Gliwice Scientific Meetings 2003

Gliwice, 21-22 XI 2003
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Organizers:

Center of Oncology - Maria Skłodowska-Curie Memorial Institute, Branch in Gliwice
Association for the Support for Cancer Research

Co-organizers:

Institute of Occupational Medicine and Environmental Health, Sosnowiec
Centre of Excellence PAGEN

Supported by:

Polish Ministry of Scientific Research and Information Technology
Polish Oncological Society
Polish Genetic Society
Polish Network of Cellular and Molecular Biology, UNESCO-PAS
Committee for Human Genetics and Molecular Pathology, Polish Academy of Sciences

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Program

Friday, November 21st

9.00 Opening of the Conference
Opening address: prof. Joanna Rzeszowska, President of the Organising Committee
Welcome address: prof. Jan Kaźmierczak, Vice-President of Gliwice

9.15-11.00 Session I
Structure and Functions of the Genome
Chairperson: **J. Rzeszowska**

S.V. Razin (Institute of Gene Biology, RAS, Moscow)
Domain organization of human dystrophin gene

J. Filipski (Institut Jacques Monod, Universite Paris VII, Paris)
Mapping of the hot spots of recombination in human DNA cloned in *S.cerevisiae*

E. Youdinkova (Institute of Gene Biology, RAS, Moscow)
Organization of the chicken domain of alpha-globin genes

V. Philimonenko (Institute of Experimental Medicine, Prague)
Nuclear myosin I and actin are required for RNA polymerase I transcription

M. Malanga (Institute for Veterinary Pharmacology and Toxicology, Zurich)
Poly(ADP-ribose) reactivates stalled DNA topoisomerase I: relevance to genomic stability and cancer therapy

M. Gniazdowski (Medical University, Łódź)
Covalent binding of anthracycline antibiotics to DNA

11.00-11.30 Coffee break

11.30-14.00 Session II
Transgenic Organisms in Agrobiotechnology and Medicine -
Centre of Excellence PAGEN Workshop on Modifications of Commercially Valuable Plants
Chairperson: **J. Szopa**

B. Müller-Röber (Potsdam University, Potsdam)
Transcription factor function search: combining genomics and single-gene approaches to discover new regulatory networks in plant biology

H. Hesse (Max-Planck-Institute, Golm)
Transgenic plants as a tool to improve sulphur amino acid content in plants

U. Schlüter (Risø National Laboratory, Copenhagen)
Manipulation of flax for improved fibre extractability

M. Wróbel, Jan Szopa (Wrocław University, Wrocław)
Flax engineering for fibre improvement

M. Łukaszewicz (Wrocław University, Wrocław)
The antioxidant potential of transgenic plants

J. Hennig (Institute of Biochemistry and Biophysics, PAS, Warszawa)
Functional analysis of *gluB* gene in *Solanum tuberosum* plants

J. Zimny (IHAR, Radzików)
Transgenic crops in the world

14.00-15.30 Lunch, **Poster viewing**

15.30-17.30 Session III
Modern Trends in Diagnosis and Therapy of Cancer
Chairpersons: **B. Maciejewski, B. Jarząb**

B. Utracka-Hutka (Center of Oncology, Gliwice)
Targeted chemotherapy. Principles, new targets, challenges for the future

A. Maciejewski, B. Mąka (Center of Oncology, Gliwice)
Free flaps in reconstructive surgery in head and neck region

R. Tarnawski (Center of Oncology, Gliwice)
Radiosurgery

B. Białas (Center of Oncology, Gliwice)
SWIFT system for 3D real time brachytherapy planning in prostate cancer

B. Jarząb (Center of Oncology, Gliwice)
Expression profiles in papillary thyroid cancer analysed by DNA microarrays

M. Małecki, P. Janik (Center of Oncology, Warszawa)
Bicistronic strategy in angiogenic therapy

M. Wierzbicka (Institute of Human Genetics, Poznań)
Biological implications of failure in solid tumours treatment

19.00 - **Social Party**

Saturday, November 22nd

9.00-11.00 Session IV
DNA repair and their role in carcinogenesis
Session supported by Committee for Human Genetics and Molecular Pathology, PAS
Chairperson: **M. Chorąży**

R. Oliński (Medical Academy, Bydgoszcz)
The molecular links between oxidative damage to DNA and cancer

M. Kruszewski (Institute of Nuclear Chemistry and Technology, Warszawa)
Labile iron pool, oxidative DNA damage and carcinogenesis

M. Chovanec (Cancer Research Institute, Bratislava)
DNA double-strand break repair and its implication in cancer

R. Goncharova (Institute of Genetics and Cytology, Minsk)
Some aspects of applying antimutagens as anticarcinogens

J. Rzeszowska (Center of Oncology, Gliwice)
Nucleotide excision repair – new role in oxidative damage repair?

M. Rusin (Center of Oncology, Gliwice)
DNA repair genes: polymorphisms and risk of cancer

M. Obolenskaya (Institute of Molecular Biology and Genetics, Kiev)
Glutathione transferase activity and genotoxic damage in human placentas from environmentally exposed pregnancies

11.00-11.30 Coffee break

11.30-13.00 Session V
Mathematical Models in Biology and Medicine
Chairperson: **A. Polański**

J. Leluk (Warsaw University, Warszawa)
Wrong assumptions and misinterpretations in molecular biology, biochemistry and bioinformatics

A. Masny, A. Płucienniczak (Institute of Biotechnology and Antibiotics, Warszawa)
PCR performed at low denaturation temperatures – PCR melting profiles

J. Polańska (Silesian Technical University, Gliwice)
Using Gaussian mixtures to infer structure in microarray data

T. Magdziarz (University of Silesia, Katowice)
Designing drugs by docking ligands into proteins - chemical problems and technical challenges

M. Pacholczyk (Silesian Technical University, Gliwice)
Analysis of differences in protein substitution patterns based on statistical tests

13.00-14.00 Lunch

14.00-16.00 Session VI
Satellite workshop: Cytogenetic Markers in Assessment of Human Exposure to Carcinogenic Substances
Workshop supported by Ministry of Health and Polish Genetic Society
Chairperson: **K. Szyfter**

M. Sąsiadek (Medical University of Wroclaw, Wrocław)
Chromosomal aberrations and cancer risk

M. Dušinská (Institute of Preventive and Clinical Medicine, Bratislava)
Assessment of biomarkers of exposure, effect and individual susceptibility in workers exposed to mineral fibres

F. Marcon, R. Crebelli (Istituto Supriore di Sanita, Rome)
Can current biomarkers be applied in the monitoring of low environmental exposures? Results from an investigation on traffic wardens of Rome city

G. Motykiewicz (Gliwice)
Application of cytogenetic markers in the polluted region of Upper Silesia

E. Skrzydlewska (Medical Academy, Białystok)
Green tea in cancer prevention

16.00-16.15 Closing Ceremony

16.15-17.00 Coffee/Discussion

Lecture abstracts

SWIFT system for 3D real time brachytherapy planning in prostate cancer

B. Bialas, J. Bystrzycka, M. Fijalkowski, K. Ślosarek

Aim: The aim of the study is to present treatment-planning procedures in brachytherapy of prostate cancer based on US examination with use SWIFT system (Nucletron).
INTRODUCTION: Brachytherapy Department in Institute of Oncology Gliwice is equipped with computed treatment planning system (TPS) SWIFT. The SWIFT system consists of transrectal US machine, stopper - use to fix probe and template, treatment planning system. All these elements are integrated and allow planning brachytherapy dose distribution in „real time".
Material and method: Patient has to be anaesthetized (spinal anaesthesia) before planning procedures and treatment start.
First step of procedure is to perform US examination, to place probe on the depth of prostate base and to send next slices (1mm thickness) to prostate apex (through the whole prostate)
Slices are sending to TPS automatically by network (stepper encoder, US machine and TPS are connected).
The next step is to prepare pre - plan:

- the prostate base and apex have to be identified,
- system localizes the reference scan automatically (scan between base and apex),
- the doctor contours the prostate – PTV (Planning Treatment Volume) and organ of risk (urethra, rectum)
- the prescribed dose and catheter placement is established
- the source „dwell times" are optimised and normalized
- the dose distribution is calculated (prostate volume covered by reference isodose 100%, dose in urethra is taken from - volume histogram)

According to the pre - plan doctor inserts needles and transrectal US examination with implant in place is performed.
The live planning procedure starts:

- the pre – planning slices (contours and catheter localisation) are put on the live plan
- real needles localisation (virtual - live catheter) is modified in TPS
- according to new real implant placement, optimisation and dose –volume histogram is verified

After acceptance by staff, treatment plan is send to treatment control station by network and treatment is started.
Conclusion: dose distribution based on US examination make possible verification of implant placement in PTV (prostate) in relation to organ of risk (urethra).
Integrated treatment planning system enables modification of catheter location in prostate, directly in real time of procedure (pre - planning; live planning).

DNA double-strand break repair and its implication in cancer

M. Chovanec

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DNA double-strand breaks (DSBs) are considered the most lethal form of DNA damage. They can be induced either exogenously by such agents as ionising radiation (IR) and a wide range of chemical compounds, or endogenously by such agents as reactive oxygen species. Moreover, DSBs arise in DNA as intermediates during several cellular processes.

Unrepaired or misrepaired DSBs can lead to cell death, genomic instability, and hence cancer. To combat these detrimental effects, two main pathways have evolved for the repair of DSBs: homologous recombination (HR) and non-homologous end-joining (NHEJ). In vertebrates, NHEJ pathway is represented by the DNA-dependent protein kinase (DNA-PK), consisting of the catalytic subunit (DNA-PKcs) and the DNA targeting subunit (Ku70/80 heterodimer). Another NHEJ factor is DNA ligase IV, which functions in a tight complex with the Xrcc4 protein. The two main candidates for nucleolytic processing stage of NHEJ are the Mre11-Rad50-Nbs1 complex and the Artemis protein. The Mre11-Rad50-Nbs1 complex presumably functions also in early event in vertebrate HR. Moreover, vertebrate HR involves Rad51 protein along with its five paralogues (Rad51, Rad51C, Rad51D, Xrcc2 and Xrcc3), as well as Rad52, Rad54, Rad54B proteins, and the breast cancer susceptibility proteins, Brca1 and Brca2.

Vertebrate cells deficient in components of DSB repair display sensitivity to IR and cross-linking agents, spontaneous and IR-induced chromosomal instability, centromere abnormalities and altered kinetics of DSB rejoining. Moreover, many HR and NHEJ components are essential for viability in vertebrates. The essential role of the HR or NHEJ in vertebrates is further strengthened by the discovery that mutations in some of the HR or NHEJ genes are associated with cancer-prone syndromes, e.g. Nijmegen breakage syndrome, Ataxia telangiectasia-like disorder, LIG4 syndrome, severe combined immune deficiency, and radiosensitive severe combined immune deficiency. Molecular basis of these syndromes and their relation to cancer predisposition will be discussed in more detail.

Assessment of biomarkers of exposure, effect and individual susceptibility in workers exposed to mineral fibres

M. Dušinská, M. Barančoková, A. Kažimírová, A. Horská, Z. Džupinková, K. Volkovová, M. Staruchová, A. Kočan, L. Wsólová, A. Collins[2], S. Kyrtopoulos[3]

[1]Institute of Preventive and Clinical Medicine, Bratislava, Slovakia, [2] Institute for Nutrition Research University of Oslo, Norway, [3]National Hellenic Research Foundation, Athens, Greece

An occupational biomonitoring was conducted in 3 factories producing asbestos, glass fibres and rockwool. Personal as well as workplace exposure for PAHs and fibres was measured together with personal dosimetry. The levels of asbestos in asbestos factory 3 - 5 times overstepped the Slovak occupational limit. Just presence of basalt glass fibres was confirmed in both rockwool and glass fibre factories. Individual PAH congener levels in occupational atmosphere range from tenths to several hundreds mg/m^3.

Altogether 239 exposed, and 148 controls were investigated. Subjects were clinically examined; questionaired for life style and diet, blood and 24h urine were sampled. DNA damage (strand breaks [SBs], formamidopyrimidine glycosylase [FPG] -, endonuclease III [EndoIII] -, and 3-Methyladenine DNA glycosylase [AlkA]- sensitive sites), using the comet assay in peripheral blood lymphocytes were assessed. Micronuclei and chromosome aberrations; genetic polymorphisms of xenobiotic-metabolising and DNA repair enzymes; individual DNA repair capacity in lymphocyte extracts; intrinsic antioxidants, antioxidant enzymes; proinflammatory mediators and immune markers, were also measured.

Exposed asbestos workers, especially men, had higher level of oxidative DNA damage (EndoIII sites, P=0.005). Similarly, in glass fibre factory exposed men had more oxidised pyrimidines than non-exposed. The difference between exposed and controls in SBs was significant in glass fibre (P=0.03) as well as in rockwool factory (P=0.05); this was again more pronounced in men, and in group of non-smokers (P=0.004). DNA damage correlated with age in exposed groups combined (R^2=0.16, P=0.000, n=239), which may reflect the length of exposure to the fibres, rather than age itself as there was no such correlation found in controls (R^2=0.004, P=0.000, n=100).

The only biomarker of genotoxicity that has been shown to predict cancer risk is chromosome aberrations. Chromosome aberrations were elevated in the asbestos-exposed workers, and especially in those who smoked (P<0.05) but not in glass fibre or rockwool factories.

Level of micronuclei was not affected by exposure to any fibres. However, in the three populations combined (n=388), micronucleus frequency correlated positively with EndoIII- (r=0.21, P<0.001), FPG- (r=0.29, P<0.001), and AlkA-sensitive sites (r=0.22, P<0.001). Similar correlation was seen in all subgroups: controls; exposed; men; women. It is unexpected that markers of DNA damage correlate so strongly with the 'downstream' marker of chromosome stability. There was also an interesting inverse correlation between DNA repair rate and micronucleus frequency especially in the rockwool factory.

Associations between DNA damage, exposure, smoking, sex and genotype were found in all three populations.

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Transgenic plants as a tool to improve sulphur amino acid content in plants

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Essential amino acids and cysteine represent an indispensable component in the diet of all mammals including man. Plants represent the only source widely available to meet this demand. Major crops, such as cereals and legumes, are low in Met and an attempt to manipulate the biosynthetic pathway is a major interest of molecular plant breeding. According to this it can be assumed that Met synthesis, accumulation and consumption are under high regulatory control. Amongst the amino acids underrepresented sulphur containing amino acids such as cysteine and methionine are by far the most important with respect to world nutrition. It is therefore of utmost importance to understand the physiological, biochemical, and molecular mechanisms that contribute to their transport, synthesis and accumulation in plants. This knowledge can be used to develop strategies allowing a manipulation of crop plants, eventually improving their nutritional quality.

This article is intended to serve two purposes. The first is to provide a brief review on the physiology of cysteine and methionine synthesis in higher plants. The second is to highlight some recent findings linked to the metabolism of methionine in plants due to its regulatory influence on the aspartate pathway and its implication in plant growth. Recent studies suggest that Met synthesis in plants has to be controlled at the level of competition between CgS and TS for their common substrate OPHS. This information can be used to develop strategies to improve methionine content of plants with a higher nutritional value. In this paper, a summary of our current understanding of the regulatory network with the focus on efforts to understand and manipulate the carbon flux into Met is given.

Labile iron pool, oxidative DNA damage and cancerogenesis

Marcin Kruszewski

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Department of Experimental Haematology and Cord Blood Bank, Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, 02-781 Warsaw, Poland

The unique abilities of iron to change its oxidation state and redox potential in response to the changes of liganding environment makes this metal essential for almost all living organisms. Iron-containing enzymes are the key components of many essential biological reactions, such as energy metabolism, oxygen transport, DNA synthesis and repair, detoxification of reactive oxygen species (ROS) and its reaction products and numerous other reactions catalysed by oxygenases, peroxygenases, etc. However, the same biochemical properties that make iron beneficial in many biological processes might be a drawback in some particular conditions, namely, when improperly shielded iron can catalyse one-electron reductions of oxygen species that lead to production of very reactive free radicals. Trace amounts of "free" iron can catalyse production of a highly toxic hydroxyl radical via Fenton/Haber-Weiss reaction cycle. Iron-driven generation of oxygen–derived free radicals is known to induce oxidation of proteins, lipids and lipoproteins, nucleic acids, carbohydrates and other cellular components. An oxidative damage to the vital cellular components might have in turn a deleterious effects at cellular and tissue levels, leading to the cell death, tissue necrosis and degenerative diseases or cell phenotype changes and cancer formation. The critical factor appears to be the availability and abundance of cellular labile iron pool (LIP) that constitutes a crossroad of metabolic pathways of iron-containing compounds and is midway between the cellular need of iron, its uptake and storage. To avoid an excess of harmful "free" iron, the LIP is kept at the lowest sufficient level by transcriptional and posttranscriptional control of the expression of principal proteins involved in iron homeostasis. The putative sources of cellular LIP, its homeostasis and its role in the cellular response to oxidative stress and cancerogenesis are discussed.

