XIIIth Gliwice Scientific Meetings 2009

Gliwice, November 20-21, 2009

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Organizers:

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

The Silesian University of Technology

German Cancer Research Center (DKFZ), Heidelberg

Association for the Support of Cancer Research

The Silesian Voivodship Office

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

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XIIIth Gliwice Scientific Meetings
November 20 – 21, 2009
Program

20.XI.2009
2nd Polish-German Cancer Workshop

dkfz. GERMAN CANCER RESEARCH CENTER IN THE HELMHOLTZ ASSOCIATION

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

9.00 – 9.10 Opening ceremony

Session I: *Molecular Epidemiology of Cancer*

(K. Hemminki and J. Lubiński – chairpersons)

9.10 – 10.50 (Part I)

Kari Hemminki (Heidelberg): Genetic basis of familial aggregation of cancer (25')

Jan Lubiński (Szczecin): The latest advances in clinical genetics of tumors including gastric cancer (25')

Ute Hamann (Heidelberg): Search for modifiers of hereditary breast cancer risk (25')

Asta Försti (Heidelberg): Evidence for genetic basis in breast cancer survival (25')

10.50 – 11.10 coffee break

11.10 – 13.00 (Part II)

Ewa Grzybowska (Gliwice): Molecular epidemiology of hereditary breast and ovary cancer in Silesia, clinical course of breast and ovary cancer in respect to polymorphisms of PGR and MDR1 genes (22')

Annekatrin Lukanova (Heidelberg): Pregnancy hormones and maternal cancer (22')

Dorota Butkiewicz (Gliwice): Genetic influences on survival in lung cancer (22')

Federico Canzian (Heidelberg): Genome-wide studies in cancer (22')

Krzysztof Szyfter (Poznań): Identification of laryngeal cancer-related genes using high resolution array-CGH technique (22')

13.00 – 14.15 Lunch and Poster Session (Part I)

Session II: *Functional Genomics in Cancer Research*

(J. Hoheisel and P. Widłak – chairpersons)

14.15 – 16.00 (Part I)

Stefan Wiemann (Heidelberg): Modeling and testing of cell cycle regulation via the ErbB protein and miRNA network in breast cancer (22')

Aurelio Teleman (Heidelberg): Identification of a phosphatase regulating insulin signaling (22')

Marcin Szaumkessel (Poznań): Pyrosequencing-based DNA methylation profiling of Fanconi anemia/BRCA pathway genes in head and neck squamous cell carcinoma (15')

Sandra Steinbrink (Heidelberg): Genome-wide functional screens to identify novel factors in cancer pathways (22')

Joanna Polańska (Gliwice): New mathematical approaches in analyses of genomic and proteomic data (22')

16.00 – 16.20 coffee break

16.20 – 18.10 (Part II)

Barbara Jarząb (Gliwice): Thyroid carcinoma as a model for gene expression profiling of cancer (22')

Jörg Hoheisel (Heidelberg): Functional genomics and proteomics in pancreatic cancer research (22')

Jolanta Kupryjańczyk (Warszawa): Prognostic and predictive factors in ovarian cancer-results of verification of DNA microarray data (22')

Magdalena Skrzypczak, Jerzy Ostrowski (Warszawa): Potential and challenges of microarray data analyses for predicting oncogenic signaling in colon tumors (22')

Anna Fiszer-Kierzkowska (Gliwice): Molecular profiling of histopatologically normal prostate tissue adjacent to cancer (15')

Piotr Widłak (Gliwice): MALDI-TOF MS-based serum proteome pattern analysis in molecular diagnostics of breast cancer (22')

20:00 – Party - social event

21.XI.2009
Molecular Biology, Biotechnology and Bioinformatics in Cancer Diagnostics and Therapy

Session III: *Bystander Mechanisms as Targets for Cancer Radiotherapy*

(Carmel Mothersill, Joanna Rzeszowska – chairpersons)

9.00- 11.05 (Part I)

Carmel Mothersill (Hamilton, Canada): Radiation-induced non targeted effects of low doses –what, why and how? (25')

Marie Boyd (Glasgow, UK): Bystander effects elicited by different radiation qualities - therapeutic opportunities? (25')

Elisabeth Schueltke (Freiburg, Germany): Bystander mechanisms as targets for cancer radiotherapy (25')

Mansoor Ahmed (Miami, USA): High-dose lattice radiation therapy: clinical, physics and biological perspective of bystander effects (25')

Wojciech Jurczak (Cracow, Poland): Haematologic toxicity of radioimmunotherapy (25')

11.05-11.30 Coffee break

11:30 – 13:45 (Part II)

Colin Seymour (Hamilton, Canada): What are non-targeted radiation effects? (25')

James Murphy (Ballinode Sligo, Ireland): Low LET radiation and bystander factor damage to mammalian mitochondria (25')

Bruce C. McKay (Ottawa, Canada): Targeting transcription-coupled repair as an anti-cancer strategy (25')

Anna Marciniak-Czochra (Heidelberg, Germany): Characterization of the hematopoietic stem cells using mathematical models (25')

Krzysztof Puszyński (Gliwice, Poland): Crosstalk between p53 and nuclear factor-kB systems: pro- and anti-apoptotic functions of NF-kB (25')

Joanna Rzeszowska (Gliwice, Poland): Can blood cells be important in bystander effects? bystander effects in lymphoblastoid cells (15')

13.45 – 15.00 Lunch and poster viewing

Session IV: *Ion Channels and Cancer*

(Zbigniew Grzywna – chairperson)

15.00 – 16.40

Miquel Rubi (Barcelona, Spain): Thermal effects in the Ca^{2+}-ATPase protein (25')

Walter Stühmer (Göttingen, Germany): Ion channels are increasingly being linked to cancer and tumour progression (25')

Maria Mycielska (London, UK): Citrate transporter from prostate epithelial cells (25')

Marek Los (Winnipeg, USA): Proapoptotic, pro-autophagy and proliferatory effect of calprotectin (25')

16:40 – 17.15 Rewards and selected poster presentation

17: 15 Closing remarks

Lecture abstracts

Session I:

Molecular Epidemiology of Cancer

GENETIC BASIS OF FAMILIAL AGGREGATION OF CANCER

Kari Hemminki and Asta Försti

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The recently published large genotyping studies, mainly based on genome-wide scans, have identified a new repertoire of cancer susceptibility genes and loci which are characterized by a high frequency of the risk allele and a low relative risk, in line with the common disease-common variant paradigm (1). A reason for these discrepancies is that the platforms used for genome-wide studies have been built for relatively common variants (minor allele frequency >0.1) constraining the results to variants with high population attributable fraction (PAF) and low familial relative risk (FRR). PAFs have been used extensively for environmental risk factors of cancer in order to rank them and to assess the prospective gains in disease prevention. Their use in cancer genetics is relatively new, probably because the mutant variants of the 'classical' high penetrant cancer genes are so rare that their contribution to the population burden is low compared to the high individual risks. Some recent studies on low penetrant genes do cite PAFs and point to the high conferred population burden in spite of the low relative risks. Differences between high penetrant (relative risk some 5 or more) and low penetrant (relative risks below 1.5 or 2.0) genes have recently been illustrated by 'molecular landscaping'. The PAF of a gene variant integrates any unmeasured gene-gene and gene-environment interactions for the particular study population. With the current volume of genetic data on susceptibility genes, PAFs are useful in putting the findings into an etiologic perspective. The calculation of joint PAFs for several genes gives a progress report into the limits of understanding of the genetic basis of a disease (2). For breast cancer, the nine established loci gave a joint PAF of > 60%, but explaining only some 8% of the empirical FRR. Recent publications on colorectal cancer include the chromosome 8 locus, represented by SNP rs6983267 and shared by prostate cancer, accounted for 0.4% of the empirical FRR of 2.7. Another locus close to *SMAD7* conferred a marginally lower risk and it accounted for 0.3 % of the empirical excess FRR. The joint PAF for these two loci was 27.8%; their FRR would be 1.01, accounting for 0.7% of the empirical excess FRR of colorectal cancer. The available data suggest that the majority of the familial aggregation of these cancers cannot be explained by the known genes/loci.

References:

1. Hemminki K, Försti A, Lorenzo Bermejo J. Etiologic impact of known cancer susceptibility genes. Mut Res Rev 2008;658:42-54.

2. Hemminki K, Försti A, Lorenzo Bermejo J. New cancer susceptibility loci: population and familial risks. Int J Cancer 2008; 123:1726-9.

THE LATEST ADVANCES IN CLINICAL GENETICS OF TUMOURS INCLUDING GASTRIC CANCER

J. Lubiński

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In order to solve problem – be successful, it is critical to work hard and wise, but also to be lucky. Polish society is a lucky one for effective performance of studies on clinical–genetic correlations, because Poland is relatively big country – with almost 40 mln population additionally showing high level of genetic homogeneity.

This is the main reason why in the field of genetic–clinical correlations, at least in oncology, studies conducted in Poland are frequently of unique international value.

Historical milestone in our understanding of genetic characteristic of Polish population were studies performed 10 years ago, in which we sequenced BRCA1/BRCA2 genes in almost 70 families with strong aggregations of breast/ovarian cancers. Gorski B. et al. showed that Poland is dominated by BRCA1 mutations and, additionally, only 3 of them constitute around 90% of all BRCA1 mutations in Poland. DNA test designed especially for Polish population allowed to detect BRCA1 mutations a few dozen times quicker and cheaper than in rich but genetically heterogenous Western countries. This is why in our centre only we performed almost 200 000 of BRCA1 tests detecting almost 5 000 carriers. This is the largest worldwide registry of females with mutations and under surveillance of cancer genetic outpatient clinics from almost all regions of Poland.

Genetic homogeneity of Polish population has been confirmed in studies of other genes associated with predisposition to cancers. As a consequence, we noted very rapid progress in identification of genetic markers for almost all sub-groups of tumours. In 2008, we published the first panel of genetic markers covering more than 90% of breast cancers. This work suggests directly for the first time that carcinogenesis of all tumours is caused by both groups of features–environmental and genetic, however their relative contribution in tumorigenesis of particular case can be variable ranging from a few to a few dozen of per cent. In last years almost all genes associated with monogenic high risk of cancers characterized by strong aggregation of tumours such as i.e. BRCA1/BRCA2 (~80% risk of breast/ovarian cancers), MSH2/MLH1/APC (~80% risk of colorectal cancers) or E-cadherin (~80% risk of diffuse stomach and lobular breast cancers) have been identified (Tab. 1). At present, investigations aimed to identify markers on moderate/low cancers risk are the most frequently performed. Their identification seems to be very important also from clinical practice perspective because these markers:

1. can interact – i.e. women carrying CHEK2 mutation and some BRCA2 variants can be at 6-fold increased risk of breast cancer.
2. can identify high risk persons if combined with family history – i.e. ~10 fold increased risk of prostate cancer occurs in men–carriers of NBS1, CHEK2 or some BRCA1 mutations if even one prostate cancer was diagnosed among relatives.
3. are identifying multiple site predisposition – i.e. CHEK2 mutations are associated with increased risk of cancer of the: breast, ovaries, colon, kidney, stomach, prostate and thyroid and decreased risk of cancers of the lung and larynx and are associated with distinct clinical characteristics of cancers – i.e. breast cancers in families with NOD2 or CDKN2A changes are characterized by occurrence of microcalcifications and significantly increased risk already at age 35 yrs, and cancers dependant on CHEK2 are ER(+) what suggests potential value of tamoxifen in their chemoprevention.

Spectacular progress was noted recently in chemotherapy of breast cancers dependent on BRCA1. In 2007 Byrski T. et al. published retrospective observation on the lack of effectiveness of taxans – in 9 out of 15 females – mutation carriers with breast cancers treated using AT scheme remissions were not seen in neo-adjuvant therapy. Such results were in accordance with observation of British researchers who found that cell lines from breast cancers in BRCA1 carriers are resistant to taxans. These scientists in the same publication reported high sensitivity of cancer cells lines to cis-platinum. This is why, we launched clinical trial on cis-platinum efficiency in treatment of breast cancers among BRCA1 carriers. Results of this first completed clinical trial have been published in July 2008. In all 10 recruited patients, we observed clinical and pathologic remissions which was complete in 9 of them.

Recently, another paper by Byrski T. et al. has been accepted for J Clin Oncol in which efficiency of different schemes of neo-adjuvant therapies in BRCA1 carriers with breast cancers was compared retrospectively. Frequency of complete remissions in monotherapy with cis-platinum was above 80%, in AC scheme – 30% and using CMF or AT (taxans) – 8%. At present, we perform clinical trial on the use of cis-platinum for affected BRCA1 carriers independently on cancer site thus including gastric cancer patients with germline mutations.

Table 1. Syndromes with familial susceptibility to gastric cancers (GC)

Syndrome	Gene(s)	Evidence of association of GC	Sites of other primary cancers
Hereditary gastric/breast cancer	E-cadherin (CDH1)	high risk of diffuse GC among mutation carriers	lobular breast cancer
BRCA2 breast/ovarian cancer	BRCA2	RR of GC increased 2.5–5 times	breast, ovary, prostate
BRCA1 breast/ovarian cancer	BRCA1	RR of GC increased ~4 times	breast, ovary
Peutz-Jeghers	STK11	RR of GC increased ~200 times	breast, intestine, pancreas
Cowden	PTEN	GC reported in a patient with Cowden syndrome	breast, thyroid, endometrium
Li-Fraumeni	TP53 CHEK2	Germline TP53 mutations in GC families CHEK2 truncating mutations confer ~2.5 fold risk of GC	breast, adrenal cortex, connective tissue, kidney, nervous system, pancreas, white blood cells
Familial adenomatous polyposis	APC	30 reported GC cases among published FAP families	colon, rectum duodenum, thyroid, pancreas
HNPCC/Lynch	MSH2, MLH1, MSH6	GC risk increased in carriers with atrophlcans gastritis	colon, rectum, endometrium, small bowel, urothelium, kidney, ovary
Ataxia telangiectasia	ATM	Excess risk – RR 3.5 of GC in heterozygotes	skin, breast, eyes
Werner	WRN	GC has been reported in association with the syndrome	connective tissue, skin, thyroid

SEARCH FOR MODIFIERS OF HEREDITARY BREAST CANCER RISK

Ute Hamann[1], Stefan Wilkening[1], Michael Gilbert[1], Chen Bowang[1], Kari Hemminki[1], Anna Jakubowska[2], Jan Lubinski[2]

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[2]International Hereditary Cancer Center, Department of Genetics and Pathology, Pomeranian Medical University, Szczecin, Poland.

Germline mutations in *BRCA1* confer high risks of breast and ovarian cancer. The risk varies by the age at diagnosis and the type of cancer in the index patient suggesting that breast cancer risk in mutation carriers is modified by other genetic or environmental factors that cluster in families. In this study we search for genetic modifiers of hereditary breast cancer risk in Polish women carrying the 5382insC *BRCA1* mutation using a genome-wide association study (GWAS) on DNA pools. DNA samples were collected from 124 young breast cancer cases (age of diagnosis $\leq$ 45 years) and 119 older disease-free controls (age at interview $\geq$ 50 years) all harbouring the 5382insC *BRCA1* founder mutation. Equimolar amounts of each DNA sample were added to either the case or control pool. Pools were genotyped using Illumina HumaCNV370-Duo arrays. Single nucleotide polymorphisms (SNPs) for individual genotyping were selected using three methods including the cluster method, allele frequency difference method and combined Z-test. Twenty-seven loci showing the largest significant differences were selected for individual genotyping on 1,500 carriers. Genotyping analyses are currently performed. Preliminary results will be presented.

