Gliwickie Spotkania Naukowe 2002
Gliwice Scientific Meetings 2002
November 22-23, 2002

Friday,22. November

9.00 – 9.10 ***Opening Address:***
Bogusław Maciejewski (***Head of Center of Oncology*** *Gliwice)*
Joanna Rzeszowska-Wolny *(Head of Dept. Exp. & Clin. Radiobiology, Center of Oncology, Gliwice)*
Janusz Moszyński *(vice-Mayor of Gliwice City)*

9.10-11.30 **Session I** ***New trends in cancer therapy***
Chairman: **Mieczysław Chorąży**

Beata Utracka-Hutka (Center of Oncology, Gliwice)
Chemotherapy - where are you going? Principles, new targets, challenges for the future

Jan Walewski (Center of Oncology, Warsaw)
Therapy for lymphoma: current concepts and new approaches

Jerzy Konopa (Technical University, Gdańsk)
Mechanisms of action as a basis for the development of new antitumor compounds

Maciej Siewiński (Medical Academy, Wrocław)
Inhibitorotherapy of cancer

Rafał Tarnawski (Center of Oncology, Gliwice)
How molecular biology may improve outcome of radiation oncology interactions

11.30-11.50 Coffee break

11.50-13.30 **Session II** ***Transgenic organisms in agrobiotechnology and medicine***
Chairman: **Jan Szopa**

Mieczysław Chorąży (Center of Oncology, Gliwice)
Genetically modified organisms - introduction

Marcin Łukaszewicz (Wrocław University)
Transgenic plants overexpressing biologically active compounds

Agnieszka Sirko (Institute of Biochemistry and Biophysics, PAS, Warsaw)
The potential of transgenic plants for subunit vaccine production

Jerzy Jurka (Genetic Information Research Institute, Mountain View, USA)
Transposable elements: horizontal transfer and mechanisms of integration

13.30-15.00 Lunch

15.00- 18.30 **Session III** ***Genomics in medicine***
Chairman**:** **Barbara Jarząb**

Catharina Larsson (Pharmacology Biovitrum, Stockholm)
Positional cloning of tumor suppressor genes in parathyroid tumorigenesis

Patrick Gaudray (CNRS, Nice)
Deciphering Menin's interaction networks as a tool to tackle its biological role

Helena Sztajer (Gesellschaft für Biotechnologische Forschung, Braunschweig)
Genomics and proteomics as a tools for analysis of phospholipid hydroperoxide glutathione peroxidase expression and transformation during spermatogenesis

Barbara Jarząb (Center of Oncology, Gliwice)
Expression profiling by DNA microarray in endocrine – related cancer

Włodzimierz Krzyżosiak *(Institute of Bioorganic Chemistry, PAS, Poznań)*
Translational regulation of the BRCA1 gene

Marek Figlerowicz (Institute of Bioorganic Chemistry, PAS, Poznań)
Discovery of RNAi phenomenon – turning-point in functional genomics

20.00-24.00 **Get together party**

Saturday, 23 November

9.00-14.00 **Sesion IV** ***DNA repair, radiobiology and medicine***
Chairman: **Joanna Rzeszowska-Wolny**

Marcel Blaese (University of Tübingen, Tübingen)
Role of cytoplasmic retinoic acid binding proteins CRABPI and II in regulating sensitivity of tumor cells to retinoids and ionizing radiation

Kai Rothkamm (Universität des Saarlandes (Homburg/Saar)
Cell-cycle-dependent contribution of non-homologous end joining and homologous recombination to the repair of radiation-induced DNA double-strand breaks in mammalian cells

Ingo Brammer (University of Hamburg, Hamburg)
Individual radiosensitivity: molecular mechanisms and clinical impact

Elisabeth Ortmann (University of Vienna, Vienna)
Modulation of radiation induced apoptosis by antioxidant vitamins

Antonina Cebulska-Wasilewska *(Institute of Nuclear Physics, Kraków)*
Predictive assays – comparison between various biological end-point and various doses

Rose Goncharova (Institute of Genetics and Cytology, Minsk)
Genetic efficiency of low-dose ionizing radiation in small mammals under chronic irradiation

Barbara Tudek (Institute of Biochemistry and Biophysics, PAS, Warsaw)
Long-chain adducts of fatty acids derivatives to DNA bases, mutations and repair

Krzysztof Szyfter (Institute of Human Genetics, PAN, Poznań)
Phenotypic and genotypic indications for an impaired DNA repair capacity in laryngeal cancer subjects

Ryszard Oliński (Medical University, Bydgoszcz)
Oxidative DNA damage and repair; insight from determination of 8-oxoguanine and 8-oxo-2'-deoxoguanosine in extracellular fluids

Grazyna Motykiewicz (Columbia University, New York)
DNA repair capacity in lymphoblasts from sisters discordant for breast cancer

14.00-15.00 Lunch

15.00-15.30 **Poster session**

15.30-17.10 **Session V** ***Miscellanea***
Chairman: **Maria Wideł**

Wiesława Widłak (Center of Oncology, Gliwice)
Why spermatogenic cells are sensitive to elevated temperature. The role of heat shock proteins

Dorota Rybaczek (Łódź University)
Premature mitosis (PCC) – a preserved ability of chromatin to replicate the DNA

Olgierd Unold (Technical University, Wrocław)
Towards DNA based computer – the initial state

Sergey Razin (Institute of Gene Biology, Moscow)
Chicken domain of alpha-globin genes: organization and regulation of globin genes expression

17.10-17.30 **Closing ceremony**

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LECTURES

ROLE OF CRABP I AND II IN REGULATING SENSITIVITY OF TUMOR CELLS TO RETINOIDS AND IONIZING RADIATION

Blaese Marcel A., Santo-Hoeltje Lan, Rodemann H. Peter

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Aim: Retinoids like all-trans retinoic acid (ATRA) and 13-cis retinoic acid (13c-RA) inhibit proliferation, induce differentiation and enhance radiosensitivity of many tumor cells *in vivo* and *in vitro*. The effects of retinoids are mediated by two cytoplasmic retinoic acid binding proteins (CRABPs) as well as nuclear retinoic acid receptors RARs and RXRs, which regulate the expression of several target genes.Because of its minor side-effects retinoids could be an alternative to standard tumor therapy protocols for patients with high risk of normal tissue reaction. Therefore it is necessary to identify prognostic factor(s) which enable us to predict a possible successful retinoid/irradiation therapy.

Methods: To elucidate the relation of RA- ± IR-treatment and clonogenic inactivation we measured the basic and modulated mRNA and protein level of all RA-receptors as well as cytoplasmic RA-binding proteins (CRABP I, II) by RT-PCR and Western blotting and the clonogenic survival by using colony formation assay on 7 tumor cells and two normal human fibroblasts. To prove whether CRABPI is involved in determination retinoid sensitivity and radiosensitization in tumor cells we transfected the high RA-sensitive cervix carcinoma cell line HTB35 with CRABP I by using the regulable Tet-on over-expression vector system.

Results: No correlation could be observed between clonogenic inactivation and basic or modulated RA-receptor expression in all cell lines used. Furthermore, also the RA-sensitive cell lines showed no induction of tumor-suppressorgene RARβ2 as it was described in the literature. In contrast we could demonstrate a strong correlation between clonogenic survival and the basic expression of CRABP I (RNA and protein) under RA- ± IR-treatment. Moreover, retinoid sensitive HTB35 cells could be switched to retinoid insensitive cells by transfection with CRABPI gene. These cells where insensitive against both retinoid treatment alone or in combination with irradiation.

Conclusion: The response of tumor cells to RA- ± IR-treatment seems to be directly dependent on basic CRABP I level. Therefore, CRABP I could be a possible important prognostic factor in alternative tumor therapy for clinical use especially in patients with high risk of normal tissue reaction.

INDIVIDUAL RADIOSENSITIVITY: MOLECULAR MECHANISM AND CLINICAL IMPACT

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Institute of Biophysics and Radiobiology, University Hospital Hamburg-Eppendorf, Germany

The individual radiosensitivity as measured with lymphocytes or fibroblasts show a broad variation. This variation might play a role in the individual risk of normal tissue complications after radiotherapy. The reason for the variation in the individual radiosensitivity are only partly understood. For fibroblasts, there is a clear correlation between the cellular sensitivity as measured by colony assay and the repair capacity for DNA double-strand breaks. The number of non-repaired double strand breaks is directly correlated with the number of lethal chromosomal aberrations. The differences in repair appear to be rather small; the repair capacity varied only between 95 and 98 %. The differences appear not to be regulated by different basic levels in the expression or the activity of DNA-repair genes as studied for the key proteins of the DNA-PK complex. There are also no differences in the localisation of these proteins or the induction by ionising irradiation. The repair capacity is also not affected by differences in the differentiation status of the fibroblast strains as determined by the fraction of terminally differentiated fibrocytes. The repair capacity, however, was found to be related to the extent of the acute radiation-induced permanent G1-arrest, which makes up a considerable part of the inactivation of fibroblasts. The data indicate that both processes, double-strand break repair and G1-arrest, are influenced by a common upstream signal protein, probably p53. This is presently under investigation.

PREDICTIVE ASSAY COMPARISON BETWEEN VARIOUS BIOLOGICAL END-POINTS AND VARIOUS DOSES

Cebulska-Wasilewska Antonina [1, 2]

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The challenge for molecular epidemiology and environmental studies is to improve the process for assessing risk to human health from exposure to genotoxic agents. Human biomonitoring, as a tool to identify and potentially quantify the risk of environmental exposures, has gained increasing interest especially in the area of cancer risk assessment and diseases treatment. There are several major reasons for which biomarkers may be used in epidemiological research: to improve the assessment of health risk associated to exposure; to identify subgroups of persons of different susceptibility altering the effects of the exposure or treatments; to measure early health outcome with some predictive significance; and to differentiate disease subtypes with potentially different etiologies and strategies of treatment. In order to define genotoxic effectiveness of any agent or action and to realize genetic or carcinogenic hazards, it is very important to search for correlation between evidence of the induced genotoxic damage in exposed living organisms or exposed humans with accurate measures of treatments or exposures.The desirable features of biological markers of genotoxic action are that biomarker measure should indicate a quantitative reaction to the action and should be associated with a health risk related to the genotoxic outcome. That is why, characterization of the dose response process is often done by the use of biomarkers detected in assays predictive of potential toxic outcomes and pathological changes. This creates necessity to extrapolate from high doses (experimental, occupational or accidental) to low dose region. There may be many confounding factors affecting the simple extrapolation. On the other side, an individual's genetic constitution and lifestyle, e.g., diet and levels of physical activity, can also affect the body's response to exogenous agents. In this paper are discussed influence of the shape of dose/exposure effect relationship, polymorphism and competence of DNA damage repair on the decency of the searched association between a genotoxic outcome and health risk.

Chromosome aberrations as structural or numerical chromosome changes, resulting from direct DNA breakage or from inhibition of DNA repair or synthesis, measured in peripheral blood lymphocytes have been used in occupational health surveillance programs in order to assess genotoxic risks. The concept for this biomarker assumes that the extent of genetic damage in peripheral lymphocytes reflects similar events in the precursor cells for carcinogenic processes. Many results of cytogenetic damage detected in our human monitoring studies have shown both an association with adverse health outcome on one side and the influence of confounding factors related to the life style on the other. The full potential of the molecular techniques, that have had a dramatic effect on the many insights in the clinical and research applications, lies in deriving quantitative outcomes of cytogenetic investigations. In our studies, for the purpose of retrospective biological dosimetry, in the first cellular division are applied both, unstable chromosome aberration frequencies (dicentrics, rings, fragments) and translocations (analyzed with FISH techinque), and in the second cellular division are studied sister chromatid exchanges and high frequency cells. Susceptibility to the environmental agent actions is evaluated in studies on the variation between responses to the challenging dose of UV or X-rays followed by the evaluation of the repair capacity of the DNA damage induced by a challenging dose. The induced and residual DNA damage is analyzed with the use of SCGE assay (also known as a Comet) assay. Susceptibility and repair capacities of healthy donors and cancer patients are also compared. Studies have shown a good correlation between various measures of the induced *in vivo* or *in vitro* DNA and cytogenetic damage levels. In the paper are also discussed results from studies on susceptibilities and effectiveness of the induced damage repair performed in groups of occupationally exposed and unexposed healthy donors (475 samples investigated) on one hand, and patients with diagnosed cancer on the other. The possible effects of occupational exposures, and influence of the diet and other confounding factors is shown. Prospective use of a challenging dose of radiation combined with the comet assay as a predictive assay is suggested and limitation discussed.

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GENETICALLY MODIFIED ORGANISMS - INTRODUCTION

Chorąży Mieczysław

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Modifying the genotype by introduction of a particular gene or gene sets into the genome of several animal and plant species opened new research possibilities in basic biological research, biomedicine, microbiology, agriculture, forestry and animal breeding. For dozens of years transgenic bacteria have been widely used for making bioproducts for use in medicine, agriculture, households, animal breeding, etc.

Genetically modified organisms (GMOs) have caused a worldwide debate about benefits and risks, especially with reference to plants. Large areas have been planted with genetically modified (GM) soybeans, corn, wheat, barley, rice, canola and cotton. Proponents of GM plants claim that bioengineering of plants is the only way to feed hungry people from the "Third world" and make life in less developed countries easier. GM plants are made resistant to disease and herbicides, they can resist extreme weather conditions, can grow on a high salt-containing soil, etc. Other GM plants can thrive on heavy metal-rich soil (copper, cadmium, mercury, etc.) owing to genes coding for phytochelatins – peptides that immobilize metals in nonsoluble complexes deposited in plant cells. More evident benefits include: GM plants synthetizing higher level of proteins, vitamins (A, E), monounsaturated fatty acids, fruits and vegetables suitable for long-term storage, fast growing trees with modified lignin to yield better paper, orange trees that initiate flowering and fruit production in the first year of growth, etc. The humanitarian and charitable perspectives seem a bit cynical and are shadowed anyway by intense and fruitful research, animated and supported by big corporations. These aim at developing miracle GM plants with a "technology protection system", as a way to protect their investments and future interests. These efforts resulted in development of "terminator technology" that make feasible at will a GM plant bearing sterile seeds, useless for the successive sowing. GM plants can contaminate with pollen wild species, thus posing a real danger of eliminating wild type species. Field trials with GM plants bearing Bt transgene harboring toxin and therefore resistant to insects appear to render soil sterile and less valuable for agriculture. Besides, several species of insects and larvae feed on Bt plants became resistant to Bt toxin. Use of herbicide Rundup by farmers cultivating GM beets resistant to this herbicide caused after a few years deep disturbance in the fields: some insects living on weeds disappeared and together with them birds which fed on weed's seeds. There is more convincing data indicating that genetically engineered plants pose a real risk for ecology. There is a risk for invasiveness of GM plants into native plant ecosystems, the effect upon soil micro-ecosystems, and fauna. There is a risk of developing weeds tolerant to herbicides by transfer of herbicide–tolerant traits from GM plants. Eating GM food constituting risk for human health cannot be excluded: allergic response to new types of protein (e.g. cryo protein in frost-resistant GM maize), transfer of DNA fragments stemming from virus vectors used for gene transfer into plant cells. No major objections have been imposed on experiments aiming at GM plants' use for manufacture of pharmaceuticals.

