

Gliwice Scientific Meetings 2006



The 10th Anniversary of Gliwice Scientific Meetings

Gliwice, 17-18 XI 2006

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

Organizers:

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

Association for the Support for Cancer Research

The Silesian University of Technology

Supported by:

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

European Association for Cancer Research

Polish Academy of Sciences, Branch in Katowice

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GLIWICKIE SPOTKANIA NAUKOWE GLIWICE SCIENTIFIC MEETINGS

10th Anniversary

1997-2006

21-22th November, 1997

27- 28th November, 1998 *“50th Anniversary of Institute of Oncology In Gliwice”*

26- 27th November, 1999

24-25th November, 2000

23–24th November, 2001

22- 23th November, 2002

21-22th November, 2003

19-20th November, 2004

18-19th November, 2005 *„80th Anniversary of Professor Mieczysław Chorąży”*

17-18th November, 2006

Invited Speakers that contributed to our previous Meetings (1997-2005)

Baer-Dubowska, Wanda	Medical Academy, Poznań
Barciszewski, Jan	Institute of Bioorganic Chemistry, Poznań
Bartnik, Ewa	University of Warsaw, Warszawa
Blaese, Marcel	University of Tübingen, Tübingen
Brammer, Ingo	University of Hamburg, Hamburg
Bujnicki, Janusz M.	International Institute of Molecular and Cellular Biology, Warszawa
Campos-Lima, Pedro	Laval University, Quebec
Cebur, Stanisław	University of Wrocław, Wrocław
Cebulska-Wasilewska, Antonina	Institute of Nuclear Physics, Kraków
Chekhun, Vasyl F.	Institute of Experimental Pathology and Oncology, Kiev
Choraży, Mieczysław	Institute of Oncology, Gliwice
Chovanec, Miroslaw	Cancer Research Institute, Bratislava
Dadlez, Michał	Institute of Biochemistry and Biophysics, Warszawa
Dąbrowski, Michał	M. Nencki Institute of Experimental Biology, Warszawa
Drouin, Regen	Laval University, Quebec
Dusinska, Maria	Institute of Preventive and Clinical Medicine, Bratislava
Dux, Kazimierz	Institute of Oncology, Warszawa
Dziadkowiec, Dorota	University of Wrocław, Wrocław
Erenpreisa, Jekaterina	University of Latvia, Riga
Fajkus, Jiri	Institute of Biophysics, Brno
Figlerowicz, Marek	Institute of Bioorganic Chemistry, Poznań
Filipski, Jan	Institute of Jacques Monod, Paris
Formanowicz, Piotr	Institute of Bioorganic Chemistry, Poznań
Fujarewicz, Krzysztof	Silesian University of Technology, Gliwice
Garrard, William T.	UT Southwestern Medical Center, Dallas
Gaudray, Patrick	CNRS, Nice
Gniazdowski, Marek	Medical University, Łódź
Goc, Anna	Nicolaus Copernicus University, Toruń
Goedecke, Wolfgang	University of Essen, Essen
Goncharova, Rose	Institute of Genetics and Cytology, Minsk
Grażewicz, Maria	Institute of Biochemistry and Biophysics, Warszawa
Hancock, Ronald	Laval University, Quebec
Hansen, Lise L.	University of Aarhus, Aarhus
Hennig, Jacek	Institute of Biochemistry and Biophysics, Warszawa
Hennig, Wolfgang	Shanghai Institutes for Biological Sciences, Shanghai
Hesse, Hans	Max-Planck-Institute, Golm
Jałoszyński, Paweł	Institute of Human Genetics, Poznań
Janik, Przemysław	Institute of Oncology, Warszawa
Jarząb, Barbara	Institute of Oncology, Gliwice
Jarząb, Michał	Institute of Oncology, Gliwice
Jerzmanowski, Andrzej	Institute of Biochemistry and Biophysics, Warszawa
Jurka, Jerzy	Genetic Information Research Institute, Mountain View
Kasten-Pisula, Ulla	Eppendorf University, Hamburg
Kimmel, Marek	Rice University, Houston
Konieczny, Igor	University of Gdańsk, Gdańsk
Konopa, Jerzy	Gdańsk University of Technology, Gdańsk
Kruszewski, Marcin	Institute Nuclear Chemistry and Technology, Warszawa

Krzyżosiak, Włodzimierz	Institute of Bioorganic Chemistry, Poznań
Lachowicz, Mirosław	University of Warsaw, Warszawa
Larsson, Catharina	Pharmacology Biovitrum, Stockholm
Leluk, Jacek	University of Zielona Góra, Zielona Góra
Lesyng, Bogdan	University of Warsaw, Warszawa
Limon, Janusz	Medical Academy, Gdańsk
Los, Marek	University of Manitoba, Winnipeg
Lubiński, Jan	Pomeranian Medical Academy, Szczecin
Makałowski, Wojciech	Pennsylvania State University, University Park
Malanga, Maria	Institute for Veterinary Pharmacology and Toxicology, Zurich
Markiewicz, Wojciech	Institute of Bioorganic Chemistry, Poznań
Marszałek, Jarosław	University of Gdańsk, Gdańsk
Matter, Jean-Marc	University of Lausanne, Lausanne
Mirowski, Marek	Medical University, Łódź
Motykwicz, Grażyna	Institute of Oncology, Gliwice
Muller-Rober, Bernd	University of Potsdam, Potsdam
Nakielski, Jerzy	Silesian University, Katowice
Ninio, Ewa	INSERM, Curie University, Paris
Ninio, Jacques	Ecole Normale Supérieure, Paris
Obolenskaya, Maria	Institute of Molecular Biology and Genetics, Kiev
Oliński, Ryszard	Nicolaus Copernicus University, Bydgoszcz
Ortmann, Elisabeth	University of Vienna, Vienna
Ostrowski, Jerzy	Institute of Oncology, Warszawa
Pawlak, Andrzej	Institute of Human Genetics, Poznań
Philmonienko, Vlada	Institute of Experimental Medicine, Prague
Pingoud, Alfred	Justus-Liebig-University, Giessen
Plucienniczak, Andrzej	Institute of Biotechnology and Antibiotics
Polańska, Joanna	Silesian University of Technology, Gliwice
Polański, Andrzej	Silesian University of Technology, Gliwice
Radzikowski, Czesław	Institute of Immunology and Experimental Therapy, Wrocław
Razin, Sergey V.	Institute of Gene Biology, Moscow
Rothkamm, Kai	University of Saar, Homburg
Rusin, Marek	Institute of Oncology, Gliwice
Rzepecki, Ryszard	University of Wrocław, Wrocław
Rzeszowska-Wolny, Joanna	Institute of Oncology, Gliwice
Sasiadek, Maria	Medical Academy, Wrocław
Schluter, Urte	Riso National Laboratory, Copenhagen
Shertan, Harry	Institute of Radiobiology, Muenhen
Shiek, Valentin	Institute of Molecular Biology, Moscow
Sidorenko, Svetlana	Institute of Experimental Pathology and Oncology, Kiev
Sidorik, Lyudmila	Institute of Molecular Biology and Genetics, Kiev
Siedlecki, Janusz	Institute of Oncology, Warszawa
Siewiński, Maciej	Medical Academy, Wrocław
Sikora, Ewa	M. Nencki Institute of Experimental Biology, Warszawa
Simek, Krzysztof	Silesian University of Technology, Gliwice
Sirko, Agnieszka	Institute of Biochemistry and Biophysics, Warszawa
Sjakste, Nicolai	University of Latvia, Riga
Skrzydłowska, Ewa	Medical Academy, Białystok
Smółka, Bogdan	Silesian University of Technology, Gliwice
Staroń, Krzysztof	University of Warsaw, Warszawa

Stratling, Wolf H.	Eppendorf University, Hamburg
Szopa, Jan	University of Wrocław, Wrocław
Sztajer, Helena	Technical University of Braunschweig, Braunschweig
Szumiel, Irena	Institute Nuclear Chemistry and Technology, Warszawa
Szyfter, Krzysztof	Institute of Human Genetics, Poznań
Świerniak, Andrzej	Silesian University of Technology, Gliwice
Tarnawski, Rafał	Institute of Oncology, Gliwice
Tudek, Barbara	Institute of Biochemistry and Biophysics, Warszawa
Walewski, Jan	Institute of Oncology, Warszawa
Węgrzyn, Grzegorz	Gdańsk University of Technology, Gdańsk
Węsierski-Gądek, Józefa	Medical University of Vienna, Vienna
Widział, Maria	Institute of Oncology, Gliwice
Widział, Piotr	Institute of Oncology, Gliwice
Widział, Wiesława	Institute of Oncology, Gliwice
Wójcik, Andrzej	Institute Nuclear Chemistry and Technology, Warszawa
Zakrzewska, Jolanta	Institute of Immunology and Experimental Therapy, Wrocław
Zdzienicka, Margaret	University of Leiden, Leiden
Zimny, Janusz A.	Plant Breeding and Acclimatization Institute, Radzików
Żylicz, Maciej	International Institute of Molecular and Cellular Biology, Warszawa

Gliwice Scientific Meetings 2006

Friday, 17th November, 2006

9.00 - 9.15	Opening Ceremony
9.15 - 10.45	Session I (part 1)
10.45 - 11.00	Coffee break/poster viewing
11.00 - 12.40	Session I (part 2)
12.40 - 14.00	Lunch break/poster viewing
14.00 - 16.00	Session II
16.00 - 16.15	Coffee break/poster viewing
16.15 - 18.45	Session III
20.00 -	Social event/party

Session I: Cell Signaling and Responses to Stress

Carmel Mothersill (*McMaster University, Hamilton*): Radiation induced bystander effects in vivo – are they protective or destructive?

Maciej Żylicz (*IIMCB, Warszawa*): Role of molecular chaperones in cell transformation.

Anna von Mikecz (*HH University, Dusseldorf*): Quality control in the cell nucleus by the ubiquitin-proteasome system.

Ryszard Oliński (*NC University, Bydgoszcz*): Oxidative DNA damage; cause or consequence of cancer?

Barbara Tudek (*IBB, Warszawa*): Modulation of oxidative DNA damage repair by oxidative stress and neoplastic transformation.

Jolanta Barańska (*Nencki IEB, Warszawa*): Nucleotide receptors and their role in cellular signaling in glioma C6 cells.

Joanna Rzeszowska (*Institute of Oncology, Gliwice*): Intercellular communication in X-irradiated cells and its influence on gene expression profiles.

Jarosław Śmieja (*Silesian University of Technology, Gliwice*): What mathematical modeling can help with in analysis of signaling pathways? (Lessons from modeling of IFN- α pathway).

Session II: Cell death and aging

Ding Xue (*University of Colorado, Boulder*): Coordination of apoptotic DNA degradation and cell corpse engulfment in *C. elegans*.

Ewa Sikora (*Nencki IEB, Warszawa*): What if not apoptosis?

Andrzej M. Skladanowski (*University of Technology, Gdańsk*): Too many ways to die: cell death-cell survival dilemma in cancer chemotherapy.

Gregor Meiss (*JL University, Giessen*): Structure-function relationships in DNA-fragmentation factor.

Piotr Widlak (*Institute of Oncology, Gliwice*): Does active DFF need regulation? (How many steps for regulation of apoptotic nucleases?)

Session III: Modern Trends in Cancer Diagnostics and Therapy

Barbara Jarzab (*Institute of Oncology, Gliwice*): Cancer transcriptome: factors determining gene expression profiles.

Marek Jakóbiński (*Medical Academy, Warszawa*): Prospects of cancer immunotherapy.

Marek Los (*University of Manitoba, Winnipeg*): Hijacking cell survival pathways to selectively kill cancer cells.

Hanna Rokita (*Jagiellonian University, Kraków*): Immunotherapy of neuroblastoma with peptide vaccines based on molecular mimicry of GD2 gangliosides.

Aleksander Sieroń (*Silesian Medical Academy, Katowice*): Zinc-dependent metalloproteases and their inhibitors in biology and diagnostics of cancer.

Rafał Tarnawski (*Institute of Oncology, Gliwice*): Modern radio-oncology.

Frank Koltrowitz (*Miltenyi Biotec - MEDianus*): The experimental power of MACS molecular gene expression analysis products

Sobota, 18 listopada 2006

9.00 - 11.00	Sesja: Biotechnologia w Polsce (część 1 - Nowe Technologie)
11.00 - 11.15	Przerwa na kawę
11.15 - 12.30	Sesja: Biotechnologia w Polsce (część 2 - Edukacja)
12.30 - 12.45	Przerwa na kawę
12.45 - 13.45	Sesja: Biotechnologia w Polsce (część 3 - Perspektywy)
13.45 - 14.15	Prezentacje wybranych plakatów
14.15 - 14.30	Wręczenie Nagród i zamknięcie konferencji
14.30 - 15.00	Lunch

Biotechnologia w Polsce – perspektywy rozwoju i problemy dydaktyczne

Technologia:

Andrzej Pilc (*Instytut Farmakologii PAN, Kraków*): Nowe leki antypsychotyczne

Stanisław Bielecki (*Politechnika Łódzka, Łódź*): Możliwości zewnętrznego i wewnętrznego stosowania celulozy mikrobiologicznej w medycynie

Andrzej Plucienniczak (*Instytut Biotechnologii i Antybiotyków, Warszawa*): Produkcja ludzkiego hormonu wzrostu

Jan Szopa (*Uniwersytet Wrocławski, Wrocław*): Genetyczne modyfikacje roślin uprawnych

Bernard Korzeniewski (*Uniwersytet Jagielloński*): Analiza kontroli metabolicznej a biotechnologia

OLYMPUS Optical: FluoView 1000 – nowa jakość w mikroskopii konfokalnej

Edukacja:

Jacek Leluk (*Uniwersytet Zielonogórski, Zielona Góra*): Zdalne nauczanie wczoraj, dziś i jutro.