EVIDENCE FOR GENETIC BASIS IN BREAST CANCER SURVIVAL

Asta Försti

Division of Molecular Genetic Epidemiology, German Cancer Research Center (DKFZ), Heidelberg, Germany.

Several earlier studies have assessed survival in breast cancer based on familial risk of this disease. The results have been conflicting and suggest that the risk and prognostic factors of cancer are largely distinct. As a novel concept, we searched for familial clustering of survival, i.e. concordance of survival among family members. We used the nation-wide Swedish Family-Cancer Database to estimate hazard ratios (HRs) for cause-specific and overall survival in breast cancer. The study covered 1277 mother-daughter pairs with familial breast cancer. The results were consistent in showing that both good and poor survival in breast cancer aggregated in families, suggesting that the prognosis in breast cancer is in part heritable. Accordingly, we searched for genes that control heritability of prognosis among genes involved in metastatic cascades, including angiogenesis-related genes, adhesion and extracellular matrix degradation related genes, chromosomal instability genes and telomere length related genes. For this study, we used a large Swedish study population with detailed clinical data and up to 15 years of follow-up. The results showed that variants in the angiogenesis-related genes, VEGF, KDR and POSTN correlate with traditional prognostic factors in breast cancer. Variants of the gene for PAI-1, which is an important factor for the invasive capacity of tumours, had prognostic bearing in breast cancer. Integrins have similar functions, also carrying genetic variants governing prognosis in breast cancer. Genes with implications for chromosomal instability also had variants associated with prognosis in breast cancer. As a next step, we are carrying out a genome-wide association study on survival, in which we compare the genotypes of women with short survival (< 5 years) with women who have survived 11 years or longer, in collaboration with the Nordic countries and Germany.

MOLECULAR EPIDEMIOLOGY OF HEREDITARY BREAST AND OVARY CANCER IN SILESIA, CLINICAL COURSE OF BREAST AND OVARY CANCER IN RESPECT TO POLYMORPHISMS OF PGR AND MDR1 GENES

Karolina Tęcza[1], Jolanta Pamuła-Piłat[1], Sylwia Jędruś[2], Ewa Telka[3], Ewa Grzybowska[1]

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Aim: We wanted to analyze the influence of modifying genetic factors on the risk of BRCA(+) and BRCA(-) breast and ovary cancers and on clinical parameters as the age of onset and survival.

Materials and Methods: 348 anonymous healthy women (control group), 229 persons with ovary cancer, 45 persons with breast and ovary cancer (case group). The patients under study developed breast cancer prior to ovary cancer. RFLP and ASA PCR were used to analyze mutations in BRCA genes and polymorphisms in PGR and MDR1 genes.

Results: Alleles AA PGR +331G/A and TT PGR20 slightly decreased the risk of ovary cancer. The presence of allele T of 660 PGR significantly decreased the risk of second malignancy (breast cancer) OR=0,44, p=0,039. Allele T of PGR 20 decreased also the risk of second malignancy. Heterozygotes for PGR V660L and 20 were at statistically significant decrease of second malignancy, OR=0,35, p=0,04. Heterozygotes CT of MDR1 gene C3435T and genotypes AG and AT of G2677T/A were at lower risk of developing ovary cancer. Genotype CT of C3435T polymorphism had protective effect against developing the second malignancy while allele TT increased the risk of breast cancer. Heterozygotes for both polymorphisms were at higher risk of developing malignancy.

Age of onset of ovary cancer for GT heterozygotes of PGR V660L did not depend on the status of BRCA mutation. For heterozygotes GA of PGR +331 polymorphism the age of onset for breast and ovary cancer were lower in the group with negative BRCA status. Carriers of TT and AT alleles of MDR1 G2677A/T and carriers of TT allele together with negative status of BRCA had earlier onset of ovary cancer than carriers of the same alleles, with mutation in BRCA genes. Carriers of T allele of MDR1 G2677A/T had later onset of ovary cancer regardless the status of BRCA genes.

Alleles GA of PGR +331G/A and V660L shortened the survival of ovary cancer patients with negative BRCA status. Alleles GG of PGR +331G/A and GT for V660L polymorphisms increased the survival of ovary cancer patients with mutation in BRCA genes. Patients with mutation in BRCA genes, two primary malignancies, carrying allele GA of PGR+331G/A polymorphism had the longest survival.

Conclusions: Polymorphic variants of PGR and MDR1 genes modified more the clinical course and the risk of second malignancy than the risk of developing ovary cancer alone.

PREGNANCY HORMONES AND MATERNAL CANCER

Annekatrin Lukanova, Rudolf Kaaks

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Pregnancy is the strongest natural protection against breast and ovarian cancers. Nevertheless, particularly for breast cancer, the effect appears to be complex. Before the long-term protective effect is established, there is a transient increase in risk, which is especially pronounced in women who delay childbearing until after age 30-35 or in women with a strong family history of the disease. The mechanisms underlying the association of pregnancy with cancer risk are poorly understood, but animal experiments strongly suggest that the hormonal changes during gestation are involved.

Using the resources of large Maternity Cohorts from Finland and Northern Sweden the association of first trimester pregnancy human chorionic gonadotropin, estrogens, progesterone, testosterone, SHBG and IGF-I with maternal breast cancer were explored. Initial preliminary results strongly suggest that hormone concentrations during the first trimester of a first full-term pregnancy play an important role for both the beneficial and adverse effect of pregnancy on maternal risk of breast cancer.

In the past century dramatic changes in the reproductive pattern has taken place world-wide, with a delay of the onset of childbearing and reduction of the average number of children per woman. While the societal and economic forces that are at the roots of such profound, rapid changes are not likely to be modified, a better understanding of the factors mediating the protective association of childbearing could ultimately lead us to the design and launching of preventive interventions seeking to mimic nature's plans.

GENETIC INFLUENCES ON SURVIVAL IN LUNG CANCER

Dorota Butkiewicz

Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice, Poland.

Lung cancer remains the leading cause of cancer-related mortality among men and women worldwide. Despite recent advances in therapy, the overall 5-year survival rate for the patients is still low. Molecular genetic studies have shown that polymorphic variations (SNPs) in genes participating in many pathways may modulate susceptibility to lung cancer. Also, they have been suggested to affect response to therapy, its toxicity and prognosis. While it is unlikely that a single SNP can adequately assign cancer treatment options, predict survival or allow early detection, a better understanding of the mechanisms and pathways involved in lung carcinogenesis may lead to improved clinical outcomes in the future. In the present report several biomarkers, mainly associated with DNA repair, and their role in lung cancer prognosis will be evaluated.

GENOME WIDE STUDIES IN CANCER

Federico Canzian

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In the last few years, genome-wide association studies (GWAS) have proven to be a powerful tool to explore the role of common polymorphisms on risk of common diseases, including cancer.

Existing GWAS results are still far from showing a complete picture of genetic susceptibility to cancer. New GWAS on specific types of common cancers, as well as on rare cancers, are needed.

The NCI-sponsored Cohort Consortium provides excellent opportunities to perform these studies. In particular, the Breast and Prostate Cancer Cohort Consortium (BPC3), which includes large prospective cohorts from Europe and the USA, is performing two new GWAS on estrogen receptor negative (ER-) breast cancer and aggressive prostate cancer, respectively.

Estrogen receptor negative (ER-) breast cancers have specific biological and epidemiologic characteristics and greater lethality. Aggressive forms of prostate cancer differ epidemiologically from the more common indolent forms of prostate cancer and are of greater clinical importance. The current generation of GWAS is underpowered to discover gene variants associated with these tumors. Over 2,000 cases of ER- breast cancer and of aggressive prostate cancer, and matched controls, are being currently scanned within BPC3.

In parallel, by pooling all available cases from the cohorts, we can study over 10,000 cases of both breast and prostate cancer. This enables us to further study polymorphisms shown by previous GWAS to be associated with risk of these cancers. We have so far genotyped 16 SNPs for breast cancer and 29 for prostate cancer. We use these data to 1) replicate previously reported associations, 2) examine interactions between SNPs and established non-genetic risk factors and 3) set up multifactorial scores to predict cancer risk.

Finally, a GWAS on pancreatic cancer is ongoing within the Cohort Consortium. By using nearly 4,000 cases from cohorts and case-control studies, we have identified for the first time 4 genomic regions harboring polymorphisms that influence the risk of pancreatic cancer. We are performing studies on pancreatic tumor samples to elucidate the functional relevance of these associations.

IDENTIFICATION OF LARYNGEAL CANCER-RELATED GENES USING HIGH RESOLUTION ARRAY-CGH TECHNIQUE

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Tobacco smoking and alcohol abusing are the dominant causable factors in laryngeal cancer. However, as only a fraction of exposed persons develops laryngeal cancer, a significance of genetic factor is to be studied. Further, a progression of cancer also seems to be associated with expression of specific oncogenes and tumor suppressor genes (TSG). Recently, molecular cytogenetics and biology provide promising techniques to identify an involvement of particular genes not considered yet in a given type of cancer.

It was assumed that highly amplified region contain oncogenes, whereas chromosome deletions indicate for tumor suppressor genes. The study was done on 24 cell lines derived from laryngeal cancer with variable characteristics concerning TNM, grading, survival time, treatment etc. To get orientation in genome changes related to cancer the cells were analysed by classical cytogenetics, FISH technique and array-comparative genomic hybridization. Gene copy number and gene expression were examined for the selected candidates.

An involvement of three known genes in laryngeal cancer was reinvestigated to attribute them to particular stages of cancer progression. Tumor suppressor gene *Rb* [chromosome localisation 13q14] seems to promote metastasis to the adjacent lymph nodes. *TP16* [9p21] deletion was observed only in early tumors. Oncogene *CCND1* [11q13] amplification was observed in late stages and is connected with a short survival. Two other potential oncogenes (*FGF3* and *FGF4*) amplified in the same regions are not expressed. However, 5 other genes harboured in the same region including cortactin are highly amplified and expressed. Next, for *GNG7* [19p13] a TSG activity not reported yet was proven and shown to be correlated with tumor undifferentiation. Homozygotic deletion in 3p17 region indicates for TSG activity of *SLC6A6* and *GRIP2* genes. Oncogenic function of *CRKl*, *MAPK-1* [22q11-12], *PGCP* and *SDC2* [8q22.1] is still under study.

Altogether, 5 TSG and 17 oncogenes have been studied until now. Some of them were linked to progression of laryngeal cancer. At least two novel genes could serve as progression markers.Genes involved in cancer progression could be further studied in respect to targeted therapy.

Session II:

Functional Genomics in Cancer Research

MODELING AND EXPERIMENTAL TESTING OF CELL CYCLE REGULATION VIA THE ERBB PROTEIN AND miRNA NETWORK IN BREAST CANCER

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Division Molecular Genome Analysis, German Cancer Research Center, Im Neuenheimer Feld 580, 69120 Heidelberg, Germany.

Overexpression and mutation of transmembrane ERBB tyrosine kinases are adverse prognostic markers in several cancer entities. A causative relation of ERBB signaling with cancer development and progression has been established and, therefore, molecules of the respective pathways are common targets of antibody and small molecule therapies. In breast cancer, ERBB2 is targeted by the monoclonal antibody trastuzumab, however, frequently observed de-novo resistance to this drug requires a thorough understanding of the ERBB signaling network in order to improve prognosis and therapeutic outcome. To this end, we combined computational simulations, experimental testing, and reverse engineering of the ERBB protein and miRNA interaction network in a breast cancer cell system. We first established the technologies and bioinformatic means required to quantitatively analyze ERBB signaling that links extracellular growth-factors with the cell cycle. To this end, we connected ERBB signaling with G1/S transition via two major cell signaling pathways and two key transcription factors, to model an interaction network that allows for the testing of perturbations and the prediction and analysis of induced effects on the phenotype. Individual components were then systematically knocked down in the system and effects on G1/S transition were recorded employing quantitative proteomic and molecular assays. Based on this quantitative data, the original literature-based network could be refined and extended. Additional protein and miRNA components as well as novel connections could be integrated based on experimental validation also of miRNA-gene interactions and the identification of feedback and feed forward loops regulating the signaling network in proliferation as well as in cell migration and invasion. While our understanding of ERBB-signaling is still far from being complete, our data already suggests several proteins and one miRNA (family) as potential novel targets for therapy.

IDENTIFICATION OF A PHOSPHATASE REGULATING INSULIN SIGNALING

Aurelio Teleman

German Cancer Research Center (DKFZ, Heidelberg, Germany.

Insulin signaling is an important regulator of organismal metabolism, tissue growth, and aging in animals. The intracellular insulin signaling pathway consists of a large number of kinases that activate each other. Since insulin signaling is a homeostatic signaling pathway, it needs to be both activated and inactivated. Phosphatases responsible for inactivating the insulin pathway remain to be characterized. We will present data on the identification and characterized of a phosphatase responsible for dephosphorylating and inactivating an important component of the insulin pathway, S6 Kinase (S6K).

PYROSEQUENCING-BASED DNA METHYLATION PROFILING OF FANCONI ANEMIA/BRCA PATHWAY GENES IN HEAD AND NECK SQUAMOUS CELL CARCINOMA

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Fanconi anemia (FA) is a complex of heterogenous genetical and phenotypical recessive disorders, characterized by numerous congenital malformations. The syndrome is featured by hypersensitivity to DNA crosslinking agents, increased chromosomal breakage, and defective DNA repair. Moreover, FA patients predispose to both hematologic malignancies and solid tumors, in particular acute myeloid leukemia (AML) and squamous cell carcinomas (SCC). Chromosomal instability in FA as the result of defect/mutation in system maintaining genome integrity leads to high susceptibility to cancer development, with a very high risk of developing aggressive forms of head and neck squamous cell carcinoma, especially in young patients. Therefore the Fanconi/BRCA pathway genes are considered as 'caretaker' tumor-suppressor genes and suggested to be the targets of inactivation in head and neck squamous cell carcinomas.

The aim of the study was to investigate the methylation profiles of Fanconi/BRCA pathway genes in the head and neck squamous cell carcinomas (cell lines and primary tumors) to assess a potential involvement of methylation mechanism in carcinogenesis regulation.

The study group consisted of 13 head and neck squamous carcinoma cell lines and 64 cases of larynx primary tumors. As a control group peripheral blood DNA from 10 men and 10 women was included, as well as DNA extracted from buccal swabs (5 men and 5 women). The basic technique used for methylation profiling was pyrosequencing.

Most of the Fanconi genes (BRIP1, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCL, FANCM, PALB2) showed no methylation or very low methylation level suggesting that methylation is not the mechanism for their deactivation. In turn FANCB (located on X chromosome) demonstrated differences in methylation levels but only in respect to gender (women with higher level than men). FANCA gene demonstrated lower methylation in most of HNSCC samples, in comparison to controls, where a total hypermethylation was observed. BRCA1 revealed hypomethylation in 3/13 (23%) cell lines and in 2/64 (3%) primary larynx cases. Moreover, BRCA2 gene appeared to be hypermethylated in HNSCC cell lines, simultaneously displaying lower methylation level in primary larynx samples but still higher than control group. BRCA2 is shown to be deactivated in HNSCCs, where methylation appears to be its mechanism.

The work was supported by the Federation of European Biochemical Societies.