The mighty mouse produced by transfer of rat growth hormone gene opened the possibility of applying this technology to growing livestock faster and on a lesser food supply. But it appeared soon those animals (pigs, cows, sheep) breeded with this technology develop several pathologies: nephritis, pneumonia, gastrointestinal disorders, ulcers, synovitis, etc. Obviously, no single growth factor (such as the growth hormone) is sufficient to the growth of animals. Breeding of "Belgian blue" monster cows by crossing animals lacking (natural knock-out) myostatin gene prompted studies on mice with experimentally knocked-out myostain gene. The encouraging results obtained with mouse create temptation to do the same with cow. Will it work, or again, some unexpected pathology would arise? Using GM animals as bioreactors for producing biofarmaceuticals (blood clotting factors, peptide hormones, enzymes immunoglobulins, vaccines, etc.) in general does not cause serious debates. Ethically acceptable are also trials to modify histocompatibility systems and tissue antigens in animals, could-be organ donors. No obstacles are posed for using transgenic laboratory animals in basic research. Knock-out and transgenic mice contributed priceless information to elucidate mechanisms of various pathways involved in induction and pathogenesis of a great number of diseases, including cancer. Yet, in my opinion, dreams and experiments leading to transgenic goats or cows that will produce silk in their milk (owing to introduction of spider silk gene into their genome) are not acceptable. Similarly, any interest in producing more virulent species of viruses and bacteria, carrying e.g. toxins, and aimed to be used as biological weapons, is not acceptable.

DISCOVERY OF RNAi PHENOMENON – TURNING-POINT IN FUNCTIONAL GENOMICS

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The sequencing of several complete genomes and the elaboration of a DNA microarray technology are the most important achievements, which have created proper ground for the development of modern functional genomics. The latter can be defined as the studies of how the entire genome works and how the expression of the individual gene influences the activity of other genes. However, there is one additional condition which needs to be satisfied if one wishes to study how the genome is functioning: an effective and simple method of selective inducing and silencing of the expression of each individual gene must be available. Unfortunately, for a long time such a technique was not at our disposal. The recent discovery of RNA interference (RNAi) totally changed this situation, providing us with a new powerful tool for genome studies.

RNAi is a post-transcriptional process of gene silencing induced by double-stranded RNA (dsRNA) homologous to the dsDNA fragment within which the silenced gene is encoded. The foundations for the discovery of RNAi phenomenon were laid by scientists working on gene co-suppression and antisense RNA. In the first case it was observed that the introduction of an additional gene copy into the plant genome often does not lead to an increase of its expression, but on the contrary, to its inhibition. Equally puzzling results were obtained while conducting antisense RNA research. It was observed that gene expression can be inhibited by introducing into the cell an oligoribonucleotide complementary to the mRNA fragment. It was believed that the oligoribonucleotide binds to mRNA or directly to a gene and thus prevents translation or transcription processes. Contrary to scientists' expectations, the same result was brought about when RNA with a sequence identical to the mRNA fragment was applied. Interestingly, the phenomenon of gene silencing was intensified when both molecules, i.e. the complementary one and that identical to a mRNA sequence were used simultaneously as dsRNA.

The first attempts to explain the above phenomena appeared towards the end of the 1990s. Research on plant gene co-suppression and antisense RNA showed that in both cases we are dealing with the same phenomenon of RNAi. It was determined that the transcription of silenced genes takes place uninterruptedly. However, the selected transcription products are identified in some way and degraded. It was also shown that, induced in one cell, RNAi can spread over the whole organism.

The discovery of RNAi provides completely new possibilities for the research on the gene functioning, without the necessity of altering the genome structure. Thus, at any moment of organism development one can inhibit the expression of a specific gene (or several genes) even if it is important for the life of the organism under study.

Literature:

1. G.J. Hannon, RNA interference. Nature 2002, 418, 244-251
2. RNA silencing and noncoding RNA- series of articles, Science 2002, 296, 1259-1273

DECIPHERING MENIN'S INTERACTION NETWORKS AS A TOOL TO TACKLE ITS BIOLOGICAL ROLE

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Multiple Endocrine Neoplasia type 1 (MEN1, OMIM 131100) is a cancer predisposition syndrome inherited as a dominant trait. It affects a variety of endocrine tissues, in particular parathyroids, endocrine pancreas, anterior pituitary, foregut-derived neuroendocrine tissues and adrenal cortex. Other tissues are affected in MEN1 patients, although less frequently: cutaneous proliferations such as angiofibroma, collagenoma, lipoma or melanoma, and peripheral or central nervous system. The MEN1 gene, which is localized onto chromosome 11q13, was identified in 1997. It consists of 10 exons, spanning 9 kb of genomic sequence, and encoding a protein of 610 aminoacids (Menin). Menin does not reveal homologies to any other known proteins. The only motifs which have been recognized in the Menin sequence are two leucine zippers, and two nuclear localization sequences (NLS) in the carboxyterminal part of the protein. Although a mouse knock-out model is available, the function of Menin is still elusive. Beside what can be learnt from animal models, Menin's function can be anticipated neither from its protein sequence nor from its mutation profile in MEN1 patients. Whatever its function could be, Menin is supposed to play a role in defined regulation pathways leading to the control of cell growth (MEN1 is primarily an hyperplastic syndrome) and/or the maintenance of genomic integrity. It is thus expected that deciphering the interaction network(s) in which Menin is implicated will enable us to tackle its biological role. Proteins of known function have been shown to interact with Menin : JunD, NF-KappaB, Smad3, Pem, Nm23H1, GFAP, vimentin, and probably P53. Their partnership with Menin may correspond to a regulation of their activity, but their relevance to the various traits of MEN1 pathogenicity is not established. Consistent with the fact that MEN1 represents a complex syndrome with -at the same time- a strict tissue specificity and a relatively broad spectrum of both associated tumors and phenotype expressivity, the presently known protein partners of Menin seem to drive it through various cellular compartments to act in different regulation pathways. In this respect, they have provided informations that will turn out to be essential in the understanding of the pathogenicity of MEN1.

GENETIC EFFICIENCY OF LOW-DOSE IONIZING RADIATION IN SMALL MAMMALS UNDER CHRONIC IRRADIATION

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Earlier we have established the genetic effects of low dose chronic irradiation in bank vole (somatic and germ cells, embryos), in pond carp (fertilized eggs, embryos, fry) and in laboratory mice (somatic and germ cells) in the range of doses from near-background to 10 cGy. These low dose effects observed in mammals and fish are not expected from extrapolation of high dose experiments. For understanding the reasons of this discrepancy the comparative analysis of genetic efficiency of low doses chronic irradiation and higher doses acute irradiation was carried out in natural populations of bank vole which inhabited two sites differing in ground radionuclide deposition. For comparing efficiency the linear regression model of dose-effect curve was used. Dose-effect equations were obtained for animals from two chronically irradiated bank vole populations. The mean population absorbed doses were in the range of 0.04–0.68 cGy, the main part of the absorbed doses consisted of external radiation by ^{137}Cs γ-rays. Dose-effect equations for acute irradiation to ^{137}Cs γ-rays (10–100 cGy) were determined for the same populations. Comparison of genetic efficiency was made by extrapolation, sing the regression coefficient β and doubling dose estimation. For chronic exposure doubling doses calculated from low-dose experiments are 0.1–2 cGy and the doubling doses determined from high-dose experiments are in the range of 5–20 cGy. Our hypothesis that the doubling dose estimate is calculated in higher-dose ionizing radiation experiments should be much higher than the deduced from the low dose line regression equation was verified. The doubling dose estimates for somatic cells of bank vole and those for germ cells of laboratory mice are in close agreement. Radiosensitivity of bank vole chromosomes was shown to be practically the same as that for human lymphocytes since doubling dose estimates for acute irradiation close to each other. For low LET radiation higher genetic efficiency of chronic low doses in comparison with the higher doses of acute gamma-irradiation (^{137}Cs source) was proved by three methods.

EXPRESSION PROFILING BY DNA MICROARRAY IN ENDOCRINE – RELATED CANCER

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Application of powerful, high - through - put genomics technologies creates new possibilities of cancer classification and therapy prediction. Oligonucleotide based DNA microarrays and cDNA microarrays constitute two possibilities of investigating tumor expression profiles. In the paper, the capabilities of high density oligonucleotide microarrays will be analyzed for expression profiling in endocrine - related cancer with the focus on breast cancer as an example of hormone - dependent cancer and thyroid cancer as an example of hormone - producing and - dependent cancer.

At present, high density oligonucleotide microarrays, synthetised in situ by photolithography and supplied by AffymetrixR, enable the analysis of expression of 22 000 human genes on one chip and are based on short, 25 mer - oligos, with 11 pairs used per gene analyzed. A similar number of genes (25 000) has been reported to be used in breast cancer expression profiling on AgilentR - produced chips, where 60 - mer oligos synthetised by ink - jet like technology were applied.

The ability to measure the expression level of thousands of genes in one tumor sample allows its characterization and classification. The analysis of tumor expression profiles may be performed in a sample chosen by macroscopic criteria, be supported by microscopic selection of frozen sections or by laser - directed microdissection. This last approach supplies the best material for investigation, requires, however, additional amplification of RNA and is difficult for clinical applications. An important issue is then, whether the expression profile of stromal and infiltrating cells will be essential for the prognostic and predictive purposes in examined tumors. In breast cancer, the clinical predictors fail to clarify accurately breast tumors according to their metastatic potential, while a supervised classification of oligonucleotide microarrays presented recently by van't Veer (2002) and colleagues enabled to identify a metastatic signature in low risk breast cancer patients. The 'prognosis classifier' used by them consisted of 70 genes and was obtained by analysis of correlation of the expression of circa 5000 differently expressed genes with the 5 years outcome. Most of the genes included were known to participate in cell cycle regulation, invasion, metastasis and angiogenesis. However, genes expressed in non-cancer cells present in tumor contributed also to the prognosis classifier and the expression pattern of the whole set of genes, not their particular role, was crucial for the prediction of outcome.

Papillary thyroid cancer constitutes another example of endocrine - related cancer, where the prediction of the outcome based on molecular features is expected to support clinical decisions, as only 15% of those cancers exhibit poor prognosis and require radical therapeutic measures, which are today supplied to a much wider group of patients. Recent analysis of Huang et al. (2002) revealed highly consistent expression profile in papillary thyroid carcinoma, despite its clinical heterogeneity. Our own experiences are still scarce. We observed also a distinct decrease of expression of thyroid specific genes, with the highest fall in TPO expression level. However, the pattern of genes with increased expression did not parallel the changes reported by Huang et al. Our attention was focused on SSX genes, which belong to cancer testis genes. In physiologic conditions their expression is limited to testis and normal thyroid and has not been described in thyroid ca, while a clear overexpression was noted in both tumors analyzed by us until now.

Microarray-based approaches for classification of endocrine – related cancer require further intensive basic and translational research to obtain new diagnostic and predictive criteria, useful for clinical purposes.

TRANSPOSABLE ELEMENTS: HORIZONTAL TRANSFER AND MECHANISMS OF INTEGRATION

Jurka Jerzy

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Transposable elements (TEs) are specialized DNA or RNA fragments capable of surviving in intragenomic niches. They are commonly and perhaps unjustifiably referred to as "selfish" or "parasitic" elements. TEs can be divided into two major classes: retroelements and DNA transposons. The former include non-LTR retrotransposons and retrovirus-like elements, using reverse transriptase for their reproduction prior to integration into host DNA. The latter depend mostly on host DNA replication, with the possible exception of rolling-circle transposons recently discovered by our team.

TEs can be transferred horizontally between different species. This applies primarily to cut-and-paste DNA transposons and LTR-retroelements. Most evidence for horizontal transfer of TEs is based on phylogenetic analysis, but more direct evidence also exists.

To be transferred between different species, TEs need to overcome several barriers and become successfully integrated into host DNA. The barriers include: immune systems, cell membranes, elimination by recombination, and intracellular silencing by DNA methylation and RNA degradation.

TEs are active mostly in germline cells. Based on our recent work, I will show how transposition in paternal or maternal germlines can lead to different distribution of TEs for different chromosomes. This may be of significance for evolution of eukaryotic systems.

Far from being selfish parasites, TEs should perhaps be viewed as relatively harmless elements continuously probing intragenomic responses to foreign DNA/RNA and contributing to eukaryotic evolution.

MECHANISMS OF ACTION AS A BASIS FOR THE DEVELOPMENT OF NEW ANTITUMOR COMPOUNDS

Konopa Jerzy

Gdańsk University of Technology, Department of Pharmaceutical Technology and Biochemistry, Gdańsk

Two examples will be presented to illustrate in what way the studies on the mechanism of action of antitumor drugs and compounds may augment the development of new antitumor drugs.

Our earlier studies on the mode of action of mitoxantrone have demonstrated that after metabolic activation this drug induces covalent interstrand crosslinks in DNA of tumor cells. In the formation of crosslinking bonds, diaminoalkylo groups present in the side chains of mitoxantrone are responsible, while anthraquinone chromophore probably facilitates the initial docking of the drug molecule within DNA. The above observations made us put forward a hypothesis that the linkage of diaminoalkylo moiety to another polycyclic system capable of DNA intercalation might result in obtaining new antitumor compounds. This hypothesis was verified by the development of several new groups of antitumor compounds, *i.e.* imidazoacridinones, triazoloacridinones and 4-methyl-1-aminoacridinones. The derivatives of imidazoacridinone have been most extensively studied. These compounds display antitumour activity towards a number of experimental tumours, especially towards colon carcinomas, including human colon tumour xenografts in nude mice. Imidazoacridinones intercalate into DNA, induce covalent interstrand DNA crosslinking after metabolic activation, and are topoisomerase II inhibitors. The most active imidazoacridinone derivative, C-1311, is currently in the process of preparation for the Ist phase of clinical studies.

In the 60ties/70ties, in our Department, a group of 1-nitro-9-aminoacridines was developed exhibiting high cytotoxic and antitumor activity towards some experimental tumours. One of these derivatives was registered in Poland as an antitumour drug under the name of Ledakrin (Nitracrine). The broader interest in 1-nitroacridines was discouraged by their considerable toxicity. The studies on the mechanism of action of Ledakrin and analogues showed that, after metabolic activation, these derivatives induce covalent DNA crosslinks and that the nitro group in position 1 of acridine played the major role in this mechanism of action as well as antitumor activity. Modulation of the properties of 1-nitro group, especially its susceptibility to reduction, by introducing appropriate substituents into acridine ring system, resulted in the development of new derivatives with decreased toxicity, but still displaying strong activity towards colon and prostate tumours, including human tumour xenografts in nude mice. One of these derivatives, C-1748, is in the process of preparation for the Ist phase of clinical studies.

TRANSLATIONAL REGULATION OF THE BRCA1 GENE

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With the rapid progress of research on transcripts of eukaryotic genes and the entire transcriptomes it has become clear that mRNAs are regulated at many levels by a variety of trans-acting factors and cis-elements hidden in their sequences and structures. A good example is the BRCA1 mRNA, which is involved in sporadic breast and ovarian cancer mainly through reduced expression. Two BRCA1 mRNAs containing different leader sequences show different patterns of expression. In a normal mammary gland mRNA with a shorter leader sequence, 5'-UTRa is expressed only, whereas in breast cancer tissue mRNA with a longer leader, 5'-UTRb is expressed also. We show that translation efficiency of transcripts containing 5'-UTRb is 10 times lower than those containing 5'-UTRa. The structures of 5'-UTRa and 5'-UTRb were determined by chemical and enzymatic probing aided by a new method developed for monitoring the number of co-existing stable conformers. Specificfactors responsible for reduced translation of mRNA containing 5'-UTRb were determined using a variety of transcripts with mutations in the leader sequence. These factors include a stable secondary structure formed by truncated Alu element and upstream AUG codons. The novel mechanism by which BRCA1 may be involved in sporadic breast and ovarian cancer is proposed. It is based on the expression patterns of BRCA1 mRNAs and differences in their translatability. According to this mechanism the deregulation of BRCA1 transcription in cancer, resulting in a higher proportion of translationally inhibited transcripts containing 5'-UTRb, contributes to the decrease in the BRCA1 protein observed in sporadic breast and ovarian cancer.

