Scientific Program

November 19-20, 2004

Friday, November 19th

9.15-9.30 ***Opening of the Conference***
Opening address: **prof. Joanna Rzeszowska, President of the Organizing Committee**
Welcome address: **Representative of Gliwice City Mayor Office**

9.30-11.45 ***Session I***

MATHEMATICAL MODELS IN GENOMICS AND CANCER BIOLOGY

Session supported by Apple IMC Poland SAD

Michał Dąbrowski (Nencki Institute, Warsaw):
Identification of potential cis-regulatory features associated with a mode of gene expression common to neuronal differentiation and hippocampal development. (30')

Krzysztof Fujarewicz (Silesian University of Technology, Gliwice):
Identification of models of cell signaling pathways. (25')

Piotr Formanowicz (Institute of Bioorganic Chemistry, Poznań):
Isothermic sequencing by hybridisation. (25')

Krzysztof Simek (Silesian University of Technology, Gliwice):
SVD as a tool for pattern discovery in gene expression data. (25')

Bogdan Smołka (Silesian University of Technology, Gliwice):
Fast denoising of cDNA microarray images. (25')

11.45-12.15 ***Coffee break + Poster viewing***

12.15-14.00 ***Session II***

STRUCTURE AND FUNCTION OF THE GENOME

Sergey Razin (Institute of Gene Biology, Moscow):

The spatial organization of DNA in eukaryotic cell nuclei plays an important role in determining positions of recombination hot-spots. (30')

Jan Filipski (Institut Jaques Monod, Paris):
Epigenetic regulation of transcription and the origin of isochors. (25')

Piotr Widłak (Center of Oncology, Gliwice):
The other face of histone H1. (25')

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Introductory lecture:
Vasyl F. Chekhun (Institute of Experimental Pathology, Oncology and Radiobiology, Kiev):
New trends in experimental oncology in Ukraine. (25')

14.00-15.00 Lunch

15.00-18.30 Session III

DNA REPAIR MECHANISMS AND THEIR ROLE IN CARCINOGENESIS

Antonina Cebulska-Wasilewska (Institute of Nuclear Physics, Kraków):
Response to challenging dose of x-rays as biomarker of susceptibility in molecular epidemiology. (25')

Wolfgang Goedecke (University of Essen, Germany):
The fate of DNA double-strand breaks: aspects of mitotic and meiotic cells. (25')

Andrzej Wójcik (Institute of Nuclear Chemistry and Technology, Warsaw):
Do interindividual differences in lymphocyte chromosome radiosensitivity exist? (25')

Ulla Kasten-Pisula (Eppendorf University, Hamburg):
Molecular mechanisms of the individual radiosensitivity: impact on DNA repair. (25')

Coffee break + Poster viewing (30')

Paweł Jałoszyński (Institute of Human Genetics, Poznan):
Fidelity or survival? How unrepaired DNA is replicated when polymerases encounter problems. (25')

Barbara Tudek (Institute of Biochemistry and Biophysics, Warsaw):
Processing of lipid peroxidation-derived DNA adducts by repair enzymes and DNA polymerases. (25')

Miroslav Chovanec (Cancer Research Institute, Bratislava)
Repair of DNA double-strand breaks of different origin in Saccharomyces cerevisiae. (25')

20.00 - Social Party

Saturday, November 20th

9.00-11.15 Session IV

CELLULAR STRESS, SIGNALING PATHWAYS AND CANCER

Session supported by Committee for Human Genetics and Molecular Pathology, PAS

Maciej Żylicz (International Institute of Molecular and Cell Biology, Warsaw):
Hsp70 and MDM2 are important for the transcriptional activity of wild type p53. (30')

Joanna Rzeszowska (Center of Oncology, Gliwice):
Gene expression profiles and cellular stress. (25')

Svetlana Sidorenko (Institute of Experimental Pathology, Oncology and Radiobiology, Kiev):
CD150-mediated signaling. (25')

Lyudmila Sidorik (Institute of Molecular Biology and Genetics, Kiev, Ukraine):
The role of molecular chaperons in cancer. (25')

Jekaterina Erenpreisa (Latvian University, Riga):
Expression of meiotic genes and phenotype by irradiated tumour cells. (25')

11.15-11.45 Coffee break + Poster viewing

11.45-13.00 Session V

NEW CONCEPTS AND METHODS

Lise L. Hansen (Aarhus University Hospital, Aarhus)
PCR amplification of highly GC-rich templates. An efficient tool in the search for new prognostic markers in cancer. (30')

Janusz M. Bujnicki (International Institute of Molecular and Cellular Biology, Warsaw)
Protein structure prediction by consensus fold recognition and assembly of fragments. (30')

Gabriel Wcisło (Military Medical Institute, Oncology Department, Warsaw):
From molecular studies to clinical practice. An EGFR (epidermal growth factor receptor) story. (15')

13.00-14.00 Lunch

14.00-16.15 Session VI

FUNCTIONAL GENOMICS IN MEDICINE

Session supported by State Committee for Scientific Research (Grant PBZ-KBN-040/PO4/2001)

Barbara Jarząb (Center of Oncology, Gliwice):
Genomic approach to thyroid cancer. (25')

Małgorzata Wiench (Center of Oncology, Gliwice):
RET protooncogene and gene expression profile of thyroid cancer. (25')

Joanna Polańska (Silesian University of Technology, Gliwice):
Applications of mixture models to gene expression profiles. *(25')*

Katarzyna Lisowska (Center of Oncology, Gliwice):
Gene expression profiling in hereditary breast cancer; preliminary results. (25')

Michał Jarząb (Center of Oncology, Gliwice):
From microarray data to the biological relevance. (25')

16.15-16.30 Closing Ceremony
Coffee/Discussions

Lecture abstracts

PROTEIN STRUCTURE PREDICTION BY CONSENSUS FOLD RECOGNITION AND ASSEMBLY OF FRAGMENTS

Janusz M. Bujnicki

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In the age of structural genomics, the computational assignment of three-dimensional structures to newly determined protein sequences is becoming an increasingly important complement to experimental structure determination. In particular, fold-recognition methods aim to build 3D models for proteins bearing no evident sequence similarity to any protein of known structure by identification of structural "templates" compatible with the modeled sequence. Meta-servers run a variety of primary methods to perform the fold-recognition analysis, collect and compare the results, and select one of the models as the most representative. On the average, this approach leads to much better predictions of protein structure than using any single "primary server".

Recently, a new generation of prediction methods appeared, which build hybrid models from fragments, either obtained from the experimentally solved protein structures, or from the fold-recognition models. The general premise of this approach is that the protein conformation is reasonably well approximated by the distribution of local structures adopted by known, not necessarily homologous, protein structures. The "fragment splicing" methods are very powerful, as the resulting hybrid models are on the average more complete and more accurate than the input models. They can also correctly predict the 3D structure of a protein with a completely new fold, which could not be modeled based on any single template. Currently, the accuracy of models built by the fully automated meta-servers and "fragment splicing" methods approaches the models built by best human modelers; however, it still lags behind the quality of high-resolution structures obtained by X-ray crystallography. I will review the state-of-the-art in the field of protein structure prediction, with the emphasis on what is currently possible and what is not (yet) possible.

REFERENCES

1. Proteins, Structure, Function, and Genetics: Volume 53, S6, 2003 Supplement 6: CASP
2. Practical Bioinformatics, Janusz M. Bujnicki (ed.), Springer-Verlag, 2004

RELEVANT LINKS

http://predictioncenter.llnl.gov/
http://www.genesilico.pl/meta/
http://robetta.bakerlab.org/

RESPONSE TO CHALLENGING DOSE OF X-RAYS AS BIOMARKER OF SUSCEPTIBILITY IN MOLECULAR EPIDEMIOLOGY.

Antonina Cebulska-Wasilewska[1, 2]

[1] Department of Radiation and Environmental Biology, the H. Niewodniczański Institute of Nuclear Physics, PAN, Kraków, [2] Chair of the Epidemiology and Preventive Medicine, CM UJ, Kraków, Poland

The importance of various environmental exposures has been evident in variation in cancer incidence and mortality. Elevated frequencies of chromosome aberrations or sister chromatid exchanges in human lymphocytes are recognisable biomarkers of hazardous genotoxic effects caused by environmental or other exposures. Results of the cytogenetic damage detected in our monitoring studies have shown both an association with an adverse health outcome on one side and the influence of confounding factors related to the lifestyle on the other. The single cell gel electrophoresis assay, the alkaline version in particular, has become a very popular method for the analysis of the DNA damages caused by various chemical and physical agents. Comparison between DNA damage investigated by the SCGE assay and cytogenetic damage induced in human lymphocytes by chemicals or ionising radiation revealed a strong correlation between the two assays. Obtained results suggested that the SCGE assay provides a good prediction of cytogenetic damage, and because of its simplicity and rapidity, the assay would appear to be a useful tool for the predicting the individual susceptibility to the induction of the DNA damage or for determining the genotoxicity of environmental agents. This review is based on studies in which radiation was applied as a challenging dose and DNA damage induced and repaired was analyzed with the use of the single cell gel electrophoresis (SCGE) assay. Results from studies on susceptibilities and repair competence carried out in various groups of exposed workers, controls, and cancer patients (more than 700 donors) show variability between donors both in a response to challenging treatment and in the efficiency of repair process. Influences on cellular capacities of the occupational exposures and other factors depending on genotypes or life style are observed. On the basis of presented results it could be suggested that application of ionizing radiation as a challenging treatment and SCGE as the method for DNA damage measurement in combination with repair competence assessment might be used in a molecular epidemiology or in preclinical studies as a fast biomarker predicting for various donors a cellular susceptibility to various genotoxins and exposures (environmental, occupational or therapeutic).

Acknowledgments

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NEW TRENDS IN EXPERIMENTAL ONCOLOGY IN UKRAINE

V.F.Chekhun

R.E.Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology NAS of Ukraine, Kiev, Ukraine

Currently, knowledge of tumorigenesis biology does not allow precise diagnosis of the disease based on traditional diagnostics methods as they do not permit characterizing biological peculiarities of the tumor nor detecting micrometastases. Presently, the combination of cytogenetic, immunohistochemical, immunocytochemical and molecular-biological methods, along with classic morphological methods, should be applied thus allowing determination of biological characteristics of the tumor and its metastatic potential.

Novel molecular biological technologies that are developed in IEPOR allow detection and correction of pathogenetic alterations in transformed cells via system of antigenic determinants and cytokine spectrum. In particular, use of monoclonal antibodies (MoAbs) allows diagnosing hemoblastosis in cancer patients (adults and children) in 95-98% of cases, to describe 7 variants of acute leukemia and 8 variants of acute myeloid leukemia that forms the basis for modern classification of lymphoproliferative diseases according to WHO requirements. Such precise diagnosis allows more effective therapeutic applications, i.e. to apply conjugates of MoAbs with antitumor drugs. The technology created in IEPOR is based on the use of MoAbs of IEPOR series (IPO-4) that initiates programmed death of tumor cells.

Another research direction is the development of methods for immunotherapy of cancer based on construction of antitumor vaccines using substances of microbial origin (i.e. products of bacterial synthesis, receptors etc). The latter cause devitalization of autologic tumor cells, effective stimulation of effector cells thus providing antitumor resistance. Such autovaccines may be used either as additional remedy, or as immunomodulators.

In conclusion, one may suppose that the priority in the development of new strategies for treating cancer patients will belong to early diagnosis and individualization of therapy based on the achievements of modern biotechnology. The determination of markers for early diagnosis, production of highly specific cytostatics and vector systems for their transportation, generation of new vaccines, probiotics and MoAbs that directly influence the key chains of cell differentiation and apoptosis will form a way to success in solving problems of clinical oncology.

REPAIR OF DNA DOUBLE-STRAND BREAKS OF DIFFERENT ORIGIN IN *SACCHAROMYCES CEREVISIAE*

Miroslav Chovanec[1], Viera Vlčková[2], Eva Marková[1], Zuzana Dudášová[1], Andrej Dudáš[1], Danuša Vlasáková[1], Katarína Hermanská[2], Daniela Gabčová[2] and Jela Brozmanová[1]

[1]Laboratory of Molecular Genetics, Cancer Research Institute, Vlárska 7, 833 91 Bratislava 37, Slovak Republic and [2]Department of Genetics, Comenius University, Faculty of Natural Sciences, 842 15 Bratislava, Slovak Republic

DNA double-strand breaks (DSB) are considered the most severe type of DNA damage, because if left unrepaired, they can cause cell death. If misrepaired, they can lead to genomic instability and, ultimately, the development of cancer in multicellular organisms. Two main pathways have evolved in eukaryotic cells for the repair of DSB, homologous recombination (HR) and non-homologous end-joining (NHEJ).

DSB can be induced by a variety of agents. To address the question how oxidatively induced DSB are repaired in *S. cerevisiae*, we have studied survival, mutagenesis and DSB repair in cells debilitated in HR and NHEJ after treatment with hydrogene peroxide (H_2O_2), bleomycine (BLM) and menadione. Our results indicate that defect in HR or NHEJ increases sensitivity of yeast cells to BLM, but not H_2O_2 or menadione. Only if HR and NHEJ were impaired simultaneously, yeast cells were also sensitive to H_2O_2 and menadione. Pulsed-field gel electrophoresis experiments showed no DSB induction in HR- or NHEJ-defective cells after H_2O_2 or menadione treatment, although after BLM exposure DSB were extensively induced. Therefore, toxicity of H_2O_2 or menadione does not seem to be primarily caused by DSB induction as is case of BLM exposure. BLM-induced DSB were not repaired in HR-defective cells, suggesting that HR is the main mechanism involved in their repair in yeast. Mutation frequency induced by all oxidising agents was significantly increased only in HR-defective cells, supporting an existing view that DSB repair by HR is error-free process.

DSB can also be induced indirectly as intermediates during interstrand cross-link (ICL) repair. In *S. cerevisiae*, there is a group of mutants that are specifically sensitive to ICL-inducing agents. Of them, *pso2*, seems to be defective in the repair of DSB that are induced by these agents. Since ICL repair involves the DSB repair proteins to complete the process, we have verified a possibility of interaction between the Pso2 and HR or NHEJ proteins. These results will be discussed.

IDENTIFICATION OF POTENTIAL CIS-REGULATORY FEATURES ASSOCIATED WITH A MODE OF GENE EXPRESSION COMMON TO NEURONAL DIFFERENTIATION AND HIPPOCAMPAL DEVELOPMENT

M. Dabrowski[1], S. Aerts[2], Y. Moreau[2], and B. Kaminska[1]

[1]*Nencki Institute, Warsaw, Poland*
[2]*Katholieke Universiteit Leuven, Belgium*

Availability of genomic sequence data of several species and of results of gene profiling from related biological systems permits a comparative approach to analysis of gene regulation. We analysed temporal gene expression profiles from published gene profiling experiments in two related biological systems: developing mouse hippocampus *in vivo*, and neurons undergoing differentiation *in vitro*, in conjunction with the genomic sequence of putative cis-regulatory regions, identified by mouse-to-human homology. We sought to identify features of the cis-regulatory regions common to many genes and predictive of particular expression pattern(s). We assumed that cis-regulatory regions of many genes share functional modules that have independent and additive (in log representation) effects on temporal expression profiles. Motivated by the above („mode-module") hypothesis, before analysis of cis-regulatory regions, we orthogonalized the expression data by the singular value decomposition (SVD). We demonstrate that a particular SVD mode is highly conserved between two experimental systems. The conserved mode reflects, depending on the sign, a component of continuous up-, or continuous down-regulation, in the course of either experiment. Using a candidate features approach, with pairs of motifs representing transcription factor binding sites as the candidates, we identified ten features, all conserved between the mouse and the human, that were predictive of the sign of the conserved mode corresponding to down regulation, when tested on the other half of the data than the half originally used to identify them. Two of the identified features were predictive of down-regulation in both experimental systems (a cross-system test). The relationship between the (unknown) gene expression space, the measurement space, and the subspaces of the SVD modes, will be discussed in view of the mode-module hypothesis.

EXPRESSION OF MEIOTIC GENES AND PHENOTYPE BY IRRADIATED TUMOUR CELLS

J. Erenpreisa [1], M. Kalejs [1], A. Ivanov [2], M. Cragg [2]; B. Liebe [3], H. Scherthan [3]

[1]Biomedical Centre Latvian University, Riga; [2]Cancer Science Division, Southampton; [3]Max Planck Institute for Molecular Biology, Berlin.

Many malignant tumours are expressing the so called cancer-testes associated genes and explanation for such a selective genome dysregulation is lacking. Our interest in this issue was stimulated by previous findings that radioresistant irradiated lymphoblastoid cell lines produced after mitotic catastrophe polyploid cells, which displayed some features of meiotic prophase and were capable to undergo DNA repair by homologous recombination and resist apoptosis. In this study we investigated in this model the expression of several meiotic genes and associated phenotype. It was shown that a principal meiotic regulator Mos/MEK/MAPK pathway is activated in p53 mutants by irradiation at the peak of arrest in a spindle checkpoint, proportionally to it, to the number of subsequently emerged polyploids, and to clonogenicity. In turn, inhibition of MEK/MAPK prevents catastrophic mitoses, reduces polyploidy and clonogenicity. Meiotic cohesin Rec8 is activated and appear in the structured form in the chromatin of arrested metaphases restituting into polyploid cells. The pattern of Rec8 immunofluorescence in most giant tumour cells corresponds that of preleptotene in the positive rat testes control. Correspondingly, we observed location of telomeres on the nuclear envelope characteristic for leptotene. The central and lateral elements of synaptonemal complexes (SC), SCP1 and SCP3 are transcribed, however at low levels. SCs and serial mitoses within the same giant cells, reducing the DNA content from 16C to 2C, were occasionally observed. Expression of all meiotic genes mentioned above was found in non-treated cells at the very low level. Conclusion: some kind of a meiosis-like program, although abortive in most emerged polyploid cells is activated by mitotic catastrophe in lymphoblastoid p53 mutants.