Functional analysis of *gluB* gene in *Solanum tuberosum* plants

Magdalena Krzymowska[1], Anna Barabasz[1], Agnieszka Mac[1], Kamil Witek[1], Beata Bieniak[2], Maria Charzyńska[2], Jacek Hennig[1]

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There is considerable evidence that 1,3-∃-glucanases (glucan endo-1,3-∃-glucosidases; EC 3.2.1.39) are part of plant defence systems. Although the major interest in 1,3-∃-glucanases stems from their possible role in the response of plants to microbial pathogens, there are some reports that these enzymes are also involved in diverse developmental processes of healthy plants.

We isolated the *gluB* gene coding for a novel 1,3-∃-glucanase from potato (*Solanum tuberosum* cv. Désirée). cDNA and corresponding genomic sequence were characterized. Northern blot hybridization showed the presence of gluB mRNA at a very low level in mature leaves of uninfected plants. A considerable increase of expression was observed after infection with potato virus Y (PVY) or *Phytophtora infestans* and after treatment with salicylic acid or BTH. We also found that transcript accumulation of gluB increases during flower development, reaching a maximum in stigmatic cells during anthesis.

To study the function of the GluB protein we have generated transgenic potato plants expressing *gluB* under 35S promoter in sense and antisense orientation. The susceptibility of the plants, with elevated levels of glucanase, was reduced upon powdery mildew and *P. infestans* challenge. Interestingly, the changes in the *gluB* gene product level correlated with some changes in the appearances of the plants. The plants overproducing GluB were more compact and stunted in comparison with nontransgenic control. The cells of internodes of *gluB* plants were characterized by reduced elongation (mainly in pith parenchyma) in direction of the main shoot axis and simultaneously were radially extended. We did not observe any changes in tuber size and production. Our data suggest that the *gluB* gene product may play an important role both in plant development and in the defence response against pathogen infection.

Wrong assumptions and misinterpretations in molecular biology, biochemistry and bioinformatics

Jacek Leluk

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The interdisciplinary character of research work became typical in many research studies of present century. The disciplines such as bioinformatics, computational biology, genomics, proteomics or molecular modelling assemble researchers representing various fields of science and specialization. The dialogue between biologist, computer scientist, physician, mathematician, chemist and physicist is now an ordinary feature that occurs in performing the common projects of their interest. As a consequence there appears important problem of proper mutual understanding within the interdisciplinary group of researchers. It is especially important while interpreting the results of theoretical analysis, modelling and simulation performance, and correct further application of the results.

The problem of effective dialogue and understanding between collaborators starts at very early stage of cooperation. It concerns diversity and imperfection at terminology and nomenclature level. The terminology and definitions used in biological sciences are often unclear, misused, mistaken or misinterpreted. The same problem appears with respect to the tools elaborated by computer scientists and applied to accomplish the molecular biology and bioinformatics projects. The algorithms and software are often constructed in a perfect way from the programist's or mathematician's point of view, they have perfect logical architecture, but they do not comprise all significant parameters that describe a biological process. It may happen, that some algorithm that is free of any internal inconsistency becomes useless because of purpose and subject of its application. It may happen that the examined processes do not correspond to the rules of the theoretical approach. As a consequence there is a risk of making wrong or at least incomplete hypotheses and theories, which are far from actual processes that are to be described by the theoretical models. The presented examples concern the errors in fundamental assumptions of many theoretical approaches and wrong way of their application. The examples refer to the statistical analysis of protein evolutionary variability based on stochastic matrices, which ignore the genetic background of mutational protein variability, Markovian interpretation of amino acid replacement within homologous protein sequences, hypothesis of correlated mutations, Monte-Carlo methods for computing tertiary structure of the protein, interpretation of X-ray structural data and theoretical protein folding methods. The special attention was focused on the most popular methods, commonly assumed as reliable and trustworthy.

The antioxidant potential of transgenic plants

Marcin Łukaszewicz[a] and Jan Szopa[b]

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There is a growing interest in the probiotic properties of various natural antioxidants having beneficial effects on human health. Oxidation products such as hydroperoxides or superoxide radicals formed during free radical reactions are the causative agents of many diseases. Data gathered so far strongly indicate further need to conduct a research on impact, mechanisms of function and degradation pathways of various antioxidants. Plants are rich source of such compounds. Specific only for plants are flavonoids which are supposed to play an important role in protection against UV irradiation and osmotic, oxidative or heat shock stresses. As ingredients of animal (human) diet, flavonoids have been shown to have great impact on human health. They show antimicrobial, antiviral, antiphlogistic, antioxidant, antisclerosis, analgesic and anticancer activity. Positive impact on cardiovascular, digestive and respiratory systems has been also well documented. As flavonoids are used as pharmaceuticals in the form of purified compounds or as components of plant tissue mixtures, there is a growing interest in the impact of flavonoid doses and their quality present in the food, as well as in the possibility of modification of the food composition to promote human health. In this context the flavonoids biosynthesis pathway in potato and flax plants have been modified by expression modulation of three different groups of enzymes: regulatory protein (14-3-3), biosynthetic pathway genes (chalcone synthase, chalcone isomerase, dihydroflavanone reductase) and end-product modifying enzyme (glucosyltransferase). For this purpose, a special construct enabling introduction of up to five genes has been prepared.

Plants with overexpression and repression of flavonoids have been obtained for each group of expressed enzymes. In potato plants the most effective was overexpression of DFR, and have been shown to produce mainly one anthocyanin compound. Flax plants with greatly increased antioxidant capacity have been obtained by simultaneous introduction of three flavonoid biosynthetic pathway genes. Fibres of these plants are potentially very interesting from pharmaceutical point of view.

Free flaps in reconstructive surgery in head and neck region

Adam Maciejewski, Janusz Wierzgoń, Cezary Szymczyk, Bogusław Mąka

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The main goal of surgical treatment of head and neck tumours is to achieve macro- and microscopically radical margins and to restore or preserve high quality of life. Based on own experience Reconstructive Surgery Group in Cancer Center in Gliwice introduced free flaps techniques after major resections in head and neck region.

Since November 2001 till August 2003 over 80 patients underwent surgical procedures based on microvascular free flaps. In 60% Radial Forearm Free Flap was the technique of choice. In 25 cases were mandibular reconstruction was needed Fibula Free Flap was performed and in two cases iliac crest free flap was harvested. In case of middle face defects reconstruction Rectus Abdominis Free Flap was chosen. In 12 cases were postressective defects were large and composed Anterolateral Thigh Free Flap was harvested and insetted. Twice the complex defects after extended maxillectomy was reconstructed with Subscapular Composite Free Flaps. Overall survival rate based on periodic control and imaging diagnostics was about 90%. 40% of these patients underwent pre- or postoperative radiotherapy and it did not affect flap healing and survival. Based on authors experience the progress on the field of reconstructive surgery will depend on individually chosen free flaps and its goal is to achieve optimal functional and aesthetic outcome.

Poly (ADP-ribose) reactivates stalled DNA topoisomerase I: relevance to genomic stability and cancer therapy

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DNA topoisomerase I (topo I) plays an essential role in controlling the level of DNA supercoiling and releasing the torsional stress that is generated during DNA transactions. In the course of the topo I catalytic cycle, a DNA single strand break is produced that, under normal conditions, is very short lived and escapes damage surveillance systems. However, when acting on damaged DNA, topo I may get trapped in the vicinity of DNA lesions leading to an accumulation of enzyme-linked nicked DNA (stalled topo I). If unrepaired this may cause genomic instability or cell death. In fact, the potency of the anticancer drug camptothecin and its analogues (commonly known as topo I poisons) is directly related to their ability to stabilize such topo I-DNA complexes.

We have found that poly (ADP-ribose), the catalytic product of poly (ADP-ribose) polymerases (PARPs), targets specific domains of topo I and reprograms the enzyme to remove itself from DNA and close the resulting gap. In particular, two nuclear members of the PARP family, PARP-1 and PARP-2, act as poly (ADP-ribose) carriers to stalled topo I sites and induce repair of enzyme associated-DNA strand breaks. In our studies, camptothecin was used to stabilize topo I cleavage complexes, mimicking topo I stalling in the vicinity of DNA lesions. In addition, the DNA nicking activity of topo I was completely blocked by poly (ADP-ribosyl)ated PARPs, both in the presence and absence of camptothecin. Thus, by counteracting topo I-induced DNA damage, PARP-1 and PARP-2 act as positive regulators of genomic stability. Moreover, the observation that poly (ADP-ribose) antagonizes camptothecin action may explain the cytotoxicity potentiation effect of PARP inhibitors used in combination with topo I poisons.

Bicistronic strategy in angiogenic therapy

Maciej Malecki, Przemyslaw Janik

Department of Cell Biology, Center of Oncology, Warsaw, Poland

Angiogenic gene therapy is a very promising procedure but requires large amounts of pharmaceutical-grade plasmid DNA. Overexpression of angiogenic genes like VEGF, FGF causes new vessel formation and improves the clinical state of patients. In this regard a bicistronic plasmid DNA vector encoding two proangiogenic factors, VEGF165 and FGF-2 have been constructed. The construct (pVIF) contains the internal ribosome entry site (IRES) of the encephalomyocarditis virus (ECMV) which permits both genes to be translated from a single bicistronic mRNA. The IRES sequence allows for a high efficiency of gene expression *in vivo*. The pVIF vector was characterized in *vitro* and *in vivo*. *In vivo* angiogenesis studies showed that the bicistronic vector encoding two proangiogenic factors induces the formation of new vessels significantly more than pVEGF165 or pFGF-2 alone. It is worth noticing that the combined proangiogenic approach with VEGF165 and FGF-2 is more powerful and efficient than single gene therapy.

Can current biomarkers be applied in the monitoring of low environmental exposures? Results from an investigation on traffic wardens of Rome city.

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Air pollution from vehicle exhaust is a relevant health problem in urban areas. The composition of the pollution is complex, including many chemicals known to have high potential genotoxic effects, such as benzene, 1,3-butadiene, benzo[a]pirene, in addition to particulates and traditional air pollutants (i.e. carbon monoxide and nitrogen monoxide). In recent years, in Italy, there was a progressive reduction of the levels of exposure and the doses encountered today are in most cases quite low. However, the risk for human health associated to this low exposure is not yet clear, mainly because there is a lack of information on the biological effects produced by the low doses. In this respect, the biomonitoring of human population occupationally exposed to low levels of urban air pollutants could be useful to evaluate the adverse effects resulting from the low dose exposure. With this aim, our laboratory was involved in a study on a group of traffic policemen showing a profile of exposure comparable to the one of other outdoor workers and the general population of Rome city. The sample included 206 subjects, consisting of 143 exposed individuals working in the urban traffic and 63 control policemen employed in the offices, matched by age, gender and smoking habits. The study included the analysis of markers of external and internal exposure, of genotoxic effects and susceptibility. The assessment of baseline chromosomal damage was performed using classical cytogenetic endpoints, sister chromatid exchanges (SCE), micronuclei (Mn), and the Comet assay in subjects genotyped for CYP1A1, CYP2E1, GSTM1, GSTT1, and DT-diaphorase polymorphisms. The average benzene exposure detected during the workshift was 9.5 and 3.8 $\mu g/m^3$ in exposed individuals and controls, respectively. The frequency of SCE was significantly influenced by smoking habits, but no differences were observed between exposed and control individuals. Similarly, the Mn frequency was unaffected by the occupational exposure to traffic fumes, whereas it was mainly modulated by the age and gender of the study subjects. In order to improve the sensitivity of the method to excision-repairable lesions, a modified protocol with the exposure of the cells to the DNA polymerase inhibitor cytosine arabinoside (Ara-C), was applied to 78 subjects, but the results failed to demonstrate any significant effect of chemical exposure (occupational or related to smoking habits). Moreover, the analysis of DNA damage by comet assay did not highlight any statistical significant difference between exposed and control workers. A mutagen sensitivity assay was also performed on a subgroup of 31subjects with the aim to investigate the relative role of genetic and environmental factors on individual susceptibility to genotoxic agents. The analysis of chromosomal aberrations and the assessment of the kinetics of DNA repair by comet assay revealed a modulator effect of smoking habits and GSTM1-null genotype on DNA sensitivity to γ-radiation, likely to be due to the higher expression of enzymes involved in the repair of oxidative DNA damage in heavy smokers and GSTM1-null subjects. On the whole, the results obtained in this work are in agreement with those of previous investigations on populations with low or moderate exposure to atmospheric pollutants, which indicate that the contribution of environmental pollution to background levels of genetic damage may be barely detectable, while important non-occupational factors, such as smoking habits, may have a dominant role in determining the results of biomonitoring studies. However, in the near future, genomic based new technologies such as microarray will probably be useful to identify early, sensitive biomarkers of xenobiotic exposure by examining changes in gene expression profile, thus improving the efficiency of risk assessment.

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PCR performed at low denaturation temperatures - PCR melting profiles

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We show that using low denaturation temperatures (80-88 degrees C) during ligation mediated PCR (LM PCR) of bacterial DNA leads to the amplification of limited sets of less stable DNA fragments. A set of electrophoretic patterns of such fragments obtained at different denaturation temperatures forms the PCR melting profile (PCR MP). A single pattern obtained for a given temperature and a set of patterns arising after application of several denaturation temperatures (PCR MP) are very specific for the given bacterial genome and may be used for strain characterisation and differentiation. The method may also be used for amplification and isolation of the less stable DNA fragments in a genome.

Application of cytogenetic markers in the polluted region of Upper Silesia

Grażyna Motykiewicz

Upper Silesia is a heavy industrialized region of southern Poland. It encompasses over 12 thousand square kilometres and is inhabited by almost 5 million people. Environmental pollution mainly originates from coal-based heavy industry, gasoline exhaust from automobile traffic, and combustion of coal for domestic heating. As a result of coal burning and/or processing, among the most prevalent mutagenic and carcinogenic air pollutants in Silesia are the polycyclic aromatic hydrocarbons (PAH) and heavy metals. At the second half of last century Silesia was one of the most polluted regions in the world. It has been postulated that increased incidence of cancer and other genetic diseases including infertility and congenital malformations, observed in residences of Silesia, is the result of significant damage to genetic material from long-term exposure to environmental pollution.

Over last ten years, cytogenetic methods were successfully used in Silesia to reveal DNA damage in human samples. Studies were performed on populations exposed to airborne pollutants at low-dose environmental and extremely high occupational levels. The control populations from mostly rural north-eastern Poland were also included using the same criteria for subject enrolment. Both male and female populations were studied. A preliminary study on children from Silesia was also performed but was lacking control population. The employed methods included sister chromatid exchanges (SCE), chromosomal aberrations (CA) and micronuclei formations (MN). All the methods were performed on cultured white blood cells. The data obtained with cytogenetic methods were compared with markers of exposure performed on urine samples including the Ames test, the level of 1-hydroxypyrene, and cotinine. As a part of the study, we also tested the level of aromatic adducts *in situ* and in isolated DNA samples. The blood lead and cadmium levels were also controlled but only among children.

In three independent projects we were able to show statistically significant increase of the levels of both SCE and CA in Silesian populations as compared to controls. The data were analysed by conventional statistics, ANOVA method and a multiple regression model. A dose-response, seasonal variation in marker level, and additive effects of smoking and exposure were found using the SCE method. The highest levels of SCE were seen among coke oven workers and the lowest in control samples. There was statistically significant influence of the season on SCE level. The highest values were found in samples collected in winter, when air pollution originates from both industrial and domestic heating sources. Using a multiple regression model, smoking was a major factor influencing the level of SCE. There was a clear additive effect of exposure and smoking in the group of coke oven workers. Although the CA method has also significantly revealed the damage caused by environmental pollution there was no correlation between those two markers. A borderline correlations were found between the level of aromatic adducts, tested by ^{32}P-postlabelling method, and CA as well as SCE, $p=0.05$ and $p=0.03$, respectively. A positive correlation ($p=0.003$) was found between blood lead level and formation of MN in children population.

Based on our data performed on Silesian populations exposed to airborne pollutants, cytogenetic markers are adequately sensitive, providing valuable information on potential risk of genetic-based diseases including cancer.

Mapping of the hot spots of recombination in human DNA cloned in *S. cerevisiae*

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The crossing over and gene conversion events occurring during meiosis in eucaryotic organisms are clustered in the short segments of chromosomes called hotspots of recombination. It has been demonstrated, that in yeast and mammals, these clusters are located in the vicinity of initiation sites. Initiation of meiotic recombination is associated with the formation of DNA double strand breaks (DSB) followed by DNA strand exchange, repair of heteroduplexes and resolution of Holliday junctions.

The location and activity of the hotspots of recombination depend on several factors such as: local DNA sequence features, local chromatin characteristics, distance from the chromosomal loop attachment sites or distance from the centromere and telomere.

It has been shown that the DNA segments corresponding to the cold spots of recombination in mammalian cells scarcely recombine in yeast cells if cloned as Yeast Artificial Chromosomes (YAC). The segments frequently recombining in mammalian cells conserve this property when cloned in artificial chromosomes or plasmids. The aim of our project was to identify the DNA features, which make a mammalian DNA segment recombination-prone in yeast.

We studied several YACs, most of them quite stable in yeast. The YAC 745D12 carrying the HLA class I antigen gene cluster contained six CpG islands and two long minisatellite sequences, the YAC 225A3 contained a single CpG island located 166 kb from telomere C and a region rich in GC nucleotides, YAC XY206 contained also a single CpG island located 8 kb from the telomere C, YAC A85D10 contain a long minisatellite sequence and no CpG island.