Amalia Guzdek (*Uniwersytet Jagielloński, Kraków*): Biotechnologia w Uniwersytecie Jagiellońskim - dekada nauczania.

Andrzej C. Składanowski (*Gdańska Akademia Medyczna, Gdańsk*): Patologia molekularna - jak są nauczani studenci Międzyuczelnianego Wydziału Biotechnologii Uniwersytetu Gdańskiego i Akademii Medycznej w Gdańsku - analiza SWOT

Perspektywy:

Korneliusz Miksch (*Politechnika Śląska, Gliwice*): Rola biotechnologii w zrównoważonym rozwoju województwa śląskiego

Ludwik Tomiałojć (*Uniwersytet Wrocławski, Wrocław*): Ekologiczne i ekonomiczne źródła obaw przed szybkim wprowadzaniem upraw GMO

Mieczysław Chorąży (*Instytut Onkologii, Gliwice*): Modyfikacje genetyczne – nadzieje i obawy

Lecture abstracts

NUCLEOTIDE RECEPTORS AND THEIR ROLE IN CELLULAR SIGNALLING IN GLIOMA C6 CELLS

Jolanta Barańska

Nencki Institute of Experimental Biology, 3 Pasteur St., 02-093 Warszawa, Poland

Extracellular nucleotides (ATP, ADP, UTP, UDP, and ATP metabolite, adenosine) induce a variety of responses and regulate a variety of functions in many cells of different origin. They act through two large families of receptors: P1, sensitive to adenosine and P2, sensitive to ATP, ADP, UTP and UDP. The P2 receptors include the intrinsic ion channel P2X receptors and G protein-coupled P2Y receptors. Among P2Y receptors, P2Y₂ responds to ATP and UTP, whereas P2Y₁ and P2Y₁₂, both respond to ADP. P2Y₁ and P2Y₂ receptors are coupled to PLC and are responsible for Ca²⁺ release, while P2Y₁₂ is negatively coupled to adenylate cyclase. P2Y₁, P2Y₂ and P2Y₁₂ receptors are all expressed in rat glioma C6 cells. They are not only positively coupled to PLC and negatively to adenylate cyclase but are also able to modulate activity of ERK1/ERK2 and phosphatidylinositol 3-kinase (PI3-K). These effects depend on physiological conditions of the cell. When the cells are incubated in a medium devoid of serum, nucleotides cause transient activation of ERK1/2. In non-starved cells, ADP markedly decreases the PI3-K activity, whereas in serum starved it causes an increase of the enzyme activity. Blocking of the P2Y₁ receptor by MRS2179 additionally increases this ADP response, suggesting that P2Y₁ contributes to glioma signalling by negative regulation of P2Y₁₂ action, whereas P2Y₁₂ has an opposite effect. During long-term (up to 96h) serum starvation, the cells change fibroblast-like flat morphology to a rounded one. This process is not a differentiation toward astrocytes since glial fibrillary acidic protein (GFAP) expression, standard astrocyte marker, decreases during serum deprivation. Moreover, in the cells starved for 72 and 96h, the P2Y₁ receptor is low or almost undetectable and under such conditions P2Y₁₂ receptor is the main player, responsible for ADP-evoked signal transduction. The P2Y₁₂ receptor activates ERK1/2 kinase phosphorylation, known cell proliferation regulator, and stimulates Akt activity. These effects were reduced by AR-C69931MX, specific antagonist of the P2Y₁₂ receptor. The shift in expression of nucleotide receptors from P2Y₁ to P2Y₁₂ seems to be a new and important self-regulating mechanism acting not in favor of differentiation but promoting cell growth and adapting for survival under inhospitable conditions.

PERSPECTIVES OF CANCER IMMUNOTHERAPY

Marek Jakóbsiak

Department of Immunology, Medical University of Warsaw, Banacha 1a, building 1F, 02-097 Warsaw, Poland

Tumor immunotherapy is based on application of selected cytokines demonstrating antitumor activity, monoclonal antibodies directed against tumor antigens, cytotoxic T lymphocytes, and active immunization using tumor vaccines.

As efficacy of most of these therapies remains unsatisfactory alternative therapeutic approaches are being developed. Among cytokines, only interleukin 2, interferon α , and tumor necrosis factor have found limited application in cancer patients. Monoclonal antibodies, including radioimmunoconjugates, are gaining a place in tumor therapy. T cytotoxic lymphocytes engineered to express chimeric T cell receptors are able to destroy tumor cells including those which have lost the expression of major histocompatibility complex molecules. Promising immunotherapeutic strategies are based on application of cancer vaccines consisting of gene-modified tumor cells. Tumor cells engineered to express major histocompatibility molecules, costimulatory proteins, and/or various cytokines induce effective immune response against tumor antigens.

CANCER TRANSCRIPTOME: FACTORS DETERMINING GENE EXPRESSION PROFILES

Barbara Jarzab

Nuclear Medicine and Endocrine Oncology Department, Maria Skłodowska-Curie Memorial Center and Institute of Oncology, Gliwice Branch, 44-101 Gliwice, ul. Wybrzeże Armii Krajowej 15, Poland

The clinical applications of microarray-based analyses in oncology raise much interest among both biologists and clinicians and require many specific questions to be solved. There are some important issues which should be considered for realization of this goal, among them the question of quality assurance of microarray analysis, which includes validation of the biological quality of the supplied samples (percentage of cancer cells in tumor samples) as well as analytical points (quality and quantity of RNA available, quality control of microarray analyses) . For the validity of final conclusions, the problems related to the biological heterogeneity of tumors and other clinical and biological factors influencing gene expression need to be understood. Also, data mining and statistical analysis require special tools, designed for microarray-derived data. Their understanding by their end-users – biologists and clinicians –is still far from being perfect. These aspects have to be taken into consideration at evaluation of the results. In the presentation examples of analysis will be shown in laryngeal cancer, thyroid cancer, lung cancer, pancreatic cancer and breast cancer.

HIJACKING CELL SURVIVAL PATHWAYS TO SELECTIVELY KILL CANCER CELLS

Subbareddy Maddika, Tadeusz J. Krocak, Srilekha Maddika, Emilia Wiechec, Anne Zuse, Nina Poric, Iran Rashedi, Mehdi Eshraghi, Soumya Panigrahi, and Marek Los.

Dept. Biochemistry and Medical Genetics, Dept. Human Anatomy and Cell Science, Manitoba Institute of Cell Biology, CancerCare Manitoba, 675 McDermot Ave. Rm. ON6010, Winnipeg, MB R3E 0V9

We have recently identified a novel role for the PI3-K/Akt pathway during programmed cell death induced by apoptin, as well as selected anticancer drugs. We show for the first time that apoptin interacts with the p85 regulatory subunit, through the proline-rich region of apoptin and the SH3 domain of PI3-K, leading to the constitutive activation of PI3-K. Downstream of PI3-K, Akt is activated and translocated to the nucleus together with apoptin, most likely through a piggy-back mechanism. Nuclear Akt phosphorylates p27^{kip1} at the Thr-157 site, which in turn mediates p27^{kip1} downregulation by proteasome-dependent degradation. Cyclin A-associated CDK2 is activated aberrantly upon p27^{kip1} downregulation and activated CDK2 translocates to the cytoplasm. CDK2 directly phosphorylates Bcl2, thus mediating its degradation and further activation of mitochondrial death pathway. Apoptin-facilitated nuclear Akt, in contrast to its cytoplasmic pool, appears to be a positive regulator, rather than the repressor of apoptosis. This also holds true for apoptosis induced by anticancer drugs, such as methotrexate, taxol, doxorubicin and cisplatin. Our observations indicate that PI3-K/Akt pathways have a dual role in both survival and cell death processes depending on the stimulus. The implicated link between the survival and cell death pathways during apoptosis opens new pharmacologic opportunities to modulate apoptosis in cancer.

STRUCTURE-FUNCTION RELATIONSHIPS IN DNA-FRAGMENTATION FACTOR

Gregor Meiss

*Institute of Biochemistry Justus-Liebig-University Gießen Heinrich-Buff-Ring 58
35392 Gießen Germany*

Fragmentation of chromosomal DNA is a biochemical hallmark of apoptotic cell death. Among candidate proteins involved in this process, the DNA-fragmentation factor (DFF) plays a major role. It is a heterodimeric complex of the nuclease DFF40/CAD and its inhibitor and chaperone DFF45/ICAD-L. Using sequence- and structure-based mutational analyses in combination with biochemical and biophysical characterization of protein variants, we have identified functionally important regions in the nuclease subunit DFF40/CAD. Determining the catalytic centre and the DNA binding region allowed us to assign specific roles for particular amino acid residues involved in DNA binding and cleavage by this enzyme. Here, I present structure-function data relating to the regulation of DFF40/CAD at the level of protein folding, nuclear transport as well as DNA binding and cleavage.

BIOACTIVE SMALL MOLECULES CAN MODULATE RADIATION-INDUCED BYSTANDER EFFECTS IN HUMAN CELLS AND TISSUE EXPLANTS

Carmel Mothersill, Nalini Agnihotri and Colin Seymour

Juravinski Cancer Centre, and the Dept of Medical Physics and Applied Radiation Sciences, McMaster University, Hamilton, Ontario, Canada L8S 4K1

Bystander effects including apoptosis and chromosomal instability in unirradiated cell populations receiving signals from irradiated cells are now accepted. Neither the nature of the signals nor the pathways involved in signal propagation or transduction are fully understood. Previously we have shown that very low concentrations of the monoamine oxidase inhibitors (MAOI's), *l*-deprenyl and clorgyline can change bystander responses although probably not by inhibiting MAO. We tested the hypothesis that other small signaling or bioactive molecules such as serotonin, L-DOPA, glycine or nicotine can also modulate or simulate bystander signal production by irradiated cells. We now have data which suggest that nano to micromolar concentrations of these agents can change bystander induced cell death. Zofran and Kitryl, inhibitors of 5HT type 3 receptors, and reserpine, also blocked the bystander effect although it is not clear that the target for the bioactivity of the drugs tested is the cell membrane. Evidence from measurements apoptosis frequencies and of bcl 2 expression in surviving colonies, could suggest mitochondrial targets have a role to play. The results may be important in the search for novel drugs for optimizing radiotherapy treatment or in the prevention of second cancers following therapy.

OXIDATIVE DNA DAMAGE; CAUSE OR CONSEQUENCE OF CANCER?

Ryszard Olinski, Rafal Rozalski, Daniel Gackowski, Marek Foksinski, Jolanta Guz, Tomasz Dziaman, Anna Szpila, Agnieszka Siomek

Department of Clinical Biochemistry, Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Torun

Reactive oxygen species (ROS) can cause oxidative damage to DNA resulting in potentially enhanced cancer risk. Commonly used biomarkers of oxidative DNA damage include measure of 8-oxo 7,8-dihydro-2'-deoxyguanosine (8-oxodGua) and its corresponding base, 8-oxoGua. These modifications can be determined either in cellular DNA or in urine where their excretion represents the average rate of oxidative DNA damage in the total body. Using recently developed methodology ((LC-GC-MS) we have found that urinary excretion of 8-oxoGua and 8-oxodG does not depend on diet in the case of humans and mice. Our study with cancer patients undergoing chemotherapy strongly suggest that cell death does not contribute to urinary excretion rate of the modifications. These data would appear to rule out various confounding factors, leaving DNA repair pathways as the principal source of urinary purine, if not DNA, lesions enabling such measurements to be used as indicators of repair.

In order to assess the role of oxidative DNA damage in cancer development we decided, for the first time, to analyse the broad spectrum of oxidative DNA damage biomarkers; urinary excretion of the base/nucleoside modification as well as the level of oxidative DNA damage and repair. In our recently published works have been found that the levels of oxidative DNA damage in leukocytes were significantly higher while the concentrations of the antioxidant vitamins were significantly lower in colon and lung cancer patients than in control group. Moreover, the same direction of the changes has been found in patients with adenoma. This, in turn, suggests that the changes in aforementioned biomarkers of oxidative stress are characteristic for cancer development. Although there is a little room for doubt on the basis of available experiments that oxidative DNA damage has some role to play in the pathogenesis of cancer, the quantitative relationship between the measured DNA damage and the rate of mutation and cancer is still lacking.

Age is a single the most important factor which may contribute to cancer development. There is a possibility that a common link of these pathological conditions is oxidative damage to DNA. We have demonstrated that the level of 8-oxodG in leukocytes' DNA showed statistically significant correlation with the age of the examined subjects (n = 256), and the level of urinary 8-oxoGua and 8-oxodG followed the same pattern. On the basis of the presented correlative association between oxidative DNA damage parameters and age it seems reasonable to state that the damage may be one of the substantial factors in human ageing.