EPIGENETIC REGULATION OF TRANSCRIPTION AND THE ORIGIN OF ISOCHORES

Jan Filipski

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The results obtained by density gradient centrifugation in early 70-es indicated that DNA in eucaryotic genomes (especially in mammals) is compositionally heterogeneous at high molecular weight level. In particular, it was found that the composition of large DNA segments (50 kb long and longer) vary between 35 and 65% GC in human genome. The long genomic regions, which showed some compositional homogeneity, were called isochors (from Greek "equal landscape"). The evolutionary origin of the isochors became understood only recently. The likely explanation suggests that the frequently arising new alleles containing AT base pairs in the GC-rich genomic segments have a short life and are being eliminated from the population in part by selection and in part by a process independent from selection. The process which can eliminate the AT carrying alleles from a population without physically eliminating their carriers could be the biased gene conversion (non crossover recombination) occurring during meiosis. Indeed, the hotspots of recombination in eucaryots coincide with the GC rich DNA segments. These DNA sequences showing increased recombination frequency are located in the euchromatic regions of the chromosomes, in the vicinity of the transcriptional factories in the nucleus, and contain the genes available to transcription. The rarely recombining AT-rich DNA carries the genes located in the heterochromatic chromosomal regions, frequently at the periphery of the nucleus. The transcription of the genes belonging to this class is accompanied by their transportation into the vicinity of the transcriptionally active nuclear sites. This displacement is associated with epigenetic changes of the chromatin fibre carrying the activated genes.

ISOTHERMIC SEQUENCING BY HYBRIDIZATION

Piotr Formanowicz

Reading of DNA sequences remains one of the most important problems in molecular and computational biology. Although human genome has been almost fully sequenced and also genomes of some other species are already known there is a need for an efficient and inexpensive method for DNA sequencing.

The two main methods for determining DNA sequences are shotgun sequencing and sequencing by hybridization (SBH). The latter one consists of two phases: the biochemical one and the computational one. At the biochemical stage, hybridization experiment is performed, in which an oligonucleotide library is compared with many copies of one of the strands of the examined DNA molecule. Usually, the library consists of all 4^l oligonucleotides of length l. During the hybridization reaction fragments of the target DNA attach to oligonucleotides from the library in their complementary places. After the reaction one obtains a set of sequences being subfragments of the examined DNA molecule. This set is called a spectrum. In the second phase of the method a permutation of spectrum elements corresponding the the target DNA sequence is determined.

In the case of an ideal hybridization experiment (i.e. without experimental errors) the target DNA sequence may be reconstructed in polynomial time. On the other hand, if there are errors in the experiment (positive ones – excess of oligonucleotides in spectrum or negative – lack of some oligonucleotides), the computational phase of the method becomes a strongly NP-hard problem. Hence, a challenging problem is to design some new approach reducing a number of errors in the biochemical stage of SBH. Such an approach is isothermic SBH, which is based on the usage of oligonucleotide libraries consisting of oligonucleotides of equal melting temperature, instead of oligonucleotides of equal length.

It is known that duplexes of C/G rich oligonucleotides are more stable than A/T rich ones. This phenomenon may result in numerous errors of positive type. At the same time a modification of hybridization conditions directed towards diminishing the number of imperfect duplexes of C/G rich oligonucleotides may result in increasing a number of missing perfect duplexes of A/T rich oligonucleotides. The duplex formation depends on base composition and on its length. Thus, at least formally, it is possible to compensate lower stability of A/T rich duplexes by increasing their length. The key idea of isothermic SBH is to obtain a set of oligonucleotides that differ in base composition and length and are characterized by a predefined relation between base composition and length of oligonucleotides. In a specific case, where an increment of C or G is twice that of A or T and the sum of increments for each oligonucleotide in the set is constant, the set is called isothermic oligonucleotide library. It may be shown that two such libraries are sufficient to reconstruct DNA sequences by SBH approach.

IDENTIFICATION OF MODELS OF CELL SIGNALING PATHWAYS

Krzysztof Fujarewicz

This work concerns the problem of fitting mathematical models of cell signaling pathways to real data. We propose the adjoint sensitivity analysis as a very efficient tool for solving such a problem.

Mathematical models of cell signaling pathways frequently take form of a set of nonlinear ordinary differential equations. To compare different models and to test their ability to model processes, for which experimental data are given, an effective method of parameter fitting is needed. Unfortunately, while the model is for time continuum, all available measuring techniques, for example: blotting techniques, electrophoretic mobility shift assays or gene expression microarrays give measurements only at discrete time moments. Adjoint sensitivity analysis is a technique frequently used in practical optimization problems such as identification or optimal control tasks. It calculates a gradient of performance index very effectively and decreases computational costs when compared to straight (tangent linearized) sensitivity analysis. In literature devoted to adjoint systems such systems are defined as continuous in time systems or discrete in time systems and cannot be directly applied to solve the problem of parameter fitting presented above due to its hybrid, continuous-discrete time nature. In this work we present rules for creating the modified adjoint systems for continuous-discrete systems (i.e. systems containing continuous and discrete time parts, pulsers and samplers). Then it is used to solve the problem of finding the gradient of a quadratic performance index, utilized during parameter fitting procedure. The approach is illustrated on mathematical model of NF-κB regulatory module. Presented results show very good convergence of solutions in the obtained models to real data.

THE FATE OF DNA DOUBLE-STRAND BREAKS: ASPECTS OF MITOTIC AND MEIOTIC CELLS.

Wolfgang Goedecke

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Double-strand-breaks (DSB) represent a major threat to genomic DNA. Possible consequences of these lesions include repair of the DSB, the gain of new mutations in cases of misrepair, cell-cycle arrest and apoptosis. We are interested in exploring how the cell is able to integrate these different physiological responses caused by DSBs.

A protein involved in many aspects of DSB processing is MRE11. Initially it has been identified in yeast as a member of the RAD52-epistasis group, required for processing of meiotic DNA-DSB breaks using Homologous-Recombinational-Repair (HRR). In order to find proteins that are involved in the processing of DSBs, a two-hybrid-screen was performed using MRE11 as a bait. Beside expected interactors, for example the spo11-protein, a topo-II like enzyme creating DSBs for the initiation of homologous recombination during meiosis, we have also identified the Ku70-protein, suggesting a role of MRE11 in Nonhomologous-End-Joining (NHEJ). Recently, such a function of MRE11 in by the inhibition of NHEJ-activities in cell-free systems using anti-MRE11 antibodies was found. Obviously, MRE11 is involved in homology-dependent as well as homology-independent DSB-repair mechanisms. The question came up, how MRE11 could participate in such different aspects of DSB-repair.

In order to address this question, immunocytology on rat testis sections was used to investigate the localisation of Ku70 and MRE11 in premeiotic and meiotic cells. It turned out, that in cells entering meiosis, the Ku70 protein is no longer detectable. Our current working hypothesis is, that (i) NHEJ is dominant over HRR in mammalian cells and (ii) that the NHEJ activity has to be switched-off during meiosis to allow HRR of the meiotic DSBs to take place.

These data are consistent with earlier experiments in extracts from oocytes and eggs of the clawed frog *X. laevis*, where we could show, that NHEJ activity indeed becomes lost during egg-maturation.

THE SPATIAL ORGANIZATION OF DNA IN EUKARYOTIC CELL NUCLEI PLAYS AN IMPORTANT ROLE IN DETERMINING POSITIONS OF RECOMBINATION HOT-SPOTS

O. V. Iarovaia and S. V. Razin

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We have previously found that the major recombination hot-spot of the human dystrophin gene is localized to the nuclear matrix (Iarovaia et al., 2004, Nucleic Acids Res. 32, 2079-2086). In the present study we have analysed the spatial organization in interphase nuclei of the breakpoint cluster regions (*bcr*s) of the *AML*-1 and *ETO* genes frequently participating in reciprocal t(8;21) translocations. Both *bcr*s were found to be localized preferentially, but not exclusively, to the nuclear matrix, as shown by hybridization of specific probes with nuclear halos. This association was not related to transcription as the transcribed regions of both genes located far from *bcr*s were located preferentially in loop DNA, as shown by in situ hybridization. The sites of association with the nuclear matrix of the intensely transcribed *AML*-1 gene were mapped also using the biochemical PCR-based approach. Only the *bcr* was found to be associated with the nuclear matrix while the other transcribed regions of this gene turned out to be positioned randomly in respect to the nuclear matrix. The data are discussed in the framework of the hypothesis postulating that the nuclear matrix plays an important role in determining the positions of recombination-prone areas, and that inhibition of DNA topoisomerase II of the nuclear matrix may trigger a chain of events resulting in illegitimate recombination.

FIDELITY OR SURVIVAL? HOW UNREPAIRED DNA IS REPLICATED WHEN POLYMERASES ENCOUNTER PROBLEMS.

Paweł Jałoszyński

Institute of Human Genetics, Polish Academy of Sciences, Poznań

Recent studies on the translesion DNA synthesis provided much interesting data on particular role of non-replicative DNA polymerases in mutagenic events. Enzymes belonging to the Y-superfamily of polymerases (i.e. pol. eta, kappa, iota) were found to replicate past various DNA base modifications with efficiency and fidelity strongly depending on the type of lesion. Such enzymes are proposed to participate in the "two polymerases affair" action, which assumes a dissociation of replicative (i.e. pol. alpha, delta, epsilon) or repair (i.e. pol. beta) polymerase from damaged template and continuation of the reaction by less sensitive (and often more mutagenic) translesion polymerases. Our study focused on the role of 8-oxoguanine (8-OH-G) in mutagenesis occurring during DNA synthesis catalyzed by polymerase alpha, beta, eta and kappa. To measure kinetic parameters of nucleotide incorporation, we used synthetic fragments of human and mouse *ras* genes containing one or two 8-OH-G moieties in the hypermutagenic codon 12. Mutations in this particular codon are responsible for permanent activation of the gene, leading to a neoplastic transformation, and the sequence is widely used in translesion DNA synthesis studies. Polymerase alpha was found to be extremely sensitive to 8-OH-G, being strongly inhibited by a single lesion and completely blocked by two oxidized bases in a tandem arrangement; it was able to misreplicate with low efficiency, misincorporating AMP. Polymerase beta was also remarkably inhibited, but not blocked by 8-OH-G, misincorporating AMP opposite the lesion. Polymerase kappa, also inhibited by oxidized guanine, appeared to be most mutagenic in the system applied; the efficiency of misinserting AMP opposite 8-OH-G was highest amongst the studied enzymes. Pol. kappa showed also an interesting effect of "action-at-a-distance" mutagenesis, misinserting AMP opposite non-modified guanine 3'-flanked by 8-OH-G. Polymerase eta, in turn, appeared to be completely insensitive to inhibition by the lesion, even by two 8-OH-Gs in a tandem arrangement. Moreover, this enzyme misincorporated AMP opposite the lesion and (with low efficiency) opposite G 3'-flanked by 8-OH-G. Interestingly, two lesions in a tandem arrangement strongly relaxed the enzyme specificity; in this situation all four dNMPs were incorporated. This unique property may be responsible for increased mutation rates when pol. eta replaces replicative or repair polymarases, which are strongly inhibited or blocked. This action may ensure survival of cells with massive DNA damage, but it leads to dramatically increased mutagenicity.

GENOMIC APPROACH TO THYROID CANCER

Barbara Jarząb

Department of Nuclear Medicine and Endocrine Oncology, Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch

Gene expression profiling is valuable both looking for multigene classsifiers as potent diagnostic tools in oncology and dividing tumor histotypes into new subclasses, which may fit better to cancer clinics, therapy and outcome. In this way microarray-based analysis may outperform traditional histopathology, paralelly supplying information about the mechanisms of neoplastic transformation and cancer progression.

Until now we have analyzed over 70 thyroid tumors and nearly 40 benign thyroid tissues by high density oligonucleotide DNA microarrays (U133 GeneChip, Affymetrix). These results will be presented in comparison to other datasets available in the public domain to address the questions related mainly to papillary thyroid ca (the most frequent form of thyroid cancer). Sources of variance in gene expression profile, its relation to the most important clinical and pathological prognostic features of cancer disease as well as gene expression signature for diagnostic purposes will be addressed

The question of the major sources of variance in gene expression profiles of papillary thyroid carcinoma (PTC) as compared to non-tumor thyroid tissue obtained in the same patients was analyzed by Singular Value Decomposition. SVD confirmed the prevalent role of tumor/normal difference and revealed that immunity-related gene expression varied significantly between tissues and constituted the strongest confounding signal (chemokines, T cell receptor genes etc within the 2^{nd} mode and immunoglobulin transcripts prevailing in the 3^{rd} mode). Inter-individual variability played a minor role in our samples.

Relations between PTC gene expression profile and clinical and pathological features were analyzed by SAM (Significant Analysis of Microarrays), then by other techniques. Clinical factors related to stage and course of the disease (lymph node metastases, presence of distant metastases or quick relapse) revealed much closer relation to the gene expression profile than classical pathological factors of poor prognosis (tumor diameter, multifocality, capsule infiltration, angioinvasion).

By means of a Support Vector Machine-based method developed at the Silesian University of Technology (Recurrent Feature Replacement) a 20-gene classifier was selected to support PTC diagnosis. The validation step was performed on 50 thyroid samples.The classifier correctly differentiated PTC and non-malignant thyroid tissues in 90% of cases. The analysis of misclassified samples showed a subgroup of PTCs which exhibited different expression profile than the majority of tumors. This implies a necessity to include in a classifier a wider variety of gene expression profiles and raises the question of the clinical relevance of the putative PTC subtypes.

FROM MICROARRAY DATA TO THE BIOLOGICAL RELEVANCE

Michał Jarząb

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Vast amount of information, delivered by microarray-based studies, requires special methodology for adequate analysis. Until now the main attention has been devoted to the development of algorithms appropriate for biostatistical and bioinformatical evaluation of these data (preprocessing, multiple testing-related problems, redundancy, multivariate analysis, machine learning, etc.). However, the question how to extract the biological information from the results of microarray studies is still a big challenge for the biomedical community. Some approaches to extract biologically-relevant information from the microarray data will be described and discussed. All presented problems will be discussed in the context of microarray data obtained in our laboratory.

Gene ontologies - formal classification systems of various gene and protein properties – form today a key to analysis of large groups of genes. The best known ontology, developed by Gene Ontology Consortium and used by Affymetrix to annotate probes, classifies more than 10,000 human genes. Analysis of the lists of selected "significant" genes is based on classifying them according to their ontology and comparing the number of genes present in the list to the whole class. The most overrepresented ontology classes are then considered relevant from the biological point of view.

In my presentation I will show an alternative and simple approach to this task. Before the bioinformatical analysis, the whole dataset is divided according to selected ontology classes. Then, gene selection algorithms are applied to each class separately and the contents and the power of classification are evaluated for each obtained list separately. This approach allows a direct comparison of the relevance of functional gene classes to the problem of interest.

The other important point is the comparison of gene expression in obtained lists with publicly available microarray data. Visualizing gene expression in other tissues and other pathologic conditions helps to understand the role of selected genes. The public domain microarray data are growing rapidly and the role of this approach will probably increase in future.

MOLECULAR MECHANISMS OF THE INDIVIDUAL RADIOSENSITIVITY: IMPACT OF DNA REPAIR

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The extent of the normal tissue reaction of patients after radiotherapy show huge variations. The molecular reasons for this individual radiosensitivity are not clarified in detail. So far, it is known that the cellular capacity to repair DNA double-strand breaks (dsbs) affects radiosensitivity, as the number of residual dsbs and the resultant lethal chromosome aberrations correlate with cell survival of human fibroblasts. Furthermore, it was shown that most of all dsbs induced (95-98%) are repaired and that very small variations in dsb repair capacity of only a few percent are sufficient to result in a clear change of cell survival (factor about 2). Therefore, it was of interest to test the impact of dsb repair proteins, especially that of the non-homologous end-joining (Ku70, Ku80, DNA-PKcs, XRCC4), which represents the main dsb repair pathway in mammalian cells. For these experiments different human fibroblasts with clear differences in radiosensitivity (SF3.5Gy: 0.03 - 0.28) were investigated. It could be demonstrated that neither the extent of repair gene / protein expression nor of the DNA-PK activity correlate with cell survival. This indicates that the dsb repair capacity, and also the extent of radiosensitivity, is defined by more complex mechanisms.