GENOME-WIDE FUNCTIONAL SCREENS TO IDENTIFY NOVEL FACTORS IN CANCER PATHWAYS

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A limited number of highly conserved signaling pathways control cellular homeostasis, growth and development of almost any organism. These pathways control various biological functions and their dysregulation can cause developmental defects, aberrant cell growth and cancer. With the availability of the human genome sequence, a systematic analysis of genes implicated in signaling pathways is now possible. Approaches such as high-throughput RNA interference (RNAi) screens have proven to be a valuable tool for the functional analysis of biological processes and pathways in C. elegans and Drosophila and in recent years as well in cultured mammalian cells. The further modification of such pathways with compounds of low-molecular weight, so called small molecules, enables the functional interference on a level that can be used to target hyperactivated signaling pathways in the treatment of diseases such as cancer. We combine systematic analysis of signaling pathways using genome-wide RNAi screens with high-throughput compound screenings. The screens can be used to identify novel targets and lead substances interfering with these targets. Furthermore, we developed computational methods to analyze complex screening data sets and to predict gene function by phenotypic similarity as well as possible mechanism of action and side effects of small molecules.

In a genome-wide siRNA approach we systematically screened for modifiers of cellular growth and survival by applying the death-inducing ligand TRAIL (TNF-related apoptosis-inducing ligand) to human cervix carcinoma cells (HeLa). Our analysis identified several novel genes that upon an RNAi-mediated knockdown resulted in resistance of different human cancer cell lines to apoptosis induction by TRAIL and in their enhanced clonogenic survival. Our data gives insight into the complexity of signaling pathway integration and regulation. The example of RNAi screens combined with therapeutic molecules such as TRAIL, demonstrates the power of the approach to analyze synthetic interactions and identify potential side effects and markers of treatment resistance.

NEW MATHEMATICAL APPROACHES IN ANALYSES OF GENOMIC AND PROTEOMIC DATA

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The rapid growth of technology, we have been observing in last decade, allows for collecting a huge amount of data in one experiment. Such massive information cannot be analyzed without a help of data mining algorithms. Mathematical modeling is one of these. It is especially useful while the experimental conditions are multifactorial and the sample size is too small to obtain the satisfactory power of statistical testing.

We propose to use the modified Gaussian mixture model (GMM) for the analysis of time-course and dose-response-pattern DNA microarray data. The dataset consisted of the Human fibroblasts cells' expression profiles obtained from three different subjects, irradiated five different doses and measured at three time points (45 Affymetrix DNA microarray HG-U133 Plus 2.0 in total). According to EURATOM standards, the applied irradiation doses (0mGy, 50mGy, 100mGy, 200mGy, 2Gy) are classified, excluding 2Gy, as the "low dose" and the response signal is very low, hardly to be separated from the measurement noise. The application of GMM methods allows the unsupervised grouping of responses of cells to such low dose irradiation.

The procedure for data analysis involved the following steps: data preprocessing (reannotation, normalization, filtration); grouping expression levels; functional description of obtained groups (GO ontology, KEGG Pathway Analysis). The results of grouping genes according to their patterns of expressions led to highly statistically significant gene clusters. This suggests that the proposed techniques lead to development of useful bioinformatic methods of the analysis of multifactorial-course microarray data. As a result of analyses of patterns of expressions, we observe the decrease of expression level for the genes involved in cell cycle and mitosis, and DNA replication processes.

Analogous methodology was successfully applied to ChIP-on-chip data on the heat shock response of mouse spermatocytes.

Gaussian mixture modeling is also a very useful tool for defining spectral features of MALDI ToF proteomic mass spectra. Spectral features, computed as convolutions of Gaussian masks with spectral signals, have been proven to be more efficient than commonly applied spectral peaks, in the aspect of specificity and sensitivity of classifiers.

Acknowledgement:
This work was supported by the European Program FP6 – 036452, GENEPI-lowRT

THYROID CARCINOMA AS A MODEL FOR GENE EXPRESSION PROFILING OF CANCER

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Thyroid cancer constitutes a good model for investigation of relations between the cancer transcriptome and the disease characteristics. The rare histotypes, anaplastic thyroid cancer (ATC) on one side and medullary thyroid cancer (MTC) on the other, are subjects of intensive studies on the level of both gene expression as well as array-CGH studies and the most important results will be presented briefly. The main subject of the talk will be differentiated thyroid cancer. The two DTC histotypes, papillary (PTC) and follicular (FTC), are different diseases from the molecular point of view, while their clinical course is quite similar. Despite the fact that they both arise from the same follicular cell, their gene expression profiles differ very distinctly by the expression of more than 1000 genes. In fact, the very distinct gene signatures may also be attributed to the main molecular subtypes of PTC: there is a large difference in gene expression between BRAF-positive and BRAF-negative PTCs, the RET-PTC-related PTC is characterized by a distinct gene expression profile as well.

In PTC, many tumor and patient-related features are correlated with the changed expression of multiple genes. Among patient-related features, the sex signature is very distinct, while the effect of age, also present, is less visible. Among tumor-related factors, the grade is the most significant one, while metastatic signature has not been well defined until now. In FTC, the very distinct gene signature is related to the oncocytic type of tumors.

In both types of differentiated thyroid cancer (DTC), molecular markers of poor prognosis are intensively looked for. The issue of BRAF mutation and its prognostic input for the management of PTC patients will be discussed, completed by microarray-based approaches to support the analysis. Also, the significance of NIS expression for the prognosis of DTC will be analyzed in comparison to other molecular data available.

Another very interesting issue from the clinical point of view is the molecular support for the differential diagnosis between benign and malignant follicular tumors. A survey of published attempts to solve this question will be presented, completed by own experience in this field. The molecular diagnosis of thyroid nodules will be evaluated in comparison to routine diagnostic procedures.

FUNCTIONAL GENOMICS AND PROTEOMICS IN PANCREATIC CANCER RESEARCH

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Our research aims at the development and immediate application of new technologies for an analysis, assessment and description of both the realisation and regulation of cellular function from genetic information.

Analyses on tumour material are at the centre of attention with an emphasis on pancreatic cancer. Parallel studies at a global level are under way on the epigenetic modulation of gene promoters, variations in transcription factor binding, changes of transcript levels of coding and non-coding RNAs, on the actual protein expression and the intensity of protein interactions. From the resulting data, we aim at an understanding of cellular regulation and its biological consequences. In combination with clinical facts, the knowledge is used for the creation of means of early diagnosis, accurate prognosis and the analysis of treatment results as well as the establishment of new therapeutic approaches.

A more recent line of work aims at an *in vitro* implementation of complex biological processes. Motivation is a utilisation for the production of molecules and the establishment of artificial molecular systems. Cell-free biosynthetic production will become important for many biotechnological and pharmacochemical challenges ahead.

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PROGNOSTIC AND PREDICTIVE FACTORS IN OVARIAN CANCER- RESULTS OF VERIFICATION OF DNA MICROARRAY DATA

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We aimed to verify gene expression data obtained previously in 71 ovarian carcinomas (32 treated with platinum-cyclophosphamide - PC, 39 treated with taxane-platinum - TP) with the use of cDNA oligonucleotide microarrays (HGU 133 Plus 2.0, Affymetrix). Statistical analyses (GC-RMA normalisation, test T-student, Mann-Whitney U-test, Benjamin-Hochberg procedure, false discovery rate - FDR<5% or p value <0.001) revealed relatively small numbers of genes (from 0 to 39, depending on the analysis; fold change >1.5 or <0.67) differentiating chemotherapy-sensitive and -resistant carcinomas, as well as those differentiating short and long disease-free or overall survival in the both chemotherapy groups. First, the results were verified at mRNA expression levels with the use of real-time RT-PCR on the same (PC-treated) or enlarged group (TP-treated) of ovarian carcinomas. Approximately one-third of the verified genes were confirmed as to their clinical significance in this verification. Next, clinical importance of selected genes was studied on large clinical groups of ovarian cancer patients; these analyses were performed at protein levels with the use of immunohistochemical detection. Among genes differentiating the sensitivity to chemotherapy were *TNFSF13, BCAP29, TMEM106C, FAF1* and survivin, while among those differentiating survival are *ING1*, *PTPN2*, *PCID2*, *VGLL1* and survivin. Similarly to our previous studies, TP53 status determined the clinical significance of some of these genes. On the basis of our results and data from the literature, it appears that the clinical endpoints in ovarian cancer patients are modified by subtle changes in the expression of many proteins (in addition to strong clinical factors), rather than by a stable gene expression profile.

Supported by the grant 2361/B/PO1/2008/34 from the Polish Committee of Scientific Research.

POTENTIAL AND CHALLENGES OF MICROARRAY DATA ANALYSES FOR PREDICTING ONCOGENIC SIGNALING IN COLON TUMORS

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An individual cell phenotype is a resultant of the sum of cell-specific, developmental stage-specific, and metabolism-related changes in gene expression selectively provided at the time and grouped into signaling and metabolic pathways. While cancer development results from series of somatic "driver" mutations and multiple epigenetic changes leading to growth advantage of a specific cell population over its neighbors, cancer complexity at the gene level is likely reduced to a limited number of alterations within signaling pathways.

With an introduction of high-density DNA microarrays, modern biology provides a great insight into the overall status of a cell. However, the comparative analyses of microarray-based gene expression profiles relating to carcinogenesis exhibit rather weak consistent overlap. These divergences may result from technical reasons, including a usage of different microarray platforms, different sample collection methods and different analytical algorithms. Since single standard protocol for microarray data has yet not been identified, gene expression profiles still allow rather draft and mostly indirect assumptions on oncogenic signaling.

Colorectal cancer (CRC) arises as multi-step process in which the morphologic counterpart of molecular alterations leads to progressive cytological and architectural derangement recognizable as the adenoma-carcinoma sequence. To clarify the oncogenic pathway alterations underlying the colorectal adenoma-carcinoma sequence, we designed an integrative genomics approach. Studies were performed on samples of normal mucosa, adenomas and carcinomas obtained during surgery or colonoscopy. The collections of cryostat sections prepared from tissue samples were evaluated by the pathologists to control the relative cell type content, and RNA was isolated from macro- and microdissected specimens. The measurements were done using the Affymetrix GeneChip HG-U133plus2, and the data were evaluated using pair-wise comparisons, clustering analyses and data decomposition into SVD modes and ICA independent components, followed by selection of potential alteration within signaling pathways. This presentation will addressed the potential and challenges of a translation of microarray-based gene expression profiling into the functional aspects of carcinogenesis.

MOLECULAR PROFILING OF HISTOPATOLOGICALLY NORMAL PROSTATE TISSUE ADJACENT TO CANCER

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Cancer begins with multiple genetic alterations that sequentially transform a cell, or a group of cells in a particular organ. As a result of this transformation, according to field cancerization concept, genetically altered but histologically normal appearing cells predate the development of neoplasia or coexist with malignant cells. Prostate cancer is often multifocal, and it is likely that multiple tumors arise from an organ which has been earlier genetically altered by a particular carcinogen. Aim of our study was to identify molecular signature of genetically changed but histologically normal prostate cells.

In our study we performed a comprehensive gene expression analysis on 45 human prostate biopsy samples including prostate cancer tissue, prostate tissue adjacent to tumor and benign prostatic hyperplasia, using U133 Plus 2.0 Affymetrix arrays.

In the first step of analysis genetic profiles of prostate cancer samples and benign prostatic hyperplasia samples were compared. We have found 279 genes which differentiate the groups, among them were genes found in other studies as changed in prostate cancer: AMACR, hepsin, EZH2, which demonstrates that microarray analysis of biopsy specimens gives similar results to the studies performed using prostatectomy specimens. In the next step we compared the genetic profiles of benign prostatic hyperplasia and normal-appearing prostate tissue adjacent to cancer. We obtained 129 geneset differentiating those two groups, and this difference was significant ($p=0.013$) according to the global test of difference. Biocarta database analysis revealed that pathway: "Chromatin Remodeling by hSWI/SNF ATP-dependent Complexes" seemed to be particularly down-regulated in prostate tissue adjacent to cancer ($p<0.0001$), with seven genes showing expression decrease ($p<0.05$). Genes identified by us has yet to be validated by RT-PCR and immunohistochemical analysis.

Molecular changes in prostate tissue adjacent to cancer found in our study appear to have potential utility as early diagnostic markers.

MALDI-TOF MS-BASED SERUM PROTEOME PATTERN ANALYSIS IN MOLECULAR DIAGNOSTICS OF BREAST CANCER

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Mass spectrometric analysis of the blood proteome is an emerging method of clinical proteomics. The approach exploiting multi-protein/peptide sets (fingerprints) detected by mass spectrometry that reflect overall features of a specimen's proteome, termed proteome pattern analysis, have been already shown in several studies to have applicability in cancer diagnostics. Here we aimed to identify serum proteome patterns specific for the early stage breast cancer patients using MALDI-ToF mass spectrometry.

Blood samples were collected before the start of therapy in a group of 92 patients diagnosed at stages I and II of the disease, and in a group of age-matched healthy controls (104 women). Serum specimens were purified and the low-molecular-weight proteome fraction (2-10 kDa) examined by spectrometry after removal of albumin and other large serum proteins, and then registered mass spectra were analyzed using new bioinformatic tools created in our group.

We identified the classifier built of four spectral components that differentiated healthy persons and breast cancer patients with ~85% specificity and sensitivity. Spectral components (i.e., protein ions) that were the most frequent in such classifier had approximate m/z value of 2866, 3579, and 2303 Da. The classifier intentionally built of above three components showed 80% specificity and 88% sensitivity. Interestingly, we have also observed significantly ($p=0.0003$) increased level of osteopontin in blood of analyzed group of cancer patients. However, the classifier built of osteopontin level showed 28% specificity and 88% sensitivity, and thus was outperformed by the classifier built of the most frequent spectral components identified in serum by mass spectrometry. In addition, we have identified several components whose levels in serum of individual patients were significantly changed in the course of therapy. Our data clearly indicates that MALDI-ToF-based serum proteome pattern analysis has an obvious potential in diagnostics and monitoring of therapy of breast cancer patients.

This work was supported by the Ministry of Science and Higher Education, Grant 2 P05E 067 30.

Session III:

Bystander Mechanisms as Targets for Cancer Radiotherapy

RADIATION-INDUCED NON TARGETED EFFECTS OF LOW DOSES – WHAT, WHY AND HOW?

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Ever since the grudging acceptance that non-targeted effects (NTE) can be measured in unirradiated cells or distant progeny of irradiated cells, the discussion has raged about the relevance of these effects. For the purposes of this presentation, NTE are defined as effects not associated with DNA lesions due to energy deposition in the cell showing the effect and so include genomic instability and bystander effects. Obviously, it is important to consider relevance for practical applications such as radiation protection and radiotherapy. To this end this paper will review data from in vivo experiments, which address questions about risk after medical and environmental exposures. However a major area of interest is the intrinsic relevance of these mechanisms in biology. Arguments will be made in this paper, that non-targeted effects (NTE) may call into question not only radiation effects paradigms but may also have relevance to wider mechanisms in cancer biology, population ecology and evolutionary biology concerning process of selection, the transmission of heritable traits, the relevance of "social" interactions between cells, organisms and populations and the mechanism by which cells/organisms respond rapidly to environmental stress. This paper will also argue that a key consequence of findings in NTE biology is that at any given level of organization, from gene to ecosystem – communication of stress signals and heritability of stress adaptations provide the bridges linking one hierarchical level to the next and enable the rapid propagation of change triggered by stress at one level, resulting in change at a higher (or lower?) level. This addresses a major problem in evolutionary biology because while the molecular mechanisms of natural selection are fairly well understood a major knowledge gap exists in translating mutational drift at the level of the individual cell to natural selection at the ecological level where sociobiological factors are so important. The existence of the mechanism discovered in the NTE field provides a glimpse of a major way that evolution could be regulated through communicated signals between cells, individuals, and populations, These control and optimize responses at the level of the population and coordinate the emergence of exquisitely tuned systems which can adapt rapidly to micro or macro environmental change. It is likely that consideration of these mechanisms could also be of benefit in cancer biology providing new insights into the regulation of cancer cell social groups and how these interact with the host.