IMMUNOTHERAPY OF NEUROBLASTOMA WITH PEPTIDE VACCINES BASED ON MOLECULAR MIMICRY OF GD2 GANGLIOSIDES

Hanna Rokita

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

Neuroblastoma remains the third most frequent cancer of childhood. Despite of the application of intensive treatment regimens, the majority of high-risk patients are eventually relapsing. Therefore new therapeutic approaches are needed to eradicate residual tumour cells that might improve the survival of the high-risk group neuroblastoma patients. Immunotherapy of neuroblastoma is one of the currently developed strategies. They include the passive and active therapies targeting a neuroblastoma antigen – GD2 ganglioside.

Gangliosides expressed on neuroectodermally derived tumours, including neuroblastoma and melanoma, remain weakly immunogenic in tumour-bearing organisms and induce predominantly immunoglobulin M antibody responses in the immunised host. However, the gangliosides GM2, GD2 and GD3 make the cells susceptible to immune attack by antibodies. Therefore, approaches are made to convert the GD2 ganglioside into a peptide mimetic to induce GD2 cross-reactive IgG antibody responses in mice. Peptide mimics of GD2 ganglioside constitute surrogate antigens of the glycolipid, able to elicit immunity to the self-antigen, although the original one may not.

We are working on application of a peptide vaccine to GD2 ganglioside that is T-cell dependent, highly immunogenic, and specifically directs the antibody response to a protective epitope. First, we searched through LX-8/f88-4 phage displayed peptide library with the 14G2a monoclonal antibody specific to GD2 ganglioside. A number of phage clones binding to 14G2a was identified. Within those clones five families bearing distinct peptide sequences were found. The peptides were synthesised and used in further experiments. Thus, the immunogenicity of the peptides and the ability to elicit cross-reactive humoral response against GD2 were tested *in vivo*. Additionally, effector functions of the sera from the peptide immunised mice were investigated in the CDC assay on the IMR-32 cells.

Concomitantly, sera of 16 patients who were diagnosed with neuroblastoma at the Jagiellonian University Children's Hospital, were collected and tested in ELISA for reactivity with GD2 ganglioside and selected peptides.

The work was supported by the grants 3P05A 00124 and 2P05A 03429 from the Ministry of Education and Science.

INTERCELLULAR COMMUNICATION IN X-IRRADIATED CELLS AND ITS INFLUENCE ON GENE EXPRESSION PROFILES

Joanna Rzeszowska-Wolny

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Cells exposed to ionising radiation (IR) transmit signals which induce DNA and chromosome damage, mutation, and apoptosis in non-irradiated cells, termed bystander effects, which are also induced by growth in medium from irradiated cells (irradiation conditioned medium, ICM). To examine possible changes in transcript profiles following transfer of cells into ICM we used oligonucleotide microarrays, sampling after 36 h to detect persistent effects which could be relevant to radiotherapy. The levels of <200 transcripts in Me45 (melanoma) changed >2-fold after ICM and IR, but using the criterion of a >1.1-fold change 6037 and 5616 showed increased or decreased levels, respectively after growth in ICM and the corresponding figures after IR were 6867 and 4785; more than half of those whose level changed were common to both ICM and IR. Grouping of transcripts into functional pathways (Kyoto Encyclopedia of Genes and Genomes) revealed significant differences ($p < 0.01$) in the numbers which were up- or downregulated by both ICM and IR in certain groups, most markedly in the neuroactive ligand-receptor interactions (up), oxidative phosphorylation (down), and proteasome component (down) groups. These differences were confirmed by real time RT-PCR. Signaling factors in the medium of irradiated cells therefore cause reprogramming of transcript levels, revealing a new facet of the bystander effect.

ZINC-DEPENDENT METALLOPROTEASES AND THEIR INHIBITORS AXIS IN BIOLOGY AND DIAGNOSTIC OF CANCERS

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Matrix metalloproteases are believed to play an important role in carcinogenesis via the degradation and remodelling of tumour surrounding extra cellular matrix, which could explain their association with survival. Although, many in vitro studies, animal models, and clinical research clearly showed involvement of MMPs in a number of critical steps during tumour growth and invasion, most synthetic MMP inhibitors, designed as anticancer agents, failed to improve patients outcome in clinical trials. The problems show that our understanding of the working mechanisms of MMPs in tumour biology is still poor. The most studied MMPs in tumourigenesis are MMP-2 and -9. A decade ago, for example it was reported that the levels of MMP-2 and MMP-9 in human gastric carcinoma tissues were enhanced and related to the survival of the patients. Since then the prognostic value of MMPs for carcinoma patients has been confirmed, however with variable success, in an ample of different studies and clinical trials testing the effect of MMP inhibitors for patients with various types of cancer. The major obstacle to develop valuable tests is that most of proteases is produced and secreted as inactive pro-enzymes, activated by proteolytic cleavage, and controlled in their activity by interaction with tissue specific inhibitors of MMPs (TIMPs). Disturbances in these processes are of eminent importance in tumour invasion and metastasis. Therefore, for diagnostic approaches it is critical to determine if the MMPs are at latent or activated state and what is the ratio of MMPs to TIMPs.

Degradation of extra cellular matrix is always linked to its remodelling required synthesis of new matrix involving action of numerous molecules including zinc-dependent procollagen C- and N-endopeptidases. Their activity is essential for providing self assembling collagen monomers by proteolytic removal of globular propeptides flanking the triple-helix. Also, at least for procollagen C-endopeptidase, it has been shown that this enzyme has broad spectrum of substrates including, up regulated in tumours, laminin V gamma 2 chain, prollysyl oxidase, and related to angiogenesis perlecan. The role of procollagen converting enzymes in tumourigenesis is still poorly understood and their natural inhibitors yet have to be identified. In this presentation I will discuss the current knowledge on structure and function of metzincins, TIMPs, and other inhibitors, their expression in cancers and already available, as well as potential diagnostic clinical tests for detection of metzincins including MMPs/TIMPs status in cancer patients.

WHAT IF NOT APOPTOSIS?

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Induction of apoptotic cell death with anticancer drugs or irradiation is correlated with tumor response. In addition, failure in anticancer treatment is due to drug resistance in cancer cells, and the phenomenon of drug resistance is considered to be almost equal to resistance to apoptosis. However the strong correlation between induction of apoptosis and drug sensitivity is not necessarily correlated with overall tumor sensitivity. Even if apoptotic cell death is blocked, non-apoptotic cell death could be achieved, leading to cell death. Anticancer drugs can induce other cell death mode, such as autophagy, mitotic catastrophe or proliferative cell death (cellular senescence), which have different characteristic from apoptosis. Moreover, the cell death mode strongly depends on cancer cell type and drug concentration. The main hallmark of apoptosis is activation of executor caspases followed by DNA fragmentation and apoptotic bodies formation. We showed that curcumin, a natural dye, induced caspase-3 activity and subsequent oligonucleosomal DNA fragmentation in human HL-60 cells, HL-60 cells with multidrug resistance phenotype (MDR), and resistant to undergo apoptosis upon etoposide treatment differentiated HL-60 cells. However, in human Jurkat cells curcumin used at the same concentration failed to cause DNA fragmentation despite the significant reduction of cell survival and activation of caspase-3 sufficient to cleave DFF45 factor which is the DFF40 endonuclease inhibitor. Inhibitory effect of curcumin on DFF40 activity resulted from curcumin binding to the active center of DFF endonuclease. In other resistant to apoptosis cells, namely human HCW-2 derived from HL-60 cells and Bcr/Abl-transfected mouse 32 progenitor cells, which are a model of chronic myeloid leukemia, curcumin strongly inhibited cell proliferation and affected cell viability, induced caspase-3 activation and DNA fragmentation. However, both caspase-3 activation and DNA fragmentation followed G2/M cell cycle arrest, together with increased mitotic index and cellular and molecular morphology resembling not apoptotic ones, but those described for mitotic catastrophe. Relatively low concentration of curcumin did not induce cell death in human colon cancer HCT116 cells. Instead, the symptoms of cellular senescence were observed in these cells. Upon curcumin- or doxorubicin-treatment HCT116 cells became giant/polyploid and expressed active β -Sagalactosidase, a common marker of cellular senescence. Altogether, our results show that the same drug can induce many different cellular responses and apoptosis blockade can be overcome in cancer cells by inducing other types of cell demise.

TOO MANY WAYS TO DIE: CELL DEATH-CELL SURVIVAL DILEMMA IN CANCER CHEMOTHERAPY

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Cell death by apoptosis induced by chemotherapeutic treatment attracted enormous attention during the last 15 years. It has been long believed that apoptosis is the major, if not the only, pathway leading to death of tumor cells as a result of antitumor therapy. However, recent studies provide cumulative evidence that other cell death types and pathways are activated by antitumor agents such as paraptosis, mitotic catastrophe, autophagy, and even programmed forms of necrosis. To add more confusion, some cell death pathways seem not to be mutually exclusive e.g. apoptosis and autophagy or mitotic catastrophe and apoptosis may partially overlap or integrate, providing in this way a variety of different cellular responses to drug action. The best described examples are insufficient caspase activation and ATP levels which may function as molecular switches between apoptotic and necrotic death. The molecular mechanisms of apoptosis have been studied for many years and are currently known in great details. In contrast, molecular and biochemical phenomena which are associated with other death pathways have only started to be characterized.

Survival of tumor cells after drug treatment depends not only on the ability of these cells to activate different cell death programs but also on survival signaling mediated by e.g. PI3K/Akt/PKB, MAP kinases and inhibitors of apoptosis or IAPs (e.g. survivin). Both cell death and survival pathways can be defective or abnormally active in tumor cells which may lead to higher drug sensitivity in one case or drug resistance in the other. Modern therapeutic approaches are based on the modulation of these pathways by chemical inhibitors which could be combined with other antitumor agents to restore the ability of cells to die and improve drug efficacy.

In this presentation, the different cell death and survival signaling pathways induced in tumor cells after treatment with antitumor agents will be reviewed and their role in the cytotoxic and antitumor action of chemotherapeutic agents will be discussed. In particular, the issue will be raised whether induction of a particular cell death pathway could be predictive for the final therapeutic effect of drug treatment.

WHAT MATHEMATICAL MODELLING CAN HELP WITH IN ANALYSIS OF SIGNALLING PATHWAYS?

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Following rapid developments in new experimental techniques, mathematical modeling of signaling pathways that control intracellular biological and chemical processes is gaining increasing interest in biomedical research. Though the models are unavoidably much simplified, they can significantly contribute to the biological field. Knowledge about dynamics of the processes involved in a given pathway facilitates better planning of experiments. Mathematical models can help to formulate or reject new hypotheses about unknown processes underlying results observed in experimental work. As a result, directions to be taken in experimental work may be suggested by mathematical models. Moreover, modeling can be used to analyze perturbed behavior even before experiments are undertaken, and answer the question if the desired effects are possible. Finally, analysis of dynamics can indicate time points, at which measurements should be taken to gain maximum information from experiments.

This work presents how mathematical analysis influenced investigation of Interferon-beta stimulated signaling pathway. The model includes several feedback loops, and comprises both early and late responses of a cell to IFN- β treatment. The early response involves phosphorylation of STAT proteins, their subsequent dimerization and nuclear import of newly formed complexes. Once in the nucleus, those complexes act as transcription factors for early genes. Also in nucleus they undergo dephosphorylation followed by nuclear export of products of this process. Among the early genes is IRF1, and IRF1 protein is a member of complexes activating late gene expression.

Here, experimental work yielded surprising results that could not be explained by already known mechanisms regulating the pathway. Two main questions arose from these results: 1) Why, despite unperturbed phosphorylation of STAT1, STAT1 homodimer levels decrease very rapidly, and 2) What causes cytoplasmic accumulation of IRF1 protein, known to be an active transcription factor for late genes activated in the investigated pathway. Careful analysis of the mathematical model made it possible to state hypotheses about molecular mechanisms that could be the basis for the observed behavior and was subsequently used to plan a series of experiments, currently in progress, that should expand our knowledge about interferon-induced pathways.

This work is partially supported by BW-409-RAu1/2006.

MODERN-RADIOONCOLOGY

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Intensity modulated radiotherapy (IMRT) is an emerging concept of high-precision radiotherapy, a modality characterized by adaptation to patient and tumor characteristics. Modern radiotherapy has unprecedented technical capabilities to create conformal dose distributions allowing for avoidance of critical structures. Precision of IMRT is based on precise immobilization, advanced imaging, computer optimized treatment planning. Functional imaging may potentially help in delineation of target volume, tumor staging, visualization of possible microscopic tumor infiltration, estimation of radioresistance (Biological Target Volume). Modification of radiosensitivity nowadays is based on common use of radio-chemotherapy, but many molecular targeted drugs are used in clinical trials. Better understanding of tumor biology may potentially introduce molecular biology techniques to combined treatment (radio-chemotherapy) as prognostic/predictive factors or response modifications.

