombination mechanism required for immunoglobulin and T cell receptor gene expression is the main source of the enormous variety of these immune receptors. This process consist in the selection and assembly of one of many germline variable (V), diversity (D) and joining (J) gene segments into a single exon coding for the variable domain of the receptors. The V genes pool is the largest. The aim of this study was to propose a statistical method to compare V gene content in TCR sequences within samples differing strongly in size and estimating whether low doses of radiation affect the selection of V gene segment.

The study was carried out on data obtained from high-throughput sequencing with focus on murine T cell receptor sequences from whole blood. The material was collected from 30 mice treated with no radiation, 0.1Gy and 1Gy at three time-points (1, 3 and 6 months post irradiation). As a result more than 0.5 million unique sequences for all mice were obtained giving a 12.5 fold change between two extreme samples. Each input was carrying information about the sequence frequency and the content of V gene. To compare the frequencies of V genes between the samples a biodiversity approach was proposed using Pielou's index. To estimate the significance of the differences between the samples a modification of Hutcheson t-test was applied with use of bootstrap method drawing numbers from multinomial distribution, which allowed correct estimation of variance. 95% confidence intervals were computed from standard error of bootstrap statistics received from 2000 iterations. A Bonferroni correction for multiple testing was applied.

As a result nine Pielou's indices with confidence intervals were obtained indicating that all samples differ with respect to V gene content ($p<10^{-7}$). The results range from 0.807 to 0.844 pointing rather even distribution of V genes. What may be concluded is the impact of both time and dose on V gene content showing fixed relations between groups. The dose seem to make V gene distribution less homogenous, which may be observed in weakening or favoring single genes, where 0.1Gy strengthen the observation. Similar conclusions might be drawn for time impact, where with increase of mice age the distribution of V genes became less even.

The Pielou's index allowed to compare samples differing strongly in size. Both size and time factors seem to affect selection of V gene segments during VDJ recombination process.

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(F17) IDENTIFICATION AND MODELLING OF BLOOD FLOW PROCESSES IN SECTION OF LARGE ELASTIC BLOOD VESSEL USING FLUID STRUCTURE INTERACTION APPROACH

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The numerical modeling of the human blood flow through the arteries is very complex due to presence of many physical and mechanical effects that act on the arterial wall. The main scope of the presented work is to develop a model which will be capable to solve complex blood flow in a selected section of human artery.

The blood arteries due to their natural tendency for deformation, caused by pressure pulsation, requires special attention during modeling process. In presented work the blood flow within arteries will be modeled using the Computational Fluid Dynamics (CFD), whereas the elastic nature of the blood vessels will be taken into account applying Fluid Structure Interactions (FSI). This technique allow to model the effect of pressure and fluid stresses which acts on the arteries boundary cause their deformation. The FSI technique has been already used by other researchers for modeling blood vessels deformations, nevertheless the combination presented in this work i.e. the hybrid Euler-Lagrange approach used for modeling blood flow together with FSI technique is unique in the discussed field of research.

The developed numerical model will be capable to solve complex multifluid flow within selected arteries sections taking simultaneously into account deformation of the blood vessels wall due to the blood pressure fluctuations and wall shear stress caused by fluid flow.

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(S31) DIFFUSION ON TWO-DIMENSIONAL MEMBRANES AND NUMERICAL SIMULATIONS OF LEVY PROCESS

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Two-dimensional diffusive motion of a gas particle on membrane structure was studied. The examined membranes are composed of ethylcellulose and magnetic powder. The structure of pattern formed by the magnetic powder used in the membrane matrix depends on many parameters such as polymeric matrix used, type of powder, its amount, granulation and molecular clusters (if any).

Diffusion on two dimensional membranes was studied using numerical simulations of Lévy process. Lévy flights are a particular class of a generalized random walk in which the step lengths during the walk are described by a 'heavy-tailed' probability distribution. Within membranes structure, a gas molecule is allowed to perform a Cauchy random walk with uncorrelated steps. In the case of obstacle-free space a superdiffusive type of particle motion was observed.

Our analysis shows that the presence of magnetic particles in the membrane matrix significantly influences the type of motion.

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(S5) SPECIFIC ALLELE COMBINATIONS IN TUMOR PROGRESSION GENES ARE ASSOCIATED WITH LUNG CANCER PROGNOSIS

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Lung cancer is the neoplasm with very poor prognosis. Its development, progression and metastasis is influenced by factors regulating tumor angiogenesis. Matrix metalloproteinase MMP-2 regulates tumor microenvironment and contributes to angiogenesis by releasing VEGF and FGF from extracellular matrix. The VEGF receptor 2 (VEGFR2) is a key player in complex process of new vessels formation. The cyclooxygenase COX-2 is an inducible enzyme participating in prostanoid biosynthesis and its overexpression is associated with enhanced angiogenesis, invasion and metastasis, host immunity suppression and apoptosis resistance. Some variants of genes coding for above mentioned pro-angiogenic and pro-metastatic molecules may modulate the gene/protein expression, cancer risk and patient survival.

The aim of our study was examination of the combined effects of functional polymorphisms in *VEGFR2, COX-2* and *MMP-2* genes on overall (OS) and progression-free survival (PFS) in lung cancer. A group of Caucasian patients with inoperable non-small cell lung cancer (NSCLC) treated with radio- and chemoradiotherapy was investigated using PCR-RFLP. Survival curves were determined with Kaplan-Meier method. Uni- and multivariate analysis using Cox proportional hazards regression was also performed.

Our results indicate that rs2071559, rs689466 and rs243865 variants may significantly influence clinical outcome in the group. The rs243865 T, rs689466 G and rs2071559 C were found to be unfavorable alleles for OS and/or PFS. In addition, number of adverse alleles for variant combinations showed strong association with worse OS and PFS.

These findings suggest that the examined polymorphisms may be potential prognostic markers in inoperable NSCLC.

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(S6) POTENTIAL PROGNOSTIC MARKERS IN OVARIAN CANCER: Q-PCR AND IMMUNOHISTOCHEMICAL VALIDATION OF CLASP1, FN1, MFAP5 AND POSTN EXPRESSION

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Ovarian cancer is a relatively rare female cancer but it is the most deadly among gynecological malignancies. Because of minimal improvement in effective treatment there is a constant need to find new predictive and prognostic molecular markers that will enable individualization of therapy of this type of cancer.

In our previous microarray study we identified a set of genes related with different survival of the patients [1]. Four genes were selected for further validation by Q-PCR and by immunohistochemistry (IHC). These were three genes connected with the structure and function of extracellular matrix: fibronectin (FN1), periostin (POSTN), microfibrillar associated protein 5 (MFAP5) and one gene related with microtubule function - Cytoplasmic linker associated protein 1 (CLASP1). By Q-PCR method we confirmed that CLASP1 is related with overall survival (OS) and disease-free survival (DFS) while MFAP5 with OS only. Currently we analyze an independent set of 100 ovarian cancer samples by IHC. Our results indicate that high expression of FN1 and POSTN (measured by IHC) correlates with shorter overall survival (the Kaplan Meier survival analysis, log rank test $p=0.003$ and $p=0.036$ respectively for FN1 and POSTN). Correlation with DFS was not confirmed for these genes.

The association between expression level of these proteins and other clinico-pathological and molecular features (tumor grade, FIGO stage, p53 protein accumulation in cancer cells, etc) will be also analyzed.

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(S51) PRIVILEGED STRUCTURES CONCEPT AND MACHINE LEARNING METHODS AS TOOLS IN DRUG DISCOVERY RESEARCH: APPLICATION IN LIGAND-BASED VIRTUAL SCREENING IN A SEARCH FOR NOVEL ANTI HIV-1 CHEMOTYPES

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Virtual screening (VS) is an important approach in the drug discovery process. Methods used in VS can be divided into two classes: structure- or ligand-based techniques. So far in both structure- and ligand-based VS protocols, machine learning (ML) procedures were often successfully applied. The most common practical ML usage is to classify or prioritize databases of molecules in terms of biological activity, specific ADMET properties or various other chemical purposes. ML classification can be performed as binary classification of molecules (as active or inactive) or as numerical predictions of certain property values determining which compounds possess more "promising" properties than others. This second approach is in fact the ML-based scoring function, which may be used as a decision function (e.g., for distinguishing between active and inactive compounds).

Yet another ligand-based approach for VS is the concept of privileged structures (PS), which was introduced by Evans[1] and further developed by others. The PS concept is based on the assumption that certain structural features produce biological effects more often than others. The theory of PS evolved from a pragmatic tendency to simplify the complexity of drug design through fragmentation and has previously been used in explaining structure-activity relationships in diverse groups of drugs. If PS is shown to be useful, then molecular motifs that enrich biological activity can be used when designing new drugs.

In a search for new anti HIV-1 chemotypes, we developed a multistep ligand-based virtual screening protocol combining machine learning methods with the privileged structures concept.[2] In its learning step, the VS protocol was based on HIV integrase (IN) inhibitors fetched from the ChEMBL database. The performances of various ML methods and PS weighting scheme were evaluated and applied as VS filtering criteria. Finally, a database of 1.5 million commercially available compounds was virtually screened using a multistep ligand-based cascade, and 13 selected unique structures were tested by measuring the inhibition of HIV replication in infected cells. This approach resulted in the discovery of two novel chemotypes with moderate antiretroviral activity, that, together with their topological diversity, make them good candidates as lead structures for future optimization.

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(F22) A SIMULATION STUDY OF THE RELATIONSHIP BETWEEN REPLICATION STRESS DETECTION PATHWAY AND THE CELL CYCLE

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Ataxia telangiectasia mutated and Rad3-related (ATR) kinase is activated by the presence of single-stranded DNA (ssDNA) fragments. Such forms occur due to spontaneous replication errors, resection of double-strand breaks during repair or after blockade of replication forks on lesions caused by ultraviolet irradiation. After detection of ssDNA ATR module activate p53 transcription factor, which is responsible for cell fate decision. P53 regulates such cellular processes, like apoptosis, DNA repair or cell cycle progression.

In this work we studied the impact of ssDNA detection on the cell cycle. We also tried to examine how cell cycle phase influences DNA damage detection process. We developed deterministic mathematical model, which we use to investigate the dependencies between these two important cellular pathways.

Our results indicate that response of ATR module to ssDNA formation is correlated with cell cycle phase. Genetic material condensation level and cell volume have an impact on DNA susceptibility to damage and strength of detection signal. It may be an explanation why heterogenous population of cells exhibit different responses to irradiation during biological experiments. Our model shows also how big is the impact of DNA damage detection on cell cycle progression.

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(S45) TEMPORAL CHANGES IN MN-SOD EXPRESSION IN RESPONSE TO DOXORUBICIN TREATMENT AND HYPOTHERMIA

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Manganese-dependent superoxide dismutase (MnSOD, SOD2) is the primary, indispensable anti-oxidant enzyme responsible for neutralization of superoxide anion. MnSOD *knock-out* mice die before 2 weeks of age due to cardiac, neurological and metabolic disorders. Expression level of MnSOD varies among tissues. Different types of tumors also show variation in MnSOD expression. Induction of overexpression of MnSOD has been proven to suppress tumorigenicity of many cancer cell lines. Cultivation of cells in sub-physiologicial temperature has been shown to slow metabolism, arrest cell cycle or induce apoptosis. It has been shown that hypothermia decreases amount of reactive oxygen species in cells. Hypothermia is used to preserve organs during transportation and operation.

The rationale behind the study was to check whether MnSOD mRNA and protein level change in the course of doxorubicin treatment. Additionally, it was examined if hypothermia had any modulatory effect on MnSOD expression in the presence of doxorubicin. Mouse embryonic fibroblast cells, C3H/10T1/2, were grown in normal (37°C) and mild hypothermic conditions (33°C). Cells were treated with doxorubicin and collected for analysis at different time-points (0.5 to 72 hrs). Expression of MnSOD mRNA was quantified in Q-RT-PCR. MnSOD protein was visualized by Western blotting.

Here we show that MnSOD transcription started decreasing soon after addition of doxorubicin to culture medium and remained decreased throughout the course of the treatment. MnSOD protein level increased in the initial phase of treatment and decreased after 3 hours of incubation with doxorubicin. Mild hypothermia preserved MnSOD mRNA up to 6 hours of incubation with doxorubicin. After 6 hours transcription of MnSOD started to decline but remained at slightly higher level than transcription in 37° C culture. After 48 hours of incubation transcription in hypothermic culture decreased below the level of 37° C culture. In hypothermic conditions protein level of MnSOD increased slowly peaking at 6 hours of treatment. It remained elevated until 48 hours of incubation. Afterwards it reached the same level as in 37° C culture. We assume that hypothermia has a preventive effect on MnSOD expression by delaying downregulation of MnSOD expression by doxorubicin.

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(F55) P53 PROTEIN-DEPENDENT CHANGES IN THE GLYCOSYLATION PATTERNS OF CANCER CELLS

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Alterations in glycosylation patterns of apoptotic cells were reported many times in scientific literature, however the mechanism that induces changes in the glycosylation pathway is still poorly understood. The aim of this work was to verify whether the expression level of p53 protein, which is a well-known apoptosis inducer, is correlated with the presence of specific glycans on the surface of human cancer cells.

Using FITC labeled lectins we determined the glycosylation pattern of cancer cells with wildtype and knocked out or damaged p53 protein. We than induced changes in the p53 level with ionizing radiation and identified glycosylotransferases (GT) whose expression level may be altered by the p53. This was possible using our custom GlycoGene application which based on results of FITC lectin labeling, and both GT and glycan structure databases, was able to identify relationship between individual lectins and glycan structures leading to specific GT coding genes involved in their synthesis.

Our study shows that p53 can interact with glycosylotransferases involved in the synthesis of complex glycans, leading to changes in the glycan repertoire on the cell surface.

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(F49) ALGORITHM FOR DETECTION AND CORRECTION FOR BATCH EFFECTS IN LARGE DNA MICROARRAY DATASETS

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Datasets containing gene expression profiles obtained with the use of DNA microarrays have been supporting studies on numerous aspects of biological and biomedical problems. Algorithms for their analysis include processing steps oriented towards extracting most important and reliable information. Due to complexity, large size and high level of noise in the DNA microarray data a lot of efforts have been undertaken to improve efficiency of extraction of useful biological information from gene expression datasets. Important areas of research towards improving efficiency of extraction of information are projects related with creating large DNA microarray datasets obtained either by integration of smaller DNA microarrays datasets or by parallelizing many DNA microarray scanners in one large experiment.

Aims of the presented study were:

(i) To develop appropriate methodologies of analysis of large DNA microarray datasets,

(ii) To verify the developed methodology on a publicly available, large DNA microarray dataset, and to compare the obtained conclusions to the existing, published results of analyses of this DNA microarray dataset.

In our study we show that large DNA microarray datasets are very likely to exhibit inhomogeneity, which may lead to biases in results of standard analyses. We interpret inhomogeneity of DNA microarray datasets as a "batch effect", following from different (albeit very difficult to ascertain) conditions. We demonstrate that even in a very carefully designed experiment, involving parallelized DNA microarray scanners, batch effects are significant. We developed a method of batch effect detection by using PCA analysis. Using the results of PCA analysis, we were able to incorporate the Combat algorithm for batch effect correction, based on Bayesian statistics, as the second step of our method.