BYSTANDER EFFECTS ELICITED BY DIFFERENT RADIATION QUALITIES - THERAPEUTIC OPPORTUNITIES?

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Radiotherapy is currently utilised in the treatment of a significant number of cancers, however its efficacy is hampered by limited dose administration due to normal tissue toxicity. Several approaches are currently under consideration for improved efficacy and one particularly promising approach is the utilisation of radiation induced biological bystander effects (RIBBE) for tumour cell kill. It has long been accepted that when cells are irradiated, both direct effects and indirect effects are consequences of such irradiations. It has become apparent however that different radiation sources result in a variety of indirect (bystander) effects that differ with respect to their magnitude and nature. In particular RIBBE following irradiation of cells with targeted radionuclides conjugated to alpha-–beta- and auger emitters are quantitatively and qualitatively different to those achieved following irradiation with external beam sources. It appears that when cells are irradiated with the lower dose and dose rate radiopharmaceuticals, RIBBE are more toxic to recipient cells and the effect on recipient non-irradiated cells is more dependant on dose. When pharmaceuticals are conjugated to high LET radiopharmaceuticals, recipient cells succumb to toxic bystander effects in a dose dependant fashion, superior to direct irradiation and with higher target cell dose this effect is perturbed (U-shaped survival curve). Thus RIBBE following targeted radionuclide therapy is a complex and novel phenomenon which offers a new tool for optimisation of tumour cell kill if its mechanism and nature can be determined. Our studies are aimed at investigating such effects to determine optimum radiopharmaceuticals for maximisation of both direct and indirect effects. Our studies are suggestive of a mechanism that involves several unique components including generation of Reactive Oxygen and Nitrogen Species and complex cellular signalling pathways. As it is likely that clinical translation of targeted radionuclide therapy will involve combinations with radiosensitisers and other chemotherapy drugs, it is vital to understand the RIBBE process at play in such strategies to ensure that drug/radiation combinations are complementary to the production of RIBBE so that their potential for eliciting cell kill in a tumour specific fashion can be maximised. These indirect effects thus offer a novel approach for cancer cell treatment utilising targeted radionuclide therapy.

BYSTANDER MECHANISMS AS TARGETS FOR CANCER RADIOTHERAPY

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In collaboration with Carmel Mothersill, Colin Seymour,Richard Smith, Jean Laissue, Hans Blattmann and Elke Bräuer-Krisch

When new radiotherapy concepts are developed, the first question, of course, is whether survival times can be increased, compared to already established therapy concepts. Having established survival advantage, the next important task is to investigate the new therapeutic concept for unwanted side effects and to identify, where possible, the pathways of action.

Since 2005, we have conducted a series of studies using an experimental radiotherapy concept called microbeam radiation therapy (MRT) at the biomedical beamline ID17 of the European Synchrotron Radiation Facility (ESRF) in Grenoble (France). The concept of MRT has been developed during the 1990s at the NSLS in Brookhaven (USA) and at the ESRF. In the hospital setting, a broad beam in the MeV range is used for tumour radiotherapy. For MRT, highly collimated synchrotron beam in the keV range is split with the help of a special collimator into an array of small near-parallel beams in the micrometer range. This creates a characteristic irradiation profile and also permits the deposition of X-ray doses into the tumour that can be higher by two orders of magnitude compared to the doses used in hospital-based radiotherapy programs. This could prove a therapeutic advantage especially where the target tumours are extremely radioresistant.

Our research group has studied survival, effects on new memory formation as well as bystander effects of MRT in two small animal models of malignant brain tumour. Data from those studies and the potential for transfer of MRT studies into a clinical trial phase will be discussed.

HIGH-DOSE LATTICE RADIATION THERAPY: CLINICAL, PHYSICS AND BIOLOGICAL PERSPECTIVE OF BYSTANDER EFFECTS

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The standard paradigm for ionizing radiation (IR) effects involved DNA damage with dsDNA breaks as triggers for mutation, cell death and transformation. However, a growing body of evidence reported non-targeted effects, including genomic instability, gene induction, adaptive responses and low dose hypersensitivity. One such non-targeted effect often observed is deleterious signaling exerted to either neighboring or distal cells by radiation-induced damage cells. These signals can be classified into two independent phenomena that are (i) Abscopal effect and (ii) Bystander effect. This presentation focuses on the potential utilization of bystander/abscopal effects in different therapeutic settings to eliminate malignant cells while protecting the normal tissue in lung cancer. Our media transfer experiments and analysis of serum samples from high-dose GRID radiation-treated patients demonstrated that such high-dose radio-inductive therapy caused the release of TNF-□, TRAIL and ceramide as bystander factors mediating the killing of unirradiated tumor cells. We found that reduced bystander response in A549 lung cancer cells was due to activation of NF□B signaling by TNF-□, whereas, enhanced response to IR-induced bystander signaling in H460 lung cancer cells was due to release of TRAIL associated with nuclear translocation of PAR-4. Using the low energy GRID for animals, high-dose Spatially Fractionated Grid Radiation Therapy (SFGRT) caused regression of the neighboring untreated tumor and the regression was significantly enhanced with addition of 2 Gy. This was evident in both nude mice xenograft as well as in Lewis Lung Carcinoma (LLC) model in C57Bl/6 mice. Further, in serum of SFGRT-treated patients, ceramide was significantly induced in response to high-dose radiation of 15 Gy and targets the endothelial cells of tumor microenvironment. Although, clinically, relatively small in number of patients receiving SFGRT treatment either with early ortho-voltage machines or more recently with MV X-rays, tumor regressions were reported, leaving a perplexed paradox contradicting to the principle of conventional radiation therapy, in which a total tumor volume irradiated with a rather uniform dose is called for. Thus, this evidence strongly suggest that SFGRT would induce a rapid and higher rate of tumor cell apoptosis in bulky tumors. This leads to the new notion of using high-dose SFGRT as an induction therapy to enhance therapeutic outcome of subsequent conventional radiation therapy. It is not unreasonable to anticipate that such new approach may open a new paradigm in radiation therapy, if the clinical efficacy proven positively. Most importantly, the highest-dose regions of the SFGRT are superficial, and often are outside of the tumor target itself. Unnecessary high dose exposure to the surrounding normal tissue can be significantly reduced by reconfiguring the SFGRT treatment into a 3D-SFGRT dose LATTICE, a new approach to spatially fractionated radiation which takes advantage of modern-era technology. This technique can also be used to place high-dose islands within the tumor target only, not outside of the target. Using this technique, high doses of radiation are concentrated at vertices within the tumor volume, with drastically lower dose between vertices (peak-to-valley effect) and leaving anything outside of tumor volume minimally exposed. Because more pronounced radiation dose peaks and valleys are generated using LATTICE technique compared to SFGRT, it may be more radiobiologically effective, with significantly less radiation dose to adjacent normal tissues, and therefore should confer less additional toxicity. This new concept is adopted in clinical settings as well as in animal models. Further, current studies are in focus to study the synergizing effect of high-dose radiation-induced bystander factors such as TNF-α and TRAIL with standard chemotherapeutic drugs (such as Taxanes, Carboplatin and 5-FU), signaling agents (such as TKIs, proteosome inhibitors and 2-Deoxy-Glucose) and natural herbal agent such as Curcumin.

HAEMOTOLOGIC TOXICITY OF RADIOIMMUNOTHERAPY

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Background: Radioimmunotherapy (administration of ^{90}Y-Zevalin) is registered by FDA for Follicular Lymohoma Pts (FL) refractory/ relapsing to previous Rituximab containing regimens. In Kraków University Hospital, there were 101 radioimmunotherapy procedured performed, 85 either as monotherapy or consolidation to previous chemotherapy regimens: 20 in FL, 53 in MCL (Mantle Cell Lymphoma) and 12 in DLBCL(Diffuse Large B cell Lymphoma). Further 16 procedures were elements of autologous transplant (ASCT) conditioning (3 –in MCL, 13 in DLBCL). A short clinical background and our clinical results will be presented

Methods: Patients were given 250 mg/m^2 of R followed 1 wk later with a second dose of R + Zevalin (11 or 15 MBq = 0.3 or 0.4 mCi/Kg based on PLT count; maximum dose = 32 mCi). Cytopenia was assessed with full blood counts every wk until recovery. In a subset of patients trephine biopsies were performed before Zevalin dosing and at 2 and 6 wks after Zevalin to assess marrow cellularity and stromal cells.At the same time points, mantle cell (CD19+CD20+CD5+CD23-) infiltration of BM was assessed by flow cytometry; myeloid clonogenic capacity (CFU-GM, BFU-E, CFU-GEMM, CFU-Meg) was evaluated by cell culture; and BM stroma function was measured by assaying 5 cytokines: GM-CSF, EPO, TPO, IL-3, and SCF. Results were compared between MCL and FL patients who received Zevalin alone following relapse after chemoimmunotherapy.

Results: The level of neutro- and thrombocytopenia was greater when fludarabine was given before Zevalin than in patients who received only Zevalin. Patients given fludarabine pretreatment experienced a 2- to 4-fold greater degree of clonogenic capacity impairment compared with those that received only Zevalin, except CFU-Megs, whose profiles were similar in both groups. Seven- to 10-fold ↓ in myeloid stem and progenitor cells in response to FCM → RIT were associated with ↓ in PMNs and PLTs. Stromal cells experienced minimal, transitory (fully reversible by 2 wks) impairment, suggesting a negligible adverse effect of RIT on BM stroma. Compared with pre-Zevalin values, GM-CSF levels ↓ 2 fold at wk 4, and TPO and IL-3 ↓ 30% and 3-fold, respectively, at wk 2. EPO levels ↓ 3 fold during the first 4 wks, and paralleled decreases in BFU-Es.

Conclusions: Delayed pattern of cytopaenia after radioimmunotherapy is not characteristic for any chemotherapy regimens. It depends from the diagnosis (pattern of infiltration of BM by lymphoma) and the intensity of preceeding therapy. It is most likely secondary to the stem cell damage, as indicated by clonogenic capacity studies, lack of impairement of the stroma cell assessment and lack of any additional perturbations in transplanted patients with the stem cells reinfused.

WHAT ARE NON-TARGETED RADIATION EFFECTS?

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Non targeted effects suggest an unpredictable response, as opposed to the predictable response of target theory. Experimental data though produces fairly robust responses, which are reproducible even if they do not follow a direct dose response relationship.

For non targeted effects to be exploited in therapeutic situations a basic understanding of mechanisms is required. Even if a response is reproducible the underlying mechanisms may be unclear.

In this paper we will examine potential mechanistic theories in terms of our fish model which uses water borne transmissible factors from irradiated to non irradiated fish.

LOW LET RADIATION AND BYSTANDER FACTOR DAMAGE TO MAMMALIAN MITOCHONDRIA

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Radiation damage incurred by nuclear DNA is well documented and interest is increasing in the properties of "bystander" factors though its effects and those of direct low LET irradiation on the mitochondria, and more particularly mitochondrial DNA (mtDNA) are less well understood. The current study characterised the mitochondrial response to both direct irradiation and bystander factors in human keratinocytes (HPV-G) and Chinese hamster ovarian cells (CHO-K1).

Cells were exposed to either γ radiation (0Gy, 5mGy, 0.5Gy or 5Gy) or Irradiated Cell Conditioned Medium (ICCM) and analysed 4 to 96 hours post exposure. MtDNA damage analysis included mutation and deletion analysis. Mitochondrial (dys)function was analysed by a range of approaches including polarography, i*n organello* mitochondrial protein synthesis and kinetics of the oxidative phosphorylation (OXPHOS) enzyme complexes. Mitochondrial mass was determined using MitoTracker FM.

Results demonstrate mtDNA damage in HPV-G mtDNA was induced as early as 12 hours post direct exposure 24 hours post exposure to ICCM. Furthermore, low dose exposure appeared most potent in inducing the mtDNA^{4881} deletion. Significant increases in mitochondrial mass were observed after exposure to both direct radiation and ICCM in both cell types and mitochondrial dysfunction varied greatly post exposure and was non-uniform between OXPHOS enzyme complexes

Findings show mitochondria are prone to even very low-level ionizing radiation-induced stress and medina damage load may accumulate with time post exposure. The multi-heterogeneous medina population of cells represents a sensitive barometer for low-level radiation exposure damage.

TARGETING TRANSCRIPTION-COUPLED REPAIR AS AN ANTI-CANCER STRATEGY

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Many cancer therapeutics and drugs undergoing clinical trials for cancer treatment elicit their anti-neoplastic effects by inducing DNA damage. There is considerable interest in the development of strategies to target specific DNA repair pathways to increase the efficacy of these agents. We have used RNA interference to target several proteins involved at specific steps in transcription-coupled subpathway of nucleotide excision repair (TC-NER). Notably, Cockayne syndrome (CS) group B protein (CSB) is an ATP-dependent chromatin remodeling protein of the SWI2/SNF2 family that plays an essential role in the rate limiting step of the transcription-coupled subpathway of nucleotide excision repair (TC-NER). RNA interference against CSB resulted in a marked increase in the sensitivity of a variety of tumour cell lines to cisplatin and this was independent of p53 and DNA mismatch repair. These genetic alterations, common in cancer, have been linked to cisplatin resistance in a variety of model systems and may contribute to resistance in the clinic. Our results suggest that targeting this DNA repair pathway may represent an effective anti-cancer strategy even in drug resistant tumours.

CHARACTERISATION OF THE HEMATOPOIETIC STEM CELLS USING MATHEMATICAL MODELS

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Stem cells behaviour is an important field of research with promising clinical impacts. Due to the revolutionary new technologies of biological data collection, an enormous amount of information on specific factors and genes responsible for cell differentiation is available. However, the mechanisms controlling stem cell self-renewal, maintenance and differentiation are still poorly understood and there exists no general characterisation of stem cells based on measurable cell properties. We address these problems with the help of new mathematical models. Results of numerical simulations are compared with the experimental data obtained from patients with multiple myeloma after high-dose chemotherapy and stem cells transplantation. It leads to the conclusion that the regulation of the asymmetry of cell divisions is significantly more efficient that the regulation of the proliferation rates. Moreover, analysis of the model equations leads to a generalization of the concept of self-renewal potential, which might be helpful to define the stem cell compartment.

CROSSTALK BETWEEN P53 AND NUCLEAR FACTOR-KB SYSTEMS: PRO- AND ANTI-APOPTOTIC FUNCTIONS OF NF-κB

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Nuclear factors p53 and NF-kB control many physiological processes including cell cycle arrest, DNA repair, apoptosis, death, innate and adaptive immune responses, and inflammation. There are numerous pathways linking these systems and there is a bulk of evidence for cooperation as well as for antagonisms between p53 and NF-kB. In this theoretical study, the authors use earlier models of p53 and NF-kB systems and construct a crosstalk model of p53-NF-kB network in order to explore the consequences of the two-way coupling, in which NF-kB upregulates the transcription of p53, whereas in turn p53 attenuates transcript_ion of NF-kB inhibitors IkBa and A20. We consider a number of protocols in which cells are stimulated by tumour necrosis factor-α (TNFα) (that activates NF-kB pathway) and/or gamma irradiation (that activates p53 pathway). The authors demonstrate that NF-kB may have both anti- and pro-apoptotic roles. TNFa stimulation, preceding DNA damaging irradiation, makes cells more resistant to irradiation-induced apoptosis, whereas the same TNFa stimulation, when preceded by irradiation, increases the apoptotic cell fraction. The finding suggests that diverse roles of NF-kB in apoptosis and cancer could be related to the dynamical context of activation of p53 and NF-kB pathways.