TRANSGENIC PLANTS OVEREXPRESSING BIOLOGICALLY ACTIVE COMPOUNDS

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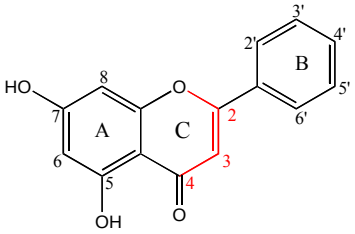
Flavonoids are a large group (about 7 000) of secondary metabolites ubiquitous in all vascular plants. New compounds are being identified in nature and chemically synthesized. The core structure of flavonoids (C6-C3-C6) is two aromatic rings (A and B) joined by 3 carbon bridge, which most often forms the third C-ring. Flavonoids are classified into various groups according to the modification (saturation, hydroxylation) of the C ring. The main structure (aglycone) very often carries one or more sugar residues. The glycosides are stored mainly in vacuoles. The multitude of structures implicates variable functions. In plants, flavonoid compounds are supposed to play an important role in protection against UV irradiation, and osmotic, oxidative or heat shock stresses. As pigments determining the colour of flowers and fruits flavonoids may be either attractants or repellents. They are also active in the plant-micro organism interactions (promotion of symbiosis and protection against pathogens).



Biological activity of flavonoids is not limited to plants. 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. Thus, 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 on human health, 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. Constructs coding regulatory gene (14-3-3 protein) and structural genes of flavonoids biosynthesis pathways, i.e. chalcone synthase (CHS), chalcone isomerase (CHI), dihydroflavanone reductase (DFR); have been introduced in sense and antisense orientation under strong, constitutive promoter from Cauliflower Mosaic Virus (CaMV35S). CHS, CHI and DFR genes have been introduced either separately, or two or all the three genes simultaneously. 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. Plants with higher amounts of flavonoids show higher resistance to pathogens attack. Potato plants with overexpression of DFR have been shown to produce mainly one anthocyanin compound.

OXIDATIVE DNA DAMAGE AND REPAIR; INSIGHT FROM DETERMINATION OF 8-OXOGUANINE (8-oxoGua) AND 8-OXO-2' DEOXOGUANOSINE (8oxodGuo) IN EXTRACELLULAR FLUIDS

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8-oxoguanine has been recognized as a biomarker of oxidative DNA damage by endogenously generated oxygen radicals. Following excision from DNA the modified base (8-oxoGua) or nucleoside (8-oxodGuo) is excreted into urine (or another extracellular fluid) where their presence has been acknowledged to be reflective of overall oxidative stress. The presence of these compounds can also mirror an involvement of different DNA repair pathways, namely base excision repair and nucleotide excision repair towards removal of these two lesions.

These aspects will be discussed during the presentation and our published results (1,2,3) as well as the recently obtained results concerning experiments with mOGG1 knock out mice will be described.

1. Gackowski D., Różalski R., Roszkowski K., Jawień A., Foksiński M., Oliński R., 8-oxo-7,8-dihyrdroguanine and 8-0xo-7,8-dihydro-2'-deoxyguanosine levels in human urine do not depend on diet. *Free Radical Res*. 35: 825—832, 2001.

2. Oliński R., Gackowski D., Foksiński M., Różalski R., Roszkowski K., Jaruga P., Oxidative DNA damage: assessment of the role in carcinogenesis, atherosclerosis and acquired immunodeficiency syndrome. *Free Rad.Biol.Med*. 33: 192- 200, 2002.

3. Rozalski R., Gackowski D., Roszkowski K., Foksinski M. and Olinski R.
The level of 8-oxoguanine – possible repair product of oxidative DNA damage - is higher in urine of cancer patients than in control subjects. *Cancer Epidemiology, Biomarkers &Prevention*. 2002 Oct;11(10):1072-5.

EFFECT OF ANTIOXIDANT VITAMINS ON RADIATION INDUCED APOPTOSIS

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Among the various forms of radiation-induced cell death, apoptosis was found to be important in the cellular radio-sensitivity of tumors and normal tissues alike. Radiation-induced reactive oxygen species (ROS) as well as ROS produced during signal transduction events play a role in the process of apoptosis.. Anti-oxidant vitamins have been used in clinical settings to suppress normal tissue toxicities due to their radical scavenger capacity. Therefore we investigated how a-tocopherol, ascorbic acid, and b-carotene influence radiation-induced apoptosis.

We studied the human lymphoblastic leukemia cell line MOLT-3 which undergoes apoptosis within 8 hours after exposure to ionizing radiation. MOLT-3 cells were incubated with 0.01mM, 1mM or 100mM of the vitamins mentioned above as well as with all combinations of vitamins. Cells were treated with the vitamins prior or after irradiation with 3 Gy. Eight hours after irradiation apoptosis was scored morphologically. Vitamin A, C, and E as well as all combinations thereof were found not to influence apoptosis in unirradiated cells when given at concentrations mentioned. When given prior to irradiation vitamins A and E showed a significant radio-protection at concentrations of 0.01mM as well as 1mM. Combinations of vitamins did not influence this behavior significantly. When given immediately after irradiation we observed no alteration of the cellular radio-sensitivity at concentrations of 0.01mM and 1mM while we observed a radio-sensitization at 100mM. Anti-oxidant vitamins apart from vitamin C show a significant radio-protective effect in the low dose range while high concentrations of vitamins tend to work as radio-sensitizers. Therefore clinical administration of anti-oxidant vitamins should consider the time and dose effect found to obtain the desired effect.

CHICKEN DOMAIN OF ALPHA-GLOBIN GENES: ORGANIZATION AND REGULATION OF GLOBIN GENES EXPRESSION.

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Chicken domain of alpha-globin genes represents a classical example of "weak" domains with poor-defined boundaries. It is located in permanently open chromatin area and overlaps a house-keeping gene expressed in cells of different lineages. Nevertheless, the globin genes are expressed only in erythroid cells. In non-erythroid cells the whole domain is repressed by mechanisms which, apparently, have nothing to do with a change in the mode of chromatin packaging. We have identified two blocks of regulatory elements that might contribute to the repression of alpha-globin genes in non-erythroid cells. The first one includes matrix attachment region, CTCF-dependent insulator and elements blocking the continuous transcription of the whole domain. The second one is a differently methylated region located upstream to the whole cluster of alpha-globin genes. These CpG-rich region is extensively methylated in non-erythroid cells and remains non-methylated in erythroid cells. In a model experiment we have demonstrated that, being methylated, this region suppress activity of promoters of alpha-globin genes. In erythroid cells, productive expression of alpha-globin genes seems to be controlled at the post-transcriptional level. We studied, by in situ hybridisation and confocal microscopy, the distribution of the transcripts of the chicken alpha A gene in the course of induced terminal differentiation of the erythroleucemic AEV cells. Furthermore, we analyzed the size distribution and overall quantity of transcripts of this gene present in nuclei and cytoplasm using Northern-blot hybridization. The results obtained suggest that globin genes are actively transcribed at both early and late stages of erythroid cell differentiation. Most surprisingly, we found that, prior to induction, the globin RNA accumulates mainly in the peri-nucleolar areas. Transport of globin mRNA from nuclei to cytoplasm seems, thus, to occur only in terminally differentiated cells, whereas in the dividing erythroleukemic cells (pre)mRNA is retained in nuclei.

CELL-CYCLE-DEPENDENT CONTRIBUTION OF NON-HOMOLOGOUS END JOINING AND HOMOLOGOUS RECOMBINATION TO THE REPAIR OF RADIATION-INDUCED DNA DOUBLE-STRAND BREAKS IN MAMMALIAN CELLS

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Two enzymatically distinct pathways, non-homologous end-joining (NHEJ) and homologous recombination (HR) contribute to the repair of DNA double-strand breaks (DSBs) in eukaryotic cells. Although most of the enzymes involved are surprisingly conserved from yeast to man, the relative importance of NHEJ and HR shows considerable variation. Yeast cells primarily rely on HR for the repair of radiation-induced DSBs, while NHEJ is believed to be the major repair pathway in mammalian cells. The objective of the present work was to assess the relative contribution of NHEJ and HR to the repair of radiation-induced DSBs in synchronized populations of Chinese hamster ovary cells. To that end, we employed an immunological approach based on microscopic detection of fluorescent foci of the phosphorylated histone□-H2AX that was recently shown to detect individual DSBs induced by ionizing radiation (Rogakou et al., JCB 146:905-916, 1999) and provides a quantitative measurement of the repair of individual breaks in single cells. We show that V3 cells compromised in NHEJ exhibit strongly impaired DSB repair after irradiation with 1 Gy in all phases of the cell cycle. irs1SF cells deficient in homologous recombination, in contrast, show no repair defect in the G_1 phase, a partial impairment in S but a substantial defect in G_2. Furthermore, the radiosensitivity of irs1SF cells is marginal in G_1 but dramatically increases in G_2, while V3 cells are highly sensitive throughout the cell cycle. This shows that NHEJ is important in all cell cycle phases while HR is restricted to late S/G2 where both pathways contribute equally to DSB repair and radioresistance. In contrast, breaks introduced by the replication inhibitor aphidicolin are repaired by homologous recombination, and irs1SF but not V3 cells show hypersensitivity to aphidicolin treatment.

PREMATURE MITOSIS (PCC) – A PRESERVED ABILITY OF CHROMATIN TO REPLICATE THE DNA

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To preserve genomic stability, nuclear DNA must be replicated once and the resulting sister chromatids partitioned equally between each of the daughter cells. Temporal ordering of these events is controlled tightly by the so-called 'cell cycle checkpoints', which are signal-transduction pathways specific for either abnormal or incompletely assembled cellular structures. The checkpoint-mediated inhibitory pathways block or slow down cell cycle progression until the abnormalities are repaired and the molecular components assembled properly, thus providing means to assure a correct and adequate genetic inheritance to each of the cells produced by mitosis. One of such responses, the S-M checkpoint delays the onset of mitosis (M) while DNA synthesis (S) is underway. Our studies on root meristems of *Vicia* indicate that, following the hydroxyurea (HU)-mediated arrest of DNA replication, molecular signals generated by caffine, 2-aminopurine, or sodium vanadate may invoke mechanisms allowing the cells to override the S-M dependency control system. The ensuing events result in a variety of aberrant mitotic divisions including chromosomal breaks and gaps, lost and lagging chromatids and chromosomes, acentric fragments, chromosome bridges and micronuclei. Electron microscopic studies indicate that, depending on the chemical agent applied to induce premature condensation of incompletely replicated chromosomes (PCC), interphase and mitotic cells reveal some characteristic ultrastructural features, comprising both chromatin and cytoplasmic structures. Long-term experiments show thay root cells displaying PCC become transformed into apoptotic cells. Furthermore, using Feulgen cytophotometry and ^{3}H-thymidine autoradiography, we have evidenced that the frequency and appearance of PCC vary significantly depending on the stage at which hydroxyurea-blocked S-phase cells were enforced by caffeine to enter mitosis. Despite the condensed state, pulverized chromosomes in HU/caffeine-treated S-phase cells stimulated to PCC still preserve the ability to incorporate ^{3}H-thymidine. However, the validity of acquired licence for DNA replication is limited merely to anaphase and telophase cells. This result suggests that resumption of S-phase activities in cells displaying symptoms of PCC is strictly dependent on the Anaphase Promoting Complex/Cyclosome (APC/C)-mediated decline in the amount of B-type cyclins and, consequently, on the degradation of protein kinases indispensable for the metaphase-anaphase transition.

INHIBITOROTHERAPY OF CANCER

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The hypothesis that cysteine endopeptidases play a fundamental role in tumor malignancy becomes well established. Activity of these enzymes can be regulated on the level of the enzyme synthesis and by inhibitors. Specific endogenous cysteine peptidase inhibitors (CPI) serve as an ultimate control mechanism for tumor cysteine endopeptidases activity *in vivo*. In the light of the hypothesis the aim of therapeutically procedures should be an attempting to reduce activity of these enzymes.

1. Correlation between tumor invasiveness, metastasis and neoplastic transformation and activity of cysteine endopeptidases particularly cathepsins B and L in "cascades of proteases and caspases enzymes.
2. The activity of cysteine proteinases, their activators (marker of tumor aggresivity) and their autogenously inhibitors - total, active and in complex forms (marker of organism defense again cancer aggressivity) are investigated in body fluids and homogenates of tissues
3. Inhibition in vivo and in vitro cysteine peptidases using inhibitors from human urine, placenta and white egg.
4. Administration of vitamin E on stimulation on macrophages, interleukin 1, lymphocytes, interleukin 6 levels and increased level of T-kininogen (cysteine peptidases' inhibitor).

THE POTENTIAL OF TRANSGENIC PLANTS FOR SUBUNIT VACCINE PRODUCTION

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Subunit vaccines are defined as those containing one ore more pure or semipurified antigens. Availability of complete sequences of many pathogens allows now for production of the recombinant antigens in heterologous production systems. The traditional systems used for the production of subunit vaccines include bacterial, yeast and mammalian cells cultures. Recent advances in plant transformation techniques made it possible to employ plant cells and plants as vaccines production systems. Using plants for these purposes is extremely attractive for a number of reasons, including economics of production, purification and delivery - especially if edible plant parts will be used for oral vaccination, possibility to extend the shelf life - if the vaccine will be stored in plant organs (e.g. seeds) and purified only when required, as well as safety for vaccinated individuals since plant pathogens generally do not infect humans and animals. The plant systems have also some disadvantages, including low expression levels and a lack of correct (animal-specific) posttranslational glycosylation. However, several strategies successfully adopted to increase protein expression level in plants have been reported. During the last decade many bacterial and viral antigens have been produced in plants. The immune responses to plant-derived antigens and protection against pathogens observed in many cases confirmed efficacy of this system for subunit vaccine production.

The aim of experiments conducted in our laboratory is to produce the selected antigens of *Helicobacter pylori* (UreB, HspB and HspA) and of equine herpes virus 1, EHV-1, (gC and gD) in transgenic plants. The respective plant expression cassettes have been prepared and their functionality confirmed in a transient *in planta* expression system. A novelty of our current approach is: (i) production of antigens as fusion proteins with mammalian cytokines that are supposed to act as immunological adjuvants and (ii) targeting the products into endoplasmic reticulum in order to increase their yields. Selection of transgenic tobacco plants with the sufficient level of antigens expression is in progress. The next planned steps are transformation of other plant species (carrot or lettuce) and verification of the efficacy of the potential vaccines in experimental models. A short review of the experimental data from various laboratories will be shown and discussed along with our results.

