XIIth Gliwice Scientific Meetings 2008

Gliwice, November 21-22, 2008

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

Organizers:

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

The Silesian University of Technology

Association for the Support of Cancer Research

The Silesian Voivodship Office

Polish Academy of Sciences, Committee for Human Genetics and Molecular Pathology

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XIIth Gliwice Scientific Meetings
November 21 – 22, 2008
Program

Friday, November 21, 2008

9:00 – 9:10 Opening Ceremony
9:10 - 13:35 Session 1: Responses to Ionizing Radiation and DNA Damage
9:10 – 10:50 Part I
Chairman: Carmel Mothersill
(30') **Kevin Prise** *(Gray Cancer Institute, Northwood, UK)*: New insights on bystander signalling: The role of DNA damage sensing and repair.
(30') **Carmel Mothersill** *(McMaster University, Ontario, Canada)*: Bystander effects and adaptive responses induced by radiation exposure.
(30') **Marie Boyd** *(Beatson Institute, Glasgow, Scotland)*: Radiation induced biological bystander effect elicited *in vitro* by targeted radiopharmaceuticals labeled with α–, β– and auger electron emitting radiohalogens.
(10') **Joanna Rzeszowska-Wolny** *(Institute of Oncology, Gliwice)*: Transcription profile change after irradiation or in bystander cells; differences between cell lines.

10:50 – 11:20 Coffee break

11:20 – 13:35 Part II
Chairman: Marek Los
(30') **Rachel Martin** *(Shimadzu Biotech Kratos Analytical)*: Characterizing protein post translational modifications using MALDI mass spectrometry.
(15') **Beata Walczak** (*Silesian University, Katowice*): Proteomics: automated analysis of electropherograms.
(30') **Chryso Kanthou** *(Sheffield University, UK)*: Response of tumour vasculature to vascular disrupting agents and radiation.
(30') **Walter Mier** *(Universitätsklinikum Heidelberg, Heidelberg)*: The targeting of melanoma for endoradiotherapy.
(30') **Micheline Giphart-Gassler** *(Leiden University Medical Center, Leiden, The Netherlands)*: Towards a gene expression profile to classify patients for normal tissue toxicity.

13:35 – 14:35 Lunch

14:35 – 15:35 Poster Session

Session 2: Molecular channels
15:35 – 17:05
Chairman: Zbigniew Grzywna
(30') **Mustafa B.A. Djamgoz** *(Imperial College, London)*: Voltage-gated ion channels as mediators of growth factor effects in metastatic disease.
(20') **Adam Szewczyk** *(Nencki Institute of Experimental Biology, Warsaw)*: Mitochondrial potassium channels.
(20') **Maria Mycielska** *(Imperial College, London)*: Citrate transport in human prostate cancer cells: regulation by functional voltage-gated Na^+ channel expression.
(20') **Krzysztof Dołowy** *(Warsaw University of Life Sciences – SGGW)*: The role of mitochondrial ion channels in ischemic preconditioning.

17:05 – 17:25 Coffee break

Session 3: System Biology, Bioinformatics and Signaling Pathways
17:25 – 19:25
Chairman: Piotr Widłak
(30') **Miguel Rubi** *(Universitat de Barcelona)*: Somatic exocytosis of serotonin mediated by molecular motors.
(20') **Jerzy Mozrzymas** *(Wrocław Medical University)*: Impact of acidosis on modulation of GABAergic IPSCs by benzodiazepine receptor agonists.
(30') **Jerzy Jurka** *(Mountain View, USA)*: Mobile DNA elements throughout evolution.
(20') **Maria Obolenskaya** *(Institute of Molecular Biology and Genetics, NAS, Kiev,Ukraine)* Folate, aminothiols and cystathionine-β-synthase in human placenta.
(20') **Jan Poleszczuk** *(Inst. Appl. Math. Mech.,University of Warsaw)*: Tumor development model under angiogenic signaling with dependence on vessel impairment.

20:30 – 23:00 Social event: Concert and get-together party

Saturday, November 22, 2008

Session 4: New drugs and treatments
9:00 – 10:50
Chairman: Jarosław Polański
(20') **Ryszard Oliński** *(Nicolaus Copernicus University, Bydgoszcz)*: Oxidative processes and cancer.
(30') **Jean Francois Mouscadet** *(Ecole Normale Supérieure de Cachan, France): in silico* study suggests that Raltegravir-resistant mutations modify the DNA recognition properties of HIV-1 integrase.
(20') **Marek Los** *(Manitoba Institute of Cell Biology, Winnipeg, Canada)*: Apoptin and its derivatives as molecular templates for the development of Bcr-Abl kinase inhibitors.
(20') **Alexa Schieck** *(Universitätsklinikum Heidelberg, Heidelberg)*: Identification of the determinant of hepatitis B virus liver tropism and its implications for hepatocyte-specific drug targetting.
(20') **Martin Dolezal** *(Charles University in Prague, Czech Republic)* Biologically active pyrazines, the past and the future.

10:50 – 11:20 Coffee break

Session 5: Experimental Biotechnology
11:30 – 13:05
Chairman: Wiesław Szeja
(15') **Wiesław Szeja** *(Silesian University of Technology, Gliwice),* Synthetic glycomix for mining natural products as drug leads. Part I
(15') **Grzegorz Grynkiewicz** *(Pharmaceutical Research Institute, Warsaw)*: Synthetic glycomix for mining natural products as drug leads. Part II
(15') **Aleksandra Rusin** *(Institute of Oncology, Gliwice)*: Genistein derivatives and their biological activity.
(15') **Krystiana Krzysko (***University of Warsaw, Poland)* Binding of genistein derivatives to abl and lck protein kinases, and to microtubules - modelling studies.
(20') **Andrzej Gamian** *(Institute of Immunology and Experimental Therapy, Wrocław)*: Glycation of proteins as their modification in physiology and disease.
(15') **Olena Palyvoda** *(Wayne State University, Detroit)*: Molecular organization in self-assembled monolayers used for neuronal cell growth.
(15') **Joanna Polańska** *(Silesian University of Technology, Gliwice)*: Statistical processing of DNA microarray data - detecting subtle changes of gene expressions.

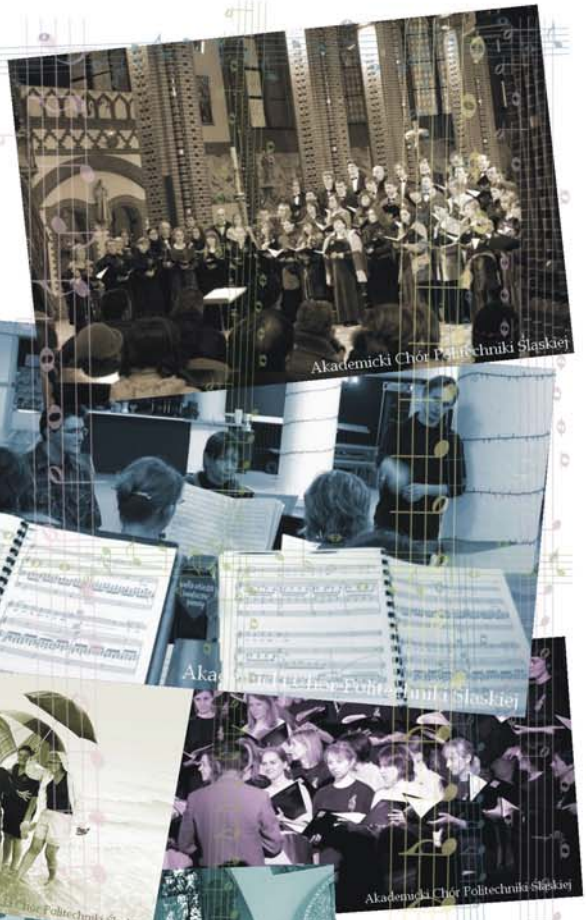
13:20– 14:20 Lunch

14:20 – 15:20 Presentation of winning posters and Closing Remarks

THE ACADEMIC CHOIR
OF THE SILESIAN UNIVERSITY OF TECHNOLOGY IN GLIWICE

The Choir was established in 1945 by former employees and students of Lvov Technical University who used to singin Lvov Technical Choir. In the academic year 2004/2005 the Academic Choir celebrated it`s 100th anniversary, referring to the its history and tradition. Academic Choir gives approx. 40 performances a year in Poland. On their numerous tours (over 30) to almost all European countries, Siberia, Canada, the USA and South Corea they gave over 100 concerts. They gained over 50 musical awards as well as honourable mentions for their social activities, including: Prize of the Minister of Art and Culture, Prize of the President of Gliwice City in 1996 and 2005, Decoration of Merits for Services for Silesian University of Technology granted by University Authorities in 1998 and Medal on Occassion of 60-year Anniversary of University in 2005 as a recognition of meritsfor development of University. Since 1980 the Choir has been organising national festival under the name Of Choir Meetings in Gliwice City - Gliwickie Spotkania Chóralne. They record for Polish Radio and Television. They have published 8 cassettes and 5 Cds as well as a book "50 Years of Academic Choir of Silesian University of Technology in Gliwice" and three books of a magazine "Silesian Singer" ("Śpiewak Śląski") Which is published by Silesian Branch of Polish Association of Choirs and Orchestras (1-st and 2-nd book on the occassion of 55-th and 60-th anniversary, 3-rd one as Books of Lvov Technical Choir). The repertoire of the Choir is very rich and various. The Choir performs works of early, classical and contemporary music of both Polish and foreign composers, folk music adaptations, as well as big instrumental and vocal forms, including pieces by : Beethoven, Elsner, Haendel, Mozart, Rossini, Rutter, Twardowski, Schubert, Viern and in 2007 - "Requiem M. Durufle. On 16th of January 2006 Tomasz Giedwiłło took over artistic managment of the choir from Professor Czesław Freund. But he worked with the choir since 2001-first having student practition than, since 2003 becoming conductors assistant. First year was the time of intense work:66concerts and performances-2 in Slovakia (Namestovo, Siedliacka Dubova), 10 in Uruguay (Colonia, Minas, Montevideo, Salto, SanCarlos, San Jose; in Uruguay our choir performanced also in Montevideo`s television). In Argentina we had 6 concerts (Buenos Aires, Berisso). Recordings of the choir were presented in Argentina's music programs with the wordsof Andrzej Jezierski; 48 concerts in Poland-especially in Gliwice - 29 and in Bielsko-Biała, Chełm Śląski, Katowice, Łodz, Myślenice, Rabka, Rybnik, Szczyrk, Tychy, Wisła, Wrocław and Zabrze. We participate in 11 choral festivals. In 3 competition festivals we won Grand Prix of XIV National Festival of Polish Choral Songs with the cup from Polish President, 1st prize in category of academic choirs on IX Choral Festival in Łódź - Cantio Lodziensis, 1st prize in category of mixed Choir in XVI Festival of choral songs in Myślenice.We recordered and published CD with Christmas Carrols in Henryk Botor`s arrangement. On 11th May 2007 our choir took part in VII International Choir Festival in Prijedor (Bosnia i Herzegovina) where we got The 1st prize in the category of national composition. On 13th May we were invited by Teachers Choir of City Kecskemet to give a concert in Kecskemet (Hungary). On 5-8th September we gave concert on 34 International Choral Meetings "Citta di Fano"in Italy.

Tomasz Giedwiłło - He graduated The Karol Szymanowski Academy of Music in Katowice, majoring in Composition, Theory and Education of Music in the Class of Conducting of Professor Warzecha. During his studies he was granted a scholarship by the Minister of Art and Culture. He brushed up his conducting skills in Academic Choir of Silesian University of Technology under the supervision of professor Czesław Freund. In his professional career he works for Silesian Philharmonic as a choir artist, for the Academy of Music in Katowice where he conducts a mixed choir at the Instrumental Faculty (extramural studies), for the Department of Jazz Music as conductor and for the Group of Schools of Music in Tychy. He cooperates also with Theater"A"from Gliwice. He took part in many International Courses and Meetings of Choral Condactors: In 2003 he participated in an international course for choral conductors (he was conducting Swedish Voices Chamber Choir from Stockholm) at the Z. Kodaly Institute in Kecskemet (Hungary). In 2005 he took part in the International Symposium of Choral Music in Gdańsk (he was conducting Polish Chamber Choir - Schola Cantorum Gedanensis) and in 2007 in Gent (Belgium) under the supervision of Professor Frieder Bernius.



THE ACADEMIC CHOIR
OF THE SILESIAN UNIVERSITY
OF TECHNOLOGY IN GLIWICE

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Akademicki Chór Politechniki Śląskiej GLIWICE

POLITECHNIKA ŚLĄSKA

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Akademicki Zespół Muzyczny Politechniki Śląskiej

Academic Music Ensemble (AZM in short) of the Silesian University of Technology is a group consisting of choir and chamber orchestra. Probably it is a unique such group acting at Polish technical universities. The ensemble was established in 1996, and within a very short time period acquired a very high level of artistic performance. The first trophy was gained in 1998 (Chairman Award of the Polish Association of Choirs and Orchestras at the International Festival and Competition of Choir Music in Międzyzdroje). AZM has won numerous prizes. Among them the most important are Silver Band in 'chamber choir category' at 'Carol and advent song tournament' in Prague '99 (the conductor Krystyna Krzyżanowska - Łoboda achieved there a prize for being the best conductor of the tournament); Golden Medal at 'Sacral Music Category' in June 2001 Olomouc, Czech Republic; Silver Medal at the XXXVI Contemporary Music Tournament in June 2001, Międzyzdroje; Triple Silver Medal at the Academic Choir Tournament in July 2002, Pardubice, Czech Republic; Triple Silver Medal, one Gold Medal and GRAND PRIX at the Academic Choir Tournament in July 2004, Pardubice, Czech Republic; first place at Franz Schubert's Tournament in November 2005, Vienna, Austria; Silver Medal at the Choir Tournament in July 2007, Wernigerode, Germany; third place at XXXIX Choir Tournament Legnica Cantat in May 2008. AZM is also an International Cultural Ambassador of People to People International Organization.

AZM released several CD's: "JAZZM", "Anioł pasterzom mówił" (carrolls), "GOSPEL" as a result of IIIrd International Musical Workshops "Musica pro Europa". AZM also took part in the recording of "Sound of Europe" for the American organization People to People International. Since 2000 AZM hosts an annual event of "International Musical Workshop – Musica pro Europa" In 2006 AZM celebrated its 10th anniversary with a magnificent performance of G. Verdi's Requiem at the VIIth IMW. AZM has paid visits to numerous countries: Germany, Hungary, Finland, Ukraine, Czech Republic, Portugal, Greece, Spain, and Malta. Every year, the Ensemble performs around 45 concerts throughout Poland and abroad.

Lecture abstracts

VOLTAGE-GATED ION CHANNELS AS MEDIATORS OF GROWTH FACTOR EFFECTS IN METASTATIC DISEASE

Mustafa B A Djamgoz

Imperial College, London, UK.

A 'neuroscience' approach to understanding the pathophysiology of cancer has revealed that acquisition of metastatic potential in carcinomas, including prostate cancer (PCa) and breast cancer (BCa), involves up-regulation of voltage-gated sodium channels (VGSCs) and concomitant down-regulation of potassium channels. Thus, metastatic cancer cells are electrically excitable and, indeed, respond to depolarizing stimuli with regenerative activity. VGSCs are also expressed in vivo and blocking VGSC activity suppresses a range of cellular behaviours integral to the metastatic cascade. Thus, VGSCs are a novel cancer biomarker and therapeutic target.