The CpG islands, with one exception, were hypersensitive to nuclease. The island accompanying the human *G6PD* gene has also been subcloned from YAC XY206 into a yeast plasmid in front of the cDNA segment coding *ADE2HI* gene. This construct complemented the auxotrophic mutation *ade2* of the yeast. Apparently the island was acting as a transcription promoter. Its function was dependent upon the integrity of the sequences present in the island. We interpret this finding as suggesting that the sequences present in the island are recognised by yeast transcription factors.

Three classes of DNA sequences were associated with meiotic DSB. First class contained some, but not all of the CpG islands. The second class contained the GC-rich region in the YAC 225A3. The DSB formed in this region showed spacing corresponding to the length of the chromatin loops in yeast. The third class was associated with minisatellite sequences. We suggest that meiotic DSB are formed at least in some in of these sites during meiosis in human germ-line cells.

Transcription factor function search: combining genomics and single-gene approaches to discover new regulatory networks in plant biology

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Transcription factors (TFs) regulate the expression of downstream target genes and thereby contribute to the establishment of complex traits in higher plants, including developmental features, cell differentiation, biosynthetic pathways and adaptation to environmental stresses. The evolution of higher plants is tightly coupled to TF evolution and the high TF number in plants places them on the same level of complexity with e.g. *Drosophila*. Several examples demonstrate a large biotechnological potential of plant TFs for the modification of traits. We use genomics and single-gene approaches to investigate the function of currently under-explored plant-specific TF genes, using *Arabidopsis thaliana* as a model. More than 150 TF genes were over-expressed in transgenic plants, including those of the DOF and NAC families. TF function is deduced by microscopic and macroscopic phenotype analysis, the identification of potential target genes and interacting protein factors. RNAi lines are generated for a selected number of TF genes. Functional analysis is enhanced through in-house development of suitable bio-informatics tools.

Glutathione transferase activity and genotoxic damage in human placentas from environmentally exposed pregnancies

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Introduction: The early protection of children from environmental hazards has become of particular importance in the Ukraine in view of the industrial pollution and the accident at Chernobyl. Also the increasing Ukrainian cancer incidences support the notion that the lifetime environmental exposures may contribute to cancer risk in exposed individuals. Extracorporally the foetus is protected from environmental factors by maternal (mainly hepatic) detoxification and by placental detoxification. However, genotoxic exposures that occur transplacentally are evidenced in the form of macromolecular damage measurable in the placenta. Damage to placental DNA cannot only document individual exposure and the efficiency of detoxification but can serve as a surrogate for DNA damage occurring in the foetal tissues.

The underlying hypothesis of this study was that placental detoxification efficiency reflects the individual genotype of detoxifying enzymes and the environmental exposure and influences on the foetus development. The objectives of this study were to compare the biomarkers of metabolism with: individual genotype of polymorphic detoxifying enzymes in placenta – cytochrome P4501A1 (CYP1A1) and glutathione transferase P1 (GSTP1); polycyclic aromatic hydrocarbon (PAH) adducts in DNA; clinical status of mother and newborn.

Material and methods: The placentas (186 samples) were obtained during the period 1991 – 2002 from several areas of the Ukraine and neighbouring region of Byelorussia, some exposed to high levels of radioactivity, some exposed to high levels of PAH and others exposed to low levels of both. They were classified into 8 groups according to areas of origin: the radioactively contaminated areas with higher (Group 1) and lower (Groups 2, 3) Summary Effective Equivalent Annual Exposure Dose (in mSv), where the samples were obtained in 1991 – 1993 (Groups 1, 2) and 1999 (Group 3); the chemically polluted areas monitored for ambient levels of benzo(a)pyrene (BP, ng/m^3) and arranged in the order of decreasing pollution (Groups 4 – 6) where the samples were obtained in 1992 (Group 4), 1992 – 1993 (Group 5) and 2002 (Group 6); the area judged as clean (Group 7, 1992 – 1993); the group with variable pathologies of pregnancy (Group 8, 1995) from area with combined radioactive and chemical pollution at intermediate level.

The parameters that were studied: GST[1] and glutathione reductase (GSSG-R) [2] activities in placental cytosol; thiobarbituric acid reactive substances (TBARS) [3] and reduced low molecular weight (rLMW) thiols[4]; ethoxycoumarine de-O-ethylase (ECOD) activity in microsomes[5]; relative amount of GSTP1-specific mRNA in total placental RNA by Northern hybridisation; immunohistochemical detection of GSTP1 specific antigen; CYP1A1 Ile462Val[6] and GSTP1 Ile104Val[7] polymorphism; PAH-DNA adducts by competitive chemiluminescence immunoassay[8].

Questionnaires and clinical data concerning mother and newborn are used in analysis.

Results and discussion: The comparison of metabolism biomarkers at the carriers of different genotypes has revealed the stronger impact of CYP1A1 genotype than GSTP1 one. GST and GSSG-R activities, TBARS concentration are statistically significantly higher and rLMW thiols are lower at the carriers of CYP1A1 Ile/Val genotype than at carriers of Ile/Ile genotype.

GST activity and rLMW thiols decrease and GSSG-R activity increases in the row of GSTP1 Ile/Ile, Ile/Val and Val/Val isoforms.

The higher is the radioactive contamination and chemical pollution the lower is GST activity and higher is the concentration of PAH-DNA adducts. But the difference between both types of exposure is evidenced in other parameters. In radioactively contaminated area down-regulation of ECOD activity, GSTP1 specific mRNA concentration in total RNA and GSTP1 specific antigen is combined with two-fold increase of TBARS concentration as an indirect index of radioactive exposition. In chemically polluted area on the contrary up-regulation of ECOD activity and GSTP1 specific mRNA is observed as an indicator of CYP1A1 and GSTP1 induction. Reducing treatment of cytosol with dithiotreitol nonsignificantly increases GST activity in the samples from radioactively contaminated areas while nearly 2.5 times increases it in the samples from chemically polluted area. We suggest that down-regulation of GSTP1 expression plays the primary role in the radioactively contaminated areas and serves as a prerequisite of PAH-DNA adducts accumulation. Up-regulation of GSTP1 expression combined with posttranslational inhibition of GSTP1 activity manifests itself in PAH-polluted areas.

Despite the challenge of less number of individuals of different genotypes in each of classified group the data demonstrate contributions by both genotype and exposure in the detoxification. Lower GST activity in placental cytosol statistically significantly correlates with elevated frequencies of hypoxia, anaemia and risk of premature pregnancy interruption at mothers and with worse status of new-borns according to Apgar coefficient.

Thus PAH-DNA adducts and GST activity in placental cytosol may be used as more and less specific biomarkers of environmental exposure while together with genotype of detoxifying enzymes they may serve as prognostic factors for new-borns.

References: 1. Habig W. et al. (1974) J. Biol. Chem. **249,** 7130-7139. 2. Carlberg J., Mannervik B. (1975) J. Biol. Chem. **250**, 5475-5480. 3. Gavrilov V. et al. (1987) Voprosu Med. Chem. **33**, 118-122. 4. Sedlak J., Lindsay R. (1968) Analyt. Biochem. **25**, 192-195. 5. Greenlee W., Polalnd A. (1978) J.Pharmac. Exp. Ther. **205**, 596-605. 6. Shields P. et al. (1993) Cancer Res. **53**, 3486-3492. 7. Harries L. et al. (1997) Carcinogenesis **18**, 641-644. 8. Divi R. et al. (1999) Cancer Res. **59**, 4829-4833.

The molecular links between oxidative damage to DNA and cancer

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A wide variety of oxidative DNA lesions are present in living cells. One of the best known lesions of this type is 8-oxoguanine (8-oxoGua), which has been shown to have mutagenic properties. Our works demonstrated an influence of antioxidative vitamins and labile iron pool on the background level of 8-oxoGua in cellular DNA (1-4).

Our recently published results of analysis of urinary excretion of the oxidatively modified base/nucleoside combined with the determination of background level of 8-OH-dGuo in leukocytes DNA and measurement of 8-OH-Gua repair activity appears to predict an individual's susceptibility to tobacco-related lung cancer (5).

An involvement of 8-oxoGua in the origin and/or progression of cancer will be reviewed. It is concluded that a severe oxidative stress manifested as a high level of 8-oxoGua in cellular DNA as well as in urine of cancer patients is a consequence of development of many types of cancer. Although at present it is impossible to answer directly the question concerning involvement of oxidative DNA damage in cancer etiology it is likely that oxidative DNA base modifications may serve as a source of mutations that initiate carcinogenesis (i.e. they may be causal factors responsible for the process).

1/. Gackowski D., Kruszewski M., Bartlomiejczyk T., Jawien A., Ciecierski M., Olinski R., J. Biol. Inorg. Chem. **7**, 548-550, 2002
2/. Oliński R., Gackowski D., Foksiński M., Różalski R., Roszkowski K., Jaruga P., Free Rad. Biol. Med. **33**, 192-200, 2002
3/. Rozalski R., Gackowski D., Roszkowski K., Foksinski M. and Olinski R. Cancer Epidemiology, Biomarkers & Prevention. **11**(10), 1072-5, 2002
4/. Gackowski D., Banaszkiewicz Z., Rozalski R., Jawien A. and Olinski R. Intern. J Cancer, **101**, 395-397, 2002
5/. Gackowski D., Speina E., Zielinska M., Kowalewski J., Rozalski R., Siomek, A., Paciorek T., Tudek B., Olinski R., Cancer Res. **63**, 4899-02, 2003

Analysis of differences in protein substitution patterns based on statistical tests

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Protein substitution matrices are widely used in protein alignment algorithms. Protein similarities may reveal degree of evolutionary relatedness among different organisms. Comparison of substitution matrices derived using data from two groups of bacterial genomes with different GC content, makes possible analysis of differences in protein substitution patterns being possibly the result of dissimilar environmental conditions. The analysis was carried out using standard nonparametric tests with G statistic.

Nuclear myosin I and actin are required for RNA polymerase I transcription

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Recently, nuclear myosin I (NMI) was shown to be involved in RNA polymerase II transcription. We report for the first time that actin and NMI are required for rRNA synthesis. Electron microscopy reveals that both actin and NMI are localized within the nucleoli, and their distribution is highly specific. NMI colocalizes with nascent rRNA in the dense fibrillar component and its presence here is transcription-dependent. When nuclear extracts are fractionated, significant amounts of actin and NMI are detected in fractions containing partially purified Pol I and its transcription factors. Co-immunoprecipitation experiments demonstrate association of actin with murine initiation-competent Pol I holoenzyme complexes. The rate of Pol I transcription *in vitro* is dramatically reduced by antibodies directed against NMI, while addition of purified NMI stimulates transcription from mouse rDNA promoter in a dose-dependent manner. Anti-actin antibodies specifically inhibit formation of full-length transcripts, but not ACU trimers in abortive initiation assay, indicating that actin plays a role in promoter clearance or transcript elongation. Microinjections of anti-actin and anti-NMI antibodies significantly reduce the level of nucleolar transcription in cultured HeLa cells, demonstrating the requirement for these proteins for transcription *in vivo*. We conclude that the acto-myosin molecular motor can be generally required for the movement of DNA relative to transcription complexes.

Covalent binding of anthracycline antibiotics to DNA

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It has been largely documented that doxorubicin (DOX), daunorubicin (DRB) and their analogues with 3'NH_2 group in daunosamine moiety form with the exocyclic 2NH_2 group of guanine a covalent bond via a methylene group from formaldehyde (CH_2O). It is postulated that a Shiff base type intermediate is formed between CH_2O and NH_2 group in the course of the reaction. This reaction is supposed to occur in the cell. The analogues of anthracycline antibiotics with formamidine functionality bonded to daunosamine moiety and containing bulky morpholine (DRBM and DOXM) or hexamethylene (DRBH and DOXH) rings attached are studied in our laboratory. These substituents structurally hinder formation of Schiff base-intermediates with CH_2O. A decrease of DNA template transcriptional activity upon preincubation of the derivatives and DNA in the presence of CH_2O and spectrophotometric estimation upon washing out non-covalently bound drugs indicate on covalent binding of morpholine analogues of DRB and DOX. Covalent binding of hexamethylene derivatives to DNA is hardly detectable under these conditions. Electrophoretic analysis of drug-DNA complexes formed in the presence of CH_2O indicate that the analogues as their parent compounds induce labile "virtual" cross-links in DNA. Since HPLC analyses indicate that the drugs molecules remain integral in the condition of CH_2O – dependent complex formation with DNA these experiments suggest another, yet unidentified route leading to covalent binding of amidine derivatives to DNA.

Comparison of the results obtained at the subcelular level with cytotoxicity estimations indicates that there a high correlation between cytotoxicity of anthracyclines and transcriptional template activity of drug-DNA complexes formed in the presence of CH_2O ($r=0.792$; $n=9$) while no correlation was found between cytotoxicity and their non-covalent interactions with DNA measured by binding constants. These data confirm a notion that covalent attachment of anthracyclines to DNA is an essential event leading to cytotoxicity.

DNA repair genes: polymorphisms and risk of cancer

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Infrequent, mutant versions of many genes, including tumor suppressor and DNA repair genes, are associated with significant increase of cancer risk (even 1000 fold). However, the human gene pool contains probably hundreds, or even more, frequent gene alleles modulating cancer risk only slightly, what makes their identification very difficult. In order to identify the alleles, researchers use a candidate gene approach by selecting genes plausibly involved in cancer formation. One of the selected groups of genes code for the proteins of DNA repair. An allele may be regarded as the *bona fide* marker of cancer risk if its influence on cancer formation was shown in a large, well controlled case-control analysis, preferably performed on different populations and by different research teams. Equally important are the functional, mechanistic studies on the link between the change of DNA or protein sequence and alteration of protein activity as well as the influence of altered protein functioning on cell and tissue physiology. Judging by those standards, there are only a few alleles of DNA repair genes, whose influence on cancer risk have been relatively well documented. The strong candidates for the true cancer risk markers are: *hOGG1* Ser326Cys, *XRCC1* Arg194Trp, BRCA2 Asn372His polymorphisms. The major findings published by others on these polymorphisms will be presented together with the epidemiological data and results of functional experiments performed in our laboratory on polymorphic sequences of *MGMT* - DNA repair gene and *HSC70* - stress-response gene.

Nucleotide excision repair - new role in oxidative damage repair?

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Individuals differ in their reaction to the same dose of genotoxic agents and in DNA repair kinetics and efficiency (Palyvoda et al. 2002). As has been shown for aromatic DNA adducts and UV-induced DNA damage, these differences in response to genotoxic factors may depend on the genetic background and particularly on the existence of polymorphic variants of genes coding for DNA repair enzymes (Matullo et al. 2001, Qiao et al. 2002, Spitz et al. 2001). To examine this hypothesis in respect to ionizing radiation, we assessed the effect of polymorphisms of repair genes coding proteins which take part in different repair mechanisms on induction and repair of single strand breaks and on the frequency of micronuclei and of apoptosis in lymphocytes from different donors exposed to γ-radiation *in vitro*. The results concerning polymorphism of XRCC1, XPD, XPA and MGMT will be presented.

Ionizing radiation induces single and double strand breaks and a spectrum of oxidative damage in DNA molecules. While the breaks are repaired by homologous recombination or nonhomologous end joining mechanisms, oxidative damage is mainly removed by base excision repair (BER) although recently interconnections between BER and nucleotide excision repair (NER) have been shown in yeast and bacteria and mutations in genes coding for NER reduce the level and rate of global removal of γ-irradiation-induced DNA damage (Gellon et al. 2001, Doetsch et al. 2001, Weinfeld et al. 2001). Comparison of the results of DNA repair in irradiated lymphocytes obtained from donors carrying different polymorphic variants of repair genes showed that polymorphism in genes coding for XPD and XPA proteins correlates with the change in kinetics of DNA repair and influences the induction of apoptosis. XPD gene codes for a helicase, which is a component of TFIIH. This multiprotein and multifunctional factor is required for both NER and transcription initiation by RNA polymerases I and II (Weber et al. 1990, Reardon et al. 1996, Hoogstraten et al. 2002). XPA protein is also the component of NER mechanism. Polymorphism of XRCC1 protein engaged in BER did not influence the lymphocyte answer to irradiation. Our results suggest that NER takes part in repair of ionizing radiation-induced DNA damage and also that polymorphism in genes coding for proteins of this pathway can influence an individual's response to ionizing radiation.

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Chromosomal aberrations and cancer risk

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Process of carcinogenesis is closely related to the accumulation of genetic alterations in a single cell. This accumulation results from an imbalance between the induction of DNA-damage and its repair, which is one of the most important factors modulating individual susceptibility to mutagens and thus the individual cancer risk. Alterations in DNA repair machinery results in genomic instability, which manifests itself as chromosomal or molecular instability. Chromosomal instability is one of characteristic features of cancers. In solid tumours, as well as in haematological malignancies, chromosomal instability is expressed by accumulation of structural and numerical aberrations. Chromosomal aberrations can play a key role in cancer initiation and progression, or can be a feature of genetic instability that cells acquire during tumour development. The increased chromosomal instability (both, spontaneous and induced) has been observed not only in tumour cells but also in the normal tissues of cancer patients. The *in vitro* susceptibility to clastogenic effect of bleomycin has been accepted as a predictor of cancer risk. However, it can be used only for a group not for an individual prognosis. Therefore, there is still an unanswered question how to employ the cytogenetic methods in the evaluation of individual cancer risk.