In order to verify the proposed methodology we used a well examined dataset of leukemia gene expression profiles, which consisted of 16 subgroups of leukemia, myelodysplastic syndrome and one group of non-leukemia and healthy tissue. The dataset has above 2000 microarray samples, which were obtained using a carefully designed experiment in 11 institutions from around the world. Despite the careful design, after pre-processing we observed batch effect related to variation caused by sample preparation in different institutions. In the primary analysis of the dataset authors did not take this fact into account.

Using the designed algorithm, we were able to discover new tumor markers in the leukemia dataset, not reported in the study. These markers are ASIC2, GABRE, LINC00525 and CTNNA3. Validity of the discovered markers is supported by independent literature sources.

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(F48) ADJOINT SENSITIVITY ANALYSIS OF SPATIAL SYSTEMS DESCRIBED BY DIFFERENCE EQUATIONS

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Models of spatial systems are often used in biology and medicine. These models are usually described by partial differential equations. This causes that these models must be discretized before simulation on discrete machines like modern computers. In discretization process the partial differential equations are converted to difference equations. For example, many types of 1D reaction-diffusion systems after discretization can be presented as following general equation:

$$y_i^{j+1} = f(y_i^j, y_{i-1}^j, y_{i+1}^j, u_i^j), \quad j = 1, 2, \ldots, j_{max}, \quad i = 1, 2, \ldots, i_{max}$$

where: y_i^j is state of cell *y* at time *j* and in spatial point *i*, u_i^j is a value of input signal y_i^j affecting the system at time *j* and in spatial point *i* and *f* is a non-linear function describing the state of the system in nexj_{max}ation. is the number of time iterations and

i_{max} is a number of spatial points of the system.

This work demonstrates how adjoint sensitivity analysis can be used to efficiently calculate the spatiotemporal gradient of predefined objective function with respect to control signal which affects the system described by difference equations. As an example, a spatial model for tumor response to radiotherapy [1] was analysed. The calculated spatiotemporal gradient was used during optimization of the irradiation signal which may be helpful in developing new radiotherapy protocols.

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(S49) INFLUENCE OF BINDING POCKET RESHAPING ON THE ACCESS TO THE ACTIVE SITE IN LIMONENE-1,2-EPOXIDE HYDROLASE FROM *RHODOCOCCUS ERYTHROPOLIS*

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The structure of limonene epoxide hydrolase (LEH) from *Rhodococcus erythropolis* is the first representative of bacterial limonene-1,2-epoxide hydrolase (EC 3.3.2.8) deposited in Protein Data Bank. LEH participates in microorganisms defense against toxic compounds and can be a useful target for drug design. The active site of LEH consist primarily of hydrophobic amino acids, located at the bottom of the pocket. In 2015, Zhoutong and coworkers reshaped binding pocket by saturation mutagenesis of amino acids lining the deep pocket to enhance and invert stereoselectivity of wild-type LEH. This strategy resulted in R,R – and S,S – selective mutants for the hydrolytic desymmetrization of cyklohexen oxide.

The binding site reconstruction might cause modification of the entrance to the active site. In our study we were aiming to investigate in details changes in the dynamic of enzyme structure and active site accessibility by intensive *in silico* study.

Short 10ns molecular dynamics simulations were run for LEH wild-type and two mutants with changed enantioselectivity and analysed by CAVER to identify tunnels leading to active site. Analysis of MD results and recognized tunnels showed that binding pocket shape modification has significant influence on the ligand entrance to the active site.

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(S19) SYNERGISTIC EFFECTS OF NOVEL DERIVATIVES THIOSEMICARBAZONES IN COMBINATION WITH CHLORINE DERIVATIVES IN PHOTODYNAMIC THERAPY

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Photodynamic therapy (PDT) is a promising and developing approach in the treatment of cancer. At the basis of photodynamic therapy is the combined action of a photosensitizer, light and molecular oxygen within malignant tissue. Under these conditions, administration of the photosensitizer (PS) to the tumor and local exposure to light of a specific wavelength can lead to a series of photochemical reactions and, consequently, to the generation of singlet oxygen and reactive oxygen species (ROS) [1]. Accumulation of ROS may cause induction of protein damage, DNA disruption, lipids peroxidation and consequently - triggering of apoptosis. Currently, using combination therapies is a growing approach to increase the overall therapeutic efficacy of PDT. The basis of such therapy is the combination of two or more drugs, that can exert preferably additive or synergistic effects [2]. In our group, the promising results were obtained when novel thiosemicarbazones derivatives (TSC) were used in combination with 5-aminolevulinic acid (ALA) which is the precursor in ALA-PDT treatment [3].

Recently, we focused deeper on the interactions of novel highly active thiosemicarbazones with known PS - chlorine and temoporfin (Foscan) in combined PDT. We performed an in vitro assay of cell viability on human colon cancer cell lines (HCT116 +/+) to examine the dark- and photo-toxicity effects of the drugs (TSC and PS) alone and in combination, respectively. Accumulation sites for those drugs were evaluated in co-localization experiments on confocal scanning microscopy. In addition, we measured the production of singlet oxygen, as well as lipids peroxidation by TSC, PS and combination thereof as major factors leading to the induction of apoptosis.

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(F24) MODELING OF PROTEIN SPOTS ON 2D GEL ELECTROPHORESIS IMAGES

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Two-dimensional gel electrophoresis is a common technique used in proteomics for cleaning and separation of proteins or finding post-translational modifications. Recently, it has been applied to discover protein biomarkers by detection of differentially expressed proteins between samples from a series of 2D-gels. Main advantage of the technique is obtaining high resolution data, which gives opportunity to find an expression level of few thousands proteins in one gel. However, the overall quality of gel images suffers due to artifacts, inhomogeneous background and high levels of noise. An important drawback of existing software tools is that they fail to detect overlapping spots and there are dependent on manual parameter tuning and correction of obtained results, which causes subjective and non-reproducible experiment outputs.

Processing of the gel images may be divided into following steps: image pre-processing, image alignment, spot detection and spot quantification. In this work we propose to divide image into smaller segments using watershed algorithm, perform signal processing locally and aggregate final results. We remove vertical and horizontal streaks using background correction based on polynomial fitting. Then we smooth signal by matched filtering with Gaussian function and Otsu thresholding. Protein spots are detected by fitting 2D Gaussian mixture model to each segment by modified EM algorithm. Large-area and low-height components are filtrated using common outlier detection methods.

We present results of applying the developed method using Raman's dataset with annotated proteins, which appeared in many 2D gel software evaluation studies and example dataset available in RAIN software. The proposed pre-processing of gel images and application of Gaussian mixture modeling leads to improved detection of overlapping peaks and decrease of false positives. Spot quantification using Gaussian masks results in precise estimation of protein amount. Method is fully automatic since it uses properties of analyzed image to adapt main parameters of the algorithms.

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(S48) INFLUENCE OF THE MISSING LOOP REFINEMENT FOR SUBSTRATE ACCESS TO THE ACTIVE SITE IN *ASPERGILLUS NIGER* EPOXIDE HYDROLASE

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Epoxide hydrolases (EHs) are enzymes involved in detoxification of xenobiotics and synthesis of secondary metabolites. EH belong to a superfamily of α/β-hydrolases with its catalytic pocket buried deep in the protein core and connected with the environment by tunnel(s). *Aspergillus niger* epoxide hydrolase is the member of soluble microsomal EHs family. The active site consists of catalytic nucleophile (Asp124) and a charge-relay system formed by histidine (His289) and another aspartic acid (Asp260). The enzyme shows also higher activity and improved enantioselectivity when the substrates are present at high concentrations.

The *Aspergillus niger* epoxide hydrolase crystal structure is deposited in Protein Data Bank database (PDB ID: 1QO7). The sequence is 394 amino acids long and the structure is missing 9 amino acids in a loop 320TASAPNGAT328. That loop is located near the active centre, so it might have influence on substrate access and selectivity. The aim of the study is to find, how the refilling of missing loop influences the shape and size of tunnels leading to the active site.

10 000 models with refilled loop were created using Modeller9v14. Created models were clustered according to Modeller score and geometrical parameters reflecting size, shape and position of the loop. Amber14 was used to run 10 ns molecular dynamics simulations to observe dynamic of the refiled loop. Finally CAVER 3.0.2 was used to facilitate tunnel identification and analysis.

Our preliminary results show that the shape and conformation of amino acids in the refilled missing loop could play the key role in substrate accessibility. Next step will be to examine the influence of adjacent amino acids mutations on loop behaviour.

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(S43) ENHANCING THE EFFECTIVENESS OF CHEMOTHERAPY WITH CISPLATIN USING A MICROSECOND ELECTROPORATION IN PANCREATIC ADENOCARCINOMA *IN VITRO*

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In spite of the considerable progress in the diagnosing and treatment of various cancers, the number of cancer deaths among patients burdened with pancreatic carcinoma is still increasing. Electrochemotherapy (ECT) is a novel technique combining a standard chemotherapy with an electromagnetic field action. The application of the short pulses during electroporation (EP) increase the cell membrane permeabilization by the formation of unstable pores in the cell membranes.

The aim of our study was to evaluate the influence of electrochemotherapy with cisplatin in pancreatic cancer cells. The human adenocarcinoma drug-sensitive cell line EPP85-181P was used for experiments. The cell culture was maintained in highly humidified conditions of 37°C and 5% CO_2 at modified Leibovitz medium (L-15). The cells were electroporated with the following parameters of electric field:800 and 1200 V/cm;8 pulses with a length of 100μs, interval length 1s. The electroporation process was performed in EP buffer containing solutions of cisplatin in concentrations: 0, 5 and 10 μM.. Cellular viability was measured using the SRB and MTT assays after 24 and 72 hours of incubation. Additionally the trypan blue staining was performed.

The obtained results show that microsecond electroporation is an effective method of enhancing the efficiency of chemotherapy with cisplatin. The most significant results were obtained after combination of 10 μM of cisplatin with 1200 V/cm. This indicate the most effective membrane permeabilization.

The use of electrochemotherapy may reduce pancreatic adenocarcinoma cell viability. However, the parameters of electroporation have to be chosen carefully to selectively support cisplatin transport directly into cancer cells.

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(S35) STATISTICAL ANALYSIS FOR MITOCHONDRIAL ACTIVITY OF MCF-7 CELL LINE TREATED WITH SUGAR-CENTERED STAR-SHAPED POLYMETHACRYLATES AND THEIR CONJUGATES WITH DOXORUBICIN

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In recent years, there has been growing research interest in developing effective drug delivery systems of known chemotherapeutic agents. Polymeric nanomaterials have gained attention since they offer a tailor-made structure, and thus improve drug solubilisation, pharmacokinetics and reduce drug toxicity. Another advantages include their capability to form nanosized vesicles with excellent storage stability as well as possibility to bound chosen drug by different spacers.

Presented work is a result of further biological analysis of non-degradable amphiphilic star-shaped carriers with methacrylic arms. Synthetized copolymers are based on three different acetal-based initiators, and arms composed of methyl methacrylate (MMA) and 2-hydroxy-3-[(2-aminoethyl)amine]propyl methacrylate (HAmPMA) repeating units with different content of amine groups. Finally we obtained 5 different nanocarriers, which were used to conjugate doxorubicin via imine bond. Copolymers and corresponding conjugates with different number of doxorubicin molecules were investigated for anticancer activity towards MCF-7/W (wild type) and MCF-7/R (doxorubicin resistant) cell lines by MTS assay. The results were processed using Statistica 12 (StatSoft, Poland). We used ANOVA with Tukey post-hoc test to evaluate: 1) the cytotoxicity of each polymer on MCF-7/W and MCF-7/R cell lines, and 2) influence of the number of arms and their molar composition on cytotoxicity. Unpaired Student's t-test was used to evaluate statistical significant result between Control group and concentration of polymers. All values are shown as the mean ± standard error. Probability values of $P < 0,05$ were considered statistically significant. Additionally, cell internalization of conjugates were monitored and proved by fluorescence microscopy.

Statistical analysis showed that V-shaped and 3-arm nanocarriers were the most cytotoxic on MCF-7/W. In the case of MCF-7/R cells, V-shaped copolymer was the most toxic and 4-arm carrier was the least toxic. We have also indicated relationship between the content of HAmPMA repeating units (F_{HAmPMA}) and cytotoxicity, on the basis of four-arm carriers. According to results obtained for conjugates, the toxicity of 4-arm conjugate with F_{HAmPMA}=0.53 was highest for wild type breast cancer cell line. Similar results were obtained for MCF-7/R, where 4-arm conjugate (F_{HAmPMA}=0.53) and 3-arm copolymer (F_{HAmPMA}=0.49) were the most toxic. In both cell lines, 4-arm conjugate with F_{HAmPMA}=0.77% was the least toxic.

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(S26) EVALUATION OF CYTOTOXIC ACTIVITY OF 1-THIOGLYCOSYL DERIVATIVES OF URIDINE AND URACIL

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Glycosyltransferases (GTs; EC 2.4.x.y) represent a large group of enzymes that are involved in the biosynthesis of oligosaccharides, polysaccharides, glycoproteins and glycolipids. They catalyze glycosidic bond formation by transferring a monosaccharide unit from an activated sugar donors to an acceptor substrate [1,2]. Glycoconjugates play important roles in a lot of biological processes such as cell growth, cell-cell adhesion, tumorigenesis, immune function, inflammation, bacterial, viral and fungal infection [2,3]. These are the reasons of creating the potential therapeutic agents that could regulate the biosyntheses of glycoconjugates.

In the present study the biological activity of twenty compounds was determined using colorectal cancer cell line (HCT116) and human prostate cancer cell line (DU145). The 1-thioglycosyl derivatives of uridine or uracil were synthesised as a potential competitive inhibitors of glycosyltransferases. Structure of tested compounds were based on the similarity to the natural donor type substrate of these enzymes. The cells were treated with compounds at range from 0.01 to 100 μM concentrations for 48 hours. The evaluation of cytotoxic activity was performed using MTT assay.

Unfortunately, the results show that none of the testing compounds is significantly cytotoxic relative to the HCT 116 and DU 145 cells. In addition, some glycoconjugates stimulate proliferation of the cells. This issue was visible after using the 1-thioglycosyl derivatives of uracil and uridine containing D-galactose with acetyl protecting groups. We should note that the synthesised glycoconjugates can be cytotoxic to other cancer cell lines but this requires additional research.

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(S7) CHANGES IN PLASMA ASCORBATE AND 2-KETOGLUTARATE LEVELS AMONG PATIENTS WITH COLORECTAL CANCER COMPARED TO CONTROLS

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The oncometabolites are suspected to inhibit the hydroxylation of DNA which occurs by TETs (*Ten-eleven translocation*), that convert 5mC to 5hmC. The level of 5mC in many tissue types is quite constant, whereas the tumor cells have a very low content of 5hmC. Emerging data suggest a role of a reduced level of 5hmC in carcinogenesis. TETs belong to the group of dioxygenase enzymes. These enzymes use Fe^{2+} as a cofactor and 2-ketoglutarate (2-KG) as a cosubstrate and some of them require ascorbate as another cofactor. Isocitrate dehydrogenases (IDHs) convert isocitrate to 2-KG. Mutations in IDH1 and IDH2 have been discovered in glioma, acute myeloid leukemia and other solid tumors. They result in the simultaneous loss and gain of the ability to produce 2-KG and 2-hydroxyglutarate (2-HG), respectively. Excess of 2-HG accumulates in IDH mutant tumors and promote the reduction of 5hmC by TETs inhibition. Biochemical and genetic evidence support the view that changes in TETs' activity contribute to carcinogenesis.