In on-going work, we are investigating the possible underlying cause(s) of the VGSC upregulation in BCa and PCa in a parallel approach since these cancers share many similarities, including hormone sensitivity and metastatic sites (eg bone). Our working hypothesis is that VGSC upregulation occurs at the transition from hormone to growth factor dependency. Most work has been done on epidermal growth factor (EGF) [1,2]. Inhibiting endogenous EGF receptor tyrosine kinase activity with AG1478 suppressed VGSC expression whilst exogenous EGF had the opposite effect. Importantly, the effect of exogenous EGF effect was blocked significantly by co-treatment with the highly specific blocker of VGSCs, tetrodotoxin (TTX), consistent with the following basic scheme:

EGF → VGSC upregulation → enhancement of metastasis

A markedly different effect was obtained with insulin/insulin-like growth factor receptor. In the presence of insulin, TTX increased migration of MDA-MB-231 human BCa cells. Thus, VGSC activity would accelerate or decelerate metastatic cell behaviour depending on the biochemical constitution of the extracellular medium. It is concluded (i) that functional VGSC expression is controlled by growth factor, especially EGF receptor signalling and (ii) that VGSC activity can influence metastasis by enhancing cancer cells' metastatic behaviour as well as homing into metastatic sites.

References:

1. Ding Y, Brackenbury WJ, Onganer PU, Montano X, Porter LM, Bates LF & Djamgoz MBA (2008). Epidermal growth factor upregulates motility of Mat-LyLu rat prostate cancer cells partially via voltage-gated Na^+ channel activity. *J Cell Physiol*. In the press.
2. Uysal Onganer P & Djamgoz MBA (2008). Epidermal growth factor potentiates in vitro metastatic behaviour of human prostate cancer PC-3M cells: Involvement of voltage-gated sodium channel. *Mol Cancer*. In the press.

BIOLOGICALLY ACTIVE PYRAZINES, THE PAST AND THE FUTURE

Martin Doležal

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In plants or insects, pyrazines play the roles of attractants, feromones and signal substances. Similar substances were found in food and therefore their sensoric properties were investigated. Pyrazines are also synthesized by a number of fungi, such as antibiotic aspergillic acid and fungicidal pigment pulcherrimin.

Synthetic pyrazines are used as additives in food manufacture and in tobacco industry.

Some of them are important pharmaceuticals such as sulfonamides sulfapyrazine and sulfalene, antituberculotic pyrazinamide, potassium-sparing diuretic amiloride, drug for smoking addiction treatment varenicline, hypolipidemid acipimox, peroral antidiabetic glipizide, hypnotic eszopiclone, potential anticancer pyrazine diazohydroxide and bortezomib, antiviral 6-fluoro-3-hydroxypyrazine-2-carboxamide.

Supporting grant: MSM0021620822

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1. Doležal, M. Biologicky aktivní pyraziny přírodního a syntetického původu. Chem. Listy *100 (11)*, 959-966 (2006).
2. Brown D.J.: The Pyrazines. J. Wiley & Sons, Inc., New York, 2002.

THE ROLE OF MITOCHONDRIAL ION CHANNELS IN ISCHEMIC PRECONDITIONING

Krzysztof Dołowy

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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). The molecular mechanism of ischemic preconditioning is not known. The surprising observation that the mitochondrial ATP regulates potassium channel opener protects heart from apoptosis due to ischemia pointed to mitochondria as a key player in the phenomenon. Three mechanisms were suggested. Reactive oxygen species (ROS) production in mitochondria after ischemia is one. The failure of volume regulation of mitochondria during ischemia is the second. And the release of calcium from mitochondria is the third. It is suggested that activation of mitoK_{ATP}, mitoBK_{Ca} and blocking of mitoCl channels decrease production of ROS after restoration of oxygen supply due to decrease of electric component to proton motive force.

This work was supported in part by the Polish Mitochondrial Network MitoNet.pl and the grant from the President of WULS-SGGW

GLYCATION OF PROTEINS AS THEIR MODIFICATION IN PHYSIOLOGY AND DISEASE

Gamian Andrzej [1,2], Pietkiewicz Jadwiga [2], Szeja Wiesław [3], Bartyś Arkadiusz [1]

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[2]*Department of Medical Biochemistry, Wrocław Medical University, Poland.*
[3]*Department of Chemistry, Silesian Technical University, Gliwice, Poland*

The advanced glycation end products (AGE) formed during spontaneous reaction of aldehydes and proteins accumulate in tissues and modify several proteins. Glycation is currently considered as a physiological process, whereas the excessive level of accumulating AGEs is correlated with diabetic complications. The group of glycation products is very heterogeneous and only few of them are identified so far, like pentosidine, argpyrimidine, carboxymethyllysine, contributing to only few percent of total products [1]. There are no tests practically that could be in routine use in analytical laboratories for measuring the AGEs in biological samples, while their quantization may serve as a useful marker for monitoring progression of certain pathological processes. Usually, model glycation end products are synthesized from glucose or from such degradation products like glyoxal, methylglyoxal, 3-deoxyglucosone [2]. However, the sera obtained after immunization with a mixture of glycation products possess antibodies in low titer against AGEs [3]. Therefore it is necessary to find the common and specific products of glycation that are formed in human tissues. Synthesis of such products would allow to obtain antibodies for test for glycation [1,2].

In our laboratory a few methods were applied to obtain a panel of model glycation products from several sugars [4]. These procedures involved high pressure glycation (HPG), high temperature (HTG) and also ultrasonic waves (UWG) and microwaves reactor (MWG). The fractionated products were used to prepare rabbit anti AGE sera, which did not recognize products formed from routinely used sugars for glycation and intermediates of Maillard reactions. It appeared in the immunoblotting experiments, that the epitopes on cross-linked glycation products formed in water solutions differ from those originating in dry conditions [3]. The specific immunochemical tests have been elaborated for the determination of protein AGE, anti-AGE antibody and immune complexes. Immunochemical experiments revealed the distinct lower level of circulating serum AGEs in patients with Alzheimer's disease, in relation to healthy controls [5].

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1. Al-Abed Y, Kapurniotu A, Bucala R, Meth. Enzymol. 1999, 309, 152-171.
2. Biemel KM, Reihl O, Conrad J, Lederer MO, J. Biol. Chem. 2001, 276, 23405-23412.
3. Turk Z, Ljubic S, Turk N, Benko B, Clin. Chim. Acta 2001, 303, 105-115.
4. Staniszewska M, Jarosz S, Jon M, Gamian A, Arch. Immunol. Ther. Exp. 2005, 53, 71-78.
5. Staniszewska M, Leszek J, Małyszczak K, Gamian A, Int. J. Geriatr. Psychiatry 2005, 20, 896-897

TOWARDS A GENE EXPRESSION PROFILE TO CLASSIFY PATIENTS FOR NORMAL TISSUE TOXICITY

Micheline Giphart-Gassler

Department of Toxicogenetics, LUMC, The Netherlands

Radiation is an effective anti-cancer therapy but leads to severe late radiation toxicity in 5%–10% of patients. These late effects limit the dose of radiotherapy. We postulate that variation in the incidence and severity of late complications is at least partly determined by intrinsic genetic differences between individuals. Such variation might be reflected by a difference in genetically regulated responses to radiation such as transcription. Our ultimate goal is to predict the severity of normal tissue damage after radiotherapy by transcription profiling. Assuming that genetic differences between individuals affect the transcriptional response to radiation of all cells, we have chosen T-lymphocytes as a surrogate tissue. We have determined the differences in gene expression after 2Gy in lymphocytes of patients treated for prostate cancer. This difference was used to discriminate between patients with severe late radiation toxicity from patients without complications following radiotherapy. By using a random cross validation strategy, a gene expression profile (classifier) was found that correctly classified 63% of the patients. A better performance was obtained by taking functional gene sets based on gene ontology for classification. Although the results were promising additional studies are needed, also because less correct classification was obtained of patients in an independent validation set.

Classical cellular responses to in vitro radiation at a dose of 2Gy are dominated by cell killing and a p53-dependent transcriptional response. The *in vitro* response to 2Gy in lymphocytes shows large variations between individuals. Interestingly it is not the p53-dependent response that is different between patients groups differing in late toxicity. We propose that late radiation toxicity reflects more sub-lethal effects of radiation in normal tissue. Therefore we hypothesize in the Genepi-lowRT program that *in vitro* irradiation with low dose ≤100mGy evokes a transcriptional response that relates closer to the late complications to radiotherapy. We intend to identify by micro array analysis, genes or gene sets of which expression after low dose radiation can discriminate between breast cancer patients of the Genepi cohort that differ in normal tissue toxicity. As a first step towards this goal we have analysed gene expression patterns of T-lymphocytes of 2 normal individuals after radiation with a range of low doses to detect possible low-dose specific pathways, dose response relationships and to compare low and high dose transcriptional responses.

MOBILE DNA ELEMENTS THROUGHOUT EVOLUTION

Jerzy Jurka

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Eukaryotic genomes contain vast deposits of so-called “repetitive DNA,” derived mostly from active or extinct transposable elements (TEs). Analysis of genomic sequences revealed a wealth of information about the origin, diversity and genomic impact of these elements. DNA sequence analyses revealed new classes of TEs, including *Helitrons*, *Polintons,* and numerous superfamilies. TEs are evolutionary precursors of many genes, including the RAG1 protein gene in our immune system. They are the driving force in the evolution of epigenetic regulation and they have long-term genetic impact on genomic stability and evolution. Remnants of ancient TEs appear to be overrepresented in regulatory modules, which raises considerable interest in their impact on gene regulation envisioned decades ago by Britten and Davidson. I will present an overview of recent studies of TEs with emphasis on their evolutionary role in gene regulation and speciation.

RESPONSE OF TUMOUR VASCULATURE TO VASCULAR DISRUPTING AGENTS AND RADIATION

Chryso Kanthou, Sara Jane Lunt, Gillan M. Tozer

Cancer Research UK Tumour Microcirculation Group, Academic Unit of Surgical Oncology, School of Medicine and Biomedical Sciences, University of Sheffield, Beech Hill Road, Sheffield, S10 2RX, United Kingdom.

The tumour vessel network is essential for tumour growth and metastasis and is therefore an important target for therapy. Vascular Disrupting Agents (VDAs) are a relatively new class of low molecular weight drugs that cause necrosis of the tumour mass by inducing selective, rapid and profound disruption of established tumour blood vessels. Combretastatin A-4-Phosphate (CA-4-P) is a lead tubulin-binding VDA, with tumour selectivity currently undergoing advanced clinical development. CA-4-P leads to a rapid rise in vascular permeability within the tumour, which is thought to be central to the mechanism by which blood flow shutdown and vascular collapse occur. Susceptibility to CA-4-P and other VDAs is ascribed to the "immature" nature of blood vessels within the tumour, which have defective pericyte coverage and are highly permeable. Although VDAs induce substantial tumour necrosis, on the whole they are ineffective at halting tumour growth when used as single agents, as tumour re-growth initiates from the peripheral tumour outer rim, which is generally resistant to this type of therapy.

Recently, progress has been made when using VDAs in combination with conventional radiotherapy, which targets tumour cells directly. Significant improvements in tumour growth delays have been reported in pre-clinical models and this combination is also undergoing clinical testing with some positive outcomes. The success of this treatment combination is thought to be due to selective targeting of cells within the viable and better-oxygenated tumour outer rim by radiation, while VDAs are more effective within the central hypoxic regions of a tumour. Optimal responses have been shown to be dependent on scheduling. Indeed, radiation is more effective if administered before the VDA, which inevitably results in hypoxia. In addition to targeting tumour cells, radiotherapy also damages blood vessels, and therefore potential interactions between radiation and VDA effects on the tumour vasculature exist. At the cellular level, both CA-4-P and radiation cause rapid remodelling of the endothelial cytoskeleton, and a rise in permeability, and these events occur through similar signaling mechanisms involving activation of RhoA-GTPase signaling pathways.

This presentation will evaluate current knowledge of interactions between VDAs and radiotherapy and will address issues of resistance and susceptibility. Understanding the mechanisms by which radiation and VDAs interact to target tumour blood vessels is important in order to maximise the efficacy of this therapeutic strategy.

Funded by Cancer Research UK

CHARACTERIZING PROTEIN POST TRANSLATIONAL MODIFICATIONS USING MALDI MASS SPECTROMETRY

Rachel Martin

Shimadzu Biotech, Manchester, UK

Post translational modifications (PTMs) are critical to the understanding of proteins in biological systems. They can include, amongst others, the addition of functional groups, the addition of other peptides or proteins, structural changes or substitution/delete/inclusion of amino acids.

They are particularly relevant for study as they may alter physical and chemical properties, folding, conformation distribution, stability, activity, and consequently, function of proteins. Examples include phosphorylation for signal transduction, ubiquitination for proteolysis, attachment of fatty acids for membrane anchoring and association, and glycosylation for protein half-life, targeting, and cell:cell and cell:matrix interactions.

In this presentation, we investigate the use of MALDI mass spectrometry for the analysis of post translational modifications. We demonstrate not only how the nature of the modification, for example oxidation, phosphorylation or glycosylation, can be determined, but also how the position of the modification can be identified. We will show examples using MALDI TOF, MALDI TOF/TOF and MALDI Ion Trap TOF data on a wide variety of PTMs.

THE TARGETING OF MELANOMA FOR ENDORADIOTHERAPY

Walter Mier, Michael Eisenhut, Uwe Haberkorn

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The incidence of malignant melanoma continues to rise. In the USA, the American Cancer Society predicts that there will have been 62190 new patients and 7910 deaths in 2006. Because of the lack of efficient chemotherapeutic agents, the treatment of metastatic melanoma with currently available drugs like nitrosoureas and the triazene dacarbazine results in low response rates. The primary treatment of melanoma usually involves surgical removal of the tumor. However, surgery may not be an option when the tumor exceeds three millimeters in diameter, as it may have already spread to other areas. For those melanoma patients who are not candidates for surgery or whose disease has spread or metastasized, therapeutic approaches such as external beam radiation, chemotherapy and immunotherapy (treatment to modulate the body's immune system) to elicit a therapeutic response, are often used.

Benzamides have been found high and long lasting uptake in melanoid structures of the uveal tract of pigmented C57Bl6 mice. This observation was especially prominent using [^{125}I]BZA (*N*-(2-diethylaminoethyl)-4-[^{125}I]iodobenzamide) and the B16 melanoma mouse model. It was, therefore, attractive to investigate whether the carrier characteristics of *N*-(2-(diethylamino)ethyl)benzamide derivatives or compounds combined with the 2-diethylaminoethylamine pharmacophore can be exploited for selective cytostatic drug delivery. Indeed, radiolabeled conjugates of chlorambucil with procainamide (CHL-BZA) and 2-diethylaminoethylamine (CHL-DEAE) revealed high uptake in B16 melanoma cells. The selectivity was confirmed with biodistribution studies in the B16/C57Bl6 mouse melanoma model. The reason for benzamide-melanoma selectivity may be found in the embryonic origin of melanocytes. They are derived from the neural crest, which is a completely different source than that of the surrounding keratinocytes. Melanoma cells resemble, therefore, nerve cells with similar transporters for basic drugs. Owing to our clinical results that radioiodinated BZA can be used as an imaging agent for metastatic melanoma screening experiments with a number of benzamide derivatives have been performed.

BYSTANDER EFFECTS AND ADAPTIVE RESPONSES INDUCED BY RADIATION EXPOSURE

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This presentation reviews our current knowledge of the mechanisms underlying the induction of bystander effects by low dose low LET ionizing radiation and discusses how they may be related to observed adaptive responses or other protective effects of low doses exposures. Bystander effects appear to be the result of a generalized stress response in tissues or cells. The signals may be produced by all exposed cells but the response appears to require a quoram in order to be expressed. The major response involving low LET radiation exposure discussed in the existing literature is a death response. This has many characteristics of apoptosis but is p53 independent. While a death response might appear to be adverse, the position is argued in this paper, that it is in fact protective and removes damaged cells from the population. Since many cell populations carry damaged cells without being exposed to radiation (so called “background damage”, it is possible that low doses exposures cause removal of cells damaged by agents other than the test dose of radiation. This mechanism would lead to the production of “U-shaped” dose response curves. In this senario, the level of “adaptive” or beneficial response will be related to the background damage carried by the cell population. This model may be important when attempting to predict the consequences of mixed exposures involving radiation and other environmental stressors.