MODULATION OF OXIDATIVE DNA DAMAGE REPAIR BY OXIDATIVE STRESS AND NEOPLASTIC TRANSFORMATION

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Activity of DNA repair enzymes depends on many factors, such as gene polymorphisms, mRNA and protein level, as well as enzymes activation and inhibition. We report here on the modulation of repair activities of base excision repair enzymes eliminating from DNA oxidatively formed lesions by inflammation and neoplastic transformation. In newborn piglets, which on the third day after birth were supplemented with iron (75 or 200 mg in a single sc injection) increased repair activity for 8-oxoguanine (8-oxoG), and two lipid peroxidation-derived DNA damages, 1,*N*⁶-ethenoadenine (ϵ A) and 3,*N*⁴-ethenocytosine (ϵ C) was transiently observed. This was probably due to de novo enzyme synthesis, since 8-oxoG glycosylase (OGG1) quantity in the intestine epithelium increased on the 7th day after supplementation, and subsequently gradually decreased. Inflammation induced in newborn rats by i.p. injection of *E. coli* lipopolysaccharide (LPS) increased the repair activity for 8-oxoG in their intestines and the effect was observed even two months after LPS administration. This coincided with the stimulation of mRNA synthesis of abasic sites endonuclease, APE1, which can activate OGG1 glycosylase, *in vitro* up to 400-fold. LPS induced also preneoplastic changes - aberrant crypts foci (ACF) in colons of rats. Inflammatory processes probably change also repair capacity in humans. Both repair activity for 8-oxoG and mRNA level of APE1, but not OGG1, was higher in blood leukocytes of colon cancer patients in comparison to healthy controls. Cancer tissues also differ from unaffected surrounding in repair capacity of oxidative DNA damage. Lung tumors reveal lower 8-oxoG repair activity than normal lung tissue, however this is neither correlated to OGG1 and APE1 mRNA level, nor OGG1 protein level, but may depend on interaction with other Base Excision Repair regulating proteins, like XRCC1, TP53 or tuberine or direct oxidation of repair proteins. Modulation of repair enzymes activities may be a cell response to oxidative stress on its way to neoplastic transformation.

QUALITY CONTROL IN THE CELL NUCLEUS BY THE UBIQUITIN-PROTEASOME SYSTEM (UPS)

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Compartmentalized proteolysis provides an efficient tool for controlling degradation of specific proteins by regulating their subcellular localization. Considering the central role of the ubiquitin-proteasome system (UPS) in cellular processes, detailed knowledge of the time and place of substrate ubiquitination and proteolysis proves to be essential to our understanding of the molecular mechanisms that regulate cell structure, function, development and disease. Consistent with this idea we and others have shown that the UPS is an active player in the cell nucleus. Accumulating evidence suggests involvement of the protein ubiquitination machinery in both, epigenetic regulation of gene expression and nuclear quality control. We analyse nucleoplasmic protein clusters that contain components of the UPS with respect to their (i) protein composition, (ii) ubiquitination capacity, and (iii) proteasomal activity in order to define functional versus pathological protein aggregates / clusters. A tight balance of ubiquitination and proteolysis within or near such nucleoplasmic clusters may decide whether the cell nucleus sustains gene expression or the development of diseases, in particular those of neurodegenerative and autoimmune nature.

DOES ACTIVE DFF NEED REGULATION? (HOW MANY STEPS FOR REGULATION OF APOPTOTIC NUCLEASES?)

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One of the biochemical hallmarks of programmed cell death is DNA breakdown. The major apoptotic nuclease, DNA fragmentation factor (DFF), also termed Caspase-activated DNase (CAD), is primarily responsible for internucleosomal DNA cleavage during the terminal stages of apoptosis. In non-apoptotic cells, DFF exists in the nucleus as a heterodimer, composed of a 45 kD chaperone and inhibitor subunit (DFF45/ICAD) and a 40 kD latent nuclease subunit DFF40. Activation of caspase-3 results in DFF45 cleavage and release of active DFF40 that forms homo-oligomers. Binding of additional factors to such DFF40 homo-oligomers further regulates the nuclease activity. To date, histone H1, HMGB1/2 and TOPO2 have been reported to stimulate the nuclease activity, while nucleophosmin and CIITA have been identified as the nuclease inhibitors. It has been recently reported that DFF40/CAD is involved in maintaining genomic stability apparently playing a role of tumor suppressor (Yan *et al*, 2006, PNAS 103: 1504-9). This unexpected yet fascinating finding boosted efforts aimed to fully decipher mechanism involved in regulation of this nuclease. Currently, we have set up appropriate yeast two- and three-hybrid system in aim to identify novel partners of DFF40.

In conclusion, DFF is regulated by multiple pre- and post-activation fail-safe steps, which include requirements during translation for DFF45 (and HSPs) to mediate appropriate folding to generate a potentially activatable nuclease, the synthesis in stoichiometric excess of the inhibitor, and the presence of additional activators/inhibitors that regulate the nuclease after removal of its inhibitor.

COORDINATION OF APOPTOTIC DNA DEGRADATION AND CELL CORPSE ENGULFMENT IN *C. ELEGANS*

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Programmed cell death or apoptosis is a fundamental aspect of animal development and tissue homeostasis. Abnormal apoptosis may underlie many human diseases including cancers, autoimmune disorders and neurodegenerative disorders. We use the nematode *Caenorhabditis elegans* as a model system and a combination of genetic, biochemical, molecular biological, and functional genomic approaches to study how apoptosis is regulated, activated and executed. Using a functional genomic approach, we have identified nine cell-death related nucleases (CRN nucleases) that act in two independent pathways and in both dying cells and phagocytes to promote chromosome fragmentation during apoptosis and complete DNA degradation after dying cells are engulfed. Interestingly, defects in either DNA degradation pathway in the dying cell can compromise engulfment of apoptotic cell corpses in sensitized genetic backgrounds, suggesting that the apoptotic DNA degradation process and the cell corpse engulfment process are intrinsically connected. Further molecular genetic and biochemical analyses of these new cell-death nucleases and their interactions with other cell death factors in *C. elegans* will help elucidate how the apoptotic DNA degradation process and the cell corpse engulfment process are coordinated, regulated, and executed.

ROLE OF MOLECULAR CHAPERONE IN CELL TRANSFORMATION

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The p53 tumour suppressor gene encodes a sequence specific transcription factor, which is mutated in the vast majority of human cancer. One of the foremost characterized target genes of p53 is the *mdm2* gene. MDM2 protein possesses E3 ubiquitin ligase activity towards p53. Through its ability to ubiquitinate p53 and target it for proteasomal degradation, MDM2 plays key role in retaining p53 at very low levels in non stress conditions. MDM2 oncoprotein also possesses numerous p53-independent activities, which contribute to the development of tumours where *mdm2* is overexpressed, mostly by gene amplification. It should be stressed that not all MDM2 client proteins are targeted by it for proteasome degradation; hence not all involvements of MDM2 can be explained by its E3 ligase activity. Recently we have shown that Hsp90, in an ATP-dependent reaction, retains wt p53 in the conformation, which allows binding to the specific promoter sequence (Walerych et al., 2004). Hsp90 binds also to the mutant p53, but this interaction is indirect. With the use of highly purified proteins, we identified intermediate reactions that lead to the assembly of the multi chaperone complex (mutp53-Hsp40-Hsc70-Hop-Hsp90) (King et al., 2001). Given that MDM2 protein can bind ATP, interact with the HSP90 chaperone, and play a role in nascent p53 protein biosynthesis, we have evaluated and have found that MDM2 protein possesses an intrinsic molecular chaperone activity. MDM2 can function like the Hsp90 chaperone in the protection of citrate synthase and firefly luciferase from aggregation. MDM2 can also promote ATP-dependent *in vitro* p53 DNA-binding activity to the *p21* derived promoter sequence. Although the E3-ligase inactive MDM2 mutant still maintains the chaperone-like activity, the ATP-binding mutant MDM2 protein (K454A) lacks the chaperone activity. The *mdm2* cotransfected with wild-type *p53* stimulates efficient p53 protein folding *in vivo* and this effect is abrogated when using the ATP-binding defective form of MDM2. This is the first demonstration that MDM2 possesses an intrinsic molecular chaperone activity and indicates that the ATP-binding function of MDM2 can mediate its chaperone function towards p53 (Wawrzynow et al., 2006).

Poster abstracts

INVESTIGATION OF EXPRESSION AND CHROMOSOMAL LOCALIZATION OF GENE ENCODING TUMOR SPECIFIC PROTEIN WITH MOLECULAR WEIGHT 65 kDa

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P65 gene encodes 65 kDa tumour-associated protein. They seem to be a potential, non-specific tumor marker expressed in many neoplasms. P65 was first time isolated from the MCF-7 human breast cancer cell line. The presence of 65 kDa protein was observed not only in tumor tissue but also in the serum of patients diagnosed with different types of neoplasms. The blood level of P65 in patients suffering from cancer was significantly higher in comparison to healthy men. Also 150 bp fragment of *P65* gene expression was detected by multiplex semi-quantitative analysis in many different types of tumors. In majority of investigated carcinomas presence of this gene expression appeared in advanced clinical stages. Quality results were confirmed by real-time PCR analysis. We also established that high levels of *P65* are connected with shorter survival time.

The whole nucleotide sequence of *P65* gene is unknown so in the next step, fragment of gene detected in PCR analysis was elongated to 900 bp. Obtained fragment was sequenced and cloned into *E. coli* by TOPO system where expression of protein was stimulated. The expressed protein was isolated and digested by trypsin. The peptides spectra were compared with protein NCBI data base with the use of MASCOT program. The sequenced *P65* gene fragment revealed strong homology to the retrovirus by HERVd FASTA search, other computer analysis by Blat search using the UCSC Genome Browser showed that sequenced fragment is similar to the region of human chromosome 1. These results would be confirmed by hybridization procedures with the RPCI-11 library.

Supported by Grant KBN 2 P05A 098 27
Supported by Grant KBN 2 P05A 098 27

1. ANTITUMOR ACTIVITY OF CARRIER-METHOTREXATE CONJUGATES

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Methotrexate (MTX) has been used in chemotherapy of malignancies for several decades. However, low plasma half-life, toxicity to normal proliferating cells and other limitations impel scientists to search for improved forms of MTX. Conjugation of the drug with macromolecular carriers is one of the strategies applied to improve therapeutic properties of anticancer drugs.

We tested several conjugates of MTX, namely with fibrinogen, albumin, glycosylated proteins, dextrans and mannan. They were studied both *in vitro* and *in vivo*. It allowed us to assess their advantages and disadvantages, and also determined directions for further research. Almost all carrier-MTX conjugates revealed stronger antitumor activity *in vivo* against i.p. transplanted P388 mouse leukemia as compared to free MTX.

2. DEFECT IN DOUBLE-STRANDED DNA BREAKS REPAIR IN L5178Y-S CELLS IS NOT ASSOCIATED WITH ALTERATIONS IN THE AUTOPHOSPHORYLATION SITES OF DNA-DEPENDENT PROTEIN KINASE

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The DNA-dependent protein kinase catalytic subunit (DNA-PKcs) plays an essential role in the repair of double-stranded DNA breaks through the nonhomologous DNA end joining pathway (NHEJ) by initially recognizing and binding to DNA breaks. It has been shown that DNA-PKcs undergoes autophosphorylation at six sites tightly clustered within 38 residues (ABCDE cluster), and that this autophosphorylation event is critical for its function. Cells with mutated autophosphorylation sites in the ABCDE cluster are defective in the repair of ionising radiation-induced double strand breaks, but show in an *in vitro* test the same DNA-PK activity as the cells possessing wild type enzyme. This characteristics correspond well with the phenotypic features of the L5178Y-S (LY-S) cell line that is defective in double strand breaks repair, shows a pronounced G1 phase radiosensitivity, but in which the level of DNA-PK activity present in total cell extracts is similar to that of its radioresistant counterpart L5178Y-R (LY-R) cell line.

In the present study the possible alterations in the sequence encoding the cluster of autophosphorylation sites in the DNA-dependent protein kinase in LY-S cells were examined. Total RNA was extracted from LY-S and LY-R cells and partial cDNA containing ABCDE cluster was synthesized, amplified and sequenced. Obtained sequences were aligned and analysed.

Despite the presence of phenotypic features indicating the possibility of such alterations, no differences were found between the sequences encoding the autophosphorylation sites in L5178Y-R and L5178Y-S cells. In conclusion, the repair defect in LY-S cells is not related to the structure of the DNA-PKcs autophosphorylation sites in the ABCDE cluster.

This work was supported by the Institute of Nuclear Chemistry and Technology Statutory Grant.

3. WRN EXONUCLEASE ACTIVITY IS BLOCKED BY OXIDATIVE DNA BASE LESIONS

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Werner syndrome (WS) is a premature aging disorder caused by mutations in the WS gene (*WRN*). Loss of WRN is associated with genomic instability and an elevated incidence of cancer. Although WRN has been suggested to play an important role in DNA metabolic pathways, such as recombination, replication and repair, the precise role of WRN still remains to be determined. WRN is a member of the RecQ family of DNA helicases and possesses ATPase, helicase, and exonuclease activities. Previous studies have shown that the WRN exonuclease is inhibited by certain oxidative lesions, including 8-oxoguanine and 8-oxoadenine, positioned in the digested strand of the substrate. The presence of the Ku heterodimer alleviated WRN exonuclease blockage imposed by these lesions. In the current study, we analyzed several other oxidative lesions, which block WRN exonuclease progression and carefully analyzed their blocking abilities in contrast of two different substrates. We have also shown that Ku stimulates the WRN exonuclease to bypass these lesions. In addition, we also show that the sensitivity towards oxidative lesions is not a general feature of 3' to 5' exonucleases, as the exonuclease activity of AP endonuclease 1 (APE1) was not blocked by oxidative lesions or stimulated by Ku. This study extends the spectrum of lesions which block WRN exonuclease progression and supports a possible function for WRN and Ku in a DNA damage processing pathway.