There are now additional data indicating that also secondary dsbs arising during the repair of clustered base damage might affect cell survival. It could be demonstrated that the repair kinetics of radiation-induced oxidative base damage (8OH-guanine) is clearly faster in rodent compared to human cells ($t_{1/2}$ 20min vs $t_{1/2}$ 80min) and that this corresponds with cellular radiosensitivity ($D_{0.1}$ rodent 5.2Gy vs human 2.7Gy). Further, a stimulating effect of Tp53 on the repair kinetics of oxidative base damage was shown in Tp53 proficient mouse embryonic fibroblasts ($t_{1/2}$ 21min) compared to Tp53 deficient fibroblasts ($t_{1/2}$ 49min), which again corresponds to the radiosensitivity ($D_{0.1}$ Tp53+/+ 6.6Gy vs Tp53-/- 4.8Gy). These data indicate that not only directly induced dsbs but also base damage (cluster) and their repair kinetics might play a role in defining cellular radiosensitivity.

GENE EXPRESSION PROFILING IN HEREDITARY BREAST CANCER; PRELIMINARY RESULTS.

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Gene expression profiling allows better understanding of cancer biology and offers potential practical clinical applications. DNA microarray analyses have been successful in finding potential new molecular markers as well as molecular signatures characteristic for cases with different clinical and pathological properties. Promising findings were achieved e.g. in studies of lymphoma, breast and prostate cancer where distinct sets of genes were identified, which expression was significantly associated with prognosis.

Our aim was to analyze differences in gene expression profiles between hereditary ($BRCA1^{mut}$ linked) and sporadic breast cancer and try to find molecular basis underlying clinical differences in those diseases observed by some authors. We used HG U133 Plus 2.0 oligonucleotide microarrays allowing detection of 47 000 transcripts. Gene expression profiling was performed in following samples: i. - eight cases of medullary breast carcinoma, five with mutations in BRCA1 gene and three without mutations; ii - six cases of ductal carcinoma, three with mutated BRCA1 and three with wild type BRCA1; iii - four samples of normal breast tissue. Samples were normalized by RMA (Robust Microarray Analysis) method. At first step, we performed Principal Component Analysis (PCA), which confirmed that the distance of breast tumors to normal tissues is large enough to be detected by unsupervised method. We did not observe such a division in PCA results in respect to BRCA status and histopathology. Thus, supervised methods were applied. Among the genes that we found to be linked to BRCA status, three were previously reported by Hedenfalk et al. (N. Engl. J. Med. 2001, 344, 539-48), namely: selenium-dependent glutathione peroxidase 4 (GPX4), transducer of ERBB2 1 (TOB1; two transcripts related to BRCA status) and ARVCF (Armadllo repeat gene deleted in VCFS, catenin family). GPX4 and TOB1 were decreased in BRCA1-positive tumors, while ARVCF gene expression was slightly increased in this group.

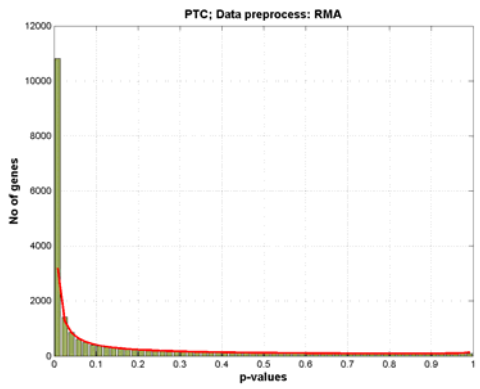
APPLICATIONS OF MIXTURE MODELS TO GENE EXPRESSION PROFILES.

J. Polańska, M. Wiench, M. Jarząb, B. Jarząb, J. Rzeszowska-Wolny, M. Kimmel, A. Świerniak, A. Polański

A very important topic in the research concerning DNA microarray data processing is modeling distribution of gene expression levels. One possible approach is using Gaussian mixtures for logarithms of expression levels. Approximating probability density functions (pdfs) of log expression levels by Gaussian mixtures can be used to solve several issues in interpretation of DNA microarray data. Decomposition of data into normal components is setting thresholds to classify expression levels as "change", "no change", "overexpressed", "underexpressed" etc., based on pdfs of estimated components. A related area is mixture modeling of values of sets of significance levels obtained from DNA microarray scanners and classical statistical analyses applied to gene expression profiles. It is formulated as the problem of estimating False Discovery Rate (FDR) to properly address the multiple testing phenomena in DNA microarrays. Recently a technique to solve the FDR problem by decomposing the distribution of significance levels to (at least) two components, beta and uniform and was described in the paper by Gadbury et al. (2004). The p-value distribution is modelled as a mixture of ν+1distributions on the interval [0, 1]:

$$f(p) = \prod_{i=1}^{k} \sum_{j=0}^{v} \lambda_j \beta\left(p_i | r_j, s_j\right)$$

where p_i is the p-value from a test on the *ith* gene, p is the vector of k p-values, and λ_j is the probability that a randomly sampled p-value belongs to the *jth* component. If no genes are differentially expressed, the sampling distribution of p-values will follow a uniform distribution on the interval [0,1]. If some genes are differentially expressed, additional beta distribution components will be required to model a set of p-values clustering near 0. Maximum likelihood estimates of the parameters can be obtained using numerical methods. The decomposition applied to data on thyroid cancer is shown in the plots below.



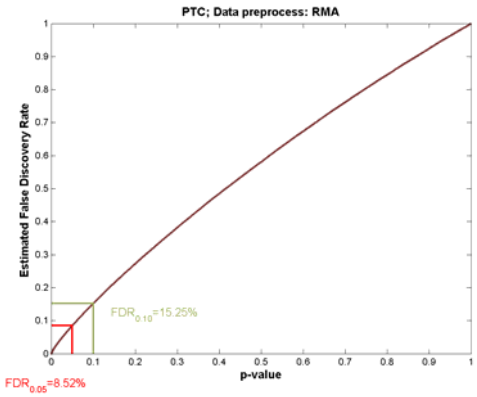


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Figure in the left hand side shows the quality of the decomposition into beta and uniform components (red curve versus histogram). Using the decomposition the plot in the right can be computed which allows the following conclusion to be drawn: setting the threshold for a single test equal to 0.05 will result in 8.5% of FDR. Further analysis will lead to estimating TP and TN levels equal to TN=65% and TP=97%. Increasing the threshold to 0.1 will result in increasing FDR to 15.25% and TN to 75% and changing TP to 95%.

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GENE EXPRESSION PROFILES AND CELLULAR STRESS

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Using oligonucleotide microarrays we studied the changes of gene expression in response to ionizing radiation treatment of human erythroleukemic cells K562. In analyzing the results we used a new approach utilising Gaussian mixture model to describe total gene expression profiles obtained in all hybridizations and to compare expression in differentially treated cells. Metropolis-Hastings algorithm was used to determine the best fitting Gaussian components that describe experimentally obtained histograms. The Gaussian component patterns were characteristic for cell type and changed after irradiation with the dose of 4 Gy. The histograms describing frequencies of hybridization signal level in control K562 were decomposed into five fractions. Among genes found in low signal level fractions #1, #2 and #3 prevailed receptor genes, signal transduction proteins and transcription factors. Fractions #4 and #5 contained mainly genes for proteins taking part in translation process. Gaussian mixture model applied in two-sample comparisons allowed to identify genes that significantly changed their expression after irradiation. Ionizing radiation caused synchronous down-regulation of more than 2700 genes mainly from fraction #2. The mean transcription level of genes in this fraction is about 2 molecules per cell. The model of populations with non-uniform gene expression will be discussed.

CD150-MEDIATED SIGNALING

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The CD150 is a prototypic receptor of a subfamily of dual-function coreceptors within CD2 family. The CD150 subfamily consists of CD150 along with CD84, CD229, CD244, NTB-A/SF2000, and CS1/CRACC. The genes of CD150 subfamily are clustered together on a long arm of chromosome 1 at bands 1q21-24. The criteria for defining this subfamily are the presence of at least two characteristic signaling motifs TxYxxV/I (ITSM – immunoreceptor tyrosine-based switch motif) in the cytoplasic tail of the receptor and binding of adaptor proteins SH2D1A and/or EAT-2 to these ITSMs. The ITSM is different from immunoreceptor tyrosine-based motifs ITAM and ITIM. Like ITIM, ITSMs are tyrosine phosphorylated by Src-family kinases and serving as binding sites for SH2-containing phosphatases: SHP-1, SHP-2 and SHIP. Unlike ITIM, ITSMs bind directly adaptor proteins SH2D1A and EAT-2, Src-family kinases Fyn(T), Lyn, Fgr and the p85 subunit of PI3-kinase. The paired ITSM within cytoplasmic tails of the receptors contribute to the dual-function of CD150 subfamily receptors. At least two signal transduction pathways are initiated via CD150 subfamily receptors. Erk1/2 pathway requires Ras, Raf, MEK1/2 and SHIP, but not SH2D1A. Both SHP-2 and SHIP may contribute to CD150-mediated Erk-activation. CD150 ligation also results in PI3K-dependent Akt phosphorylation. This pathway depends on SH2D1A and Syk expression; however it is SHIP-independent and is negatively regulated by Lyn, Btk and SHP-2. Taken together, CD150 subfamily receptors mediate different functions depending on the availability of downstream molecules within their signal transduction pathways, and SH2D1A functions as a molecular switch. Analysis of CD150 and SH2D1A expression in non-Hodgkin's and Hodgkin's lymphomas revealed stages of B cell differentiation where these molecules are expressed alone or coexpressed. Signaling studies in Hodgkin disease cell lines showed that CD150 is linked to the ERK and Akt pathways in neoplastic B cells. Our data support the hypothesis that CD150 and SH2D1A are coexpressed during a narrow window of B cell maturation and SH2D1A may be involved in regulation of B cell differentiation via switching of CD150-mediated signaling pathways.

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THE ROLE OF MOLECULAR CHAPERONS IN CANCER

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Physiological stresses (heat, hemodynamics, genetic mutations, oxidative injury and myocardial ischemia) produce pathological states in which protein damage and misfolded protein structures are a common denominator. The specialized proteins family of antistress proteins – molecular chaperons (HSPs) – is responsible for correct protein folding, dissociating of protein aggregates and transport of newly synthesized polypeptides to the target organelles for final packaging, degradation or repair. They are inducible in different cellular processes such as division, apoptosis, signal transduction, differentiation and hormonal stimulation. HSPs are involved in numerous diseases including cancer, revealing changes of expression and cell localization.

The investigation of molecular and cellular mechanisms of anticancer immune response has led to the discovery that some members of HSPs family induce specific protection against cancer.

Recently it has been observed in rodent models that autologous tumor-derived HSP-peptide complexes were able to induce a specific anti-tumor immune response, i.e. were effectve in adjuvant setting. Moreover, the overexpression of some HSPs (as Hsp70, Hsp60 and Hsp27) in membranes of some tumors (such as breast cancer, endometrial-cervix cancer) correlated with poor prognosis and resistance to therapy while in osteosarcoma, squamous cell carcinoma of the esophagus and renal cell carcinoma the Hsp70 over-expression was associated with improved prognosis and response to chemotherapy. The specific anti-HSPs autoantibodies level correlated in some cases with the target organ injury degree.

We studied changes of the main mitochondrial chaperon Hsp60 expression level and re-localization in cells of thyroid cancer as well as accompanying autoimmune processes using Western-blot and immunohistochemical analysis. Positive correlation between Hsp60 expression level, anti-Hsp60 autoantibodies level and thyroid gland injury degree has been observed. These changes correlated with re-localization of Hsp60 (anomal expression on the tumor cells surface) and (in many cases) with drug-resistance of patients investigated.

Based on the presented and literature data we have proposed a new approach to the development of an effective therapeutic and diagnostic tool for cancer treatment.

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SVD AS A TOOL FOR PATTERN DISCOVERY IN GENE EXPRESSION DATA.

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Singular Value Decomposition (SVD) is a matrix factorization known from linear vector algebra, which reveals many important properties of a matrix. It is a standard tool in many areas of physical sciences, and many algorithms in matrix algebra make use of SVD. Recently, gene expression data were analyzed using Singular Value Decomposition. In gene expression data analysis the principal aim of application of SVD is to detect and extract internal structure existing in the data and corresponding to important relationships between expression of different genes.

We aim to investigate the effectiveness of SVD as a preprocessing step to cluster analysis of gene expression data. In the clustering literature, SVD is sometimes applied to reduce dimensionality of the data set prior to clustering. The idea behind using SVD prior to cluster analysis is that SVD may extract the cluster structure in the data. Since characteristic modes are uncorrelated and ordered, the first few that are the most significant and reflecting most of data variation are usually used in cluster analysis.

Our approach differs from that known from the literature, where characteristic mode coefficients (gene coefficient vectors), are used for clustering instead of original variables. We propose to apply SVD to select a set of original genes and then apply them for clustering samples by one of standard algorithms.

The proposed gene selection algorithm inspects gene coefficient vectors corresponding to the set of the most significant characteristic modes. Each coefficient is compared to the threshold value, the meaning of which is similar to a 3σ statistical significance cutoff. If the magnitude of the element is greater than the threshold, the corresponding gene is selected to the clustering set. In practice we choose genes having sufficiently big coefficients for the most important characteristic modes. Variation of the threshold value gives possibility of changing a number of selected genes. In the result we obtain a set of genes having patterns ‘similar’ to the dominant modes.

We test our approach on three different sets of biological gene expression data aquired in in the Comprehensive Cancer Centre Maria Skłodowska-Curie Memorial Institute Branch Gliwice, Poland, using Affymetrix Human Genome U133A arrays.

This work was partially supported by grant BW-424/RAu1/04.

FAST DENOISING OF cDNA MICROARRAY IMAGES

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The cDNA microarray is an effective tool for simultaneously assaying the expression of large numbers of genes and is perfectly suited for the comparison of gene expressions in different populations of cells. A cDNA microarray is a collection of spots containing DNA, deposited on the surface of a glass slide. The spots occupy a small fraction of the image area and they have to be individually located and isolated from the image background prior to the estimation of its mean intensity.

The fluorescent intensities for each of the spots are measured using two light sources of different wavelength, producing a two-channel image. The image is false colored using the red and green color for each image components, which represent the light intensity emitted by the two fluorescent dyes used in this method.

The quantitative evaluation of microarray images is a difficult task. The major sources of uncertainty in spot finding and measuring the gene expression are variable spot sizes and positions, variation of the image background and various image artifacts. Additionally the natural fluorescence of the glass slide and non-specifically bounded DNA or dye molecules add a substantial noise floor to the microarray image. To make the task even more challenging, the microarrays are also afflicted with discrete image artifacts such as highly fluorescent dust particles, unattached dye, salt deposits from evaporated solvents, fibers and air-borne debris. So, the task of microarray image enhancement is of paramount importance.

In this work a novel, ultrafast noise reduction method for the enhancement of multichannel images is presented. This novel technique can be successfully applied for the denoising of the cDNA microarray images, as those pictures consist of two channels and are of huge dimensions, which makes their processing very time consuming.

The proposed method is a simplification of the so called *Peer Group Filtering*. Instead of calculating the adaptive threshold, which determines the number of pixels similar to the central pixel in the filtering window, a global threshold for the similarity function can be experimentally determined. Additionally, for not too high noise intensities, the number of pixels belonging to the peer group can be established. In this way the algorithm calculates the number of pixels in the filtering window, which satisfy a certain similarity criterion and checks if this number is below a certain, experimentally established threshold value. If this is the case, then the central pixel is being replaced by the local vector median of the samples, otherwise the central sample is retained unchanged.

The new technique is capable of reducing various kinds of noise present in the microarray images and it enables efficient spot localization and estimation of the gene expression level due to the smoothing effect and preservation of the spot edges. The presentation will include comparison of the new technique of noise reduction with the standard procedures used for the processing of vector valued images, as well as examples of the efficiency of the new algorithm when applied to typical cDNA microarray images.

PROCESSING OF LIPID PEROXIDATION-DERIVED DNA ADDUCTS BY REPAIR ENZYMES AND DNA POLYMERASES

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Lipid peroxidation leads to the formation of a large family of alkenals, among which *trans*-4-hydroxy-2-nonenal (HNE) is one of the most ubiquitous. HNE forms adducts to all four DNA bases, however with different efficiency: G>C>A>T. These are cyclic propano- or ethenoadducts bearing a hexyl- or heptyl- side chains. Due to the fact that reaction of aldehyde group with the nitrogen atom of DNA base is reversible, HNE can also form inter-strand DNA cross-links, and these constitute around 5% of damaged DNA. Interaction of HNE with DNA is sequence-specific. *In vitro* studies on *p53* gene sequence show that the major hot-spots for *p53* mutations are readily modified by HNE, with the possibility of cross-links formation within three hot-spot codons 175, 248 and 249.

HNE-induced modifications block DNA synthesis, and reveal mutagenic properties in bacterial and mammalian cells. In bacteria, the major target for mutations are HNE adducted cytosine residues. HNE-DNA lesions when present in single-stranded DNA, are highly recombinogenic. Removal of these lesions from the double-stranded DNA is realized mainly by the nucleotide excision repair pathway. Equally important mechanism of avoiding deleterious effects of HNE-adducts presence in DNA is translesion synthesis by damage-specific DNA polymerases. *E.coli* DNA polymerase IV is engaged in lesions bypass, probably in an error-free manner. DNA polymerase V is indispensable for induction of mutations.