The goal of this study was to investigate the changes in the concentrations of blood plasma L-ascorbic acid and 2-ketoglutarate in patients from the Department of General Surgery, Gastroenterology, Colorectal and Oncology and Interventional Endoscopy Center, as compared to controls, using highly sensitive LC-UV/MS/MS method, offering the hope of a promising potential diagnostic option.

The preliminary results show, in contrast to vitamin C, higher level of 2-KG among patients with colorectal cancer and polyps, as compared to the controls. These differences were statistically significant ($p<0.05$).

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(F47) NOVEL IK-MEANS ALGORITHM COMPARISON WITH PCA-BASED APPROACH FOR DETERMINING HETEROGENEITY IN MALDI-MSI TUMOR SAMPLES

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The aim of our study was to develop MALDI-MSI signal analysis pipeline in order to distinguish heterogeneous regions of tumor tissues basing on their molecular spectra. Standard approach uses PCA transformation on data obtained during the experiment and detailed analysis of PCA heatmaps. This approach is however not good enough to be able to automatize discovery of different tissues regions in sample and therefore cannot handle heterogeneous regions in single tissue differentiation with respect to peptide signature.

Material from five patients who underwent surgery because of Oral Squamous Cell Carcinoma (OSCC) was collected. Tissue samples which contained tumor and surrounding tissues were evaluated and resected by an experienced pathologist from fresh postoperative material. The collected samples were frozen and kept at -70^0C. Subsequently, each tissue specimen was cut in a cryostat (10 μm sections) and placed onto a glass slide coated with indium tin oxide (ITO). The prepared slides were stored at -70°C until analysis.

The presented results of our research were based on all five datasets of 9492, 12957, 12963, 5232 and 5094 spectra with 109,564 mass channels. The data was preprocessed and normalized to express information as a set of 3202 features representing peaks corresponding to different proteins with abundance above noise level.

The first problem to be solved was microarray background identification and removal. Since whole procedure was demanded to be fully automatic, using the unsupervised clustering method was proposed. The microarray background spectra were detected and filtered out resulting in data reduction to: 8005, 11869, 11823, 4505 and 3958 spectra respectively for each sample.

Then, unsupervised grouping method was applied in recursive manner with adaptive feature selection on each level of recursion in order to adjust it to spectra distribution over specified kind of tissue. At every level (for whole sample and every obtained subregion), feature expression variance GMM decomposition was used to select only the most informative ones. Finally, in space reduced from 109,564 to just few thousands (depending on sample), optimally adjusted k-means algorithm was used for segmentation.

Pathologist review of these results led to tissue identification, and this made possible to use ANOVA test for biomarkers discovery. For tumor region found, Dice index of similarity with respect to pathologist decision was 49.4%, 59.6%, 78.7%, 70.3% and 38.2% respectively, however the ‘undiscovered’ part was found to be prominent lymphoid infiltration, molecular signature of which is truly different.

Those results were compared with PCA-generated heatmaps. When using PCA-based approach any of the regions found were hardly visible (despite microarray removal step), Moreover, transformed features space was used what made original peptides identification much more complex, while our method allows to work on original dataset with only proper filtration applied.

Acknowledgements: The work was partially supported by SUT grant BK/227/RAU1/2015/10 and NCBiR GeCONiI project (POIG 02.03.01-24-099).

(S18) A NOVEL PRO-APOPTOTIC ROLE OF ZINC-PHTHALOCYANINES IN MELANOMA ME45 CANCER CELLS PDT THERAPY

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Phthalocyanine compounds are an important class of organic materials. They have found many applications in industry than include semiconductors device, photosensitizers, gas sensors, electrochromism, Langmuir-Blodgett (LB) films, liquid crystals and nonlinear optics [1]. Many applications of phthalocyanines require water-soluble derivatives. Water-soluble phthalocyanines serve as an important class of photosensitizers which can be used in photodynamic therapy in the treatment of a range of cancers [2]. The subject of our research is zinc phthalocyanine, that has eight carboxylic groups attached to the benzene rings (fig. 1). The compound is well soluble in water. Zinc phthalocyanines have been widely studied for PDT therapy due to his high triplet quantum yields and long triplet lifetimes and high fluorescence yield [3].

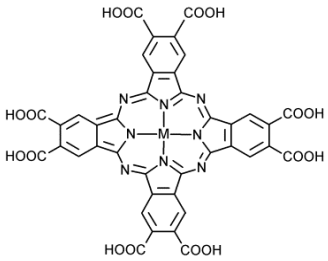

Fig. 1. Structure of octacarboxyphthalocyanine, M – Zn.

Our preliminary studies show a potential role of Zinc-phthalocyanines in Me45 cancer cells apoptosis pathway induction. Although far-red irradiation (690 nm), followed by 2h compound pre-incubation (30 μM), seemed not to be connected with significant reactive oxygen species (ROS) production, pro-apoptotic signals are well distributed in melanoma cancer cells. Flow cytometric Anexin-V apoptosis assay confirmed well Me45 cancer cells death by apoptotic, but not necrotic way.

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Acknowledgments: This work was supported by grant SUT BK-277/Rau1/2015 t.3 from Silesian University of Technology in Gliwice, Poland (M.S.), all biological experiments were performed in the Biotechnology Center of the Silesian University of Technology using equipment financed by the ''Silesian Biofarma'' program.

(S17) NOVEL ALUMINIUM PHTHALOCYANINES AS POTENTIAL PRO-OXIDATIVE AND PRO-APOPTOTIC DRUGS AGAINST MELANOMA ME45 CANCER CELLS

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Metallophthalocyanine (MPc) complexes are a class of synthetic compounds, which consists of macrocyclic tetraazaporphyrin structure and they are similar to porphyrin complexes. More recent research developments include applications of phthalocyanine complexes in materials science, medicine, electrochemistry, and photocatalysis [1]. In the last years phthalocyanines have been intensively studied, as second-generation photosensitizers for PDT [2]. This is due to the molar absorptivity of these compounds and at wavelengths 680 nm permitting greater penetration of light in normal tissues, when compared with photofrin® [3]. Aluminium phthalocyanine has in particular received considerable attention, because they exhibits high triplet life times and gives high quantum yields for singlet oxygen formation [4]. Metallophthalocyanine complexes are soluble in different solvents, peripheral substitution with eight carboxylic groups leads to Mpc derivatives soluble in water (fig. 1).

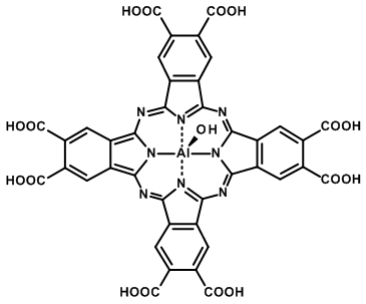

Fig. 1. Structure of octacarboxyphthalocyanine, M–Al.

Our studies showed pro-oxidative potential of aluminium phthalocyanines, after far red irradiations (690 nm). Free oxygen species (ROS) were monitored by flow cytometry, followed by 2 h of compounds pre-incubation (30 μM) with cancer melanoma Me45 cells, as well as normal human fibroblasts NHDF and keratinocytes HaCaT. The obtained results indicate ROS activation after PDT treatments in cancer cells, and apoptosis induction connected with mechanism based on oxidative stress in cells.

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Acknowledgments: This work was supported by grant SUT BK-277/Rau1/2015 t.3 from Silesian University of Technology in Gliwice, Poland (M.S.), all biological experiments were performed in the Biotechnology Center of the Silesian University of Technology using equipment financed by the ''Silesian Biofarma'' program.

(S28) GOLD NANOPARTICLES – NEW METHODS OF BEATING CANCER

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Gold nanoparticles (GNPs) are good biocompatible materials due to their special physical and chemical properties. There is more and more research where GNPs are used in the medical imaging and in the cancer therapy.

Gold nanostructures can be used in photothermal therapeutic applications or in non-invasive bioimaging due to their high absorption levels in the near infrared tissue transmission window. Although GNPs can act alone as a drug, nowadays the most interesting and promising approach to the usage of GNPs is their conjugation with many types of drugs or biomarkers. One of such biomolecules can be an antibody, which can help to supply the drug directly to the tumour.

The particular advantage of anti-cancer GNP-conjugates is their selective accumulation inside the tumour due to their increased permeability and retention.

Indeed, there are many examples described in the literature of chemically and biologically functionalized GNPs; however, these conjugates are very often instable. Although GNPs are rather stable in period of many months, modified nanoparticles – conjugates GNPs to drug, dye or antibody are often instable in a long period, particularly in the presence of high salts and proteins, which is the essence of human body.

Gold nanoparticles represent a potent, versatile, selective and also highly multi-functional technology that seems useful for anti-cancer strategies. In this report we wanted to show many important issues connected with GNP and their conjugates: the synthesis of GNPs and their conjugation, the stability of these particles as well as their application in photothermal therapy or in drug delivery.

(F34) CHANGES IN CELLULAR SIGNALING AFTER HEAT STRESS IN MCF7 BREAST CANCER CELLS

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The cellular response to hyperthermia includes the transcriptional HSF1-dependent activation of genes encoding heat shock proteins (HSPs) as a part of an internal repair mechanism which results in the activation/deactivation of several cascade pathways. NF-κB - dependent signaling is one of the signaling pathways which is altered by heat stress. Heat shocked cells do not exhibit typical NF-κB induction after cytokine (e.g. TNFα) stimulation.

To investigate the role of HSF1 and dependent on it HSPs in NF-κB signaling following heat shock we constructed MCF7 cells with a downregulated expression of HSF1 (by specific shRNA). We monitored the kinetics of TNFα-induced NF-κB activation by measuring of the p65 (the most common NF-κB subunit) phosphorylation level on Ser536. Cells with a silenced HSF1 responded to TNFα in a similar way that cells with a normal level of HSF1 protein. Also heat shock applied directly before TNFα led to a similar time delay and inhibition of the p65 phosphorylation in both types of cells. Then we asked if thermotolerance, which was acquired due to HSF1-dependent HSPs accumulation one day after pre-exposure to elevated temperatures, could change NF-κB signaling. Thermotolerance acquisition in cells with normal level of HSF1 partially rescue NF-κB signaling after heat shock applied directly before TNFα. This effect was not so evident in cells with a silenced HSF1. It needs further studies on cells with completely removed HSF1.

Activation/deactivation of cascade pathways strongly depends on kinases activity. Thus, we used Human Phospho-Kinase Arrays to investigate the influence of heat shock and TNFα treatment on the intracellular kinases profile. Heat shock resulted in the enhanced phosphorylation of p38α, ERK1/2, JNK1/2/3, AKT1 S473, and CREB in MCF7 breast cancer cells. We also observed a slightly weaker phosphorylation of p53, RSK, and PYK2. TNFα treatment resulted in similar changes in kinases phosphorylation (although slightly weaker). The only difference was lack of AKT1 S473 phosphorylation. Heat shock pretreatment had no additional influence on the kinase phosphorylation pattern.

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(F19) STUDY OF TREE-DIMENSIONAL HYBRID MODEL OF SOLID TUMOUR GROWTH AND SPROUTING ANGIOGENESIS

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During tumour growth, the increase of tumour mass is accompanied by increased demands of oxygen and nutrients. These components are supplied by a network of blood vessels. Nevertheless, for tumour cells exhibiting fast metabolism the existing vasculature often becomes insufficient. In this situation, in areas with low oxygen levels hypoxic regions appear. Further growth of the tumour depends on expansion of the blood vessel network. Hypoxic cells secrete factors stimulating vasculature for growth. Angiogenesis is a physiological process of growth of new vessels from pre-existing vasculature. Very often, angiogenesis occurs in pathological conditions associated with cancer. One of the main angiogenic factors present in this process is vascular endothelial growth factor (VEGF).

The aim of this study was to develop the model that would allow three-dimensional simulations of solid tumour growth and pathological angiogenesis driven by a tumour. Developed model has a hybrid structure and can be divided into continuous and discrete parts. In order to model the dynamics of growing tumour continuous multiphase theory was applied. The model distinguishes tumour and normal cells of three types: proliferating, quiescent and necrotic. This part of the model is complemented by reaction-diffusion equations for oxygen and vascular endothelial growth factor. The process of angiogenesis is modelled by discrete model. It enables modelling growth and sprouting of the new vessels, creation of functional vessel loops and simulation of the blood flow. Vascular network adapts to the situation during tumour development by sprouting of new vessels, pruning inactive vessels and changes in vessel diameter.

The developed model captures the behaviour of tumour growth and angiogenesis. It may be used to analyse different aspects of tumour progression processes, like perfusion and oxygenation of the tissue. A number of simulations were performed with different parameters in order to show, on one hand, their influence on angiogenesis and tumour growth, and on the other hand, to find ranges corresponding to reality.

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(S12) THE INFLUENCE OF DNMTI ON THE *DIRAS3* ACTIVITY IN OVARIAN CANCER CELLS

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The basis for the initiation of carcinogenesis is the coexistence of multiple mutations in oncogenes and tumor suppressor genes. During the carcinogenesis changes in epigenotype are also observed, what among others results from abnormalities in DNA methylation within tumor cells. DNA methylation plays a significant role in genes expression control and as a result aberrant hypermethylation of tumor suppressor genes is commonly associated with the development of cancer. DNA methylation is mediated by DNA methyltransferases (DNMTs), and it has been established that the inhibition of DNA methyltransferase activity can strongly inhibit the tumors formation.

The aim of this study was to evaluate the influence of the DNMTi (DNA methyltransferases inhibitor) – 5-aza-2'-deoxycytidine (in concentrations between 50 μM – 0,5 μM) for the *DIRAS3* and *TBP* methylation and expression level. *DIRAS3* – DIRAS Family, GTP-Binding RAS-Like 3, is a putative tumor suppressor gene whose function is abrogated in ovarian and breast cancers, and *TBP* – TATA Box Binding Protein, is a housekeeping gene, and has been chosen as a control gene in our studies. Tests were performed on ovarian adenocarcinoma cell line – A2780. Cells were treated with DNMTi for 72 hours and 7 days respectively. The methylation of the *DIRAS3* and *TBP* promoter region was determined by MS-PCR (Methylation-specific PCR) technique, after the bisulfite DNA conversion. Quantitation of gene expression *DIRAS3* and *TBP* in the A2780 cell line treated with 5-aza-2'-deoxycytidine was estimated by Real-time™ PCR.

The results confirmed the partial methylation of the *DIRAS3* gene in the tumor control cells. In cells treated with 5-aza-2'-deoxycytidine relative balance between both methylated and unmethylated variants was shifted towards the unmethylated one. The level of *DIRAS3* gene expression in A2780 cell line after treatment with 5-aza-2'-deoxycytidine was higher compared to the expression level of the gene in control cells. The greatest difference in *DIRAS3* gene expression relative to control cultures was observed for cells treated with 50 μM solution of 5-aza-2'-deoxycytidine for 7 days ($p = 0.000174$) and the lowest was found for cells treated with the concentration of 0,5 μM 5- aza-2'-deoxycytidine for 72 hours ($p = 0.030575$).

Our studies have confirmed the effectiveness of 5-aza-2'-deoxycytidine as a DNA methyltransferases inhibitor, which resulted in increasing the level of expression and in changing the methylation level of the studied gene.