CAN BLOOD CELLS BE IMPORTANT IN BYSTANDER EFFECTS? BYSTANDER EFFECTS IN LYMPHOBLASTOID CELLS

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All tissues of multicellular organisms are permanently in contact with cells of the blood, including lymphocytes, macrophages and other cells of the immune system. These cells are naturally programmed to produce signaling molecules and molecules inducing oxidative stress, and to interact and influence the fate of other cells. Because during anticancer therapy they always receive some dose of radiation or drugs, their ability to communicate with other non-irradiated cells and the processes which they induce i may be of importance for therapy. We have studied the bystander effect in lymphocytes isolated from peripheral blood and in lymphoblastoid cells. At different times after direct irradiation or co-culture with irradiated cells, oxidative DNA damage and DNA breaks, apoptosis, micronuclei, and clonogenic survival were assessed. Changes of the transcriptome were also studied by a microarray approach. All our results suggest that lymphoblastoid cells express bystander effects, and that after irradiation they release signals which may sometimes counteract the induction of apoptosis and support the survival of neighboring cells.

Session IV:
Ion Channels and Cancer

THERMAL EFFECTS IN THE Ca^{2+}-ATPase PROTEIN

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To know the microscopic mechanism controlling energy transport in bio-molecules constitutes a considerable challenge which could provide a basis to understand complex biological processes. The time scale associated with the energy relaxation throughout the molecule can give information about the kinetics of bio-molecule reactions. Moreover, the pathways followed by the energy during the relaxation process can provide important clues to understand basic mechanisms of the molecule. In order to analyze these processes it is necessary to know how perturbations occurring at a specific spot in the bio-molecule, namely, binding of small molecules at receptor sites, may impart a conformational change at a distant spot, lying several nanometers away. We apply non-equilibrium thermodynamics and computer simulations to analyze thermal effects in the Ca2+-ATPase.

1. Kjelstrup, S.; Rubi, J.M.; Bedeaux, D. Physical Chemistry Chemical Physics ***2005****, 7, 4009–4018.*
2. A. Lervik, F. Bresme, S. Kjelstrup, D. Bedeaux, J. M. Rubi, preprint

ION CHANNELS ARE INCREASINGLY BEING LINKED TO CANCER AND TUMOUR PROGRESSION

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Here we describe a voltage-gated, potassium selective channel (Eag1, Kv10.1) with novel electrophysiological properties, whose normal physiological function is yet unknown but which shows oncogenic transforming potential if expressed ectopicaly. Strikingly, the expression of the human Eag1 is restricted to brain, but it is also present in several tumour-derived cell lines. More importantly, the protein can be detected in more than 72% of human tumour samples, while the corresponding normal tissues are devoid of the channel. Experiments under in vitro conditions have demonstrated decreased proliferation of Eag1-expressing cells by inhibition of expression and/or function of this channel. This inhibition of Eag1 is accomplished using RNA interference, functional anti-Eag1 antibodies, or (unspecific) EAG1 channel blockers. We have also used in vivo models to visualise the distribution of Eag1 in tumour-bearing mice using specifically designed recombinant antibodies. We conclude that Eag1 is a widely distributed tumour marker with diagnostic and therapeutic potential.

CITRATE TRANSPORTER FROM PROSTATE EPITHELIAL CELLS

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Prostate gland is a unique organ that produces and releases large amounts of citrate into prostatic fluid (up to 180 mM). This is necessary to sustain sperm vitality and motility. Importantly, citrate levels drop dramatically when prostate becomes metastatic. Determination of the mechanisms of citrate synthesis and release in prostate epithelial cells are therefore important for understanding crucial aspects of male infertility and prostate cancer.

Large amounts of citrate are produced in prostatic cells because of the rate-limiting role of mitochondrial aconitase which expression and activity is regulated by hormones and Zn^{2+}. Surprisingly, the way citrate is released from prostatic epithelial cells has not been known until now. We used cDNA library screening and RACE PCR to determine the molecular nature of the citrate release transporter from PNT2-C2 cells. We obtained a functional clone, expressed it in HEK cells and evaluated the role of the newly cloned transporter in prostatic cells using siRNAs and functional assays. We also produced an antibody for the novel transporter and showed that the cloned protein co-localised with the plasma membrane of prostatic cells.

PRO-APOPTOTIC, PRO-AUTOPHAGY AND PROLIFERATIVE EFFECT OF CALPROTECTIN

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The complex formed by two members of the S100 calcium-binding protein family, S100A8/A9, known also as calprotectin, may exert pro-cell death effects, growth-promoting activity, and immunomodulatory functions, depending on concentration, cell type, and local micro-environment. An element of the complex, S100A9 gained recently a significant attention due to its tumor protecting activity against immune response. S100A8/A9 triggers proliferation at low micromolar concentration, whereas at intermediate or high micromolar concentrations it is toxic. Cell death induction by S100A8/A9 is independent of RAGE-mediated signaling, but both proliferative effects and immunomodulatory properties require RAGE. The underlying molecular mechanisms of cell death induction are both programmed cell death I (PCD I, apoptosis), and PCD II (autophagy). Treatment of cells with S100A8/A9 caused the increase of Beclin-1 expression as well as Atg12-Atg5 formation. S100A8/A9-induced cell death was partially inhibited by the specific PI3-kinase class III inhibitor, 3-methyladenine (3-MA), and by the vacuole H^+-ATPase inhibitor, Bafilomycin-A1 (Baf-A1). S100A8/A9 provoked the translocation of BNIP3, a BH3 only pro-apoptotic Bcl2 family member, to mitochondria. Consistent with this finding, ΔTM-BNIP3 over-expression partially inhibited S100A8/A9-induced cell death, decreased ROS generation, and partially protected against the decrease in mitochondrial transmembrane potential in S100A8/A9-treated cells. In addition, either ΔTM-BNIP3 over-expression or N-acetyl-L-cysteine co-treatment decreased lysosomal activation in cells treated with S100A8/A9. Our data indicate that S100A8/A9-promoted cell death occurs through cross-talk of mitochondria and lysosomes via ROS.

Poster abstracts

Numbers beside the abstract title correlate with poster numbers.

1. IMAGE PROCESSING BASED METHOD USED IN AUTOMATED ANALYSIS OF CELL GLYCOSYLATION PATTERNS

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Glycosylation is involved in many biological processes and its alterations is associated with some diseases. Quantification of oligosaccharide molecules and estimated theirs cell surface localization is important to understanding one of mechanisms of diseases development.

The aim of this work was to develop algorithms for automatic recognition FITC-labeled lectin bound to K562 cells in fluorescence microscopy images. The mean fluorescence intensity of each cell, corresponding to amount of cell-bound lectins, was measured too.
For detecting of cells regions in the image the new adaptive global image thresholding method was developed. After segmentation stage numbers of image processing operations are used for improvement of image segmentation results.

The new method was using to estimate the level of glycosylation in irradiated and control (non-exposed) K562 cells at different time points after exposure to 4 Gy of X-rays. Obtained results showed changes in protein glycosylation elicited by ionizing radiation. The data were compared with the level of K562 cell glycosylation estimated with CometScore software.

This work was supported by the grant PBZ/MEiN/01/2006/49

2. CHEMOSENSITIVITY OF BLADDER CANCER CELLS AFTER CHEMICAL OR RNAi-MEDIATED MODULATION OF MULTIDRUG RESISTANCE (MDR) GENES EXPRESSION

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Multidrug resistance (MDR) is the lack or the loss of sensitivity of cancer cells to chemotherapeutic agents and it is the main obstacle in successful treatment in most patients. Bladder cancer is one of the malignant cancers where MDR contributes significantly to treatment failure. The origin of most MDR mechanisms lies in the overexpression of cell membrane transporters, such as ABC (ATP-binding cassette) proteins, involved in xenobiotic efflux.
The aim of our study was to develop an efficient molecular method, based on RNA interference, capable of modulating expression of genes responsible for multidrug resistance in bladder cancer. Six MDR genes overexpressed in bladder cancer were subjected in the study: MDR1 (*P-gp*), MRP-1, -2, -3, -5, and LRP (*lung resistance protein*). Two strategies of post-transcriptional silencing were used: enzymatically prepared esiRNA (*endoribonuclease-based siRNA*) and vector-based strategy of short-hairpin RNA prepared by cloning shRNA sequences into pSUPER plasmids. Experiments were carried out *in vitro* using primary cancer cells isolated from bladder cancer resection tissue obtained from patients of Urology Division, (Małachowski Hospital, Katowice), and established T24 cell line (ATCC: HTB-4).

Lipid-based transient transfection of both primary, and T24 cultures revealed suppression of the target genes expression at the mRNA level as was confirmed by Real-Time RT-PCR. mRNA levels were reduced by 80% (MRP1) - 99% (MRP5). Stronger effects were observed in T24 cultures than in primary cultures; also shRNA modulation was more effective than esiRNA. In the next step, different chemotherapeuticals were used as substrates of MDR proteins: etoposide, doxorubicin, bleomycin, vinblastine, cytarabine. Results showed significantly reduced viability in T24 cultures compared with the non-transfected: from 8,45% (LRP + vinblastine) to 43,6% (MDR1 + etoposide).Finally, both esiRNA, and shRNA constructs were tested for their efficacies as molecular sensitizers in etoposide treatment. The results were compared with chemical modulators of MDR (cyclosporine A and probenecid). IC_{50} values showed significant reduction in etoposide concentrations necessary to induce cancer cells death. In T24 cultures resistance-modifying factor (RMF) values were from 24.07 for MDR1 to 6.29 for LRP (shRNA modulation), and from 6.25 for MDR1 to 2.35 for LRP (esiRNA). In primary cells these values ranged between 31.2-6.53 (shRNA), and 7.06-2.68 (esiRNA), respectively. Chemical modulation by cyclosporine A and probenecid showed significantly lower RMF values (only 1.16 – 1.69).

Altogether, these results show that both types of our RNAi designs significantly suppress expression of all studied multidrug resistance genes in a sequence-specific manner. PTGS with esiRNA helps to obtain rapid effects, while that mediated by shRNA *in situ* expression enables to perform stable suppression (especially when *neo* selection is used). This molecular modulation allows us to decrease concentrations of chemotherapeutic drugs and it is significantly more efficient than using chemical modulators of MDR genes.

The study was supported by the funds of Medical University of Silesia (KNW-1-145/08, KNW-1-117/09). Results were partially shown in I. Bednarek postdoctoral habilitation thesis (ISBN: 978-83-7509-094-9).

3. ZINC OCTACARBOXYPHTHALOCYANINE – A POSSIBLE PHOTOSENSITIZER FOR PHOTODYNAMIC THERAPY

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Photodynamic therapy (PDT) is a form of cancer treatment which requires three factors: molecular oxygen, light of specific wavelength and a photosensitizer - a substance which is activated by this light. The activated photosensitizer produces reactive oxygen species and free radicals as a result of energy transfer from its excited triplet state to ground state of molecular oxygen or organic substrate (tissue).

Treatment of different kinds of cancer requires various photosensitizers. This is why chemists, physicists and biologists continue search for new substances which can act as photosensitizers and are activated by light of different wavelengths. Longer waves can penetrate deeper through the tissue and can activate photosensitizers which accumulate not only directly under the skin but deeper.

Phthalocyanines are interesting candidates for PDT because their chemical structure is closely related to that of porphyrins. Phthalocyanines have four nitrogen atoms in *meso* positions and bind four pyrrole rings forming one molecule. Phthalocyanines have also four benzene rings bound to pyrrole rings (one benzene ring is bound to one pyrrole ring) which shift their absorption spectrum towards longer wavelengths. Zinc octacarboxyphthalocyanine has, in addition, two carboxyl groups bound to each benzene ring. Their presence makes this compound soluble in polar solvents. Zinc atom causes the quantum yield of singlet oxygen to be twice as high as that of unsubstituted phthalocyanines.

Zinc 2,3,9,10,16,17,23,24-octacarboxyphthalocyanine, ZnPcOC (Fig. 1), was obtained according to the general procedure described in Ref. [1]. We synthesised free-base octacyanophthalocyanine (**1**) by the cyclotetramerization of 1,2,3,4-benzenetetracarbonitrile and purified it, exactly as described in this procedure. Next, from **1** and purified zinc, we synthesised the 2,3,9,10,16,17,23,24-octacyanophthalocyanine, $ZnPc(CN)_8$ (**2**). Finally, we hydrolyzed **2** to ZnPcOC. A portion of **2** was added to the deoxidized ethylene glycol-water solution containing NaOH. The reaction was carried out in dark, at 110–120°C, with constant nitrogen bubbling. When ammonia stopped to evolve, the reaction mixture was cooled down, diluted with deoxidized water and filtered. The product was precipitated by addition of concentrated HCl, purified according to Ref. [1] and dried. Finally, Soxhlet extraction with acetone and methanol was performed.

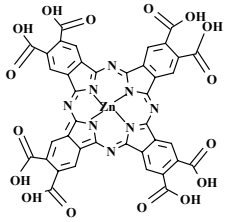

Fig. 1 Zinc 2,3,9,10,16,17,23,24-octacarboxyphthalocyanine, ZnPcOC

In the study, efficiency of zinc octacarboxyphthalocyanine in the absence of light was measured. The studies were carried on the cell line HCT 116- Human Colon Carcinoma. The solutions with concentrations of octacarboxyphthalocyanine: 0.4 [μM/l]; 0.75 [μM/l]; 1 [μM/l]; 1.5 [μM/l] were administered to the cells. The chemical compound is soluble in DMSO. Maximum concentration of DMSO in solutions was no higher than 0.3% volume. After 24 hours of incubation the MTS test was measured. The percentage of survival cells makes the zinc octacarboxyphthalocyanine a good candidate as photosensitizer for PDT.

The image of surface of this compound was obtained by the use of an electron microscope (SEM). The chemical composition was also measured by the SEM and it shows that obtained percentage of the chemical composition confirms the probable composition of zinc octacarboxyphthalocyanine.

[1] Wőhrle D, Meyer G. Reaktive oktafunktionelle phthalocyanine aus1,2,4,5-tetracyanbenzol. Makromol Chem 1980;181:2127–35.

This work was supported by the Polish Ministry of Science and Higher Education (grant No. 0538/R/T02/2007/03).

4. FUZZY ANALYSIS IN RISK FACTORS OF CANCER

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Cancer is a major public health problem. Currently, one in four deaths in the United States is due to the cancer. In many papers we can find information about risk factors which show how significant problem it is. A risk factor is a variable associated with an increased risk of disease. Factors that increase cancer risk can be external such as personal lifestyle choices or substances such as chemicals and asbestos, present in the environment, those are known to cause cancer. Some people also have internal risk factors for cancer such as a genetic predisposition or those that develop as a result of aging.

Using fuzzy set theory, we have created a system which predicts the risk factors for different kind of cancer. We have taken into account lung, colon, breast, cervical and prostate cancer. We used the Mamdani model which is implemented in the Fuzzy Logic Toolbox in Matlab. As inputs in our system we have taken following risk factors: genetic, biological (race, age, sex) and behavioral (overweight, alcohol consumption, tabacco smoke). Better estimates of cancer risk probability will direct more intensive clinical services and research. By means of the fuzzy set theory this risk factors of cancer can be chosen very quickly.