Human cells deficient in nucleotide excision repair, both global (XPA) and transcription-coupled (CSB) are more sensitive, and develop higher level of sister chromatid exchanges (SCE) than their repair proficient counterparts under low, micromolar HNE concentrations. Similar are Chinese hamster cells deficient in nucleotide excision repair (XPD) and non-homologous end joining (NHEJ). Interestingly, transcription-coupled repair deficient line (CSB) appear to be more sensitive (10-100 fold) to HNE than any other tested cell line. Thus NER and NHEJ pathways are engaged in processing of the HNE-induced DNA damages in mammalian cells. HNE-induced DNA damages, if not repaired, may block replication, transcription and trigger recombination in mammalian cells.

Hsp90 AND MDM2 ARE IMPORTANT FOR THE TRANSCRIPTIONAL ACTIVITY OF WILD-TYPE p53

Bartosz Wawrzynow, Dawid Walerych, Grzegorz Kudla, Malgorzata Gutkowska, Aleksandra Helwak, Joanna Boros, Alicja Zylicz and Maciej Zylicz

International Institute of Molecular and Cell Biology in Warsaw, Institute of Experimental Biology PAS, Institute of Biochemistry and Biophysics PAS

The p53 tumour suppressor protein is a transcription factor which regulates cellular response to stress, abnormal cell proliferation and DNA damage. In a non-stress situation, the level of p53 in the cell is low, mainly regulated at the posttranslational level by the E3 ubiquitin ligase, MDM2. Hsp90 is known to interact with both p53 and MDM2 but the role of this interaction is not clear.

Immortalized human fibroblasts were used to investigate the interactions of the Hsp90 molecular chaperone with the wild-type p53 tumor suppressor protein. We show that geldanamycin or radicicol, specific inhibitors of Hsp90, diminish specific wild-type p53 binding to the *p21* promoter sequence. Consequently, these inhibitors decrease *p21* mRNA levels. This leads to a reduction in cellular p21/Waf1 protein, known to induce cell cycle arrest. To support our *in vivo* findings, we used a reconstituted system with highly purified recombinant proteins to examine the effects of Hsp90 on wild-type p53 binding to the p21 promoter sequence. We show that the incubation of recombinant p53 at 37^{o}C decreases the level of its wild-type conformation and strongly inhibits the *in vitro* binding of p53 to the *p21* promoter sequence. Interestingly, hHsp90 alfa in an ATP-dependent manner can positively modulate p53 DNA binding after incubation at physiological temperature of 37^{o}C. Consistent with our *in vivo* results, geldanamycin can suppress Hsp90 ability to regulate *in vitro* p53 DNA binding to the promoter sequence. We have shown that Hsp90 partially unfolds p53. Unfoldase activity of Hsp90 requires the presence of ATP. Surprisingly, MDM2 accelerates this reaction. Moreover, MDM2 alone possesses the chaperone activity. We propose that partial unfolding of p53 catalysed by Hsp90 and MDM2, and the subsequent spontaneous refolding of p53 back into a wild-type-like conformation, may prevent p53 aggregation thus increasing the p53-DNA binding. These events would ultimately promote the p53 transcriptional activity and allow for the ubiquitination and degradation of p53 protein.

THE OTHER FACE OF HISTONE H1

Piotr Widłak, Magdalena Kalinowska, Missag H. Parseghian, Xu Lu, Jeffrey C. Hansen and William T. Garrard

Histone H1 is a multigene protein family, consisting of six different proteins in somatic cells of mammals. Histone H1 is an essential protein involved in regulation of chromatin structure and gene expression. Most recently a specific histone H1 subtype was demonstrated to be a messenger from the nucleus to the cytoplasm, signaling DNA damage and triggering apoptosis. Upon DNA damage, histone H1.2 was shown to leak to the cytoplasm, trigger the release of cytochrome c from mitochondria and initiate apoptosis. The apoptotic nuclease, DNA fragmentation factor (DFF40/CAD), is primarily responsible for internucleosomal DNA cleavage during the terminal stages of programmed cell death. Previously we demonstrated that histone H1 greatly stimulates naked DNA cleavage by this nuclease.

In this work we investigate the mechanism of this stimulation with purified, recombinant, and synthetic mouse and human histone H1 species. We find that each of the six somatic cell histone H1 subtypes, which differ in primary sequence, equally activate DFF40/CAD. Using a series of truncation mutants of recombinant or synthetic mouse histone H1-0 species, we demonstrate that nearly as efficient stimulation can occur with different ~50- to 72-amino acid long fragments of the C-terminal domain (CTD). We show further that histone H1 C-terminal segments bind to DFF40/CAD and confer upon it an increased ability to bind to DNA. We conclude that the interactions identified here between histone H1 segments and DFF40/CAD effectively target linker DNA cleavage during the terminal stages of apoptosis.

RET PROTOONCOGENE AND GENE EXPRESSION PROFILE OF THYROID CANCER

Małgorzata Wiench

Structural rearrangements of the *RET* gene leading to its oncogenic activation cause the neoplastic transformation in about 30% of papillary thyroid cancers (PTC). They are linked to radiation history on the one hand and to the young age of presentation on the other. It remains to be determined whether the same signal transduction pathways are stimulated in both *RET*-positive and *RET*-negative tumors, whether they occur on different levels of the same pathway (MAP kinase cascade), or rather there are quite different mechanisms underlying the follicular cell transformation. The purpose of this study was to evaluate whether *RET*-positive and *RET*-negative tumors show distinct patterns of gene expression.

For gene expression studies the cRNA obtained from 12 *RET*-positive and 17 *RET*-negative PTC tumors were hybridized to Human Genome U133A arrays according to Affymetrix recommendations. The presence of *RET* rearrangement was confirmed by RT-PCR study.

All data were obtained using MAS 5.0 software (Affymetrix). Comparison of gene expression between groups was performed by SAM (Significance Analysis of Microarrays). For further analysis, the list of 343 genes differentiating *RET*-positive and *RET*-negative tissues was chosen [5,02% False Discovery Rate (FDR) implies 17 falsely positive genes]. Among them there were 71 genes up-regulated in *RET*-positive and 272 genes up-regulated in *RET*-negative tissues. The Gene Ontology analysis revealed the striking over-representation of the cell communication-related and morphogenesis-related genes in the *RET*-positive cancers. In the group of *RET*-positive up-regulated genes there were 21 transcripts with at least two-fold change whereas only two transcripts (*TPD52L1, RRAGD*) fulfilled this criterion in the *RET*-negative over-expressed genes. A special attention was paid to signal transduction genes frequently represented in *RET*-positive PTCs: *NFKBIA, RGS2, SLC2A3, GEM, NR4A2, NR4A3, OPCML, PASK* as well as *RET* itself.

The putative difference between *RET*-positive and *RET*-negative papillary thyroid cancers is not distinct enough to be deciphered by an unsupervised method (i.e. SVD). Nevertheless, the supervised selection is able to identify a panel of differentially expressed genes, mainly contributing to cell communication and signal transduction processes in tumors initiated by *RET* rearrangement.

DO INTERINDIVIDUAL DIFFERENCES IN LYMPHOCYTE CHROMOSOME RADIOSENSITIVITY EXIST?

Andrzej Wójcik

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Individuals show marked differences in radiation sensitivity. This has considerable consequences both in the fields of radiation protection and radiation therapy. Despite numerous studies no single, reliable assay has been established that reflects the intrinsic, individual sensitivity. Human peripheral blood lymphocytes make up the biological system of choice. The endpoints used most frequently are chromosomal aberrations, micronuclei or kinetics of DNA repair. Each assay has its advantages and pitfalls.

We are presently studying the frequencies of radiation-induced aberrations in lymphocytes of patients with larynx carcinoma. Furthermore we search for a possible correlation between the kinetics of DNA repair measured by the alkaline comet assay and the frequency of chromosomal aberrations. Finally, we study, by chromosome painting, the extent of interindividual differences in the sensitivity of selected chromosomes in lymphocytes of healthy donors. Results will be presented and discussed with reference to published data.

Poster abstracts

1. THE EFFECT OF INHIBITION OF PARP ON THE FREQUENCY OF HOMOLOGOUS RECOMBINATION IN CHO-K1 WILD TYPE AND XRS-6 MUTANT CELL LINE

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The aim of this project was to examine the role of PARP-1 (poly(ADP-ribose) polymerase-1) in repair of DNA double strand breaks. In mammalian cells DSB are repaired by nonhomologous end-joining (NHEJ) and by homologous recombination (HR). CHO-K1 wild type and xrs-6 mutant cell line were transfected with pLrec plasmid carrying two nonfunctional copies of the galactosidase (lacZ) gene in a tandem array. As a result of recombination they give rise to a functional copy of beta-galactosidase. Isolated transfected clones were used to examine the effect of ADP-ribosylation inhibition on the frequencies of spontaneous and X-ray- (2 Gy) induced recombination. The cells were incubated with the PARP-1 inhibitor (AB) and recombination frequency was determined with histochemical or flow cytometry methods. Cells were cultured in the medium containing G418 or left to grow without G418 (5 days before and 2 days after treatment). This treatment allows to distinguish between reciprocal gene exchange/HR (loss of neo gene) and gene conversion (neo gene is still present and cells are resistant to G418). The level of -galactosidase activity (reflecting the frequency of spontaneous recombination) in transfected CHO-K1 cells was 2-3 times lower than in xrs-6 cells, whereas frequency of recombination per generation measured by flow cytometry was one order of magnitude lower. However, significant differences between individual clones have been observed. Irradiation of the cells with 2 Gy of X-rays insignificantly elevated the level of the enzyme in both cell lines. As expected, in non-irradiated cells, release of the selective pressure (7 days without G418) significantly elevated the level of beta-galactosidase activity as compared with that in cells cultured with G418. These results suggest that the defect in NHEJ-mediated DSB repair pathway results in elevated frequency of HR. No effect of inhibition of poly(ADP)rybosylation was observed in this experimental model.

2. ETOPOSIDE-INDUCED APOPTOSIS OF HL-60 CELLS MAY BE ENHANCED BY PYRROLIDINE DITHIOCARBAMATE (PDTC).

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Chemotherapy is one of the main strategies used for medical intervention in cancer treatment. Most of antineoplastic drugs induce programmed cell death – apoptosis. Agents that interfere with the ability of an antineoplastic drug to induce apoptosis may limit drug efficacy. Reactive oxygen species activate cell death but mostly by non-apoptotic mechanism causing damage to the surrounding tissue. According to the published data cells killed *in vivo* in the presence of hydrogen peroxide are not phagocytosed until after they have begun to leak their contents into the extracellular space, what may induce a cycle of chronic inflammation and further interference with chemotherapy action. The overall effectiveness of chemotherapy might by improved by co-administering antioxidants with chemotherapeutic agent.

The aim of the presented study was to determine, whether the antioxidant: *pyrrolidine dithiocarbamate* (PDTC) enhances etoposide-induced apoptosis of cancer cells. Using HL-60 cells, human acute promyelocytic leukemia cells, we show that PDTC used at 12.5 – 25 μM concentration administrated with *etoposide* increases the apoptotic cell death. Proliferation of HL-60 cells treated with *etoposid*e dropped down with increasing dose of drug, the values of cytotoxic index (IC50) was estimated as 17 μM (24 hrs incubation). The highest apoptotic cell death induction was observed in drugs combination: 12.5 μM of PDTC with 4 μM of *etoposide*.

Quantitative analysis of *bcl-2* and *bax* mRNA showed changes in *bcl2/bax* ratio: from 0.525 for *etoposide*-treated HL-60 cells to 0.334 in HL-60 treated with PDTC and *etoposide*.

On the basis of presented results a conclusion can be made that administration of antioxidant *pyrrolidine dithiocarbamate* increases *etoposide* antineoplastic efficiency regarding to human HL-60 leukemia cells apoptosis.

3. PLASMID DNA COMBINED WITH CYCLOPHOSPHAMIDE INHIBITS B16(F10) MELANOMA METASTASES SPREAD INTO MOUSE LUNGS.

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The aim of the presented study was to examine if gene therapy using plasmid DNA constructs encoding genes of anti-angiogenic proteins can be effectively applied to inhibit experimental B16 (F10) melanoma metastases in mouse lungs. Two different therapy approaches were used. The first was based on transferring plasmid DNA into muscle tissues via electroporation; second involved systemic transfer of polyplexes formed by the plasmid construct and PEI 750 kDa or PEI 25 kDa.

In experiments involving constructs' transfer via electroporation the number of lung metastases was decreased two-fold only in mice treated with endostatin and sFlt-1 genes. No prolonged mice survival was observed as compared to controls. Both experimental and control animals died at approximately the same time.

In therapy mediated by polyplexes two-fold decrease in the number of metastases was seen in both mice treated with constructs encoding antiangiogenic proteins as in mice treated with constructs containing empty plasmid. Neither this solution resulted in prolonged animal survival. The decreased number of metastases resulting from use of polyplexes was a result of CpG motifs present in plasmid backbone and not of therapeutic genes' presence in the tested constructs.

Survival rates were improved by treatment of mice with a combination of cyclophospamide and plasmid DNA. Our data indicate for the first time that only specific combinations of plasmid DNA with cyclophosphamide can inhibit metastatic spread and significantly prolong survival rates.

4. DNA ADDUCTS FORMATION AND DNA CROSSLINKING BY C-1748, A POTENT ANTITUMOR 4-METHYL-1-NITROACRIDINE IN HUMAN COLON CANCER CELLS

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4-Substituted 1-nitroacridines represent a new group of acridine derivatives synthesized at Gdansk University of Technology. Compared to parent 1-nitroacridines, these compounds exhibit lower toxicity and enhanced antitumor efficacy especially against colon and prostate cancers. Initiation of the first phase of clinical evaluation is proposed for the leading derivative, 4-methyl-1-nitroacridine, denoted C-1748. The introduction of an electron donating methyl group into position 4 (*para* to 1-nitro) decreased the susceptibility of 1-nitro group to reduction. This 1-nitro group is crucial for biological activity and also for the ability of 1-nitroacridines to form DNA adducts and induce interstrand DNA crosslinking. In the present study, we compare DNA covalent binding properties of C-1748 and its parent 4-unsubstituted analogue – C-857. Two radioactive methods were used: ^{32}P-post-labelling technique for detection of covalent DNA binding and modified Parsons method for detection of interstrand DNA crosslink formation. The maps of ^{32}P-labelled adducts formed by C-1748 displayed more chromatographic spots (adducts) than those obtained for C-857 under experimental conditions in human colon carcinoma HT29 cells. The chromatographic pattern of DNA adducts observed in cell-free system resembled the ones observed in cell system. Using Parson's method it was shown that C-1748 and C-857 were able to form DNA crosslinks in human colon cancer HCT-8 cells in a dose-dependent manner. The DNA crosslinking bonds induced by 1-nitroacridinones were stable in alkaline conditions but they were sensitive to high temperature. In conclusion, obtained results suggest that this new generation of 1-nitroacidines is able to bind covalently to DNA and crosslink DNA. Thus DNA seems to be their major molecular target for covalent modification.

5. SECOND PRIMARY TUMORS OF HEAD AND NECK – LOH PATTERN ANALYSIS AND ASSESSMENT OF THEIR CLONALITY STATUS.

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The reason of treatment failures in head and neck tumors is often linked to the appearance of second primary tumors (SPT). There are three mechanisms of SPT development of clonal or non clonal secondary tumors: 1^{o} via micrometastases (clonal); 2^{o} from a common carcinogenic field (SFT) (partially clonal); 3^{o} via independent events (from different carcinogenic fields – "true" SPT) (not clonal).

In this study a set of 12 microsatellite markers was used to find similarities and/or differences in allelic imbalance patterns between 22 pairs of tumors (index and SPT) obtained from the Department of Otolaryngology, K. Marcinkowski Medical University, Poznań, Poland and to confirm if two tumors originated and progressed from a single cell indicating clonal progression or independently in different locations as a result of unrelated initiations of carcinogenesis.

The results allowed to distinguish unrelated second primary tumors in 9 (41%) cases and in 4 (18%) cases particular tumors carried clonal genetic changes. In 9 (41%) cases the results were insufficient or ambiguous to state the clonality status.

Assessing the clonality of diagnosed tumors is not only a matter of theoretical dispute but carries important clinical implications including chemoprevention, radiotherapy and general patient management and thus should become a routine molecular diagnostic tool.

6. ENERGY CHARGE AND CELL PROLIFERATION ACTIVITY AS MARKERS OF THE MALIGNANCY IN GLIOMAS.

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Molecular analysis is currently being used in clinical diagnostics of tumors. One of the methods for assessment of tumor malignancy is determination of transcriptional activity of histone H3 genes. Expression of H3 encoding genes correlate with DNA synthesis both in normal and malignant cells while H3 transcripts are absent in cells that do not divide. Tumor cells are characterized by high proliferation, inhibition of differentiation and changes in metabolism. ATP concentration, which reflects balance between synthesis and hydrolysis in reactions requiring energy is directly associated with cell metabolism. The depletion of ATP synthesis influences adenylate energy charge of the cell.

The aim of our study was to investigate proliferation activity in gliomas of different malignancy and to compare expression level of histone H3 genes with energy metabolism in gliomas.