GENOMICS AND PROTEOMICS AS A TOOL FOR ANALYSIS OF PHOSPHOLIPID HYDROPEROXIDE GLUTATHIONE PEROXIDASE EXPRESSION AND TRANSFORMATION DURING SPERMATOGENESIS

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It was found that selenoprotein phospholipid glutathione peroxidase (PHGPx) is abundantly formed in spermatids as an enzymatically active and soluble protein and is transformed into inactive form in mature spermatozoa. As such it accounts for at least 50% of the keratin-like material that embeds the mitochondrial helix in the midpiece of speremato zoa. Preliminary clinical data reveal that the PHGPx contents of human sperm samples correlate with fertility related parameters such as morphological integrity, motility and, less evidently, with viability.

Additionally it was observed that glutathione (GSH), the normal PHGPx reducing substrate, drops in late spermatogenesis to undetectable level. The mechanism leading to the burst of PHGPx synthesis and transformation during spermatogenesis are being investigated by RNA expression analysis and proteome analysis of defined stages of spermatogenesis in rats. It is anticipated that the data will ultimately provide a key to understand and possibly intervene with male fertility problems.

PHENOTYPIC AND GENOTYPIC INDICATIONS FOR AN IMPAIRED DNA REPAIR CAPACITY IN LARYNGEAL CANCER SUBJECTS

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The presented studies are a part of long-lasted interest in biology and genetics of tobacco smoke-associated laryngel cancer.

Interindividual differences in susceptibility to pathogens and drugs have been recently extensively studied because of an association with an (i) estimation of an individual risk to develop cancer, (ii) a variable progression of cancer and (iii) a clinician's claim to individualise cancer therapy.

Concerning laryngeal cancer, a slight significance of genetic risk factor was shown in numerous studies on genes/enzymes involved in the processes of metabolic activation and detoxication of tobacco smoke carcinogens. Following the same line we have focused our attention an impact of DNA repair process.

First, DNA repair damage induced by bleomycin or S9-activated benzo(a)pyrene was determined in peripheral blood leukocytes using alkaline comet-assay. Laryngeal cancer subjects (n=52) were shown to have higher levels of spontaneous and mutagen-induced DNA damage as compared to healthy controls (n=56). A level of spontaneous DNA damage tended to increase with tumour aggressiveness. A percentage of individuals with the arbitrary chosen efficient DNA repair was higher for controls for the both used mutagens.

Then, a genotyping in the group of laryngeal cancer subjects (males, n=293) and in the matched controls (n=322) was performed for the genes coding one activating enzyme, 3 detoxifying enzyme and 3 DNA repair enzymes was performed. The latter group included the genes *XPD*, *XRCC1* and *XRCC3* representing, respectively, NER, BER and non-homologous end rejoining. All three mechanisms are involved in removal of DNA lesions induced by tobacco smoke. The following polymorphisms were studied by PCR-RFLP: transversion A-C at 35931 for *XPD*, transition C-A at 28152 for *XRCC1* and transition C-T at 18067 for *XRCC3*. There were found only two *XPD* alleles significantly over-represented in laryngeal cancer that could be interpreted as an increase of genetic risk. Besides, there was discovered an accumulation of gene defects in laryngeal cancer that substantially contributed to the genetic risk.

Recently, a group 46 subjects with the second primary tumour was included to the study. Preliminary results seem to indict for a higher frequency of defects of DNA repair genes in case of second primary tumour.

HOW MOLECULAR BIOLOGY MAY IMPROVE OUTCOME OF RADIATION ONCOLOGY I NTERACTIONS

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Recent progress in molecular biology have broadened our knowledge about DNA repair and cell cycle control. Nowadays we better understand the mechanisms of interactions between inonizing radiation and cell survival or death. Radiation therapy had been developed as an empirical skill and its effectiveness has not been changed by the advances basic biological sciences. Although the major improvement in radiotherapy outcome has been driven by technology and physics, the combination of promising new biological modifying drugs with radiation therapy has significant potential for improving anti-tumor responses over radiation treatment alone. In general these modifiers may be classified according to mechanims of their action:

I Modifiers of tumor response:
1) Proliferation
a. Growth factor receptors (EGFR. Her2Neu)
2) Radiosensitivity
a. Modification of intrinsic radiosensitivity
    i Increase apoptotic threshold
    ii. Decrease DNA repair
b. Complementary cell kill
    i. S-phase specific cytotoxins
c. Additive cell kill
3) Hypoxia
    a. Hypoxic radiosensitizers (nimorazole, etc)
    b. Oxygenators (EPO, oxygen carriers, etc)
    c. Decrease oxygen consumption hyperglycemia, etc)
    d. Hypoxia directed therapy
        i. cytotoxins, bioreductive drugs (tirapazamine, etc}
        ii. hyperthermia
        iii bacteria gene therapy vectors (clostridia, salmonella, etc)
    e. Hypoxia response modifiers (Glycolysis, VEGF, HIF, etc...)
    f. Vascular targeting drugs, inhibitors of angiogenesis (combretastatin, etc)

II Modifiers of normal tissue
1) Modifiers of survival or the number of clonogenic cells
    a. Increase protiferation (KGF, etc)
    b. Block apoptosis (pifithrin, etc)
2) Modifiers of the function-differentiation of normal cells
    a. Stem cell therapy
    b. Cytokine therapy

LONG-CHAIN ADDUCTS OF FATTY ACIDS DERIVATIVES TO DNA BASES, MUTATIONS AND REPAIR

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Oxidative stress enhances lipid peroxidation (LPO), implicated in the promotion and progression stages of carcinogenesis, particularly under conditions of chronic inflammation and infections. One of the most abundant products of LPO, *trans*-4-hydroxy-2-nonenal (HNE), was shown to be mutagenic and carcinogenic, however, its oxidised derivative, 2,3-epoxy-4-hydroxynonanal (EH), is much more potent mutagen and carcinogen. Our studies on M13 phage have revealed that all four DNA bases, A, C, G and T, are targets for HNE. DNA synthesis by T7 DNA polymerase is stopped at A, C, G and T sites in M13 DNA pretreated with HNE, albeit the reactivity of bases is different and follows the order G≥C>A≥T. Mutagenicity studies in M13 phage system showed increased mutation rates, among which mainly recombination events were observed, followed by base substitutions and frameshifts, the latter occurring in runs of A, C or G. Over 50% of base substitutions were C→T transitions, followed by G→C and A→C transversions. In *E.coli* strain *recA* deficient the frequency of recombination events decreased significantly, suggesting that recombination repair system is involved in removing HNE/EH adducts to DNA bases. Since at present, only reaction of HNE with dG was described in literature, we have undertaken studies on all four deoxynucleosides. The HPLC analysis of reaction mixtures showed formation of several products in each case, and reactivity follows the order dG>dC>dT≈dA. MS of the reaction mixtures showed peaks corresponding to HNE-dN 1:1 adducts, and in lower abundance, to 2:1 and 3:1 adducts. In dA, dC and dG reactions peaks corresponding to heptyl-substituted ε-adducts were detected, what indicates that during reaction HNE is oxidized to EH, probably by oxygen from air. The four most abundant products of the HNE-dC reaction were temporarily characterised on the basis of MS, UV and pK_a evaluation: A is N3-substituted HNE-dC (cyclic or linear), B and C are N^4-substituted HNE-dC, whereas D is dehydrated heptyl-substituted etheno-dC adduct. Thus, these long chain adducts to DNA bases arrest DNA synthesis, initiate recombination, base substitutions and frameshift mutations.

TOWARDS DNA BASED COMPUTER – THE INITIAL STATE

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This lecture presents the possibility to build DNA based computer using the available equipment of the laboratory in Medical University of Wroclaw. Our approach is based on the experiment conducted by Profesor Ehud Shapiro of the Weizmann Institute of Science [reported in the November 22, 2001 issue of *Nature*]. Shapiro has found a way to use DNA as a truly general-purpose computer, suitable for solving any kind of problem. The computation method proposed by a group of scientists headed by Shapiro uses two types of DNA molecules: software, or computation molecules, which are about 40 base pairs long and contain the instructions for the computation, and input molecules, which contain strings of six bases that represent the problem to be solved. A computation happens when these two types of molecules interact. Each has a sticky end, meaning one strand of the double helix is longer than the other, exposing a sequence of bases that are not paired. When the sticky end of an input molecule bumps into a software molecule that has a sticky end that fits, the bases of the two sticky ends join together, and an enzyme present in the solution seals them together. The molecule is then cut in a different place by a second enzyme, exposing another sticky end so that a second step can take place. The number of steps depends on the number of computations coded into the software DNA strand. Up to now a group of scientists from Wroclaw University of Technology and Medical University of Wroclaw has worked out the program which simulates the experiment performed by Shapiro in a test tube. During the simulation virtual DNA molecules are hybridized and divided like in the normal, biological experiment. Experiments with DNA-manipulating enzymes different then used by Shapiro are currently in progress.

CHEMOTHERAPY – WHERE ARE YOU GOING? PRINCIPLES, NEW TARGETS, CHALLENGES FOR 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.

THERAPY FOR LYMPHOMA: CURRENT CONCEPTS AND NEW APPROACHES

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There is a common belief that we are approaching a new era in diagnosis of lymphoma. Impressive advances in molecular techniques provided new tools for investigating multiple genetic abnormalities in distinct lymphoma subtypes and hold promise of generating data that will make possible establishing a classification based on disease pathogenesis. Current WHO classification based on combined analysis of all diagnostic data available, i.e. morphology, immunophenotype, cytogenetics, and clinical information is successful in defining disease entities that are clinically relevant.

Chemotherapy, although declared as having reached plateau of efficacy some time ago, continues to be a mainstay of treatment. Intensified regimens developed over the last decade offer cures for majority of patients with highly aggressive disease types like Burkitt's and T-cell lymphoblastic lymphoma. High-dose chemotherapy with autologous stem cell support evolved as a routine and safe treatment for recurrent aggressive lymphoma that is two-fold as effective as conventional chemotherapy in terms of survival. Allogeneic stem cell transplantation is being widely investigated with ultimate goal of reducing morbidity and mortality due to graft versus host disease and enhancing graft versus lymphoma effect.

Monoclonal antibodies are entering clinical practice as powerful new agents and they are new hope for patients with indolent lymphoma. Evolving science of cytokines, receptors and signal transduction pathways has already reached the bedside with new options of targeted therapy. Accumulating results of pharmacogenomic studies open new insights in mechanisms of treatment successes and failures.

Variety of emerging options implies that most of lymphoma patients should be considered as possible candidates for prospective clinical trials.

WHY SPERMATOGENIC CELLS ARE SENSITIVE TO ELEVATED TEMPERATURE? THE ROLE OF HEAT SHOCK PROTEINS

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Somatic cells are protected from thermal insult and other stress conditions by inducing a set of heat shock proteins (HSPs). Major function of HSPs is to maintain other proteins in their native folded state, facilitate proper folding of nascent polypeptides and repair or promote degradation of unfolded proteins, thus HSPs are called "molecular chaperones". In contrast to somatic cells, mechanisms of the response to stress conditions in germ cells are not clear at the moment. In majority of mammals testicular temperature is lower than the core body one, and its elevation disrupts spermatogenesis leading to infertility. Pachytene spermatocytes of the first meiotic division are the most thermo-sensitive cells, and mild hyperthermia will induce gross apoptosis within one day.

Spermatogenic cells specifically express two members of the mammalian multigene *hsp70* family. One of them, named *hst70* in rat and *hsp70.2* in mouse, is activated in pachytene spermatocytes during prophase of meiosis I. Second one, called *hsc70t* in mouse, is expressed in spermatids. Both genes are developmentally regulated. Both are not stress-inducible and do not protect spermatogenic cells against heat shock. The *hsp70.2* gene codes for protein involved in desynapsis of synaptonemal complexes as well as chaperoning of CDC2 kinase required for completion of the meiotic division. Homozygotic removal of the *hsp70.2* gene leads to male infertility (due to spermatocytes apoptosis), whereas females remain fertile. The function of spermatid-specific HSC70t protein remains unknown.

The expression pattern and function of the stress/heat inducible *hsp70i* (*i* – inducible) genes in the testis is more elusive. According to our observation heat inducibility of the *hsp70i* genes in the rat testes significantly decreases during postnatal development. One can postulate that inducible *hsp70* genes are not activated in spermatogenic cells and an observed expression originate from somatic cells, which are in minority in mature testes. However, it has been shown by others that HSP70i can be synthesized in spermatocytes, which is correlated with induction of apoptosis in such cells.

Hsp genes are activated during thermal stress by heat shock transcription factor 1 (HSF1). In unstressed cells of higher eukaryotes HSF1 exists as an inactive monomer, whose basal activity is negatively regulated by heat shock proteins. In response to stress, HSF monomers aggregate into homotrimers that bind avidly at conserved nGAAn repeats (HSE – heat shock element) and activate target *hsp* genes. A previous study suggested, that activation of HSF1 would be a major trigger for the induction of apoptosis in germ cells. Our preliminary data shows that in transgenic mice which constitutively express in spermatocytes active trimeric form of HSF1 (human HSF1 gene is under control of the *hst70* gene promoter) the germinal epithelium of seminiferous tubules is severely affected.

Sperm quality is directly linked to embryo quality and injury caused in spermatogenic cells by thermal stress is passed on the next generation. The mechanism that actively eliminates germ cells exposed to thermal stress might be an important factor involved in maintaining the quality of the progeny.

POSTERS

EFFECT OF TNF ON PROLIFERATIVE ACTIVITY OF HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS (HUVECs) IN NON-HODGKIN LYMPHOMA

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Angiogenesis plays a key role in tumor growth, expansion and metastasis. The critical step in angiogenesis is the proliferation of endothelial cells, which is induced and regulated by network of mitogens and cytokines such as tumor necrosis factor (TNF) a. o. TNF level is heightened in biological liquids in many pathological conditions and plays an important role in pathogenesis of malignant lymphomas. However the role of TNF in angiogenesis of non-Hodgkin lymphoma (NHL) is not clear. The aim of our study was to determine the proliferative activity of HUVECs after influence of recombinant human TNF (rhTNF, Sigma) and supernatants from primary cultures of mononuclear cells from lymph nodes (MNLN), peripheral blood (MNPB) and plasma of 10 patients with NHL.

Methods. HUVECs were obtained from human umbilical vein by digestion with 0,2% collagenase and were cultivated as described (Jaffe E.A., 1973). TNF level was detected by bioassay. TNF activity concerning HUVECs proliferation was measured by incorporation of ^{3}H-thymidine and estimated by index proliferation (IP). The NHL patients (Low-Inter Grade) were not treated before investigation. Results. Obtained data indicated that in NHL patients, plasma as well as supernatants samples of MNLN, where TNF concentrations were 0,944±0,265 ng/ml and 0,142±0,067 ng/ml respectively, have comparatively strong proliferative activity on HUVECs (IP=1,507±0,360 and IP=1,460±0,213 respectively). MNPB more slowly induced proliferation of HUVECs (IP=1,072±0,215) and the TNF level was lower in these supernatants (0,082±0,020 ng/ml); $p<0,05$. We found that low concentrations of rhTNF (from 0 – 1,550 ng/ml) inhibit the ^{3}H-thymidine incorporation in HUVECs (IP<1). In a presence of intermediate rhTNF concentrations (1,550 ng/ml up to 13,630 ng/ml) the significant growth of endothelial cells was revealed (IP>1; r=0,966). However in higher concentrations of rhTNF the cytolysis of HUVECs was observed. Conclusion. Both endogenic and rhTNF *in vitro* influence on HUVECs proliferative activity in dose-dependent manner. These events may bear some relation to angiogenesis control in malignant lymphomas.