IN SILICO STUDY SUGGESTS THAT RALTEGRAVIR-RESISTANT MUTATIONS MODIFY THE DNA RECOGNITION PROPERTIES OF HIV-1 INTEGRASE

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Raltegravir is the first effective antiretroviral agent belonging to the novel class of HIV-1 IN inhibitors. Virologic failure under Raltegravir treatment was associated with IN mutations following at least two distinct genetic pathways which involve mutation at either Q148(H/R/K) or N155(H) and one or more secondary mutations unique to each pathway, in particular the G140S mutation in the Q148 pathway. To determine the origin of the resistance, we compared the structural and recognition properties of the wild-type and mutant INs. 2D prediction and 3D molecular modelling were performed: (i) to establish the folding of the 140-149 catalytic loop; (ii) to probe the influence of the Mg^{2+} co-factor and its binding mode on the catalytic core structure; and (iii) to study the structural effects of the drug-induced mutations.

We observed that the native and mutant INs show a similarity for general enzyme folding. We found that the 140-149 catalytic loop is characterised by a striking conservation of a Ω-shaped hairpin, a structural element involving 144-148 residues, stabilised by multiple H-bonding spanning across the loop. Folding of the hairpin is due to the strong conformational preferences of the N, P, Q, S and G residues. This hairpin which can move from 16 to 4.5 Å towards the active site as a rigid body in a gate-like manner remains topologically invariant with respect to the presence of mutations selected under raltegravir. In contrast, there was a striking difference between the wild-type and mutant INs in regard to their specific recognition by DNAbases. The native N155 and Q148 show a clear preference for binding with adenine, interacting by pair of strong H-bonds whereas the R, K and H mutant strongly favor pyrimidines. Furthermore, we observed that the secondary mutation G140S which is readily observed following the selection of the primary Q148(H/R/K) mutation, modified the mobility of the catalytic loop, thereby probably reflecting an adaptation to the change of base specificity induced by Q148R/H/K mutation. This alteration provides a molecular explanation for Raltegravir inhibition. Indeed, we observed that this compound is an adenine bioisoster, thus capable to compete with the 5'-AC overhang for the close contact with Q148 that was experimentally observed. The loss of this contact is compensated by the H/R/K148 mutant which retains DNA recognition, while these mutations impairs Raltegravir binding to IN.

Finally, we observed that two Raltegravir fragments act as Adenine thereby suggesting two possible independent contacts with Q148 and N155, thus providing a rational for the two independent resistance pathways.

CITRATE TRANSPORT IN HUMAN PROSTATE CANCER CELLS: REGULATION BY FUNCTIONAL VOLTAGE-GATED NA^+ CHANNEL EXPRESSION

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Prostate gland is a unique mammalian organ which produces and releases large amounts of citrate into prostatic fluid (up to 180 mM). This is a necessary energy substrate for sperms' motility and vitality. Importantly, citrate level in prostate cancer (PCa) drops significantly and this drop is considered to be a necessary step in malignant transformation. We have determined that citrate is released from normal human prostate epithelial (PNT2-C2) cells by a K^+-dependent transport mechanism designed primarily to transport citrate in the outward direction. Interestingly, PCa (PC-3M) cells were found to express a Na^+-dependent citrate transport mechanism which facilitates uptake of citrate. A K^+-dependent transport mechanism was also present in PCa cells but with reduced activity. It was shown earlier that, unlike normal prostate epithelial cells, metastatic PCa cells expressed functional voltage-gated Na^+ channels (VGSCs) which potentiated the cells' metastatic behaviour. Instantaneous application of TTX, specific blocker of VGSCs, on PC-3M cells had no effect on citrate transport. However, long-term (24 h) incubation resulted in significantly decreased Na^+-dependent citrate uptake. It was concluded that VGSC activity is involved in expression of the Na^+-dependent transport mechanism in PCa cells. This conclusion is consistent with functional VGSC expression being an early event in metastatic progression in PCa.

FOLATE, AMINOTHIOLS AND CYSTATHIONINE-B-SYNTHASE IN HUMAN PLACENTA

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Folate-mediated one-carbon unit metabolism has been a topic of discussion in the obstetrical community for several decades since maternal folate deficiency was related to fetal central nervous system malformations and periconceptual folate supplement was assessed to diminish these manifestations [Ueland, Vollset 2004; van der Put et al., 2001; Finkelstein 2000a]. Folate is present in the organism as a family of structurally related derivates of tetrahydrofolate (THF). In mammalian tissues folate, the generic term, functions as substrate in series of interconnected metabolic cycles involving thymidilate and purine (adenine and guanine) biosynthesis, methionine (Met) cycle, serine and glycine interconversion and metabolism of histidine and formate [van der Put 2001; Suh et al, 2001]. Thus, folate indirectly is involved in essential cell functions, including biosynthesis of nucleic acids and proteins, transmethylation reactions, maintenance of cellular redox status by folate-dependent aminothiols. The mammals cannot synthesize folate *de novo* and obtain it from the diet and intestinal flora.

Methionine cycle is a heart of folate-related metabolism. It is tightly associated with THF cycle. 5-MeTHF after donation of its CH_3 group to Hcy remethylation converts into THF that accepts one-carbon unit in result of serine to glycine interconversion catalyzed by serine hydroxyl methylase while synthesis of glycine and 5,10-methylene THF. 5,10-methyleneTHF is reduced to 5-MeTHF by the key enzyme of this cycle - methylenetetrahydrofolate reductase. MTHFR is a polymorphic enzyme. Its alleles with C677 $\rightarrow$ T677 or alanine 222 to valine substitutions are highly representative in Caucasian population [Frosst 1995]. This mutation is associated with thermolability and lower catalytic activity of MTHFR, elevated Hcy and lower plasma folate and increased risk of neural tube defects [De Franchis 1995, van der Put 2000, Shields 1999].

Methionine cycle is intimately connected with transsulfuration pathway via homocysteine (Hcy) that may follow either remethylation in methionine cycle and conserve methioine or irreversible catabolism by consequent activities of cystathionine β-synthase (CBS) and cystathionase γ-lyase while synthesis of cysteine used for protein synthesis and/or catabolized to taurine and sulfate. The inability to eliminate Hcy by transsulfuration may make the corresponding tissues especially vulnerable to elevated concentration of Hcy that is considered as a potential marker and cause or contribute to a wide range of obstetrical disorders.

The active role of folate related metabolism in human placenta will be discussed on the basis of our own research.

MOLECULAR ORGANIZATION IN SELF-ASSEMBLED MONOLAYERS USED FOR NEURONAL CELL GROWTH

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The main goal of the presented project is to develop material surfaces that may be recognized as biological and can form truly biocompatible interfaces with the tissue. We are developing two-dimensional (2-D) and three-dimensional (3-D) scaffoldings to connect neurons over long distances and to investigate neuron interfaces with microsystems by using biocompatible, self-assembled monolayers (SAMs) to direct and control the growth of neurons. To accomplish this goal we need to be able to control the growth of neural tissue as well as the locations and patterns in which the cells grow.

A significant finding in our preliminary research on the patterned growth of neurons on the surface of micro electrode arrays has shown that we have the ability to encourage the selected growth of neural cells into specific micro patterns. Neuron adhesion efficiency was studied for amino-terminated, carboxy-terminated and 1:1 mixed alkanethiol SAMs deposited on gold substrates. By varying both the specific chemistry and pattern size we have been able to precisely control the growth of the neurons to predetermined 2-D locations. Using photolithography techniques we have been successful in finding optimal features for positioning cells. To assess the suitability of SAMs for neuronal growth, we have investigated correlations of neuron adhesion with the chemical structure at the SAM surface characterized by Sum Frequency Generation (SFG) vibrational spectroscopy and with the roughness of gold substrate and thickness of SAM monolayers studied by X-ray spectroscopy (XPS) and atomic force microscopy (AFM). We conclude that the neuronal cells adhesion is not critically affected by the surface roughness within 0.7-2 nm range or details of the molecular ordering and orientation of the terminal amino groups, but only on the chemical functionality displayed at the surface. This approach can be used to create new methods that help map structure-property relationships of biohybrid systems and lead to the possibility of utilizing this method to direct neurons in 3-D scaffoldings to interconnect over relatively long distances in a controlled and precise manner.

This complex system will be designed to aid in the understanding the neuronal growth and behavior and may enable precise and localized neuron stimulation and surveillance for both biological research and medical applications.

APOPTIN AND ITS DERIVATIVES AS MOLECULAR TEMPLATES FOR THE DEVELOPMENT OF BCR-ABL KINASE INHIBITORS

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The *BCR-ABL* chimeric oncoproteins are key factors responsible for development and progress of Philadelphia positive (Ph+) chronic myeloid leukemia (CML) and some other hematologic malignancies. Gleevec/Imatinib, the clinically used inhibitor of BcrAbl kinase is not sufficiently effective to cure these malignancies in a single-agent therapy. In the search for a more potent inhibitor of Bcr-Abl, we tested a naturally occurring molecule, called apoptin. Apoptin is a 14 kDa protein derived from chicken anemia virus, and is known to induce apoptosis in a wide range of transformed, but not primary cells. We found by an array-based analysis that apoptin interacts with the SH3 domain of Abl. The specificity of these interactions were further confirmed by high stringency pull-down and co-immunoprecipitation assays. Subsequently, a set of apoptin and Bcr-Abl deletion mutants were used to precisely map this interaction site that mainly occurrs between a proline rich domain of apoptin and the SH3 domain of Bcr-Abl. Furthermore, apoptin was able to modify the activities of a series of targets downstream of Bcr-Abl kinase (e.g. CrkL, STAT5, c-Myc).

Imatinib and apoptin had comparable effects on $BcrAbl^+$ CML cells, whereas apoptin was also active on Imatinib-resistant cells. Finally, a computational algorithms for protein modeling to study the 3D structure of apoptin and its docking with Bcr-Abl at the molecular level was applied to gain further insight into mechanism of inhibitory action of apoptin. Our work provides important insights towards the development of peptide based tyrosine kinase inhibitors as new anti-cancer agents.

STATISTICAL PROCESSING OF DNA MICROARRAY DATA - DETECTING SUBTLE CHANGES OF GENE EXPRESSIONS

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For some DNA microarray datasets, standard methods of gene expression profile analyses fail to provide statistically significant conclusions, yet there is evidence that these datasets might potentially lead to discovering interesting genetic mechanisms. An example of such datasets are the results of experiments related to low dose irradiation which contain only subtle differential expressions and therefore need special methods of analyses. In the presentation we overview methods of tuning methodologies of DNA data analyses to the problem of detection of subtle (low level) differences and signals in DNA microarray data.

The results are based on the analysis of existing DNA microarray datasets related to sensitivity of patients and cells to irradiation.We studied several possible hypotheses concerning developing procedures for detecting low level signals and/or differences in DNA microarray data. These hypotheses concerned different levels of DNA microarray data processing: (i) the level of probes and cells and the level of DNA microarray data quality control, (ii) the level of annotations of genes corresponding to probes, (iii) the level of signal processing and classification algorithms. In the research we have composed and verified algorithms of different structures, based on the analyzed datasets. The methodology for verification of the efficiency of different approaches was based on comparing predictive powers of classifiers with the use of multiple random validation tests.

We observed that introducing various changes to constructions of classifiers, such as introducing rules of selections for genes, selecting genes by using quality control procedures or fitting classifiers to hypotheses concerning probability distributions of expression signals, may lead to differences of predictive powers of classifiers of the order of 10-20% of errors in the verification step.

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TUMOR DEVELOPMENT MODEL UNDER ANGIOGENIC SIGNALING WITH DEPENDENCE ON VESSEL IMPAIRMENT

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In adults the normal physiological role of angiogenesis is restricted to wound healing, menstrual cycle and pregnancy. Unfortunately, it is also essential for the successful growth and development of solid tumours. After reaching avascular dormant state tumour can grow further only by inducing host tissue to sprout capillary tubes which migrate towards and ultimately penetrate the tumour, providing it with a circulating blood supply and, therefore, an additional source of nutrients.

Based on the idea that carrying capacity for any solid tumour depends on its vessel density Hahnfeldt, et. al created a mathematical model of tumour growth under angiogenic signalling. Dr. Judith Folkman who discovered process of tumour angiogenis proposed that tumour can be treated by influencing that process. On the basis of the Hahnfeldt, et. al model some protocols of antiangiogenic treatment were proposed.

Unfortunately, recent studies show that tumour angiogenesis is highly pathological. Long lasting overexpression of proangiogenic factors (like VEGF) causes impairment and malfunction of newly formed vessels. We propose the model of vessel impairment in the process of tumour angiogenesis, which is based on the idea of the Hahnfeldt, et al. model. In the proposed model carrying capacity depends also on the process of vessel impairment. Simulation of the model solutions shows that tumour vascular dormant state can be reached in two different ways. In addition, in each case efficiency and effects of standard chemotherapy, antiangiogenic treatment and combined treatment are different.

NEW INSIGHTS ON BYSTANDER SIGNALLING: THE ROLE OF DNA DAMAGE SENSING AND REPAIR

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Cells have evolved complex processes to maintain the stability of their genomes. In response to genotoxic stress, the DNA damage response (DDR) is activated whereby a series of interlinked sensor processes signal to a panel of repair pathways which can attempt to repair the damage. Recent studies have shown compelling evidence for the activation of DDR by ionising radiation even when radiation is not directly deposited in the DNA within the nucleus. Our own studies, using both charged particle and X-ray microbeams, have shown that bystander responses are activated when cells are not directly irradiated through the nucleus. A key modulating role is played by mitochondria under these conditions, probably acting as a source for reactive oxygen species production. In a range of studies we have started to elucidate the relevance of DNA damage and repair pathways in both irradiated and bystander cells.

In previous work we have shown formation of γH2AX foci in bystander cells ~ 30 minutes after irradiation and its persistence for up to 48 hours in a range of cell types. This may be due to persistent formation of DNA damage in bystander cells, lack of removal of the γH2AX signal and/or compromised repair. From studies with repair deficient mutants it is clear that dsb repair type processes are involved in bystander cells as cells deficient in components of the non-homologous end-joining pathway show increased bystander responses. In further work, we now have evidence in bystander cells that the initial phosphorylation of H2AX is performed by the ataxia telangiectasia and Rad3-related protein (ATR) rather that ataxia telangiectasia mutated protein (ATM) or DNA dependent protein kinase (DNA-PK). This occurs predominantly in S-phase cells and supports the assertion that damage accumulation in bystander cells leads to stalled replication forks. After this initial ATR-dependent response, ATM dependent signalling plays a role downstream in determining overall biological response.

Overall these studies are changing our views of radiation responses in cells and tissues leading to a more comprehensive appreciation of DDR in the maintenance of genomic stability.

GENISTEIN DERIVATIVES AND THEIR BIOLOGICAL ACTIVITY

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Among health benefits of high genistein intake are lowered incidence of cardiovascular diseases, prevention of osteoporosis, attenuation of post-menopausal problems and cancer chemoprevention. Antitumor activity of this isoflavonoid can manifest itself through a spectrum of effects including cytotoxic, cytostatic and antiangiogenic action, induction of tumor cell differentiation, prevention of metastasis, attenuation of multidrug resistance, reactivation of relevant genes silenced by methylation and others. Genistein sensitizes cells to radiotherapy and selected cytostatics, thus it may be considered as a drug for use in combination with routine cytostatics or radiation. Due to its recognized chemopreventive and antitumor potential, genistein is thus a molecule of great interest as a lead compound in drug design. In our approach genistein was modified by glycosylation with lipophilic sugar moieties. Some of these novel synthetic derivatives of genistein were found to posses a significant cytostatic and cytotoxic activity against multiple cancer cell lines.