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(F37) MCPIP1 OVEREXPRESSION DOWNREGULATES CHOLINE UPTAKE AND POSSIBLY INDUCES DIFFERENTIATION IN NEUROBLASTOMA CELLS

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MCPIP1 is a recently discovered multidomain protein, that has been described as a ribonuclease and a member of a deubiquitinase complex. Its major function is the downregulation of inflammation due to degradation of proinflammatory cytokines transcripts and deubiquitination of TRAF proteins. However its role in cancer is yet to be fully recognized. Temporary overexpression of MCPIP1 protein in BE(2)-C neuroblastoma cell line, obtained through transfection of plasmid constructs bearing *MCPIP1wt* or a mutant form lacking the RNase domain (*MCPIP1ΔPIN*), caused a potent decrease in proliferation and cellular ATP content.

We have used expression microarrays to further investigate the role of MCPIP1 in neuroblastoma. We examined the changes of expression of three solute carrier family genes. We found significant downregulation of *SLC44A1* gene, which encodes membrane choline transporter and its protein product as assessed by western blot. We also performed functional analyses, by measurement of [^{3}H]-choline uptake, which confirmed the decrease in choline transport through cell membrane in neuroblastoma cells with MCPIP1 overexpression as compared to control. The downregulation of choline uptake is often associated with inhibition of proliferation, cell cycle arrest and differentiation. In order to investigate whether MCPIP1 overexpression could lead to differentiation of neuroblastoma cells we measured the changes in expression levels of genes belonging to inhibitor of differentiation family: *ID1, ID2* and *ID3* and some of their downstream genes. We also checked expression levels of the nerve growth factor receptor *TRKA*, which promotes cell cycle arrest and differentiation, and *BDNF*, a member of neurothrophin family that promotes proliferation of neural cells. We found the decrease of *ID1* gene as well as upregulation of expression of its downstream gene - *P27*, while no significant changes in expression of *TRKA* and *BDNF* were observed. Expression level of investigated genes was checked by RT-qPCR.

Our data suggest that MCPIP1 overexpression in neuroblastoma results in cell cycle arrest and differentiation via intracellular pathways.

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(S41) EFFECT OF SELECTED POLYPHENOLS, GALLIC ACID AND EPIGALLOCATECHIN GALLATE, ON P-GLYCOPROTEIN LEVEL IN SENSITIVE AND MULTIDRUG RESISTANT MCF7 BREAST CANCER CELLS

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Multidrug resistance (MDR) constitutes the major problem in cancer chemotherapy. Tumour cells become resistant to a wide array of chemotherapeutic agents structurally diverse and having different mechanisms of action. The occurrence of MDR is conferred by multiple mechanisms, especially it is associated with the overexpression of membrane transporters (e.g. P-glycoprotein, P-gp; MRP1; BCRP/MXR1) responsible for the active ATP-dependent efflux of drugs.

It is proposed that several signal transduction pathways (e.g. NF-κB, HIF-1, PI3K/Akt and p53 pathways) play a crucial role in the regulation of *mdr1* gene encoding P-gp. Recently, increasing interest of many investigators has been focused on the use of dietary polyphenols in cancer prevention and chemotherapy of multidrug resistant tumours. They interrupt cellular signalling, mainly NF-κB and AP1 pathways, by scavenging reactive oxygen species (ROS) responsible for their activation.

In our laboratory we obtained two human breast adenocarcinoma sublines, MCF7/DOX_{200} and MCF7/DOX_{500}, selected from multidrug resistance subline MCF7/DOX with the use of 200 nM and 500 nM doxorubicin (DOX), respectively. They are characterized by an important increase in P-gp level. The aim of this study was to investigate the effect of selected polyphenols: gallic acid (GA) and epigallocatechin gallate (EGCG) on the level of this drug transporter.

The cellular level of P-gp was determined by direct immunofluorescence assays with the use of anti-P-gp antibodies conjugated with phycoerythrin (PE) as a fluorophore. They were carried out with the aid of BD FACSCalibur flow cytometer. We have found that GA and EGCG used at non-toxic concentration (120 μM and 20 μM for GA and EGCG, respectively) did not significantly change the level of P-gp in resistant MCF7/DOX_{200} and MCF7/DOX_{500} cells during 24 h- and 72 h-incubation.

Further studies are needed to investigate the effect of GA and EGCG on the expression of P-gp in MDR cells using higher concentrations and prolonged times of incubations. It will be also interesting to study the effect of other dietary polyphenols on the expression of drug transporters in MDR tumour cells.

Acknowledgements: This study was supported by the Faculty of Biology, University of Szczecin, Poland

(F21) THE EFFECT OF BIOLOGICAL SWITCHES ON CELL RESPONSE

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Cellular processes base on biochemical reactions between organic macromolecules such as carbs, proteins, lipids and nucleic acids and many different non-oragnic molecules and ions. The constant rate of the reactions in cells similarly to any chemical reactions depends on physical and chemical conditions such as temperature or pH. The well-known example of biological switch occurs in every cell is change of gene state which influences the mRNA production rate. Activation or deactivation of the allels in the chromosomes results in significant alterations in mRNA levels and subsequently proteins levels and the total cell response. The other example is cells growth. Gradual volume and size increase causes changes in intracellular reactions conditions. Mathematical modelling of biological processes requires strict determination of the model parameters, which can be estimated or calculated using biological experiments results. In our work we focused on influences of the changes in parameters values on the model response. We were interested how small parameters switches affected the nominal state of the model. The example model responds to the hypothetical therapy aiming to decrease proteins levels in abnormal or infected cell. We examined how time of the parameter switch and quantity of the change influence on the cell response and the cells ability to reach therapeutical aim.

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(S24) DETERMINATION OF GENISTEIN DERIVATIVES PERMEABILITY ACROSS CACO-2 MONOLAYERS AND PAMPA SYSTEM

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Caco-2 cell line and PAMPA (parallel artificial membrane permeability assay) are *in vitro* models used for studying drug transport and absorption. Caco-2 model is used to determine active transport, efflux, and metabolism of compounds in the cell monolayer, while PAPMA method is used to evaluate passive transport across artificial phospholipid membrane [1].

In this study we determined the relationship between the structure of genistein derivatives (structure of the linker between the sugar moiety and genistein, and position of phenol group substitution) and their transport across Caco-2 monolayer and artificial membranes. To determine transport of tested compounds across monolayers Caco-2 cells were cultured in 24-well plates with porous membranes (Millicell PCF, 0.4 μm pore size). First, the cells were pre-incubated with a Hanks' Balanced Salt solution containing calcium and magnesium (HBSS) at 37ºC for 30 min, then the buffer was replaced with the proper solutions of genistein or its derivatives (25 μM). Compounds were added either to the apical (transport AB) or to the basolateral (transport B-A) compartment. Fresh HBSS without analyzed compounds was added to basolateral (transport A-B) or apical (transport B-A) compartment in a well serving as a blank sample. The samples were collected from basolateral (transport A-B) or apical (transport B-A) chambers after 1, 2, 4, 8 and 24 h incubation.

To determine transport of these compounds across phospholipid membrane we used PAMPA system. PAMPA plates (96-well Corning® Gentest™ Pre-coated PAMPA Plate System) were warmed for 60 min at room temperature. Next, the compound solutions in phosphate buffer saline were added to the wells of the receiver plate, while phosphate buffer saline to the wells of the pre-coated filter plate. Then, the filter plate was coupled with the receiver plate and was incubated at room temperature for 5 h.

The concentration of genistein derivatives in medium collected from apical and basolateral chambers was determined using a Dionex UHPLC system connected to a 4000 Q TRAP triple quadrupole linear ion trap mass spectrometer.

Our result indicate that both structure of the linker between the sugar moiety and genistein, and position of genistein substitution are important determinants of transport of the compounds across monolayers and membranes. The derivatives containing sugar moiety connected with genistein via *C*-glycosidic bond were not transported either through Caco-2 layer or PAMPA membrane. In contrast, the compounds containing *O*-glycosidic bonds, instead of *C*-glycosidic bond, were transported only across PAMPA membrane, but their permeability coefficient was lower or similar to the permeability of genistein. Derivatives substituted only with an alkyl chain were transported through both Caco-2 monolayer and PAMPA system with high permeability coefficient, even higher than genistein.

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(S23) CONJUGATES OF URIDINE AND ARYL 1-THIOGLYCOSIDES: EVALUATION OF THE IMPACT OF STRUCTURAL ELEMENTS ON THEIR BIOLOGICAL ACTIVITY TOWARDS B-1,4-GALACTOSYLTRANSFERASE

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Glycosyltransferases (GTs) constitute a large group of enzymes that are responsible for the formation of the glycosidic bond in living system [1]. These enzymes received the most attention because they are responsible for the synthesis of glycoconjugates that play major role in recognition or signaling events, cell adhesion, cell differentiation, glycoprotein folding, targeting organelles and bacterial/viral infections [2]. For this reason GTs are targets for the development of method for control their activity. Selective GTs inhibitors can provide the control of glycosylation. There are different types of inhibitors based on their structural similarity to natural GTs substrates. Some of them are sugar donor analogues (analogues of NDP-sugar). 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 would like to present a wide range of synthesized uridine glycoconjugates in which 1-thioglycosides derivatives of D-glucose and D-galactose are connected to uridine or uracil through the various linkers creating an amide bond. These compounds have been subjected to the biological evaluations of their inhibitory activity against commercially available *β*-1,4-galactosyltransferase (β-1,4-GalT) from bovine milk. To evaluate the activity of tested compounds concentrations of substrate and product of enzymatic reaction in the reaction mixtures was determined by RP-HPLC method.

The results indicate that activity against β-1,4-GalT affected by the type and configuration at the anomeric center of attached sugar, type and length of the linker and the presence of the protecting groups in the sugar moiety. It was observed that the absence of ribose ring in uridine part is not necessary for inhibitory activity.

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(F57) GAME-THEORETICAL IMAGE SEGMENTATION ALGORITHM FOR NUCLEAR MEDICINE IMAGES

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Image segmentation is often used in medical image processing. This procedure is not trivial and can influence all results obtained from the next steps of image analysis. Many image segmentation methods were proposed. We present one of them which uses the notion of evolutionary games theory. Segmentation is often used for delineation of the region of interest (for example tumour), especially in nuclear medicine imaging.

In this work, we tested previously implemented [1] and adapted method, on nuclear medicine images. Data has been obtained with Philips Gemini or Siemens Biograph hybrid PET/CT device at Department of PET Diagnostics, Maria Sklodowska-Curie Memorial Cancer and Institute of Oncology, Gliwice Branch. Phantom objects were used to evaluate the potential of proposed algorithm. One of the phantoms was a standard cylinder with five spheres of different radius and volume (diameter from 1.3 cm to 4 cm) filled with F-18 with constant concentration.

For segmentation purpose, an algorithm, introduced by M. Pellilo [2-4] was implemented and tested. In this algorithm, the image is represented as an edge-weighted graph, where vertices correspond to individual pixels and their weights reflect similarity between them. The clustering algorithm uses discrete replicator dynamics equations. Pixels are allocated to the cluster that is removed from the graph. Described process is repeated until all pixels are assigned to corresponding clusters. Results of this algorithm were compared with other methods used in nuclear medicine imaging (threshold based: absolute SUV 2.5 threshold, fixed and adaptive threshold range of 41-70% of maximum value and contrast-oriented method). As a reference mask, CT image of the phantom was used. The final comparison of each segmentation method was done using the Jaccard index.

The algorithm gives satisfactory results, comparing with other methods and determines the number of clusters in the image. However, it is a graph-based method and the size of analysed images could be problematic. For bigger images inefficient graph representation causes insufficient memory problem and requires a large amount of computer memory to perform calculations.

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(S15) IL-6 – A DOMINANT CYTOKINE RELEASED BY MESENCHYMAL STROMAL CELLS ISOLATED FROM ADIPOSE TISSUE

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Mesenchymal stromal cells (MSCs), isolated from many sources, are plastic-adherent cells with multipotent differentiation capacity. MSCs display immunological properties, although the exact mechanisms underlying these effects remain largely unknown. Existing data suggest that MSC cells may be involved in two processes which can be used for therapeutic purposes:
- they inhibit inflammatory response through secreted inflammatory factors;
- they can stimulate regeneration of damaged tissues and organs through secreted cytokines and growth factors.

Interleukin 6 (IL-6) is now regarded as a prominent target for clinical intervention. IL-6 is a proangiogenic cytokine, which has a broad effect on cells of the immune system. This cytokine has context-dependent pro- and anti-inflammatory properties.

Adipose tissue is a readily accessible good source of mesenchymal stromal cells. The amount of the MSC in the adipose tissue reaches about 1% while in the bone marrow it is only 0.001-0.002%. Adipose-Derived Stromal Cells (ADSC) due to availability of material, simplicity of isolation and tremendous therapeutic potential (eg immunomodulating properties) raise great hopes for the use in regenerative medicine.

The aim of the project was to optimize the isolation methods and characteristics of human Adipose-Derived Stromal Cells (hADSC) from fragments of subcutaneous adipose tissue. To obtain the desired cell population we digested adipose tissue with collagenase solution. The characteristics of cells *in vitro* were investigated by monitoring cell morphology with a light microscope. Cell phenotype was verified by flow cytometry. The established cell line has the following phenotype: $CD105^+$ / $CD90^+$ / $CD73^+$ / $CD44^+$ / $CD29^+$ / $CD146^{+/-}$ and $CD45^-$ / $CD34^-$ / $HLA\text{-}DR^-$ / KDR^- / lin^- / $CD31^-$.

To identify the main cytokines secreted by hADSC Human Cytokine Antibody Array C5 was used. For evaluation of the amount of secreted IL-6 by the cells ELISA assay was used. hADSCs were plated at high density in DMEM medium without serum. After 2, 4 and 8h the medium was analyzed. In this medium the levels of IL-6 secreted Human IL-6 ELISA Read by MSCs were determined by ELISA. The amounts of secreted IL-6 were calculated for 1mg of total protein content. After 48h the medium was analyzed using Human Cytokine Antibody Array C5 Kit. Densitometric analysis indicates that MSC secrete mainly IL-6.

(S47) BALCONY – BETTER ALIGNMENT CONSENSUS ANALYSIS

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Protein engineering can substantially benefit from computer aided rational design methods. Identification of amino acids essential for the intrinsic or engineered activity of the enzyme is one of many possible preliminary steps of the enzyme functionality improvement. There are number of approaches aiming at identification of amino acids of this kind. Following the hypothesis that functionally important amino acids are relatively highly conserved, conservativeness analysis seems to be the best suited method for this purpose.

Multiple alignment coupled with computation of a consensus sequence are the most straightforward methods to estimate conservativeness of amino acids within a group of predefined proteins. However, results of such an approach strongly depend on input parameters and both alignment and consensus computation influence eventual results. In the presented work we have focused our attention on consensus calculation only.

Hence, BALCONY, a tool facilitating visualization, analysis, and computation of consensus sequences was created. For a set of aligned proteins it computes series of consensus sequences by varying input parameters. Accuracy of each of consensus sequences is calculated for all analyzed proteins. This results in a collection of histograms. Inspection of these histograms allows selection of the most reliable consensus. Moreover, detection of outlying sequences can be easily done by removing proteins placed far at the head or tail of the histogram. Additionally, our tool can also estimate variations of amino acids at given position of the consensus.

Ultimately, BALCONY correlates amino acids on certain positions with their function (described in FASTA-like text file for example) and consensus sequences. This functionality allows for a quick insight into amino acids functions within a group of analyzed proteins. This can substantially facilitate deliberated protein manipulations during the protein engineering processes.