The studied material consisted of brain tissue obtained form stereotactic biopsy. Biopsy specimens were divided into two parts: one part was used for RNA extraction and another one for extraction of phosphorylated nucleotides. Total RNA extracted form the material served as a template in Real-Time RT-PCR (TaqMan) for determination of the transcriptional activity of histone H3 genes using ABIPRISM 7700 Sequence Detector System and β-actin as an internal control. Each result was normalized on the basis of β-actin transcript content. HPLC analyses of ATP, ADP and AMP were carried out using a Hewlett Packard 1100 system with a diode array UV-VIS detector. The extracts kept at -70^{o}C were melted just before analysis, immediately filtered (0.22 μm, Millipore) and injected onto the C_{18} Eurospher column (4 x 250 mm, 5 μm, Knauer). A gradient mixture of methanol, ammonium acetate (0.2 M) and water was used for elution. Separations were performed at 30^{o}C using 1 ml/ min flow rate. Determination of ATP, ADP and AMP was based on retention times and spectral characteristics comparison with the corresponding standards. A library search function of the HPLC ChemStation software was used in all experiments. Amounts of the adenylate nucleosides were calculated using appropriate calibration curves.

Our study indicates that the histone H3 genes were expressed in all examined samples. The highest level of mRNA H3 was found in glioblastoma. This corresponded to the low energy charge. On the contrary, astrocytoma II and III showed lower levels of mRNA H3 and higher energy charge.

7. THE ROLE OF ERCC3/XPB IN GLOBAL AND TRANSCRIPTION-COUPLED DNA REPAIR

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Mutation of the *ERRC3/XPB* gene in humans gives rise to the distinct autosomal recessive disorders with a striking clinical heterogeneity: xeroderma pigmentosum associated with Cockayne's syndrome (XP/CS) and trichothiodystrophy (TTD). XPB is a subunit of a multifuctional RNA polymerase II general initiation factor TFIIH and codes for 3'–5' DNA helicase essential for both nucleotide excision repair (NER) and transcription. In NER, it unwinds DNA around the damage by about 25-30 base pairs forming an open complex. In transcription, XPB functions at multiple steps to promote efficient initiation and promoter escape by RNA polymerase II. There have been only five XP-B patients with three naturally occuring mutations identified so far. The *XPB* gene was shown to be a homologue of the hamster *ERCC3* gene. Hamster cell lines belonging to the third complementation group are a unique source for the structure – function analysis of the ERCC3/XPB protein.

To study the role of the ERCC3/XPB protein in the removal of variuos DNA damages, we focused on the role of NER and TCR in the removal of DNA damage caused by ultraviolet (UV-C)-irradiation and hydrogen peroxide (H_2O_2) in the wild type and 9 UV-sensitive ERCC3 hamster mutant cell lines. All the tested mutant cell lines are very sensitive to killing by UV-C and are unable to recover RNA synthesis after 10 Jm^{-2} UV-C, suggesting a defect in TCR. They also have limited global NER capacity measured by the single cell gel electrophoresis assay (0.25 Jm^{-2}). Their sensitivity to H_2O_2 is the same as in the wild type cell lines suggesting they possess functional global BER.

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8. HUMORAL IMMUNE RESPONSES INDUCED IN MICE WITH 12-AA CONSTRAINED PEPTIDES WHICH MIMIC GD2 GANGLIOSIDE

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Neuroblastoma is the most common extracranial tumour in childhood. Despite applying standard treatment including intensive induction, surgery, radiotherapy, myeloablative therapy with stem cell transplantation the majority of the high risk patients eventually die of relapsing disease. This stresses the need for new therapeutical approaches to treat minimal residual disease, which might improve the survival of high risk group neuroblastoma patients. Immunotherapy of neuroblastoma is one of the currently developed strategies. It aims to mobilise innate and adoptive immunity of patients for recognising and eradicating tumor cells.

Our goal was to develop active specific immunotherapy of neuroblastoma, which targets neuroblastoma associated carbohydrate antigen, namely GD2 ganglioside. Gangliosides have been shown to be weakly immunogenic, since they belong to a group of T cell independent antigens. For this reason, we are working on application of peptide vaccines, which could structurally and functionally mimic GD2 ganglioside.

We have panned phage display peptide library LX-8, which encodes for 12 aa peptides constrained with disulphide bridge, using mouse monoclonal anti-GD2 ganglioside antibody 14G2a. Five peptides sequences have been identified. The synthesised peptides have been shown to bind to 14G2a antibody in competition assay using GD2 ganglioside-positive human neuroblastoma cell line IMR-32.

Using BALB/c mice model we tested the peptides for their ability to induce GD2 ganglioside specific immune responses. Animals were immunised with 3 doses of peptides conjugated to carrier protein KLH (keyhole limpet hemocyanin) in combination with adjuvants: Freund's adjuvant and GM-CSF. GD2 ganglioside boosting has been applied for the final immunisation step. Three weeks after the last dose of our vaccine sera samples were collected and analysed for anti-GD2 ganglioside humoral responses. In order to find the best epitope that mimics functionally GD2 ganglioside we have analysed the sera for the level and isotype of the antibodies that bind to the immobilised GD2 ganglioside with ELISA. In addition, flow cytometry technique has been utilised to show the cross-reactivity of the murine sera with the glycolipid expressed on neuroblastoma cells.

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9. THERAPEUTIC EFFECT OF VASOSTATIN IN THE TREATMENT OF B16(F10) MURINE MELANOMA

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It has become almost indisputable that angiogenesis i.e. formation of new blood vessels out of pre-existing capillaries, is essential to development of the tumor vasculature. The discovery of specific antiangiogenic inhibitors would have important therapeutic applications in the treatment of cancer. Vasostatin, the N-terminal domain of calreticulin, is a potent endogenous inhibitor of angiogenesis and tumor growth. In this study we compared vasostatin gene therapy strategy with administration of purified recombinant vasostatin in B16(F10) murine melanoma model. We applied the combination of intramuscular gene transfer of vasostatin by electroporation and cyclophosphamide using an antiangiogenic schedule of drug dosing. In such combined therapy we observed synergistic antitumor as well as extended survival of treated mice. However, when melanoma-bearing mice received i.t. recombinant vasostatinwe observed a more significant suppression of tumor growth and prolonged survival. These initial results suggest that vasostatin protein applied in cancer therapy is more effective than combination of vasostatin gene and cyclophosphamide and that recombinant vasostatin might find potential therapeutic use in the future.

Key words: vasostatin; gene therapy; angiogenesis; cyclophosphamide

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10. EXPRESSION OF EXTRACELLULAR MATRIX METALLOPROTEINASES : MMP-1, MMP-3, MT1-MMP (MMP-14) AND MT2-MMP (MMP-15) IN LIVER BIOPSIES OF PATIENTS WITH CHRONIC HEPATITIS C

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Prolonged activation of hepatic stellate cells caused by HCV infection together with accumulation of extracellular matrix components may result in liver fibrosis developing into cirrhosis. Removal of factor which caused liver injury may lead to regression to the initial stage of liver fibrosis as a result of activating extracellular matrix metalloproteinases, which may degrade components produced by activated hepatic stellate cells.

Liver biopsies and blood of sixty patients with chronic hepatitis C were examined in this study.

The aim of this study was to determine the transcriptive activity of genes encoding extracellular matrix metalloproteinases in liver biopsy in patients with chronic HCV infection and to determine the transcription activity according to presence or absence of RNA HCV in liver biopsy, genotype of HCV, stage and grade in histopathological picture QRT-PCR was performed using sequence detector ABI PRISM^{TM} 7700. SSCP and reversed hybridization (test INNOLiPA HCV II) were used.

It was shown that the presence of RNA HCV in liver biopsies of patients with chronic hepatitis C irrespective of genotype HCV: 1b or 3 are accompanied by overexpression of genes encoding MMP-3 and MMP-15 but without influence on the expression of genes encoding MMP-1 and MMP-14. No changes of the expression of genes encoding extracellular matrix metalloproteinases: MMP-1, MMP-3, MMP-14 and MMP-15 in liver biopsies of patients with chronic hepatitis C with stage: F1, F2 and grade: G1, G2 in histological pictures of liver biopsies were shown. Level of HCV viraemia in blood was directly proportional to the level of HCV viraemia in liver biopsies and may allow determining the level of HCV viraemia in liver. On the basis of RNA HCV in blood the level of viraemia in the liver can be estimated.

11. REGULATION OF THE HUMAN APOPTOTIC NUCLEASE ENDONUCLEASE G

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Endonuclease G (EndoG) is a mitochondrial enzyme that turns to be an apoptotic nuclease when released from mitochondrial intermembrane space. EndoG is a DNA/RNA non-specific nuclease. However, at physiological ionic strength, RNA is a much more favorable substrate of EndoG, as compared to chromatin. This indicates that (i) EndoG could be an apoptotic RNase and/or (ii) for efficient cleavage of double-stranded DNA *in vivo* EndoG requires additional co-activators. In the present study we have searched for factors that affected activity of human EndoG. EndoG forms *in vitro* complexes with AIF and FEN-1 but not with PCNA. Interestingly, heat shock proteins 70 interact with EndoG and are involved in regulation of its activity. Purified Hsp70 prevented stimulation of EndoG activity by other nuclear factors in the ATP-dependent manner.

12. DNA DAMAGE IN CHILDREN EXPOSED TO LEAD

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Environmental exposure to lead in childhood remains a serious environmental health problem in the Silesia region. Lead is known to be a toxin mostly affecting nervous and hematopoietic systems, however its genotoxic potential has also been shown.

The objective of the study was to confirm contribution of lead to the development of cytogenetic damage and to assess selected genotoxic effects of environmental exposure to lead in children. Additional aim was to examine if the micronuclei formation results from a break of chromosome or damage of karyokinetic spindle.

Examined population was composed of 67 nine year old children, living in the region where non-ferrous ores are extracted and processed and 15 control children of the same age. Exposure to lead was assessed by lead in blood (PbB) determination (AAS). The following biomarkers of DNA damage were included: (1) frequency of micronuclei (MN) - to confirm the relationships between PbB and MN levels observed in our previous study, (2) MN-FISH with specific pan-centromeric probe - to distinguish between MN caused by chromosome breakage and MN cause by chromosome malsegregation, (3) single strand breaks (SSB) by comet assay (alkaline version) expressed as tail moment-TM, (4) FPG sensitive sites by comet assay modified by use of DNA glycosylase expressed as TM – to estimate DNA bases oxidation, as well as (5) mutation frequency in (a) locus TCR in lymphocytes (number of $CD4^+/CD3^-$ cells) and (b) locus GPA in erythrocytes (number of N0 and NN cells) in MN heterozygotes.

Environmental exposure to lead resulted in significantly increased levels of PbB, although average level was much below the value of biological exposure limit = 10 μg/dl. Median value in exposed children was 4.8 μg/dl compared to the controls: 2.5 μg/dl. The results showed significant difference in SSB level between exposed and control groups: median value was 1.81 vs. 0.92, respectively. Environmental exposure caused significant induction of MN (2.8 vs. 1.0). The level of micronuclei with centromeric signals was also significantly increased in exposed children. Examined children had higher level of FPG sensitive sites and mutation frequency in GPA locus compared to control children while no effect of exposure was found in relation to the frequency of mutations in locus TCR.

In conclusion, our results indicate that although environmental exposure to lead was not high, it resulted in measurable biological effects in examined children (SSB, MN, MN-FISH, FPG, GPA). Higher level of micronuclei with centromeric signals suggests that the formation of micronuclei probably results from a damage to karyokinetic spindle.

13. THE ROLE OF *rrp1* IN THE REPAIR OF DNA DAMAGE IN *SCHIZOSACCHAROMYCES POMBE*.

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Homologous recombination is an important process assisting in the repair of DNA double-strand breaks. In eukaryotic cells, proteins belonging to the Rad51 (Rhp51 in fission yeast) group, functionally homologous to the E*scherichia coli* RecA protein, take part in homologous pairing and strand exchange, essential to the DNA repair process. It was previously shown that *sfr1* gene in *Schizosaccharomyces pombe* participates in the Rhp51-dependent recombination repair pathway that does not require the Rhp57 protein. Here we report that another protein, Rrp1, possibly involved in chromatin remodelling and DNA repair, may also take part in this pathway. The strain devoid of the *rrp1* gene is viable and does not show any apparent growth defect upon exposure to UV or MMS. It is also proficient in mating-type switching. The double mutant *Δrrp1Δrhp57* but not *Δrrp1Δsfr1* or *Δrrp1Δrhp51* is more sensitive to UV and MMS than respective single mutants, suggesting that, as *sfr1*, *rrp1* is epistatic to *rhp51*, but not *rhp57*. Further studies will be needed to determine the exact role of these genes in different aspects of recombination DNA repair in fission yeast.

14. CHARACTERIZATION OF A SILENCER LOCATED WITHIN CpG ISLAND UPSTREAM TO THE CHICKEN ALPHA-GLOBIN GENE CLUSTER

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It was shown that the DNA fragment of 200 base pairs size located within the CpG island at 2.5 – 4.5 kbp distance upstream of the chicken alpha-globin gene cluster contains binding site for multifunctional protein factor CTCF and possesses a silencer activity, but not an insulator or promoter activity. The formation of complex between CTCF and the DNA fragment under study was demonstrated using electrophoretic mobility shift assays. The data are discussed with underlying hypothesis suggesting that the discovered silencer plays role in regulation of the expression of ggPRX gene (chicken analogue of human housekeeping gene "-14"), transcribed in the direction opposite to the alpha-globin genes transcription.

15. THAMINE PREVENTS X-RAY INDUCTION OF GENETIC CHANGES IN HUMAN LYMPHOCYTES *IN VITRO*

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Oxidative stress influences DNA and other biomolecules damage *via* oxidative changes to their chemical structure. These changes are believed to increase the risk of cancer, heart disease and aging processes. It has been demonstrated that antioxidants such as ascorbic acid, tocopherols and flavonoids give protection against oxidative damage and several degenerative diseases, including cancer. Little is known about whether B group vitamins give protection against DNA damage in human cells. The water-soluble vitamin B1 (thiamine) is commonly included in multivitamin preparations and it is nontoxic for humans.

In the present study the effects of thiamine on the extent of spontaneous as well as irradiation-induced DNA damage was measured in cultured human lymphocytes. Cultures were exposed to increasing concentrations of thiamine (0-500 μg/ml) and irradiated with X-rays. The DNA damage was estimated as the frequency of micronuclei and apoptotic or necrotic morphological changes in fixed cells.

The results show that thiamine alone did not induce genetic changes. A significant decrease in the fraction of apoptotic and necrotic cells was observed in lymphocytes irradiated in the presence of vitamin B1 at concentrations between 1-100 μg/ml compared to those irradiated in the absence of thiamine. Vitamin B1 at 1 and 10 μg/ml decreased also the extent of radiation-induced formation of micronuclei. Vitamin B1 had no effect on radiation-induced cytotoxicity as measured by nuclear division index. The results indicate that vitamin B1 protects human cells from radiation-induced genetic changes.

16. THE FUNCTIONAL IMPACT OF POLYMORPHISMS AND MUTATIONS IN SELECTED DNA REPAIR GENES: *XPA*, *XPB* AND *RECQ1* ON THE PROTEIN LEVEL, LOCALIZATION AND ACTIVITY.

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The inter-individual variation in DNA repair efficiency and cancer susceptibility are associated with polymorphisms of DNA repair genes. Previously, we and others have found sequence alterations within the coding and non-coding regions of genes involved in DNA metabolism. It is not known whether and how these particular sequence alterations change the functioning of the encoded proteins. We focused on two genes involved in nucleotide excision repair system – *XPA*, *XPB* and on the *RECQ1* gene encoding one of the human helicases from the RecQ family. This gene family contains at least three genes (*WRN, BLM* and *RTS)*, whose mutations are associated with cancer-prone genetic diseases (e.g. Werner syndrome, Bloom syndrome, Rothmund-Thomson syndrome).

For our functional analyses we have selected the common polymorphism of *XPA* (-4G>A) located four residues upstream the start codon, which in previous molecular epidemiological analyses showed significant association with lung cancer risk. Two relatively infrequent *XPB* polymorphisms (117:Lys>Arg; 402:Gly>Cys) were selected due to their localization within the evolutionary conserved regions of the protein. Finally, we studied the functional impact of three sequence alterations of *RECQ1* helicase gene: 248:Ala>Pro, 487:Lys>Thr (polymorphic allele), 566:Thr>Ala (sequence alteration found in HeLa cells) arranged as 8 different sequence combinations. RECQ1 helicase is not well studied protein and its cellular function remains unknown. Our luciferase reporter assay showed that co-transfection of *RECQ1* expression vector with the reporter vector causes fourfold increase of the reporter gene activity when compared with the co-transfection of the LacZ control expression vector, indicating that RECQ1 facilitates gene expression. The high *RECQ1* expression in the lung suggests that its sequence alterations may influence lung cancer risk.

Using the luciferase reporter assay, host cell reactivation assay, *in vivo* protein labeling by the fluorescent tags and Western blotting we assessed the influence of the sequence alterations on the expression level, activity and cellular localization of the studied DNA repair proteins. The detailed results of the analyses will be presented.