We report here the results of our study aimed to determine the molecular mechanism of cytotoxicity of the highly active synthetic glycosidic derivative of genistein, termed G21. We show that, in contrast to genistein, G21 exhibits aneugenic activity and has the ability to alter tubulin dynamics, as well as to destroy mitotic spindles. Cancer cells exposed to G21 arrest in mitosis at G2/M phase and are eliminated through apoptosis and mitotic death. Polymerization of tubulin in an *in vitro* assay was found to be inhibited by G21, indicating that this compound interacted with tubulin directly. To our best knowledge, G21 is the first derivative of genistein able to efficiently affect microtubule dynamics. These promising results prompted us to synthesize a novel series of derivatives in which glycosidic moieties were conjugated with genistein through 2-5 carbon linkers at C-7. Antiproliferative potential of these new glycoconjugates of genistein is currently under study. Our results indicate that some glycosides and glycoconjugates of genistein exhibit higher cytotoxicity, as compared to genistein, and exert their antiproliferative action through mechanisms different from those of parent compound, genistein.

TRANSCRIPTION PROFILE CHANGE AFTER IRRADIATION OR IN BYSTANDER CELLS; DIFFERENCES BETWEEN CELL LINES

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Cells exposed to ionizing radiation release factors which can induce DNA damage, chromosomal instability and apoptosis in neighboring untreated cells, phenomena known as bystander effects. We used genome-wide microarrays to compare changes in transcript profiles of human cells grown in culture medium from irradiated cells (irradiation conditioned medium, ICM) with those which occurred in melanoma Me45 and lymphoblastoid K562 cells after IR.

Under both conditions, among Me45 transcripts there were more than 10 000 genes present at increased or decreased levels, using the criterion of a >±10% change; and >85% of these were common to growth in ICM and after IR.

For K562 cells more than 6 000 genes showed a changed transcript level under bystander as well as irradiated growth conditions, as compared to control cells; the level of more than 3 000 transcripts was higher than that in control cells and in more than 70% of these transcripts the level showed the same response (increase, decrease or no change) and only 0.6% showed an opposite response.

Grouping of transcripts into functional pathways showed that, under both examined conditions, it was neuroactive ligand- and cytokine-cytokine-receptor interactions as well as Jak-STAT, MAPK, and other signaling pathways that contained the largest number of up- or down-regulated transcripts. The similarities between the responses of the transcriptome in bystander and irradiated cells and the kinetics of some transcripts' change were confirmed by quantitative RT-PCR. The level of transcript groups in some cell-cell communication and signaling pathways was decreased less during growth in ICM or after irradiation compared to control cells, an effect of possible importance for long-term bystander effects such as genome instability.

SOMATIC EXOCYTOSIS OF SEROTONIN MEDIATED BY MOLECULAR MOTORS

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We quantified somatic exocytosis of serotonin in Retzius neurons and explored the possible contribution of molecular motors and the cytoskeleton on the mobilization of vesicles induced by stimulation with trains of electrical impulses. Secretion was quantified from the increase of fluorescence of FM1-43 in response to sequences of *20 mV* trains of *10* impulses at *2 min* intervals, produced by intracellular current injection. Somatic secretion was also evoked by a pulse of *10 mM* caffeine applied to the bathing solution.

Stimulation of neurons produced a gradual increase in FM1-43 fluorescence for over the next five minutes. The kinetics and latencies of these increases varied from one neuron to another but usually maintaining a sigmoidal shape in one or two steps. Neurons stimulation in the presence of colchicine to uncouple microtubules, failed to evoke fluorescence increases, thus suggesting that vesicle mobilization depended on tubulin-based motor.

The kinetics of the fluorescence increase in individual neurons was accounted for by using a model based on a diffusion equation in the presence of external forces, consistent with the contribution of molecular motors to the mobilization of the vesicle clusters towards the membrane in response to electrical activity. Our data show that somatic serotonin secretion in Retzius neurons depends on a motor-based cytoskeletal mobilization of vesicles induced by electrical activity.

IDENTIFICATION OF THE DETERMINANT OF HEPATITIS B VIRUS LIVER TROPISM AND ITS IMPLICATIONS FOR HEPATOCYTE-SPECIFIC DRUG TARGETING

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A hallmark of Hepatitis B Virus (HBV) infection are the extraordinary specificity and efficiency by which virions target hepatocytes in the liver. This property has been attributed to (a) specific interaction(s) of (a) viral envelope protein(s) with a yet unknown receptor. We have recently shown that the subcutaneous administration of low doses of a myristoylated peptide corresponding to the N-terminal 47 amino acids (a.a.) of the preS1-domain of the HBV large surfaceprotein blocks HBV infection in an animal model (Nat. Biotech., 2008). Our observation that this peptide selectively targets the liver of non HBV-susceptible mice suggests that it encompasses a species-independent determinant of hepatotropism.

We characterized the compulsory a.a. sequence and the role of the fatty acid modification with respect to their contribution to target the peptide to hepatocytes and analyzed the kinetics of uptake of fluorescently labeled derivatives in vivo. Using a series of HBVpreS lipopeptides carrying deletions, point mutations, D-amino acid exchanges, sequence permutations and lipid variations we found that (i) N-terminal acylation prevents renal secretion of the peptide and leads to systemic retention, (ii) a highly conserved 7 a.a. sequence motif is the pharmacophor required for liver-targeting (iii) peptides containing this sequence are taken up by hepatocytes and accumulate within the cells. This process is highly specific and differs from constitutive hepatic delivery via the blood, since single amino acid exchanges within the conserved motif resulted in a disperse distribution of the peptide in many organs.

Aside from important implications concerning the species specificity of receptor recognition of HBV, HBVpreS-mediated drug targeting opens a highly selective approach to deliver drugs to hepatocytes or hepatoma cells. Possible applications include the delivery of interferons, inhibitors of HCV or HBV replication, cell cycle inhibitors for HCC treatment, inhibitors of plasmodium falciparum, siRNAs or peptides for MHC-mediated antigen presentation.

SYNTHETIC GLYCOMIX FOR MINING NATURAL PRODUCTS AS DRUG LEADS

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Majority of proteins are post-translationally modified by glycosylation, which enhances their proper folding and stability, and also influences their functioning. With all due proportion, it can be postulated that low molecular weight ligands, designed as drugs, can also be advantageously modified by chemical glycosylation using natural or synthetic sugars. Suitably selected sugar moieties can render pharmacophoric structures properties desirable in a pro-drug category. In particular, they can serve as: i) an active transport and biodistribution enhancers; ii) protection against phase II metabolism bioconjugation leading to excretion; iii) providers of a lipophilicity element needed for membrane, receptor and binding pockets docking [1]. Several classes of plant polyphenols (flavonols, catechins, isoflavones etc.) are currently studied as antioxidants, detoxicants, chemoprotectants, immunomodulators, antitumor agents, regulators of lipids metabolism and cardiovascular health promoters, but only few lead compounds have reached clinical trials level [2]. Apparently, the main reasons for slow progress in pharmaceutical development of these secondary metabolites in their native state are their sub-optimal physicochemical properties, low bioavailability and unfavorable metabolism.

We focused our attention on soy isoflavonoids which are drug candidates in antitumor therapy and started a research program extending from chemical derivatization of genistein to biological activity studies of its new derivatives. From this perspective, of particular interest are regio- and stereoselective syntheses of O-glycosides, and glycoconjugate derivatives of 2,3-unsaturated mono- and disaccharides with genistein.

The effect of synthesized compounds on proliferation of selected cancer cell lines will be discussed in some detail.

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MITOCHONDRIAL POTASSIUM CHANNELS

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Mitochondrial potassium channels are believed to contribute to cytoprotection of injured cardiac and neuronal tissues. The following potassium channels have been described in the inner mitochondrial membrane: the ATP-regulated potassium channel, the large conductance Ca^{2+}-activated potassium channel, the voltage-gated Kv1.3 potassium channel, and the twin-pore domain TASK-3 potassium channel. The putative functional roles of these channels include changes in mitochondrial matrix volume, mitochondrial respiration, and membrane potential. In addition, the activity of these channels modulates the generation of reactive oxygen species by mitochondria. In this presentation, we discuss recent observations on three fundamental issues concerning mitochondrial potassium channels: (i) their molecular identity, (ii) their interaction with potassium channel openers and inhibitors, and (iii) their functional properties.

PROTEOMICS: AUTOMATED ANALYSIS OF ELECTROPHEROGRAMS

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Two dimensional gel electrophoresis is a powerful technique of protein separation, based on two independent properties of proteins, i.e., on the differences in their isoelectric points and molecular weights. This method is used as a main tool for protein separation in proteomics, its goal being determination of protein composition in cells and tissues.

In order to study differences in protein expression in healthy and diseased tissues, many samples ought to be compared in order to eliminate differences caused by natural diversity of samples. Identification of proteins responsible for a disease studied can be performed based on a comparison of the 2D gel digital images. This, however, requires effective approaches of image enhancement, warping, normalization and significance analysis. In the start-to-end approach proposed by us [1-4], all steps of data analysis are automated. Moreover, data analysis is performed at the pixels level, instead of the spots level [4, 5], thus eliminating main drawbacks of the existing software packages, i.e. with missing elements in the data table (due to the requirement of spots detection). Additionally, multivariate significance analysis is proposed, based on Partial Least Squares discriminant analysis with the randomization tests, which allows minimization of the false discovery rate.

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Poster abstracts

Posters with underlined titles participate in poster competition.
Numbers beside the abstract title are poster numbers.

1. TYPE OF HPV 16/18 INFECTION, PROLIFERATION RATE, MICROVESSEL DENSITY AND EXPRESSION OF P53 PROTEIN AS PREDICTORS OF TUMOUR RESPONSE TO CONCURRENT CHEMORADIOTHERAPY IN CERVICAL CANCER

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Results concerning predictive significance of type of HPV 16 and 18 (Human Papilloma Virus) for concurrent chemoradiotherapy of cervical cancer are conflicting. Some data indicate that presence of integrated or episomal HPV DNA in tumour cells can influence treatment outcome. Also, little is known about biological characteristics of cervical tumours with different type of HPV infection. Therefore, the aim of presented study was to assess the influence of HPV 16/18 infection type, proliferation rate, microvessel density and P53 status on treatment outcome (disease free survival - DFS) of patients with cervical cancer undergoing concurrent chemoradiotherapy (CRT).

Tumour biopsies were obtained before treatment from 64 patients with squamous cell carcinoma (SCC) of the cervix. Median patients` age was 52 years (ranging from 37 to 80). There were 4 tumours at FIGO stage IB, 13 with IIA, 20 with IIB and 19 with IIIB. All biological parameters were assessed using formalin fixed, paraffin-embedded tumour samples. HPV 16/18 infection type was detected by *in situ* hybrydization. To assess the type of HPV infection, two parameters were analyzed: (1) the percentage of infected tumour cells (%HPV) and (2) type of HPV signal: diffuse (DS) - representing episomal HPV DNA, and punctuate (PS) - representing integrated HPV DNA. Proliferation rate (Ki67 labelling index - Ki67 LI), microvessel density (MVD) and P53 proteins status (P53 labelling index – P53 LI) were assessed on the basis of immunohistochemical staining. Apoptotic level (apoptotic index - AI) was evaluated by TUNEL method.

In the examined group, the percentage of HPV positive tumours was 98.4, with mean % HPV value of 44.0 ± 3.0. There were 45.0% of tumours with both types of HPV signal (punctuate and diffuse) in tumour cells and 55.0% with only punctuate signal. In the studied group, no tumours showing diffuse HPV signal only were observed. There were no correlations between type of HPV infection and clinical features (patients' age, menopausal status, clinical stage FIGO and histopathological grade). The mean values of Ki-67 LI, MVD, P53 LI and AI ± SE were: 48.8%±1.5, 80.7 microvessel/mm^2 ± 5.4, 11.9% ± 2.0 and 0.8% ± 0.09, respectively. Significantly faster proliferation rate was found in case of tumours from younger patients (≤ 52 years) and a woman before menopause ($p = 0.028$, $p = 0.029$, respectively). No correlations between clinical stage (FIGO) or histopathological grade and assessed biological features were found. There were also no significant associations between type of HPV infections and biological parameters. Significantly higher probability of DSF was found for patients with faster proliferating tumours ($p = 0.005$) and those with tumours showing higher apoptotic index ($p = 0.002$). Additionally, tumours with lower percentage of HPV 16/18 infected cells and with both types of HPV signal and higher MVD showed a trend for better DFS ($p = 0.087$, $p = 0.06$, respectively). However, Cox multivariate analysis showed that only higher apoptotic index was an independent favourable prognostic factor for DFS.

The data presented here indicate that the apoptosis level may be used in cervical cancer clinical practice as a predictor of tumour response to concurrent chemoradiotherapy.

2. SYMMETRICAL AND UNSYMMETRICAL AMIDE-CONJUGATED α,ω-NUCLEOSIDES – THE SYNTHESIS AND POTENTIAL INTERCALATING ACTIVITY

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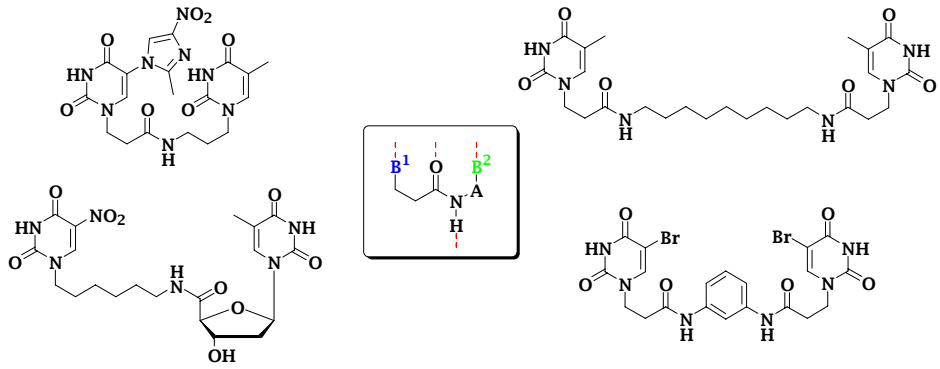
Intercalators are molecules that insert perpendicularly with respect to the axis of DNA, generally forming all types of weaker bonds than covalent ones. They include van der Waals, hydrogen bonding, hydrophobic, charge transfer forces and, presumably, frontier orbital interaction. The intended chemical structures designate the nature of reversible interactions DNA strand–intercalator. As the consequence, a large number of diverse classes of compounds is used in medical treatment, *e.g.* acridines, alkaloids, anthracyclines, anthracenediones, arylaminoalcohols, coumarins, indoles, phenanthridines, quinolines, quinoxalines, *etc.* [1].

Herein we report a synthesis of potential intercalators – symmetrical and unsymmetrical α,ω-nucleosides based upon aliphatic or aromatic linkages containing one or two amide bonds. The title compounds (Figure), subdivided into four groups, were synthesized in the final stage, via condensation of various units bearing carboxylic and amine groups, in the presence of 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM). Moreover, for α,ω-diaminic aliphatic linkages, a selective aminolysis was achieved.



The units containing carboxylic group were obtained by acidic hydrolysis of Michael N^1-adducts of 5-substituted uracil derivatives to methyl acrylate [2]. The main reactant bearing primary amine group on short aliphatic linkage was synthesized by reduction of Michael adduct of thymine to acrylonitrile [3]. Additionally, as the amine reactants 1-(ω-aminoalkyl)-5-nitrouracils [2], 5'-amino-5'-deoxy-*β*-thymidine, 5-aminouracil and adenine have been applied.