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(F31) EPIGENETIC REGULATION OF *STAT3* GENE ACTIVITY IN HUMAN BREAST AND OVARIAN CANCER CELL LINES

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The epigenetic alterations, such as DNA methylation, can play an important role of regulation gene expression in various cancer cells. DNA methylation, a well-known epigenetic change, occurs in CpG islands, are often located within promoter regions of genes. Methylation and hypermethylation are also known to inactivate of tumor suppressor genes in cancer cell lines, but hypomethylation or methylation failed usually could be associated with activation of proto-oncogenes expression. STAT3 is a latent cytoplasmic transcription factor. Activated STAT3 is oncogenic, antiapoptotic, proinflammatory, proangiogenesis and promigration.

The aim of the study was to search for correlation between the *STAT3* gene expression and methylation pattern in the promoter region of *STAT3* gene in cancer cell lines.

Analyses of the expression and methylation *STAT3* gene were carried out on two different human cell lines: MCF7 (human breast adenocarcinoma cell line) and TOV-21G (human ovarian adenocarcinoma cell line). Cancer cells were cultured in standard conditions (37°C, 5% CO_2, complete growth medium: MCF7 - RPMI-1640 medium supplemented with 10% FBS and 10µg/ml gentamycin, TOV-21G - mixture of MCDB 105 medium and Medium 199 (1:1) supplemented with 15% FBS and 10µg/ml gentamycin). DNA and RNA was isolated from examined cell lines by using ZR-Duet™ DNA/RNA MiniPrep (Zymo Research). The methylation of bisulphite-modified DNA was amplified by Methylation-Specific PCR (MSP-PCR). The changes in methylation level of *STAT3* were estimated by Q-MSP (Quantitative Methylation-Specific PCR) and the expression level of analyzed gene was determined by Real-Time™ RT-PCR.

Methylation-specific PCR and Q-MSP revealed changes in the methylation pattern of *STAT3*. Both methods confirmed demethylation of the promoter region (681-926pz) *STAT3*. Real-Time™ RT-PCR revealed changes in the expression of analyzed gene. The results show positive correlation between *STAT3* gene expression and methylation in the promoter region of tested cancer cell lines. Moreover, the results of our study suggest that the observed correlation may be an important mechanism for regulation of analyzed gene expression in cancer.

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(F51) AUTOMATIC CELL DETECTOR: A MICROSCOPE SOFTWARE MODULE FOR AUTOMATIC ACQUISITION, OBJECT DETECTION AND ANALYSIS

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The acquisition of a series of microscopic experiment images is highly time-consuming and in most cases requires user supervision. A variety of solutions supporting automatic collection is commercially available, although this process is mostly realized by taking pictures without considering the presence of observed objects. This leads to a significant increase in required time as well as memory. A lot of inconvenience is also caused by analysis of the data which is not relevant.

The automation of data analysis plays nowadays a key role in scientific research upgrading. Accurate processing of microscope image data is a particularly difficult domain of automation, despite the fact that a supporting system of automated image acquisition and analysis has been created. Our program is especially directed to biologists working on microscopic research of human cell structures and processes that are taking place inside them. It is worth to point out that in contrast to commercial solutions, our program is based on Micro-Manager, a free and open-source software package for control of automated microscopes which can be used on different brands of microscopes.

Acquisition and evaluation of an image for further biological analysis, segmentation of nuclear areas, and parameter analysis are the capabilities of the programme. The system is fully automated and does not require user supervision. Its viability has been tested on a human cells, particularly *HCT116 human colon cancer cells which are especially difficult to automatically segment due to their clustering tendency.*

At the data acquisition stage the images obtained by a brightfield technique are evaluated in the first instance. The method is based upon calculation of the eigenvalues of a structure tensor of an input image and binarization by the method based upon an IsoData algorithm. In a confirmatory analysis, Laplacian's method of edge detection has been used to process images of DAPI-stained nuclei. Only when suitability for further biological analysis is confirmed, the acquired images are saved on disk.

The segmentation process has been focused mainly upon correcting the areas of touching nuclei using an adaptive local thresholding with a Phansalkar method and with a watershed method.

The system is working in a fully automated way starting from the moment of acquisition to the final segmented areas analysis. The software allows to acquire images with various microscope settings, accordingly to user preferences. Such parameters as mean grey value, area or position coordinates can be calculated during a final analysis of segmented areas for all images obtained during the process.

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(S22) SYNTHESIS AND CHARACTERIZATION OF NOVEL THIOSEMICARBAZONES BASED ON 2-NITROBENZALDEHYDE

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Thiosemicarbazones (TSC) are an important class of organic compound of great pharmaceutical value. They exhibit anticancer, antibacterial and antifungal activities. Some of them could be used as antitubercular drug and for the treatment of malaria. In the structures of TSC there are sulfur and nitrogen donor atoms used by chelation of metal - especially transition metal ions. The presence of multiple donor atoms within the same ligand multiplies coordination modes and affects the properties of ligand and complexes.

Considering all of the biological properties of thiosemicarbazones, it is important to be able to synthesize new series of of TSC which shows biological activities without any side effects [1,2].

As a result, ten thiosemicarbazides were prepared using a reflux method (2h in ethanol). Ten thiosemicarbazones were synthesized using a microwave-assisted methodology; eight of them are novel compounds.

The obtained thiosemicarbazides were used in further synthesis with 2-nitrobenzaldehyde. The reaction mixtures were irradiated in a scientific microwave reactor at 83˚C for 20 min at 50W, when ethanol was used as a solvent and 2 drops of acetic acid as a catalyst.

This method permits to obtain products in high-purity and satisfactory yields in a short time. The thiosemicarbazides and thiosemicarbazones were fully characterized by ^{1}H- and ^{13}C-NMR spectroscopy, TSC were characterized also by HMQC and COSY spectroscopic method. The structures of the received thiosemicarbazones were confirmed by using Mass Spectrometry and the purity were confirmed by using TLC and HPLC technique.

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(F9) COPY NUMBER VARIATION ANALYSIS AS A METHOD FOR FINDING OUT BIOMARKERS OF RADIOSENSITIVITY

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Radiosensitivity is defined as a relative susceptibility of cells, tissues, organs or organisms to the harmful effect of ionizing radiation. Different level of radiosensitivity is the main reason for individual differences in effects of radiotherapy and its side effects among patients. This is a particularly important issue in process of cancer radiotherapy personalization. Some studies show that individual resistance to environmental factors can be caused by the existence of copy number variations which are the alterations of the DNA that results in the cell having an abnormal variation in the number of copies of one or more sections of the DNA (longer than 1kbp).

The aim of the study was to develop the signal analysis pipeline for analyzing changes in genome structure depending on ionizing radiation dose. The goal was also to search for differences in radiation effect between cell lines with different radiosensitivity level.

Affymetrix CytoScan HD microarrays were used for genome modification detection. Two cell lines of human fibroblasts: CCF71 classified as normal responders to radiation and CCT39 classified as radiosensitive (derived from cancer tissue) were studied. Each cell line was irradiated with 0.5Gy, 1Gy, 2Gy, 3Gy, and 4Gy acute doses. Additionally, control samples with no irradiation were included to the study as reference signals. The signal log2 ratios (SLR) referenced to array internal standard and cell line control level were calculated. Their distributions were approximated by Gaussian mixture model and the component representing “no significant response to irradiation” was used in one-versus-sample t-test. The obtained p-values, corrected for multiple testing, allowed for selection of significant CNVs. Depending on the SLR value, every significant CNV was classified as deletion or amplification. The functional analysis of genes linked to the selected sequences was performed.

Our study confirmed different response to irradiation between analyzed cell lines. The cell line responses differ in number of significant CNVs (both deletions and amplifications) in general and per doses of irradiation. We indicated significant CNVs characteristic for chosen irradiation dose. We searched for significant CNVs located within coding sequences and we performed a functional analysis of genes being modified.

The performed study proved that it is possible to indicate sequences in human genome where genomic changes are more likely to occur after absorption of the radiation dose. This sequences, in particularly within coding sequences, are important for functionality and regulation of processes in cells. Significant CNVs, specific for no irradiation experimental condition, indicate the differences in genome structure between analyzed radiosensitive and regular cell lines.

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(S34) EPOTHILONE A IN COMBINATION WITH THE ANTIDIABETIC DRUGS METFORMIN AND SITAGLIPTIN: THE ROLE OF TRANSCRIPTIONAL FACTORS NF-κB AND P53

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Type 2 diabetes mellitus patients are at increased risk of many forms of malignancies, especially of the pancreas, colon and hepatocellular cancer. Unfortunately, little is known of the possible interaction between antidiabetic drugs and anticancer agents. The present study investigates the influence of metformin (MET) and sitagliptin (SITA) on the *in vitro* anticancer activity of the microtubule depolymerization inhibitor agent epothilone A (EpoA). Hepatocellular liver carcinoma cell line (HepG2) apoptosis was determined by double staining with PO-PRO-1 and 7-aminoactinomycin D, respectively after treatment with EpoA, metformin or sitagliptin. The levels of nuclear factor (NF)-κB and p53 were evaluated in the presence and absence of inhibitors.

EpoA and SITA induced higher p53 levels than MET. All tested drugs increased the level of NF-κB. Only MET enhanced the proapoptotic effect of EpoA. The EpoA+MET combination led to apoptosis independent of p53, decreasing the level of NF-κB.

These findings support the link between NF-κB and p53 in the modulation of apoptotic effect in HepG2 cells treated by EpoA. The therapeutic advantages of the combination of EpoA with MET may be valuable in the treatment of patients with diabetes mellitus type 2 (T2DM) and liver cancer.

(F25) COSTS OF HEALTHCARE FOR PATIENTS UNDER 14 YEARS OLD DIAGNOSED WITH TYPE 1 DM IN UPPER SILESIA REGION – PRELIMINARY RESULTS

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Diabetes mellitus (DM) is chronic metabolism illness, in which the most characteristic symptom is a high blood glucose level. There are two main types of DM: type 1 that results from pancreas' failure to produce enough insulin, and type 2 that begins with insulin resistance, where cells fail to respond to insulin properly. Prevalence in Poland is around 5% of population, where over 90% of all cases is DM Type 2, whereas rest is Type 1.

Illness like diabetes mellitus is not only a problem of patients but also their families and government of country where they live. It is estimated that over 5% of total finances of Health Department is earmarked for treatment of different types of diabetes. Patient's health condition is the result of treatment, when he is diagnosed correctly he would be able to work and earn money for himself. In other hand, if money for diagnosis were saved, there would be necessary to pay more for treating complication of DM. It could lead patient to become unable to work, who would require to get financial support from the government or medical insurance. The problem of DM is very serious, which touch all society directly or indirectly. Our project is dedicated to better understanding an epidemiology of DM within the Upper Silesia region and costs related to the long-life treatment. The research is conducted in cooperation with National Heath Fund - Silesia Branch. The data about patients suffering from different types of metabolic diseases in years 2008-2013 in Upper Silesia was analyzed.

We studied the costs of medical services and drugs prescribed to the patients under 14 years old diagnosed with type 1 DM. Data included: costs of services provided during the control visits in outpatient clinic, outpatient treatment, hospitalizations and insulin pumps. The costs of blood glucose test strips were also included. Costs of prescribed drugs were split into two parts: refund and patient-covered costs.

During the first months after diagnosis total costs of type 1 DM treatment are much higher than the treatment costs during the second and later years. The average monthly total costs of treatment of DM Type 1 remain at the same level independently of year of observation. In general, each year the treatment costs are about 0.05% of total medical coverage by National Health Found. It was noticed that the ratio between the costs refunded by National Health Fund and covered by patient himself decreases with years.

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(S30) GAS TRANSPORT PROPERTIES OF MODIFIED MAGNETIC POLY(2,6-DIMETHYL-1,4-PHENYLENE OXIDE) MEMBRANES

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Development and characterization of modified polymers for gas separation is an important factor for the future of membrane processes used in various medical and industrial applications (air separation, hydrogen recovery and CO_2 removal).

The major issue of current membrane research is developing highly permeable and selective membrane materials, that also show sufficient resistance. For this purpose, the polymers can be modified by various substitutions, insertion of the metal cations, production of copolymers or carbon membranes. The next strategy for improving the mass transport through polymer films is the incorporation of inorganic materials (zeolites, carbon molecular sieves, silica nanoparticles, carbon nanotubes, metal organic framework and clay layered silicates) into a polymer matrix [1, 2].

This work is the continuation of our earlier research [3-6], that concentrated on magnetic membranes used for the air enrichment in oxygen. We have found that incorporation of magnetic micropowders into the polymer matrix improved the gas transport properties of membranes. We have examined the gas separation properties of homogeneous HSPPO, NaSPPO and FeSPPO membranes and the magnetic hybrid inorganic-organic membranes (with dispersed magnetic powders, like MQP-14-12, MQP-16-7 and MQP-B with various granulations) based on these modified polymer matrices. The results showed that the sulfonation of PPO causes the increase in a separation coefficient and decrease in O_2 and N_2 permeabilities. The sulfonated polymers modified further by substitution with Na and Fe cations were more permeable to gases than HSPPO and showed no change or small increase in ideal selectivity. In turn, the magnetic hybrid membranes with modified matrices (HSPPO, NaSPPO and FeSPPO) were characterized by higher gas permeability and diffusivity, while their permselectivity and solubility were rather maintained or slightly increased. It was found that the magnetic membrane's gas transport properties were improved with the increase of magnetic neodymium particle filling and decrease in powder particle size. The most improved gas transport properties had the magnetic hybrid membrane based on FeSPPO matrix.

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(F2) ROLE OF AUTOPHAGY IN SURVIVAL OF EMBRYONAL CARCINOMA CELLS TREATED WITH ETOPOSIDE

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Although autophagy is considered a hallmark of cellular senescence, it was shown to be important in tumour maintenance and resistance to therapy. The chromatin macroautophagy in the cultures of human tumour cells after DNA damage has been described previously however the biological significance of this phenomenon remains unclear [1]. Here we studied its role for the balance between self-renewal and senescence in TP53 functional PA1 embryonal carcinoma cells after etoposide (ETO) treatment. Induced autophagy was monitored by markers - LC3B, p62/SQSTM1, and LAMP2. ETO induced upregulation of autophagic flux in about 70% of cells, while 30% displayed signs of the autophagy exhaustion. We found often extrusion and accumulation of the damaged gH2AX/CHK2/DAPI positive DNA in perinuclear autophagic vacuoles. Serum starvation induced abundant release of self-renewal transcription factor OCT4A from ETO-treated cells accompanied by increase of the senescence marker p21cip1, and delay of clonogenic recovery. On the other hand, p16ink4a, the inducer of terminal senescence, underwent autophagic sequestration in the cytoplasm of ETO-treated cells, allowing suppression of senescence. Inhibition of autophagy with Bafilomycin A1 caused accumulation of p16ink4a in cell nucleus, nuclear disintegration, and loss of cell recovery [2]. These data indicate the importance of autophagy for sorting of the damaged DNA and support of the balance between self-renewal and senescence regulators, on behalf of cell survival.

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(S16) THE PRESENCE OF PORCINE ENDOGENOUS RETROVIRUSES IN LESCS IN ASPECT OF XENOTRANSPLANTATION SAFETY

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Cornea disorders leads to blindness or vision dysfunction. Development of regenerative medicine brings hope for therapy of corneal damage but there is still no treatment for bilateral limbal stem cells deficiency. The lack of corneal allografts for transplantation inclines to look for alternative solutions. Porcine corneal cells might be the best choice. Recent studies showed that xenotransplantation of porcine cornea may be applied. The Gal antigens which are responsible for induction of graft versus host reaction is low or totally absent in porcine cornea. Another serious problem are PERVs (porcine endogenous retroviruses) which are present in porcine tissues.