Manipulation of flax for improved fibre extractability

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Flax (*Linum usitatissimum* L.) is a multipurpose crop of great antiquity. The flax fibres are long, thin and lustrous and can produce textiles and linen cloth of great quality. More recently flax also provoked interest as a source for novel industrial purposes including fibre reinforced plastics and pulp and paper applications.

Unfortunately, the processing of flax still has to rely on the initial microbial decomposition of the fibre cell walls (retting) – a method that is time- and area-consuming and often rather unreliable. During the retting process hemicelluloses, pectins and lignins are loosened from the cellulose fibres and manipulation of the fibre cell wall composition could help to improve extractability of the fibres. To test this, two different approaches have been chosen. Firstly, fungal pectinases will be introduced into the flax plant and expressed in the stem in order to weaken the pectin bondage between the fibres. Secondly, the fibre cell wall composition will be manipulated by downregulation of hemicellulose and lignin related enzymes. The obtained transformants will be tested for their retting characteristics and fibre quality.

Green tea in cancer prevention

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The critical action during carcinogenesis is transformation of normal cells by genotoxic carcinogens [chemicals, radiation or viruses], which affect specific codons in DNA and represent a somatic mutation of oncogenes or tumor suppressor genes. Growth and development of cells with such a modified DNA and additional alterations of the genetic elements, leads to typical neoplastic cells with the characteristic gene structure and phenotypic expression. Some epidemiological studies have found an inverse association between green tea consumption and the risk of cancer. It has been shown that green tea can block the formation of mutagens and carcinogens from precursor. Besides that green tea and their polyphenols inhibit the biochemical activation of genotoxic carcinogens. Moreover green tea increases their detoxification through the induction of metabolic enzymes – related phase I enzymes and phase II enzymes. Tea polyphenols also influence molecular events at the level of the gene. EGCG effects an action of tumor promoters on transcription factors such as AP-1 or NF-κB, what lead to control of the activity of transforming growth factors TGF-α and TGF-β. Genotoxic carcinogens and also oxidative processes enhanced during carcinogenesis in cells lead to formation of reactive oxygen species that alter DNA. Green tea, as a nonoxidized-nonfermented product receiving from tealeaves, contains several polyphenolic components, mainly flavonoids such as epicatechin, epicatechin gallate, epigallocatechin and epigallocatechin gallate, which possess antioxidant properties. The possible mechanism of green tea as a typical natural antioxidant is connected with inhibition of activity of enzymes participating in free radicals generation, such as xanthine oxidase, lipooxygenases and cyclooxygenases and connected with induction of antioxidant enzymes such as superoxide dismutase. Subsequent steps in the development of neoplasia involve the growth control of the early neoplastic cells. The active ingredients of tea decrease effectively these sequences. Last results suggest that the gallate structure of catechins is important for growth inhibition of tumor cell lines by these compounds. The effect of promoters involves blockage of cellular growth control messages through gap junctions and tea polyphenols restore effective gap junction communication and hence inhibit the action of promoters. Despite of proved antioxidant and cancer chemoprotective properties of main components of green tea i.e. catechins, studies of last years on several human cohort and case-control have indicated significant positive relationships between green tea consumption and cancers of various organs. Thus the problem of participation of green tea in cancer prevention is still open.

Targeted chemotherapy. Principles, new targets, challenges for the future

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The effective use of cancer therapy requires an understanding of the principles of tumor biology, cellular kinetics, pharmacology and drug resistance. Thanks to the development of the new effective chemotherapeutic agents coupled with our expanding knowledge about the administration and combination of these agents we are now able to cure almost 20% of all new cases by chemotherapy along. Combination of chemotherapy with other modalities of treatment like radiotherapy and surgery improves greatly the chance of curing.

The lecture focuses on the principles responsible for the development of modern combination regimens. This is followed by description of new chemotherapeutic drugs, and description of the new exciting agents as angiogenesis, COX-2 and epidermal growth factor receptor inhibitors. Many of them are already used in the clinic improving patients outcome and giving new hope for the future.

Biological implications of failure in solid tumors treatment

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Objective: Loco-regional relapses, distant metastases and second tumour development constitute majority of head and neck cancer (HNSCC) treatment failures. Occurrence of second primary tumours after curative treatment is current problem in contemporary oncology. The mutagen sensitivity is well known marker to predict patient proneness to develop the second tumour. Another aspect is influence of genes on individual susceptibility. *GST, CYP* and *CCND1* genotypes have been associated with HNSCC outcome. In this report a prospective, case-control study in which we determined the induced mutagen sensitivity i.e. frequency of mutagen-induced chromatid breaks (breaks per cell; b/c) in peripheral blood lymphocytes (PBLs) in patients with multiple primary tumours (MPT) compared with patients with single HNSCC and healthy controls is presented. The relationship between spontaneous and induced mutagen sensitivity and age, smoking, alcohol consumption, index and second primary tumour site and interval between both tumors occurrence was estimated for MPT group. *GSTM1, GSTT1, GSTM3, CYP1A1, CYP2E1XPD, XRCC1* and *XRCC3* genotypes were analysed.

Material and method: 36 patients with MPT and two control groups: 52 patients with one malignancy and 47 healthy individuals were analysed. The differences between examined patients and control groups were estimated and differences among the patients with MPT spontaneous and induced b/c level were compared using U Mann-Whitney and Spaerman Rank Correlation.

Results: The b/c level in PBLs of patients with MPT ranged from 0,26 to 4,12 (mean 1,53) and was significantly higher ($p<0,000006$) both compared with patients with one malignancy (b/c ranged from 0,02 to 3,08; mean 0,74) and healthy controls (b/c ranged from 0,04 to 1,14; mean 0,41). The spontaneous b/c level in PBLs of patients with MPT ranges from 0 to 0,22 with mean score b/c=0,84. Induced b/c scores between tumors deriving from one tissue, i.e. HNSCC and tumours of different sites and histologicaly divergent were not statistically significant (p=0,051) whereas spontaneous mutagen sensitivity in patients with SCCHN (b/c=0,07) have been statistically lower then in patients with MPT of different sites and histology (b/c=0,13; p=0,012). Spontaneous mutagen sensitivity in patients with smoking-related tumours (b/c=0,07) has been lower than in patients with not smoking-related MPT (b/c=0,11; p=0,05). An increase of b/c index was observed in almost all chromosomal arms. The majority of chromosomal locations with the increased proportion of breaks in the group of patients with multiple tumours were identified as regions where loci involved in DNA repair, cell cycle regulation suppressor genes and oncogenes were found. *GSTM1+, CYP1A1 1/4* and combinations of: *GST T1+/GST M1+, GST M null/CYP 1A1* and *GST M null/CYP 1A1* were also associated with MPT group.

Conclusions: Statistically higher induced individual susceptibility in MPT patients compared with single tumour and healthy controls were confirmed. Comparable induced mean b/c was found in patients with two smoking-related cancers as well as with not smoking related tumours. In opposite, baseline spontaneous b/c in MPT localization distinguished according to exogenous factors compliance were found; more sensitive occurred to be individuals who develop not smoking related tumour or second primary different then HNSCC.

Flax engineering for fibre improvement

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Flax (*Linum usitatissimum* L.) is an annual plant cultivated in temperate climate. Although the whole genus *Linum* has about 230 species, the only one, *Linum usitatissimum*, remains a very important plant of commercial use, serving as a fibre donor for the textile industry and linseed oil production.

In order to improve the properties of flax fibres, which are of lower quality than those of cotton origin, transgenic plants synthesizing polyhydroxybutyrate (PHB) in stem tissue were generated and analysed.

Poly-β-hydroxybutyrate (PHB) is a hydrophobic, thermoplastic polymer produced by numerous bacteria as a source of carbon and energy.

PHB can be called 'green' plastic, because it is completely degraded by microorganisms in the different environmental conditions, like soil or seawater.

In this study bacterial pathway of PHB synthesis was transferred into flax by *Agrobacterium* mediated transformation. Three genes, used throughout this study, encoding enzymes necessary for PHB production derived from *Ralstonia eutropha* cells. We have prepared two constructs, a multigene construct that contains three genes for PHB production (*phb A, phb B, phb C*) and a single construct bearing only *phb A* gene encoding ∃-ketothiolase, the first enzyme of PHB biosynthetic route. ∃-ketothiolase was suggested to be rate-limiting enzyme for PHB production and it was placed under the control of recently isolated and characterized 14-3-3 promoter. Acetyl-CoA is the substrate needed in the PHB synthesis. Since there is a high amount of acetyl-CoA in chloroplasts, the PHB enzymes were directed into flax plastids. Transgenic plants obtained after transformation with single construct mainly served as a transformation and regeneration control.

The level of PHB in selected transgenic lines was analysed using the GC-MS method. Transgenic plants produced over 70-fold higher level of polymer than untransformed, wild type plants.

The PHB accumulation in plastids caused changes in their shape and size, which were confirmed using transmission electron microscopy.

Biomechanical parameters of obtained transgenic plants were characterized. Young's modulus (E) was measured and compared to control plants. The E parameter ranged from 24.1 to 54.4 MPa in plants transformed with the triple construct (26.9 MPa for control plants). Thus the increase in PHB level affects the mechanical properties of flax stem.

Since acetyl-CoA is a key compound in many metabolic processes occurred in cells, the metabolite profile of transgenic plants was determined. Polymer production caused the alterations in the metabolite composition, for example: some amino acids (e.g. lysine), fatty acids (e.g. 18:0, 18:2, 16:0) and citrate cycle intermediates (e.g. citrate, isocitrate) were decreased in transgenic plants. However the PHB accumulation did not have any negative effect on fertility or growth of transgenic lines, which were observed in other species and when 35S CaMV promoter was used for the expression of PHB genes.

The obtained transgenic plants with higher PHB content and improved mechanical properties can serve as a source of industrially important flax cultivars.

Transgenic crops in the world

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World population, now 6.0 billion, has doubled during last 40 years and is expected to grow to 10 billion, by 2050. Some 2 billion people already lack food security and about 800 millions are suffering hunger. There is a hope that genetically modified crops could help to solve this problem.

Genetically modified crops were introduced in agriculture in 1994. According to Clive James[1] between 1996 and 2002 the global area of GM crops cultivation, has increased from 1.7 million hectares to 58.7 million hectares. The use of GM crops was in 2002 limited mainly to four countries: USA (66%), Argentina (23%), Canada (6%) and China (4%). Only two European Union countries Spain and Germany were growing GM crops in 2001 in very small amount. However other European countries (Rumania and Bulgaria) started to grow GM crops also. The number of countries growing GM crops was 16 in 2002, with new users like India, Colombia and Honduras.

Since 1996 adoption of transgenic plants in agriculture has been very crop-specific. Mainly herbicide tolerant crops such as soybean and cotton and insect resistant crops such as Bt cotton and Bt maize have been cultivated.

Although it seems that the use of GM crops is expanding rapidly there is still a very controversial discussion around potential environmental and health risks and unclear benefits. With respect to the above, it is a need to create a biological safety system that among others should result from the Convention on Biological Diversity and the Protocol on Biological Safety signed in Cartageny. The biological safety system is a global issue and its goal is to ensure a safe use of present and future GMO's.

[1] C. James, Preview: Global status of commercialised transgenic crops: 2002. ISAAA Briefs 27 2002

Poster abstracts

Posters marked with red numbers (or underlined) were qualified to competition for Award of Association for the Support for Cancer Research due to on time registration.

1. Salivary epidermal growth factor levels decrease in oral cavity cancer patients

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Epidermal growth factor (EGF) is a cytokine that contributes to the maintenance of mucosal integrity: an intact oral epithelial barrier and its proper function. It is responsible for epithelial regeneration by means of growth induction and cellular revival. EGF has additional potent and diverse effects on cell migration and matrix synthesis. One of the major sites of EGF synthesis in humans are parotid salivary glands. There is now good evidence that development and progression of epithelial malignancy is associated with the abrogation of normal growth control mechanisms but salivary EGF effect on tumorigenesis and oral cancer biology is unknown.

The goal of our study was to investigate the changes of EGF concentration in the whole resting ($_r$) and stimulated ($_{st}$) saliva in healthy volunteers (K_r, K_{st}) in comparison with oral cancer patients before (C_r, C_{st}) and after (C'_r, C'_{st}) chirurgical tumour excision.

Whole resting and stimulated saliva of 10 patients with oral squamous cell carcinoma was investigated before and two weeks after chirurgical treatment. Volume of saliva was volumetrically determined. Concentration of EGF in saliva was evaluated by sandwich ELISA technique (Quantikine® Human EGF Immunoassay kit, R&D Systems).

Salivary EGF concentration in patients before treatment (C_r=1,7939±0,57ng/ml, C_{st}=1,3047±0,92ng/ml) was lower ($p>0,05$) than after surgery (C'_r=2,0128±1,66ng/ml, C'_{st}=2,1947±1,52ng/ml) both in the whole resting and stimulated saliva. As against the control group (K_r=3,3182±1,83ng/ml, K_{st}=1,7524±1,13ng/ml) the values of EGF levels were considerably lower in the whole resting saliva of the patients before surgery ($p<0,05$). As for the whole stimulated saliva in patients before tumour excision the value of this parameter was inconsiderably lower than in the control ($p>0,05$). After surgery salivary EGF concentration in the whole resting saliva was still decreased in comparison with the control group ($p>0,05$). EGF level in the whole stimulated saliva in postreatment cancer patients was higher than in the volunteers group ($p>0,05$).

Our data (especially decreased levels of EGF concentration in saliva before and its contrary tendency after chirurgical treatment) may suggest important role of EGF in oral cancer biology. This problem needs to be more profoundly examined including more numerous groups of patients and longer postreatment time.

2. Treatment planning in prostate cancer based on transrectal USG examination

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Aim: The aim of the study is to present treatment-planning procedures in brachytherapy of prostate cancer based on USG examination with use to SWIFT system (Nucletron).

Introduction: Brachytherapy Department in Institute of Oncology Gliwice is equipped in computed treatment planning system (TPS) SWIFT. The system component is transrectal USG machine, stepper – use to fixed probe and template, and treatment planning system. These elements are integrated and allow to "real time" brachytherapy treatment.

Material and method: Before start treatment planning procedures and treatment, patient has to be anaesthetized (spinal anaesthesia).

First step is to perform USG examination, to place probe on the depth of prostate base and to send next slices (1mm thickness) until prostate apex.

Slices are sending to TPS automatically by network (stepper encoder – connect USG machine and TPS). The next is to prepare pre – plan. First, the prostate base and apex have to be identified. System localized the reference scan automatically (scan between base and apex). Than the doctor contours the prostate – PTV (Planning Treatment Volume) manually or automatically based on interpolation between scans and the organ at risk – urethra. Successively, prescribed dose and catheter placement in prostate is established.

The next step is the source "dwell times" optimisation and normalization. Prostate volume covered by reference isodose (100%) and dose in urethra is taken from dose – volume histogram. The doctor inserts needles according to catheter placement. The next is to perform USG examination with implants and to start live planning procedure. Pre – planning slices (contours and catheter location) is to lie on live plan scans. TPS enable to modify needles location (virtual $\rightarrow$live catheter). The next is the source "dwell times" optimisation (according to new, real implant placement) and dose – volume histogram verification. After acceptance, treatment plan is send to treatment control station by network and patient treatment is started.

Conclusion: Dose distribution based on USG examination enable to verified implant placement in PTV (prostate) relation to organ at risk (urethra).

Integrated treatment planning system enable to modifies catheter location in prostate, directly (pre - planning $\rightarrow$ live planning).

3. Cisplatin with aminoflavone wings as an improved apoptosis inducer of cancer cells

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In this work we have compared biological properties of *cis*-diamminedichloroplatinum (cisplatin) and its new analogue cis-[$Pt(AF)_2Cl_2$] (AF stands for 3-aminoflavone) containing two bulky aminoflavone wings, as non leaving ligands, instead of ammine groups. Both compounds were tested for their antiproliferative activity against cultured L1210 cells, their DNA damaging properties and ability to induce apoptosis. The new analogue was found to be less more cytotoxic than cisplatin. In terms of IC_{50} (the drug concentration inhibiting 50% of L1210 cell growth after 72 hours exposure) cisplatin was about 4 times more active. Both complexes reacted with purified calf thymus DNA in a cell-free system producing of DNA interstrand crosslinks. Kinetics of crosslinks formation was very similar for both compounds but maximal level of crosslinks was higher for cisplatin (crosslinked DNA fractions were 0.59 and 0.40 for cisplatin and *cis*-[$Pt(AF)_2Cl_2$], respectively). In cells, however, as assayed by DNA alkaline elution, crosslinks formation was very similar for both compounds.