17. INDUCTION OF UNIQUE STRUCTURAL CHANGES IN GUANINE-RICH DNA REGIONS BY THE TRIAZOLOACRIDONE C-1305 RESULTS IN ISOFORM-SPECIFIC STABILIZATION OF TOPOISOMERASE II-DNA CLEAVABLE COMPLEXES

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C-1305 is a triazoloacridone with potent activity in lung and colon cancer models. We have recently reported that C-1305 is a topoisomerase II poison which is able to induce topoisomerase II-mediated cleavable complexes *in vitro* as well as in living cells [Lemke et al., 2004]. An unusual feature of C-1305 is the induction of low levels of very toxic cleavable complexes in tumor cells. Topoisomerase II is a nuclear enzyme which can catalyze catenation/decatenation reaction as well as knotting/unknotting of DNA. This protein exists as two different isoforms: the alpha isoform which is restricted to proliferating tissues and which frequently is deregulated in human tumors and the beta isoform which constitutively is expressed in all tissues. Unexpectedly, only the alpha isoform of topoisomerase II was found to be covalently associated with DNA following exposure of living cells to C-1305 in marked contrast to amsacrine, a classic topoisomerase II inhibitor, which stimulated cleavable complexes with both isoforms. To explore the molecular mechanisms underlying this phenomenon, we have investigated the sequence specificity of DNA binding for C-1305 and other triazoloacridones in comparison with classic topoisomerase II inhibitors and other DNA interacting compounds. These studies have revealed that C-1305 is able to induce structural distortions in DNA regions containing guanine tracts. Studies with other triazoloacridone derivatives as well as topoisomerase inhibitors did not give similar results. The experiments carried out with isolated enzymes (topoisomerase IIα and β) and DNA have shown strong DNA cleavage site within the guanine-rich region as well as differences between topoII isoforms in terms of enzyme binding to DNA in presence of C-1305. One of the explanations of these findings might be the interaction of the drug with specific regions/sequences of DNA or distortion of DNA structure in the way that only one of the isoforms of DNA topoisomerase II would be able to bind to its substrate. Based on our results, we propose a model of the complex between C-1305 and a short oligonucleotide containing 5'-AGGGT-3' sequence, where drug-DNA complex is stabilized by hydrogen bonds between triazoloacridone and neighbouring bases. The whole structure could also be stabilized by the π-π interactions between triazoloacridone chromophore and guanine as well as interactions of positively charged side chain within the minor groove of DNA.

Together, we here show that the topoisomerase II inhibitor triazoloacridone C-1305 binds strongly to DNA at guanine-rich regions resulting in unique conformational alterations. This effect leads to specific DNA cleavage by only the α isoform of topoisomerase II. Our results suggest that C-1305 might specifically influence the expression of genes that are regulated by guanine-rich elements in the promoter regions.

Lemke K, Poindessous V, Larsen AK, Skladanowski A. The antitumor triazoloacridone C-1305 is a topoisomerase II poison with unusual properties. Mol Pharmacol. 66 (4): 1-9 (2004)

18. NEW ANTITUMOUR DERIVATIVE OF CIS-PLATINUM: STRUCTURE AND BIOLOGICAL ACTIVITY OF PT(II) COMPLEX WITH METHYL 3,4-DIAMINO-2,3,4,6-TETRADEOXY-α-L-LYXO-HEXOPYRANOSIDE

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Cationic platinum-based antitumor drugs containing the DMSO molecules appear to be of special interest, since as a rule their therapeutic activity is of the same magnitude as that of *cis*-platinum and they are believed to be only marginally nephrotoxic [1-4].

Herein, we report our structural, spectroscopic and biological studies of a novel *cis*-platinum-like, DMSO containing complex with expected antitumor properties. This cationic complex consists of one chloride anion, methyl-3,4-diamino-2,3,4,6-tetradeoxy-□-L-lyxopyranoside ($C_7H_{16}N_2O_2$) and dimethylsulfoxide (DMSO) molecules forming a square-plane. The sugar moiety is a modified carbohydrate part of the commonly used in therapy antineoplastic anthracycline antibiotics, daunorubicin and doxorubicin, where it acts as a minor groove binder contributing close to 40% to free energy of DNA-drug binding [5-7]. It has been obtained by courtesy from prof. Priebe (Texas University, Houston, USA).

Biological tests performed using leukemia L1210 cells for the investigated platinum(II) complex or the free lyxo-hexopyranoside, respectively, have shown that toxicity of the title complex is similar to that of *cis*-platin. No significant toxicity of the aminosugar was found, up to the highest concentration tested. Although the ID_{50} values for the title complex and the *cis*-platin (studied as the reference drug) appeared to be similar within the experimental system applied, the ID_{90} values show that *cis*-platin is about two times more toxic than the investigated complex.

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19. PROGNOSTIC VALUE OF LYMPHOCYTES IN CERVICAL CARCINOMA

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Objective: The aim of our study was to test whether DNA damage in peripheral blood lymphocytes may be a factor predicting development of cancer and results of local treatment.

Methods: The tested group consisted of 56 patients with cervical cancer in clinical stage IIB- IIIB and the control group of 38 healthy women. Lymphocytes were isolated from fresh blood, taken from patients before treatment (radiotherapy or radiochemotherapy). DNA damage and its repair were measured after irradiation with γ rays (2Gy) using alkaline comet test. Lymphocyte subpopulations were detected by monoclonal antibodies and flow cytometry.

Results: Lymphocytes of patients with cancer presented a higher level of background DNA damage ($p<0.00001$), increased radiation induced damage ($p<0.00005$) and reduced repair capacity ($p<0.00001$) in comparison with healthy donors. In both groups broad interindividual differences in DNA repair kinetics were observed.

Blood of patients and normal controls contained comparable numbers of T, B, CD4+, CD8+ and NK lymphocytes. Testing the relationship between the distribution of subpopulations and DNA repair kinetics we found that patients who were characterized by more efficient repair in lymphocytes (lower level of residual DNA damage after 180 min of repair) had a higher number of T, CD4+ lymphocytes and a lower number of NK cells than patients with less efficient DNA repair ($p=0.021$, 0.021, 0.005 respectively)

The data obtained using the comet test were compared with clinical results of treatment. A higher efficiency of DNA repair in lymphocytes was characteristic for patients with faster regression of tumour. About 93% individuals whose lymphocytes repaired DNA damage better, exhibited complete or almost complete reduction of tumour mass after termination of radiotherapy. A faster regression of tumour was characteristic for patients with a higher number of T lymphocytes ($p=0.013$) and a lower number of NK cells ($p=0.017$). This suggests that lymphocytes which repair DNA damage more efficiently probably survive better genotoxic effects of radiotherapy and participate in tumour elimination.

Conclusions: DNA repair capacity of lymphocytes may be an important factor in determining susceptibility to cancer and response to cancer therapy. Differences in response of lymphocytes to genotoxic factors may result from different sensitivity of their subpopulations.

20. EXPRESSION OF PORCINE ENDOGENOUS RETROVIRUSES SUBTYPES (PERV-A, PERV-B AND PERV-C) IN IMMUNOSUPPRESSED PIGS

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Possibility of breaking the cross-species barrier by some infectious particles is the main obstacle for developing xenotransplants. Expression of porcine endogenous retroviruses (PERVs) does not pose any threat for their host (pig) in a physiological state. However, immunosuppressed recipient is vulnerable to numerous infectious agents including endogenous viruses or latent viruses that normally do not manifest their presence. This may play an important role in post-transplantational complications.

The aim of this study was to assess if immunosuppression influences PERV-A, PERV-B and PERV-C expression in domestic pig (*Sus scrofa domestica*). The studied material consisted of peripheral blood samples collected after transplantation from pigs treated with cyclosporin A (Sandimmum Neoral; 4mg/kg body weight) and Solu-Medrol (500mg/kg). We used genotype-specific PCR with primer sets described earlier by Bösch et al (2000).

The results indicate that immunosuppression with the drug set used in this study (immunosuppressant + steride) causes a significantly elevated PERV-A expression ($p=0.028$) compared with a control group, while it does not affect expression of PERV-B ($p=0.203$) or PERV-C ($p=0.817$). These results are in concordance with literature data showing that PERV-A has the strongest capability to express and propagate (its full-length-cDNA is most frequently found in the pig genome). Since it is difficult to eliminate the hazard of PERVs expression in xenotransplantation by conventional methods (e.g. protease inhibitors), PERVs silencing by means of antisense oligonucleotide strategy should be developed.

The study was supported by the grant from the State Committee for Scientific Research (No. KBN 048/PO5/2001/10).

21. INDUCTION OF APOPTOSIS BY 1-NITROACRIDINE DERIVATIVE – C-1748 (4-METHYL-1-NITROACRIDINE) IN HUMAN COLON CANCER HCT-8

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The derivatives of 4-methyl-1-nitroacridine are a group of antitumor compounds developed in our Department. The most active derivative of 4-methyl-1-nitroacridines, 9-(2'-hydroxyethylamino)-4-methyl-1-nitroacridine denoted C-1748, displays very high antitumor activity against a number of human prostate cancer xenografts in nude mice: LnCaP, JCA, PC3 and TSU, as well as towards human colon cancer HCT-8. This compound shows a decreased general toxicity compared to other derivatives of 1-nitroacridine and exhibits high cytotoxic activity *in vitro* towards cells derived from solid tumors. The 4-methyl-1-nitroacridine C-1748 has been selected for the I phase clinical trials.

We studied in HCT-8 human colon cancer cells the mechanism of apoptosis induced by C-1748 in biologically relevant concentration, corresponding to EC_{90} value. The fluorescence microscopy studies of HCT-8 cells incubated with C-1748 showed morphological changes such as chromatin condensation and formation of apoptotic bodies, after 12 hr of treatment. The induction of internucleosomal DNA fragmentation, characteristic for apoptosis, was observed in HCT-8 cells after 12 hr of exposure to C-1748 by agarose gel electrophoresis. The ability of 4-methyl-1-nitroacridine to induce apoptosis in HCT-8 cells was also demonstrated by flow cytometry methods. The disruption of mitochondrial membrane potential ($\Delta\Psi m$) appeared after 12 hr of incubation HCT-8 cells with C-1748. The loss of phospholipid asymmetry of the plasma membrane in HCT-8 cells was evident starting from 24 hr of treatment. Percentage of HCT-8 cells with active caspase-3 amounted to 6% after 12 hr of incubation of cells with the drug and after 72 hr it reached about 15%. The TUNEL assay confirmed the occurrence of DNA fragmentation in HCT-8 cells. However, this DNA fragmentation was not specific to any phase of the cell cycle. The 4-methyl-1-nitroacridine studied induced apoptosis in HCT-8 cells in a time dependent manner. Nuclear DNA fragmentation and morphological changes induced by C-1748 in HCT-8 cells occurred in the same time as the decrease of mitochondrial membrane potential and appearance of activated caspase-3.

22. MITOGENIC SIGNALLING BY BRADYKININ RECEPTORS IN CERVICAL CANCER

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In a large survey of cancers, receptors for bradykinin were most commonly found on tumor cells and on established cancer lines. Bradykinin plays an important role in enhanced vascular permeability in tumor tissue, improves transportation of nutrients to tumor cells, facilitates metastasis and sustains tumor growth. The two characterized bradykinin receptors are designated B1 and B2. The B1 receptor shows greater preference for the bradykinin metabolite [des-Arg^9]bradykinin than for bradykinin itself.

It has been suggested that mitogenic signalling may display a high degree of cellular specificity. The precise role of each bradykinin receptor in controlling of both normal and cancer cell division needs to be clarified. Moreover, the importance of various intracellular pathways in transduction of signals generated by bradykinin receptors has not been properly elucidated.

The purpose of this study was to characterize the cellular mechanisms underlying mitogenic activity exerted by bradykinin in cervical cancer.

The analyzed caterial was biopsy samples of cervical cancer.

The genes related to mitogenic pathways were selected. The relationship between studied genes was established and illustrated as a gene map. The map will be used in analyses of transcripts of the studied genes based on oligonucleotide microarray HU 133 A.

23. INCREASE OF ER-BETA5 mRNA COPY NUMBER CORRELATES WITH HIGH PROLIFERATIVE ACTIVITY IN HUMAN COLON CANCER

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Introduction: Research conducted on colon cancer cell lines show that growth of tumour cells is influenced by the action of estrogen receptors. The working efficiency of "wild-type" estrogen receptors depends not only on the transcriptional activity of estrogen receptors (ERs)-encoding genes but also on the part of posttranscriptional ERs mRNA modification in total pool of ERs RNA. The tumour ability for metastasis depends on proliferation potential. Therefore, proliferative activity of tumour centre cells of the copy number of posttranscriptional mRNA ER-beta variants was analyzed in accordance with the standards of the American Joint Committee on Cancer (AJCC).

Material and Method: Colon cancer tissues were obtained from 41 patients after surgery. The patients were divided into groups taking into account the classification of colon adenocarcinoma according to the AJCC. The RNA from 164 segments of colon tissues was extracted using the modified phenol-chloroform method. The tissue sample of each patient consists of the margin, centre of tumour as well as normal tissue. QRT-PCR reaction was performed for all RNA extracts. RT-QPCR was also performed using the Quanti Tect SYBR Green Kit and sequence detector ABI PRISM7000.

Results: Positive correlation among mRNA H3j copy number and mRNA ER-beta5 copy number showed stages I and IV of colon adenocarcinoma clinical classification (the rang correlation R Spearman test).

Conclusion: Existence of positive correlation among transcriptional activity of H3 gene and ER-beta5 mRNA copy number indicated the unquestionable role of this variant in the control of the proliferative potential in colon cancer tissue.

24. RADIATION THERAPY GEL DOSIMETRY

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The main aim of radiation therapy is to deliver a high radiation dose to the tumour while on the same time keeping the dose at surrounding healthy tissue minimal. Recent advanced techniques of conformal radiotherapy, as Stereotactic Radiosurgery, Intestity Modulated Radiation Therapy and Brachytherapy, manage to deliver dose using high dose-gradient radiation fields so that the dose distribution closely fits the shape of the target. Due to these complex shapes and high dose gradients used, new precise three-dimensional dose verification methods are necessary. The only such unconventional method nowadays, capable of measuring three-dimensional dose distributions directly, is the polymer gel dosimetry.

The polymer gel dosimetry was established to overcome the limitations of up-to-date techniques and to obtain a full three-dimensional dose distribution from a single measurement with high spatial resolution of dose. For this method polymer gel dosimeters have been developed that consist of a gelatin matrix in which monomers are embedded. Since the major ingredient of these systems is water, their exposition to radiation causes water radiolysis products formation. The radical products initiate polymerisation of monomers, the degree of which directly relates to the absorbed dose. The process results in the change of chemical and physical properties of the irradiated part of the gel. The appropriate imaging techniques like optical, ultrasound, and in particular three-dimensional nuclear magnetic resonance gives a precise three-dimensional image of the dose distribution and allows the reconstruction of dose distribution in any preferred plane of the gel.

Nowadays in the clinical environment the new irradiation techniques urge for such new methods of dosimetry capable of precise evaluation of the calculated dose distributions. Therefore, gel dosimetry paired with MRI scanning appears as a promising tool. In this study we provide basic characteristic of selected gel compositions and the results of applying the method in conformal radiotherapy techniques.

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25. THE 1,4-DIHYDROPYRIDINE DERIVATIVE PROMOTES DNA REPAIR THROUGH STIMULATION OF POLY(ADP-RIBOSYLATION)

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Among 1,4-dihydropyridine derivatives (1,4-DHPs), the analogs of nicotinamide and remote analogs of NAD^+ and $NADP^+$ attract a special attention because of a crucial role of the mentioned coenzymes in cells. It seems probable that such type analogs can influence NAD^+ and $NADP^+$ metabolism and, in this way, the excision repair through poly(ADP-ribosylation) [Kuzhir, 1999] in which NAD^+ is used as a substrate. In our studies, the data of alkaline single cell gel electrophoresis (Comet Assay) had shown that one of such 1,4-DHPs (AV-153) reduced significantly (up to 70%) the endogenous, radiation- (X- and gamma-rays) and chemically-induced (oxidized and alkylated) DNA damage in lymphocytes of healthy donors and lymphoblastoid cell lines *HL-60* and *Raji*. This effect of AV-153 was observed in a wide range of concentrations, from 10^{-9} to 10^{-6} M, it had reverse dose-effect relationship and it appeared from first minutes of repair. The fact that the most effective concentrations of AV-153 (10^{-9} and 10^{-8} M) correspond to the intracellular concentrations of NAD^+, ATP and nicotinamide that take part in poly(ADP-ribosylation), supports the hypothesis that 1,4-DHPs could modulate the synthesis of poly(ADP-ribose) (PAR). The reverse relationship observed between the AV-153 concentration and DNA damage reduction suggests that AV-153 may stimulate or inhibit poly(ADP-ribosylation) depending on its concentration.

We compared the level of PAR induced by H_2O_2 treatment in presence or absence of AV-153. The level of PAR was measured by immunocytochemical test with anti-PAR polyclonal antibody and computer quantification of PAR as pixel intensity per cell square unit. We found that AV-153 at concentration of 10^{-9} M stimulated synthesis of PAR up to 120% during the first minute of repair process after H_2O_2 treatment, the concentrations of 10^{-8}–10^{-6} M were less effective and 10^{-5} M had slight inhibitory effect. The level of PAR in presence of AV-153 inversely correlated with AV-153 concentration ($R^2 = 0.99$, $P < 0.05$). The efficiency of DNA repair in the presence of AV-153 showed positive correlation with the inducible level of PAR at the first minute of repair process ($r > 0.9$, $P < 0.05$). The AV-153 stimulated increase of PAR level correlated with increase of DNA repair rate.