The ability of dedicated molecules to form highly ordered assemblies was indirectly confirmed using electron microscopy (STM, TEM). The intercalating properties are under investigation at the Nucleic Acid Centre in Odense, Denmark.

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3. ON THE GALVANOTAXIS OF PROSTATE CANCER CELLS

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The Mat-LyLu prostate cancer cells exhibit a galvanotaxis that is independent of external Ca^{2+} concentration. This should be the case in the cathodal galvanotaxis. We explain a possible mechanism of galvanotaxis in prostate cancer cells which does not depend on extracellular Ca^{2+} concentration but relies on the action of sodium channels.

4. KARYOMETRIC FEATURES OF CELL NUCLEI IN PAPILLARY THYROID CANCER AND THEIR CORRELATES IN GENE EXPRESSION PROFILE

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Diagnostic criteria of papillary thyroid carcinoma (PTC) are defined by the presence of characteristic cell nuclei features. We hypothesize that these features are driven by specific molecular mechanisms and assess whether karyometric parameters of PTC nuclei do correlate with gene expression profile assessed by microarray analysis.

Post-operative thyroid frozen samples and respective paraffin blocks from 41 patients with PTC were analyzed. Images were obtained by light microscopy with a semiautomatic computer image analyzer. We calculated parameters directly related to nuclear size, shape and chromatin distribution. For each karyometric parameter, mean value and its variance were assessed and correlated with gene expression patterns by permutation-based test. Global significance was calculated for relation of each parameter to gene expression profile (evaluated by Affymetrix HG-U133A). Validation was carried out in the independent group of 36 PTCs by QPCR; in the same specimens karyometric analysis was carried out as described previously.

We found out that the variability of nuclear size is the most significantly associated with PTC gene expression profile in PTC. Nucleus area variance was positively correlated with expression of 55 genes and showed inverse correlation to 95 genes. Among chromatin distribution parameters Margination (marg) and Granularity (clump) was correlated with genes expression prifile. One of the genes related to nuclear size variability (HDAC1) was validated by QPCR. An observed trend for the relation of HDAC1 with nuclear size was confirmed.

Among karyometric parameters investigated, it is anisotropy of PTC nuclei which significantly correlate with its gene expression profile. The association of specific transcripts with karyometric features of PTC shall be carried out in the extended set of samples.

5. FUNCTIONAL ANALYSIS OF THE HUMAN *GSTP1* GENE PROMOTER REGION IN CULTURED CANCER CELLS

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Glutathione S-transferases (GSTs) are a multigene family of enzymes that play an important part in the second phase of drug detoxification in cells. One of the most important GST izoenzymes is glutathione S-transferase P1 (GSTP1), which catalyzes conjugation with a number of electrophilic hydrophobic compounds such as xenobiotic drugs, toxins and carcinogens. Expression of this gene is associated with resistance to some antineoplastic drugs and genotoxic carcinogens. We have examined the level of *GSTP1* gene expression in human normal and neoplastic cells. The highest level of *GSTP1* mRNA was found in the non-tumorigenic mammary epithelial cell line MCF10a, while human breast adenocarcinoma MCF7 and human hepatocellular carcinoma cell line HepG2 lacked its expression.

GSTP1 is transcriptionally silenced by promoter hypermetylation in several human cancer cells. GSTP1 promoter was hypermethylated in breast cancer cells MCF7 and partly methylated in BeWo (human choriocarcinoma cell line). The loss of glutathione S-transferase P1 expression in MCF7 cells resulted from hypermethylation of CpG sites in the GSTP1 promoter region.

Functional analysis of the proximal promoter indicated that *GSTP1* gene is positively regulated by NF-kB element and negatively regulated by NF-kB-like element and CRE in BeWo, Hbl-100 and Me45 cells.

The cytotoxic effects of radiation therapy are mediated primarily through increased formation of hydroxyl radicals and reactive oxygen species, which can damage cells, proteins and DNA; the glutathione S-transferases (GSTs) function to protect against oxidative stress. Cells of the examined cell lines, irradiated with 2Gy showed reduced GSTP1 expression from the beginning until 3h after exposure and increased transcription from 5h to 12h hours after irradiation.

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6. STRUCTURE PREDICTION OF A PROTEIN CHANNEL BASED ON PROBABILISTIC FORMAL GRAMMARS AND THE CONTINUOUS ION FLOW MODEL

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Structure prediction of protein channels remains a challenge mostly due to the small number of already solved structures and conformational space size. The former restricts homology based methods, while the latter limits *de novo* techniques. A promising strategy to make *de novo* approach capable of dealing with large channel proteins is to constrain the search space on the basis of primary and secondary structure features and local spacial homologies.

In particular, molecular interactions between distant residues, which tend to determine the overall global protein structure, seem to be suitable for this purpose. We propose to use the framework based on Probabilistic Context Free Grammars (PCFG) to detect certain characteristic points in order to build a graph of those interactions and constrain the 3D structure prediction. In our previous work aimed at detecting protein regions involved in binding sites, the PCFGs were induced automatically using a genetic algorithm from a set of unrelated protein sequences that shared a common feature. This framework can be adapted for robust detection of characteristic points by implementing *a priori* knowledge about transmembrane proteins, eg. by appropriate grouping of aminoacids, introducing specific grammar rules or restricting parsing tree.

Prediction of ion channels structure without known homologous proteins can also be improved by comparing functional characteristics obtained from the model with experimental data. The most accurate Molecular Dynamics technique is currently limited to nanoseconds time scale due to the high computational cost. The ion flux phenomenon requires longer time and therefore other frameworks are needed, e.g. Poisson-Nernst-Planck (PNP) model. The PNP, although an averaged theory, is capable of reproducing biologically valid characteristics. We have already optimised the PNP method in the terms of computational cost and prepared the pipeline for obtaining the current-voltage and conductance-concentration characteristics for a given channel structure. The criterion of compatibility of the experimental and simulated characteristics can be then used to choose the structural model of the channel or even to provide some feedback into the 3D modelling process.

7. CARRIERS OF *BRCA1* MUTATIONS HAVE ELEVATED LEVEL OF OXIDATIVE DNA DAMAGE; REVERSAL OF THIS EFFECT IN ADNEXECTOMISED PATIENTS DUE TO SELENIUM SUPPLEMENTATION

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A mutation in the BRCA1 gene predispose women to a high risk of breast and ovarian cancers. Although the precise biological functions of the BRCA1 tumor suppressor are still unknown it is widely accepted that the proteins encoded by the gene participate in the monitoring and repair of DNA damage.

Selenium has several anticancer properties which are linked with protection against oxidative stress. Namely, Se is required to maintain the activity of some antioxidant enzymes and was found to scavenge free radicals

Therefore, to asses a involvement of *BRCA* in oxidative damage to DNA and to have a further insight into the issue concerning a role of the damage in cancer development in the present study all the above mentioned parameters reflecting oxidative DNA damage were analyzed in three groups of subjects; i/ the group of healthy subjects; ii/ patients with BRCA1 mutation without symptoms of the disease and iii/ patients with breast or ovarian cancer with the mutations.

The aim of the present study was two-fold; aside from aforementioned aim we also wanted to know whether supplementation of *BRCA1* carriers with selenium have beneficial effect concerning oxidative stress/DNA damage.

Collectively the results of our study demonstrate that *BRCA1* carriers have elevated level of promutagenic 8-oxodG in cellular DNA. Moreover, the results demonstrate that supplementation of supranutritional selenium doses to high risk group of subjects with *BRCA1* mutations resulted in significant decrease of oxidative damage to DNA. However, this effect was restricted only to the patients who underwent adnexectomy. Therefore selenium supplementation may be recommended to the carriers with adnexectomy without symptoms of the disease. Our results also demonstrated that selenium supplementation of the breast cancer patients is responsible for decreased oxidative damage to DNA what in turn may slow down the disease progression.

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8. STAPHYLOKINASE PRODUCTION IN PLANT SYSTEMS

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Recombinant protein production *in planta* is recognized as a promising alternative to microbiological synthesis and human or mammalian cell systems. During the past decades, several efficient methods of plant transformation have been developed. This resulted in construction of numerous transgenic plant systems for *in planta* production of plant antibodies, vaccines and various recombinant proteins of medical and industrial value. However, the recombinant protein expression level still appears to be too low from industrial (biotechnology) point of view. For this reason, the optimization of transgene expression has recently become one of the major goals of molecular biotechnology research.

Our current research is focused on optimizing the production of recombinant proteins in plants and on developing efficient methods of extraction and purification of these proteins. As a model protein we used a bacterial anticoagulant factor – staphylokinase, which is an effective activator of human plasminogen. It is well documented that high level expression of bacterial genes in eukaryotic cells depends on a large number of factors. So far, we attempted to obtain a high level of staphylokinase expression in three model plants: *Nicotiana tabacum*, *Solanum tuberosum* and *Arabidopsis thaliana*. For genetic transformation we used a gene construct consisting of either constitutive (CaMV 35S) or facultative (P*phas*) promoter and various regulatory elements and signal sequences (KDEL, ss, *acr*5-I or TMV Ω leader sequence).

These experiments resulted in constructing transgenic plants characterized by whole plant or seed specific expression of staphylokinase transgene. However, our system requires further optimization to become economically viable.

9. 5-(4-NITROIMIDAZOL-1-YL- AND 1,8-DICARBOXYNAPHTHALIMID-2-YL)-2'-DEOXYURIDINE AS SPECIFIC GUANOSINE SINGLE NUCLEOTIDE POLYMORPHISM PROBE

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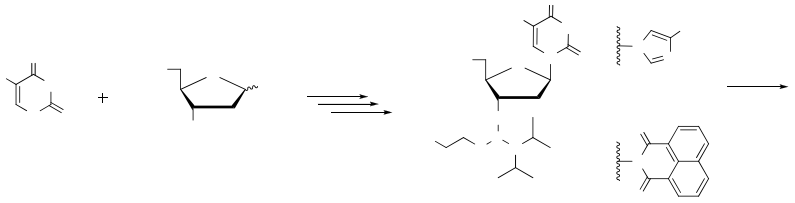
Single nucleotide polymorphism (SNPs), where one of the bases is substituted by other, is recently one of the major interests in the oligonucleotide chemistry [1]. Base pairing mutations of a gene sequence can occur resulting in switched-off gene activity or induction of disease [2]. There are two main approaches of determining SNPs. Most available methods are based on differences in hybridization efficiency of the matched and mismatched oligonucleotide probe and the target sequence [3]. Second group of SNPs probes contains the nucleotide bearing the fluorescent reporter group. The reporter group can be attached to the sugar or nucleobase moiety or flurophore which can mimic the base [4].



Single nucleotide polymorphism (SNPs) oligonucleotide probes containing the C-5 substitued 2'-deoxyuridine modified by 4-nitroimidazol-1-yl (**X** modification) and 1,8-dicarboxynaphthalamid-2-yl (**Y** modification) moiety were synthesized and the effect on duplex stability upon incorporation of monomers **X** or **Y** into mixed 21-mer sequence (**ON11** and **ON12**) was evaluated. Fluorescent incorporation of **Y** opposite to **G** in mismatched duplex displays significant hipsochromic shift of fluorescence spectra.

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10. ANALYSIS OF EXPRESSION OF GAS41 AND RELATED PROTEINS IN HUMAN CANCERS

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Yaf9 protein is a *S. cerevisiae* protein involved in remodeling of chromatin structure as well as in controlling stability of the yeast genome. Human ortholog of Yaf9 is called GAS41 and contain a structural domain called YEATS. This domain has been identified in several other human proteins, which comprised YEATS protein family: MLLT1/ENL, MLLT3/AF9 and BRDT. YEATS protein family members are involved in regulation of gene expression, yet their exact functions are not clear at the moment. However, their similarity to yeast Yaf9 suggested involvement in controlling genomic stability and possible importance for cancer development.

Here we aimed to analyze the levels of GAS41 and other YEATS protein family transcripts in human cancer tissues. Data regarding levels of YEATS protein family transcripts have been extracted from global gene expression profiles obtained by means of expression microarrays. We have examined datasets from Affymetrix microarrays analyzed at the Institute of Oncology in Gliwice or present in publicly available databases. Analyses were performed on material from breast cancer, papillary thyroid cancer, bladder cancer, lung cancer, mesothelioma and melanoma, matched with corresponding non-malignant tissues. We observed that expression of MLLT3/AF9 gene was two-fold lower in thyroid cancer as compared to non-cancerous thyroid. The change has a high statistical significance ($p<0.000001$). Some other cancer/control differences were also detected, however they didn't have a high statistical significance. Additionally, we have analyzed levels of YEATS protein family transcripts in a dataset from ovary cancer samples. Higher levels of MLLT1/ENL and BRDT gene transcripts correlated with better responses to chemotherapy, yet statistical significance of the difference was only moderate ($p=0.015$ and $p=0.029$, respectively). Considering impact of studies that suggested possible interactions between GAS41 and N-MYC oncoprotein, we have additionally analyzed putative correlation between the levels of GAS41 and N-MYC gene transcripts in human brain tumor tissues. Obtained data suggest collectively that members of YEATS protein family might be involved in processes related to cancer.

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11. MOLECULAR SWITCH OF ENZYMATIC ACTIVITIES: THE CASE OF TOPOISOMERASE I

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Human DNA topoisomerase I (topoI) exhibits two different enzymatic activities depending on the substrate. Interaction with DNA facilitates the relaxation activity of topoI, whereas contact with SF2/ASF protein results in its phosphorylation catalyzed by topoI. Primarily, SF2/ASF protein acts in spliceosomes influencing the determination of splicing sites. It works antagonistically to hnRNP A1 protein that, similarly to SF2/ASF protein, has two RRM domains. For this reason, both proteins interact with topoI in the same manner. Interestingly, the *in vitro* tests carried out in our study confirm that the interaction of hnRNP A1 protein with topoI promotes its DNA relaxation activity even in the presence of SF2/ASF.

What is responsible for switching of the topoI activity? We looked for structural differences in both SF2/ASF and hnRNP A1 proteins. The most obvious dissimilarity is the linker region located between two RRM domains. The former is equipped with a flexible linker consisting of 9 glycine residues, while the latter has a comparatively short and rigid linker that seems to prevent unrestricted movements of RRM domains.

In order to answer the above question, we constructed two recombinant proteins with swapped linkers: $SF2/ASF_{hard}$ and $UP1_{flex}$ that have native linkers from hnRNP A1 and SF2/ASF proteins, respectively (UP1 stands for the shortened hnRNP A1 protein commonly used in the *in vitro* studies). Unlike the native UP1 protein, the $UP1_{flex}$ was not able to fully promote the DNA relaxation activity of topoI, which continued to phosphorylate SF2/ASF. On the other hand, using $SF2/ASF_{hard}$ protein, we confirmed that the linker region has no impact on the interaction of SF2/ASF with topoI nor on its phosphorylation efficiency. Moreover, presence of $SF2/ASF_{hard}$ protein left the DNA relaxation activity of topoI unaffected. Taking together these results, we suggest that the linker region of SF2/ASF protein is predominantly responsible for inhibition of the relaxation activity of topoI. Further, the antagonistic action of SF2/ASF and hnRNP A1 in the determination of splicing sites possibly may result from the different structure of the linker region between two RRM domains.

12. OPTIMISATION OF PROCEDURES OF ISOLATION AND CULTURE OF MURINE HEART MICROVASCULAR ENDOTHELIAL CELLS

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The aim of the study was to optimise previously published methods for isolation of endothelial cells from mouse hearts to establish pure cell cultures. The isolation of microvascular endothelial cells was achieved by releasing cells from hearts by mechanical mincing of the tissue and incubating with proteolytic enzymes, followed by selection with antibodies targeting endothelial-specific cell-surface antigens CD31, magnetic selection and culturing in specialised media. Optimal tissue digestion and release of single cells was achieved by incubation with collagenase followed by mechanical treatment (syringing). Although dissociation of tissue into single cell suspension was improved after trypsinization, this treatment was found to compromise subsequent binding of CD31 antibodies and reduced cell yields. Two different commercial systems employing magnetic cell sorting of antibody-labelled endothelial cells were evaluated: MACS and Dynal system.