5. STAT3 IS A PHOSPHO-SPECIFIC BINDING PARTNER FOR FIBROBLAST GROWTH FACTOR RECEPTOR THAT IS ACTIVATED BY AMPLIFIED RECEPTOR EXPRESSION

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Fibroblast growth factor receptors (FGFRs) play many roles in development, cell proliferation, differentiation and physiological function. Potentially aberrant manifestations of FGFR signaling have been implicated in a variety of tumor cell types including the acquisition of FGFR amplicons and potentially elevated levels of FGFR expression. However the oncogenic mechanisms associated with amplified receptor expression are not known. FGFRs possess intrinsic tyrosine kinase activity which, via direct or indirect phosphorylation of target substrates, leads to activation of other signaling proteins, formation of multiprotein signaling complexes and concomitant downstream responses. Using a proteomics approach we here identify signal transducer and activator of transcription 3 (STAT3) as a phosphorylation-dependent partner for Tyr677 of FGFR1. Association of STAT3 with activated FGFR is essential for the subsequent tyrosine phosphorylation of STAT3, nuclear translocation and activation of downstream targets. Tyrosine phosphorylation of STAT3 is also dependent upon the concomitant FGFR-dependent activity of SRC and JAK kinases. We also show that tyrosine (but not serine) phosphorylation of STAT3 requires amplified FGFR protein expression generated either by forced expression or associated with gene amplification in tumor cells lines such as SUM-52PE. These findings show that engagement of the STAT3 pathway resulting from amplified FGFR expression merits consideration as a potential therapeutic and diagnostic target.

6. IDENTIFICATION OF NOVEL (HYPOTHETICAL) PROTEIN PARTNERS OF THE MAJOR APOPTOTIC NUCLEASE DFF; YEAST TWO-HYBRID BASED STUDY

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The major apoptotic nuclease, DNA fragmentation factor (DFF), also termed Caspase-activated DNase (CAD), is primarily responsible for internucleosomal DNA cleavage during the terminal stages of apoptosis. In non-apoptotic cells, DFF exists in the nucleus as a heterodimer, composed of a 45 kD chaperone and inhibitor subunit (DFF45/ICAD) and a 40 kD latent nuclease subunit DFF40/CAD. Activation of the nuclease depends on caspase-3-mediated cleavage of DFF45/ICAD inhibitor and formation of DFF40/CAD homooligomers. Caspase-activated DFF40/CAD homo-oligomers can further interact with additional activators or inhibitors; however, only few of them had been identified so far.

Here we used a yeast-two and -three hybrid system in aim to identify novel proteins that potentially interact with DFF40/CAD. *S. cerevisiae* AH105 strain was transformed with human DFF40 (cloned into pGBT9 vector) and then mated with *S. cerevisiae* Y187 strain carrying human brain embryo or HeLa cell cDNA libraries. Alternatively, to identify proteins potentially interacting with the DFF heterodimer, *S. cerevisiae* AH105 strain was transformed with both human DFF40 and DFF45 (cloned into bi-cistronic pBridge vector); an approach called a yeast-three hybrid system. In addition, *S. cerevisiae* AH105 strain was transformed with human DFF45 to search for possible partners of this protein alone.

The screening revealed DFF45 as the only partner of DFF40 when expressed separately. However, screening of HeLa cDNA library revealed that DNA sequences present in chromosome 10 (ESTs CV366200.1 and DB313950) potentially encode for protein that interact with the DFF heterodimer. Interestingly, silencing of corresponding transcript in HeLa cell by shRNA affected proliferation rate and resistance to apoptosis. In addition, the screening revealed several proteins that potentially interact with DFF45, including GFAP, FHL1, FBXO28, FOSL1, PGK1 and PCNT.

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

7. A COMBINATION OF ENDOGLIN-BASED DNA VACCINE AND INTERLEUKIN-12 GENE ENHANCES ANTIANGIOGENIC AND ANTITUMOR EFFECTS

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Endoglin (CD105), a marker of endothelial cells, is a receptor for transforming growth factor β. CD105 is overexpressed on proliferating endothelial cells in tumor blood vessels and thus it offers an attractive target for antiangiogenic therapy.
In this study we made use of an oral DNA vaccine encoding murine endoglin, carried by attenuated *Salmonella typhimurium* SL7207 (aroA$^-$). It has been reported that CD8+ T cell-mediated immune response induced by this vaccine effectively suppressed tumor growth by eliminating proliferating endothelial cells in the tumor vasculature. Our own results showed that this endoglin-based DNA vaccine effectively inhibited growth of murine renal carcinoma as well as murine melanoma tumors, both in prophylactic and therapeutic settings.

To improve the therapeutic effects achieved in murine tumor models we combined oral administration of this endoglin-based DNA vaccine with direct intratumoral injection of plasmid DNA vector encoding the murine IL-12 gene, an immunomodulatory cytokine with antiangiogenic properties. Interleukin 12 plays a prominent role in activating the immune system, *inter alia* by enhancing the cytotoxicity of CD8+ T cells. We observed that a combination of endoglin-based DNA vaccine and IL-12 gene significantly inhibited growth of established murine melanoma, leading to complete regression of tumors (without recurrence) in 33% of cases, compared to mice receiving single-agent therapy. As a consequence, lifespan of animals treated with the combination was significantly extended. In addition, the combined therapy appeared more effective at reducing the density of tumor microvessels than either treatment alone.

In summary, a combination of endoglin-based DNA vaccine carried by *Salmonella typhimurium* and interleukin-12 gene therapy showed a synergistic effect in suppressing tumor angiogenesis, leading to significant tumor regression.

8. ENDOTHELIAL CELLS ARE RESISTANT TO RADIATION-INDUCED BYSTANDER EFFECT

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Radiation-induced damage of cardiovascular system is one of reported side effects of radiotherapy. Heart failure related to radiotherapy most possibly involves long-term effects of damage of cardiac microcirculation. Here we aimed to analyze radiosensitivity of cardiac endothelial cells.

Three types of endothelial cells were used: primary cells isolated from hearts of C57BL/6J mice, H5V cells (isolated from mouse embryo heart) and b.END3 cells (isolated from mouse brain). In addition, primary cardiomyocytes were isolated from the same mice strain. Cells were irradiated in vitro with 2 Gy dose of ionizing radiation. Radiosensityvity of cells was measured using □H2A.X staining, induction of apoptosis and clonogenic survival. We have analyzed effect of either direct irradiation or so called bystander effect (in different combination of cell types). In addition, permeability of EC monolayer was analyze to determine possible effect of irradiation upon inter-cellular interaction. We observed that in vitro radiosensitivity of tested endothelial cells was similar to radiosensitivity of other cell types. However, endothelial cells were resistant to radiation- induced bystander effect.

This work was supported by the FP7/EURATOM Grant CARDIORISK.

9. ANALYSIS OF FREQUENCY *CHEK2, P53, NOD2/CARD15 AND RET* GENE POLYMORPHISMS IN POLISH PATIENTS WITH DIFFERENTIATED THYROID CANCER

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Thyroid carcinomas amounts to 1% of general and are the most often carcinomas of endocrine system with still growing up frequency. The most often papillary and follicular thyroid cancer occurs (80-90%), which belongs to group of tumors well prognoses, slowly progress and benignity. Very serious problems in this cancer disease are recurrences and regional or remote metastasis. There were observed numerous cases of osteolytic, cerebral and pulmonary metastasis and moreover, well differentiated thyroid cancer can progress to malignant anaplastic.

In this focus, very important seems to be searching for molecular markers of disease course, good or poor prognosis and response on medical treatment as well. It is expected that SNP polymorphisms research in genes demonstrating association with neoplastic diseases will be helpful in understanding of molecular mechanisms of thyroid gland tumors development and allow improving diagnosing.

The dependence of differential thyroid cancer occurrence on DNA variation: I157T in *CHEK2* gene, R72P in *P53* gene, 1007fs in *NOD2/CARD15* gene and synonymic G2497T substitution in *RET* protooncogene was examined. 296 patients with differentiated thyroid cancer and 200 individuals from population group were examined. I157T, G2497T and R72P variants were analyzed by pyrosequencing and 1007fs by PCR-SSCP and DNA sequencing.

There were no significant differences in allele or genotype frequencies in analysis of RET G2497T substitution and R72P in *P53* gene but polymorphic allele frequencies of 1007fs and I157T was 8,95% and 4,90% in patients with thyroid cancer, compared with 2,92% and 2,1% in control individuals respectively. Frequencies between patient and control groups were tested using Pearson's χ^2 statistics. This analysis shows that 1007fs and I157T mutations are associated with susceptibility to differential thyroid cancer. Our findings indicates that particular characteristics of cancer risk genes on RNA level as well as DNA changes, which may influence on transcription is necessary. Additionally a summary effect of different SNP changes as a cancer predisposing factor is possible, so further analysis will be performed.

10. INTERFERENCE BETWEEN THE HEAT SHOCK RESPONSE AND NFκB-DEPENDENT SIGNALING PATHWAY

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NFκB is a family of transcription factors that regulate numerous genes important for pathogen- or cytokine-induced inflammation, immune response and cell proliferation. NFκB also activates several genes that promote cell survival, which contributes to aggressive tumor growth and resistance to chemotherapy and radiation in cancer treatment. HSF1 is the primary transcription factor responsible for cellular response to different forms of stress (e.g., a heat shock), which upon stress-induced activation binds regulatory DNA elements, termed heat shock elements (HSE), present in promoters of heat shock proteins (HSPs) genes, and activates their expression. In general, HSPs function as molecular chaperones in regulation of cellular homeostasis and promoting survival. HSPs over-expression is frequently found in many types of cancer, and is usually associated with poor prognosis. On the other hand, however, hyperthermia is an adjuvant treatment used to sensitize cancer cells to radio- and chemotherapy, possibly affecting pathways that promote cell survival.

Here we aimed to address possible mechanisms by which hyperthermia and HSF1-dependent signaling interfered with NFκB-dependent pathways. The U_2OS osteosarcoma human cell line was used as an experimental model. The heat shock response was induced by mean of hyperthermia (incubation at 43^oC for one hour). Alternatively, cells were transfected with mutated constitutively active HSF1 with deletions in regulatory domain (HSF1ΔRD) to activate HSF1-dependent signaling in the absence of the heat shock. Cells were incubated with TNFα cytokine to activate the NFκB pathway, and then expression of NFκB-regulated genes was assessed by RT-PCR. The activation of the NFκB signaling pathway was monitored by mean of degradation of IκBα inhibitor and appearance of active DNA-binding NFκB forms in nuclear extracts.

We have observed that TNFα-induced activation of NFκB was inhibited in cells subjected to hyperthermia, and four hours recovery in physiological temperature was necessary to allow full activation of NFκB. On the other hand, NFκB remained to be fully activatable by TNFα treatment in cells containing constitutively active mutated HSF1 at normal temperature. Interestingly, however, expression of several TNFα-activated and NFκB-dependent genes, including genes encoding TNFα and IL-6, was down-regulated in the presence of active HSF1. Our findings clearly indicates functional interference among hyperthermia, HSF1- and NFκB-dependent signaling pathways.

This work was supported by the Ministry of Science and Higher Education, Grant PBZ-MNiI-2/1/2005.

11. ANTITUMOR POTENTIAL OF NOVEL SYNTHETIC GENISTEIN GLYCOCONJUGATES

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Genistein have been found to block the uncontrolled cell growth associated with cancer via different molecular mechanisms, which encourages chemical modification of this leader compound for further drug development. There is evidence that some sugar derivatives of genistein may have stronger inhibitory effect than genistein itself.

We decided to investigate biological effect of new synthesized genistein sugar derivatives on human prostate cancer cells DU145 and human colon cancer cells HCT116. Antiproliferative activity of a series of novel synthetic genistein glycoconjugates was studied *in vitro* with use of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay. Genistein glycoconjugates showed diverse antiproliferative potency against tested human tumor cell lines. The structure activity relationship is not clear yet, and the role of a sugar moiety for molecular targeting still remains to be found. However, the series with genistein combined with acetylated rhamnal seems to be the most potent. Among analyzed derivatives the most active compound revealed Ram-3 which inhibit growth of cancer cell lines through different cellular mechanisms: inhibition of EGFR phosphorylation, cell cycle inhibition in the G2/M phase, mitotic arrestment and induction of apoptosis.

The results show that the compound Ram-3 demonstrates strong antiproliferative activity against human cancer cells. Further chemical modifications and structure – activity relationship studies should be performed in order to find optimized molecule for chemotherapeutical use.

12. SYNTHESIS OF THE DERIVATIVES OF VITAMIN C AND THEIR LIPOSOMES FOR A POSSIBLE ANTICANCER TREATMENT

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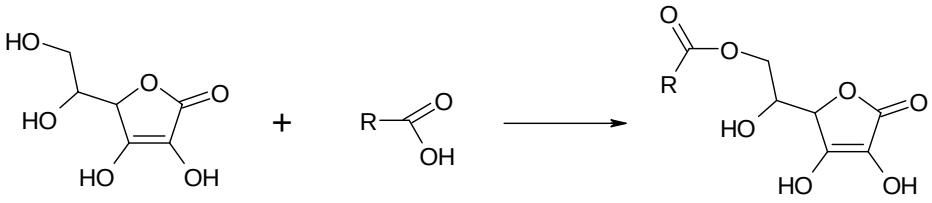
Observational studies showed that ascorbate at pharmacologic concentrations generates hydrogen peroxide-dependent cytotoxicity toward a variety of cancer cells in vitro without adversely affecting normal cells [1]. 6-O-ascorbic acid derivatives were synthesized with the main purpose of preparing vitamin C analogues that combine the prooxidant properties of ascorbic acid and its anticancer activity with good solubility in lipophilic media [2]. Compared to ascorbic acid, solubility of 6-O-ascorbic acid analogues in oils and fats is greatly enhanced [3]. These compounds (Scheme 1) were synthesized and studied for their anticancer activity.



Scheme 1.

Products were obtained in good yields, and structures were confirmed by spectral data (NMR, MS). Antiproliferative activity was estimated on HCT 116 cell line (human colon carcinoma) and anticancer activity was evaluated using MTS - reduction colorimetric survival assay. However first results indicated that compounds under study if tested alone did not indicate cytotoxic effect. The next thing we wanted to achieve was to obtain liposomes containing 6-O-ascorbic acid analogues to evaluate their antiproliferative activity. The results showed that this compounds did not form liposomal structures.

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The study has been financed by a grant from the Ministry of Science and Higher Education, No. N N 405 1787 35, 0538/R/T02/2007/03 and PB-W-03-036-00-08.

13. THE USE OF MOSTBIODAT FOR RAPID SCREENING OF MOLECULAR DIVERSITY

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MoStBioDat is a uniform data storage and extraction system with an extensive array of tools for structural similarity measures and pattern matching which is essential to facilitate the drug discovery process. Structure-based database screening has recently become a common and efficient technique in early stages of the drug development, shifting the emphasis from rational drug design into the probability domain of more or less random discovery. The virtual ligand screening (VLS), an approach based on high-throughput flexible docking, samples a virtually infinite molecular diversity of chemical libraries increasing the concentration of molecules with high binding affinity. The rapid process of subsequent examination of a large number of molecules in order to optimize the molecular diversity is an attractive alternative to the traditional methods of lead discovery. This poster presents the application of the MoStBioDat package not only as a data management platform but mainly in substructure searching. In particular, examples of the applications of MoStBioDat are discussed and analyzed.

14. DOSE-DEPENDENT GLYCOSYLATION CHANGES IN IRRADIATED HUMAN K562 AND ME45 CANCER CELLS

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Aberrant glycosylation is associated with a number of diseases, including all cancer types. One of the most popular tools for characterizing cell surface glycoconjugates are lectins – ubiquitous proteins which specifically bind defined oligosaccharide structures.