Limbal epithelial stem cells are insulated from corneal limbus. Those cells are present at the edge of cornea, and create a thin layer between cornea and the sclera of eye. LESCs are important for continuous reconstruction of cornea. They are responsible for Its proper efficiency. Implantation of those cells to the eyes with limbal stem cells deficiency may improve the eye functions. In aspect of xenotransplantation safety, we have to find out if there in LESCs insulated form porcine cornea, the PERVs are present.

In this study we used the quantitative reverse transcriptase polymerase chain reaction (Q-RT-PCR) to diagnose limbal epithelial stem cells against the presence of porcine endogenous retroviruses in three variants: PERV A, PERV B, PERV C.

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(F13) COMPARISON OF TRANSCRIPTIONAL ACTIVITY MARKERS OF ADSC FROM DIFFERENT SOURCES

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Mesenchymal stem cells (MSC) are multipotent cells which at appropriate conditions they have pluripotent properties. Sources of MSC are bone marrow, umbilical cord tissue and blood and primarily adipose tissue. Adipose tissue donation is the easiest and not invasive for patients. It is proved that MSC have ability to differentiate to adipocytes, chondrocytes and osteoblasts. They also adherence to plastic and have expression cell surface antigens: CD73 (ecto-5′-nucleotidase), CD90 (Thy-1) and CD105 (endoglin).

Unfortunately, properties of stem cells differ, depending on place of donation. Probably also markers gene expression change at adipose stem cells from different sources. Main aim of the study is to verify differences in transcriptional activity of adipose derived stem cells markers. We examined expression of three markers CD73, CD90, CD105. Experiment was carried out on porcine stem cells from orbital fat tissue and subcutaneous fat. Identification of mesenchymal stem cells markers was evaluated with quantitative reverse transcription-PCR (RT-qPCR) analysis.

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(F8) EVOLUTION OF CORE PROMOTER STRUCTURE

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The core promoter is the genomic region immediately surrounding the transcription start site (TSS), playing an essential role in regulation of gene transcription. Studies in yeast, *Drosophila* and mammals have identified a number of core promoter functional elements, including the TATA box, DPE (the downstream promoter element) and Inr (the initiator) [2,3]. However, a comprehensive survey that would allow us to trace the evolution of regulatory elements across animal phyla is lacking.

Nematostella vectensis (starlet sea anemone) is a basal metazoan organism belonging to the Cnidarian phylum which forms a sister group to all bilaterian animals. Thanks to its phylogenetic position and slowly evolving genome it is particularly informative as an out-group, allowing us to infer gains and losses of core promoter features in metazoa. In our study we used full transcript RNA-sequencing data with tagged start sites to identify 9000 promoters positioned -100 to +50 bp around *Nematotella vectensis* TSSs at single nucleotide resolution. We integrated the results of several motif finding tools (including RSAT, PEAKS and meme), to generate a non-redundant set of more than ten motifs with positional overrepresentation in these core promoters. Several of these were canonical elements that have previously observed in other metazoa (e.g. Sp1, YY1, TATA box, NRF-1), but we also identified a motif previously only observed in yeast, as well as several novel motifs. The relative position of canonical motifs were compared to other model species (human, mouse *Drosphila* and yeast), revealing a high degree of similarity. Notably, *Nematostella* core promoters display a greater similarity to mammalian promoters than do *Drosophila*. This mirrors observations made on the level of gene complement and intron structure, and again suggests that *Drosophila* is a quickly evolving lineage with derived characteristics. A few notable differences in *Nematostella* is the position of TATA elements which is found quite far upstream of the TSS, and the apparent low complexity of motif element combinations, perhaps suggesting a simple mode of regulation.

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(S8) ARE VIRALLY MODIFIED CELLS A RELIABLE MODEL FOR STUDYING THE ROLE OF THE *HSPA2* GENE IN RESPONSE OF CANCER CELLS TO CISPLATIN?

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Human HSPA2 is a member of the heat shock protein family HSPA (HSP70), highly expressed in the testis. Recently, it was revealed that HSPA2 is also expressed in various somatic tissues and different types of tumors. Available evidence suggests that HSPA2 can exert a cytoptotective function in cancer cells. In our previous experiments we have found that HSPA2 is highly expressed in non-small cell lung carcinoma (NSCLC) cell lines and high HSPA2 expression in NSCLC tumors correlates with shorter overall survival in patients.

This study was aimed at searching for biological role of HSPA2 in NSCLC cells. In particular, we intended to confirm our preliminary results suggesting that HSPA2 may modulate response of NSCLC cells to cisplatin, an anticancer drug. For this purpose we used RNAi technology to suppress HSPA2 expression in NCI-H1299 and NCI-H358 cells (both cell lines produce endogenous HSPA2 protein at high level). Using retroviral and lentiviral gene transfer of shRNA sequences (sh-3 and sh-4) we established stable cells lines characterized by massive and comparable reduction of the *HSPA2* gene expression. The effect of HSPA2 suppression on cells sensitivity to cisplatin was evaluated by MTS or MTT assays and by propidium iodide exclusion test. We found that in retrovirally transduced cells HSPA2 silencing exerted cell-type dependent effect on cells response to cisplatin. HSPA2 reduction was associated with increased sensitivity of NCI-H358 cells, whereas in NCI-H1299 cells the opposite effect was observed. However, when the *HSPA2* gene expression was suppressed in these cell lines by lentiviral transfer no differences in response of control and HSPA2-depleted cells to cisplatin treatment were observed.

Our current work showed that different types of viral vehicles used for delivery of RNAi-inducing shRNA sequences into NSCLC cells can lead to contradictory results. At present, it is not clear whether retroviral or lentiviral vectors alone could affect cells response to cisplatin. Our results also prevented us from clarifying the potential impact of the *HSPA2* gene on cisplatin sensitivity. This unsolved issues will be explained by further studies cells modified by non-viral methods of DNA delivery or by knock-out of target gene mediated by CRISP-R/CAS9 system.

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(F11) RAREVARIANTVIS: NEW TOOL FOR IDENTIFICATION OF CAUSATIVE VARIANTS IN RARE MONOGENIC DISORDERS USING WHOLE GENOME SEQUENCING DATA

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The search for causative genetic variants in rare diseases with unknown etiology of presumed monogenic inheritance has been boosted by the implementation of whole exome (WES) and whole genome (WGS) sequencing. In many cases, WGS seems to be superior to WES, but the analysis and visualization of the vast amounts of data is demanding.

To aid this challenge, we have developed a new tool – RareVariantVis – for analysis of genome sequence data (including non-coding regions) for both germ line and somatic variants. It visualizes variants along their respective chromosomes, providing information about exact chromosomal position, zygosity (i.e. percentage of variant reads) and frequency, with point-and-click information regarding dbSNP IDs (if relevant), gene association (if relevant) and whether the variant is inherited from the mother, father or both. Rare variants with no dbSNP ID or frequency below a certain user-defined threshold as well as de novo variants can be flagged and visualized in different colors. The RareVariantVis tool accepts vcf files and annotated variant tables. It can be run on a desktop computer.

The tool with documentation is available for download under the following link:http://bioconductor.jp/packages/3.2/bioc/html/RareVariantVis.html. We have tested the usefulness of the RareVariantVis tool in two WGS data sets, namely the Genome in a Bottle Ashkenazim Trio sample (Complete Genomics data) and an in-house collection of 27 WGS samples (Illumina X Ten data) obtained from 9 families with rare inherited disorders. The tool performed technically well regarding filtering and visualization of the variants, with focus on rare, non-synonymous coding variants. We also show that the software could be used successfully to identify probable causative variants for three hitherto undisclosed monogenic disorders. RareVariantVis is therefore a new and user-friendly tool to aid manual inspection and analysis of human WGS data in a diagnostic setting.

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(S53) FRACTIONAL DIFFUSION APPLICATIONS IN THE PHYSICAL AND BIOLOGICAL SCIENCES

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For three centuries fractional calculus developed mainly as a pure theoretical field of mathematics, however, over the past fifty years, it has found applications in diverse fields ranging from biological and physical sciences, engineering to internet traffic and economics. Many scientists indicate that derivatives and integrals of non-integer order are very suitable for the description of properties of various real materials. One of the reasons for fractional calculus popularity is that fractional derivatives provide an excellent instrument for the description of memory and hereditary properties of various materials and processes. Furthermore, another field which requires the use of derivatives of non-integer order is theory of fractals. The development of the fractal theory has opened further prospects for modelling dynamical processes in self-similar structures. Herein, we review some chosen scientific problems from physics and bio-medical sciences. At the beginning, we provide a short discussion on the theory of differentiation of arbitrary order and the theory of fractional differential equations. Survey of applications covers mainly viscoelasticity and modelling of diffusion in the biological and synthetic membranes of fractal structure.

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(S13) INFLUENCE OF XANTHONE DERIVATIVES ON THE EXPRESSION OF THE TRANSCRIPTION FACTOR *NANOG* IN TUMOR CELL CULTURES

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One of the recently discovered crucial factors involved in tumor progression is *NANOG*. Transcription factor *NANOG* is responsible for cancer development including tumor cell proliferation, motility, and drug resistance. In many cancers overexpression of *NANOG* has been demonstrated, especially in tumor cell side populations. Based on the folk medicine compounds termed *xanthones* may provide us with successful new agents for the treatment of cancer. These compounds are obtained from plants belonging to the family of *Clusiaceae* Lindl. that have used as antioxidant, antibacterial, anti-inflammatory, antipyretic and antifungal substances. The most popular natural xanthones are α-mangostin and gambogic acid. Antitumor activity of those compounds has become a point of interest to develop molecular studies and research directed to synthesis of a variety of new xanthone derivatives.

The aim of the study was to investigate expression of *NANOG* gene in human cancer cells exposed to xanthones. The study included two natural derivatives (α-mangostin and gambogic acid) and four synthetic derivatives (1, 2, 3, and 4) synthesized at the Department of Bioorganic Chemistry of the Jagiellonian University, Kraków. The study was conducted on the following cell lines: A549 (lung carcinoma), HeLa (cervical cancer) and T24 (urinary bladder carcinoma). *NANOG* expression was analyzed using techniques Real-Time^{TM} RT-PCR and ELISA. Additionally, in HeLa cell line silencing of *NANOG* expression was also carried out by RNAi technique followed by the treatment with xanthones. The quantity of living cells and apoptosis-necrosis balance were estimated using by microscopic examination (phosphatidylserine translocation) and by DNA fragmentation ELISA assay.

The obtained results demonstrate that *NANOG* gene expression was significantly higher in A549 and T24 cell cultures than in HeLa. We found a correlation between the impact of xanthones on the activity of *NANOG*, and their chemical structure. Moreover, influence of xanthones on the *NANOG* expression depended on tumor origin: HeLa cell cultures demonstrated the greatest decrease in gene expression after treatment with compound 4 (72%, $p = 0.000188$), and the greatest decrease in protein expression after treatment with α-mangostin (64.53%, $p = 0.002997$). The combination therapy in HeLa cell line (silencing of NANOG expression mediated by RNAi technique and treatment by xanthones) led to stronger proapoptotic results in comparison to the independent use of individual factors. The optimal results were obtained for cell cultures with silenced gene expression and treatment with compound 1.

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(F28) INHIBITION OF THE MIR-155 TARGET NIAM PHENOCOPIES THE GROWTH-PROMOTING EFFECT OF MIR-155 IN B-CELL LYMPHOMA

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MiR-155 is an important regulator of B-cell development and immune response. Deregulation of miR-155 leads to hematologic malignancies such as leukemia and lymphoma. High miR-155 levels were indeed observed in GC B cell-derived lymphomas like Hodgkin lymphoma (HL), chronic lymphocytic leukemia, primary mediastinal B-cell lymphoma and diffuse large B-cell lymphoma. In contrast, miR-155 levels were very low in Burkitt lymphoma (BL). The molecular mechanisms that underlie the oncogenic role of miR-155 in B-cell lymphoma are yet to be fully elucidated.

To identify the miR-155 targets that are relevant for B-cell lymphoma, we performed RNA immunoprecipitation of Argonaute 2 in Hodgkin lymphoma (HL) cells upon miR-155 inhibition and in BL cells upon ectopic expression of miR-155. We identified 54 miR-155-specific target genes in BL cells with overexpressed miR-155 and we confirmed miR-155 targeting of DET1, NIAM, TRIM32, HOMEZ, PSIP1 and JARID2 in luciferase reporter assay. Five of these targets were also regulated by endogenous miR-155 in HL cells. Next, we tested which of the identified targets were involved in the enhanced growth phenotype that was observed in GFP competition assay upon miR-155 overexpression in BL cells. We showed that downregulation of NIAM by shRNA reproduced this oncogenic effect of miR-155. NIAM-positive cases were also shown to have significant lower miR-155 levels as compared to NIAM-negative cases, suggesting that NIAM is also inhibited by miR-155 in primary B-cell lymphoma.

In conclusion, we identified NIAM as a novel miR-155 target gene in B-cell lymphoma. Our data together with the recent observation that NIAM-deficient mice are predisposed to malignant transformation suggest that NIAM is a crucial target for the oncogenic effects of miR-155 in B-cell lymphoma.

(S39) DERMATOLOGICAL AND COSMECEUTICAL BENEFITS OF PLANT POLYPHENOLS

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Polyphenols are produced by all higher plants in order to protect them against biotic and abiotic stress such as UV radiation, temperature changes, infections, wounding, and herbivores. They have antioxidant, anti-inflammatory and antibacterial effects (1)

Polyphenolic extracts are attractive ingredients for cosmetics and pharmacy due to their beneficial biological activities such as anti-inflammatory, collagen stimulating effect, potent anti-oxidant scavenging peroxyl radicals, inhibition of lipid peroxidation and protection against UV radiation (2).

When in contact with human skin, polyphenols exert either curative or damaging action depending on their physical-chemical properties and used concentration as well as bioavailability through cutaneous barrier, metabolism in the skin, and individual sensitivity.

In the case of topical delivery of the polyphenols, the penetration of polyphenols into the skin is limited and successful delivery of plant polyphenols requires cream-based, organic solvent-based or lipid soluble topical formulations that can enhance the penetration of the polyphenols

Immunomodulating properties of polyphenols are used in wound healing (2). In mice after induction atopic dermatitis showed that quercetin inhibited hyperkeratosis, parakeratosis, acanthosis, mast cells and infiltration of inflammatory cells. Furthermore, quercetin treatment downregulated cytoplasmic HMGB1, RAGE, nuclear p-NFκB, p-ERK1/2, COX2, TNFα, IL-1β, IL-2Rα, IFNγ and IL-4 and upregulated nuclear Nrf2 (3)

Polyphenols are a large group of compounds with multidirectional action. These compounds, on the one hand, help to maintain the good condition of the body, while on the other prevent a variety of diseases, as well as support the treatment of disease states already arisen.

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(S21) [11C]-CHOLINE AS AN ACCURATE AND EFFECTIVE PET/CT DIAGNOSTIC TOOL AND ITS COMPARISON TO [18F]-FLUOROMETHYLCHOLINE

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[^{11}C]-choline-PET/CT may offer new hope to patients with prostate cancer and lymph node metastasis as current imaging modalities (including transrectal ultrasound, MRI, CT, and bone scan) demonstrate poor performances in the diagnosis and staging of this disease.
In the study involving 72 patients with prostate cancer the [^{11}C]-choline-PET/CT was shown to be 89.4% accurate.