The endothelial cell recovery by MACS technique was very low and therefore subsequently the Dynal system was evaluated. This system employs large visible magnetic beads, which are first coated with specific antibodies and then applied to tissue digests. Selection of antibody-bound cells was achieved by applying the cell suspension into a tube placed in a magnet followed by removal of unbound cells by aspirating the supernatant. Primary endothelial cells were found to adhere efficiently on 1% gelatin coated dishes and endothelial colony expansion was optimal in the presence of 100 □g/ml endothelial cell growth supplement (ECGS). To reduce fibroblast contamination we used media in which L-valine was substituted for D-valine, which is reported to be poorly metabolised by fibroblasts. A second magnetic selection procedure was also employed once the original primary cells reached confluence. The resulting microvascular endothelial cells were positively identified by their morphology and expression of specific antigens. Successful isolation of endothelial cells was achieved from hearts of animals up to 16 weeks of age.

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13. EXPRESSION OF ELP-GUSPLUS FUSION PROTEIN IN TRANSGENIC TOBACCO PLANTS

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Elastin-Like Polypeptides (ELP) are synthetic polypeptides composed of repeatig pentapeptide Val-Pro-Gly-Xaa-Gly where Xaa can by any amino acid except proline. This polypeptides are characterized by reversible inverse phase transition in response to the changes of environmental parameters such as temperature, pH or ionic strength. This transition from a soluble form to insoluble coacervates is fully reversible by changing the solution parameters to the initial state. Due to this specific properties, application of ELP in biotechnology, bioengineering and biomedicine is the subject of extensive research. In this report we present expression of the transgene coding for an ELP-GUSPlus fusion protein in transgenic *N. tabacum* plant obtained by an *A. rhizogenes* mediated transformation. Based on histochemical (GUS activity assay) and molecular (Western blot analysis) analyses we report on targeting of ELP-functional enzyme fusion to ER.

This research was supported by the Ministry of Science and Higher Education (grant No 2 P04B 029 29) and the University of Lodz (grant No 506/040996)

14. STUDY OF THE MOLECULAR MECHANISM AT THE BASIS OF THE ANTI-INFLAMMATORY ACTION OF CYCLIC-AMP

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Cyclic-AMP is a second messenger that operates through the activation of PKA or the more recently discovered guanine exchange proteins directly activated by cAMP (epac). Alterations of intracellular cAMP levels have been shown to have profound effects on cytokine secretion. Intracellular cAMP concentrations are predominantly increased via G-protein-mediated activation of adenylyl cyclase, which converts ATP to cAMP. In addition, cAMP levels are affected via breakdown of cAMP by phosphodiesterases. We and others have observed that pharmacological or physiological (using serotonin or the β-adrenergic agonist isoproterenol) elevation of intracellular cAMP levels represses the expression of several pro-inflammatory cytokines by human monocytes. In this project we wanted to investigate the molecular basis of this anti-inflammatory effect. A major issue was the assessment of the effects of cAMP and cAMP-inducing agonists on the activation of the NF-kB transcription factor, which is pivotal in the expression of a multitude of inflammation-related genes.

As a model system we have used the human THP-1 leukemic monocytic cell line. Their inflamatory response was checked via expression of interleukins six and eight.

Interestingly, we observed that LPS, but not other pro-inflammatory stimuli such as PMA or TNF, induced IL-6 secretion. PMA and TNF however did induce the secretion of IL-8, another NF-κB -dependent gene, indicating gene-specific effects of these pro-inflammatory stimuli. In addition, whereas cAMP also inhibited LPS-induces IL-8 expression, it did not affect PMA-induced IL-8 and even synergized with TNF to induce IL-8. On the other hand we could not detect any inhibitory effects of cAMP on IL-6 expression in Raw264.7 mouse macrophages and in human astrocytes, we explored whether the inhibitory effect of cAMP was perhaps cell type specific. These observations indicate the effects of cAMP are not only stimulus-specific, but also cell-type specific.

We also observed that whereas cAMP inhibits IL-6 expression in THP-1 cells, it also induced phosphorylation of p65 at the serine 276 residue, a modification that has previously been demonstrated to be associated with transcriptional activation of NF-κB-dependent genes such as IL-6. We also show that both cytokines we investigated are differently regulated and the regulation is changing with the developmental stage of the cells.

In summary, our findings indicate that cAMP can inhibit pro-inflammatory gene expression via different gene- and stimulus-specific mechanisms. Further experiments, perhaps using a combination of classical gene expression tools in combination with genome-wide bio-informatics approaches, will be required to elucidate the multiple targets via which cAMP-dependent pathways can modulate pro-inflammatory gene expression.

15. HEAT SHOCK INDUCES DOWN-REGULATION OF SEVERAL TRANSCRIPTION FACTORS EXPRESSION PRIOR TO THE INDUCTION OF APOPTOSIS IN SPERMATOGENIC CELLS

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Elevated temperature and other stress conditions cause denaturation of cellular proteins, which induces activation of the heat shock transcription factor 1 (HSF1) and leads to elevated expression of heat shock genes. Heat shock proteins (HSPs) are then rapidly synthesized and their accumulation can result either in refolding of proteins to their native state or in degradation of abnormal proteins, which is called the heat shock response. However, not all cell types respond to cellular stressors in the same manner. In the male germ cells (spermatocytes) activation of HSF1 does not lead to HSP synthesis and cytoprotection. Instead, caspase-3 dependent apoptosis is induced and spermatogenic cells are actively eliminated.

To elucidate a mechanism of pro-apoptotic activity of HSF1 in spermatogenic cells we carried out genome-wide transcriptional analysis in control and heat-shocked cells, either male germ cells (where HSF1 activation leads to apoptosis) or somatic cells (that survive in elevated temperature). Spermatocytes and spermatids were isolated from mouse testes by unit gravity sedimentation. Hepatocytes isolated by collagenase perfusion of a mouse liver exemplified the somatic cells. RNA was isolated from control and heat-shocked cells, either directly after treatment or after 1-3 hrs recovery at physiological temperature. Approximately 36 000 transcripts, representing the entire murine genome, were monitored using the Affymetrix GeneChip system before and after the heat shock and 2 hrs recovery. In addition, expression of selected genes was tested by RT-PCR. We identified genes that are differentially expressed during hyperthermia in somatic and male germ cells. In hepatocytes, the heat shock stimulates expression of some genes involved in inflammatory and immune response, like p38 (Mapk 14), CD14 antigen, interleukin 1 and chemokine ligand 1. These genes, however, are not stimulated in spermatocytes. More importantly, expression of many transcription factors, for example subunits of AP-1 (Jun and Fos), Egr1 and 2, Zfp361l, Klf6, Atf3, Nfkbiz, that is strongly induced by the heat shock in hepatocytes is down-regulated in spermatogenic cells. Thus it seems possible that negative regulation of transcription could be most essential for HSF1-dependent induction of apoptosis in spermatogenic cells.

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16. MOLECULAR SIGNATURE OF THE *BRCA1* MUTATION IN BREAST CANCER, TRUE OR MYTH?

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Since wide implementation of mutation screening and genetic counseling, breast cancer has been frequently regarded either as a sporadic or a hereditary disease (hereditary breast cancer, HBC). There is an ongoing debate whether pathology and clinical behavior of HBC is distinct from those of sporadic breast cancer. Undoubtedly, patients with HBC develop the disease at a younger age, but it is not clear whether they have worse prognosis, as suggested in some studies. Pathologically and immunophenotypically, *BRCA1* mutation-linked breast cancer is regarded as the most distinct category. Among its characteristics are: high tumor grade, high mitotic index, pushing margins, elevated lymphocyte infiltration and low estrogen receptor expression. The question of putative molecular differences between hereditary *BRCA1* or *BRCA2* mutation-linked and sporadic tumors was first analyzed by Hedenfalk et al. who claimed that these three categories of breast cancer could be easily distinguished on the basis of distinct gene expression pattern (Hedenfalk et al., 2001).

The aim of our study was to verify the magnitude of difference in gene expression profile between *BRCA1*-associated and sporadic breast cancers. In our analyses we took into account estrogen receptor status and molecular subtype of the tumor, the two most significant features affecting global gene expression pattern in breast cancer. We also considered that *BRCA1* gene inactivation may be caused not only by its mutation but also by the epigenetic silencing. Thus we checked for *BRCA1* promoter methylation in the tumor samples and analyzed the gene expression profile in all tumors with inactive *BRCA1* gene.

Our results show that a marked difference between *BRCA1*-mutation linked and sporadic breast cancer, previously reported by others, was probably due to uneven stratification of ER(+)/ER(-) and basal-like/luminal tumor samples. Apparent difference between *BRCA1*-linked and other types of breast cancer observed in univariate analysis is diminished when data are corrected for these features in multivariate analyses. In fact, the difference in gene expression pattern of *BRCA1*-mutated and sporadic cancer is very discrete. These conclusions were supported by the Q-PCR validation. We also found that BRCA1 gene inactivation due to promoter methylation had similar effect on general gene expression profile as mutation-induced protein truncation. This suggests that in the molecular studies of hereditary breast cancer, *BRCA1* gene methylation should be recognized and considered together with gene mutation.

17. ROLE OF ELECTROSTATICS IN INACTIVATION OF KV 1.2 POTASSIUM ION CHANNEL. RANDOM WALK SIMULATIONS

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Inactivation of the potassium ion channel is the process in which the ball-like peptide blocks the pore of the ion channel. In the intact (wild type) channel, inactivating peptides (four of them) are bounded to the protein by the tethers. In the excised channel, inactivating balls are removed, yet it is still possible to induce the inactivation by adding peptides to the surrounding water-solution.

In our model, inactivating ball behavior is subjected to the overdamped Langevin dynamics. The influence of electrostatic interactions of balls with channel protein and cellular membrane is included. Comparison of predicted and measured (literature data) rates of inactivation is presented. Common features and differences between different modes of inactivation experiment are discussed.

18. PEAK ALIGNMENT IN PROTEIN SPECTRA. A COMPARISON OF DIFFERENT ALIGNMENT TECHNIQUES

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In protein spectra features of scientific interest include peaks which represent relative levels of each protein within the mixture. However, as measurements are affected by error, this causes peak shifts between spectra. These shifts are nonlinear and persist even when we use technical replicates. Concurrent analysis of many spectra is possible only after their alignment. Alignment is a sequence of operations which settles localization of common peaks in individual spectra.

In recent years mass spectrometry has been used to find disease related patterns in complex protein mixtures. Detected markers need to be qualified,verified and validated. This processes rejects almost all candidates. One of the reason of this is that the true biochemical composition of the samples used in the experiments is not known, so accuracy of spectra processing techniques used can't be estimated. Creating three different data sets with 100 realistic spectra each, using a virtual mass spectrometer, developed by Morris and Coombes [1], enabled valuable comparison of efficiency, reliability, and accuracy of alignment algorithms. From all the alignment algorithms, four of them were chosen. First two are warping methods which received recognition in chromatogram and NMR spectra alignment. Next two are methods used in two different protein spectra processing software: SpecAlign [2] (method using Fast Fourier Transform) and PrepMS [3] (method based on Matlab msalign function). They were a good reference to old methods.

Results of our research is presented using both visual (heatmatps, mean spectrum, all spectra view) and numerical (working time, precision) methods. For alignment precision measurements peaks obtained after processing were compared with expected values. Such operations describe advantages and disadvantages of the tested algorithms.

The important thing that was observed concerns complexity and difficulty level with respect of peak alignment of the investigated spectra and strictly linked to concentration of the peaks in small areas. The fastest one and having very good accuracy of alignment was the algorithm used in SpecAlign. However, when the spectrum was too complex (considerable peak shifts) it didn't align all spectra. One of the warping methods, the slowest one, managed this problem. However, after using this method accuracy of alignment was worse.

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19. ANALYSIS OF INDIVIDUAL RADIOSENSITIVITY ON THE BASIS OF EXPRESSION PROFILING

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Current guidelines for radiation dose limits are based on linear extrapolation of population-wide biological effects (like cancer risk) obtained at relatively high doses of radiation (above 100 mGy). However, it is unclear whether such linear relationship models are valid at low radiation doses. Other relationships between dose and effect are imaginable: for example a threshold dose may exist below which little or no effects occur. As a measure of biological consequences to low dose and dose rate radiation we characterized genome-wide transcriptional responses. Human lymphocytes from two different donors were isolated and subjected in triplicate to various (low) doses of irradiation (0.4 mGy, 2 mGy, 50 mGy, 100 mGy and 200 mGy). All doses were administered either acutely (high dose rate for short amount of time) or chronically (low dose rate for long time) after which gene expression profiling was performed using Affymetrix GeneChips HGU133plus2. From the outcome of these gene expression data we will determine the lowest dose or dose rate for which a specific gene expression response can be found and will determine whether this response follows a threshold or linear dose relationship.

Additionally we performed a time course experiment on material from the second donor, in which we investigate gene expression profiles 3h, 8h and 24h after irradiation with acute low doses (25mGy, 50mGy and 200mGy). The goal of this experiment is to obtain information about differences between genes acting at distinct time points post irradiation.

In analogy to the observed inter-individual variation in sensitivity to high dose radiotherapy, we expect a similar variation in biological effects to low dose radiation. We will irradiate lymphocytes from two groups of breast cancer patients displaying either late normal tissue toxicity to radiation therapy or non-toxicity with the most optimal low dose determined in the experiment described above. We hope to find an intrinsic difference in gene expression between these two groups, enabling to predict an individual's radio-sensitivity to low-dose radiation.

20. NOVEL QUINAZOLINE DERIVATIVES, THEIR SYNTHESIS, STRUCTURE-ACTIVITY RELATIONSHIP AND ANTIPROLIFERATIVE PROPERTIES

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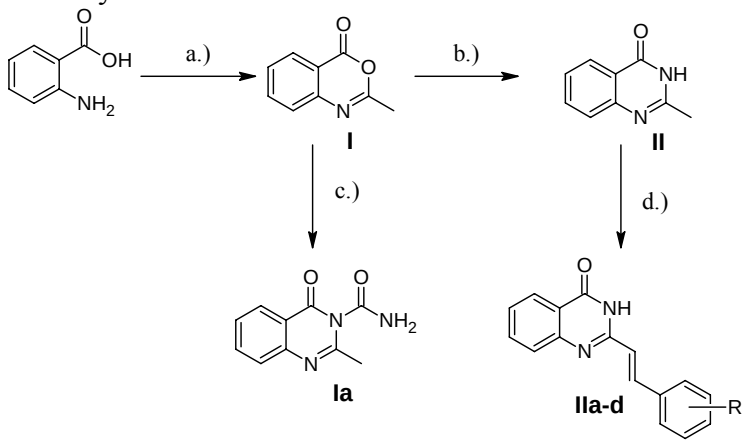
Quinazolinone moiety exists in number of bioefectors. This molecular scaffold has been studied as antiamnestic and antioxidant compounds (1), and antimitotic anticancer agents (2). Series of quinoline and quinazoline derivatives (Scheme 1) were synthesized and evaluated for their anticancer activity.