At higher concentrations of drugs, strong degradation of DNA was observed in L1210 cells treated with *cis*-[$Pt(AF)_2Cl_2$] but not in the cells incubated with *cis*-DDP. This DNA degradation seems to reflect very efficient apoptosis induction by *cis*-[$Pt(AF)_2Cl_2$] as electrophoretic patterns of DNA from cells incubated with this drug showed a ladder typical for apoptotic cells. An additional confirmation of this result was obtained by flow cytometric analysis of drug treated cells. Our results suggest that *cis*-[$Pt(AF)_2Cl_2$] is much more effective apoptosis inducer than its parent compound cisplatin.

4. Identification of nuclear proteins binding to human topoisomerase I

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Human topoisomerase I is a multifunctional protein possessing two distinct enzymatic activities. Its relaxing activity contributes to basal processes like transcription, replication and recombination. Additionally, the enzyme participates in splicing by acting as SR proteins kinase.

The purpose of the work was to identify protein partners of human topoisomerase I. To get more detailed information about the sites of binding, the enzyme was divided into four functional domains. Each of these domains was fused with GST and used as a bait in a pull down assay with nuclear protein extract from HeLa cells. Bound proteins were identified by mass spectrometry.

The catalytic domain as well as the linker domain did not bind proteins in this assay. On the contrary, the N terminal and core domains bound distinct subsets of proteins.

The N terminal domain is expected to be the major site of protein-protein interactions. Within proteins revealed bound to N terminal domain two groups were distinguishable. One group are SR proteins, either playing role in splicing or not. Remaining proteins can be clustered on a base of possessing acidic stretches similar to nucleolin, known protein partner for topoisomerase I.

The core domain is the largest part of the enzyme containing almost all catalytic aminoacids. The core domain seemed to bind proteins from two complexes. One is pre-ribosomal complex, the other one pre-spliceosome complex. This result suggests the potential role of topoisomerase I in both pre-RNA splicing or assembly and its contribution to spliceosome complex.

5. Towards identification of features of cis-regulatory regions associated with common patterns of gene expression during hippocampal development

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We employ a combination of computational and experimental approaches to identify features of cis-regulatory regions that underlie common patterns of gene expression during hippocampal development. We analysed the published data from Affymetrix gene profiling of the hippocampal development in mouse (Mody et al., 2001, PNAS 98: 8862-7). Our analysis revealed that most of variations in expression can be explained by the difference in the amplitude of expression, and pattern of up- and down-regulation in the course of development. We sought to identify features of the cis-regulatory regions specifically associated with these two characteristics of expression profiles. Current computational work constitutes of identification of putative regulatory regions and annotating them with motives for consensus binding sites for transcription factors. Then we build higher order features from these motives using purpose-built software. Such features are scored for their statistical association with the amplitude or up/down regulation. The role of the most promising features identified *in silico* will be tested by perturbation experiments in the hippocampal neuronal culture. This culture system is a good model of the gene regulation in the developing hippocampus (Dabrowski et al., 2003, J. Neurochem. 85: 1279-88). Our strategy will be to interfere with the function of a binding site, or of the transcription factor, and to monitor the effects of such perturbations on the expression of the putative target genes.

6. Expression of recombinant staphylokinase gene in transgenic potato (*Solanum tuberosum* L.) plants

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Over the past decade, due to progress made in genetic transformation methodology, transgenic plants have become a convenient eukaryotic system for the expression of synthetic genes coding for recombinant proteins of different origin. Moreover, molecular farming that utilize transgenic plants, have become an inexpensive alternative for commercial production of valuable recombinant proteins. This technology has many potential advantages as compared to industrial facilities using fermentation or bioreactor systems for generating recombinant proteins on a large scale. Among the recombinant proteins produced by transgenic plants, those of therapeutic value attract attention of scientists as well as pharmaceutical and biotechnological companies. Here we report on the construction of fusion gene that consists a recombinant staphylokinase (plazminogen activator protein, a promising thrombolytic agent of bacterial origin) and sequences coding for mGFP and ß-glucuronidase markers and its expression in potato (*Solanum tuberosum* cv. Desiree) transgenic plants. The Western blot analysis and biochemical assay were used to confirm the presence of staphylokinase domain in total protein extract from transgenic plants and its amidolytic activity, respectively.

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7. DNA double strand break rejoining in M059J and K human glioma cells X-irradiated and treated with signaling pathways inhibitors

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The aim of this work is verification of the hypothesis that radiosensitization of cancer cells by inhibitors of signalling pathways initiated at growth factor (mitogen) receptors is due to their effect on DNA repair systems. We used two related human glioma cell lines: M059K and M059J, the latter lacking in the catalytic subunit of the DNA-dependent protein kinase (DNA-PK_{cs}) expression. This results in slower DNA double strand break (DSB) rejoining and a substantial increase in M059J cells sensitivity to ionising radiation and bleomycin as compared to M059K cells. Conversely, M059J cells are more resistant to signalling pathways inhibitors: tyrphostine AG 1478 – specific for epidermal growth factor receptor (EGFR) and PD 098059 – acting on MEK 1/2 kinases. In clonogenic growth ability assay, PD 098059 sensitises both glioma cell lines to X-radiation whereas tyrphostine AG 1478 exerts only an additive effect. However, investigation of DSB rejoining efficiency by DNA pulse field gel electrophoresis (PFGE) gives contrasting results. Tyrphostine AG 1478, added before X-irradiation (10 Gy) of the cells, significantly elevates residual DSB levels measured after 30-min (M059K) or 60-min (M059J) repair period, the effect being much more pronounced in M059J cells. Under the same conditions, PD 098059 has no influence on the DSB rejoining rate. The PFGE results may indicate that homologous recombination (HR) system rather than non-homologous end-joining (NHEJ, non-functional in M059J cells) is affected by inhibition of signalling that starts at the plasma membrane. It should be taken into account that cell death processes may accompany DNA repair in a cell culture exposed to growth factor inhibitors and X-radiation and make it difficult for interpretation. Thus, examination of apoptosis/necrosis kinetics may explain inconsistency of cell survival and DNA rejoining rates in M059K and M059J cells treated with X-radiation and signalling pathways inhibitors.

8. Identification of genes important in modulation of radiosensitivity in human melanoma cells

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Melanomas are very malignant cancers capable of forming distant metastases. Usually, albeit not always, they are ionising radiation-resistant. During periods between therapy sessions as well as during metastatic spread, selective expansion of neoplastic cell clones occurs. Due to great genetic instability of these neoplasms both genotype and phenotype of arising clones may differ (for example in their radiosensitivity). The goal of our experiments was to check gene expression pattern changes during growth of a new cell population and whether clones obtained differ indeed in their radiosensitivity. Answer to these questions is essential for understanding mechanisms of metastasis. The present study is the first of the planned series.

Starting with Me45 melanoma line, three subclones were obtained. Ionising radiation sensitivity of the starting line and that of the subclones was compared. Cell survival tests indicated no statistical differences in radiosensitivity. Total RNA was then isolated from the starting cell line as well as from the subclones and expression levels of some 22 thousand genes were checked using high-density DNA microarrays from Affymetrix.

Among genes with significantly altered expression level were those coding for G antigens and other cell surface proteins, transcription factors, protein membrane receptors and other signalling proteins.

The study was financed through KBN grants no. PZB-040/PO4/2001 and 4PO5015190.

9. Ionising radiation-induced changes of gene expression patterns in human melanoma cells

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Ionising radiation is a factor both increasing the risk of malignancy as well as an important antitumour therapy tool. Damages induced by radiation trigger in cells various signalling pathways, repair mechanisms as well as cause changes in gene expression patterns. Knowledge of processes induced in cells by radiation should help in understanding the basis of different radiosensitivity of various cell types.

The goal of this study was to compare gene expression patterns in non-irradiated control cells and cells exposed to ionising radiation as well as to monitor the time course of these changes. Material used in our study was human melanoma Me45 cell line (obtained at the Gliwice Center of Oncology). Cells were irradiated with 4Gy dose and total RNA was then isolated from the cells (a) immediately; (b) after 12 h and (c) after 24 hours following radiation exposure. As a control, non-irradiated cells were used. Expression levels of some 22 thousand genes were then assessed using high density oligonucleotide microarrays, hybridisation equipment and procedures from Affymetrix.

Groups of genes were selected for which gene expression pattern differed in a specific manner following various exposure times. Among genes for which expression was substantially increased or decreased in irradiated cells were those coding for:

- transcription factors
- cell cycle control proteins
- proteins participating in metabolic processes
- signal transduction proteins
- repair proteins

The study was financed through KBN grants no. PZB-040/PO4/2001 and 4PO5015190.

10. Analysis of antibody responses induced by peptides mimicking neuroblastoma antigen GD2 ganglioside

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Neuroblastoma is the third most frequent cancer of childhood. Although having the highest spontaneous remission rate among all human malignancies, the disease in advanced stages often cannot be tackled sufficiently. Hence novel therapeutic strategies need to be put forward. One of the new approaches is immunotherapy directed against a glycolipid antigen present on the surface of neuroectodermal cells. The antigen – GD2 ganglioside – is abundant on neuroblastoma cells but not on normal cells. However, due to chemical properties of glycolipid antigens their immunogenicity is low and they are generally difficult to apply in immunotherapy. Yet these antigens can be successfully imitated with mimeotopes – peptide sequences found using phage display technology.

The aim of our studies was to investigate the immunogenic potential of peptide sequences mimicking GD2 ganglioside. The peptides of 15 aa in length were isolated by panning of phage displayed peptide libraries with mouse monoclonal antibody specific to GD2 ganglioside (14G2a). We carried out immunizations of BALB/c mice with 2 doses of three different peptide-KLH conjugates in combination with Freund's adjuvant to set the optimal immunization schedule. We showed that the peptide-KLH conjugates posses the ability to elicit humoral response in BALB/c mice and measured the level and reactivity of anti-peptide antibodies with ELISA. Immunotyping of sera samples was also performed to further characterize the immune response induced with our peptide vaccines.

In a separate set of experiments we investigated whether sera samples from animals immunized with the peptide mimics recognize the GD2 ganglioside preparations (coated on microtiter plates). We also carried out experiments with human GD2 ganglioside-bearing neuroblastoma cell lines to show the cross-reactivity of the murine sera with the glycolipid expressed on tumour cells.

The work was supported by 3P05A 00124 grant from the Polish Ministry of Scientific Research and Information Technology (former State Committee for Scientific Research).

11. Aclarubicin induces the production of reactive oxygen species in human cell lines

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Aclarubicin (ACL) is one of the anthracycline antibiotics, widely used in chemotherapy of solid tumours and leukaemia. Several mechanisms of anthracycline toxicity have been proposed: intercalation into DNA and inhibition of DNA-topoisomerases, activation of caspases, alteration of the structure and function of mitochondrium and production of the reactive oxygen species (ROS). ROS generated by anthracyclines can induce a number of perturbations in cells, including lipid peroxidation, apoptosis and glutathione depletion. In this report we investigated the ability of aclarubicin to produce ROS in the human cell lines. ROS production was estimated in human fibroblasts derived from the skin of a healthy donor (S-2 cell line), the skin of diabetic patients (C5 cell line) and from the skin of Down's syndrome patients (BB cell line). The level of ROS was studied spectrofluorometrically using the fluorescent probe C2938 - [6-carboxy-2',7'-dichlorodihydrofluorescein diacetate, di(acetoxymethyl ester)] with and without antioxidant PDTC (pyrrolidine dithiocarbamate). This reagent is cleaved by intracellular esterases and releasing product fluoresces in the presence of ROS, in the live cells. Our results have showed an increase of ROS production in aclarubicin-treated cells in comparison to the control cells (drug untreated cells). The addition of antioxidant PDTC inhibits the production of ROS in the fibroblasts treated with drug.

12. The level of chromosomal aberrations in Polish refinery workers

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The objective of the project was to evaluate occupational exposure to benzene of workers of two Polish petroleum refineries using selected indicators of individual exposure to benzene (airborne benzene), internal dose (blood and urinary benzene, BB and UB; urinary phenol, UP; trans,trans-muconic acid, t,t-MA; S-phenylmercapturic acid, S-PMA), early effects (chromosomal aberrations, CA) and susceptibility (genetically-based polymorphisms: CYP2E1, NQO1). Determination of urinary cotinine was used to examine an expected confounding effect of tobacco smoke.

101 workers currently exposed to benzene from two Polish refineries: A and B located in different cities were enrolled in the study. 99 controls were selected from two workplaces in the same geographical area and were matched on age, sex and smoking habits. Benzene exposure during work shift was measured using active personal samplers. Biological samples were collected at the end of work shift. Data collected in a standardized questionnaire form included age, sex, work duration, category of job (in refineries), current and lifelong tobacco use, medical history, history of diagnostic, x-ray exposure etc.

Significantly higher levels of benzene in air were measured in Refinery A (median 0.61 mg/m³) comparing with Refinery B (median 0.11 mg/m³). Workers in Refinery A also had detectable BB levels in highest percentage (37% vs 8% in Refinery B and 1% among controls). UB levels were significantly associated with airborne benzene, while UP, t,t-MA and S-PMA concentrations did not reflect the levels of airborne benzene. Polymorphisms investigated were not associated with the levels of biomarkers of dose. Higher levels of CA were found in occupationally exposed groups with the highest frequency of CA in Refinery A where chromosomal aberrations were detected in 33% of workers vs. 14% in controls. Smoking habit had a significant effect on the frequency of CA.

13. Radiation - induced genetic changes in directly exposed and neighbouring cells in vitro

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One of the biological effects of ionising radiation is a phenomenon termed '*bystander effect*'. It involves genetic changes occurring in cells that were not directly irradiated but responded to signals transmitted from irradiated cells. It is now well established that irradiated cells secrete cytokines or other soluble factors into the culture medium. The medium from irradiated cells (ICM-irradiation conditioned medium) can initiate genetic changes in non-irradiated cells such as apoptosis, chromosomal aberrations, mutations and modulations of specific proteins` expression. It has been demonstrated that medium from irradiated human epithelial cells induced the bystander response in non-irradiated cells while medium from irradiated human fibroblasts did not. Since this effect depends on the type of cells it seems to be interesting to test the medium-mediated bystander effect in cells *in vitro*.

In the present study the cellular responses to direct irradiation and to treatment of conditioned medium were compared in human leukaemic K562 cells.

The cultures were exposed to X-radiation doses between 0 – 8 Gy. After one hour incubation at 37^{o}C the medium (ICM) was removed, filtered and transferred to non-irradiated flasks containing cells from the same line. The cultures were incubated for 36 h prior scoring. The cultures irradiated only, without change of the medium, were incubated in parallel. The genetic changes in cells exposed to radiation or conditioned medium were estimated as a frequency of micronuclei and apoptotic-like bodies. The genetic expression profiles were examined using oligonucleotide arrays that contained probes representing 22 283 human genes. The Affymetrix high density microarrays and equipment were used for hybridisation and read out of the results.

The results indicate that X-radiation and conditioned medium induced micronuclei and apoptosis. Both treatments (X-radiation and ICM) induced the change in gene expression patterns. Most of the changes in expression of particular genes were similar; they either increased (for example ORCL5, E2F2, E2F5, MADH6) or decreased (CASP2, MADH1, MADH3, CDK6). Some of genes expressed differentially after X-ray and ICM treatment (BCL2, CDC14A, BUB1).

14. Studies on apoptosis and necrosis in A549 cells caused by cis-Pt(II) complex of 3-aminoflavone in comparison with cis-DDP

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Non-small cell lung cancer (NSCLC) includes a group of tumours, which respond poorly to drugs. *Cis*-DDP toxicity still remains a problematic feature, not completely solved by the improvement of supportive care. Therefore, *cis*-Pt(II) complex of 3-aminoflavone was selected out of *cis*-DDP analogues as being less toxic towards normal cells and having at least similar or better antitumour activity in comparison with *cis*-DDP. The aim of this research is to compare *cis*-Pt(II) complex of 3-aminoflavone and *cis*-DDP abilities to induce apoptosis and necrosis in human non-small lung cancer cell line A549. Trypan blue dye, fluorescent dyes (acridine orange/ethidium bromide and Hoechst 33258/propidium iodide double staining), MTT and TUNEL assays were used. After *cis*-Pt(II) complex of 3-aminoflavone application cell viability was only 49% at 4xIC_{50} after 72-h incubation whereas it was 72.5% at the same time point after cells treatment with *cis*-DDP. The morphological symptoms of apoptosis were also noticed after cell incubations with fluorescent dyes. The results obtained showed that *cis*-Pt(II) complex of 3-aminoflavone had higher ability to induce apoptosis in a concentration-dependent manner in cancer cell line A549 despite its lower cytotoxicity (IC_{50} was 23 μM) in comparison with *cis*-DDP (IC_{50} was 8.5 μM). These observations were confirmed by the results from TUNEL assay, which detects early stages of apoptosis.

It suggests beneficial properties of *cis*-Pt(II) complex of 3-aminoflavone as a possible chemotherapeutic drug.

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15. Evaluation of the genotoxic properties of *cis*-Pt(II) complex of 3-aminoflavone in comparison with *cis*-DDP in A549 cells

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Lung cancer remains one of the most common causes of cancer-related death worldwide. In view of the central problem of drugs sufficient efficiency in chemotherapy, efforts have focused on the development of alternative platinum-based analogues that can be more effective in cancers treatment. *Cis-bis*(3-aminoflavone)dichloroplatinum(II) (*cis*-Pt(II) complex of 3-aminoflavone) represents a novel class of potential antitumour agents. The aim of this research was to assess genotoxicity of *cis*-Pt(II) complex of 3-aminoflavone and *cis*-DDP in human non-small lung cancer cell line A549.