The main aim of this work was to investigate the influence of different X-ray radiation doses on radiation-induced changes in cell surface glycosylation. For these studies, we were used two human cancer cell lines (K562 and Me45) and several fluorescently-labeled lectins (LCA, PHA-E, UEA, PNA, ConA, DBA, WGA, RCA, PSA, PHA-L, SWGA, GSL-I). Control (non-irradiated) cells were compared with cells irradiated with different doses of ionizing radiation (in the range 4-16Gy). Samples of cells were collected 7 days after exposure, incubated with a lectin, and the mean fluorescence intensity of each sample was measured using a fluorescence microplate reader (Infinite M200, Tecan). The results suggest that exposure to ionizing radiation had profound effect on the level of all of antigens recognized by lectins mentioned above and irradiation had different effect on glycosylation in both cell lines. What's more, we observed that ionizing radiation can change sugar chain antigens in cancer cells in dose-dependent manner.

This work was supported by the grant PBZ/MEiN/01/2006/49

15. APPLICATION OF PROTON MICROBEAM TO STUDIES OF APOPTOSIS AND NECROSIS IN PC-3 CELL LINE

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Scientific research carried out using the ion microprobes delivers valuable data for clinical oncology. Microprobe enables targeted irradiation of small and well-defined area of tissue (or even single cells) by the fully controlled number of ions (down to a single ion). Therefore, the results are not biased with statistical effects inherently present in traditional, random irradiation experiments and can be useful for optimization of radiotherapy or for medical bioimaging.

The human prostate adenocarcinoma PC-3 line is derived from bone metastases. The studies of death (apoptosis or necrosis) induced in the PC-3 cell line by a controlled number of protons are presented. The high degree of invasive characteristics of this cell line makes it an interesting subject of study. The 2 MeV horizontal focused proton microbeam from the Van de Graaff accelerator at the Institute of Nuclear Physics Polish Academy of Sciences was used as the irradiation source. For comparison, the cellular response to damage induction by UVA, UVC, and staurosporine was also examined. Necrotic and apoptotic cells were generally visualized using fluorescence microscopy.

The results show that „older" PC-3 cells after about 50th passage are much more sensitive to proton irradiation than „younger" ones (few passages only). Already 50 protons per cell (~1,3 Gy dose) causes apoptosis in the „older" PC-3 cells. Induction of apoptosis in younger cells was not successful. Necrosis occurs at 800 protons per cell (~20,9 Gy dose) in „older" PC-3 cells and 3200 protons per cell (~83,6 Gy dose) in cells after about 11th passage. In complementary experiments it was proved that incubation with 2,5 μM staurosporine causes apoptosis in this cell line. Necrosis occurs after 10 min of UVC (23,6 mW/cm^2) irradiation in „older" PC-3 cells and after about 20 min in „younger" cells.

16. ANTIPROLIFERATIVE STUDIES OF THIOSEMICARBAZONE DERIVATIVES

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A series of di-2-pyridyl ketone and 2-benzoylpyridine thiosemicarbazone ligands showed a broad antitumour activity in vitro and in vivo against a wide spectrum of tumours [1]. This class of iron chelators could overcome resistance to established antitumor agents [2]. Recent research points to antifungal effects of this family of compounds [3]. Iron is critical for cell cycle progression and DNA synthesis. Thiosemicarbazones derivatives are tridentate active Fe chelators with high Fe mobilization efficacy and low toxicity [4]. These compounds were synthesized and evaluated for their anticancer activity. The products were obtained in good yields, and the identification of the structure was based on spectral data (NMR, MS). Antiproliferative activity was estimated on HCT 116 cell line (human colon carcinoma), LLC cell line (murine Lewis lung carcinoma) and anticancer activity was evaluated using MTS - reduction colorimetric survival assay. The latter purpose of our studies was to obtain liposomes containg thiosemicarbazone derivatives and comparison anticancer activity of free ligands or ligands locked in liposomes.

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The study has been financed by a grant from the Ministry of Science and Higher Education, No. N 405 1787 35, 0538/R/T02/2007/03 and PB-W-03-036-00-08.

17. GENE EXPRESSION SIGNATURE OF HYPOXIA IN MELANOMA CELLS IN VIVO AND IN VITRO

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Melanoma is the most aggressive skin cancer largely refractory to current therapies. Lack of effective therapeutic strategies and shortcomings of traditional classification systems result in high mortality among melanoma patients. One of the most important features of tumor microenvironment is low oxygen tension. Transformation of melanocytes and melanoma progression *in vivo* indeed appears to be influenced by hypoxia. In our previous study we had determined global gene expression profile of B16-F10 murine melanoma cells cultured under hypoxic conditions and identified 430 hypoxia-responsive genes [Olbryt *et al* (2006) *Gene Expression* 13:191-203].

In the present study we have investigated whether expression of 23 selected genes from the above-mentioned signature is also hypoxia-affected in murine melanoma experimental tumors (B16-F10) as well as in human melanoma cell lines exposed to hypoxic conditions *in vitro*.

Localization pattern of hypoxic areas in B16-F10 tumors was detected immunohistochemically using pimonidazole. The hypoxic areas (perinecrotic regions), as well as normoxic ones (in the vicinity of blood vessels), were isolated from frozen tumor slices using laser microdissection technique. For *in vitro* study, six melanoma cell lines were cultured under hypoxic conditions (48h at 1% O_2). Expression of the selected genes in cell cultures as well as microdissected material was analyzed by quantitative real-time RT-PCR and/or semiquantitative RT-PCR.

We found excellent correlation between the hypoxic environment-induced expression patterns of majority of the studied genes (22), both *in vitro* and *in vivo*. The results obtained using human cell lines revealed significant variability in expression patterns of the analyzed genes. Nevertheless, 10 genes were proved to be hypoxia-regulated in most of the studied cell lines (≥4). Among them are those with well-documented links to melanoma biology (NME1, STAT3, MXI1, FN1) as well as known hypoxia-responsive genes (BNIP3, ADM, NPPB, NDRG1)

To sum up: 1) molecular response to hypoxia under *in vitro* conditions appears to reflect the response observed *in vivo*, at least for the same cell line; 2) the selected genes (NME1, FN1, STAT3, ADM, BNIP3, MXI1, CCNG2, CDC6, NPPB, NDRG1) are new hypoxia-responsive genes in melanoma; 3) B16-F10 murine melanoma tumor model seems to be appropriate for further analyses of the selected genes.

18. CYTOGLOBIN OVEREXPRESSION EXERTS TUMOUR SUPPRESSOR ACTIVITY IN LUNG CANCER CELL LINES

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Cytoglobin (CYGB) is a novel globin, whose molecular function remains unclear. It has been shown that CYGB is engaged in cellular response to hypoxic and oxidative stress conferring cytoprotective features. In human cancers, CYGB level is frequently reduced due to promoter hypermethylation and loss of heterozygosity. Recent data suggested that CYGB might act as a tumour suppressor gene (TSG). However, more thorough investigations are needed in order to elucidate the involvement of CYGB in tumour biology. The aim of this study was to evaluate the impact of CYGB on cell phenotype (growth & death rate, migration, invasion and transformation abilities) in the lung cancer setting.

Two lung cancer cell lines: Calu1 and H358 were utilised to obtain stable tranfectants overexpressing CYGB. Cell proliferation was measured with MTT assay and haemocytometer counting method. Cellular death was assessed by quantification of ADP:ATP ratio. Transformation abilities were studied with the soft agar assay, migration with the wound healing assay and invasion properties were analysed with the Matrigel-coated chambers.

Our data showed that restoration of CYGB expression did not trigger cellular death (neither apoptosis nor necrosis). Haemocytometer cell counting showed no change in the growth rate of Calu1/cygb+ clone. However, we observed higher values in the parallel MTT assay. This potentially suggests a switch from glycolysis to oxidative ATP production. In case of H358 cygb+ clones, both MTT assay and haemocytometer counting revealed diminished growth rate. CYGB overexpression reduced migratory potential of all Calu1 and H358 clones in the wound healing assay. Also, their invasive properties were diminished up to 80% ($p<0.02$). We observed striking depletion (up to 98%, $p<0.02$) in transformation efficiency of CYGB+ clones as assessed in the soft agar assay.

These results strongly support the hypothesis that CYGB acts as a TSG and indicate that CYGB might be considered as a potential therapuetic target. CYGB knock-down clones, as well as in vivo studies, will further improve our understanding of this novel TSG. It would be also interesting to test CYGB influence on tumour behaviour after exposure to hypoxia and radicals outburst.

19. SYNTHESIS AND DETEMINATION OF PHOTOCHEMICAL AND PHOTOPHYSICAL PROPERTIES OF SOME MESO - SUBSTITUTED PORPHYRINS AS POTENTIAL PDT AGENTS

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Porphyrin derivatives have recently been the subject of numerous theoretical and experimental studies owing to their potential usefulness as sensitizers in photodynamic therapy (PDT). Examination of photophysical and photochemical properties of porphyrins is an essential part of research preceding biological applications. The purpose of our presentation is to show details of synthesis and investigation of properties of several meso-substituted porphyrin derivatives that appear suitable for PDT.

The investigated compounds were obtained according to the classical Adler-Longo condensation procedure. Extraction and chromatographic methods were used to purify the products. All samples were characterized by mass spectrometry (ESI-MS) and nuclear magnetic resonance (^{1}H NMR) to confirm purity and structure.

Electronic absorption spectra of the investigated compounds in diluted solutions were recorded on a Genesis Spectrophotometer using 1-cm path length cuvettes. Measurements in the 350-800 nm range enabled detecting long-wave characteristics of free-base porphyrins: Q bands and, a much more intensive short-wave Soret band. Molar extinction coefficients and localization of UV/VIS bands are valuable parameters characterizing prospective PDT agents.

Singlet oxygen quantum yields were estimated by flash photolysis using an Applied Photo-physics LKS.60 Laser Flash Photolysis Spectrometer and a Nd:YAG laser. The decay curves of singlet oxygen emission were recorded at room temperature following third harmonic (355nm) laser excitation. Phosphorescence of singlet molecular oxygen was detected at 1280 nm for oxygenated toluene solutions (absorbance at 355 nm was equal to 0.35 cm^{-1}). Actual values of singlet oxygen efficiencies were estimated by comparing decay curves interpolated to zero time flash and areas under corrected decay curves with values obtained for a reference sensitizer.

The obtained results for singlet oxygen quantum yields lie in the 0.59-0.62 range. These values are similar to pophyrin singlet oxygen efficiencies that were reported in the literature and are comparable to those of agents used in clinical practice.

This work was supported by the Polish Ministry of Science and Higher Education (grant No. 0538/R/T02/2007/03).

20. NON-HOMOLOGOUS DNA END JOINING AND DNA REPAIR GENES EXPRESSION IN MO59 HUMAN GLIOMA CELLS AFTER ANTICANCER AGENTS TREATMENT

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Cisplatin and etoposide provide considerable synergy during treatment of glioma, although the mechanism of this synergy is not well defined. We previously showed that wortmannin, the catalytic subunit of DNA-dependent protein kinase (DNA-PK_{cs}) inhibitor, enhances cytotoxic effect of these drugs in combination in MO59K cells (reduction factor R=4,5). No such effect was observed in MO59J cells, lacking DNA-PK_{cs} expression, the main protein in non-homologous DNA end joining (NHEJ). The goal of this study was to test the hypothesis that chemosensitization of cancer cells by wortmannin is due to their effect on DNA repair system, especially NHEJ.

Investigation of DNA repair efficiency was done by *in vitro* NHEJ assay. Wortmannin at the concentration of 5 μM did not significantly decrease NHEJ repair after combined cisplatin and etoposide treatment in comparison to untreated control. This effect corresponded to a decrease in *DNA-PK_{cs}* gene expression at the mRNA level as determined by the QuantiGene Plex branched DNA method. Under the same conditions *Ku80* and *Ku70* NHEJ genes expression was increased. In MO59J cells we did not observe NHEJ activity suggesting that our plasmid-based NHEJ assay is apparently dependent on DNA-PK_{cs}.

These results suggest that sensitization by wortmannin in human glioma cells following combined treatment with etoposide and cisplatin could be attributed to an inhibition of DNA-PK_{cs}.

This work was supported by grant 401-117-32 from the Polish Ministry of Science and Higher Education and by grants 502-19-677 and 503-00-78-3 from the Medical University of Lodz.

21. RANDOM WALK MODEL OF POTASSIUM ION TRANSPORT THROUGH BIOLOGICAL MEMBRANE

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We are presenting a random walk model of a potassium ion transport through mammalian voltage gated potassium ion channels. Potassium channels are one of the most widely distributed type of ion channels. They can be found in virtually all kinds of living organisms from simple bacteria to eukaryotic cells of mammalian organisms and play an important role in many biophysical processes. X-ray crystallographic measurements have shown atomic structures of many different potassium ion channels. Three main processes seem to control a potassium ion transport: voltage gating, conduction of current through a selectivity filter and inactivation mechanism (ball on chain model). All these factors are taken into account in our model and allow us to compare obtained results with experiment data.

22. MASS SPECTROMETRY-BASED SERUM PROTEOME PATTERN ANALYSIS ALLOWS IDENTIFYING BLOOD COMPONENTS SPECIFIC FOR PATIENTS WITH NON-SMALL CELL LUNG CANCER

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Mass spectrometry-based analysis of the blood proteome is an emerging method of clinical proteomics and cancer diagnostics. Although no single peptide is expected to be a reliable bio-marker in such analyses, multi-peptide sets of markers selected in numerical tests have been already shown in a few studies to have potential values in cancer diagnostics. Here we performed mass spectrometry-based serum proteome pattern analysis aimed at identifying features specific for patients with non-small cell lung cancer (NSCLC).

Blood samples were collected before the start of therapy from 49 patients with locally advanced NSCLC, 39 patients with head & neck cancer and 36 patients with colon cancer, as well as in a group of age and sex-matched healthy controls (48 donors). Serum was isolated after blood clotting and the low-molecular-weight proteome fraction (2-14 kDa) was analyzed using MALDI-ToF mass spectrometry. Registered mass spectra were analyzed using bioinformatic tools created and optimized in our group.

We have identified cancer classifiers built of multiple spectral components (peptide $[M+H]^+$ ions) that differentiated sera from analyzed groups. Classifiers built of 16-18 components differentiated healthy persons and patients with NSCLC with about 90% specificity and sensitivity. Furthermore, we have compared similarity of proteome patterns specific for analyzed types of cancer. Peptide profiles characteristic of samples from blood of patients with NSCLC and head & neck cancer were the most similar, while samples of colon cancer were the most different. Spectral components that are the most characteristic of specific types of cancer have been also identified. We concluded that MALDI-ToF MS-based serum proteome pattern analysis has an obvious potential to differentiate samples between NSCLC patients and healthy controls or patients with other type of cancers.

23. SEARCHING FOR DYNAMICS' PATTERNS IN TIME-COURSE GENE EXPRESSION PROFILES

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Measurements of gene expression profiles in certain time points are commonly used for modeling the dynamics of cell reaction to a predefined factors. The application of a modified Gaussian mixture modeling (GMM) technique to the analysis of this type of data was proposed. The modification includes the assumption that parameters of Gaussian components, means and variances, can differ between time points, but the gene composition of components must be unchanged. The number of obtained components is related to the number of subgroups of genes with similar dynamics of gene expression. Solution to such problem requires the adaptations of a standard Expectation-Maximization (EM) algorithm and the reformulation of a likelihood function. The evaluation of the obtained subgroups of genes was done by a comparison of gene ontology terms related to the genes classified as members of particular subgroup. The hypothesis on non-random functional composition of gene subgroups was checked with the use of hypergeometric test.

We validate our method by applying it to two different datasets. The first one contains 45 Affymetrix DNA microarray data of human fibroblasts cells from three different subjects irradiated with different doses and measured in three time points. Second dataset include the data on cellular responses of human leukemic cells K562 to irradiation in five time points.

Both studies proved that the modified GMM outperforms standard decomposition techniques with respect to statistical meaning of the obtained gene groups, measured by p-values of the statistical tests. The application of our technique to time course data allows for precise division of genes into functional classes.