As reveals from the presented example [^{11}C]-choline can be an accurate and effective diagnostic tool.

24 patients with prostate cancer were examined in our site using [^{11}C]-Choline-PET/CT (activities used: 300 - 450 MBq). The acquisition protocol consisted of low dose CT for attenuation correction and whole-body PET scan with acquisition time of 2 min. per bed. In all cases the results of [^{11}C]-choline PET were found to be consistent with the patients' follow up.

Another radiotracer with a similar use as [^{11}C]-choline is [^{18}F]-fluoromethylcholine. Due to [^{18}F]-labeling the half-life of [^{18}F]-fluoromethylcholine is longer (109 minutes versus 20 minutes in case of [^{11}C]), whereas the [^{18}F] positron range is shorter. The longer half-life makes it possible to distribute the product away from the manufacturing site. However, the [^{11}C]-choline is preferred for prostate PET/CT imaging due to a better distribution and higher assimilation of this tracer in the patient's body as compared to [^{18}F]-fluoromethylcholine.

[^{11}C]-Choline-PET/CT can be an accurate and effective diagnostic tool in centers equipped with cyclotron.
All cyclotron-PET centers can consider using [^{11}C]-choline as it has better diagnostic properties over Fluorine-18 analogue.

(S20) SYNTHESIS, ISOLATION AND PURIFICATION OF [11C]-CHOLINE, A PET TRACER

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[11C]-choline is an effective PET tracer used for imaging of neoplastic lesions and metastases of the prostate cancer. However, its production can be a challenge for manufacturers, as it has not yet been described in Polish or European pharmacopoeia. In this study, the technical aspects of [11C]-choline production are described and detailed process parameters are provided. The quality control procedures for releasing [11C]-choline as solutio iniectabilis are also presented. The purity and quality of the radiopharmaceutical obtained according to the proposed method were find to be high enough to safely administrate the radiopharmaceutical to patients.

Application of an automated synthesiser in the production of [11C]-choline makes it possible to carry out the entire process of production, isolation and purification within 20 minutes. It is crucial to maintain all aspects of the process as short as possible, since the decay half-time of carbon-11 is 20.4 minutes. The resulting radiopharmaceutical is a sterile and pyrogen-free of a high chemical, radiochemical, and radionuclide purity proved by chromatographic techniques. The yield of the process is up to 20%.

(S4) GENETIC POLYMORPHISMS AND CLINICAL RESITANCE TO FAC CHEMOTHERAPY IN BREAST CANCER PATIENTS

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The SNPs in genes encoding drug metabolizing enzymes, drug transporters, drug-induced damage repair, involved in regulation of DNA damage response and cell cycle control can influence the pharmacokinetic and pharmacodynamic profile of anti-cancer drugs, leading to differences in response and development of severe toxicities. The aim of our study was to analyze the possible impact of polymorphic variants in genes of known or potential role in activity of FAC drugs. In this study were analyzed the genetic determinants (SNPs) of non-responsiveness to FAC chemotherapy in breast cancer patients.

We aimed to evaluate the clinical response to FAC chemotherapy and treatment outcome of 324 women diagnosed with breast cancer. All women had been treated with FAC first-line chemotherapy regime which combines doxorubicin, 5-fluorouracil and cyclophosphamide. In this study we genotyped 22 variants in 15 genes belonging to main pathways and cellular mechanisms engaged in transport and activity of three FAC drugs in order to select genetic changes that are linked to unfavorable reaction to treatment. The polymorphic variants (SNPs) in *ABCB1*, *ABCC2*, *ABCG2, MTHFR*, *GSTP1*, *GSTT1/M1* deletion, *CYP1B1*, *CYP2C19*, *TYMS*, *ERCC1*, *ERCC2*, *XRCC1*, *TP53, SLC22A16*, *DPYD* and *ATM* genes were analyzed in this study.

The results of our study suggest significant association between concurrent polymorphic variants in genes responsible for drugs' transport and DNA repair and the responsiveness of the breast cancer patients to FAC chemotherapy. The polymorphisms in two transporter genes *ABCB1*, *ABCC2* and ERCC2 radically decreased the risk of lack of response. Our study indicates the existence of chemo- resistant subpopulation of breast cancers in general, as well as of triple negative subtype. This subpopulation seems to be characterized by altered efflux and DNA repair systems. The carriers of all three high-risk genotypes within *ABCB1, ERCC2, ABCC2* genes harbor an extremely high risk of FAC treatment non-responsiveness. This observations suggested that multifactorial polymorphic models could be an useful contribute to the future design of personalized cancer treatment in breast cancer patients.

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(F46) THE APPLICATION OF THE MICROARRAY ANALYSIS METHODS IN SEARCH OF CANDIDATE GENE SIGNATURES OF RADIOSENSITIVITY

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Radiotherapy is one of the common methods of cancer treatment. It uses the high-energy radiation to damage the DNA. However, radiotherapy affects not only cancer cells, but also healthy cells and for that reason it causes side effects, for instance skin problems. It was observed that some patients are more sensitive to such adverse effects, while others are more resistant. Better understanding of the reasons of these differences may be helpful in radiotherapy planning, including the choice of radiation doses that would be the most beneficial for a particular patient. The aim of this study was to select the genes where expression levels vary in the most significant way between patients who are sensitive or resistant to the radiation side effects. This study includes also a presentation of data mining methods which can be used for dividing patients into sensitive and resistant group on the basis of gene expression before and after the treatment.

An analysis was performed on the microarray dataset from the experiment involving a group of women afflicted with breast cancer. Their gene expression was investigated ex-vivo in lymphocytes before receiving radiation, after receiving the dose of 0.2 Gy radiation and after receiving the dose of 2 Gy radiation. The data microarray experiment was normalized with the use of Frozen Robust Multichip Average method. Differential genes were selected with the use of statistical methods including: Lilliefors test, F test, Student's t-test and U Mann-Whitney test. The functions of obtained lists of genes were collected from the KEGG database and the Gene Ontology database. The dividing patients into groups was conducted on the basis of three data mining methods: PAM, bottom-up hierarchical clustering and top-down hierarchical clustering.

All three data mining methods performed satisfactorily. The accuracy values vary from 89.47% to 94.12%, while sensitivity values vary from 88.89% to 100%. This fact leads to the conclusion that it is possible to predict whether a patient is sensitive or resistant to the side effects of radiotherapy both before and after the treatment. According to results from both KEGG and Gene Ontology databases the significant differences in expression levels between two groups of patients are related to genes responsible for basic biological processes, for instance RNA and proteins synthesis. What is more, some of the differential genes are connected with the ubiquitin mediated proteolysis and p53 signaling pathway. Both ubiquitin and p53 are proteins involved (indirectly or directly) in the process of repairing DNA damage. Some of the differential genes were also found to be connected with the Alzheimer and Huntington diseases. This may indicate a relationship between the side effects of radiation and the neurodegeneration process.

Further studies of variances in the gene expression may result in developing a reasonable way for planning the doses of radiation and the length of intervals between them directly for a particular patient. The analysis of biological processes to which differential genes are related may lead to discovering new ways of preventing patients from suffering from adverse effects of radiotherapy.

(F44) THE MIGRATION EFFICIENCY OF MELANOMA AND BREAST CANCER CELLS IS NOT CORELATED WITH THE LEVEL OF HSF1 PROTEIN EXPRESSION AND PHOSPHORYLATION

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Heat Shock transcription Factor 1 (HSF1), the main regulator of the heat shock response, facilitates cell migration and metastasis which are important hallmarks of tumor progression. Our previous experiments revealed that constitutively active HSF1 supports motility, anchorage-independent growth and *in vivo* metastasis of the mouse B16F10 melanoma cells via down-regulation of vinculin. In many tumor types HSF1 is overexpressed. It could be activated by proteotoxic stress and an altered kinase signaling characteristic for cancer cells. Thus, we examined if there is any positive correlation between the level of HSF1 expression or phosphorylation and the migration efficiency of cancer cells.

The study was performed on a broad panel of human cancer cell lines: six melanoma and five breast cancer lines. The level of HSF1 expression and its phosphorylation status at 326 or 320 serine residues (which are the most important for HSF1 activation) and at Ser 303 (which is responsible for HSF1 repression) was assessed by Western Blot technique. The ability of cells to migrate was examined in the Boyden Chamber Assay. Although we observed differences in the level of HSF1 expression/phosphorylation and in migration efficiency between cell lines, we did not notice any positive correlation between these values. Moreover, the level of vinculin did not correlate with migration efficiency. It indicates that other mechanisms, distinct from HSF1 activity, are more essential for cancer cell movement in the tested cell lines.

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(S37) INITIAL CHARACTERIZATION OF A NEWLY ESTABLISHED OVARIAN CARCINOMA CELL LINE OVPA8

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Established cancer cell lines are widely used in research, although in many cases they are poorly characterized according to their cellular origin and histological type. We decided to establish our own series of ovarian cancer cell lines derived from distinct histological types of tumors. Now we present OVPA8 cell line, derived from the ascitic fluid of 48 years old patient with ovarian papillary serous carcinoma, FIGO IIIC. The cells were passaged 65 times so far. They represent epithelial morphology, according to classification by Beaufort et al. [1]. We characterized our new OVPA8 cell line in comparison to three widely used ovarian cancer lines: OAW42 (epithelial morphology), ES2 (spindle morphology), A2780 (round cell morphology). We found no p53 or BRCA1 mutations in either cell line, except a known polymorphism c.639A>G in OAW42 cell line. Then, we performed immunophenotypic characterization using following markers: WT1, HBME-1, calretinin, CD68, CD44, luminal cytokeratin 19 and EpCAM. WT1 serves as a nuclear marker in ovarian cancer. We detected moderate expression of WT1 in majority of cells from OVPA8 cell line. Strong staining was observed in the subpopulation of cells characterized by small nuclei and forming cell groups. Calretinin and HBME-1 are regarded as a markers of mesothelial cells. Surprisingly, HBME-1 could be detected both in early and late passsages of OVPA8, while no calretinin staining was observed at any stage. CD68 is used as a marker of macrophages. We detected very low levels of CD68 positive cells in OVPA8 line. CD44 is supposed to be related with higher malignant potential of cancer cells. We detected strong expression of CD44 in OVPA8, suggesting high malignancy of these cells. The epithelial markers: EpCAM and luminal cytokeratin 19 were also highly expressed in OVPA8 cells. High expression of these markers as well as CD44 indicate very aggressive tumor as a source of this cell line. These results are in line with medical history of a patient from which the OVPA8 cell line was derived (early onset, very aggresive, high grade serous cancer).

In addition, we checked expression of eight genes that were found in our previous microarray study as significantly related with patient's survival [2]: fibronectin (FN1), periostin (POSTN), MFAP5 (microfibrillar associated protein 5), SFRP2 (secreted frizzled-related protein 2), ITGLB1 (integrin, beta-like 1), FAP (fibroblast activation protein), LOX (lysyl oxidase) and PLAU (plasminogen activator, urokinase) by semiquantitative RT-PCR (reverse-transcription polymerase chain reaction). Two genes, POSTN and FN1, were also assayed in OVPA8 cell line by immunocytochemistry.

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(F36) CROSSTALK BETWEEN ESTROGEN AND HSF1 IN HUMAN MAMMARY CELLS

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Heat Shock Transcription Factor 1 (HSF1) is activated under proteotoxic stress (e.g. heat shock) which leads to an enhanced expression of heat shock genes, encoding heat shock proteins (HSPs). HSPs function as molecular chaperones, which help cells to survive stressful conditions. Activation of HSF1 is connected with its phosphorylation. Therefore, HSF1 could be also activated due to altered kinase signaling frequently observed in cancer cells. In such cases HSF1 supports a lethal phenotype of cancer. It is well documented that a high level of HSF1 is associated with increased mortality of estrogen receptor (ER)-positive breast cancer patients and endometrial cancer patients.

Here we aimed to study whether HSF1 is important for estrogen (E2) signaling in human estrogen-responsive cell lines. We found that estrogen treatment led to increased HSF1 phosphorylation on S326 (which is the final activation step) in mammary epithelial MCF10a and breast adenocarcinoma MCF7 cells. In spite of increased HSF1 phosphorylation, the expression of HSPs was not changed. To find out signaling mechanisms leading to HSF1 phosphorylation under E2 treatment (which was not correlated with estrogen receptors expression), we used Human Phospho-Kinase Arrays. We found some connections between signaling activated by E2 and heat shock in MCF7 cells. Both treatments induced the phosphorylation of ERK1/2, JNK1/2/3, and CREB kinases. They also slightly stimulated mTOR signaling, which was manifested by an increased phosphorylation of TOR and p70 S6 kinases. To further study associations between E2 and HSF1 signaling we silenced HSF1 in MCF7 cells using specific lentivirus-delivered shRNA. Preliminary analysis of a transcriptome (by RNA-Seq) showed that HSF1 silencing had an influence not only on the heat shock response but also on response to E2. A lower level of HSF1 was connected with an extension of E2 action: E2 was able to activate additional genes which were not activated in cells with a normal HSF1 level. Gene Ontology analysis revealed that genes involved in angiogenesis and the negative regulation of cell proliferation were overrepresented among them.

A functional role of HSF1 activity under E2 treatment has to be elucidated.

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(S2) IDENTIFICATION OF SERUM PROTEINS ASSOCIATED WITH THE RISK OF METASTASIS OF BREAST CANCER PATIENTS

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Breast cancer diagnosed at early clinical stages is relatively well cured, yet even in this group some patients are at high risk of metastasis and failure of the treatment. Optimal selection of adjuvant treatment for these patients would be facilitated if reliable prognostic markers of risk of metastasis were available in clinical practice. Serum proteomics allows to characterize processes related to progression of cancer and its influence at patient's organism. Hence, serum proteome might be a source of knowledge about factors reflecting or enhancing spread of cancer cells.

The major aim of this work was to analyze serum proteome of breast cancer patients in order to identify proteins that reflect high risk of metastasis and characterize systemic processes involved in spread of cancer cells. Analysis of complete serum proteome was performed using mass spectrometry approach: LC-MS/MS shotgun. We selected a group of 15 patients who suffered from cancer relapse and metastasis during 5-year follow-up, and 45 patients who benefited from successful treatment. Patients with successful treatment were selected from larger group to provide relevant control/background for patients with failure (considering age, clinical features of the tumor and the scheme of the treatment). Blood samples were collected before the start of the therapy, after the surgical resection of tumors and one year after the end of the therapy.

We have identified 26 proteins whose abundance were different in samples collected before and after treatment. Those proteins are associated in different cellular processes e.g. apoptosis, cell death as well as response to hormones and chemical stimulation.

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(S52) MECHANISTIC STRUCTURE-BASED MODELING OF KV 1.2 POTASSIUM CHANNELS

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Kv 1.2 channels regulate the flow of potassium ions into and out of cells in response to changes in the electric potential across cell membrane. They are broadly distributed in human body e.g. in neuronal and muscle cells, where they play crucial role in many processes like maintaining resting potential or generation muscle spasms.