SEMINIFEROUS EPITHELIUM DEGENERATION IN TRANSGENIC MICE THAT CONSTITUTIVELY EXPRESS TRANSCRIPTIONALLY ACTIVE HEAT SHOCK FACTOR (HSF1) IN SPERMATOCYTES

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Somatic cells exposed to elevated temperature and other non-physiological conditions respond to such a stress by a rapid induction of the heat shock proteins (HSPs), which major function is to protect cells from the harmful effects of denaturation of cellular proteins. The stress induced expression of hsps is mediated by the heat shock transcription factor 1 (HSF1). HSF1 exists in unstressed cells in a monomeric form that does not bind DNA. Stress conditions induce its conversion to trimeric DNA-binding form. Mechanisms of the stress-response in mammalian germ cells are not clear at the moment. Mammalian testes must descend from the abdominal cavity for normal development to occur and consequently the elevated testicular temperature disrupts spermatogenesis and causes infertility. The role of heat shock proteins and HSF1 in this process is not known. A previous studies suggested, that activation of HSF1 in testes would be a major trigger for the induction of apoptosis in germ cells.

The aim of this work was to study the role of HSF1 in germ cells. We constructed transgenic mice that constitutively express active trimeric form of HSF1 in spermatocytes. For this purpose, expression of the human HSF1 with deletion of the regulatory domain (amino acids 221-315) was driven by the rat *hst70* promoter. *Hst70* gene is a unique member of the multigene *hsp70* family that is specifically activated during meiosis in pachytene spermatocytes. We obtained three transgenic founders (only females) and established three lines. We confirmed the transgene expression in testes by RT-PCR.

Transgenic male mice mated with wild-type females did not sire any offspring despite normal copulatory behavior (transgene-positive females were fertile). At autopsy, the testes of transgene-positive male mice were grossly smaller than those of transgene-negative mice. Histopatological analysis of the testes of transgene-positive mice showed that the size of the seminiferous tubules was reduced. Tubules of wild-type mouse contain mitotic spermatogonia at the periphery of seminiferous tubules, spermatocytes, round spermatids and elongating spermatids being released into the tubule lumen. Seminiferous tubules from transgenic mouse contain mitotic spermatogonia and disorganized mix of pachytene, leptotene/zygotene spermatocytes as well as vacuoles and giant cells but no postmeiotic spermatids or spermatozoa. Apoptotic cells were detected *in situ* by TUNEL assay. Such analysis revealed that seminiferous tubules in cross-sections of testes from transgene-positive mice contained a cluster of apoptotic cells, whereas few apoptotic cells were detected in wild-type mice.

In somatic cells HSF1 induces expression of HSPs and is an essential survival factor in cells exposed to thermal stress. We concluded that in germ cells HSF1 has an opposite role. It inhibits spermatogenesis and induces death of germ cells by apoptosis. Apoptosis of germ cells depends on death receptors and Bcl2-family proteins. We postulate that HSF1 acts as a transcriptional regulator of genes (yet unknown) involved in this process.

INHIBITION OF 8-OXO-2'-DEOXYGUANOSINE 5'-TRIPHOSPHATE PYROPHOSPHOHYDROLASE (8-OXO-dGTPase) ACTIVITY OF HUMAN MTH1 PROTEIN BY PHYSIOLOGICAL INHIBITORS, NUCLEOSIDE 5'-DIPHOSPHATES

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Human homologue of *E. coli* MutT protein (hMTH1), an enzyme decomposing 8-oxo-2′-deoxyguanosine 5′-triphosphate (8-oxo-dGTP) to 8-oxo-2′-deoxyguanosine 5′-monophosphate (8-oxo-dGMP) and inorganic pyrophosphate, is thought to play an antimutagenic role by preventing the incorporation of promutagenic 8-oxo-dGTP into DNA. As we found in our previous studies, 8-oxo-2′-deoxyguanosine 5′-diphosphate (8-oxo-dGDP) is a strong inhibitor of 8-oxo-dGTPase activity (Białkowski, K. and Kasprzak, K.S., 1998, Nucleic Acids Res. 26, 3194-3201). In the present study we have tested the inhibitory effects of canonical ribo- and deoxyribonucleoside 5'-diphosphates (NDP's and dNDP's) on the activity of human 8-oxo-dGTPase trying to answer the question, whether the enzyme can effectively decompose 8-oxo-dGTP in the presence of physiological concentrations of NDP's and dNDP's. Among eight tested nucleoside diphosphates the strongest inhibitors of human 8-oxo-dGTPase were dGDP (*Ki* = 74 μM) > dADP (*Ki* = 147μM), and GDP (*Ki* = 502 μM). Other dNDP's and NDP's as dCDP, dTDP, ADP, CDP, and UDP appeared to be equally very week inhibitors of 8-oxo-dGTPase activity. A potential of diphosphonucleosides to inhibit 8-oxo-dGTPase activity *in vivo* is discussed. We also present a novel approach to investigating a substrate specificity of MTH1 proteins that is based on 8-oxo-dGTPase activity competitive inhibition studies.

THE EXPRESSION OF A DNA REPAIR PROTEIN – XPA IN NON–SMALL CELL LUNG CANCERS

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Lung cancer is the most common cause of cancer death in industrialized countries. The procees of cancer development is connected with damage in DNA repair pathways and accumulation of somatic mutation in genes coding for protein involved in regulation of cell proliferation, migration and apoptosis. Deficiences in DNA repair correlate with an increased sensitivity to the cytotoxic effect of particular DNA damaging agents, as well as the tendency for chromosomal abnormalities and mutations in specific oncogenes and tumor suppresor genes. Studying the proceses preventing carcinogenesis e.g. DNA repair pathways may lead to better ways of cancer prevention. There are several systems that repair damaged DNA in human cells. Nucleotide excision repair (NER) is considered to be a major DNA repair mechanisms, involved in the removal of wide spectrum of bulky DNA adducts modyfying DNA double helix conformation. The XPA protein is a component of NER and plays a central role in DNA lesion recognition. Regulation of XPA gene expression has not been extensivelly studied. We analyzed expression of XPA protein in non-small cell lung cancer (NSCLC) tissue using immunohistochemical metod. We detected high expression of XPA protein in cancer cells. This was surprising. We expected low XPA expression in cancer cells because null mutation in XPA gene cause cancer-prone human disorder *xeroderma pigmentosum*. We also compared the expression of XPA gene in primary human diploid fibroblasts (GMO7532) and lung cancer cell line A549. Using western-blot and immunochemical metods we found overexpression of XPA in A549 line and low expression in GMO7532 cells. Additionally, we found that expression of XPA is reduced in human diploid fibroblasts after contact inhibition of their growth. It is possible that overexpression XPA in lung cancer contributes somehow to the cancerous phenotype of cells. The study on the expression in an extensive set of lung cancer cell lines is currently under way.

FUNCTIONAL ANALYSIS OF SEQUENCES UPSTREAM OF THE RAT *HSP70.1* STRESS GENE

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The HSP70 family constitutes the most conserved and best studied class of HSPs. There are two inducible histocompatibility complex (MHC)-linked HSP70 genes in rat genome, which show highly differentiated expression pattern from being strictly inducible in certain tissues to constitutively active in various tumours. Little specific knowledge is available about the regulation of tissue- and cell-specific expression of *hsp70i* genes under normal and stress conditions *in vivo*. Some studies show that beside HSE-HSF there are other cis-trans interactions regulating both basal and inducible transcription. The characterization of noncoding sequences of the *hsp70i* genes has been suggested, in order to define the novel *cis* regulatory elements that could influence the expression level of these genes.

Structure of the *locus* containing *hsp70i* genes was recently extensively investigated in rat. A relatively extensive regions surrounding the *hsp70.2* and *hsp70.3* genes have been analyzed. Here we present the promoter study of the *hsp70.1* gene.

Functional studies of the promoter region were performed by transient transfection assays with the use of series of constructs containing CAT reporter gene under control of various fragments of the *hsp70.1* gene promoter. We observed that out of the three HSE sequences present in the promoter the central one is crucial for heat inducible transcription. This observation is in agreement with others' results (Konishi et al., 1995), which proved that of two proximal HSEs only the HSE2 interacts with protein factors in heat shocked rat tissues. The level of heat inducible activity can also be modulated by sequences other than heat shock element. Sequence located between –869 and –478 which does not contain any evident regulatory elements enhances substantially heat inducible CAT activity. Sequences localized further upstream in the region between position –1024 and –869 cause significant downregulation of transcription. This effect may be reversed when more upstream DNA region containing the microsatellite sequence is incorporated into the construct. In our previous experiments we observed forming of non-B-DNA structure within this microsatellite sequence *in vitro*. It remains to be established if any non-B-DNA structure forms *in vivo* and if it is responsible for described regulation of the reporter gene.

HSP70 OVEREXPRESSION DOES NOT PROTECT MITOTIC SPINDLE IN NORMAL (V79) AND TUMOR (B16) CELLS DURING AND AFTER EXPOSURE TO BENOMYL AND GRISEOFULVIN

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Enhanced expression of hsp family genes results in increased resistance of cells to cytotoxic agents like eleveted temperature or drugs. Previously we have shown that V79 cells transfected with rat hsp70 are more resistant to cytotoxicity caused by heat shock or toxins from airborne pollutants. Interestingly, incubation with extract of airborne pollutants gave the higher level of disturbed mitotoses for hsp70 transfected than for mock transfected cells.

Now we have shown that toxicity of mitotic spindle poisons like benomyl and griseofulvin is more or less similar for hsp70 overexpressing and mock transfected V79 and B16 cells as measured by survival assay. We also did not observe very relevant differences in hsp70 overexpressing *versus* normal cells as far as mitotic spindle structure is concerned (the exception is benomyl in concentration of 5 ug/ml that results in relatively less invalid mitoses in hsp70+ clones of V79 and in B16 cell lines).

Experiments with 24h recovery after basic 24h of incubation with benomyl or griseofulvin result in increased survival of V79 (hsp70+) cells when compared to mock transfected cells. Such a difference is also observed when cells are exposed to this toxins for more than 24h. There is no difference in the percentage of disturbed mitoses between hsp70+ and hsp70- cells after 24h of recovery. It means that although mitotic spindle structure is not protected in hsp70 overexpressing cells after exposure to benomyl or griseofulvin their survival is higher than cells with no hsp70 overexpression.

REGULATION OF PHOSPHATIDYLINOSITOL 3-KINASE SIGNALLING SPECIFICITY DURING THE COURSE OF DIFFERENTIATION AND APOPTOSIS OF HUMAN ERYTHROLEUKEMIA CELL LINE K562

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The diagnostic hallmark of chronic myelogenous leukemia (CML) is the Philadelphia chromosome (Ph1), which carries the fusion gene for p210 Bcr-Abl. Leukemic blasts expressing Bcr-Abl display arrested differentiation as well as resistance to apoptosis. The induction of differentiation and apoptosis should be regarded as a promising therapeutic approach for CML. We investigated the regulation of phosphatidylinositol 3-kinase (PI 3-kinase) during the course of differentiation and apoptosis of human erythroleukemia cell line K562. Signalling protein PI 3-kinase is involved in the coordinated control of proliferation, differentiation and apoptosis and is activated by Bcr-Abl in Ph1-positive cells. Our previous studies showed that the tyrosine kinase inhibitor Herbimycin A induced mainly erythroid differentiation and quercetin caused mainly apoptic events in K562 cells. We found that the induction of erythroid and megakaryocytic differentiation, as well as apoptosis in K562 cells by Herbimycin A, phorbol 12-myristate 13-acetate (PMA) and quercetin, respectively, is characterized by a dramatic decrease in PI 3-kinase activity at 3h and by an increase at 6-12 h. Subsequently, quercetin sufficiently inhibits PI 3-kinase activity. Nevertheless, no inhibition has been observed during differentiation induced by either Herbimycin A or PMA.

We have also analysed changes in protein-protein interactions mediated by the p85α regulatory subunit of PI 3-kinase in K562 cells induced by these inducers of differentiation and apoptosis. We have used the GST-conjugated recombinant forms of the full-length p85α regulatory subunit of PI 3-kinase and its SH3 domain for *in vitro* binding assay. It was determined that both the full-length p85α and its SH3 domain bind to 210, 160, 140, 120, 70, 52, 46, 38 and 27 kDa pTyr-containing proteins to varying degrees. The dynamics of protein binding was of a wave-like character. The correlation and multiple regression analysis between the dynamics of protein-protein interactions mediated by p85α and the dynamics of PI-3-kinase activity have shown that:

1.Ubiquitin ligase p120 Cbl mediates a negative regulator effect on PI-3-kinase activity during erythroid differentiation of K562 cells induced by Herbimycin A;

2. Cbl and tyrosine kinase p140 Abl mediate the negative regulatory effect on the catalytic activity of PI 3-kinase during megakaryocytic differentiation induced by PMA in K562 cells;

3.The specific interaction of adaptor/scaffold proteins and tyrosine kinases with p85α during apoptosis induced by quercetin mediates the inhibition of PI-3-kinase activity.

Ruk ADAPTOR PROTEINS – STRUCTURAL AND FUNCTIONAL FEATURES

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Ruk_I, also known as CIN85 and SETA, is a recently characterized member of a subfamily of adaptor/scaffold proteins that potentially play essential roles in signal transduction. Ruk_I 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. Ruk_I is not the only protein product of the *ruk* gene. Northern hybridisation, direct cDNA cloning and analysis of EST clones in the data banks revealed multiple transcripts of *ruk* gene. Some of these transcripts have characteristic tissue specific or developmentally regulated expression patterns. It was shown that Ruk_I/CIN85/SETA-mediated comlexes include a set of signalling proteins such as Cbl, Grb2, Crk-I, Crk-II, $p130^{Cas}$, Sos, BLNK, SB1, Src-family tyrosine kinases, endophilin, p85, regulatory subunit of Class IA PI 3-kinases. In contrast to many other adaptor proteins that activate PI 3-kinase, interaction with Ruk_I results in the substantial inhibition of the lipid kinase activity of the holoenzyme and leads to apoptic death of PI 3-kinase pathway-dependent cells. Through the formation of Cbl-CIN85-endophilin complex CIN85 may control post-membrane events such as targeting tyrosine kinase receptors for degradation and regulation of gene expression.

Using immunofluorescence microscopy, we have been shown that recombinant Ruk_I Glu-tagged protein is distributed diffusely both in the cytoplasm and the nucleus of transfected HEK293 cells, but with punctate structures in the nucleus, which correspond in all probability to nucleolus. Ruk_I localization in the nucleus was confirmed by Western-blotting of nucleic extracts prepared from transfected HEK293 cells using monoclonal anti-Glu-tag antibodies, as well as from nontransfected HEK293 and U937 cells using polyclonal anti-Ruk antibodies. The ability of the affinity purified Ruk_I Glu-tagged preparation both bind to and hydrolize DNA was revealed by different experimental approaches. The obtained data suggest that Ruk_I may form complexes with endonucleases and by this is involved in some new, yet nonrecognized functions in the nucleus of eucaryotic cells.

MEDIUM - MEDIATED BYSTANDER EFFECTS ON K562 CELLS IRRADIATED BY X-RAYS

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Introduction

It has long been accepted that the radiation-induced DNA damage in cells is the result of either direct ionization or the production of free radicals. Over the past decade it has been demonstrated that the genetic damage occurred in cells that were not directly irradiated but responded to signals transmitted from irradiated cells. This phenomenon was termed 'bystander effect'. There are at least two mechanisms proposed for the transmission of signals from irradiated cells to non-irradiated ones. One line of evidence indicates that the bystander effect is dependent on intracellular communication through gap junctions. Another suggests a mechanism in which 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 human leukemic cells.