4. EXPRESSION OF ESTROGEN AND PROGESTERONE RECEPTORS IN THYROID CANCER

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The problem of sex steroid receptors expression and their influence on development and progression of thyroid tumors is important from the viewpoint of higher thyroid cancer incidence rate among women than in men, especially during puberty (according to different authors male/female ratio ranges from 1:4 to 1:7). This difference is less visible among patients before menarche and after menopause.

The aim of this study was to analyze estrogen receptor (ER) and progesterone receptor (PR) expression among men and women with different thyroid diseases.

ER and PR expression was analyzed at transcript level using microarray analysis and at protein level using immunohistochemical staining. 120 probes were used for microarray analysis. Among them: papillary thyroid carcinoma (PTC) - 47 cases, follicular thyroid carcinoma - 12 cases, anaplastic cancer – 6 cases, follicular adenoma – 8 cases and nodular goiter – 36 cases. Immunohistochemical examination of ER and PR expression was conducted on thyroid tissue from 61 patients with different thyroid diseases: PTC – 17 cases, follicular carcinoma – 4 cases, medullary carcinoma – 1 case, nodular goiter – 13 cases, toxic goiter – 10 cases, lymphocytic thyroiditis – 9 cases, follicular adenoma – 8 cases.

The results of immunohistochemical examination show that majority of ER and PR-positive tissues were from patients with PTC: ER-alpha was positive in 11 of 61 (18%) of examined patients, among them in 8 of 17 (47%) of patients with PTC. PR were positive in 25 of 61 (40%) of examined patients, among them in 15 of 17 patients with PTC. Among all examined cases ER-alpha was observed in thyroid tissue of 18% male and 18% female patients, PR in 45% males and 41% females.

The results of microarray analysis show that expression of ESR1 was decreased in anaplastic, follicular and medullary carcinomas in comparison with PTC and normal thyroid. ESR1 expression level in PTC and normal thyroid was comparable but range of expression values in PTC was wider. To analyze this difference, hierarchical clustering of PTC and benign thyroid neoplasms/non-neoplastic thyroids (n=91) set was performed. Two subgroups (with higher and lower ESR1 expression level) were not related to the histopathological type of sample. The difference in ESR1 expression was also analyzed in the context of the gender of patients: in men the difference in ESR1 expression level between PTC and benign thyroid tissue is more visible. All tumors with maximal ESR1 expression level were found in women, but the differences between tumors and non-neoplastic thyroid were less visible.

In conclusion, no difference between ER and PR expression among men and women was found. Increased expression of both ER and PR in PTC was observed.

5. ANTIOXIDATIVE ABILITIES AND LIPID PEROXIDATION IN KIDNEY TUMOR

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Renal cell carcinoma (RCC) represents 2-3% of all cancers, with the highest incidence in the more developed countries. The worldwide and European increase in incidence is approximately 2%. Renal cell carcinoma is the most frequently occurring solid lesion within the kidney and comprises different RCC types with specific histopathological and genetic characteristics. There is a 1,5:1 predominance of men over women, with peak incidence occurring between 60 and 70 years of age. Aetiological factors include lifestyle factors, such as smoking, obesity and antihypertensive therapy. Common element of above situations that take part in development of kidney cancer is enhanced generation of free radicals. Kidney tissue is particularly susceptible to reactive oxygen species attack and antioxidants play an important role in defence strategy against them.

The present study aims at examining antioxidant parameters and malondialdehyde – the product of lipid peroxidation as well as the marker of cancer progression – in renal cancer (RCC) patients. The activity of superoxide dismutase, glutathione peroxidase and reductase, thiol compounds, vitamin C and malondialdehyde have been determined in tumors and unchanged tissue of 24 patients with renal cell cancer. The tumor and morphologically unchanged tissue were collected during surgery. The 10% homogenates were used to examinations. Superoxide dismutase activity was determined spectrophotometrically by measuring inhibition of epinephrine oxidation to adrenochrome. Glutathione peroxidase and glutathione reductase activities were measured spectrophotometrically by monitoring the oxidation of NADPH to NADP. The level of sulfhydryl groups were measured spectrophotometrically. Lipid peroxidation product malondialdehyde, ascorbic acid were detected by high performance liquid chromatography. Statistical analysis was performed using Wilcoxon Matched-Pairs Signed –Ranks Test and values for $p < 0,05$ were considered significant.

There were no statistical differences between superoxide dismutase activity in cancer tissue and non-tumor tissues. The present study has indicated a significant increase of glutathione peroxidase activity in tumor tissues as compared with controls in all cases. The glutathione peroxidase activity in kidney tumor was increased up to 400%. There were no statistical differences among kidney tumor and morphologically unchanged tissue in glutathione reductase activity, although was decreased in comparison to control tissue. Changes in the activities of antioxidant enzymes were accompanied by changes in non-enzymatic antioxidant parameters. The present study has evidenced a significant decrease in protein SH groups in tumor tissues as compared with controls. It has been shown that the level of vitamin C insignificantly decreased in tumor kidney tissue. The level of the last of lipid peroxidation product - malondialdehyde was increased average by about 150% in kidney cancer tissue.

In conclusion, the kidney cancer cells are more exposed to oxidative stress than the surrounding noncancerous cells and further understanding of tumour biology from the point of reactive oxygen species may be helpful for establishing a new strategy for cancer therapy.

6. MOLECULAR INTERACTION BETWEEN GD2 GANGLIOSIDE-SPECIFIC ANTIBODY 14G2a AND GD2-MIMICKING PEPTIDES

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Molecular mimicry is a phenomenon of structural similarity between two or more different molecules that gives them similar antigenic properties. The molecules that demonstrate mimicry are related in respect of conformation, although can differ significantly in terms of chemical nature. Molecular mimicry is naturally observed in a range of pathogens, but is also a useful research tool in experimental immunotherapy. The phenomenon is especially interesting to provide an alternative to non-protein antigens like glycolipids. Those antigens are often aberrant on cancer cells, but due to their chemical properties are poorly immunogenic and therefore difficult to apply in immunotherapy. However, peptide sequences that mimic glycolipid structure are able to elicit immune response which is cross-reactive against the original antigen.

In our studies we search for an active immunotherapy against neuroblastoma, a malignancy of neuroectodermal origin. One of the markers of the disease is GD2 ganglioside, a glycolipid abundantly expressed on the surface of neuroblastoma cells. Using phage-displayed peptide library LX-8/f88-4 and the 14G2a monoclonal antibody specific to GD2 ganglioside, we identified five peptide sequences with high affinity to 14G2a, which was confirmed by ELISA. Further experiments demonstrated that the peptides were able to compete with GD2 for the binding site of the antibody in a dose-dependent manner. Sequence similarity analysis performed with a multiple sequence alignment program revealed two clusters of peptides: (#85, #D, #8) and (#65, #94). Subsequently, a variety of molecular modelling tools was used to further investigate the nature of interaction between peptides and 14G2a binding site.

Molecular modelling of peptide-14G2a antibody interaction was performed with a reduced lattice representation model CABS (*CA, cB and Side groups*). The receptor structure (14G2a) was generated from amino acid sequence with a template structure PDB ID: 1SVZ. Then the ligand (peptide) sequence was introduced to the resulting structure and the complex was optimised with Monte Carlo simulation method. 5000 structures of receptor-ligand complex were obtained and subsequently clustered by HCPM method (*Hierarchical Clustering of Protein Models*). Complex structure from the largest cluster underwent full-atom reconstruction and was further optimised to calculate the ligand binding energy. The same full-atom representation model was used to identify interacting amino acid residues.

The *in silico* approach provided valuable information about the structure of the peptide mimics and produced enough data to compare the binding characteristics of the five peptide sequences. Moreover, the results obtained with molecular modelling are in agreement with *in vitro* assay results. Therefore, the methods used to construct the computer model were correct and could be employed to optimise the peptide sequences for 14G2a binding. Our studies demonstrated that the computational approach and the *in vitro* methods may successfully complement one another.

The work was supported by 3P05A 00124 grant from the Polish Ministry of Science and Information Technology and N302 034 31/3063 grant from the Polish Ministry of Science and Higher Education.

7. PARTICIPATION OF THE EGF RECEPTOR IN THE RESPONSE TO X-IRRADIATION IN HUMAN GLIOMA M059 K AND J CELLS

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The role of epidermal growth factor receptor (EGFR) in ionising radiation-induced DNA double-strand break (DSB) rejoining was investigated in two related human glioma M059 K and J cell lines. The latter cells are ionising radiation sensitive due to deficiency in the catalytic subunit of DNA-dependent protein kinase (DNA-PK_{cs}) – an essential component of DNA-PK-dependent nonhomologous DNA end joining (D-NHEJ).

DSBs were induced by X-irradiation of the cells (10 Gy) in the presence (or absence) of a specific EGFR kinase inhibitor - tyrphostin AG 1478 (T). DSB rejoining, measured with the use of pulse-field gel electrophoresis, was significantly slowed down by 5 μ M T in M059 K but not in M059 J cells. This effect corresponded with a decrease in survival in M059 K but not in M059 J cells subjected to combined (X+T) treatment, as compared with X-irradiation alone. During the repair period, the levels of EGFR in whole cells and nuclei were monitored using enzyme immunosorbent assay (ELISA). The total cellular EGFR level was higher in M059 J than in M059 K cells in agreement with the sensitivity to T treatment. After irradiation, EGFR accumulated in the nuclear fraction of M059 K (but not M059 J) cells and this process could be prevented by T treatment (5 μ M, 1 h) preceding irradiation and continued until the end of the experiment. The presented results allow us to suppose that in X-irradiated M059 cells the D-NHEJ system of DSB repair is activated by EGFR. Autophosphorylation of the receptor is essential for this process. X-irradiation-stimulated translocation of EGFR to the nuclei, not observed in M059 J cells, implicates a direct involvement of the receptor in DNA repair through interaction with DNA-PK_{cs}.

IN THE SEARCH OF NOVEL PARTNERS OF THE APOPTOTIC NUCLEASE DFF40/CAD - EXPLOITING OF YEAST TWO-HYBRID SYSTEM

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The major apoptotic nuclease, DNA fragmentation factor (DFF), also termed Caspase-Activated DNase (CAD), is primarily responsible for internucleosomal DNA cleavage during the terminal stages of apoptosis. In non-apoptotic cells, DFF exists in the nucleus as a heterodimer, composed of a 45 kD chaperone and inhibitor subunit (DFF45/ICAD) and a 40 kD latent nuclease subunit DFF40/CAD. Activation of the nuclease depends on caspase-3-mediated cleavage of DFF45/ICAD inhibitor and formation of DFF40/CAD homooligomers, which further may interact with additional activators or inhibitors.

In aim to identify novel proteins that interact with DFF40/CAD we have used yeast-two hybrid system. Yeast *S. cerevisiae* strain AH105 was transformed with human DFF40 (cloned into pGBT9 vector) and then mated with *S. cerevisiae* strain Y187 carrying mouse brain embryo cDNA library. Several clones have been identified, including DFF45/ICAD ones, that are under further investigation. Currently we are screening human HeLa cell cDNA library with yeast strains either expressing DFF40 alone or co-expressing DFF40 and DFF45 (so called three-hybrid system).

This work was supported by the Ministry of Science, Grant N301 058 31/1763.

8. APOPTOSIS OF LYMPHOCYTES IN *H. PYLORI CAG A+* AND *CAG A-* INDUCED PERIPHERAL BLOOD MONONUCLEAR CELLS OF *H. PYLORI* INFECTED AND UNINFECTED CHILDREN WITH GASTRITIS.

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Background: The aim of this study was to estimate that actual *H. pylori* infection influence on apoptosis of lymphocyte in *H. pylori* (*cagA+* and *cagA-*) induced peripheral blood mononuclear cells (PBMC) of *H. pylori* infected (Hp+) and uninfected (Hp-) children with gastritis.

Materials and Methods: 1) PBMC of Hp+ (n=6) and Hp- (n=5) children and controls (n=6) were used. 2) Quantitative analysis of apoptotic lymphocytes in 72h culture of PBMC with *H. pylori cagA+* and *cagA-* (induced apoptosis) or with medium alone (spontaneous apoptosis) (staining with Annexin V-FITC and Propidium Iodide (PI) and flow cytometry).

Results: 1) Lymphocytes of both Hp+ and Hp- children showed high apoptosis in comparison to control, 2) There were not statistically differences between *H. pylori*-induced and spontaneous lymphocyte apoptosis, 3) There was higher early spontaneous lymphocyte apoptosis in PBMC of Hp+ children than Hp- ones.

Conclusions: Gastritis is connected with high sensitivity of peripheral lymphocytes to apoptosis. It is not dependent on *H. pylori* infection.