Although the state of the art concerning Kv 1.2 channels function and mechanisms of their functioning is advanced, some aspects of their activation and gating remain still ambiguous or even unknown (for example the way of motion of the voltage sensing domain in response to membrane depolarization). Our aim is to introduce a model directly connected with channel structure and show exactly probable mechanism of channel activation – including the forces between channel domains, ranges of their motion and resulting conductance through the channel. The popular way of ion channel modeling is based on molecular dynamics (MD) simulations. This approach concerns the details of the system and is supposed to give results of high accuracy. However it demands high computational effort and describes processes at relatively short time scale. In contrary, our model describes motions and objects of larger size than in MD e.g. channel domains as a whole, nevertheless the interaction between them are evaluated on the base of the forces between their functional residues. Such an approach allows for detailed description of the activation and gating phenomena over long time scale. Our results shed also new light on the probable scenario of way of movements of Kv 1.2 domains, which have been controversial hitherto.

(S33) ELECTROCHEMOTHERAPY WITH BLEOMYCIN IN BREAST CANCER CELLS *IN VITRO* AND IN CANINE BREAST ADENOCARCINOMA

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Electrochemotherapy is an innovative method of anticancer treatment based on the combination of chemotherapy and electroporation of the cell membrane. It uses a pulsed electric field for the enhancement of chemotherapeutic drug delivery. The most common drug for electrochemotherapy is bleomycin - a nonpermeant, hydrophilic antibiotic with a very high intrinsic activity.

In the present study we investigated the effects of electrochemotherapy with bleomycin *in vitro* and in canine breast adenocarcinoma. *In vitro* experiments were conducted on two human breast adenocarcinoma cell lines: sensitive (MCF-7/WT) and resistant (MCF-7/DX) to doxorubicin. Trypan blue staining was used for the evaluation of cell death. The cellular responses were also assessed via MTT assay reporting the mitochondrial activity of cells and SRB assay reporting the ability of cells to protein synthesis. All analysis were performed on cells subjected to bleomycin alone, electroporation alone and to electrochemotherapy with bleomycin. The procedure of electrochemotherapy with bleomycin was also tested in a treatment of 8 nodules of breast adenocarcinoma in an 18-year-old dog.

The obtained results show that under the controlled experimental conditions (parameters of electric pulses, drug dose and exposure time) electroporation and bleomycin alone were non-toxic to the breast cancer cells *in vitro*. However, the combination of electroporation with bleomycin significantly improved the effectiveness of a treatment in both MCF-7/WT and MCF-7/DX cell lines. Electrochemotherapy reduced the mitochondrial activity of cells and the ability to protein synthesis. The induction of a cell death was also observed. The application of electrochemotherapy with bleomycin for a treatment of breast cancer in dog resulted in the inhibition of tumor growth. Additionally, no recurrences were observed. Our study demonstrated the effectiveness of electrochemotherapy with bleomycin in the treatment of breast cancer.

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(S46) HOW DOES THE MISSING LOOP REFILLMENT INFLUENCE ON THE SUBSTRATE ACCESS TO THE CATALYTIC POCKET IN *ASPERGILLUS NIGER* EPOXIDE HYDROLASE?

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Members of epoxide hydrolases (EHs) class are important in cell defense against harmful epoxides. These enzymes convert epoxides to trans-dihydrodiols, which are less toxic. *Aspergillus niger* soluble epoxide hydrolase is one of them.

The structure is deposited in Protein Data Bank (PDB ID: 1QO7) and is missing residues from 320 to 328, which constitute loop near the active centre. The aim of the study was to analyse the influence of the refilled loop on substrate access to the catalytic pocket. A model with reconstructed loop was compared with original structure. Received results suggests that the position of the added loop might influence distribution of tunnels in protein structure and substrate access to the active site.

The refilled loop was designed by Modeller 9v14 using ab inito method. To analyse the changes in protein structure dynamics of original and refilled models a 10 ns molecular dynamic simulation were performed using Amber 14. For identification and visualization of tunnels CAVER 3.0.2 was used.

The results demonstrate that the refilled loop must be considered during investigation of *A. niger* structure dynamics.

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(S38) MOLECULAR DIAGNOSTICS OF ROUNDUPREADY® MODIFICATION IN SOYBEANS AVAILABLE IN REGULAR SALES

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Rapid technological advances have enabled development in agriculture industry especially with using genetic engineering as a tool. The main objectives of "green biotechnology" focus are the improvement of quality characteristics, yield, physiological efficiency, increased production, and thus, profits. Genetically modified plants are created by introducing a new set of genes into their genome which, by undergoing expression, leads to the creation of a product such as a new protein. This type of end product provides the organism with the desired trait without causing visible changes in the plant – detecting the presence of GMO takes place at the molecular level.

The aim of the study was to detect the RoundupReady® modification in randomly purchased soybean samples, available in regular sales. Collected material was being sold without labeling. We achieved a positive result with one of all the tested samples.

The benefits and risks of farming GM varieties require continuous in-depth analyses. Monitoring the situation is extremely important, especially in case of the impact of GM crops on non-target organisms and often a complex network of ecosystems that occur locally.

It has been already known that conventional crop breeding methods will not be able to meet the constantly growing needs of the population and meet the increasing demands. The scientific communities offer a balanced and rational approach to the problem while providing ready-made solutions in the form of modern technologies of conventional farming and innovative biotech solutions. All these efforts are aimed at achieving sustainable intensification of crop productivity while maximally conserving the environment, woodlands and biodiversity.

(F6) TARGETED SEQUENCING OF CANCER- AND EPIGENETICS-RELATED GENES IN GLIOBLASTOMA REVEALS A DEEP DEREGULATION OF EPIGENETIC MECHANISMS

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Recent whole genome studies demonstrated that epigenetics enzymes, histones and chaperone proteins harbor mutations that may result in gross alterations of the epigenome leading to genome instability. Glioblastoma (GBM, WHO grade IV) is common and most lethal primary brain tumor that remains largely resistant to current therapies. The epigenetic landscape is deregulated in GBM due to aberrant activation or inactivation of enzymes maintaining and modifying the epigenome. Dissection of the GBM genetics may lead to more targeted and effective treatments. Here we report the results of targeted next-generation sequencing of cancer- and epigenetics-related genes in 37 fresh frozen GBM samples. We employed a second generation DNA sequencing target enrichment design comprising a 600 cancer-related gene panel and 100 epigenetics-related genes, comprising the exomes +/- the promoter regions. The target region spanning 7 MB ($1x10^6$ base pairs) was designed to cover meaningful portion of genomic, cancer-related sites with a strong emphasis on epigenetic regulators (histone modifiers, chromatin modelers, histone chaperons). Several filtering steps were used to eliminate variant calling errors: an average total coverage depth >100, each variant coverage >20, a variant frequency >5%. To distinguish somatic and germ-line mutations, detected mutations were compared to variants in the 1000 Genomes Project. Targeted sequencing of GBMs demonstrated different genetic drivers (including well known *EGFR, TP53, PDGFR* and *PTEN* mutations) and numerous genetic alterations in genes responsible for histone and chromatin modifications, chromatin remodeling and DNA methylation. Discovered variants were confirmed by ultra-deep sequencing and compared to existing databases to report new variants.

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(F52) AMYLOAD - WEB SERVICE DEDICATED TO AMYLOIDOGENIC PROTEINS

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A significant growth in the number of patients with neurodegenerative diseases, such as Alzheimer's or Parkinson's disease, has been observed recently. Studies show that these diseases are related to the occurrence of specific, amyloidogenic protein sequence fragments, which are prone to aggregate. The analysis of these fragments can provide new knowledge about mechanisms of neurodegenerative diseases development. Different websites can be found on the Internet, which contain sets of amyloidogenic sequences. However, these sets are typically represented by the plain text, hence are difficult to browse or use for more advanced analysis or modeling.

We present here the AmyLoad web portal which gathers amyloidogenic sequence fragments from different sources, such as WALTZ-DB [1], AmylHex [2], AmylFrag [2], and a great number of publications reporting such sequences. AmyLoad provides easy way to filter the data, e.g. according to fragment length, subsequence occurrence, or the protein name. Selected fragments, along with more extended information and references, can be downloaded in one of several supported file formats. Also, AmyLoad allows users to add their own sequences, which can be later available in the database. Finally, our portal provides the tools for analysis of FASTA sequences with regard to the occurrence of amyloidogenic fragments. For this purpose, different methods, such as FoldAmyloid [3], AGGRESCAN [4], and FISH [5] are implemented. The AmyLoad website provides more advanced and comfortable ways of studying amyloidogenic sequences.

The website is available at http://comprec-lin.iiar.pwr.edu.pl/amyload/

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(S9) EXPRESSION PROFILE OF GENES ENCODING ENDOTHELINS AND THEIR RECEPTORS IN ENDOMETRIAL CANCER

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In 2012, endometrial cancer was the fourth most common newly diagnosed cancer and seventh leading cause of cancer death among women in Poland. Despite the high percentage of women with endometrial cancer, its mechanisms of induction and progression remain partially unknown. Endothelins are a group of multifunctional peptides, which activity depends on binding to their receptors. Published experimental studies indicate that changes in the expression level of genes encoding endothelins and their receptors may contribute to the initiation and progression of many cancers. The premise of the study was a hypothesis that genes encoding endothelins and their receptors in endometrial cancer change their transcriptional activity in cancer induction and progression.

The purpose of the study was to examine whether the expression profile of genes encoding endothelins and their receptors in endometrial cancer depends on the stage of endometrial cancer.

The research included samples of the endometrium: 6 histopathologically confirmed as normal and 12 as endometrial cancer, further divided according to the tumor grade: G1 - 3, G2 - 8 and G3 - 1. Expression profile of *EDN1, EDN2, EDN3, EDNRA, EDNRB* and *ACTB* (endogenous control) genes was performed by RT-qPCR and the results were statistically analyzed with REST 2009 software.

The analysis showed a statistically significant ($p<0.05$) decrease of the transcriptional activity of *EDN3* gene in G1, G2 and G3 endometrial cancer in comparison to normal endometrium. In addition, the results showed a statistically significant decrease of the transcriptional activity of *EDNRB* gene in G1 and G2 endometrial cancer as well as decrease of the transcriptional activity of *EDN1* gene in G2 endometrial cancer comparing to normal endometrium.

From these results the following conclusions were drawn: transcriptional activity of genes encoding ET-1, ET-2 and ETB in endometrial cancer depends on the stage of cancer and may be an additional marker in the staging of endometrial cancer.

(S42) DNA DOUBLE-STRAND BREAKS INDUCED BY NADPH CYTOCHROME P450 REDUCTASE-ACTIVATED MITOXANTRONE IN SENSITIVE AND MULTIDRUG RESISTANT LOVO COLON CANCER CELLS

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Mitoxantrone (MX), a synthetic anthracenedione antitumour drug, was developed as a doxorubicin (DOX) analogue with decreased cardiotoxicity. It is among the most effective drugs, currently available for the treatment of advanced solid tumours, lymphoma and leukaemia. Its clinical usefulness is limited by the development of multidrug resistance (MDR) by cancer cells, associated with the overexpression of genes encoding ATP-binding cassette (ABC) membrane transporters (e.g. P-glycoprotein, P-gp; MRP1; BCRP/MXR1) responsible for active efflux of drugs out of resistant cells resulting in the decreased intracellular accumulation insufficient to inhibit resistant cell proliferation.

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

The aim of this study was to examine DNA damage induced by CPR-activated MX in in cells originating from solid tumours: human sensitive LoVo and multidrug resistant LoVo (overexpressing P-gp) colorectal adenocarcinoma cells. DNA double strand break (DSB) formation was examined by determining the amount of phosphorylated histone H2AX (γ-H2AX) being a cellular marker of DSB presence. The study was carried out with the aid of BD FACSCalibur flow cytometer and real-time living cell imaging system BD Pathway 855 Bioimager.

The effect of CPR-activated MX on cellular DSB formation was examined after 24 h and 72 h of incubation. It was found that CPR-activated MX used both at IC_{50} and IC_{90} caused significant DSBs in sensitive LoVo as well as multidrug resistant LoVo/DOX cells detected by γ-H2AX-positive staining. However, these effects were similar to the level of DNA damage induced by MX alone (non-activated) used at corresponding IC_{50} and IC_{90}, respectively. It suggests that CPR-activation of MX does not significantly change the cellular DNA damage response of studied sensitive LoVo and multidrug resistant LoVo/DX cells.

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(F58) COMPARISON OF GENE SET ENRICHMENT ANALYSIS METHODS IN SINGLE NUCLEOTIDE POLYMORPHISM INVESTIGATION ON RADIOSENSITIVITY PHENOMENA

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Aim: This work was concentrated on comparing two methods of gene set enrichment analysis (GSEA) dedicated to single nucleotide polymorphism (SNP) investigation. While standard GSEA is dedicated to *gene-GeneSet* transformation there is a problem how to investigate *SNP-gene-GeneSet*. The biological motivation was based on seeking biomarkers of radiation response phenomena.

Materials and methods: The population under investigation was composed of 44 unrelated, healthy individuals from Caucasian population. For each person genotyping results of 567 095 polymorphisms (SNP) by Axiom platform, were collected. Additionally the PUMA expressions were measured after the irradiation of 2Gy and in normal conditions (0Gy) – qPCR. The standardized fold change was calculated. For every gene-SNP, statistical significance was calculated by the most powerful test and for the best model of interaction (ANOVA, t-test or Mann-Whitney). The group of relevant SNPs ($p<0.05$) were transformed to gene representation by $\min\{\text{p-value SNP}_{(\text{in gene})}\}$. Two methods of GSEA were compared Subramanian et al. [1] (GSEA Pre-Ranked based on test statistic) and MAGENTA-GSEA Sagre et al.[2] where 5th and 10th percentiles were taken as threshold.

Results: From genotype-phenotype modelling process we obtained 62 914 significant polymorphisms which further were assigned to 7 520 different genes. The three main GeneSet Collection features were investigated (hallmark, oncogenic, radiation) where the most important findings are KRAS pathway and poor survival after gamma radiation. Both of them are highly related to investigated phenomena. Moreover we show that GSEA Pre-Ranked methods have similar power to MAGENTA-GSEA on 10^{th} percentile. Additionally, we show that MAGENTA GSEA on 5^{th} percentile is the most conservative method.

Conclusions: In the group of significant SNPs some of them are highly related with radiosensitivity phenomena as the genes are in KRAS and poor survival pathways. However, independent validation is needed. The MAGENTA-GSEA on 10^{th} percentile seems to be the most powerful method from all investigated. Additionally, it has similar power to the most popular GSEA Pre-Ranked which allows comparison of results from different studies.

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Cultural event information

The Academic Music Ensemble (AZM) of The Silesian University of Technology was formed in 1996. AZMs co-founder and conductor is Krystyna Krzyżanowska-Łoboda – a Professor at the Academy of Music in Katowice. The Ensemble includes a choir and an instrumental band. From the very beginning AZM has been braking stereotypes so in the repertoire you will find works ranging from Gregorian chant to Rock'n'Roll. Even the most demanding music lovers will find something for themselves.

AZM performes in many different places depending on the occasion. The Ensemble was repeatedly awarded at several competitive events and festivals both in Poland and abroad (Czech Republic, Portugal, Spain, Germany, Estonia, Finland, Italy, Malta, Hungary, Ukraine and Austria). Recently AZM won three awards in 2nd European Choir Games in Magdeburg (one gold medal and two silver medals). Now AZM is focusing on celebrating its 20th birthday. Therefore please join AZM in this celebration.

CONCERT PROGRAM:

1. **Rorate Coeli Desuper**
2. **La, la, la, Je ne l'ose dire**
3. **Szła dzieweczka do laseczka**
4. **Sometimes I feel like a motherless child**
5. **Come as you are**
6. **Yesterday**
7. **Only you**

Silesia.

Positive energy

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