Aim

Medium-mediated bystander effect in human leukemic K562 cells was tested. The direct as well as bystander cellular responses to X-radiation were compared.

Material and Methods

The cultures of K562 cells were exposed to X-radiation doses between 0 – 8 Gy. After one-hour incubation at 37oC the medium (ICM) was removed, filtered and transferred to non-irradiated flasks containing cells from the same line. The genetic changes in cells exposed to radiation or conditioned medium were estimated as a frequency of apoptotic and micronucleated cells incubated at different times up to 36 hours. The strand breaks were measured by single cell gel electrophoresis (comet) assay. The cytotoxicity of radiation or conditioned medium was evaluated by trypan blue exclusion technique in cells cultured for 3 days.

Results

The increase of apoptotic as well as micronucleated cells both, after X-ray or conditioned medium (ICM) exposure was observed. Only X-radiation but not ICM induced the DNA strand breaks detected by the comet assay. The percent of died cells was lower for those incubated in ICM than for cells directly exposed to radiation.

Conclusions

In the presented study we observed the medium-mediated bystander response in human leukemic K562 cells.

The DNA damage induced by the bystander effect is not the same as that directly induced by radiation.

ASSESSING GENOTOXIC PROPERTIES OF *cis*-Pt(II) 3-AMINOFLAVONE COMPLEX IN COMPARISON WITH *cis*-DDP

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Cis-diamminedichloroplatinum(II) (*cis*-DDP) is one of the most commonly and widely used chemotherapeutic drug today. Despite obvious benefits, it exhibits several deleterious side effects. This has warranted search for its novel analogs, equally potent yet less toxic. Previous demonstration of anticancer properties of flavonoid compounds prompted synthesis of *cis*-bis(3-aminoflavone)dichloroplatinum(II). The *cis*-DDP analog is less toxic and has proved its anticancer properties in *in vivo* studies. To assess genotoxic properties of this compound a comet assay was performed using human peripheral blood lymphocytes. Analysis of DNA damage was done following 1h incubation of cells with *cis*-Pt(II) 3-aminoflavone complex in comparison with *cis*-DDP. Kinetics of DNA repair after 0.5h 1h and 1.5h incubation also were investigated. It is shown that *cis*-Pt(II) 3-aminoflavone complex causes increase of tail moment value compared to *cis*-DDP. Besides cross-links, DNA breaks may be induced by the investigated compound. Lower values seen, on the other hand, for *cis*-DDP seem due to the presence of DNA-DNA and DNA-protein cross-links. A similar relationship was observed for DNA repair processes. A distinct decrease in tail moment was observed for *cis*-Pt(II) 3-aminoflavone complexas soon as after 0.5h.

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DEGRADATION OF MODIFIED DEOXYNUCLEOSIDE-5'-TRIPHOSPHATES BY HUMAN TISSUE HOMOGENATES

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Bases in deoxynucleotide-5'-triphosphate (dNTP) pool can be damaged by mutagens as bases in DNA. The level of bases damage in dNTP pool is much higher than in the DNA. Modified dNTPs can be mutagenic, because they can be incorporated into DNA by DNA polymerases. The *E. coli* MutT protein is an enzyme which sanitise dNTP pool of 8OH-dGTP by its hydrolysis to 8OH-dGMP and inorganic pyrophosphate. The human homologue of MutT, hMTH1 protein, hydrolyses also oxidised forms of dATP, 2OH- and 8OH-dATP. An important role in dephosphorylation of damaged dNTPs can also play other enzymatic activities, like phosphatases and nucleotidases, and final product of dephosphorylation, the modified dN can be exerted from cells.

We studied degradation of dNTPs formed during oxidative stress, 8-OH-dGTP and etheno derivatives - εdCTP and εdATP, by homogenates from human tissues (lung tumour and healthy surrounding). The HPLC profiles of degradation of the examined dNTPs showed the sequential formation of dNDPs, dNMPs and dNs, what suggests that various enzymatic activities are engaged in this process. We found that 8OH-dGTP and εdCTP are dephosphorylated much more efficiently than their unmodified equivalents, whereas εdATP is dephosphorylated nearly as efficiently as dATP. This suggests that degradation of at least some damaged dNTPs could involve enzymatic activities more specific for the damage than these which are involved in degradation of unmodified dNTPs. In all tested patients, the rate of dephosphorylation 8OH-dGTP and εdCTP by tumour tissue homogenates was always higher than by healthy tissue ones, whereas the rate of dephosphorylation of unmodified dGTP by both types of homogenates was similar. This indicates higher level of sanitising activities in tumor than in healthy tissues.

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EXPRESSION OF HSP25 IN THE RAT LIVER AFTER TREATMENT WITH HEPATOTOXICANTS INDUCING INFLAMMATORY REACTION

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The present study was undertaken to examine the induction of HSP25 in inflammatory processes in the rat liver. For this purpose animals were treated with single intraperitoneal injections of one of the indicated hepatoxicants: tioacteamide (TAA), D-galactosamine or allyl alcohol, which cause an inflammatory reaction around the central veins, diffusely distributed throughout the entire liver lobule or localized in periportal areas, respectively. These toxicants were administered for different time lapse ranging from 6 – 96 hrs. In control animals the immunoreaction for HSP25 was never observed. Expression of HSP25 appeared only after 36 hrs after TAA administration when the centrilobular inflammatory reaction was highly pronounced. The immunostaining was localized in hepatocytes directly adjoining the inflammatory cells. Appearance of the HSP25 protein initiated the gradual regression of inflammatory reaction. After the D-galactosamine treatment the inflammatory There is an abundant literature concerning the role of high molecular weight HSPs in inflammation. However less is known about involvement of small heat shock proteins in this process. Induced expression of HSP25 was described in inflammatory cells as well as in epithelium accompanied by infiltrating inflammatory cells (in epithelial cells in pancreatitis, in epithelium of periapical lesions of endodontic origin and in bronchial epithelium).

Cells were dispersed in the lobule and HSP25 was present in many hepatocytes in centrilobular and intermediate regions. Intoxication with allyl alcohol induced expression of HSP25 in hepatocytes surrounding the areas of necrosis and inflammation in the periportal zone. Our results suggest that HSP25 was induced by inflammatory cells, but the mechanism of this induction needs further elucidation.

THE INFLUENCE OF IDA IN COMBINATION WITH GLUTARALDEHYDE ON THE HUMAN ERYTHROCYTES

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Idarubicin (IDA, 4-demethoxydaunorubicin) is a cytostatic drug, highly effective in the treatment of various types of malignancies, especially in leukemias. It is well known that cancer chemotherapy remains unfortunately still largely nonspecific, and drugs, including IDA, are toxic for tumour cells as well as for normal cells.

We have been interested in using erythrocytes as a biocompatible and biodegradable drug carriers, to diminish the toxic effect and improve therapeutic index of IDA. In our study glutaraldehyde was used as the agent linking covalently the drug to the proteins of erythrocyte membrane.

Nowadays little is known about the interactions of IDA and glutaraldehyde with the human erythrocytes.

Thus, in the present work we have compared the combined effects of IDA and glutaraldehyde on the properties of erythrocytes (GSH+GSSG and GSH concentration, conformational changes of haemoglobin). It has been observed that human erythrocytes treated with glutaraldehyde were able to take up the increased amount of IDA from the incubation medium.

The influence of IDA and glutaraldehyde on the structure of heamoglobin was examined by Electron Spin Resonance Spectroscopy. The data imply that the glutaraldehyde (in concentrations 0,0005%, 0,0025%, 0,005%) alone has stronger influence that the glutaraldehyde and IDA.

The concentration of GSH+GSSG and GSH was determined by Akerboom and Sies method (1981). A decrease of both GSSG+GSH and GSH ratio was observed in the presence of all tested concentrations of glutaraldehyde alone (0,0005%, 0,0025%, 0,005%) as well as in combination glutaraldehyde with drug.

PCC IN A PLANT CELL – THE WAY TO APOPTOSIS

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Cell-cycle checkpoints provide an efficient control mechanism to ensure that DNA synthesis and segregation of chromosomes between two daughter cells at mitosis proceed with accuracy high enough to preserve genomic integrity. A number of inhibitory pathways within G1, S, G2 and M phases give a cell both time and means needed for repair processes to occur before genetic alterations are rendered irreversible and heritable. Experiments on root meristems of *Vicia faba* indicate that, when exposed to caffeine (a putative inhibitor of Rad3-like protein kinase), 2-aminopurine (2-AP; CDK inhibitor), or sodium vanadate ($NaVO_3$; protein phosphatase inhibitor), the S phase-blocked cells may override the S-M control system and induce premature condensation of incompletely replicated chromosomes (PCC). A variety of aberrant mitotic divisions includes chromosomal breaks and gaps, lost and lagging chromatids and chromosomes, acentric fragments, chromosome bridges and micronuclei. Furthermore, electron microscopic studies demonstrate that, depending on the chemical agent, ultrastructure of interphase and mitotic cells reveals some specific features, comprising both chromatin condensation and the appearance of cytoplasmic structures. The long-term experiments show that root cells displaying PCC become transferred into apoptotic cells.

UNDERSTANDING THE IMAGES OF COMETS

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Single cell gel electrophoresis is a rapid and sensitive microscopic method for detection of DNA damage on the individual cell level. This assay is relatively inexpensive. The images of comets we can get fast, but the interpretation might be difficult. The comets can be evaluated quickly by visual scoring but for accurate measurement is necessary to use computerised image analysis. To use naked eye for comparison of images of comets with different tail moments and/or tail inertias is not possible because the human eye cannot add different intensity levels of comet image area.

Among various parameters calculated by computerised image analysis the tail moment has been regarded as one of the best and most popular indices of induced DNA damage. But the tail inertia provides more precise information about the distribution of individual DNA fragments within a tail. The tail inertia was also found to be the most sensitive indicator of DNA damage in comparison with other tail parameters. The advantage of tail inertia over tail moment for estimation of DNA damage induced by radiation and H_2O_2 will be presented.
Key words: *comet assay, quantitative image analysis*

CASPASE 3 INDUCTION AND SOME APOPTOTIC GENES MODULATION IN HUMAN ACUTE PROMYELOTIC LEUKEMIA CELL LINE HL-60 BY CARBOPLATIN IN COMBINATION WITH AMIFOSTINE

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The influence of carboplatin and carboplatin in combination with amifostine on the growth, caspase 3 activity and some apoptotic genes expression was investigated *in vitro* in human acute promyelocytic leukemia cell line HL-60. The exposure of HL-60 cells to amifostine (10^{-6} to 10^{-3} M) showed no or very little influence on number of HL-60 cells in culture. Proliferation of HL-60 cells exposure to carboplatin dropped down with increasing dose of the drug. This effect was slightly higher when carboplatin in combination with amifostine was used. The values of cytotoxic indexes (IC_{50}) was estimated as $6.6x10^{-4}$ and $4.4x10^{-4}$ M (24 hours incubation) and $3.3x10^{-5}$ and $2.5x10^{-5}$ M (48 hours incubation) for carboplatin and carboplatin with amifostine, respectively. This effect was accompanied by caspase 3 activity induction, which seems to play a key role in the apoptotic process. HL-60 cells treated with carboplatin alone showed caspase 3 activity increase about 120 times. Combination of carboplatin with amifostine intensify caspase 3 activity to 280 times. Furthermore, the expression of selected genes, which are involved in apoptosis like *bcl-2*, *c-myc, bax*, and *p65* gene, which function in this process is unknown, were determined. Semi quantitative RT-PCR technique showed decrease of *bcl-2* gene expression and increase expression of *bax, c-myc* and *p65* genes in HL-60 cells treated with carboplatin in combination with amifostine in comparison to the cells treated only with carboplatin.

On the basis of our study we conclude that amifostine may increase corboplatin therapeutic efficiency regarding to human acute promyelocytic leukemia cells.

ALTERATION OF MEMBRANE PHYSICAL STATE ACTS AS "MOLECULAR THERMOMETER" AND MODULATE THE EXPRESSION OF HEAD SHOCK PROTEINS

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The classical model on the sensing of heat shock proposes that accumulation of denatured proteins triggers the activation of the stress-response. Our alternative, but not exclusive view is that the temperature-sensing mechanism is associated with the physical state of membranes (Horvath et al, 1998; Vigh et al. 1998;Vigh and Maresca, 2002;). In the present study we made the first attempt to extend the validity of this concept to mammalian cells.
We have treated mammalian cells (K562) with benzyl alcohol and heptanol at concentrations that at normal growth temperature induce the expression of heat shock proteins, including the synthesis of the major stress proteins such as HSP70. The critical concentrations of each of the two membrane fluidizers were selected so that their addition to cells caused an identical increase in the level of plasma membrane fluidity. Formation of isofluid states resulted in almost identical downshifts in the temperature thresholds of the HSR of the treated cells. As in the case of heat stress, the initial fluidity up-shifts induced by the membrane perturbants was accompained by rise of intracellular Ca^{++} and was followed by a fluidity relaxation period. It is noted that the above treatments resulted in no measurable changes in the lipid class and/or fatty acid composition. By monitoring the enzymatic activity of luciferase expressed in mammalian cells we have demonstrated that not like the heat stress, benzyl alcohol and heptanol do not cause protein denaturation at concentrations that do induce the heat shock response.
Our results suggest, that membranes seem to act as cellular thermometer where thermal stress is sensed and transduced into a cellular signal leading to the activation of heat shock genes.

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MICROSATELLITE INSTABILITY AND DNA REPAIR IN COLON CANCER

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The main causes of colorectal carcinoma (CRC) include: (i) inflammatory processes and high fat diet resulting in the increase of DNA damage, among others - lipid peroxidation (LPO) derived etheno-DNA adducts formation; (ii) deficiency in mismatch repair (MMR) resulting in the increased genomic instability.

We have compared microsatellite instability (in 5 microsatellites: Bat26, Bat40, D2, D5 and D17), expression of MMR proteins (MLH1, MSH2, MSH6) as well as the activity of DNA glycosylases specific for 1,N^6-ethenoadenine (εA) and 3,N^4-ethenocytosine (εC) in tumour and normal colon epithelium obtained during surgical intervention on 50 sporadic CRC patients in Norway.

Out of 50 analyzed CRC cases, in 6 patients disturbances of genomic stability and/or MMR deficiency was observed. Four of them revealed MSI in tumour tissue: two in all five microsatellites, one in BAT26, BAT 40 and D5, and one in BAT26, BAT40 and D2. Disturbances in MMR proteins expression were noted in tumour tissue of only 3 out of 50 patients, and they involved exclusively deficiency of MLH1 protein expression as measured by antibody staining. Interestingly, only in one case (patient no 4) microsatellite instability coincided with the dysfunction of MLH1 protein. In one case with MSI, MMR deficiency has not been measured yet. In the other four cases there was no correlation between microsatellite instability and expression of MMR proteins, suggesting that other factors than MMR deficiency can also contribute to genomic instability in colon cancer.

DNA glycosylases were measured only in 2 out of 6 patients, who revealed either MSI or MMR disturbances. For one of them (patients No 4), neither εA- nor εC-glycosylase activity was detectable in normal and tumour colon epithelium. This patient had also defective MMR, thus high level of DNA damage could be expected. For the second one, both εA- and εC-glycosylases activities had significantly higher values in normal colon and in tumour than in other patients, and moreover the εA-glycosylase activity increased tremendously in the tumour to the value 41.8 εA pmoles/h/mg protein, which was at least 3-fold higher value than in any other tissue measured in this study. Further investigations are now in progress to evaluate the role of etheno-DNA adducts and DNA-glycosylases activities for the genomic stability in colon cancer.