In order to evaluate genotoxic properties of this chemical compound the comet assay in A549 cells was used. The analysis of DNA damage after 1-h cell incubation with *cis*-Pt(II) complex of 3-aminoflavone and *cis*-DDP was carried out including 0.5-h, 1-h and 1.5-h postincubation. The statistically significant increase in tail moments was observed after *cis*-Pt(II) complex of 3-aminoflavone application in comparison with *cis*-DDP which can indicate DNA breaks induced by the former compound. On the other hand, the decrease in these values caused by *cis*-DDP was connected mainly with the presence of DNA-DNA and DNA-protein cross-links. The distribution of tail moments after A549 cells treatment with both compounds was also different. The increase of these values was also noticed after A549 cells postincubation especially with 1 μM and 2.5 μM of *cis*-Pt(II) complex of 3-aminoflavone contrary to *cis*-DDP.

Results obtained on the basis of the comet assay could confirm the presence of different mechanisms of action of both tested compounds i.e. the occurrence of DNA breaks (besides cross-links) induced by *cis*-Pt(II) complex of 3-aminoflavone in comparison with *cis*-DDP.

This work was partially supported grants No 502-13-849; 503-316-2 and 502-12-732 from the Medical University of Lodz.

16. The examination of XPA protein interaction with selected glycosylases: NTH1, TDG, OGG1-1A and OGG1-2A

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Cancer arises as a result of multiple somatic mutations of genes involved in regulation of cellular proliferation, apoptosis, migration, angiogenesis. Attenuation of DNA repair significantly increases cancer risk. Studying the functioning of various DNA repair systems and their interactions and regulation helps to understand cancer formation and response of cancer cells to chemo- and radiotherapy.

The goal of this research project was to test the hypothesis that two DNA repair systems: base excision repair and nucleotide excision repair cooperate by physical interaction of their DNA damage recognition elements: glycosylases (BER) and XPA (NER). We selected four glycosylases: NTH1, TDG, OGG1-1A and OGG1-2A. The GST pull-down assay was employed as an experimental procedure. Glutathione-sepharose beads loaded with GST-XPA or GST only (negative control) were incubated with the cell lysates containing the glycosylases. Proteins in the bound fraction were detected using Western blotting. Antibodies used for detection were either polyclonal (recognizing native NTH1 glycosylase) or monoclonal, recognizing the FLAG epitope attached by the genetic engineering to the TDG, OGG1-1A and OGG1-2A glycosylases. These three recombinant proteins were produced in COS-7 cells after the recombinant vectors transfection. None of the glycosylases showed the detectable binding to the GST-XPA fusion protein in the in vitro system that we used. We found however that the recombinant plasmids that we constructed produce easily detectable recombinant TDG, OGG1-1A and OGG1-2A glycosylases linked to the FLAG epitope. They are valuable tools for further biochemical and biological studies on base excision repair.

17. Comparison of DNA damage and repair kinetics in lymphocytes of cancer patients and healthy donors

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Correct repair of DNA damage plays a protective role against genotoxic factors, while its impairment leads to mutation and may be a basis of cancerogenesis. Single cell gel electrophoresis (comet assay) is a sensitive method for evaluation of DNA damage and kinetics of its repair and is widely used in environmental mutagenesis and radiobiological studies.

In this study the alkaline version of the comet assay was applied for comparison of radiation-induced DNA damage and repair capacity in peripheral blood lymphocytes of 42 patients with cervical carcinoma and 30 healthy women.

The results indicate a great variation in response to radiation in both groups under the study. However, the lymphocytes of patients were characterized by significantly higher level of a background ($p=0{,}000005$), increased sensitivity to radiation ($p=0{,}002$) and reduced DNA repair potential ($p<0{,}001$). Furthermore, these features were more pronounced for patients with familial anamnesis. Significant influence of age on background damage was revealed in both groups, but age connection with DNA repair after irradiation was observed in healthy donors only. The smoking status increased the level of background damage ($p<0{,}03$) and decreased lymphocyte repair ability ($p<0{,}005$) in healthy donors. In the case of patients the inverse relationship was noticed, but significance was not achieved. The observation that tumour regression is connected with lymphocyte repair kinetics suggests that local response depends not only on tumour cell radiosensitivity but may be assisted by host lymphocytes.

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18. Atypical cytogenetic aberrations in a patient with CML accelerated phase: a case report

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Introduction: Chronic myeloid leukaemia (CML) is a clonal proliferation associated in 90-95% of cases with a specific chromosomal abnormality - Philadelphia (Ph) chromosome and/or bcr-abl fusion gene. The Ph marker is a product of balanced translocation t(9;22)(q34;q11), resulting in the generation of chimeric bcr-abl oncogene. Secondary chromosomal changes in bone marrow of patients with CML may appear before or during progression of the disease. In addition to the Ph chromosome, they include trisomy 8, trisomy 19, monosomy 7, isochromosome 17q, + der (22) (so-called extra Ph chromosome), -Y and indicate poor prognosis.

The aim: We report a case of Ph-positive CML with multiple typical and atypical secondary aberrations.

Materials and methods: A 33-years old patient has been previously treated with hydroxyurea and was in chronic phase of CML during 10 years. Cytogenetic analysis was performed for the first time because the signs of accelerated phase were observed. We initiated 48-hour unstimulated cultures from bone marrow biopsy. Cytogenetic study included karyotyping (GTG) and FISH methods.

Results: Karyotyping showed the presence of several clones with multiple numerical and structural aberrations. All the cells were found to express Ph chromosome and an unbalanced translocation t(1;2)(p36;p21). Following the literature data, t(1;2) is not typical secondary abnormality for CML. Besides these changes, we also detected del(6)(q21), +del(8q), del(11)(q23), del(18q), +der(22) and marker chromosomes as well. The most common clone was 48, XY, t(1;2)(p36;p21), del(6)(q21), +del(8q), t(9;22)(q34;q11), del(18q), +der(22). Some cells showed loss of 11, 15, 20, 21 chromosomes and absence of +der(22), but these changes were not regular. To verify the presence of t(1;2) and to estimate the percentage of cells with an extra Ph chromosome and with an additional copy of chromosome 8 the metaphase FISH was performed. t(1;2) was examined two times. We used the painting probe for the whole chromosome 2 or the painting probe for 2p in combination with the centromere probe for chromosome 1. Both of the studies identified t(1;2)(p36;p21). The detection of Ph and extra Ph markers by dual-colour interphase FISH showed the presence of extra Ph and Ph chromosomes in 95% and 4% of cells, respectively. To score the percentage of cells with trisomy 8 the centromere probe for interphase and metaphase FISH was used. We detected an additional copy of chromosome 8 in 18% of cells. The patient was treated with combined chemotherapy and later with Glivec, but the disease progressed to the blast crisis during 3 months.

Conclusions: We observed multiple secondary cytogenetic aberrations in a patient with accelerated phase of CML. One of them – t(1;2)(p36;p21) – is not typical for any of the haematological malignancies; its prognostic significance is to be discussed. The rest are usual for accelerated phase and their presence indicate a poor prognosis.

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19. Microvascular free flaps in reconstructive surgery after extension resections in head and neck region

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The main goal of surgical treatment of head and neck tumours is to achieve macro- and microscopically radical margins and to restore or preserve high quality of life. Based on own experience Reconstructive Surgery Group in Cancer Center in Gliwice introduced free flaps techniques after major resections in head and neck region.

Since November 2001 till August 2003 over 80 patients underwent surgical procedures based on microvascular free flaps. In 60% Radial Forearm Free Flap was the technique of choice. In 25 cases were mandibular reconstruction was needed Fibula Free Flap was performed and in two cases iliac crest free flap was harvested. In case of middle face defects reconstruction Rectus Abdominis Free Flap was chosen. In 12 cases were postressective defects were large and composed Anterolateral Thigh Free Flap was harvested and insetted. Twice the complex defects after extended maxillectomy was reconstucted with Subscapular Composite Free Flaps. Overall survival rate based on periodic control and imaging diagnostics was about 90%. 40% of these patients underwent pre- or postoperative radiotherapy and it did not affect flap healing and survival. Based on authors experience the progress on the field of reconstructive surgery will depend on individually chosen free flaps and its goal is to achieve optimal functional and aesthetic outcome.

20. Novel phosphonate derivatives of uracil and thymine – ability to induction apoptosis in human peripheral blood lymphocytes *in vitro*; comparison to 5-fluorouracil

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For many years 5-fluorouracil (5-Fu) has been widely used in cancer chemotherapy, especially in the treatment of colorectal, liver, ovarian, head and neck, lung and breast carcinomas. This fluorinated analogue of the uracil can be used alone in monotherapy or in combination with other cytotoxic drugs (e.g., methotrexate, cisplatin) or with agents that are themselves not toxic but that modulate 5-Fu's antitumor activity (e.g. leucovorin, folinic acid). Unfortunately clinical application of this drug is limited by many undesirable effects, such as myelosuppresion, gastrointestinal symptoms, neurotoxicity, cardiotoxicity, hyperpigmentation of skin. Due to that the aim of many studies is to search for new less toxic and more effective compounds.

In Department of Bioinorganic Chemistry (Medical University of Lodz) prepared novel phosphonate derivatives of uracil and thymine: 5-uracilmethylphosphonic acid (5-umpa), K^+/5-umpa adduct and Na^+/5-umpa adduct. Cytotoxicity and genotoxicity of this compounds were described previously.

The aim of this study was to estimate ability of new 5-fluorouracil analogues to induct apoptosis in human lymphocytes. Lymphocytes were isolated from peripheral blood of healthy, non-smoking donors. For detection of apoptotic cells we have used three methods: terminal transferase-mediated dUTP-fluorescensin nick end-labelling assay (TUNEL); double staining with propidium iodide / Hoechst 33258; double staining ethidium bromide / acridine orange.

The results indicate that in comparison to 5-fluorouracil this novel analogues poorly support induction of apoptosis in normal human lymphocytes.

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21. Mitotic instability of human BRCA1 gene cloned in yeast

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Identification and subsequent mapping and sequencing of human *BRCA1* gene has been followed by extensive studies of causes and pathways leading to breast and ovarian cancers. Hundreds of allelic forms of *BRCA1* gene have been identified, however, little is known about the molecular mechanisms causing mutations and rearrangements, exceptionally frequent in this chromosomal region.

Mutagenesis in the germline cells are mainly caused by incorrect replication and unequal crossing-over between repetitive DNA sequences. The main class of sequences involved in the processes causing chromosomal instability in the human genome are Alu repeats. Experimental data show that Alu-mediated recombination, followed by duplication and/or deletion of large portions of the gene, could be responsible for the dysfunction of *BRCA1* transcript and its protein product.

We have studied the mitotic stability of the YAC (Yeast Artificial Chromosome) carrying human *BRCA1* gene and its flanking regions. *S. cerevisiae* a good model to study the instability of Alu sequences during mitotic divisions of human germline cells as the yeast chromatin shows some similarity to the chromatin present in the early embryo cells of higher Eucaryota: the cytosine in the CpG dinucleotides is not methylated and the chromatin is less condensed than the one present in somatic cells.

Yeast cells containing YAC carrying human *BRCA1* gene have been cultivated for 10-15 generations in rich medium. The rare-cutter restriction sites were mapped in the subclones of original YAC by indirect end-labelling, using pulse-field gel electrophoresis and hybridisation with radioactive probe recognising one end of the YAC.

Comparison of the autoradiographic pattern of hybridisation bands with the restriction map based on the unmodified sequence extracted from the GenBank shows large deletions and duplications caused probably by unequal crossing-over and incorrect replication of repetitive sequences present in *BRCA1* region.

22. Damage to DNA and its repair assessed by the „comet" assay in patients with autonomous thyroid nodules receiving 131-Iodine therapy

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The purpose of this study is to evaluate the DNA breakage and base damage with the use of comet assay in radioiodine treated patients with autonomous thyroid nodule.

In all the patients thyroid scintigram was performed using 131-I. The thyroid scan showed a single "hot nodule" with suppressed radioactive iodine uptake in remaining thyroid tissue. The dose of administered 131-I was from 14 to 16 mCi. Damage to DNA was estimated in a tissue from "hot nodule" by fine needle aspiratory biopsy and in lymphocytes (PBL). Samples were taken three times: before radioiodine treatment, 12 and 54 days after.

Preliminary results (on four patients) indicate a high diversity in the level of DNA damage between individual patients. Generally, in lymphocytes 12 days after 131-I application significant level of DNA breakage and base damage was still observed. However, after 54 days the level of DNA damage in lymphocytes was even lower than in the control. On the contrary, in "hot nodule" cells DNA damage persisted till 54th day after 131-I treatment. Differences in the type of damage between thyroid cells and lymphocytes were also observed. In lymphocytes there was more base damages while in nodule cells single strand DNA breaks prevailed.

Our preliminary results indicate that the comet assay can be a valuable tool for monitoring radioiodine treated patients. It can also allowed to estimate proper for each patient 131-I dose. Differences in the type and persistence of DNA damage in lymphocytes and thyroid nodule cells might indicate the different mechanism of DNA damage induction and/or differences in DNA damage repair mechanisms.

23. Recurrent *BRCA1/BRCA2* mutations and new aberrations in BRCA1 promoter region in breast and ovarian cancer cases from Upper Silesia in Poland

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Introduction. Germline mutations in *BRCA1* and *BRCA2* tumour suppressor genes cause a hereditary predisposition to breast and ovarian carcinomas. Mutations are distributed through the gene and most frequently lead to truncation of the protein. However, detectable mutations of in those genes have only explained less than half all familial breast and ovarian cancer cases. It has be also estimated that aberrations which affect expression, splicing or stability of transcript maybe responsible for additional 15-20%.
Aberrations within BRCA1/2 promoters, which can result in BRCA1/2 protein decrease, could be also associated with an increased risk breast and ovarian cancer.

Patients and Methods. One hundred and fifty unrelated probands with strong family history of breast and ovarian cancer, bilateral or early-onset breast cancer were chosen for analysis. We screened by direct sequencing all exons and exon/intron boundaries.
Eighty seven breast/ovarian cancer patients without mutations in BRCA1/2 genes were selected for the analysis of BRCA1 promoter region.

Results. We found two families with deletions in beta-promoter of *BRCA1* (GeneBank NoAc U37574, 2223delAAAAA) one family with mutation, 1827insCdelGGAACA (GeneBank No.Ac U37574) and four polymorphisms in *BRCA1* promoter region (GeneBank No.Ac U37574, 2642A>G, 2743T>C, 1983G>C, 1873G>C). We also found five different disease predisposing mutations within *BRCA1* gene (185delAG, 300T/G, 4153delA, 5382insC, 5528del1+IV22-6). The results confirms the presence of two strong *BRCA1* founder mutations in Polish population- 5382insC and 300T>G. The BRCA1 (5528del1+IV22-6) mutation was not reported previously and might be specific to the southern Polish population while the others were recurrent. We also detected two new sequence variants in *BRCA1* introns (IVS12-4G→T and IVS21-31C→G). Two disease predisposing mutations were detected in *BRCA2* (6174delT, 9631delC). In addition, eight new unclassified sequence variants were found within *BRCA2* exons (3431T>C, 3446A>G, 3655G>C, 4846G>T, 4988C>T, 6188A>G, 8335A>T, 8341A>T) and 6 new variants in *BRCA2* introns (IVS4+67A/C, IVS4+147G/T, IVS12+157A/G, IVS12+183A/G, IVS18+13C/A, IVS24-36C/T).

Conclusion. Identified novel aberrations in the *BRCA1* promoter suggest that mutation and polymorphisms in this region might be responsible for significant fraction of breast and ovarian cancer cases. Our results lend further support to the need for more detailed functional and epidemiological studies aimed at understanding the role of *BRCA1* and *BRCA2* promoters in the etiology of breast and cancer.

24. Correlation between gene positioning and gene expression

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We have investigated the spatial relationship of transcriptionally active or inactive genes within chromosome territories in the interphase nucleus of human fibroblasts. As a model we used paternally imprinted genes located within the 15q11-q13 region of chromosome 15 (SNRPN locus), deletion of which leads to Prader-Willi syndrome. For the simultaneous detection of genes and chromosome territories, we used *in situ* hybridisation with commercially available DNA probes to SNRPN locus, and to the q arm of human chromosome 15. A computer program was written which allows the detection of the border of chromosome territory, and the distance between the border and the centre of mass (labelling) of the gene. The analysis demonstrated no significant differences in distances from gene to chromosome territory border between active and inactive genes. Significant portion of genes were found located outside of 15q territory – however, only if the allele were transcriptionally active.

In agreement with previously obtained data, we also observed a distinct substructure in interphase chromosome territories. Strongly labelled chromosomal subdomains were surrounded by less intensely labelled areas. Using a thresholding of digital images of human fibroblast nuclei we discriminated these subchromosomal domains and evaluated the distances between their borders and SNRPN loci. The SNRPN gene loci were located preferentially at the borders of chromosomal subdomains.