9. GHRELIN, A NATURAL LIGAND FOR THE GROWTH HORMONE SECRETAGOGUE RECEPTOR, SENSITIZES BLOOD MONONUCLEAR CELLS TO OXIDATIVE STRESS

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Ghrelin, a recently described endogenous ligand for the growth hormone secretagogue receptor (GHS-R), is produced by stomach cells and is a potent regulator of food intake, energy expenditure, adiposity, and growth hormone secretion. However, the functional role of ghrelin in regulation of immune responses remains poorly defined. GHS-R and ghrelin are expressed in human T lymphocytes and monocytes, where ghrelin inhibits the expression of proinflammatory cytokines. Ghrelin exerts potent anti-inflammatory effects and attenuates endotoxin-induced anorexia in a murine endotoxemia model.

In this work we investigated the effect of ghrelin addition to food on the susceptibility of peripheral blood mononuclear cells to oxidative stressors generated during inflammation. Ghrelin was administered intragastrically to newborn piglets for 7 days at two doses 7.5 and 15 µg/kg body mass. After treatment, blood was collected by heart puncture and mononuclear cells (MNCs) were isolated by density gradient centrifugation on Histopaque 1137, resuspended in RPMI1640 medium supplemented with 20% of FCS. Isolated cells were exposed to different DNA damaging agents, such as X-radiation (dose 0-3 Gy, 200 kV, 5 mA, dose rate 1.2 Gy/min), H₂O₂ (0-250 µM) in PBS for 15 min at 4°C, SIN-1 (0-50 µM) in PBS for 15 min at 4°C. The extent of DNA damage was evaluated by alkaline comet assay. To investigate the influence of ghrelin on cells' ability to repair DNA damage, MNCs were irradiated with 2 Gy of X-radiation and disappearance of DNA breaks was monitored 0.5, 1 and 2 h after irradiation.

We found that ghrelin had a marked effect on the level of X-radiation-, H₂O₂- and SIN-1-induced DNA damage. In all cases, we observed a significant increase in the level of DNA strand breaks in ghrelin treated animals as compared with untreated controls. However, no effect of ghrelin was observed on cells' capacity to repair DNA damage. Our data point to a dual role of ghrelin in inflammation. On the one hand, it is known to inhibit the expression of proinflammatory cytokines and to have a potent anti-inflammatory action; on the other hand, expression of ghrelin potentiates the genotoxicity of oxidative stressors generated during inflammation.

This work was financed from research sources for years 2006-2009 as a scientific grant No. PBZ-KBN-093/P06/2003

10. PRO-APOPTOTIC ROLE OF PI3-K/AKT PATHWAY DURING APOPTOSIS INDUCED BY SELECTED ANTI-CANCER DRUGS

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Introduction: PI3-Kinase/Akt pathway is a well-known cellular pathway, which regulates the cell survival and proliferation. This pathway is frequently activated in various cancers and is responsible for poor response to anti-cancer therapeutics. Intriguingly in this context, Akt-inhibitors have proved to be only moderately successful in experimental cancer therapy. Thus, here we provide a novel approach, where instead of inhibiting the PI3-Kinase/Akt pathway, we redirect this survival pathway towards a death pathway by using selected anti-cancer drugs.. The constitutive activation and nuclear mislocalization of Akt, the central downstream effector molecule of PI3-K plays a positive role in apoptosis induced by selected anticancer drugs.

Purpose: The overall aim of this study was to investigate the role of PI3-K/Akt signalling pathway in promoting apoptosis induced by anticancer drugs such as Methotrexate and Docetaxel.

Material and Methods: The material for this study included 293-T, MCF-7 and PC-3 cell lines undergoing chemotherapeutic treatment with selected anticancer drugs like Methotrexate and Docetaxel. The anti-Akt and anti-phospho Akt antibodies are commercially available (Cell Signalling). The level of phospho-Akt and total Akt was detected by immunoblotting with anti-Akt and anti-phospho Akt antibodies respectively. The confocal microscopy was used for visualization of mislocalized Akt in cell structure in either control, untreated cells or drug treated cells. The percentage of cell death was studied by nicoletti method followed by flowcytometric analysis.

Results: Our results indicate that activation of Akt is associated with its consequent nuclear translocation during treatment with chemodrugs. The nuclear localization of Akt is dependent on the upstream activation of the PI3-K. The PI3-K inhibition by wortmannin resulted in abolition of nuclear localization of Akt in the presence of anticancer drugs. According to this event, we observed that nuclear Akt enhanced the cell death induced by Methotrexate and Docetaxel in contrast to inhibition of cell death in the presence of drug and PI3-K inhibitor.

Conclusion: The PI3-K/Akt activation and nuclear location of Akt play an important pro-apoptotic role in cell death induced by Methotrexate and Docetaxel.

11. COCKAYNE SYNDROME GROUP B PROTEIN IS INVOLVED IN REPAIRING OF DNA ADDUCTS INDUCED BY *TRANS*-4-HYDROXY-2-NONENAL

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Cockayne Syndrome (CS) is characterized by progressive multisystem degeneration and is classified as a segmental premature aging syndrome. The CS complementation group B (CSB) protein is engaged in transcription – coupled (TCR) repair, global genome and base excision repair (BER) of some types of oxidative DNA damage and in general transcription. Here we show that cyclic propano- or ethano adducts, which are induced by *trans*-4-hydroxy-2-nonenal (HNE) may be processed by the TCR pathway. For that reason we studied CSB proficient and deficient human cell lines, as well as several cell lines containing different point mutations in the ATPase domain of the CSB protein. We show for the first time, that CSB cell lines deficient in TCR are hypersensitive to extremely low, physiological concentrations of HNE and exhibit a higher number of sister chromatid exchanges (SCEs) in comparison to their proficient counterpart. We also show that a cell line bearing mutation in motif II of the CSB ATPase is as sensitive to HNE as a CSB-null line. Cell line with a mutation in motif V resembles the wild type, while motif VI renders intermediate sensitivity to HNE. Homology modeling of CSB protein showed that amino acids mutated in motifs II and VI, but not V, were localized in the vicinity of the ATP binding site. Treatment of wild type cells with HNE causes dephosphorylation of the CSB protein, which stimulates its ATPase activity and activates the TCR pathway. In addition, we observed for the first time that un-repaired HNE-DNA adducts can block transcription *in vitro*. This data confirms that the TCR pathway is engaged in the processing of HNE-induced DNA lesions. A decreased activity or lack of the TCR pathway may cause inhibition of transcription and may lead to apoptosis.

12. MASS SPECTROMETRY BASED INVESTIGATION OF PROTEIN N-HOMOCYSTEINYLATION IN BLOOD PLASMA

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Increased concentration of homocysteine (Hcy) is important factor causing arteriosclerosis, mainspring of early mortality from coronary diseases. There are many mechanisms of toxic action proposed for homocysteine. Among others, the most cited are: induction of oxidative stress, inflammatory factors, responses for non-folded proteins and modification of proteins by homocysteine tiolactone (HTL) - N-homocysteinylation. The latter effect relies on covalent linking of homocysteine to α -amino residue of lysine. It provokes serious physiological consequences. Proteins lose their functions, are toxic and become autoantigens inducing immunological response. Antigens specific to proteins modified by HTL can be used for detection of N-Hcy-proteins and can serve as a diagnostic tool for recognizing diseases caused by increased level of homocysteine in human blood. The alternative method for detection of N-Hcy-proteins could be elaborated using both, classic analytical methods like chromatography and modern techniques like MALDI/TOF mass spectrometry.

The aim of our project is to work out the efficient method for medical assay of the level of N-Hcy-proteins in human blood. In this poster the first results on mass spectrometric evaluation of N-homocysteinylation degree for chosen proteins are presented. This stage relied on elaboration of method for assaying the localization of N-homocysteinylation sites. For this purpose four N-homocysteinylated proteins: cytochrome c, transferrin, mioglobin and albumin were digested with trypsin and analyzed using MALDI/TOF mass spectrometer. Also blood plasma was analyzed in respect of albumin presence in this body fluid. Detailed analysis of results by comparison of peptide maps of control proteins with peptide maps derived from modified samples allowed us to localize N-homocysteinylation sites. Also analysis of control and modified intact model proteins permitted estimation of possible level of N-homocysteinylation of the studied proteins. Elaboration of quantitative approach for assayed so far peptides will provide good and relevant diagnostic technique for evaluation of patients with elevated levels of homocysteine in blood.

13. INTERACTION OF DINITROSYL IRON COMPLEXES WITH DNA

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Dinitrosyl iron (II) complexes (DNIC) are an important factor in the NO-dependent regulation of cellular signalling pathways. We examined DNIC interactions with DNA, choosing two ligands: glutathione and histidine, each representing different dinitrosyl iron complex with proteins. Glutathione is a low-molecular thiol compound ubiquitous in all kinds of cells and displaying a wide range of biological activities. Dinitrosyl-dithiol iron (II) complexes are well characterised species, in which iron atom is chelated by two S⁻² atoms and two NO molecules. Histidine is an amino acid, which imidazole ring participates in DNIC formation in non-thiol proteins. In the histidine dinitrosyl iron complexes, iron is coordinated to the N7 atom of imidazole ring.

Complexes of iron with histidine and glutathione were obtained in vitro and the interactions of these complexes with DNA was studied by circular dichroism spectroscopy. Formation of DNIC complexes was monitored by UV/VIS spectroscopy, IR spectroscopy and NMR. We examined the influence of increasing amounts of Fe(II) ions, either in the form of dinitrosyl complexes or in the form of hydrated Fe(II) ions, on DNA in different pH and ionic strength conditions.

The right band of the DNA spectrum is monotonously decreased by the increase of the FeSO₄ concentration. This effect is completely suppressed in pH 7; increasing ionic strength also eliminates this effect gradually. The small decrease in the intensity of the right band of the circular dichroism spectrum of DNA, upon addition of Fe(II) up to 1:1 molar ratio indicates that the interaction between the metal complex and DNA induces only slight modifications to the native conformation of DNA. Disappearance of this effect in pH 7 illustrates that observed effect comes from an external electrostatic binding interaction, between the Fe(II) cation and the negatively charged phosphate groups of DNA. In pH 7 Fe(II) is present in the solution in the form of Fe(OH)₂, and therefore, is no longer attracted to DNA. Influence of ionic strength supports this explanation. Similar spectra were found by Silvestri et al. [Wolak M., van Eldik R.: *Coord. Chem. Rev.*, **230**, 263-282 (2002)] for Fe (Salen) complexes and also attributed to ionic interaction.

Supported by the Polish State Committee (KBN) statutory grant for the INCT

14. *ABCB1* GENE POLYMORPHISM, HAPLOTYPES ANALYSIS AND PROGNOSIS IN COLORECTAL CANCER

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The *ABCB1* (*MDR1*) gene encodes P-glycoprotein (P-gp), a 170kD member of adenosine triphosphate-binding cassette (ABC) superfamily of membrane transporters. P-gp is ATP-dependent transporter exporting xenobiotics to extracellular environment. Overexpression of P-gp correlates with high pathological grading of tumours in colon cancer.

Mutations in *ABCB1* gene may change substrates specificity of P-gp. It was also shown that single nucleotide polymorphisms (SNP) in *ABCB1* gene can influence the level of expression of P-gp.

Relation between C1236T, G2677T/A and C3435T SNPs of *ABCB1* gene remains unclear. Maybe this three polymorphisms are linkage disequilibrium (LD), but it is unknown if this genetic variants are located on the same LD-block or haplotype. The implications of genetically determined differences in P-gp function for drug disposition, therapeutic outcome and risk of development of certain diseases are intensively studied.

The aim of this study was to compare allele frequency of three SNPs in *ABCB1* gene namely C1236T, G2677T/A and C3435T, between 100 patients with colorectal cancer and 100 healthy controls of Caucasian origin. The next purpose was to find dependence between allele frequency and histopathological parameters and haplotypes analysis.

Genotyping of *ABCB1*_{C1236T}, *ABCB1*_{G2677T/A} and *ABCB1*_{C3435T} was performed by automated sequencing and by restriction fragment length polymorphism (RFLP) method, respectively.

Supported by KBN grant: 2 P05F 02628

15. INTERFERON ALPHA IN RAT LIVER AFTER PARTIAL HEPATECTOMY

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Interferon (IFN)- α treatment is a common therapy for chronic viral hepatitis that contributes to hepatocarcinogenesis prevention. Besides, IFN- α , combined with chemo- and radiotherapy, is prescribed as a prolong course after surgical removal of tumours. The cancers of different origin vary in their sensitivity to IFN- α . Along with IFN-sensitive cancers (kidney adenocarcinoma, lung sarcoma, malignant melanoma, neuroblastomas, cancers of lymphoid, endocrine and generative organs), there are resistant ones – cancers of stomach, liver and colon. Moreover, high and low concentrations of IFN- α can cause different response of the organism. The mechanisms of IFN- α action in different situations are still obscure.

The aim of our study was to investigate the expression of IFN- α , some of its targets (PKR and RNase L) and IFN-receptors during liver transition from quiescence to proliferation in the absence of viral infection. The rats after partial hepatectomy (PHE) and laparotomy (LAP) were used as the corresponding models of G₀→S transition and acute phase response. The latter is a compound of postoperative G₀→S transition. The expression of investigated genes was assessed by RT-PCR in total liver samples and in isolated hepatocytes (Hep) and Kupffer cells (KC) from intact and operated rats in 1, 3, 6 and 12 hours after surgery. The content of IFN in liver samples was evaluated by antiviral test.