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FASTER DNA REPAIR IN LYMPHOCYTES IRRADIATED IN VITRO CORRELATES WITH A LATER MAXIMAL ACUTE REACTION DURING RADIOTHERAPY

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It is commonly known that individuals show marked differences in radiation sensitivity. To study the relation between the sensitivity of healthy tissues and the side effects occuring during radiotherapy, we conducted a population study of DNA damage and repair in lymphocytes from 82 healthy donors and patients with head and neck cancers before radiotherapy. DNA damage and the kinetics of repair were determined by comet assays in isolated lymphocytes irradiated in vitro with 2 Gy. The DNA repair curves for every donor were computer fitted to an exponential equation and the para-meters reflecting induced DNA damage (a), rate of DNA repair (τ) and residual DNA damage (c) were calculated. The patient group showed a significantly higher level of background DNA damage in lymphocytes before irradiation than the healthy donors, while the parameters of repair were greatly scattered in both groups. In the patient group we compared the response of lymphocytes irradiated in vitro with the patient's acute reaction to radiotherapy estimated with the method described by Dische. We did not find a correlation of the level of the maximal acute reaction appearing during radiotherapy with any of the parameters describing the kinetics of DNA repair in lymphocytes. However, a significant difference ($p=0,029$) was observed in the time (or cumulative dose) after which the maximal acute reaction was seen between patients characterized by high and low value of the parameter τ describing the rate of DNA repair.
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BRCA1 AND BRCA2 GERMLINE MUTATIONS IN SPORADIC BREAST AND OVARIAN CANCER CASES FROM UPPER SILESIA

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Mutations in BRCA1 and BRCA2 genes are responsible for familial breast and ovarian cancer syndrome and they were found in the families with strong history of cancer cases. The aim of our study was to estimate the frequency of germ-line mutation in sporadic cases of breast and ovarian cancer. Previous studies demonstrated that screening for local founder mutations might identify the majority of BRCA1 and BRCA2 carriers in Polish population. We used allele specific oligonucleotide PCR-based assays (ASA-PCR) for pre-screening of 49 sporadic breast and ovarian cancer cases. Four different disease predisposing mutations in BRCA1 gene (185delAG, 300T/G, 4153delA, 5382insC) and two mutations in BRCA2 gene (6174delT, 9631delC) were chosen for analysis.

In total, germ-line mutations were found in five (10,2%) of 49 patients without family histories of breast and/or ovarian cancers and 5 mutation carriers of mutation were identified among their family members. Four probands with detected mutation had ovarian cancer and one breast cancer. Germline mutations were found only in BRCA1 gene (185delAG, 300T/G, 4153delA, 5382insC). There were cancer cases in these families (gastric cancer, leukemia, head and neck cancer, prostate cancer, lung cancer, colorectal cancer and breast cancer) but the families did not meet the criteria for HB(O)C. Three probands inherited mutation via male line so the observed phenotype were not typical and in one of the families only men inherited the mutation.

This percentage of mutations in sporadic cases is significant in comparison with the total of 10% among probands with the strong family history of breast or/and ovarian cancer, 27% in bilateral breast or/and ovarian cancer cases and 18% in early onset breast and ovarian cancer cases which are observed in Silesian population.

We conclude that a significant fraction of sporadic breast/ovarian cancer patients carry inherited founder BRCA1 and BRCA2 mutations. These findings indicate that not only patients with the strong family history, bilaterality, multifocality or early onset breast and/or ovarian cancer can benefit from genetic susceptibility testing.

POLYMORPHISMS AND EXPRESSION OF HUMAN *MGMT* GENE

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Genetic polymorphism of DNA repair genes may be associated with modulation of cancer risk. Our investigation focused on *MGMT* gene encoding an alkyltransferase involved in direct DNA repair. The aim of our study was to determine the frequency of four polymorphic alleles of *MGMT* gene: 84Leu>Phe, associated substitutions 143Ile>Val-178Lys>Arg, enhancer polymorphism 1099C>T (Acc. X61657) and 79G>T (Acc.U95038) in 96 non-small cell lung cancer (NSCLC) cases and 96 cancer-free individuals from Upper Silesia. We also performed genotyping of three frequent *MGMT* alleles (84Phe, 143Val-178Arg, 1099C>T) in 164 NSCLC cases to examine the association of *MGMT* alleles with basic clinical and epidemiological characteristics of the patients. We studied "differential expression" of *MGMT* alleles and its association with polymorphisms in regulatory region of *MGMT*. Material for the expression study was RNA and DNA from white blood cells of 45 healthy inhabitants of Upper Silesia. We found that no *MGMT* allele showed statistically significant frequency difference between cancer cases and controls. The enhancer polymorphism was less frequent in never smokers (4%) than in smokers (18%), in females (5%) than in males (17%) and in never smoking cancer patients (6%) compared with never smoking controls (24%). We detected strong association between 84Phe allele and the clinical stage of cancer. *MGMT* alleles in heterozygotes revealed "differential expression" in white blood cells (14% of informative samples) and this phenomenon was associated with the enhancer polymorphism of *MGMT*. To further investigate the functional significance of the enhancer polymorphism, we cloned 275 bp wild-type and polymorphic promoter-enhancer element into pGL-3 vector in a way that the element controlled the expression of firefly luciferase gene. After transfection into three different cell lines, we found that the polymorphic variant was invariably associated with significantly increased activity of the luciferase enzyme. These observations are consistent with the hypothesis that the enhancer polymorphism is of functional significance since it is associated with increased expression of the *MGMT* gene and may decrease lung cancer risk at low carcinogen exposure.

CYTOTOXIC EFFECT OF DOXORUBICIN CONJUGATES WITH POLY-3-HYDROXYBUTYRATE. PRELIMINARY STUDY

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Chemotherapy is one of the most important anticancer treatment modes. Several approaches have been undertaken to elaborate a strategy of a cancer-targeting drug delivery system that could increase the concentration of pharmaceutical drugs in tumor tissue, to reduce drug exposure and toxicity to other tissues, and to overcome drug resistance. There is an increasing interest in designing vectorized drugs in which a given chemotherapeutic is linked by a covalent hydrolysable bond to either a peptide or a polymeric substance. One of such polymeric substances, a potential vector for drug delivery, is poly-(3-hydroxybutyrate) (PHB in short). PHBs are a ubiquitous constituent of cells and have been found in bacterial cell membranes, in a variety of plant tissues, as well as in plasma membranes, mitochondria, and microsomes of animal cells. Novel methods developed at the Centre of Polymer Chemistry, Zabrze enable synthesis of tailor-made oligo- and poly-(3-hydroxybutyrate) with well-defined structure and properties. Recently, the conjugates of doxorubicin with poly-(3-hydroxybutyrate) were also synthesized.

The aim of the present study was to compare the cytotoxicity of free and conjugated doxorubicin as well as to compare the kinetics of uptake and subcellular localization of these two forms of this cytostatic drug. We first determined whether short-chain PHBs could exert a cytotoxic effect on cells grown in culture. Cytotoxicity MTT assays were performed on hamster V79 cells, murine melanoma Bl6 cells and human breast cancer cell line MCF7. We found that the majority of several PHB oligomers tested did not significantly affect cell growth; this was also confirmed in clonogenic assay. We also found that the PHBs tested do not induce cytoprotective mechanisms manifested by induction of *hsp70i* genes. In order to compare a cytotoxic effect of free and PHB-bound doxorubicin we performed MTT assays using cell lines mentioned above, and found that both forms of the cytostatic drug kill cells at a similar rate.

To study the intracellular distribution of the free and PHB-conjugated doxorubicin we used confocal laser scanning microscopy (CLSM). Owing to doxorubicin autofluorescence the CLSM was found to be a highly useful method for detecting intracellular localization of this compound. The study was performed on V79, B16 and MCF7 cells, as well as on the doxorubicin-resistant variant of MCF7 cells. We found that, in contrast to doxorubicin, which is confined to the nucleus, PHB-doxorubicin conjugates are localized predominantly in cytoplasm. A very distinct feature of the PHB-bound doxorubicin is its significantly faster cellular uptake, compared to the free drug. Of special importance is the observation that while free doxorubicin is effectively removed from doxorubicin-resistant MCF7 cell variant, PHB-bound drug is not.

Our data show that the PHB-conjugated doxorubicin is a very effective drug-carrier molecule. It can be considered a novel form of this cytostatic drug, potentially able to overcome drug resistance phenomenon.

CHARACTERISTICS OF REPETITIVE DNA SEQUENCES IN HUMAN GENOME

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Metaanalysis of simple repeated repetitive sequences in human genome requires general characteristic. Simple repeats were selected from GenBank. These repeats comprised tandemly repeated units 1-6 nucleotides (nt) long and have been extracted from genomic DNA sequences. Of the 501 theoretically possible, different types of repeat only 67 were present in the analysed database in at least two different size ranges over 12 nt. (they present ~ 1% of the total DNA taken to the analysis) [1].

The chi-square test is usually used to analyse the relation between qualitative variables (type of amino acid sequence, its localisation etc). The new Z- coefficient method for quantitative analysis of contingency tables is introduced. This method allows to put all combinations from all forms of analysed two qualitative variables in the ranking order. The information about such order allows to reveal the combinations, which are more and those which are less responsible for the presence of significant dependence between two analysed variables. It was found that in the dependence between length of repeat units and degree of dispersion the tri-nuleotides fragments represent the highest localisation stability. 1-, 2-, 4-, 5- and 6-nucleotides fragments exhibit the higher ability to be dispersed in genome.

The expandability of repetitive sequences in human genome is assumed to cause the neurodegenrative diseases so it can be important in clinical image.

THE URINARY EXCRETION OF 8-OXOGUANINE AND 8-OXO-2'-DEOXYGUANOSINE IN NON SMALL CELL LUNG CANCER PATIENTS

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Using HPLC prepurification/isotope dilution GC/MS technique, we examined whether the amount of 8-oxoguanine and 8-oxo-2'-deoxyguanosine excreted into urine is higher in cancer patients when compared with the control group. The control group consisted of 38 healthy subjects and the patient group comprised 57 non small cell lung cancer patients.

The mean level of 8-oxoguanine in urine samples of 57 cancer patients was 191 ± 102 nmol/24h. It was significantly higher ($p=0.0086$) than in the urine of the control group, where the level reached the value of 138 ± 82 nmol/24h. The mean levels of 8-oxo-2'-deoxyguanosine in the control group and in cancer patients were very similar and reached the mean values of 35 ± 21 nmol/24h and 32 ± 18 nmol/24h respectively. This difference was not statistically significant.

We have found that the amount of the modified base (but not the nucleoside) excreted into urine is about 40% higher in cancer patients than in the control group. Since the presence of the modified base in urine may represent the primary repair product of the oxidative DNA damage in vivo our results suggest an important role of DNA glycosylases (most likely OGG1) in removal of the damage induced as a result of cancer development.

HEAT SHOCK COGNATE 70 (*HSC70*) GENE SEQUENCE ALTERATIONS IN NON-SMALL-CELL LUNG CANCER PATIENTS – ASSOCIATION WITH *P53* GENE MUTATIONS AND IMMUNOHISTOCHEMICAL STAINING OF HSC70 AND P53 PROTEINS

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The loss of heterozygosity at the *locus* 11q23.3 is a frequent event in various human cancers. Recently, somatic mutations of 11q23.3-linked heat shock cognate *HSC70* gene, in coincidence with allelic imbalance, were found by others in sporadic breast carcinomas. To find out whether intragenic somatic mutations of *HSC70* occur also in lung carcinomas, we sequenced exons 2 – 8 of the gene as well as adjacent intronic sequences in a series of DNA samples isolated from non-small-cell lung cancer (NSCLC) tissues. No somatic mutations were detected, however we identified 22 polymorphic sequence changes, 19 of which were not reported previously. We found a significant association between the most frequent *HSC70* polymorphism (1541-1542delGT) and the immunohistochemical staining pattern of the HSC70 protein and p53 tumor suppressor protein, determined by us previously. Generally, carriers of that polymorphism showed weaker staining of HSC70 and p53 in tumor tissues, as compared to wild-type homozygotes. We also found that 1541-1542delGT polymorphism is associated with frequency of the deletion/insertion mutations of *p53* gene. For the latter comparison we re-examined the mutation frequency of the *p53* gene in our series of lung cancer patients. Here, we report 17 newly detected *p53* mutations. The overall *p53* mutation frequency (including the alterations that were previously found by us) reached 65%, a value that is among the highest found in NSCLC so far. Our data indicate that the 1541-1542delGT polymorphism of *HSC70* gene may be functional and associated with modulation of cellular levels of HSC70 and mutant forms of p53 protein.

*These authors contributed equally to this work.

PREMATURE MITOSIS – CHROMOSOMES REPLICATE THE DNA

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The prolonged arrest of DNA replication invokes mechanisms that relay a signal (or signals) to effector molecules which implement a number of checkpoint-dependent responses, including a decrease in the activity of CDK-cyclin B complexes. In consequence, the commitment of cells to enter mitosis become blocked. Experiments on root meristem cells of *Vicia faba* indicate that caffeine allows a number of cells to override the intra-S-phase checkpoint control and to induce mitotic condensation of incompletely replicated chromosomes (PCC). Using Feulgen cytophotometry and ^{3}H-thymidine autoradiography, we have evidenced that their appearance varies significantly depending on the stage at which hydroxyurea-blocked S-phase cells were stimulated to enter mitosis. Furthermore, prematurely condensed chromosomes maintain the ability to continue DNA replication. Since ^{3}H-thymidine incorporation is restricted merely to cells at anaphase and telophase, it seems reasonable to suppose that the commitment of chromatin to the resumption of DNA synthesis depends on the transition throughout the APC/C-mediated checkpoint, which gates the cells into the exit from mitosis.

THE COMBINED TREATMENT OF TRANSPLANTABLE SOLID MAMMARY CARCINOMA IN WISTAR RATS BY USE OF PHOTODYNAMIC THERAPY AND CYSTEINE PROTEINASE INHIBITOR

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Numerous studies showed that lysosomal proteinases play important role in carcinogenesis. Enzymatic activity of tumor-associated proteases is counter-balanced by specific inhibitors. PDT is technique which involves photoexcitation of sensitizing drug retained in neoplastic tissue that is subsequently destroyed. Intraperitoneal injections of hematoporphyrin derivative (HpD) were made in dose 20 mg/kg in rats transplanted with mammary carcinoma. Halogen lamp was used 24 hrs later at 630+/-20 nm and total dose - 200 J/sq. cm. Cysteine proteinase inhibitor (CPI) was dissolved in saline and injected subcutaneously in doses 50 μg and 200 μg per animal. Effectiveness of treatment was evaluated with regard to survival time and tumor response and to depth of necrosis. In several cases tumors completely disappeared following HpD-PDT+CPI. Number of complete tumor responses was higher when PDT+200 μg of CPI was used, i.e. 6/10 rats. Promising results have also been obtained with regard to survival time of treated animals and to induction of tumor necrosis.