Scheme 1. a) Ac_2O\ microwave, b) NH_3\NaOH, c) urea, d) aldehyde\microwave

The products were obtained in good to excellent yields, and structures were confirmed by spectral data (NMR, IR). During the synthesis microwave irradiation was successfully applied (3). Antiproliferative activity was measured using HCT 116 cell line (human colon carcinoma). We discuss the structure-activity relatioship between chemical structure and biological activities of the evaluated compounds. Several analogues have shown significant growth inhibitory activity against HCT 116 cells. Anticancer activity was evaluated using MTS-reduction colorimetric survival assay, and clonogenic cell survival method. Further studies on docking quinazolinone based compounds to reverse transcriptase are also discussed.

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21. ACTIVITY OF NFκB TRANSCRIPTION FACTOR IS AFFECTED BY HYPERTHERMIA AND ACTIVE HSF1

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NFκB is a family of transcription factors, which in resting cells are sequestered in the cytoplasm by association with IκB inhibitory protein. Activation of NFκB requires degradation of IκB, which allows nuclear translocation of NFκB and their binding to cis-acting DNA regulatory elements. NFκB regulates numerous genes important for pathogen- or cytokine-induced inflammation, immune response and cell proliferation. NFκB also activates several genes that promote cell survival, which contributes to aggressive tumor growth and resistance to chemotherapy and radiation in cancer treatment. Various reports have shown that experimental activation of NFκB results in reduced apoptosis while its inhibition promotes apoptosis and suppress tumor growth. HSF1 is the primary transcription factor responsible for the transcriptional response to different forms of cellular stress (e.g., a heat shock). The hyperthermia-activated HSF1 binds regulatory DNA elements, termed heat shock elements (HSE), present in promoters of HSPs genes, and activates their expression. In general, HSPs function as molecular chaperones in regulation of cellular homeostasis and promoting survival. HSPs overexpression is frequently found in many types of cancer, and is usually associated with poor prognosis. Such up-regulation of HSPs putatively increases resistance of cancer cells to therapy and apparently diminishes the success of anti-cancer treatment. On the other hand, however, hyperthermia is an adjuvant treatment used to sensitize cancer cells to radio- and chemotherapy, possibly affecting pathways that promote cell survival.

Here we aimed to address possible mechanisms by which hyperthermia and HSF1-dependent signaling interfere with NFκB-dependent pathways. The U_2OS osteosarcoma human cell line has been used as an experimental model. The heat shock response has been induced by means of hyperthermia. Alternatively, cells have been transfected with mutated constitutively active HSF1 to activate HSF1-dependent signaling in the absence of the heat shock. Cells were incubated with TNFα cytokine to activate NFκB, and then expression of NFκB-regulated genes has been assessed by RT-PCR. We have observed that activity of NFκB was inhibited in cells subjected to hyperthermia, and four hours recovery in physiological temperature was necessary to allow TNFα-induced activation of NFκB. On the other hand, NFκB remained to be activatable by TNFα treatment in cells containing constitutively active mutated HSF1 at normal temperature. Interestingly, however, several NFκB-activated genes were differently regulated in the presence of active HSF1. Our findings clearly indicates functional interference among hyperthermia, HSF1- and NF□B-dependent signaling pathways.

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22. CYTOTOXICITY OF ETOPOSIDE AND CISPLATIN IS SYNERGISTICALLY INCREASED BY WORTMANNIN IN HUMAN GLIOMA CELLS

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Etoposide and cisplatin treatment are used routinely in glioma patients. However, resistance to these drugs is a major problem in therapy and can be associated with accelerated repair of etoposide and cisplatin-induced DNA damage. Etoposide induces DNA double-strand breaks (DSB), and cisplatin induces DNA intra- and inter-strand cross-links (ICL). The latter agent may induce DSB as an intermediate step during ICL repair. DSB are repaired mainly by non-homologous DNA end joining (NHEJ). Important component in this repair pathway is the catalytic subunit of DNA-dependent protein kinase (DNA-PK_{cs}). The aim of our work was to answer the question if the nonspecific DNA-PK_{cs} inhibitor wortmannin can sensitize human glioma cells to DNA damaging agents: etoposide and cisplatin.

Effect of wortmannin on drugs cytotoxicity in T98G and MO59K human glioma cell lines was evaluated using XTT assay. The potential mechanism of action of wortmannin on etoposide and cisplatin cytotoxicity was examined by analysis of synergy.

The results demonstrate that wortmannin increases the cytotoxicity of etoposide and cisplatin in combination in T98G and MO59K cells (reduction factor R>1). Pre-incubation of T98G cells with 10 μM wortmannin potentiates about seven times the growth inhibition of drugs (R=7,3), whereas pre-incubation of MO59K cells with 5 μM wortmannin potentiates about four times the growth inhibition of drugs (R=4,5). Moreover, there is a synergistic interaction between these two drugs and wortmannin in both cell lines (combination index CI<1).

Our data show that wortmannin is able to modulate drugs toxicity to increase cell killing. This inhibitor, in combination with etoposide and cisplatin, may have potential benefits in cancer treatment.

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23. APPLICATION OF MALDI-TOF ANALYSIS OF THE SERUM PROTEOME IN DETECTION OF BREAST CANCER PATIENTS; SAMPLE PREPARATION METHOD-DEPENDENT CLASSIFICATION PERFORMANCE

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Proteomics is the study of the proteome – a complete protein component of the cell. In contrast to the genome, the proteome is dynamic and its fluctuations depend on combination of numerous internal and external factors. Identifying and understanding changes in the proteome related to a disease development and therapy progression is a subject of clinical proteomics. Here we aimed to identify in the circulating blood a set of polypeptide biomarkers that could be used in diagnostics and monitoring of therapy of breast cancer patients.

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

Blood samples were collected from the group of 100 breast cancer patients before the start of therapy, as well as in the group of 400 healthy controls. Specific patterns of low-molecular-weight polypeptides (1-10 kD) were identified thanks to mathematical analyses and cross-correlated between experimental groups. A multi-component set of polypeptides has been selected as a classifier that differentiate control and cancer samples.

Here we present a report from the project aimed to identify a set of polypeptide biomarkers that could be used for diagnostics and monitoring of a therapy of breast cancer patients. Preliminary data show that cancer-specific multi-component polypeptide pattern could be identified in serum of breast cancer patients. However, their importance for cancer diagnostics remains to be verified.

This work was supported by the Ministry of Science and Higher Education, grant 2P05E06730.

24. ANALYSIS OF INTRACELLULAR LOCALIZATION OF THE HUMAN HspA2 PROTEIN IN CANCER CELLS

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The human *HSPA2* gene belongs to a HSP70 multigene family of heat shock genes. The HspA2 protein is necessary for completing of meiosis and the progression of spermatogenesis. Reduced expression of this protein was found to be related to male infertility. Expression of the HspA2 is not heat inducible. The *HSPA2* transcripts were reported to be present in various human somatic tissues. However, the HspA2 expression in non-testicular cells, both at mRNA and protein level, is poorly characterized. The *HSPA2* gene activity was also observed in cell lines derived from several human cancers and in cancer surgical samples. Depletion of *HSPA2* might be involved in diminished growth and survival of cancer cells.

The first aim of the study was to establish the expression and intracellular localization of the HspA2 protein at physiological temperature. We used *qRT-PCR* to determine transcription level of *HSPA2* in human cancer cell lines of various origin. Intracellular localization of the HspA2 was analyzed using cell lines expressing HspA2-GFP and mRFP-HspA2 fluorescent fusion proteins and confirmed by immunofluorescence using cell lines naturally expressing HspA2 and the specific anti-HspA2 antibody. In cells growing under normal culture conditions the HspA2 protein was localized mainly in cytoplasm. The HspA2 protein was shifted into nucleus and nucleoli during heat shock. We also observed that HspA2 is accumulated at centrosomes of interphase and mitotic heat-shocked cells. Our results suggest, that the HspA2 protein can be involved in protecting nucleoli and centrosomes integrity in cells subjected to heat shock, and possibly to other cellular stressors. It seems that the HspA2 can be considered as chaperone protecting cells against proteotoxic stresses. We observed that overexpression of the HspA2 protein enhanced clonogenic potential of cells treated with proteasome inhibitor.

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25. CHLORITE LEADS TO FORMATION OF CHLOROHYDRINS IN PHOSPHATIDYLCHOLINE VESICLES CONTAINING UNSATURATED FATTY ACID RESIDUES

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The activity of neutrophil-derived enzyme – myeloperoxidase is associated with generation of $HOCl/OCl^-$, which protects against pathogen invasion but also brings about oxidative injuries in host tissues. It is known, that $HOCl/OCl^-$ is able to induce lipid peroxidation; however the analysis of the mechanism of this reaction and the products pattern are still to be elucidated. Therefore, we selected three phosphatidylcholines with known fatty acid content: 1-Steraroyl-2-Oleoyl-*sn*-Glycero-3-Phosphocholine (18:0-18:1 PC), 1-Steraroyl-2-Linoleoyl-*sn*-Glycero-3-Phosphocholine (18:0-18:2 PC) and 1-Steraroyl-2-Arachidonyl-*sn*-Glycero-3-Phosphocholine (18:0-18:4 PC). The lipid vesicles were prepared in sodium phosphate buffer (pH = 6.0), sonicated and treated with $HOCl/OCl^-$ (5 molar excess per one double bond). The excess of $HOCl/OCl^-$ was washed on reverse phase Sep-Pak columns. Lipids were washed with organic solvent system and after evaporation of solvents lipids were reconstituted in 20 mM sodium phosphate buffer (pH = 7.4). Analysis of $HOCl/OCl^-$ treated lipids was carried out using positive-ionization electrospray mass spectrometry as well as the liquid chromatography accompanied by mass spectrometry. Under these conditions we observed the complete conversion of lipids into chlorohydrins. However the formation of chlorohydrins was accompanied by the formation of appropriate co-products (for SLPC-HOCl: –18 m/z, for SAPC-HOCl: –18 and –36 m/z) regarded as the chlororohydrin molecule, which lost one molecule of H_2O or HCl respectively. No hydroperoxides or other products characteristic for lipid peroxidation were detected.

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26. INSIGHTS INTO DNA REPAIR SYSTEM IN COLON CANCER CELL LINES EXPOSED TO IONIZING RADIATION

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The oxidative stress generated by ionizing radiation during radiotherapy induces different type of DNA damage. The formation of DNA lesions leads to activation of DNA repair enzymes or to genomic instability and apoptosis. In this process, the tumor suppressor gene p53 plays a major role in allowing DNA repair or triggering apoptosis when DNA alteration has reached an unacceptable level.

Here we compared the level of DNA strand breaks and their repair in two cell lines differing in p53 status (HCT116 p53+/+ and HCT116 p53-/-) exposed to ionizing radiation. The level of DNA strand breaks was measured by the comet and micronuclei assay. The changes in expression of genes coding for DNA repair proteins were analyzed by quantitative RT-PCR. The level of poly(ADP-ribose) in the first minutes after exposure to IR was also assessed. Our results suggest that wild type and p53 mutated HCT 116 cells differ in mechanisms of DNA repair. The differences in DNA repair did not concern the first steps of DNA repair since the level of DNA damage and poly(ADP-ribose) production in the first minutes after exposure to IR were almost the same in both cell lines. However, transcripts of genes from DNA repair pathways analyzed by quantitative RT-PCR showed different trends in expression after exposure to irradiation in HCT p53+/+ and HCT p53-/- cells. Also DNA breaks level at 30-60 min. after irradiation was statistically higher in HCT p53-/- than in HCT p53+/+.

27. NFκB SUPPRESSES P53-DEPENDENT UV-INDUCED APOPTOSIS

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Signaling pathways that depend on p53 or NFκB transcription factors are essential components of cellular responses to stress. In general, p53 is involved in either activation of cell cycle arrest or induction of apoptosis, while NFκB exerts mostly anti-apoptotic functions; both regulatory pathways apparently interfere with each other.

Here we aimed to analyze effects of NFκB activation on DNA damage-induced apoptosis, either p53-dependent or p53-independent, in a set of human cell lines. Four cell lines, HCT116 and RKO colon carcinoma, NCI-H1299 lung carcinoma and HL60 myeloblastoma, each of them in two congenic variants either containing or lacking transcriptionally competent p53, were used. Cells were incubated with TNFα cytokine to activate NFκB and then treated with ultraviolet or ionizing radiation to induce apoptosis, which was assessed by measurement of the sub-G1 cell fraction. We observed that treatment with TNFα resulted in an approximately 2-fold reduction in the frequency of apoptotic cells in UV-irradiated p53-proficient lines (with exception of UV-resistant NCI-H1299 cells).

This anti-apoptotic effect was lost when cells were pretreated with parthenolide, an inhibitor of NFκB activation. In marked contrast, TNFα-pretreatment of p53-deficient lines resulted in an increased frequency of apoptotic cells after UV irradiation (with exception of HL60 cells). Such anti- and pro-apoptotic influence of TNFα was less obvious in cells treated with ionizing radiation. The data clearly indicates functional interference of both signaling pathways upon the damage-induced apoptotic response, yet the observed effects are both cell type- and stimulus-specific.

28. PHOTOPHYSICAL AND BIOLOGICAL STUDIES OF SOME NOVEL PHOTOSENSITIZERS WITH PROSPECTS FOR USE IN PDT

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Photodynamic therapy (PDT) is a promising approach to cancer treatment which combines light, a photosensitizer and oxygen action. However, search for novel, better photosensitizers is continuing as majority of photosensitizers that have been synthesized do not exhibit desirable combinations of chemical, photophysical and biological properties which is a perequisite for improved efficacy of PDT against varying types of cancer.

The aim of the presented study was to investigate selected properties of various photosensitizing compounds with special interest focusing on porphyrin-type and chlorin-type photosensitizers. Preliminary i*n vitro* cytotoxicity studies were performed using colon adenocarcinoma cells (Hct116) and mouse Lewis lung carcinoma cells (LLC). Cell proliferation was evaluated by MTS-tetrazolium reduction assay for both types of photosensitizers. More detailed studies were carried out for a chlorin-type photosensitizer, for which dark toxicity, PDT efficiency and some chemical as well as photophysical properties were evaluated. To verify chemical structure of the examined chlorin photoelectron spectroscopy (XPS), Raman and infrared (IR) spectroscopy were used. Photophysical properties such as absorption in the therapeutic window (600 – 800nm) were determined by UV-VIS spectroscopy (emission and excitation spectra).

The obtained Raman, IR and XPS spectra confirmed the chemical structure of the examined chlorin derivative. Chemical purity, photophysical and biological properties of this photosensitizing compound fulfill suitability requirements for application in PDT. The investigated porphyrin-type photosensitizers, on the other hand, require further study as primary results indicate that high dark toxicity of some of them makes them rather useless in PDT whereas others should be investigated thoroughly.

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29. THE INTERACTION OF TRANSFRRIN - DOXORUBICIN CONJUGATE WITH CCRF-CEM LEUKEMIA CELLS

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Doxorubicin (DOX) is a monosaccharide anthracycline anticancer drug active against a wide variety of solid tumors and hematological malignancies. Nevertheless, its using causes many systemic side effects. Transferrin (TRF) is one of the very promising proteins that can be used to transport anticancer drugs directly to the neoplastic cells.

DOX was coupled to human TRF by using glutharaldehyde crosslinking method. The mixture conjugates were separated by column chromatography and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS/PAGE). These methods confirmed that the obtained conjugate was the connection of two compounds – doxorubicin and transferrin.

T-lymphoblast leukemia (CCRF-CEM) cell is moderately sensitive to several cytotoxic agents and have huge number of transferrin receptors on their surface. Cytotoxicity studies performed with 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay reveals that TRF-DOX conjugate inhibited the proliferation of tested cells more effectively than free DOX. The kinetics of leukemia cells' growth show that DOX and conjugate significantly inhibited the cell proliferation.

Apoptosis was detected in cells using Hoechst 33258/Propidium Iodide double staining after 48 hours incubation with drugs. The results demonstrate that TRF-DOX induced a higher level of apoptosis and necrosis in cultured cells than DOX. However, more studies are needed to show if that conjugate of DOX could replace the free anthracycline in chemotherapy.