The data obtained demonstrate one of the mechanisms of 1,4-DHPs action on DNA repair through promotion of PAR synthesis. This is the first evidence of poly(ADP-ribose) polymerase-1 (PARP-1) stimulation by compound in non-genotoxic concentrations. It opens the further possibilities for modulation of PARP-1 activity and study of the mechanisms of PARP action.

This work has been carried out at the Department of Experimental and Clinical Radiobiology, M. Skłodowska-Curie Memorial Cancer Center and Institute of Oncology (Gliwice Branch) and partially supported by the fellowship grants from the Association for the Support of Cancer Research, the UNESCO (Polish Committee) and the National Cancer Institute (Bethesda, USA).

26. DNA DAMAGE INDUCED BY 1-NITROACRIDINE DERIVATIVES IS REPAIRED BY HOMOLOGOUS RECOMBINATION

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1-nitroacridines are potent antitumor compounds with high activity toward ovary, breast and prostate cancer. Previous studies have shown that 1-nitroacridines are activated by cellular enzymes and bind covalently to DNA, and that covalent binding and crosslinking of DNA correlate well with the cytotoxic and antitumor activities of biologically active 1-nitroacridines. Our studies have shown that, covalent modification of DNA by Ledakrin but not C-857 leads to topoisomerase I-associated DNA cleavage *in vitro*. We have also demonstrated in LNCaP human prostate carcinoma cells incubated with 1-nitroacridines that the level of DNA-protein complexes greatly increased for C-857 and Ledakrin but to much lesser extent for C-1748 with increasing concentrations of studied compounds. The kinetics of appearance and removal of these complexes differed from those induced by camptothecin and m-AMSA, classical inhibitorsof DNA topoisomerases. Studies using Immuno-Complex of Enzyme (ICE) assay where one can determine the existence of the covalent DNA-topoisomerase complexes have shownthat topoisomerase I and topoisomerase II are differently involved in DNA-protein complexes induced by 1-nitroacridines. All studied 1-nitroacridines induced high levels of double-strand breaks (DSB) in LNCaP cells. Interestingly, simultaneous incubation of LNCaP cellswith aphidicolin, a well known inhibitor of DNA replication, and 1-nitroacridines led to reduced levels of DSBs. This suggests that DSBs are generated by collisions of the replication fork with DNA-topoisomerase I complex stabilized by DNA adducts produced by 1-nitroacridines. We have also observed high number of sister chromatid exchanges (SCE) in CHO cells treated with C-1748 and to much lesser extent with Ledakrin and C-857 or DNA topoisomerase II inhibitor,*m*-AMSA. It is known that generation of SCE in drug-treated cells is associated with the ongoing DNA replication and/or inhibition of DNA topoisomerases.

DSBs are very toxic cellular lesions and, if left unrepaired, they lead to impaired cell function or direct cell killing. One of the DNA repair mechanisms which could repair DBSs which originate from replication fork-stalling or during attempted replication over a single strand break is homologous recombination (HR). To clarify the potential role of HR in DNA repair of DNA lesions produced by 1-nitroacridines, we exposed CHO cells which have deficient *BRCA2* gene and have only partially functional HR pathway to studied 1-nitroacridines. We observed that cells deficient in HR DNA repair are about 5-10-fold more sensitive to 1-nitroacridines compared to parental cells which are HR-proficient. Interestingly, CHO xrs-6 cells, in which *Ku80* gene is mutated and non-homologous end joining pathway is inactive, are equally sensitive to 1-nitroacridines as parental cells.

Finally, we compared gene expression profiles by DNA microarray technology in LNCaP/A40 cells which are about 40-fold resistant to C-1748 and parental LNCaP cells. Interestingly, C-1748-resistant cells exhibit about 3 times lower expression levels of BRCA1 and BRCA2 than parental LNCaP cells. Taken together, all available data suggest that DBSs induced by 1-nitroacridines are repaired by homologous recombination.

27. TRANSCRIPTION ACTIVITY ANALYSIS OF GENES REGULATING APOPTOSIS ASSOCIATED WITH TNF SUPERFAMILY RECEPTORS IN CD34- CELLS WITH DIFFERENT ADHESION CAPABILITIES USING OLIGONUCLEOTIDE MICROARRAY TECHNIQUE

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The cells presenting inherent adhesion capability have stable metabolism. Reduction of cell adhesion capability may be associated either with cell cycle stage that precedes the cell division or with cell damage leading to changes in transcription activity profile of proapoptotic and antyapoptotic genes.

The aim of the study was to estimate the transcription activity of genes associated with death receptors apoptotic pathways, as well as the analysis of genes expression profiles in cells with different adhesion capability.

The material used in this study were cell cultures of human CD34- progenitor cells with different adhesion capabilities, isolated from cord blood. The variant I were CD34-NP cells (with reduced adhesion capability) and variant II were CD34-P cells (with inherent adhesion capability, controle). Absolute and comparative analyses were performed using HG_U133A oligonucleotide DNA microarrays, instrumentation and software tools from Affymetrix. 139 genes transcripts associated with death receptors apoptotic pathways were analyzed usingthe HG_U133A DNA microarray. The transcripts were divided into three groups: GROUP I - proapoptotic genes (111 transcripts), GROUP II – antiapoptotic genes (22 transcripts), GROUP III – genes that, depending on their expression regulation, can promote as well as inhibit apoptosis (6 transcripts).

Absolute analysis allowed to determine which among 139 examined transcripts are present, marginally present or absent in CD34-NP and CD34-P cells. Comparative analysis yielded information how transcription activity of analyzed groups of genes changed in CD34-NP cells compared to CD34-P cells. The obtained preliminary results permit to assume that in CD34- cells changes of the adhesion capabilities can be associated with the changes of transcription activity of both proapoptotic and antiapoptotic genes.

28. THE LEVEL OF 8-OXO-2`-DEOXYGUANOSINE IN LEUKOCYTE DNA OF CANCER PATIENTS UNDERGOING CHEMOTHERAPY.

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The anticancer therapy involves a wide spectrum of drugs. Some of them are attributed to intracellular production of ROS which can be generated by both enzymatic and nonenzymatic mechanisms. ROS may cause damage to biological molecules, including proteins, lipids, and DNA. Production of different kinds of DNA lesions, particularly free radical- modified DNA bases are responsible for mutations. 8-Oxo-2`-deoxyguanosine (8-oxo-dG) is one of the typical biomarkers of oxidative stress.

Materials and methods: The study comprised a group of 34 cancer patients undergoing chemotherapeutic treatment with different drugs like Cis-platine, vepesid, bleomycine, adriblastine. Patients were suffering from different cancer types like ovarian cancer, breast cancer and adenocarcinoma. Blood samples were collected before chemotherapy and one day after the treatment. Leukocytes were isolated on Histopaque 1119 solution (Sigma), according to the procedure laid down by the manufacturer. HPLC with electrochemical and UV-absorbance detection was used to determine the level of 8-oxo-dG in leukocytes DNA.

Results: The mean levels of 8-oxo-dG in leukocytes DNA before chemotherapy and one day after the treatment were 10.46 ±3.96 /10 ^{6} dG molecules and 11.79± 5.03 /10 ^{6} dG, respectively. This difference was statistically significant ($p<0.02$).

Conclusion: In the present study we observed that the level of 8-oxo-dG in leukocytes DNA was higher one day after the treatment than before chemotherapy. Some reports have shown that adriamycin derivatives and some other drugs used in chemotherapy can produce ROS, what in turn may be responsible for oxidative stress at the level of the whole organism. In our study the level of 8-oxo-dG in leukocytes DNA remains elevated up to 24-th hour after the treatment. This suggests that in human leukocytes *in vivo* some of DNA lesions may avoid the repair processes. Some nucleobase modifications lead to mutagenesis. Since 8-oxo-dG posses mutagenic properties it may be responsible for secondary cancers after chemotherapy.

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29. INDUCTION OF CELL DEATH BY IMIDAZOACRIDINONE DERIVATIVE C-1311 IN T LYMPHOBLASTOID LEUKEMIA MOLT4 CELLS.

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The imidazoacridinone derivative C-1311 is a highly potent antitumor agent with a broad spectrum of activity against different experimental tumors. C-1311 has been synthesized in Gdansk University of Technology and is currently undergoing phase I clinical trials. This compound has previously been shown to display DNA-binding properties and to inhibit activity of topoisomerase II. One of the early biological effects induced by pharmacologically relevant doses of C-1311 (EC_{90} concentrations) was arrest in the G2M phase of the cell cycle. C-1311 induced apoptosis of L1210 murine leukemia cells and to much lesser extent in ostegogenic sarcoma cells as well as abortive mitosis followed by cell death of HT29 human colon adenocarcinoma cells. The aim of this work was to investigate the ability of C-1311 to induce apoptosis of T lymphoblastoid leukemia MOLT4 cells. Cell death studies were carried out over continuous exposure times varying from 3 to 72 h at EC_{90} concentration of the drug.

DAPI staining of cytospin preparations was performed to analyze the cellular morphology. Mitochondrial membrane potential was measured by flow cytometry using JC-1 fluorochrome. Caspase-3 activity and phosphatydilserine externalization was evaluated using commercially available assay kit on flow cytometry. DNA fragmentation was analyzed by 1,8% agarose gel electrophoresis.

Microscopic examination of MOLT4 cells showed alterations of the nuclear morphology after 24 h but cells with more condensed chromatin and apoptotic body-like structures appeared after 48 and 72 h. Percentage of Annexin-V positive cells increased gradually upon treatment starting from 12 h and after 72 h it reached about 70%. However, by this latter time cells were also stained red (due to PI) which indicated late stages of apoptosis and/or necrosis. Disruption of mitochondrial transmembrane potential was observed in the cells after 30 h of incubation with C-1311. Induction of caspase-3 activity was detectable in 30% of cell population after 39 h of treatment and after 72 h this percentage increased to 80%. Characteristic internucleosomal DNA fragmentation was observed only after 48 and 72 h.

These results indicate that C-1311 induced apoptosis of MOLT4 cells in a time-dependent manner and both mitochondria and caspase-3 are involved in this process.

30. ASSESSMENT OF HBV DNA AND TGF-ß1 AND ITS RECEPTORS TßRI, TßRII, TßRIII mRNA LEVELS IN THE QUALIFICATION OF PATIENTS WITH THE CHRONIC HBV INFECTION FOR THE INTERFERON α TREATMENT

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The frequency of HBV infection as well as the serious consequences for patient's health and life resulting from the insufficiency of liver and the probability of the development of cancer, force to constant searching for new techniques to complete the virological diagnosis. Such procedure permits putting the patients through an appropriate form of treating and individualizing methods of the therapy used.

The aim of the study was the estimation of the applicability of the designed QPCR for HBV DNA and QRT-PCR for TGF-β1 and its receptors: TβRI, TβRII and TβRIII mRNA in the qualification of patients with the chronic HBV infection for the interferon α treatment.

The first stage of the research embraced designing, empirical optimization and the assessment of the specificity QPCR for HBV DNA and QRT-PCR for TGF-β1and receptor's mRNA. The second stage of the study deals with analysis of HBV DNA isolates for the purpose of indication of the HBV polymorphic types the designed oligonucleotydes, specifity and the number of HBV DNA copies in patients with chronic virus hepatitis B treated with interferon α. The last stage deals with the indication of the dynamics of changes in the expression of TGF-β1 genes and the receptors TβRI, TβRII and TβRIII in peripheral blood in patients put through treating and in healthy people, and then the indication of reciprocal relations between parameters marked in blood as well as the inflammatory activity and the degree of fibrosis in the liver.

The number of DNA HBV copies in plasma conditioning the set-back of HBV replicative activity and a good prognosis of the therapy with interferon α in patients put through the treatment, was fixed on the level of 4.52×10^8 of the virus copies/ml of plasma. The transcription activity of the TGF-β1 gene in peripheral blood in patients suffering from chronic virus hepatitis B correlated with the expression of TβRI, TβRII receptors genes while it was not influencing the expression of the gene of the TβRIII receptor. Indicated by means of the QRT-PCR, the number of mRNA TGF-β1 copies in 1 μg of the total RNA in peripheral blood in patients suffering from chronic virus hepatitis B was significantly higher comparing to healthy people, and was used as a marker, differentiating patients treated with interferon α with the degree of inflammatory activity in liver $< \text{or} \geq 3$ points in 4-point Scheuer's scale.

31. PREVALENCE OF PORCINE CYTOMEGALOVIRUS (PCMV) IN PIG HERDS INBRED FOR XENOTRANSPLANTATION

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Xenotransplatation opens new perspectives for medicine, however the problem linked with these procedures is the threat of pathogen transmission. Infections with porcine cytomegalovirus (PCMV), a member of *Herpesviridae*, are latent in most cases and do not manifest any clinical symptoms. Nevertheless, when activated, PCMV causes severe systemic injury resulting, in many cases, with death. Immunosuppression used in transplantation procedures is a state that enables many latent viruses to express. What is more, porcine CMV is capable of activating recipient's latent viruses of the *Herpesviridae* family. Thus, it is crucial to exclude PCMV-carriers from herds inbred for xenotransplantation.

The purpose of the study was to monitor different herds of domestic pig (*Sus scrofa domestica*) for PCMV frequency. The analyzed material was samples of peripheral blood collected from pigs from three different herds of the National Research Institute of Animal Production, Balice/Kraków. For detection of PCMV DNA with nested-PCR primer sets described earlier by Fryer et al (2000) were used.

We found significant differences in the prevalence of PCMV genome among the herds: herd SZ 35.2% (6 positive individuals per 17 individuals in total), herd BS 84.2% (59/70), and herd NZ 71.4% (25/35). The results indicate that herds with minimal risk of PCMV spread can be established.

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32. BIOLOGICAL ACTIVITY AND SINGLET MOLECULAR OXYGEN PRODUCTION OF SYNTHETIC PORPHYRIN DERIVATIVES; POTENTIAL NEW PHOTODYNAMIC THERAPY AGENTS.

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Background and purpose: Photodynamic therapy (PDT) is a treatment method which uses the combination of photosensitiser and visible light to induce a cytotoxic effect in cancerous tissue. The therapeutic effect is mediated through two types of reactions: the first generating free radicals and other reactive oxygen species, such as superoxide radical anion and lipid peroxidation products and the second occurring solely in the presence of oxygen and generating highly reactive singlet oxygen, O_2 ($^1\Delta_g$). These species are cytotoxic and disruptive to cellular structures (DNA, lipid membranes, mitochondria and lysosomes). The high quantum yield of singlet oxygen is a critical feature for effective photosensitisation. Other chemical prerequisites for good photosensitisers are: stability, purity and high absorption coefficient in the phototherapeutic window (600-800 nm). The desired biological features of photosensitisers are: low dark toxicity, selective accumulation and prolonged retention in tumour cells. The porphyrins, as agents with good photosensitising properties are very promising for PDT.

Materials and methods: We have undertaken the complex studies involving synthesis, physico-chemical characterisation and evaluation of biological activity of new synthetic aminoacid-, cholesteryl-, pyridyl- and cetyl- porphyrin derivatives. On the basis of physico-chemical properties some of them were selected for biological studies including cellular accumulation, dark cytotoxicity and photodynamic efficiency in different lines of human tumour cells. Cationic liposomes were used as carriers for transfer of porphyrins into the cells. Cellular accumulation of drugs was assessed on the basis of luciferase activity measured in lysates of cell cultures previously transfected with liposomes complexed to plasmid DNA carrying luciferase reporter gene. The confocal microscopy was also used for visualization of drugs in cell structures. Cytotoxicity and photodynamic activity was determined by the tetrazolium colorimetric reduction assay (MTS assay). The modes of cell death after PDT treatment (apoptosis or necrosis) were studied by differential staining with acridine orange/ethidium bromide.

Results: Our results indicate that at least two probed synthetic porphyrin derivatives are relatively nontoxic without light treatment and are highly effective with regard to phototoxicity in human malignant melanoma and colon carcinoma cell lines and may be promising candidates for further use in PDT experiments. Selected data will be presented.

33. EXPRESSION OF CELL CYCLE REGULATORY GENES IN CD34$^-$ CELLS ISOLATED FROM CORD BLOOD

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Introduction: Cell cycle is responsible for most important events in the cell life. Accordingly, from cellular context signaling pathways can recruit components linked with DNA repair, activation of apoptosis, breaking of transcription, activation of arrangements checking cell walking by definite points of cell cycle, cell proliferation and differentiation. Expression profile of cell cycle regulatory genes can determine the ultimate fate of cells. Mechanism regulating self-renewal and cell fate decisions in human cord CD34$^-$ cells are poorly understood.

Purpose: The aim of the study was to determine (by oligonucleotide microarray assay) the expression profile of cell cycle regulatory genes in cord CD34- cells with different ability for adhesion.

Material and Method: Material for the study included cord CD34- cell lines with ent ability for adhesion. The CD34 cells were isolated from cord blood in Ficoll and uroproline gradient (F/U). CD34 cells were divided into CD34- and CD34+ populations using Progenitor Cell Isolation Kit and Mini&MidiMACS Starting Kit. Gene expression was evaluated using HG_U133A microarrays (Affymetrix). The absolute and comparative analyses was carried out by means of Affymetrix GeneChip Analysis Suite 5.0 software. The results obtained after comparative analysis were sorted to single out genes with changed expression, using the software Affymetrix Data Mining Tool.