DNA ADDUCTS AND REPAIR CAPACITY IN LUNG CANCER

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Oxidative stress and lipid peroxidation (LPO) generate promutagenic DNA lesions such as 1,N^6-ethenoadenine (εA) and 3,N^4-ethenocytosine (εC) that are likely to contribute to lung cancer development and progression.

We measured the levels of εA and εC by immunoaffinity/^{32}P postlabeling in the DNA of tumour and normal lung tissue of lung cancer patients (33 cases) as well as in leukocytes of the same group. Activities of DNA-glycosylases repairing εA and εC were also measured in the same tissues (57 cases) and in leukocytes of healthy volunteers (26 individuals).

High individual differences (up to 10-fold) were observed both in adduct level and glycosylase activities. No difference in εA and εC level between tumour and non-affected lung tissue was recorded. However, leukocytes accumulated statistically significant higher number of DNA adducts than lung tissues. Repair activities for both εA and εC were significantly higher in tumour than in normal lung tissue. There was inverse correlation between the level of εC and the activity of εC-glycosylase in normal and tumour lung tissue, however for εA such correlation was found only in tumours. This suggests that εC-DNA-glycosylase, is the "first choice" enzyme for removing this lesion from DNA in humans.

There was no difference in εA and εC-glycosylases activities between men and women as well as smokers and ex-smokers.

εC-glycosylase activity was decreasing gradually with age in normal lung (3.22-fold difference between groups of 40-50-years old and over 70-years old patients).

We observed differences between two histological types of lung cancer: squamous cell carcinoma (SQ) and adenocarcinoma (AD). In patients with SQ the ratio of εA/εC was higher in non-affected lung tissue than in AD patients. In AD individuals εA and εC DNA-glycosylase activities were significantly lower than in SQ, however normal lung of AD patients revealed higher deficiency in εA-glycosylase (2.72-fold decrease) than in εC-glycosylase (1.8-fold) in comparison with SQ type of tumour. This might suggest that people developing inflammation-related adenocarcinoma, might have defective "first choice" defense mechanisms against LPO-induced DNA damage.

Plasma of healthy volunteers contained higher level of vitamin E and A in comparison with that of cancer patients.

THE '*cis*' REGULATORY SEQUENCES ESSENTIAL FOR TRANSCRIPTION OF THE *hst70* GENE ARE LOCALIZED WITHIN THE 5'UTR REGION

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The rat *hst70* gene belongs to the *hsp70* family of heat shock genes and codes for a molecular chaperone protein necessary for synaptonemal complex disassembly, progression of spermatogenesis beyond meiotic phase and probably development of spermatids.

Previously, we identified a short intron in 5'UTR of the *hst70* gene and demonstrated that the transcription of *hst70* gene could be initiated from two main start sites, localized 353 bp (T1 start site) and 116 bp (T2 start site) upstream of the ATG codon. T2 start site is localized within the intron sequences, T1 precedes the untranslated exon1. Functional studies of the *hst70* gene promoter activity revealed, that a 367 bp region upstream of ATG is sufficient to direct expression of CAT reporter gene specifically to testes of transgenic mice.

In the present study we demonstrate the expression pattern of series of pHST-CAT constructs in which the *hst70* gene promoter was truncated either from 5' or 3' directions. We showed, using transfection *in vitro*, that sequences localized in exon1 and a 5' part of intron are essential for expression of the *hst70* gene. Searching for "*cis"* regulatory elements localized within this *hst70* promoter fragment we found two short stretches of almost perfect homology (described as boxA and boxB) in homologues genes of rat, mice and human. We determined that sequences containing only boxes A and B are capable to direct expression of the CAT reporter gene specifically to testis of transgenic mice. Next, we analyzed the binding of nuclear proteins from testes of immature (*hst70* gene inactive) and sexually mature rats (*hst70* gene highly active) to boxA and boxB sequences using the "band shift assay". We obtained a specific complex only between nuclear proteins from testes of immature rats and the octamer sequence within boxA. We speculate that this protein/proteins could repress the activity of the *hst70* gene in immature rats.

EFFECT OF DIFFERENT TRANSFECTION AGENTS ON THE ACTIVITY OF THE RAT *hsp70i* GENE PROMOTER

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In normal mammalian cells, under physiological conditions the *hsp70i* (i-inducible) genes are either inactive or expressed at a very low level. Stress response triggers heat shock factor (HSF) activation, its interaction with regulatory sequence HSE and finally the *hsp70i* genes expression. The *hsp70i* genes are also constitutively expressed in some primary tumors and tumor cell lines (Morimoto, 1999). However, the mechanism of that phenomenon is poorly understood. It seems that except the well-recognized HSE-HSF interaction other *cis-trans* interactions may be involved in the regulation of inducible *hsp70* genes in some circumstances.

To search for possible other cis-trans interactions that could regulate hsp70 gene expression we have constructed series of vectors in which GFP or CAT reporter genes were fused with different fragments, (up to –2.0 kbp, relative to the transcription start site) of the promoter region of the rat hsp70.1 gene. Surprisingly, when we used Lipofectin Reagent (Gibco BRL) for transient transfection of the cells with these constructs, a strong activation of the reporter gene was observed regardless of incubation temperature. High hsp70.1 promoter activity was observed between 16 and 48 hours after lipofection of B16 and FTO 2B cells. Heat shock did not significantly increase CAT activity in lipofected cells. This was in contrast to cells transfected by DEAE-dextran method that did not exhibit CAT activity at 37°C while they did at 42°. This suggested that Lipofectin itself induced hsp70 gene promoter activity. The electrophoretic mobility shift assay (EMSA) with the HSE oligonucleotide and the extracts from Lipofectin treated cells showed that Lipofectin activation is independent of the HSF-HSE interaction. Northern blotting experiment revealed that only transiently transfected hsp70 promoter sequences were susceptible to the Lipofectin treatment while the endogenous hsp70 was not induced. Preliminary search for the sequences responsible for Lipofectin induced expression showed that most important regulatory sequences are localized within 350 bp region encompassing 85 bp of the 5'UTR and 260 bp of the promoter. We also analyzed the effect of other liposomal transfection agents on the hsp70.1 promoter activity and we found that Lipofectamine PLUS (Gibco BRL) had similar properties to Lipofectin while two home-made liposomal formulations (Arg-Chol/DOPE and DDAB/DOPE) did not induce reporter gene transcription by themselves. Our results indicate that caution must be taken when performing promoter studies of hsp genes, as the type of compounds used for transfection may seriously affect the results.

GFP FLUORESCENCE IN SOMATIC CELLS OF TRANSGENIC MICE CONTAINING EGFP REPORTER GENE UNDER THE CONTROL OF THE RAT TESTIS-SPECIFIC *hst70* GENE PROMOTER

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Rat *hst70* gene (in mouse called *hsp70.2*) is a unique member of the multigene hsp70 family. The gene is activated during meiosis in spermatogenic cells.and codes for molecular chaperone protein involved in desynapsis of synaptonemal complexes as well as chaperoning CDC2 kinase required for completion of the meiotic division. Homozygotic removal of the *hsp70.2* gene leads to male infertility (due to spermatocytes apoptosis), whereas females remain fertile. We have previously shown using RT-PCR and transgenic mice with CAT reporter gene, that an expression level of *hst70* in tissues other than testis is 10-100 times lower. The highest *hst70* expression can be detected in the brain while in the liver the gene is fully repressed.

To study the expression of the *hst70* gene at a single cell level we have constructed transgenic mice bearing enhanced green fluorescent protein gene (EGFP) under the control of about 800bp of *hst70* promoter. We have obtained 17 founders of transgenic mice, and established 6 transgenic lines. All transgenic males exhibit EGFP fluorescence in testes, epididymides and vasa deferentia. EGFP is expressed in testes in cell- and stage-specific manner resembling the pattern observed for the endogenous *hst70* gene. The *hst70* gene is strongly activated in pachytene spermatocytes that appear during maturation of testes in two weeks-old mice. EGFP fluorescence is present in cytoplasm and nucleus of spermatocytes, spermatids, residual bodies and spermatozoa. EGFP fluorescence is detected (in some transgenic lines) in female reproductive organs, especially in uterus, and in one-cell and two-cell stage embryos. In all transgenic mice EGFP fluorescence is present in the brain. Depending on the line, transgene may be active in other somatic organs: pancreas, pituitary, salivary gland, kidney, adrenal glands, bladder, heart, lung, and spleen. Differences in pattern of the transgene expression observed between the founders may result from site of its chromosomal integration.

Transcription of *hst70* in restricted areas of somatic tissues may suggest novel function for its protein. However, it is unclear whether *hst70* transcripts are efficiently translated in somatic cells. In order to elucidate this problem we are going to construct transgenic mice expressing HST70-EGFP fusion protein.

LAST MINUTE LECTURE…

DNA REPAIR CAPACITY IN LYMPHOBLASTS FROM SISTERS DISCORDANT FOR BREAST CANCER

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The mutagen sensitivity assay is one of the approaches used to investigate individual DNA repair capacity. This method is based on the premise that after *in vitro* treatment with a test mutagen, DNA from subjects with defective repair will be more damaged than DNA from those with an efficient repair system. However, very little is known about unmeasured processes that occur between cell treatment and final assessment of DNA damage. To develop a more precise assay, we modified the traditional mutagen sensitivity assay to also include measurement of DNA damage after culturing cells in the absence of mutagen. First we treated apparently normal and Xeroderma Pigmentosum lymphoblastoid cell lines with various doses of benzo(a)pyrene diol epoxide (BPDE), and harvested cells at different time points. A polyclonal antiserum against BPDE-DNA was used to quantitate levels of adducts by immunoslot-blot and immunohistochemistry. Selected conditions included treatment with 10□M BPDE, a 4h culture in mutagen-free medium, and immunohistochemical measurement of BPDE-DNA adducts. The method was then applied in a pilot study to 50 lymphoblastoid lines from sisters discordant for breast cancer. There was no significant difference between cases and controls in the level of BPDE-DNA adducts in lymphoblasts harvested immediately after BPDE treatment. However, after a 4h culture in mutagen-free medium, the level of adducts was significantly higher ($p=0.006$) among cases than in controls. There was a two-fold increase in mean adduct removal in lines from non-affected as compared to affected sisters (44% and 22% decrease, respectively). DNA repair capacity was predictive of case status ($p=0.04$) in logistic regression analysis. This method, which can be easily applied to large numbers of samples should be useful in studies to investigate the role of DNA repair in cancer risk.

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1. Barilka V.A., Matlan V.L., Piddubnyak V.A.*, Volodko N.A.*, Novak S.V.*, Chervinska O.D., Bilynsky B.T.*, Loginsky V.E.
EFFECT OF TNF ON PROLIFERATIVE ACTIVITY OF HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS (HUVECs) IN NON-HODGKIN LYMPHOMA

2. Benedyk Konrad, Widłak Wiesława, Głowala Magdalena, Małusecka Ewa, Krawczyk Zdzisław
SEMINIFEROUS EPITHELIUM DEGENERATION IN TRANSGENIC MICE THAT CONSTITUTIVELY EXPRESS TRANSCRIPTIONALLY ACTIVE HEAT SHOCK FACTOR (HSF1) IN SPERMATOCYTES

3. Białkowski Karol
INHIBITION OF 8-OXO-2'-DEOXYGUANOSINE 5'-TRIPHOSPHATE PYROPHOSPHOHYDROLASE (8-OXO-dGTPase) ACTIVITY OF HUMAN MTH1 PROTEIN BY PHYSIOLOGICAL INHIBITORS, NUCLEOSIDE 5'-DIPHOSPHATES

4. Czarny Małgorzata, Chyczewski Lech*, Chorąży Mieczysław, Rusin Marek
THE EXPRESSION OF A DNA REPAIR PROTEIN – XPA IN NON–SMALL CELL LUNG CANCERS

5. Fiszer-Kierzkowska A., Wysocka A., Vydra N., Lisowska K., Krawczyk Z.
FUNCTIONAL ANALYSIS OF SEQUENCES UPSTREAM OF THE RAT *HSP70.1* STRESS GENE

6. Głowala Magdalena, Mazurek Agnieszka, Piddubnyak Valeria, Fiszer-Kierzkowska Anna, Krawczyk Zdzisław
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7. Ilnytska O., Oleksyn H., Kusen' S., Drobot L.
REGULATION OF PHOSPHATIDYLINOSITOL 3-KINASE SIGNALLING SPECIFICITY DURING THE COURSE OF DIFFERENTIATION AND APOPTOSIS OF HUMAN ERYTHROLEUKEMIA CELL LINE K562

8. Kit Yu.Ya., Drel' V.R., Shuvayeva H.Yu., Palyvoda O.Yu., Kovalyova V.A., Vovk O.I., Igumenceva N.I., Bobak Ya.P., Rzeszowska J., Gout I.T., Buchman V.L., Drobot L.B.

Ruk ADAPTOR PROTEINS – STRUCTURAL AND FUNCTIONAL FEATURES

9. Konopacka Maria, Rogolinski Jacek, Orlef Andrzej, Rzeszowska-Wolny
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10. Kośmider Beata, Osiecka Regina, Zyner Elzbieta, Ochocki Justyn,Ciesielska Ewa
ASSESSING GENOTOXIC PROPERTIES OF *cis*-Pt(II) 3-AMINOFLAVONE COMPLEX IN COMPARISON WITH *cis*-DDP

11. Lichota Katarzyna D., Tudek Barbara, Kuśmierek Jarosław T.
DEGRADATION OF MODIFIED DEOXYNUCLEOSIDE-5'-TRIPHOSPHATES BY HUMAN TISSUE HOMOGENATES

12. Małusecka E., Zborek A., Krzyżowska-Gruca S., Krawczyk K.
EXPRESSION OF HSP25 IN THE RAT LIVER AFTER TREATMENT WITH HEPATOTOXICANTS INDUCING INFLAMMATORY REACTION

13. Marczak Agnieszka, Łubgan Dorota, Jóźwiak Zofia
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14. Maszewski Janusz, Rybaczek Dorota
PCC IN A PLANT CELL – THE WAY TO APOPTOSIS

15. Matulewicz Łukasz, Chwałek Agnieszka, Wideł Maria, Rzeszowska-Wolny Joanna
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16. Mirowski M., Różalski M., Krajewska U., Balcerczak E., [2]Młynarski W., Wierzbicki R.
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17. Nagy Enikő, Balogh Gábor, Horvath Ibolya, Benkö Sándor, Gyöfry Zsuzsa, Hoyk Zsófia, Bensaude Olivier, Vigh László
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18. Obtułowicz T, Skjelbred CF, Bowitz Lothe IM, Hansteen I-L, Bock G, Nilsen B, Jørgensen H, Aase S, Tudek B, Kure EH
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19. Palyvoda O., Wygoda A., Drobot L. , Rzeszowska-Wolny J.
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22. Piddubnyak Valeria, Jedliński Zbigniew, Matuszowicz Andrzej, Głowala Magdalena, Kurcok Piotr, Juzwa Maria, Śnietura Mirosław, Lange Dariusz, Krawczyk Zdzisław

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25. Rusin Marek, Zientek Helena, Krześniak Małgorzata, Małusecka Ewa, Zborek Anna, Krzyżowska-Gruca Stefania, Butkiewicz Dorota, Lisowska Katarzyna, Grzybowska Ewa, Krawczyk Zdzisław

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GFP FLUORESCENCE IN SOMATIC CELLS OF TRANSGENIC MICE CONTAINING EGFP REPORTER GENE UNDER THE CONTROL OF THE RAT TESTIS-SPECIFIC *hst70* GENE PROMOTER

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