These data support the idea that genes are preferentially located at the outer side of domains with condensed chromatin, but no strict correlation was found between active and inactive genes within the entire chromosomal domain. The only striking difference was the presence of ~ 7% of active genes at relatively large distances from the chromosomal territories.

25. A program for the statistical evaluation of clustering and colocalization patterns in immunogold labelling experiments

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The ultrastructural localization of various antigens in a cell using antibodies conjugated to gold particles is a powerful instrument in biological research. However, statistical or stereological tools for testing the significance of non-random location of gold particles are missing. We have therefore developed a method, which allows the detection of clustering or colocalization of gold particles using the distribution of distances between them (*Philimonenko et al., 2000, J. Struct. Biol., Vol. 132, pp. 201-210*). The program or plug-ins which are based on this method can be downloaded free of charge on our web-site (*http://nucleus.biomed.cas.cz/gold*). They allow one to evaluate statistically the observed immunogold labelling patterns for clustering or colocalization. Our program and plug-ins are also a good addition for image analysis software, which accompanies most of the modern CCD cameras for electron microscopes.

26. Oligo(3-hydroxybutyrate)-drug conjugates - a novel drug delivery system. Rapid cellular uptake and multidrug resistance overcoming by OHB-conjugated doxorubicin

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Extensive search of drug delivery systems that would improve effectiveness of therapy has been performed in recent years. Among the most promising approaches is the application of drugs conjugated to polymeric carrier molecules. It is expected that in conjugated form drugs would have improved pharmacokinetics and biodistribution, and would be released in a controlled manner. In case of cytostatics using in cancer therapy, reduced toxicity to normal cells and the ability to overcome drug resistance is also expected.

Aim: The purpose of the present study was: 1) to compare the cytotoxic effect of doxorubicin, either free or conjugated to well-defined tailor made oligo-[R,S]-3-hydroxybutyrates (OHBs); 2) to compare the kinetics of uptake and subcellular localization of both forms of this drug.

Methods: The drug cytotoxicity was tested *in vitro* on the human breast cancer cell lines, doxorubicin-sensitive (MCF7/WT) and doxorubicin-resistant (MCF7/DOX) subclones, using 3-[4,5-dimethylthiazol-2-y]2,5-diphenyltetrazolium bromide (MTT) test and clonogenic-forming assay; the kinetics of drug uptake and localization within the cells were studied by the confocal laser scanning microscopy (CLSM).

Results: The OHBs chosen as drug delivery carriers, especially those having MW lower than 1000, did not affect cell viability. Our cytotoxic studies showed that the growth of MCF7/DOX cells was not affected by treatment with 5 μg/ml of free doxorubicin (concentration used for culturing this type of cells), while OHB-conjugated doxorubicin killed approx. 30% of cells after 10 h treatment. The CLSM studies showed that OHB-conjugated doxorubicin localized in the cytoplasm of both cell lines. In contrast, free doxorubicin was localized predominantly in the nuclei of MCF7/WT, whereas in MCF7/DOX the fluorescence of the drug was barely detectable in perinuclear area. Moreover, the uptake of OHB-conjugated doxorubicin by cells was much faster than the uptake of free drug. In fact, the fluorescence within the cytoplasm was clearly visible in both cell lines as soon as 15-30 sec from the addition of the conjugate into the culture medium.

Conclusions: These results suggest that OHB-doxorubicin conjugates could overcome multidrug resistance of cancer cells. Our data show that tailor-made biodegradable and biocompatible oligomers of hydroxybutyric acid can be regarded as effective non-toxic vectors for drug delivery in a conjugated form.

27. Proliferation associated gene A (PAG) protein binds to DNA damaged by N-acetoxy-acetylaminofluorene (AAAF)

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Proteins that recognize and bind to damaged DNA participate in all repair pathways. Damage recognition is the first step of all repair system. However, several proteins that preferentially bind damaged DNA but do not participate in repair systems have been identified as well. Among such proteins are chromatin proteins HMG-1/2 and histone H1 that bind preferentially DNA damaged by cisplatinum. Non-repair proteins, which specifically recognize DNA damage induced by AAAF or UV irradiation, are poorly characterized.

Using an electrophoretic mobility shift assay two different nucleoprotein complexes specific for DNA damaged by AAAF have been detected in nuclear extracts from rat tissues. We attempted to identify proteins that formed such complexes. Proteins binding to DNA damaged by AAAF were purified from nuclear extracts of rat liver using combination of an ion-exchange chromatography and an affinity chromatography on AAAF-damaged-DNA-cellulose. Specific protein binding to DNA damaged by AAAF was then detected in such preparations using Southwestern blot analysis. Major protein that bound AAAF-damaged radioactive probe, that had molecular size of about 24 kDa, was analysed using mass-spectrometry and identified as PAG protein (proliferation associated gene A protein, termed also heme binding-protein 23 kDa, macrophage 23-kD stress protein, peroxyredoxin1). The enhanced binding of PAG protein to AAAF-damaged-DNA-cellulose (and also to cisplatinum-damaged-DNA-cellulose) was further confirmed by Western-blotting. The PAG gene is constitutively expressed in most tissues (the various cell types), but its expression is higher in organs having a higher level of proliferation. The PAG protein has an antioxidant function and protects cells against the oxidative stress, and is localized in either cytoplasm and nucleus. However, the DNA-binding form of the PAG protein has been detected exclusively in nucleus.

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28. Electronic compensator in combined brachytherapy and teletherapy of gynecological patients

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Definitive radiation therapy alone combined with teletherapy (TT) and brachytherapy (BT) has been established as an effective form of treatment in patients with advanced cervical cancers.

In Center of Oncology – Institute in Gliwice, Poland the following schedule has been used: TT – total dose of 40 – 54 Gy administered in 20 – 27 fx, 2 Gy per fraction, BT – introduced after completing 20 Gy to the entire pelvis from TT. TT is continued on non-BT days with a 4 cm wide midline block to shield critical organs.

The use of standard cuboid-in-shape shield in combination with variation in patient shape as well as tissue inhomogeneity can lead to large dose variations in the irradiated volume (risk of hot or cold spots).

Dynamic technique of teletherapy and inverse planning option enable to create the individual plan based on BT dose distribution and computed tomography data with no need to use the standard block.

The aim of the study is to present the influence of individual compensating filter, instead of standard central shield, on improvement of dose distribution within irradiated area.

29. Isocenter verification in stereotactic radiosurgery using electronic portal imaging device

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Purpose: To develop a method of isocenter verification in Stereotactic Radiosurgery (SRS) in order to in precise digital way of isocenter verification by Electronic Portal Imaging Device (EPID).

Methods and Materials: Linear accelerator Varian Clinac 2300C/D equipped with EPID and BrainLab stereotactic accessory including micro-Multileaf Collimator (mMLC) and EPID were used. Digital verification method based on Winston-Lutz test, however in digital verification method it is EPID that collects images in order to precise verification of isocenter. During digital verification method mMLC leaves are set to 'H' shaped field (two pairs of leaves in the middle of the field form small square gap). Then first two portal images are taken. Using laser positioners a small metal phantom ball is located in isocenter. To check isocenter invariability several portal images are obtained at various collimator, gantry and couch positions. After each portal field acquisition a quick visual and digital check is done to control if ball is inside square formed by mMLC. The idea of digital analysis is to subtract two portal images: one with phantom ball and second without ball in the same collimator position. Digital check is performed by independent computer program – 'WinIzo'; developed in Treatment Planning Department in MSC Institute in Gliwice, Poland. Digital analyse subtract two portal images (first without ball, second with ball, both with the same collimator position) and shows optical density symmetry distribution.

Results: Isocenter verification method thanks to EPID and WinIzo application let to obtain result quickly in easy eye and digital check form. Portal images are collected in database for further analysis.

Our takes about 30 minutes. Winston-Lutz test is more time-consuming and takes about 1h.

Conclusions: Moreover in presented method images analysis is improved. The correction of ball position can be done after each single portal aqusition and there is no need to wait till the whole test is preformed as in basic winston lutz one. Our method is faster and less expensive then Winston-Lutz test based on portal x-ray film.

This method is a part of Quality Assurance (QA) in SRS procedures.

30. Radioprotective and cancer preventive effects of the 1,4-dihydropyridine derivative in human cells

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Previously it has been reported that some of the derivatives of 1,4-dihydropyridine (1,4-DHP) series are effective antimutagens and reduce the spontaneous, alkylation- and radiation-induced mutagenesis in various test-systems in *Drosophila*, mouse and fish. Several mechanisms of their actions were studied and/or discussed, among them were antioxidant activity, modulation of DNA repair and apoptosis that can play important role in cancer prevention or therapy [Goncharova et al., 1974, 2002; Kuzhir, 1999; etc.]. The chemical structure of 1,4-DHP derivatives resembles dihydronicotinamide, so it seems probable that they can influence NADH and NAD(P)H metabolism and, in this way, the excision repair and apoptosis [Kuzhir, 1999]. We were interested, if the compounds of this series modulate the levels of DNA damage and apoptosis in control and irradiated human cells. The pilot investigation was carried out using AV-153 1,4-DHP derivative, which showed the highest antimutagen activity in animal experiments [Goncharova et al., 1980; Kuzhir, 1999]. The lymphocytes isolated from peripheral blood of healthy donors were treated with different concentrations of AV-153 dissolved in culture medium. The trypan blue exclusion test did not show any evident cytotoxicity of the compound in a wide range of concentrations (10^{-11}–10^{-5}M) after 24 h of lymphocyte incubation with AV-153. In reality, the antimutagen even decreased the background and radiation-induced cell death frequencies. The alkaline single cell gel electrophoresis (Comet assay) has shown that AV-153 reduces effectively DNA damage in the control and gamma- and X-ray irradiated cells (reduction factor up to 70 %). The analysis of DNA repair kinetics revealed that AV-153 increases the effectiveness of repair process during the first 15–60 min of incubation. The data of micronucleus test in PHA stimulated lymphocytes have demonstrated anticlastogenic effect of AV-153. We demonstrated also that at concentrations which effectively decrease DNA damage AV-153 stimulated apoptosis in human lymphocytes. We conclude that 1,4-DHP derivatives should be further studied as cancer preventive and radioprotective compounds.

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31. Intra S-phase checkpoints. Induction of double strand breaks (DSB) and premature chromosome condensation (PCC) in root meristem cells of *Vicia faba*

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The conserved system of cell-cycle checkpoints provides an efficient control mechanism to ensure that DNA synthesis and segregation of chromosomes at mitosis proceed with accuracy high enough to preserve genomic integrity. The checkpoints are signal-transduction pathways specific for either abnormal or incompletely assembled cellular structures. Each of them comprises three essential parts: (1) a way of sensing that a cell cycle event is aberrant or incomplete, (2) means by which this signal is transmitted, and finally, (3) the effectors that delay or block the cell cycle transitions until the problem is resolved. The position of arrest within the cell cycle varies depending on the phase in which the damage is sensed. Since the main role of all these checkpoints is to make a decision, whether or not the cell division cycle has to be continued, particular elements of these checkpoints deserve special attention as promising targets for pharmacological treatment of cancer.

Within interphase, the checkpoint-mediated inhibitory pathways give a cell both time and means needed for repair processes to occur before genetic alterations are rendered irreversible and heritable. In yeasts and complex multicellular Eukaryotes, the G1-DNA damage checkpoint prevents from entering the new cell cycle in response to DNA damage. The S-phase damage checkpoint slows down replication when the cell exposed to ionising radiation ”senses” breaks, lesions, or modified nucleotide composition of DNA. Another discrete S-M replication checkpoint monitors nuclear DNA structure and delays the onset of mitosis until S phase is complete. The DNA damages preserved or incurred at later stages of the cell cycle are detected by the G2 DNA damage checkpoint, which restrains the onset of mitosis until the mechanisms engaged to repair DNA restore adequate genomic description for the next generation of cells.

Studies on root meristems of *Vicia faba* indicate that a lot of agents may allow the S-phase-arrested cells to override the S-M control system. First of all - caffeine, which is known to induce premature mitosis when cells stay underreplicated. In *Vicia*, S-phase-blocked cells treated with caffeine start out aberrant mitotic divisions. The full array of aberrations encountered in *V. faba includes*: chromosomal breaks and gaps representing unreplicated regions of DNA molecules, lost and lagging chromatids and chromosomes, acentric fragments, chromosome bridges and micronuclei.

The main conclusions of this work are as follows:

- Agents, including caffeine, CDK inhibitors (2-AP), protein kinase inhibitors (sodium vanadate), and activators of protein kinase C (12-meristate-13-acetate - PMA), can override the S-M (replication) checkpoint and induce premature chromosome condensation (PCC).
- Caffeine and PMA belongs to the most effective stimulators of PCC in S-phase- arrested cells of *Vicia faba*. Following HU/caffeine treatment, some cells enter “S-PCC”. A combined treatment of other chemical agents can override the S-M checkpoint and brings about the “G2-PCC”.
- Structural aspects and regulatory mechanisms of the cell cycle are similar in yeasts, animals, and plants. The checkpoint control systems sense DNA damage or incomplete duplication of the genome, and act to delay the initiation of chromosome segregation to the daughter cells (mitosis). Signalling components, transmitters, and effectors of these pathways are probably highly conserved. These comprise Chk1 kinase and H2AX, phosphorylated specifically in response to HU treatment.

32. Calcium folinate stabilizes neurochemical injuries evoked by methotrexate

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Methotrexate is widely used for the cancer treatment as immunosuppressive drug, its adverse reactions include neurological disorders. Glutathione and glutathione-dependent enzymes play central role in cellular defence against toxic agents and glutathione homeostasis can have effects on the sensitivity of cancer cells to a wide range of drugs. The aim of the present work was to study the state of glutathione system of brain tissue under the administration of methotrexate alone and combined with its antidote calcium folinate. The following neurochemical injuries under the administration of methotrexate in a doze of 2 mg/kg/day (i.p.) to Wistar rats for 4 days were established. The significant decrease of the total glutathione and its reduced form contents in forebrain homogenates was marked. The oxidized glutathione contents did not change. It was shown, that activity of the following enzymes – glutathione reductase and peroxidase, and acetylcholinesterase did not differ from control values, while the glutathione transferase activity increased significantly. The increase of the phospholipids content and antioxidizing capacity of brain tissue was also observed, which most probably might be explained by the activation of adaptation processes of an organism. The combined injections of calcium folinate (17.5 mg/kg) and methotrexate (i.p.) to rats changed the parameters investigated considerably, resulting in the norm values. Thus, the results obtained enable us to conclude that antitoxic effect of calcium folinate can be at least partly mediated by the stabilization of glutathione homeostasis of neuronal cells.

33. Synthesis and characterization of novel hydrophobic porphyrin derivatives suitable for photodynamic therapy

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Photodynamic therapy (PDT) is increasingly applied for treatment of different types of tumours not amenable to surgery, radiotherapy or other conventional treatments. Most widely used in PDT is the first generation of hematoporphyrins, among them Photophrin II. Due to some drawbacks in clinical application of these drugs a number of studies have been undertaken that aim at finding potential novel photosensitizers.

In an effort to find such an improved PDT agent we have undertaken syntheses and physico-chemical characterization of some hydrophobic porphyrin derivatives. Their *in vitro* biological activity was evaluated on human melanoma cell line (Me45) exposed to cationic liposomes that contained novel porphyrins.

The dark cytotoxicity and photodynamic efficiency of synthesized porphyrins was determined by tetrazolium chlorimetric reduction assay (MTS) which measures mitochondrial dehydrogenase activity of surviving cells. Alkaline comet assay (single–cell gel electrophoresis) was used to evaluate DNA strand breaks and potential genotoxic effects induced by PDT treatment. Analysis was performed through measuring the tail moment with a comet imaging system. Micronucleus test, apoptosis and assessment of necrosis, expressed as DNA damage, were also done.

The results obtained suggest that some of the novel porphyrins may be promising candidates for further use in PDT experiments.

34. Heat shock factor 1 (HSF1) does not activate *hsp70i* genes but induces caspase-3 dependent apoptosis in spermatogenic cells.

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Somatic cells are protected from stress-induced damage by activation of heat shock proteins (HSPs), which posses cytoprotective and anti-apoptotic functions. Heat induced expression of *hsp* genes is mediated by the heat shock transcription factor 1 (HSF1). Testicular temperature is lower than core body temperature and its elevation causes active elimination of spermatogenic cells. It has been recently shown that expression of the mutated, constitutively active form of the HSF1 leads to degeneration of the seminiferous epithelium in transgenic male mice.