Results: The HG_U133A microarray contains 607 transcripts for cell cycle regulatory genes. Increase of CCND3, CDKN1A, YWHAQ genes expression and decrease of CDC2, CCNB1 and BUB3 genes expression were observed in CD34$^-$ cells with weak ability for adhesion (CD34$^-$NP) in comparison with CD34$^-$cells with normal ability for adhesion (CD34$^-$ P).

Conclusion: In case of CD34- cells with weak ability for adhesion take place inhibition of crossing G2/M cell cycle check point.

34. LOSS OF HETEROZYGOSITY/ALLELIC IMBALANCE OF THE RGS AND RGS-LIKE CANCER SUSCEPTIBILITY GENES AT THE CHROMOSOME 1Q25.3 REGION IN PRIMARY SPORADIC BREAST CANCER.

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Background: Loss of heterozygosity / allelic imbalance in tumors plays an important role in the identification of new cancer susceptibility genes with a prognostic significance. Allelic imbalance describes either a deletion or an amplification of an allele. Recent studies have led to the identification of four genes from the regulators of G protein signalling family within a 370 kb region at chromosome1q25.3. This chromosomal region is rearranged in breast tumors and is tightly linked to the familial prostate cancer (HPC1).

Aims: The overall aim of this study was to investigate loss of heterozygosity in the RGSL2, RGSL1, RGS16 and RGS8 genes in 226 patients with primary sporadic female breast cancer and to localize the potential chromosomal breakpoints on the strength of the observed rearrangements.

Methods: Polymorphic microsatellite loci are the most important marker type used to detect loss of heterozygosity in this study. Each microsatellite locus was scored by PCR amplification with locus-specific, fluorescent-labelled primers. The amplification products were separated by capillary electrophoresis using ABI 310 Genetic Analyzer.

Results: Microsatellite markers RGS16 A and RGSL1 ex5 exhibited the highest frequency (65% and 64%, respectively) of loss of heterozygosity among all the markers analysed.
The 1q25.3 region showed a high genomic instability in primary sporadic breast cancer. LOH analysis points to the potential chromosomal breakpoint between the markers.

Conclusion: The high rate of chromosomal rearrangements at 1q25.3 region suggests the implication of RGS genes in tumor development, particularly breast cancer.

Key terms: breast cancer, loss of heterozygosity, allelic imbalance, microsatellite markers, RGS genes, chromosomal breakpoint.

35. THE EFFECT OF MISTLETOE EXTRACT (ISCADOR P) ON THE LYSOSOMAL PHYSIOLOGY

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The lysosomes are organelles responsible for a diverse spectrum of cellular functions. In physiolological conditions the lysosomal system is stable. The activity of pharmacological substances may induce a disturbance of lysosomal physiology, which is manifested by a change of activity of lysosomal enzymes. It results in cellular dysfunction and may eventually lead to a variety of diseases.

In the present study we investigated the effect of iscador P on the ultrastructure and activity of some hydrolases of the lysosomal compartment of liver cells. Iscador is the trade name of extracts of Viscum album, which are widely used as complementary cancer therapies in Europa. Preclinical data suggests immunostimulatory and cytotoxic effects of mistletoe extracts, although its mechanism of action is largely unknown. Mistletoe extracts contain a number of components considered biologically active, including lectins and viscotoxins. The extracts are biologically and biochemically standardized.The biologic effects of mistletoe lectin have been studied most extensively. Their mechanism of action is probably 2-fold. On the one hand, mistletoe lectins can stimulate immunological relevant effector cells, on the other hand, mistletoe lectins have shown direct growth inhibitory effects on tumor cells. Depending on the concentration, treatment with mistletoe lectins results in death via apoptosis or necrosis.While the clinical efficacy of iscador in cancer is being investigated, toxicity and potential interactions with standard chemoterapeutic agents are unknown.

The experiment was carried out on 5-month-old Swiss male mice. The mice serving as a control (the first group) were injected intraperitoneally with 0.9% NaCl, while the mice from the experimental groups were injected with iscador P (iscador P is extracted from mistletoe plants growing on pine) in doses 0.1mg/kg b.w., 1mg/kg b.w. and 2mg/kg b.w. After due time (24h) the animals were decapitated and segments of liver were immediately taken for biochemical and morphological studies. In the lysosomal fraction of the hepatocytes the activity of the following enzymes was estimated: cathepsin D and L (Cath D and Cath L, EC 3.4.23.5, EC 3.4.22.15), acid phosphatase (AcP, EC 3.1.3.2) and beta-glucuronidase (BGRD, EC 3.2.1.31). The activity of the enzymes were expressed in μmoles/mg of protein/hour. Data were analyzed statistically using Student's t-test.

Administration of iscador to male mice caused a dose-dependent reduction of the acivity of all examined hydrolases and protein breakdown in liver cells compared to control group. Electron micrographs showed reduction of number and sizes of primary and secondary lysosomes and decrease of autophagic vacuole formation. From our experiment we may conclude that iscador P causes lysosomal disorders.

36. METABOLIC ACTIVATION OF ANTITUMOR 9-AMINO-1-NITROACRIDINE DERIVATIVES C-857, C-1748 AS A PRELIMINARY STEP OF THE COVALENT BINDING OF THIS DRUG TO DNA

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The aim of the presented study was to explain the role of reducing transformations of 9-amino-1-nitroacridine derivatives in their biological activity and toxicity and to create a background for prediction of metabolic pathways that would be possible in the future patients.

Compounds C-857 and C-1748 belong to the new set of antitumor 9-amino-1-nitroacridine derivatives developed in our laboratory headed by Prof. Konopa. These compounds are structural analogs of the Polish antitumor drug, ledakrin. One of them, C-1748, which caused lower toxicity in animals than ledakrin was selected to phase I of clinical trials. It was shown earlier that metabolic activation of 1-nitroacridines is necessary for activity of these compounds towards tumor cells. Futhermore, the formation of the adducts between DNA and activated form of 9-amino-1-nitroacridine derivatives was demonstrated by ^{32}P-postlabeling analyses in tumor cell as well as with activation systems *in vitro*.

In the presented work the studies on the reductive transformations of these agents in the presence of dithiothreitol and various fractions of rat liver microsomes have been described. The studied processes were carried out under identical conditions, in which the covalent binding of these compounds with DNA was shown earlier. The transformations were observed spectrophotometrically and monitored by HPLC RP with MS spectra analysis. We demonstrated that C-857 was less reactive than ledakrin under the studied conditions. Nevertheless, its metabolic transformations of C-857 have led not only to the reduction of nitro group but also to the activation of the acridine ring for nucleophilic substitution in position 2 and 4 and the activation of 9-amino group for electrophilic attack. Therefore, we postulated that three reactive places in acridine ring are possible to form covalent binding of acridine to two strands of DNA. On the other hand, a new less toxic derivative C-1748 turned out to be less reactive with DTT and with microsomal enzymes than C-857. Thus, we hypothesized that high susceptibility of 9-amino-1-nitroacridines to reducing metabolism would be responsible for high toxicity of these compounds. In our investigation we also considered that metabolic transformations of the studied compounds were postulated to be the preliminary step in the covalent binding of these agents to DNA, which is crucial to their antitumor activity. In this respect, the knowledge on the metabolite pathways of the potent medicine, C-1748 will be also helpful in the design of the optimal dose schedule in the future human therapy.

37. ANALYSIS OF GENE EXPRESSION ASSOCIATED WITH IMMORTALIZATION PERFORMED IN STEM CELLS CHARACTERIZED BY DIFFERENT ABILITY TO ADHESION ISOLETED FROM UMBILICAL BLOOD

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Telomerase, a ribonucleoprotein polymerase which takes part in the maintenance of telomeric DNA length, is one of the most important and probably conditional factor in cells immortalization processes. Deregulation of telomerase activity in somatic cells is thought to be involved in oncogenesis.The aim of our study was to determine a model gene expression profile connected with telomerase - dependent telomere maintenance and immoralization in cells that are not tissue specialized, as a reference to following trials performed in neoplasm tissues. It is assumed that stem cells characterized by different adhesion ability can vary in degrees of readiness to immortalization on the transcriptional stage.

Results: Transcriptional activity of selected group of genes associated with telomerase were compared: transcripts composed the telomerase complex (9 genes), potentially activators and inhibitors of hTERT transcription – a catalytic protein component with reverse transcritase activity - (10 genes), hTERT stabilization factors in cell (4 genes), and also transcripts having an effect on telomerase complex activity in general (23 genes)

The analysis was performed by oligonucleotide DNA microarray technique using Affimetrix GeneChip U133A. This method enables parallel analysis of selected groups of genes at the same time and estimate a tendency to changes in expression, depending on examined probes. Among 25 marked activators of immortalization 11 factors revealed high and middle-high transcriptional activity, from 15 inhibitors of this process only 2 factors with high transcriptional activity were detected. The changes in transcriptional activity depending on ability to adhesion in different cells was observed in 7 cases.

Results have been shown in tables and schemes considering semiquantitative basis of method. Metabolic dependences among particular factors have been shown in diagrams.We paid attention to some interpretation problems when comparing outcomes with quantitative analysis of the same single transcripts performed by polymerase chain reaction method based on fluorescent TaqMan methodology.

Because of this ambiguity the results seem to be insufficient for making conclusion regarding a degree of readiness to immortalization in stem cells which differ in the ability to adhere. In spite of this, the study proved valuable in assessing a group of factors that may be useful in pathologic prognosing or diagnosing and in determination of genes important in some particular aspect of metabolism. It also forms a good basis for checking up a gene expression profile by conventional quantitive methods performed in parallel. Our results are a preliminary research concerned with establishing gene expression profile in neoplastic tissues.

38. VEGF ISOFORMS AND ITS RECEPTORS OF TYPE 1 AND 2 EXPRESSION IN PATIENTS WITH NON-HODGKIN LYMPHOMAS

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Vascular endothelial growth factor (VEGF) is the most important new vessel formation regulator in the process of physiological and neoplastic angiogenesis. The mRNA VEGF level and VEGF protein expression positively correlate with a tendency to tumor progression in human malignancies. Hypoxia is the most potent stimulator of VEGF synthesis. There have been four receptors for VEGF identified ; VEGFR-1, (Flt-1), VEGFR-2 (KDR/Flk-1), VEGFR-3 (Flt-4) and Neuropilin-1. It is believed that the most powerful angiogenetic stimulation is mediated by the KDR activation, with recognition of a comparatively poor role of Flt-1 in this process. It seems that it could be of considerable importance in the process of angiogenesis inhibition and inactivation of circulating VEGF protein.

In the presented study mRNA –VEGF expression and their receptors of the 1 and 2 type have been determined. in the group of 14 subjects with diagnosed malignant lymphomas of high and low malignancy grade The VEGF expression has been assessed for the whole panel of six isoforms, moreover, apart from Flt and KDR receptors, soluble receptor Flt-1 expression was also performed. Significantly, higher levels of KDR receptor in high grade malignancy lymphomas were observed, compared to lymphoproliferative disorders of lower grade malignancy. This can confirm its crucial role in the process of angiogenesis stimulation. Flt-1 expression was comparable in both subgroups of patients. Analyzing expression of six isoforms, a higher activity of $VEGF_{165}$ and $VEGF_{189}$ was found in the individuals with high grade malignancy lymphomas. Expression of other isoforms did not show any significant differences when analyzed in both subgroups of patients. Signal pathways engaged in VEGF receptors activation are schematically presented.

39. SECONDARY LIPID PEROXIDATION PRODUCTS IN CULTURED HUMAN MELANOMA CELLS

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The peroxidation of unsaturated fatty acids gives rise to short-chain carbonyl compounds, which contribute to peroxidative cell damage by inhibiting DNA, RNA, and protein synthesis, inhibiting respiration and depleting glutathione. Aldehydic lipid peroxidation products, especially the 4-hydroxylkenals (HAK), due to their high reactivity, display marked biological effects in a concentration-dependent manner. These aldehydes can initiate the processes of spontaneous mutagenesis and carcinogenesis. The two most toxic 4-hydroxylkenals are 4-hydroxynonenal (HNE) and 4-hydroxyhexenal (HHE). These aldehydic compounds can affect and modulate, at very low non toxic concentration, several cell functions, including signal transduction, gene expression, cell proliferation and differentiation, cellular growth inhibition, apoptosis induction, and more generally, response of the target cell.

Data pertaining to the amounts of different aldehydes in cancer cells after photodynamic therapy, are limited. This prompted us to determine aldehyde levels in laser irradiated and non-irradiated human melanoma cells (SK-MEL) in the presence or absence of delta-aminolevulinic acid (ALA). Caco-2 cells, which do not contain melanin, were used for comparison.

Cells were cultured under standardized conditions using Petri dishes and Cellstar 93 incubator (37^0 C, 5% CO_2). ALA(100 μg/ml) was added to the cultivation medium 1 hour before irradiation. Aldehydic products of lipid peroxidation, after extraction with acetonitrile, were detected as their 2,4-dinitrophenylhydrazone (DNP) derivates using HPLC-MS-MS. A gradient mixture of water and methanol (acidified with acetic acid) and C-18 column were used for HPLC separations. Diode-array (250 – 500 nm) detector was used to collect spectra of the separated compounds. Negative ionization was applied in TIC, SIM, product of, and MRM modes during MS analysis.

The following secondary lipid peroxidation products were present in the analyzed SK-MEL cultures: hydroxybutanal, hydroxyheptanal, hydoxyoctanal, hydroxyhexenal, hydroxynonenal, hydroxynonanal, pentanal, hexenal, and hexanal. The addition of ALA and laser irradiation, and even the simultaneous application of both factors, did not influence concentrations of the analyzed aldehydes. In SK-MEL cells, while in Caco-2 a marked increase (3-7 fold) of hydroxyhexenal, hydroxynonenal, hydroxybutanal, hydoxyoctanal, and hydroxynonanal was observed. Is it a result of protective action of melanin?

Anex

PCR AMPLIFICATION OF HIGHLY GC-RICH TEMPLATES. AN EFFICIENT TOOL IN THE SEARCH FOR NEW PROGNOSTIC MARKERS IN CANCER.

Lecture

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In the search for new molecular genetic markers with a statistical significant correlation to prognosis in cancer, it is often necessary to use large scale PCR amplification of a large variety of DNA sequences. DNA templates with a high GC content or multiple consecutive Simple Tandem Repeats (STRs) are difficult to handle using a majority of PCR related techniques and can obstruct PCR dependent analyses in the following situations:

- *Standard PCR amplification*. Highly GC-rich regions prevent template denaturation and thereby product synthesis.
- *Multiplex PCR amplification*. A preferential amplification of low GC-content sequences results in misinterpretation of the balance between the two sequences.
- *Quantitative PCR amplification*. GC-rich targets may form heteroduplexes with internal standards, which are designed to be very similar to the target, leading to misinterpretation of the results.
- *DNA sequencing*. DNA polymerase tends to stall in GC-rich regions, causing decompressing of the sequence thereby making it impossible to read.
- *cDNA synthesis* of GC-rich templates may create shorter fragments that lack GC-rich portions of the sequence. The result is a skewed representation of the original mRNA molecule.
- *Microarray (Not PCR related).*

The amino acid analogue betaine (N,N,N,-trimethylglycine) has proved very successful as an enhancer of PCR amplification of highly GC rich regions. Betaine is more efficient than other substances among low-molecular weight sulfones as DMSO and tetramethylene sulfone. For some amplifications only betaine is successful as an additive.

Reference:

Hansen, L.L. and Justesen, J. PCR amplification of highly GC-rich regions. PCR Primer: A Laboratory Manual, Second edition, chapter 5. Edited by Diffenbach, C.W. and Dveksler, G.S.New York: Cold Spring Harbor Laboratory Press. 2003

40. GENE EXPRESSION PROFILE IN B16(F10) MURINE MELANOMA *IN VITRO* UNDER HYPOHIC CONDITIONS.

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Poster

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Weak oxygenation of tumors results from disturbed blood flow or limited oxygen diffusion. Hypoxia is prevalent in tumors exceeding $1mm^3$. It bears impact upon proliferation, genetic stability, angiogenesis and apoptosis. Poor oxygenation is a serious obstacle to radio- and chemotherapy.

In this study we compared expression profile of genes in cells of B16(F10) murine melanoma cultured in a standard incubator with cobalt chloride (hypoxia mimicry) added to medium or in either a static Billups-Rothenberg chamber (nominal 1% oxygen) or hypoxic incubator with flow of a gas mixture containing 1% oxygen. Total RNA was isolated from 24-hour cultures. cDNA and cRNA was synthesized and the expression profiles were assessed using murine microarrays from Affymetrix.

Numerical fluorescence data processed by Affymetrix software were analyzed using singular value decomposition (SVD) method. Clusterization of genes chosen by SVD method shows distinct differences between experimental samples and controls. Cobalt chloride (chemical mimicry of hypoxia) induces also a set of genes distinct from those obtained using two methods of lowering oxygen concentration in cell culture.

The conditions of the experiment abruptly change the expression profile of genes when oxygen cocncentration is lowered. Among differentiating genes dominate those with increased expression (cobalt chloride – 80/109, Billups chamber – 86/97, hypoxic incubator - 60/85). Using the three approaches described a very strongly increased expression was obtained for two genes: interferon stimulated protein and galectin-3. When only lowering of oxygen concentration is considered there is also a sharp increase in the expression of vinculin, connective tissue growth factor, VEGF and Cyr61.

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