30. THE EFFECT OF PIROLIN ON THE LEVEL OF OXIDATIVE STRESS INDUCED IN HEART TISSUE OF WISTAR RATS TREATED WITH ANTICANCER DRUGS (DOXORUBICIN AND TAXANES)

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Doxorubicin (DOX) is an anticancer drug commonly used in chemotherapy of various tumours. However, antitumour therapy with this anthracycline is limited by the risk of developing heart failure. Generation of reactive oxygen species (ROS) contributes to the cardiotoxicity of doxorubicin, which is involved in the treatment. It has been shown that risk of heart disorders is higher upon taxanes therapies (especially in combined therapies with paclitaxel (PTX and DOX). It seems that addition of antioxidants may decrease side effects of anticancer drugs. Nitroxides are low molecular weight, stable free radicals reacting with free oxygen radicals and present antioxidant properties.

The aim of this study was to analyze the effect of nitroxide Pirolin (PL, 3-carbamoyl-2,2,5,5-tetramethylpirroline-1-oxyl) on the oxidative stress level induced by DOX and taxanes in the rats hearts. Rats were injected intraperitoneally with tested compounds and sacrificed four days later. The investigated compounds were injected alone or in combinations. Assessments of the amount of lipid peroxidation products (TBARS) and peroxides level were performed. The increase of the level of peroxides was found in the hearts treated with the combination of doxorubicin and Pirolin in comparison with single treatment with PL. In the case of docetaxel significant changes in the oxidative stress markers were not detected. Neither protective nor prooxidative properties of Pirolin were observed when used in combination with docetaxel. However, results obtained after paclitaxel injection have shown increase of the level of peroxides in heart tissue in comparison with control group. Pirolin in combination with PTX significantly decreased the effect induced by paclitaxel. DOX caused elevation of the level of TBARS in comparison with control group. Induction of TBARS by Pirolin and taxanes in comparison with control group was lower than that for DOX. Pirolin used simultaneously with DOX or PTX decreased production of TBARS in comparison to drugs injected alone.

The obtained results indicate that Pirolin influences prooxidative effect induced by paclitaxel. Presented data suggest that Pirolin interacts with peroxides producing thiobarbituric acid reactive substances in heart tissue. On the other hand, in combination with DOX, Pirolin lowers the level of TBARS, thus acts as an antioxidant.

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31. P53 RELATED RESPONSE OF HUMAN COLON CARCINOMA CELL LINES TO RADIATION AND BYSTANDER SIGNALS

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Radiation-induced bystander effects are biologic responses of nonirradiated cells that were not traversed by an ionizing radiation track. These bystander effects take place in the neighbors of irradiated cells, bystander cells, via cell-to-cell gap junctions or in other nonirradiated cells that have received secreted signals (soluble factors) from irradiated cells. It is likely that multiple signaling cascades involving both an initiating event and downstream signaling steps are necessary to mediate the bystander process. P53 gene seems to play an important role in the bystander effect since it coordinates cellular response to oxidative stress created by ionizing radiation through cell cycle control and apoptosis.

The aim of our studies was focused on comparing the response of two human colorectal carcinoma HCT116 cell lines differing in p53 status when they were directly exposed to radiation or exposed to soluble factors during co-culturing with irradiated ones.

As the first step of experimental cycle the radiation (0-6Gy) sensitivity of HCT116 p53+/+ and HCT116 p53-/- measured as clonogenic cell survival, induction of micronuclei and apoptosis was assessed. The dose dependent clonogenic cell survival was similar for p53 wild type and p53 knocked-out cells; however, both cell lines differed considerably in induction of micronuclei and apoptosis. Both cell lines differed also in the expression of some genes (CASP6, IRAK1,CHUK) engaged in apoptosis as measured by qRT-PCR.

The bystander experiments were then performed and micronuclei and apoptosis frequencies were measured in radiation-exposed and bystander cells. Signal molecules released from irradiated cells induced apoptosis in p53 knocked-out cells with higher efficiency than in wild type cells. The molecular nature of possible p53-independent apoptosis pathway needs further exploration.

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32. D-METHIONINE IN NOISE INDUCED HEARING LOSS

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We tested D-methionine (D-Met), a sulfur containing compound, as an otoprotectant in male C57BL/6J/Han/IMP mouse strain. D-Met was administrated 1 h before and 1 h after noise exposure. One additional dose each was given on days 1 and 2 after noise exposure.

The changes in superoxide dismutase (SOD), catalase, lipid peroxidation (LPO) were measured in the cochlea 3, 7, 14 after noise exposure (4 kHz octave band at the intensity of 110 dB SPL for 4 hours) in C57BL/6 mice. ABR were measured for each animal before and after noise exposure and after 14 days. The ABR indicates significant functional deficits due to noise exposure which stabilize in two weeks with a permanent threshold shift (PTS) of 15 dB for both 4 kHz and 8 kHz. In addition we also studied the action of an antioxidant D-methionine (D-met) to investigate its role in preventing this noise induced oxidative stress and hearing loss. D-met was able to scavenge the free radicals resulting in a significant decrease in LPO levels from noise exposure controls on the 7th day, apart from attenuating the changes in enzyme activity to control limits on the 3rd and 7th day. It also significantly reduced the PTS observed on the 14th day from 15 dB to 5 dB for 4 kHz and from 15dB it to 7 dB for 8 kHz . In summary, we established that D-met significantly protected against permanent noise-induced hearing loss.

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Addendum

RADIATION INDUCED BIOLOGICAL BYSTANDER EFFECT ELICITED *IN VITRO* BY TARGETED RADIOPHARMACEUTICALS LABELED WITH α–, β– AND AUGER ELECTRON EMITTING RADIOHALOGENS - lecture

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Recent studies have shown that indirect effects of ionizing radiation may contribute significantly to the effectiveness of radiotherapy by sterilizing malignant cells that are not directly hit by the radiation. However, there have been few investigations of the importance of indirect effects in targeted radionuclide treatment. Our purpose was to compare the induction of bystander effects by external beam γ-radiation with those resultant from exposure to three radiohaloanalogues of *meta*-iodobenzylguanidine (MIBG): [^{131}I]MIBG (low linear energy transfer (LET) β-emitter), [^{123}I]MIBG (potentially high LET Auger electron emitter), and *meta*-[^{211}At]astatobenzylguanidine ([^{211}At]MABG) (high LET α-emitter).

Methods: Two human tumor cell lines - UVW (glioma) and EJ138 (transitional cell carcinoma of bladder) – were transfected with the noradrenaline transporter (NAT) gene to enable active uptake of MIBG. Medium from cells that accumulated the radiopharmaceuticals or were treated with external beam radiation was transferred to cells which had not been exposed to radioactivity and clonogenic survival was determined in donor and recipient cultures. Other endpoints were also examined to determine the mechanisms involved in the observed effects.

Results: Over the dose range 0 to 9 Gy of external beam radiation of donor cells, 2 Gy caused 30 to 40% clonogenic cell kill in recipient cultures. This potency was maintained but not increased by higher dosage, thus indicating lack of a dose response relationship with respect to the generation of bystander signals after a particular dose administered to the donor cells. In contrast, no corresponding saturation of bystander cell kill was observed after treatment with a range of activity concentrations of [^{131}I]MIBG, which resulted in up to 97% death of donor cells. Cellular uptake of [^{123}I]MIBG and [^{211}At]MABG induced increasing recipient cell kill up to levels that resulted in direct kill of 35 to 70% of clonogens. Thereafter, the administration of higher activity concentrations of these high-LET emitters was inversely related to the kill of recipient cells. Over the range of activity concentrations examined, neither direct nor indirect kill was observed in cultures of cells not expressing the noradrenaline transporter and thus incapable of active uptake of MIBG.

Conclusion: Potent toxins are generated specifically by cells which concentrate radiohalogenated MIBG. These may be LET-dependent and distinct from those elicited by conventional radiotherapy.

BINDING OF GENISTEINE DERIVATIVES TO ABL AND LCK PROTEIN KINASES, AND TO MICROTUBULES - MODELLING STUDIES - lecture

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Genistein derivatives inhibit activity of Abl and Lck tyrosine kinases. These derivatives also cause disintegration of microtubules of the central spindle when applied at micromolecular concentration to the studied cell lines, as well as inhibit polymerization of tubulin.

In our modeling studies we optimized structures of selected genistein derivatives. Their most stable conformers were identified. Structures of the Abl and Lck kinases were also refined. Possible binding sites were found, and the genistein derivatives were docked to the mentioned targets. Also, 3D structures of microtubules were build up and optimized. Their end-parts contain GTP and GDP nucleotides. Experimental results reported by A. Rusin during this conference were accounted by our modelling studies.

During this conference novel results of our modeling studies will be reported, in particular:

- most probable specific interactions of the genistein derivatives with the mentioned above tyrosine kinases will be indicated,
- possible binding modes of the genistein derivatives with microtubules will be presented and discussed - the modeling procedures utilize existing results for colchicine interacting with microtubules.

Conclusions will be formulated in context of the experimental results obtained by the collaborating experimental groups.

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IMPACT OF ACIDOSIS ON MODULATION OF GABAERGIC IPSCS BY BENZODIAZEPINE RECEPTOR AGONISTS - lecture

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Benzodiazepine (BDZ) receptor agonists are known to increase the amplitude and duration of IPSCs. Moreover, at low [GABA], BDZs strongly enhance the current responses suggesting the up-regulation of agonist binding while their action on gating remains a matter of debate. Importantly, BDZs are widely used in clinical practice while several brain pathologies are associated with local alterations of extracellular pH (most typically acidosis) in the brain. Taking this into account, we have examined the impact of combined action of BDZ receptor agonists and acidification of extracellular medium on GABAergic currents. For this purpose, the effects of these factors on $GABA_AR$ binding and gating were investigated by a parallel analysis of mIPSCs and the current responses to rapid GABA applications. At control pH (7.2), flurazepam and zolpidem enhanced the amplitude and prolonged decay of mIPSCs. Both BDZ receptor agonists strongly enhanced responses to low [GABA] but, surprisingly, decreased the currents evoked by saturating or half-saturating [GABA]. Analysis of current responses to ultrafast GABA applications indicated that flurazepam and zolpidem enhanced binding and desensitization of $GABA_A$ receptors. These BDZ receptor agonists markedly prolonged deactivation of responses to low [GABA] but had almost no effect on deactivation at saturating or half-saturating [GABA]. Recordings of responses to half-saturating [GABA] applications revealed that appropriate timing of agonist exposure was sufficient to reproduce either a decrease or enhancement of currents by flurazepam or zolpidem. Recordings of currents mediated by recombinant ("synaptic") $\alpha1\beta2\gamma2$ receptors reproduced all major findings observed for neuronal $GABA_ARs$. Thus the results obtained at pH = 7.2 indicated that extremely brief agonist transient renders IPSCs particularly sensitive to the up-regulation of agonist binding by BDZs. Our previous studies (Mozrzymas et al. 2003, J.Neurosci.) have shown that binding and desensitization are strongly affected also by extracellular pH, the factor that may be severely altered in brain pathology. We have found that at acidic pH (6.0), flurazepam produced a stronger enhancement of mIPSC amplitudes than at physiological pH. At low [GABA], flurazepam markedly enhanced current amplitudes both at normal and at acidic pH but at the latter, the relative effect was larger. On the contrary, at saturating [GABA], flurazepam reduced current amplitudes both at pH = 7.2 and 6.0. Slowing down of deactivation kinetics by flurazepam decreased with GABA concentration but at pH = 6.0, this trend was shifted towards higher [GABA]. Pharmacokinetic analysis of current responses to ultrafast GABA applications indicated that the effects of flurazepam and protons are additive. We conclude that changes in extracellular pH not only affect the amplitude and time course of mIPSCs but also alter their susceptibility to modulation by benzodiazepine receptor agonists.

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33. COMBINED ANTICANCER THERAPY WITH ANTIVASCULAR PEPTIDE AND CHEMOTHERAPEUTIC-LOADED LIPOSOMES

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The study has sought to validate the combination of a vascular-targeting RGD-4C-GG-D(KLAKLAK)2 oligopeptide and a chemotherapeutic (doxorubicin) entrapped in long-circulating liposomes (targeted by folate ligand and non-targeted) in destroying malignant melanoma tumors in mice. The RGD-motif allows selective binding of the oligopeptide to αVβ3 integrin receptors The KLAKLAK2-motif domain mediates apoptosis of endothelial cells with subsequent necrosis of neighboring neoplastic cells. The liposomes allow the chemotherapeutic to penetrate and destroy neoplastic cells. Mice bearing B16(F10) tumors were subjected to the combined treatment to seek improvement of the therapeutic outcome.
Liposomes (DSPC/cholesterol/DSPE-PEG or DSPE-PEG-folate) were prepared using ammonium sulphate solvation procedure followed by extrusion and dialysis into sucrose solution. Doxorubicin was entrapped inside liposomes using transmembrane pH gradient. Animals were administered the peptide intratumorally and liposomes were injected intravenously. Tumor growth rate and animal survival were monitored.

Monotherapy of B16(F10) melanoma tumor-bearing C57BL6 mice with therapeutic peptide alone (4× 250μg) resulted in inhibited tumor growth but did not extend animals' survival. A single round of alternating repeated administrations of KLAK (4×250μg of peptide) and/or doxorubicin-carrying liposomes (4×40ug of drug) to tumor-bearing mice showed no sizeable differences in tumor growth inhibition or survival between groups treated with non-targeted or targeted liposomes, although both combinations were better than controls or free doxorubicin. On the other hand, when growth of tumors was rechallenged with a second round of combination therapy, tumor growth was inhibited several-fold and survival of animals was significantly extended compared to controls. Repeated combination therapy revealed differences between antivascular peptide combination with FTL and that with NTL liposomes in favor of pegylated targeted liposomes.

Peptide-mediated monotherapy resulted in multifocal cluster-type necrosis involving ca. 20% of tumors whereas liposomal doxorubicin produced multiple necrotic foci spread throughout tumors (ca. 15%). Combination therapy caused diffuse type of necrosis involving ca. 60% of tumors.

34. EFFECTS OF POLY(ADP-RIBOSE) POLYMERASE INHIBITION IN MYELOGENOUS LEUKAEMIA CELLS K562

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Poly(ADP-ribose) polymerase 1 (PARP-1 or PARP), is a nuclear enzyme catalyzing the synthesis of long and branched homopolymers of ADP-ribose from NAD^+ molecules. The enzyme is well known for its multiple regulatory functions: from DNA repair and transcription to cell survival and death. Modulating its activity, from stimulation to inhibition, is claimed to be applicable in treatment or prevention of many disease states including cardiac infarct, diabetes, inflammation, retroviral infection and cancer.

So far, several PARP inhibitors have been developed, mostly to sensitize tumor cells to chemo- and radiotherapy through inhibition of DNA repair. We used an immunocytochemical approach in our study of myelogenous leukaemia cells (K562) and mouse Lewis lung carcinoma cells (LLC) treated with 100 μM H_2O_2 to examine *in situ* the PARP inhibitory efficiency of 8-hydroxy-2-methyl-4(3H)-quinazolinone (NU1025). We also used comet assay to compare kinetics of DNA repair with the level of poly(ADP-ribose), PARP and its spatial distribution in nuclei.

According to the obtained data, NU1025 indeed inhibits PARP which is demonstrated by significant decrease in the level of poly(ADP-ribose) during DNA strand break rejoining. The results of microscopic observations suggest hat there are also differences in the distribution of PARP in cells with inhibitor as compared to untreated cells. Cells treated with NU1025 showed tendency to cumulate PARP in larger aggregates, whereas poly(ADP-ribose) polymerase in untreated cells exhibited more uniform distribution.

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