To study the mechanism of heat shock response in testes we have constructed transgenic mice in which the expression of constitutively active human HSF1 was driven by the rat spermatocyte-specific *Hst70* gene promoter. We obtained three transgenic founders (only females) and established three transgenic lines. Among transgene-positive animals only females were fertile. At autopsy, the testes of transgene-positive males were significantly smaller than those of transgene-negative mice. The transgene expression in testes, detected by RT-PCR and Western blot analysis, was observed from day 15 to 35 of postnatal development. Due to lack of spermatids which should appear at that time, evident degenerative changes in morphology of seminiferous epithelium were observed, from day 22, in transgene-positive males. However, seminiferous tubules of 18-day-old transgenic mice already contained clusters of apoptotic cells with fragmented chromatin. It seems, that appearance of active HSF1 is connected with induction of apoptotic cell death of spermatocytes. Activated caspase-3 was immunohistochemically detected in spermatocytes of 15-day-old males, immediately after appearance of mutant HSF1. Interestingly, mutant heat shock transcription factor did not activate inducible *hsp70* genes. To characterize mechanisms involved in disruption of spermatogenesis by HSF1 we employed DNA microarray technique. We compared gene expression profiles in testes of 22-day-old transgenic and wild-type males. We noticed significantly reduced expression of the 63 out of 129 genes (49%) involved in spermatogenesis, 46 out of 114 genes (40%) coding for chaperone proteins, and 92 out of 352 genes (26%) associated with cell cycle. This observation reflected, at the molecular level, the degeneration of seminiferous epithelium. Further microarray experiments are needed to elucidate the role of HSF1 at the earlier developmental stages.

35. Expression of pendrin and sodium-iodide symporter in ret-positive and ret-negative papillary thyroid carcinomas

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Altered expression of two iodine membrane transporters: NIS (sodium-iodide transporter) and PDS (pendrin) may be an important factor leading to the reduced ^{131}I accumulation in most thyroid cancers.

The aim of the study was to investigate the NIS and PDS genes expression in papillary thyroid carcinomas (PTC) by means of real-time Q-PCR and to correlate their expression with RET gene activation.

Total RNA was isolated from 64 paired normal and PTC samples using RNeasy Midi and Mini Kits (Qiagen), repurified with RNase-free DNAse I and reverse-transcribed with cDNA Cycle Kit (Invitrogene). The genes' expression was also measured in a residual material left after preparation of cytological smears from fine needle biopsy (FNAB) specimens from 24 patients with lymph node recurrence of PTC. Q-PCR was performed by means of ABI PRISM 7700 Sequence Detection System. Both NIS and PDS mRNA were amplified in multiplex reactions with GUS mRNA as control. A standard concentration curve was prepared from a single sample of hyperfunctioning goiter. In each case the results where normalized to the endogenous control.

In cancer samples both NIS and PDS were down-regulated, the expression of NIS was about 10%, of PDS – about 25% of the expression in normal tissue. In tumours NIS expression was significantly correlated with PDS, whereas such relationship was not found in normal samples. Additionally, the expression of NIS and PDS in normal thyroid cells was not predictive of the expression in tumour. In PTC metastases NIS and PDS expression was more significantly decreased than in tumour tissues and was undetectable in 8 and 6 samples for NIS and PDS, respectively. RET/PTC rearrangements were identified in 14/53 (38%) PTC samples. Statistically, we observed a difference in NIS expression in tumour tissues with RET rearrangement compared to those without this alteration.

To summarize, NIS and PDS expressions are strongly reduced in papillary thyroid carcinomas; down-regulation is more distinct in lymph node metastases. The expression of NIS and PDS in normal thyrocytes and in tumour cells is differentially regulated. Our results suggest also that NIS expression may be more distinctly reduced in RET-negative tumours.

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36. Gene expression profiling in papillary thyroid carcinoma

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The aim of the study is to evaluate expression profiles in papillary thyroid carcinomas (PTC) in order to find a set of genes separating tumours and normal tissues and to study the differences in gene expression related to the presence of RET rearrangements.

16 PTC samples and corresponding normal tissues were frozen immediately after excision. About 100-150 mg of tissue was fragmented in liquid nitrogen and homogenized. Total RNA was extracted using RNeasy Midi Kit and repurified with RNeasy Mini Kit (Qiagen), including a digestion step with RNase-free DNase I. 8 μg of RNA was taken for a ds cDNA synthesis reaction (SuperScript II), followed by the synthesis of biotin-labelled cRNA. All samples were hybridised to Human Genome U133A arrays as recommended by Affymetrix. The RET gene rearrangements were found by RT-PCR in six cases.

To analyse gene expression pattern in tumour samples the following steps were performed: 1. Preprocessing; 2. Preselection by three alternative procedures (modified Sebestyen criterion – SC, Neighbourhood Analysis – NA and Singular Value Decomposition – SVD); 3. Construction and clustering of set S (434 genes) created as a sum of three gene sets obtained by data preselection; 4. Gene selection by Recurrent Feature Replacement (choice of the best set of genes), by Singular Value Decomposition, by Single Separators and by Data Mining Tool (analysis of paired samples).

Difference in gene expression profiles between PTC and normal thyroid tissue is very clear and encompasses a significant number of 434 genes (set S). 258 transcripts were up-regulated and 176 were down-regulated in tumours. Among them there were 55 characterized by the ability to separate tumour and normal samples only by expression signal of this gene (Single Separators). By DMT analysis 49 Single Separators were found to be changed in 16/16 tumours in comparison to paired normal tissues. There were 67 increased (I) and 43 decreased (D) genes in all tumour samples. In addition, 168 genes were changed in 15/16 tumours and further 183 genes in 14/16 PTC.

Selection of sets of genes for differentiation of RET-positive and RET-negative PTC tumours was approached by RFR and SVD. RFR selected a set of 14 genes, however further analysis did not unequivocally confirm their biological significance. SVD was unable to find any trend related to the presence of RET rearrangement.

Papillary thyroid cancer, investigated in this study, shows a very strong expression profile pattern, which is confirmed also by the unsupervised method of SVD and by the existence of significant number of single separators. Our data widen significantly the list of genes characteristic for the expression profile of papillary thyroid carcinoma proposed previously by Huang et al.

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37. Differential DNA double strand break fixation dependence on poly(ADP-ribosylation) in L5178Yand CHO cells

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L5178Y (LY) sublines, LY-R and LY-S, differ in response to combined treatment with poly(ADP-ribose) polymerase inhibitor, aminobenzamide (AB) and ionising radiation: 2mM AB sensitises LY-S but not LY-R cells [Acta Radiol. Oncol. 24, 451]. The high radiation sensitivity of LY-S cells is reasonably explained by deficiency in DNA double strand break (DSB) repair [Radiat. Res., 112, 146]. Since the rejoining of DNA breaks in LY-S cells is not sensitive to DNA-PK inhibitors [Mutat. Res., 409, 31], the high radiation sensitivity is likely due to the impaired function of NHEJ.

We investigated the role of poly(ADP-ribosylation) in DSB repair in L5178Y (LY) sublines, LY-R and LY-S, and a pair of CHO lines: wild type (WT) and mutant xrs6 cells. Cells (asynchronous, logarithmic phase) were incubated with 2mM AB at 37^{o}C for 2 h, X-irradiated with 10 Gy and allowed to repair DNA breaks for 15, 60 and 120 min) at 37^{o}C or 25^{o}C. The remaining DSB were estimated by the neutral comet assay.

At 37^{o}C no effect of AB treatment on the repair kinetics was observed either in xrs6 or CHO (WT) cells. In contrast, AB inhibited the repair of DSB in LY-S line but not its parental LY-R line, in agreement with the previously observed sensitisation of LY-S cells to X-rays by poly(ADP-ribosylation) inhibition. However, DSB rejoining in the repair competent cell lines, CHO and LY-R, also was affected by AB when the post-irradiation incubation was carried out at 25^{o}C.

Analysis of these results together with some earlier data on LY-S cells allowed to interpret these results in terms of Radford's [Int.J. Radiat.Biol., 78, 1081-1093, 2002] model of radiation damage fixation. The impaired repair of DNA breaks in LY-S results in a slow repair of a sector of DSBs (presumably in transcription factories). In the repair competent cell lines, slow down repair is achieved by incubation at 25^{o}C. Then, fixation of DSB enhanced by poly(ADP-ribosylation) inhibition is revealed.

The results indicate that poly(ADP-ribosylation) can be an important modulator of the conversion of DNA damage to lethal events.

38. Automated patient positioning ExacTrack system for dynamic techniques in radiotherapy

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Nowadays, in radiotherapy various dynamic techniques are commonly used. The high precision in patient positioning during whole treatment process using these dynamic techniques is required.

In the Centre of Oncology – Gliwice automated patient positioning verification system ExacTrac is used. This system is based on markers localised on patient's skin during all pre-treatment procedures. Afterwards the same markers are read over by digital cameras set on accelerator in the course of treatment sessions.

The implementation of ExacTrack system is showed on same example of patient with prostate cancer. Treatment plan for dynamic techniques (IMRT) by means of multi – leaf micro collimator was prepared. ExacTrack system and multi – leaf collimator application in the dynamic technique enable the critical organs protection. The usage of presented patient positioning device assures quite good repeatability but is very time consuming.

39. The androgen receptor (CAG)n and (GGC)n repeats and breast and ovarian cancer risk in *BRCA1/2* carriers and non-carriers

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Introduction. About 5-10% of breast and ovarian cancers occur as a result of highly penetrant germ- line mutations in *BRCA1* or *BRCA2* tumour suppressor genes. The variation of risk between different families segregating the mutations suggests the existence of important environmental and genetic factors which can modify the cancer risk in mutations carriers and that these modifiers might be also associated with variation in risk in non- carriers.

Patients and Methods. We undertook the study of *AR* polyglutamine and polyglycine repeats length polymorphisms in 3 groups of women: 98 *BRCA1/2* mutation carriers, 55 non-carriers with the first degree family history of breast and/or ovarian cancers (*BRCAx*) and age-matched control group of 109 healthy individuals. The samples chosen for this study had been previously analysed for germ-line mutations in *BRCA1* and *BRCA2* genes. All participants were assessed for length variation in the (CAG)n and (GGC)n *AR* repeats using GeneScan analysis with fluorescently labelled primers. As in previous studies, (CAG)n repeat lengths of <22 were classified as short (S), and those of >=22 were classified as long (L). For (GGC)n repeats, those < 17 were classified as short, and those >= 17 were classified as long. The Mann-Whitney test was used to compare the lengths of alleles in cases *versus* controls.

Results. Our data show that long (GGC)n repeat (>=17 repeats) is less common between breast and ovarian cancer cases when compared to general. When the group of mutation carriers (*BRCA1/2*) was compared to healthy subjects and familial breast cancer cases (*BRCAx*), there was no observed difference in (CAG)n cumulative length, while a significant decrease in frequency of (GGC)n cumulative >=33 was revealed: 45% in the group of mutation carriers (*BRCA1/2*) vs. 67.4% in healthy subjects and 71.7% in familial breast cancer patients (*BRCAx*) (OR 0.4 and 0.32, respectively; $p<0.005$).

Conclusion. These results imply that (CAG)n and especially (GGC)n repeat length can potentially serve as a useful marker to identify a subset of individuals at higher risk of developing breast and ovarian cancer which can also modify the cancer risk in *BRCA1/2* mutation carriers.

Anex

40. Adaptor protein RukL forms protein-protein complexes with endonuclease activity in HEK293 cells (poster)

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RukL protein (regulator for ubiquitous kinase), also known as CIN85 and SETA, belongs to a subfamily of adaptor/scaffold proteins which play an integrating role in the regulation of such fundamental cellular processes and systems as survival and apoptosis, endocytosis mediated by receptor tyrosine protein kinases, and organization of the actin cytoskeleton. RukL contains three typical Src-homology 3 (SH3) domains at the N-terminus, followed by proline-rich, serine-rich regions and carboxy-terminal coiled-coil domain. Multiple modules in the RukL structure involved in protein–protein interactions can provide for formation of ligand clusters with varied properties and subcellular location. To study the nature and biological role of such complexes, the recombinant protein RukL with a Glu-epitope at the C-terminus (RukL Glu-tagged) was purified from transfected HEK293 cells by affinity chromatography on protein G-Sepharose with covalently conjugated anti-Glu-tag antibodies. By SDS polyacrylamide gel electrophoresis with subsequent staining with silver, a set of minor bands in addition to 85 kDa RukL Glu-tagged was detected in the purified preparation of the recombinant protein. Proteins with affinity for nucleic acids were also revealed in the RukL Glu-tagged preparation by retardation of electrophoretic mobility of ^{32}P-labeled oligodeoxyribonucleotides in gel. The RukL Glu-tagged preparation was also shown to hydrolyze both deoxyribonucleotides and plasmid DNA. $ZnCl_2$ and heparin inhibited the DNase activity. These findings suggest the presence of DNases associated with the RukL protein in HEK293 cells. Such complexes were isolated from lysates of HEK293 cells by chromatography on heparin-Sepharose. By elution with 0.5 and 1.0 M NaCl, two fractions with the DNase activity and containing proteins with molecular weights of 83, 80, and 72 kDa were obtained. The reaction was inhibited by $ZnCl_2$ and heparin, and previous precipitation of Ruk-related proteins with anti-Ruk antibodies resulted in the exhaustion of nuclease activity. By immunoblotting with anti-Ruk antibodies, 83 kDa protein immunologically related to the RukL protein was identified in the fractions. It was concluded that the adaptor protein RukL forms complexes with endonucleases in HEK293. cells.

Using Gaussian mixtures to infer structure in microarray data (lecture)

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The aim of the talk is to present application of the Gaussian mixture model to the analysis of DNA microarray data.

The most characterisic property of DNA microarray data on genes expressions profiles is the existence of very large number of measurements with only few related or strongly related to the result of the conducted experiment. Therefore it is necessary to develop methods to select genes that take part in the studied process. One obvious method is repeating experiments and selecting genes by using the hypothesis that expressions of genes urelated to the experiment results will take random values in the subsequent measurements, while those involved in the process will always be either over or underexpressed. However, repeating experiments many times can be very expensive. Also selecting genes by oversimplified criteria will obviously lead to loss of some information.

One of the alternative approaches, which can be used when the number of repetitions is low or even when there is only one experiment, is the method of Gaussian mixtures. Gaussian mixtures method allows approximating any probability density function with arbitrary precision, by a sum of normal pdfs. The use of Gaussian mixtures method has a twofold effect: (1) gives predictions of probabilities of results of experiments, (2) provides information about the structure of the analyzed data by estimating numbers and parameters (means, standard deviations) of normal components.

We give arguments that the Gaussian mixture method can be very useful in the analysis of DNA microarray data. The hypothesis, which can be used to select genes in experiments, is that normal components or some compositions of normal components in the observed data, correspond to the processes that take part in the studies sample. We present numerical methodology necessary to estimate normal components by maximizing likelihood function. Two main approaches to solve likelihood maximization are Expectation Maximization (EM) method and Metropolis Hastings sampling. We illustrate the method by showing the use of the Gaussian mixtures for the analysis of the expression profiles data on the Bystander effect comprising genetic changes induced in unirradiated cells by signals emitted from irradiated cells.

Expression profiling by DNA microarray in papillary thyroid cancer (lecture)

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Functional genomics offers new possibilities of looking for genes participating in malignant transformation and allows to improve cancer classification. However, only a few papers apply this technique to thyroid cancer. This seems important not only from the diagnostic point of view. The molecular mechanisms leading to different types of thyroid tumors are not completely understood. RET protooncogene activation, as a consequence of chromosomal rearrangement, is regarded at present as the most important initiating event in the development of papillary carcinomas. However, in those papillary thyroid tumors which are RET-negative the molecular mechanisms of carcinogenesis are unknown.

In the present study the expression profiling by high density oligonucleotide microarray was applied to analyze following problems:

1. Does expression profiling give a diagnostically useful set of genes for differential diagnosis between papillary thyroid carcinoma and normal/benign thyroid tissue?
2. Are there any differences in gene expression related to the presence of *RET* rearrangements which may be detected by DNA microarray analysis?

For this analysis, 23 papillary thyroid ca samples were obtained during thyroid surgery of 17 females and 6 males aged 5-71 years. 16 patients were younger than 45 years at surgery. Seven of them exhibited the presence of RET rearrangement, as judged by RT-PCR. All were euthyroid during thyroid surgery with TSH range from 0,88 to 3,06 mU/l. PTC samples and corresponding normal thyroid tissues were frozen immediately after thyroid surgery. All samples were hybridized to GeneChip U133A arrays. 16 paired tumour/ normal tissue samples were included into training set.

Different approaches were used to analyze gene expression data. Affymetrix Data Mining Tool software was used for paired analysis, an own method of unpaired analysis for the selection of non-overlapping genes was also applied. Some of the selected genes were subsequently checked by real-time Q-PCR and a good correlation was shown (correlation coefficients exceeded 0.9 for 7 of 8 analysed genes). However, none of the selected genes proved to be an ideal marker of papillary thyroid cancer at univariate analysis. Thus, a neural-network based analysis was applied. We used three different methods of gene preselection (among them two supervised methods and Singular Value Decomposition as an unsupervised method) followed by Support Vector Machine technique with a linear kernel for classification.

All the methods gave a very clear separation of gene expression profiles in tumours and normal samples, however, the sets of genes obtained showed some differences which need further analysis to choose the optimal method for diagnostic purposes. The set of 31 genes chosen by Recursive Feature Replacement was able to categorize tumour tissue properly, when the tumour contained at least 30% of cancer cells. The subdivision of analyzed PTCs into RET-positive and RET-negative tumours has not revealed a clearly distinct expression pattern.

Our results corroborate the previously postulated rather stable molecular profile of PTC and provide new clues for diagnostic purposes as well as specify new genes to be analysed for their significance in malignant transformation of thyroid cells. Simultaneously, the data obtained until now do not indicate any clear difference in expression profile dependent on the presence of RET rearrangements.

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