In the samples from intact liver the expression of all genes manifested itself at RNA level. The isolated Hep and KC differ by the amount of specific RNAs. Kupffer cells were responsible for expression of IFN- α but not hepatocytes. The level of IFN- α receptor- and PKR-specific mRNA was 2- to 3-fold higher in KC than in Hep. It corroborates the fact that KC are producers and receivers of cytokines. The amount of RNase L-specific mRNA was nearly equal in the cells of both types.

After PHE the content of IFN- α protein and mRNA increases during first 3 hours after operation with further decrease till 6–12 hours. After LAP neither IFN- α -specific RNA, nor protein is detected, pointing to the special role of IFN- α in regenerating process. This increase is less than maximally possible response for typical IFN- α inducer – poly(I)-poly(C). IFN- α specific mRNA was shown to be produced by KC and not by hepatocytes. We assume that activation of KC with cell-specific production of IFN- α and responsiveness to it is essential for G₀ → G₁ transition of hepatocytes and incompatible with the early phases of acute phase response.

16. *ABCG2* GENE AND BREAST CANCER RESISTANCE PROTEIN EXPRESSION IN COLORECTAL CANCER

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Breast Cancer Resistance Protein (BCRP/*ABCG2*) is plasma membrane glycoprotein that belongs to the family G of ABC transporters superfamily. It has the ability to pump metabolites as well as xenobiotics out of the cell and plays important protective role against toxic substances. However, overexpression of BCRP is associated with high level of resistance to a wide range of anticancer agents, e.g. mitoxantrone, anthracyclines, camptothecines and could be the reason of chemotherapy failure.

In the present study, mRNA *ABCG2* gene and BCRP protein expression levels were determined in 63 cases of colorectal cancer by real-time PCR and immunohistochemistry, respectively. Both gene and protein expression levels were compared with some histological features like grading and staging to evaluate their potential role as a prognostic marker for colorectal cancer.

17. NEW CHIMERIC PROTEIN ABRAA-VEGF₁₂₁ IS SELECTIVELY CYTOTOXIC TOWARDS CELLS OVEREXPRESSING VEGFR2 (KDR) RECEPTOR AND INHIBITS GROWTH OF PRIMARY TUMORS.

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Growth of solid tumors is dependent on successful angiogenesis, in which the key factor is the vascular endothelial growth factor (VEGF) and its receptors (VEGFRs). VEGF, following binding to a specific receptor (KDR, a.k.a. VEGFR2) undergoes cellular internalization. Endothelial cells of tumor vessels, as opposed to those lining up normal blood vessels, are characterized by an increased expression of VEGFR2 receptors.

The aim of this study was to investigate a novel two-domain protein, the cognitive part of which allows directing the chimeric protein to endothelial cells lining up tumor blood vessels whereas the other, effector domain would entail destruction of targeted cells.

We constructed a synthetic gene coding for a chimeric protein ABRAA-VEGF₁₂₁ which combines abrin A chain (ABRAA - a plant toxin that inactivates ribosomes) [Wang et al., 2004]) linked to human VEGF₁₂₁ via a short aminoacid spacer (G₄S). ABRAA-VEGF₁₂₁ expression was carried out in *E. coli* BL21(DE3) strain. The protein was isolated from insoluble fraction as inclusion bodies, then it was solubilized and purified (>95%). Additionally, LPS was removed from ABRAA-VEGF₁₂₁ preparations (<0.0025 EU/ 1µg protein). The obtained protein migrates, under non-reducing conditions, as a ~84kDa homodimer and a ~42kDa monomer; it shows immunoreactivity towards anti-hVEGF₁₂₁ monoclonal antibodies.

ABRAA-VEGF₁₂₁ shows strong cytotoxicity towards PAE/KDR cells overexpressing KDR receptor (LC₅₀ = 0.067 µg/ml), and induces in them apoptotic death, as opposed to PAE cells overexpressing VEGFR1 receptor (PAE/hFlt-1) or wild PAE cell line (LC₅₀ ≈ 27.3 µg/ml).

ABRAA-VEGF₁₂₁ inhibits protein biosynthesis in a cell-free protein translation system. Preincubation of ABRAA-VEGF₁₂₁ with anti-hVEGF₁₂₁ monoclonal antibodies eliminated cytotoxicity associated with this protein. This indicate that both domains are biologically active.

Preliminary *in vivo* studies of anticancer properties of ABRAA-VEGF₁₂₁ demonstrated inhibition of tumor growth, as observed in the B16(F10) murine melanoma tumor model.

18. CA(1-7)M(2-9) (CAMEL) PEPTIDE IN THE THERAPY OF B16(F10) MURINE MELANOMA TUMORS

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CA(1-7)M(2-9) (CAMEL) is a hybrid peptide combining fragments of Cecropin A (aminoacids 1-7) and Mellitin (aminoacids 2-9). The peptide has been known so far as an effective antibacterial drug. The aim of this study was to check how this drug would affect neoplastic cells and whether it could be useful in anticancer therapy.

We investigated the interaction of this drug with B16(F10), NIH3T3 and HeCa10 cells in culture. Irrespective of cell type studied the peptide was cytotoxic and its LC₅₀ was ca. 3 μM. Following penetration of cells in culture the investigated peptide induced their necrotic death.

We noted inhibition of primary murine melanoma tumor growth following intratumoral administration of CA(1-7)M(2-9) peptide. However, the observed inhibition of tumor growth lasted only as long as the administration of the peptide. Within 3-4 days of administration cessation, tumor growth was resumed at an increased pace.

In order to obtain prolonged survival of treated animals we applied a combined therapy consisting of CA(1-7)M(2-9) and IL-12 gene administration. The latter, when administered intratumorally, stimulates the immune system to destroy cancer cells. The applied combination caused inhibition of tumor growth and yielded a statistically significant extension of animal survival.

19. PRELIMINARY STUDIES OF NEW SYNTHETIC CHLORIN-TYPE ANTITUMOUR PHOTOSENSITISER

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The term photosensitiser is synonymous with any molecule that uses radiant energy, or light, to elicit specific biological responses. In photodynamic therapy (PDT), the photosensitiser takes the role of a drug preferentially localizing in rapidly growing cancer cells and getting activated by cells' exposure to light, generating in the presence of oxygen very reactive cytotoxic species. Each factor, required for PDT, is harmless by itself, but their combination can produce lethal cytotoxic agents that can eradicate tumor cells.

Chemical reduction of one or two of the peripheral conjugated double bonds in porphyrins' macrocycles gives rise to chlorins, with concomitant bathochromic shift of the Q-bands with higher extinction coefficients than the corresponding parent porphyrins.

Photostable water-soluble chlorins have been reported that have high singlet oxygen efficiency coupled with 10 times stronger absorption than HpD or porphyrins in the therapeutic window; hence they are expected to be good photosensitisers.

Since the mode of transfer strongly influences subsequent localization of photosensitiser in cells and, consequently, affects the percentage of killed cells, we compared the killing efficiency of an examined chlorin delivered to the cells in liposomal form or dissolved in DMSO. The study was performed using Lewis lung carcinoma cells (LLC). Cellular distribution of chlorin was studied using confocal microscopy. Dark cytotoxicity and photodynamic efficiency of the explored chlorin were determined by MTS assay.

Our preliminary results indicate that conjugation of chlorin with liposomes is an efficient means of transferring the sensitiser into the cells, leading to highly efficient photosensitization, whereas non-carrier delivery (DMSO) is rather useless in such experiments.

20. LIPOSOME-CONJUGATED NEW PORPHYRIN DERIVATIVES FOR PHOTODYNAMIC THERAPY OF CULTURED TUMOR CELLS

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In an effort to find a potential agent for PDT we have undertaken a complex studies of synthesis, physico-chemical characterization and evaluation of biological activity of some hydrophobic porphyrin derivatives: amino acid-, cetyl- and pirydy- porphyrins. We characterised these agents by light absorption and emission studies. Moreover, flash photolysis was used to measure the singlet oxygen quantum yields of porphyrins in aerated toluene. Biological studies were performed on colon adenocarcinoma (Hct116) and human melanoma cells (Me45) in culture. The dark cytotoxicity and photodynamic efficiency of the explored porphyrins was determined by the MTS assay and colony forming ability method. Some of the examined porphyrin derivatives appeared to be very efficient photosensitisers for selected cell lines, with low dark toxicity.

It has been known that the mode of transfer strongly influences subsequent localization of photosensitiser in cells and, consequently, affects cell death pathway, therefore cellular distribution and mode of cell death were also investigated. Our confocal microscopy studies indicated that use of cationic liposomes as a carrier of porphyrins enabled their efficient transfer into the cells. The mode of cell death was dependent on energy of light applied and time of post-treatment incubation, shifting from apoptosis towards necrotic death at higher light energies and longer incubation periods.

In vitro PDT experimets using liposome-conjugated novel porphyrins suggest that they might be valuable candidates for further therapeutic studies. Combination of such “second-generation” photosensitisers and targeted nanocarriers would open new routes for PDT.

NFκB SIGNALING CIRCUITS - MODELING OF INTERACTIONS WITH P53 SIGNALING PATHWAY

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Mathematical models are helpful in describing and understanding of multi-component regulatory modules related to cell signaling and control of gene expression. Mechanisms of cellular responses to stress that depend on NFκB and p53 transcription factors are of particular interest and several mathematical models have been proposed describing each of these two pathways. Both regulatory modules interfere each other, however details of such interactions are not clear at the moment. Here we aimed to build a mathematical model that will base on experimental data and describe functional interaction between NFκB and p53 signaling pathways. We have used human HCT116 cell line stimulated with TNFα or UV radiation as an experimental model. Two isogenic lines with either functional or deleted p53 gene were compared. The kinetics of changes in levels of different components of the NFκB pathway were measured by Western and gel-shift methods in such stimulated cells. The experimental data were fitted in the mathematical model built in our group to reveal the influence of the p53 status upon the NFκB regulatory circuits.

This work was supported by the Ministry of Science, Grant KBN 3T11A01929.

21. GENOME-WIDE PREDICTION AND FUNCTIONAL ANALYSIS OF RAT GENES CONTAINING ISRE IN PROMOTER

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Prediction of putative transcription factor binding sites (TFBS) has become an important resource to explore genome organization and predict transcriptional regulation. Computational TFBS prediction provides reliable results in application to prokaryotes and yeast, but in higher eukaryotes accurate and reliable TFBS prediction is an outstanding challenge.

In this study, we searched for putative interferon-stimulated response elements (ISREs) in the promoters of protein-coding genes of *Rattus norvegicus*, as defined and annotated by Ensembl (release 40), and looked at the validity of the TFBS search in terms of biological meaning.

The ISRE, a conserved regulatory element of all interferon-stimulated genes (ISGs), is the target for transcriptional activator ISGF3 (IFN-stimulated gene factor-3). Genes containing ISRE can be considered primary interferon-response genes, with the cautionary note on the ability of not only ISGF3, but also of IRFs to bind to ISRE. Finding genes which have biologically meaningful ISRE will allow to better understand the Jak-STAT activated cellular IFN response.

A total of 23286 promoters of rat genes were analyzed. Promoter was defined as a sequence extending 1000 nucleotides up the TSS (transcription start site). ISRE position frequency matrix (PFM) was taken from the TRANSFAC 7.0 Public database, accession M00258. To compute matrix-site similarity scores, PFM was converted into position-weight matrix (PWM), with added pseudocounts.

TFBS search with 80% threshold produced 5214 TFBS in 4571 promoters. In order to filter away biologically insignificant TFBS, we compared promoters of the orthologues rat and mouse genes. We looked for TFBS occurrence in orthologues genes with an additional constraint for the starts of found TFBSs to be no more than 25bp apart relative to the TSS of each gene. Applying orthology filter produced 850 TFBS in 768 promoters. Set of 768 genes was used for further analysis.

Graph of the distribution of found ISREs start sites along the length of the promoter reveals three regions of ISRE localization: 0 to -250, -250 to -550, and above -550 relative to the TSS. It is not yet known whether ISRE TFBS localization has any functional implications.

Gene Ontology (GO) categories enrichment analysis was conducted for the 768 gene set against all the rat protein-coding genes, using the GO Tree Machine (GOTM). 49 GO categories (34 in biological_process, 10 in molecular_function, 5 in cellular_component) were relatively enriched, using hypergeometric test with $p < 0.01$ and no multiple-testing correction. Of these, a number of categories were expected based on the generic knowledge about interferon effects (15 categories: cell differentiation, cell cycle, viral life cycle, etc). Relation of other enriched categories to the effects of IFN is not straightforward. However, some expected categories were not significantly enriched, e.g. immune response (enrichment ratio $R = 1.38$, $p = 0.07$).

Thus, orthology-based filtering of the initial TFBS search does increase the percent of biologically meaningful binding sites, but still is not a sufficient filter by itself. Next step of this research will be to lower the search threshold (to include more putative true-positives), to apply less stringent orthology filtering, apply learning algorithms (trained on known targets), and look for the characteristic TFBS motifs.

Genes identified in this research as ISRE-containing will be used to seed the construction of the IFN- α -induced gene regulatory network.

POLYMORPHISM OF XPD312 GENE IN COLORECTAL CANCER PATIENTS INFLUENCES A REPAIR CAPACITY OF OXIDATIVE DNA DAMAGE

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The removal or repair of DNA damage is important factor for protection of genome integrity. It has been shown that XPD312 (codon 312) polymorphism is connected with lung and other type of cancer, mainly related with environmental exposure to genotoxic agents. Less is known about XPD polymorphism in colorectal cancer, the malignancy showing permanent increase incidence in west-type countries. Our studies indicate that in this cancer XPD312 polymorphic variant plays a protective role.

The aim of current study was to look for a possible mechanism responsible for increased risk of colorectal cancer in relation to XPD polymorphism. Comparison of the different polymorphic variants capacity to repair of radiation-induced DNA damage was performed on lymphocytes (using comet assay), and simultaneously, the efficiency of removal of oxidatively modified base (8-oxoGua) and nucleotide (8-oxodG) was studied in urine in 46 patients before treatment using HPLC/GS/MS technology.

Our results indicate that wild type of XPDAsp/Asp312 gene is connected with the less efficient repair capacity of DNA damage, whereas changes of Asp to Asn in one or both alleles significantly increase it. XPD312 protein is ATPase/helicase which plays a role in nucleotide excision repair pathway (NER). The differences in the level of oxidatively damaged base and nucleotide may suggest that individuals differ in using BER or NER pathway for oxidative damage removal from the organism.

22. MODIFICATIONS OF DNA REPAIR ENZYMES

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Oxidative stress and lipid peroxidation (LPO) generates plethora of DNA lesions, among which 1,*N*⁶-ethenoadenine (ϵ A) and 3,*N*⁴-ethenocytosine (ϵ C) have high miscoding potential and may be engaged in carcinogenic process. Two major enzymes involved in excision of ϵ A and ϵ C are: ANPG (alkyl-N-purine DNA glycosylase) and TDG (thymine DNA glycosylase). Our previous studies showed that repair activity toward ϵ C was lower in leukocytes of colon cancer patients than in leukocytes of healthy volunteers.

We were looking for mutations and polymorphisms in *hTDG* as well as in *hANPG* genes of 42 colon cancer patients, in tumor and normal surrounding which did not show histological changes. *hTDG* and *hANPG* genes were screened in all exons using MSCCP method. No polymorphism was found in *hANPG* gene. In case of *hTDG* gene analysis revealed G/A substitution in exon 5 in normal and tumor tissues from 3 patients. Such substitution changes glycine199 to serine in protein sequence, but it does not change the enzyme activity as it has already been described.

Activity of BER enzymes may be regulated by protein interactions and modifications. We have examined *in vitro* influence of oxidative stress products (hydrogen peroxide) and LPO (*trans*-4-hydroxy-2-nonenal; HNE) on BER proteins- ANPG, HAP1 and Mug.

HAP1 protein as revealed by mass spectrometry was modified by HNE on residues 93Ser, 52Lys and 151His. Such modification does not have an impact on HAP1 endonuclease activity toward depurinated plasmid. HNE does not influence Mug activity either.

We observed decrease of ANPG protein activity by HNE. This can be explained by the fact that HNE adduct was found at 136His located in enzyme's active center. It is possible that such modification may be relevant in living cells. Additionally ANPG was treated with H₂O₂, which decreased enzyme's activity only at very high, non-physiological hydrogen peroxide concentrations. For this reason the H₂O₂ impact on ANPG protein might not be important *in vivo*.

These results suggest that changed activity of TDG and ANPG does not depend on mutations or polymorphisms whereas impact of LPO products on ANPG activity may be an important factor.

23. TYPE III HISTONE DEACETYLASES INHIBITION INCREASES THE RATE OF DNA-PK-INDEPENDENT DSB REPAIR

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In mammalian cells sirtuins (type III histone deacetylases, HDAC III), dependent on NAD⁺ and inhibited by nicotinamide are coded by homologues of the yeast gene SIR2. Histone deacetylases (HDAC) are an important member of a group of enzymes that modify chromatin conformation. In yeast cells, Sir2 participates in repression of transcriptional activity and in DNA double strand break repair. It is assumed that certain sirtuins may play a similar role in mammalian cells, by modifying chromatin structure and thus, altering the accessibility of the damaged sites for repair enzymes. It has been postulated by J. Zhang et al. (Bioessays, 2003, 25(8):808-814) that there is a relation between poly(ADP-ribosylation) and sirtuin function in cells with damaged DNA. Interconnections between NAD⁺ metabolism, poly(ADP-ribosylation), DNA repair and gene expression should allow to modulate the cellular response to agents that damage DNA.

We investigated the role of sirtuins in DSB repair in a pair of Chinese hamster ovary (CHO) lines: wild type (WT) and radiation sensitive, DSB repair defective mutant line, xrs-6. The latter is defective in DNA-PK-mediated DSB repair pathway due to the deficiency in Ku80 protein. Cells were incubated with sirtuins inhibitor 200 μM GPI 19015/1 (gift from prof. J. Zhang (MIT, USA)) at 37°C for 1 h, X-irradiated with 10 Gy and allowed to repair DNA breaks for 30, 60 and 120 min) at 37°C. The remaining DSB were estimated by the neutral comet assay. We observed the mild effect of GPI treatment on the repair kinetic in both cell lines, but in DSB repair defective mutant cell line, the increase of DNA repair is more pronounced. There, the effect was most marked in G1 phase and practically absent in S and G2 cell cycle phases. The decrease in number of histone γH2AX foci was consistent with repair kinetics measured with the neutral comet assay. The altered repair rate did not affect survival of X-irradiated cells, as estimated at ca 50 % level. Since in G1 xrs6 cells the DNA-PK-dependent non-homologous end-joining, D-NHEJ, does not operate, these results indicate that inhibition of sirtuins modulates DNA-PK-independent (backup) non-homologous end-joining, B-NHEJ. So, B-NHEJ is the DSB repair system affected by sirtuin inhibition to a greater extent than other DSB repair systems.

24. METHYLATION AND SITE-SPECIFIC INTERACTION OF NUCLEAR PROTEINS WITH *GSTP1* PROMOTER IN HUMAN PLACENTA

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Glutathione S-transferase P1 (*GSTP1*) plays an important role in the protection of cell against xenobiotics and organic peroxides. This gene is highly expressed in most of the human cells. Reduction of the level of *GSTP1* expression is commonly associated with carcinogenesis and neoplastic transformation. Otherwise, many tumors reveal overexpression of this gene at the latest stages of their development, which is associated with antineoplastic drug resistance. In human placenta down-regulation of *GSTP1* gene expression induces pregnancy disorders, particularly pre-eclampsia. We previously reported that *GSTP1*-specific mRNA level in human placenta obtained from radioactively exposed pregnancies was lower than from unexposed ones. Corresponding enzymatic activity was lower as well. The molecular mechanisms of regulation of this gene in intact human trophoblast as well as in exposed to environmental adverse agents are not known. Therefore the goal of this work was to unravel basic mechanisms of regulation of *GSTP1* gene transcription in human placenta. We investigated the level of promoter methylation and the pattern of transcription factors binding the defined promoter region.

The samples of human placenta from normal delivery were obtained in different regions of Ukraine, Poland and Byelorussia, frozen in liquid nitrogen and stored at -80°C. DNA was purified from placenta by Miller's salting-out method. The level of methylation was assessed by methylation-specific PCR. To unravel the expression of transcription factors potentially involved in *GSTP1* gene transcription regulation in human placenta we have screened DNA-microarray databases by on-line program "Gene Expression Atlas" for expression of NF-κB gene and all representatives of Jun, Fos, CNC, small Maf, ER, RAR and GATA families. The results were normalized and expressed in relative units. To imitate an *in vivo* binding situation the region of human *GSTP1* promoter (142 bp, -103...+39), which contains TATA-box, two Sp1-binding sites, ARE/EpRE region, NF-κB-like binding site and ARE region of the first exon was PCR-amplified and cloned in pUC19+. After excision from plasmid it was end-labeled with γ-³²P-ATP and used in EMSA. The 20bp - oligonucleotide corresponding to GATA-binding site from *GSTP1* distant regulatory element, was also end-labeled and used as a probe. Nuclear extracts from placental tissue were prepared by modified Dignam's method. In competitive EMSA nonlabeled consensus oligos for AP-1 and NF-κB and oligos corresponding to original EpRE, Nf-κB-like and GATA-binding elements, were used.

Methylation-specific PCR has not revealed CpG methylation of *GSTP1* promoter in any from 96 analyzed samples. The control reactions with commercially-available methylated and unmethylated human DNA have ascertained the validity of our procedure and results.

According to computer analysis the placental-specific pattern of chosen transcription factors is represented by highly expressed GATA2, GATA3, Fos-B, Nrf3 and MafK, and moderately expressed c-Fos, Jun, Maf, ERβ, RARα and NF-κB. It is known that all these proteins have the appropriate binding elements in human *GSTP1* promoter. Competitive EMSA provided the evidence that EpRE, NF-κB-like and GATA- sites specifically bind with placental nuclear proteins. Among these proteins binding of AP-1 and NF-κB transcription factors was identified. It seems that CpG methylation is not responsible for regulation of this gene transcription in the investigated tissue.

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¹L-lecture; P-poster;

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THE ACADEMIC CHOIR OF THE SILESIA UNIVERSITY OF TECHNOLOGY IN GLIWICE

The Choir was established in 1945 by former employees and students of Lvov Technical University who used to sing in Lvov Technical Choir (that is the reason why Academic Choir celebrated its 100-th Anniversary in the academic year 2004/05 as a continuation of this 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, Argentyna, Urugwaj 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 Occasion of 60-year Anniversary of University in 2005 as a recognition of merits for development of University. Since 1980 the Choir has been organising national festival under the name Of Choir Meetings in Gliwice City - Gliwickie Spotkania Chóralne (this year, a jubilee 25-th meeting). 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 - Spiewak Śląski - which is published by Silesian Branch of Polish Association of Choirs and Orchestras (1-st and 2-nd book on the occasion 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, Elsnier, Haendel, Mozart, Rossini, Rutter, Twardowski, Schubert, Viern.

Tomasz Giedwillo has been the conductor and artistic manager of Academic Choir of Silesian University of Technology Since January 2006. Before that, over the past 10 years this position was held by Professor Czesław Freund.

THE ACADEMIC CHOIR OF THE SILESIA UNIVERSITY OF TECHNOLOGY IN GLIWICE

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Hajnówka (Poland) 2001



Bremen (Germany) 2004



Katowice (Poland) 2006



Bremen (Germany) 2004



Wrocław (Poland) 2005



Bremen (Germany) 2004



Berlin (Germany) 2003



Busan (South Korea) 2002

The most significant artistic achievements of the Choir over the past few years are as follows:

- Main Prize in the category of academic choirs in 28-th National Tournament Legnica Cantat '97,
- Bronze Medal at 32-nd International Festival of Choral Songs in Międzyzdroje '97,
- Gold Medal and Special Award in 2-nd International Competition in Riva del Garda in Italy '97,
- First Prize in the category of lay choirs at 17-th International Festival of Orthodox Church Music in Hajnówka '98,
- Second Prize at International Choir Festival Kathaumixw in Canada '98,
- Grand Prix at Festival of Songs of Holy Mary MAGNIFICAT and Special Award granted by the Prior of the Sanctuary of Jasna Góra – Piekary '2000,
- Three Silver Medals at 1-st Choral Olympics – Linz '2000 - Austria,
- Concert at the Royal Castle in Warsaw at the invitation of Prime Minister of the Republic of Poland – 2000,
- Concerts at the Philharmonic, St. Patrick's Cathedral and United Nations Organisation in New York '2001 - USA,
- First Prize in the category of lay choirs at 20-th International Festival of Orthodox Church Music in Hajnówka '2001,
- Three Silver Medals at 2-nd Choral Olympics – Busan '2002 – South Korea,
- Three Silver Medals at 3-rd Choral Olympics – Bremen '2004 – Germany,
- Grand Prix and Cup of the President of the Republic of Poland at 14-th National Festival of Polish Choral Songs – Katowice '2006 – Poland,
- pre-performance of pieces of: Norbert Blacha, Edward Bogusławski, Andrzej Dziadek, Jan Wincenty Hawel, Benedykt Konowski, Józef Świder i Romuald Twardowski.

Emmission training of the Choir is taken care of by Lucjusz Anders who works as a senior lecturer at the Faculty of Mining and Geology at Silesian University of Technology. The position of the president and organizational manager is held by Krzysztof Chlipalski who works as a senior lecturer at the Faculty of Building Engineering at Silesian University Of Technology.

Tomasz Giedwillo (took over the artistic guidance of the Choir on 16 January 2006).

He graduated from 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.

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) and for the Group of Schools of Music in Tychy.

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 Gdansk (he was conducting Polish Chamber Choir – Schola Cantorum Gedanensis).

His independent work with Academic Choir has already resulted in the preparation and concerts of Christmas Carols in Henryk Botor's arrangement as well as their recording on a CD, concerts during Choir Meetings in Gliwice City and the latest great success on 3-rd May 2006: winning Grand Prix and the Cup of the President of the Republic of Poland at 14-th National Festival of Polish Choral Songs and a concert tour in Uruguay and Argentina.



design: architektura2@o2.pl