Acknowledgement: The work was partially supported by GENEPI low-RT FP6-036452 project.

24. DETERMINING SINGLET OXYGEN QUANTUM YIELD OF PHOTODYNAMIC AGENTS BY FLASH PHOTOLYSIS MEASUREMENTS

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Singlet oxygen is a reactive oxygen species that may be generated in biological systems. In photodynamic therapy singlet oxygen is generated by photoexcitation of sensitizers resulting in intracellular oxidative stress and induction of apoptosis. The efficiency of photodynamic therapy strongly depends on photochemical properties of photosensitizers and, in particular, on the quantum yield of molecular singlet oxygen formation.

The aim of our study was to determinate the molecular singlet oxygen quantum yield of three novel porphyrin-type compounds evaluated as sensitizers for photodynamic therapy (PDT). The quantum yield of molecular singlet oxygen formation was measured by detecting the phosphorescence decay curves in the presence of oxygen *via* flash photolysis measurements. The singlet oxygen quantum yield value was estimated using comparative method with tetraphenylporphyrin (TPP) as a reference.

Samples of the measured compounds were excited with laser pulses from Nd:YAG laser using third harmonic (355 nm) of laser irradiation and the phosphorescence intensity at 1275 nm was measured. The oxygenated solutions were prepared in toluene containing the sensitizer at a concentration suitable to produce absorbance around 0.35 at the excitation wavelength (355 nm). The phosphorescence decay curves for reference were detected under the same conditions. To confirm the sample's stability, absorption spectra of the studied compounds were compared before and after each flash photolysis experiment. Three kinetic curves of phosphorescence decay were detected in the measurements. The presented data show their mean values. After correction (light filter 880 nm) the experimental datapoints were extrapolated and plotted as a function of phosphorescence intensity vs. time. The quantum yield of molecular singlet oxygen formation was determinated by comparing the areas under emission spectra and the initial phosphorescence intensity for the studied porphyrins and TPP.

The quantum yields of the molecular singlet oxygen formation calculated based on these flash photolysis measurements were similar for all the studied porphyrins. The obtained values, ca. 0.60, are comparable with those of photosensitizers used in clinical practice.

This work was supported by the Polish Ministry of Science and Higher Education (grant No. 0538/R/T02/2007/03).

25. PRELIMINARY BIOLOGICAL INVESTIGATION OF NOVEL WATER-SOLUBLE CHLORINS

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In recent years, photodynamic therapy (PDT) has been extensively investigated as a possible treatment modality for various types of cancer. This approach is based on the combined use of light and photosensitizing agent in the presence of molecular oxygen. The energy transfer from the activated photosensitizer to oxygen present results in the formation of cytotoxic agents, such as singlet oxygen and free radicals. The efficiency of tumor destruction is crucially influenced by type of applied photosensitizing compound. A large number of photosensitizers have been investigated, but only few have shown applicable properties. Therefore, present studies have focused on the development of new photosensitizers with desirable combinations of chemical, photophysical and biological properties.

Chlorins, a class of tetrapyrrolic photosensitizers, are some of the most promising candidates for application in PDT. They are the subject of intense research due to favorable photophysical properties approaching those of ideal photosensitizers (long wavelength absorption, high molar extinction and efficient singlet oxygen formation).

In this paper we present the results of our preliminary, *in vitro* investigations of two novel water-soluble chlorins. Cytotoxicity, phototoxicity as well as subcellular localization of the novel derivatives were studied using human colon carcinoma cultured cells (HCT 116 p53+/+). The obtained results, demonstrate low cytotoxicity and high phototoxicity of the studied compounds and make them very promising agents for further PDT studies.

This work was supported in part by the Polish Ministry of Science and Higher Education (grant No. 0538/R/T02/2007/03).

26. INVESTIGATION OF POSSIBLE MEDICAL APPLICATIONS OF MAGNETIC MEMBRANES

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Air separation, or in less ambitious case, an air enrichment in oxygen, are both very important problems in medicine, industry, as well as in everyday life. Many biochemical reactions in the body depend on oxygen utilisation. Although oxygen is normally present in the air, higher concentrations are required to treat many disease processes. Oxygen therapy is a form of treatment that uses oxygen in elemental or compound forms to heal various disease conditions. It is one of the most powerful and versatile therapies known, mostly in Europe and other parts of the world. It can revitalize the practice of medicine with alternative therapies that work because of its antibacterial, anti-fungal, anti-inflammatory, anti-parasitic, anti-tumor and antiviral. We propose a new concept of air enrichment in oxygen by polymer membranes filled with neodymium powder and magnetized ("magnetic membranes"). The idea of implementing some external fields as a principal reason for gas mixtures separation (air in our case) is very promissing. The idea of "magnetic membranes" is based on the observation that oxygen and nitrogen have quite different magnetic properties i.e. oxygen is paramagnetic whereas nitrogen diamagnetic, what gives a real chance for their separation. We have got an oxygen enrichment up to the 56% in permeate for magnetic induction 2,25 mT.

27. ROLE OF DNA-REPAIR PATHWAY IN DOXORUBICIN-INDUCED CELL DAMAGE

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Background: The clinical efficacy of Doxorubicin (DOX) is greatly restricted due to the development of a severe form of cardiomyopathy and nephropathy due to induce of oxidative stress. DNA-repair and related redox-sensitive pathway may be involved in those negative effect of DOX. RNR3-lacZ reporter gene construction consist of ribonucleotide reductase promoter fusion with β- galactosidase (LacZ) and reflect the activity of DNA-repair pathway. TRX2-lacZ consist of thioredoxin gene promoter fusion with LacZ gene and may reflect the activity of related redox-sensitive pathway. The aim of our study was to investigate influence DNA-repair and related redox-sensitive pathway activation on DOX-induced oxidative stress by using eukaryotic cell model *Saccharomyces cerevisiae*.

Methods: *S. cerevisiae* strain YPH499 was transform by RNR3-lacZ and TRX2-lacZ reporter gene construction and incubate with different amount of DOX (10,30,50 μm) in SD media. The influence of DOX on strain's survival, proliferation, intracellular glutathione reduced (GSH), malondialdehyd concentration was studied. The rate of expression RNR3-lacZ and TRX2-lacZ reporter gene construction assessed through β- galactosidase activity.

Results: DOX induced dose-dependent inhibition of cell proliferation (95.5±1.5; 78.1±1.1; 70.5±0.9 per cent from control at concentration DOX in media 10,30,50 μm, respectively). Concentration of GSH was 2.82±0.5 μM̃/mg dry weight in control group. DOX at 10,30,50 μm increased GSH: 4.4±0.27 ($p<0.01$); 5.19±0.25 ($p<0.001$); 5.31±0.35 ($p<0.001$), resp. DOX is not significantly impact on malondialdehyde concentrations. DOX induced RNR3-lacZ and TRX2-lacZ expression in none dose-dependent manner. GSH concentration correlated with expression RNR3-lacZ ($r=0.69;p<0.01$), but not correlate with expression of TRX2-lacZ ($r=0.23;p>0.05$).

Conclusions: The results show that doxorubicin increase GSH concentration in cells of *S. cerevisiae* and this effect due to activation of DNA-repair pathway. We suggested that mechanisms of DOX-induced cell damage more complexes than simplicity oxidation of molecules.

28. THE NEW APPROACH TO DESIGN ANTITUMOUR IRON CHELATORS. USE OF THE KOHONEN NEURAL NETWORKS FOR MODELLING NOVEL BIOLOGICAL ACTIVE AGENTS

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Iron is one of the essential parts of human biochemical system. It plays role in a variety of physiological cellular processes such as oxygen transport, energy metabolism, electron transport and DNA synthesis [1]. The rate limiting step of DNA synthesis involves the Fe-dependent enzyme, ribonucleotide reductase (RR), which converts ribonuclotides to deoxyribonucleotides. To facilitate rapid replication neoplastic cells have significantly higher levels of RR and transferrin receptor 1 (TfR1) [2]. The higher Fe utilization by cancer cells than their normal counterparts is a good reason for modelling and synthesis the novel selective antitumour iron chelators.

In the present investigation we chose quinoline derivatives with semicarbazide scaffold as a potential Fe chelators in tumour cells. Functionalised quinoline derivatives are used as potential inhibitors of many enzymes (i.e. HIV integrase) [3]. 8-hydroxyquinoline is a well-known iron chelator, therfore we decided to design new class of iron chelators based on its moiety [4].

In our present work we used Kohonen maps of eletrostatic potencial to characterize 16 well-known iron chelators. This technique allowed us to visualize the electrostatic potencials on the molecular surfaces as a planar square map. Before building the neural map all the molecules had to be systemically aligned. The Kohonen system allowed us to compare those parts of the molecule surfaces that can be superimposed. If the surface couldn't be superimposed on reference molecule we indicated it by white colour. In these electrostatic maps we can see also red areas which indicates the most electronegative regions and the purple- the most electropositive areas.

In addition we calculated autocorrelation vectors for the set of 16 Fe chelators. It helped us to create **S**elf **O**rganized **M**aps capable of clustering active and inactive chemical compounds. The created SOM was afterwards used to screen chemical database containing 200 structures of quinoline derivatives.

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29. PHOTODYNAMIC REACTION IN COMBINATION WITH ELECTROPORATION IN HUMAN BREAST ADENOCARCINOMA CELLS IN VITRO

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The photodynamic therapy (PDT) is the method of selective tumor treatment, which uses the photosensitive substance and the suitable wavelength of light. Photosensitizer accumulation is dependent on the type of malignant cells, physical and chemical properties of the dye. It localizes in the plasma or subcellular membranes, making these structures especially sensitive to the photooxidative damage.

The application of the cell membrane electroporation in combination with cytotoxic drugs could increase their transport into cells. The combination of electroporation and application of drugs with inhibited transport is known as electrochemotherapy (ECT). ECT and PDT are low- invasive and targeted methods. Proper therapy conditions could limit necessity of surgical interventions, as well as give the better prognoses in treatment the tumors.

The current study examined an effect of combining both methods applied in vitro. Photodynamic reaction enhanced by electroporation was tested on the human breast cancer cells (MCF-7/DOX and MCF-7/WT). The photodynamic activity of an electro-photodynamic reaction with the hematoporphyrin derivative (HpD) was evaluated in relation to the standard photodynamic method by MTT assay as an examination of mitochondrial cell function. The experiments proved that electroporation effectively supports photodynamic method.

30. REGULATION REGULATION OF THE HUMAN GLUTATHIONE S-TRANSFERASE P1 GENE TRANSCRIPTION

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Glutathione S-transferase P1-1 is a widely distributed enzyme which plays a key role in protection of the cells from genotoxic damages. The aim of the present finding was to clarify the mechanisms responsible for the different levels of *GSTP1* expression observed in Me45, Hbl-100 and BeWo cells analyzing cis- and trans-acting factors implicated in regulation of its transcription. The aim of this work was to identify cis- and trans- acting factors responsible for the cell-specific levels of the human *GSTP1* gene expression and to investigate molecular mechanisms responsible for the changes in *GSTP1* expression during carcinogenesis utilizing human placenta and choriocarcinoma cells as a model system.

Level of *GSTP1* mRNA was assessed be quantitative RT-PCR and level of GSTP1 protein was assessed by Western-blotting. Truncated promoter fragments for the transient transfection assay were obtained by PCR and cloned into pGL3basic. The Me45, Hbl-100 and BeWo cells were transfected with each of recombinant plasmids and luciferase activities were measured. Transcription factors interacting with *GSTP1* promoter elements were assessed by competitive EMSA and supershift analysis. Promoter methylation was analyzed by MSP.

The highest level of *GSTP1* mRNA and protein was detected in Hbl-100 and the lowest in BeWo. Transient transfection assay provided the evidence for two positive (ARE and NF-κB) and two negative (CRE and NF-κB-like) regulatory elements similarly acting in *GSTP1* promoter in three cell types. Results of competitive EMSA and supershift analysis indicated that ERβ together with unidentified protein binds to ARE and together with Fos binds to CRE sites in all cell types, The structure of ERβ/Fos complex in Hbl-100 differs from that in Me45 and BeWo. In addition NF-κB interacting with NF-κB site was identified as a p50/p50 homodimer in BeWo and p50/p65 heterodimer in Hbl-100 and Me45cells. MSP revealed partial *GSTP1* promoter methylation in Me45 and BeWo cells and no methylation in Hbl-100.

According to the results of qRT-PCR and sqWB GSTP1 expression is down-regulaed in BeWo cells comparing to placenta.

To identify transcription factor responsible for carcinogenesis-associated GSTP1 down-regulation in trophoblast cells competitive EMSA and supershift assay were applied. In consequence with the results ARE and NF-kB sites in normal trophoblast and choriocarcinoma cells interact with the same TFs however, CRE-protein complexe in BeWo cells is different from that in normal trophoblast. Besides ERb and Fos it also contains transcription factor Jun.

Thus, we analyzed for the first time the molecular mechanisms responsible for the steady-state level of *GSTP1* transcription in Hbl-100, Me45 and BeWo cells. We assume that the absence of NF-κB p65 accounts for the lower *GSTP1* expression level in BeWo comparing to Hbl-100 cells, and the structural changes in CRE-site complex is responsible for down-regulation observed in Me45. We also demonstrated that *GSTP1* promoter becomes methylated in cell with low transcription of the gene.

31. GEFITINIB INCREASES CYTOTOXICITY OF BLEOMYCIN IN HUMAN GLIOMA CELLS

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Gefitinib (ZD1839) is a derivative of chinazolin, which suppresses the activity of the tyrosine kinase of epidermal growth factor receptor (EGFR). Signal pathways of EGFR proteins are associated with unchecked proliferation and the resistance to radiotherapy of cells of the glioma. Activation of EGFR influences mitosis, mobility associated with metastases, as well as repair of DNA damage and angiogenesis. Since in gliomas, the EGFR genes often impact gene overexpression, DNA amplification and mutations, the inhibition of tyrosine kinase of EGFR may allow the use of lower doses of drugs to treat brain tumors.

The aim of our study was to determine if gefitinib, inhibitor of EGFR, can sensitize human glioma cells to DNA damaging agents: cisplatin and bleomycin.

We performed XTT growth inhibition assay in order to evaluate the effect of the EGFR inhibitor, gefitinib, on the response of two glioma cell lines – MO59K and MO59J to cisplatin and bleomycin, the latter cell line lacking the expression of the catalytic subunit of DNA-dependent protein kinase (DNA-PK_{cs}).

Our results demonstrated that gefitinib increases the cytotoxicity of bleomycin in MO59K and MO59J cells. Simultaneous incubation with 10 μM gefitinib potentiates two times (R=2) the growth inhibition of the drug in both cell lines. Additionally, MO59J cells are twice more sensitive than MO59K to the cytotoxic effect of bleomycin, what is manifesting itself in IC_{50} values. In contrast, no sensitization to cisplatin was observed in MO59K and MO59J cells. We also found that cytotoxic effect of cisplatin and bleomycin in combination is higher than that of every drug individually (synergism, combination index CI<1).

In conclusion, our data suggest that the inhibition of EGFR activity may increase the effectiveness of bleomycin therapy in human glioma.

32. CYTOTOXICITY OF ETOPOSIDE IN CANCER CELL LINES *IN VITRO* AFTER SIMULTANEOUS *BCL-2* AND *C-RAF* GENE SILENCING WITH ANTISENSE OLIGONUCLEOTIDES

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BCL-2 and *C-RAF* genes are overexpressed in most types of cancers. Although these genes are mediators in different molecular pathways, their main characteristic is the antiapoptotic activity thus, cells that overexpress either *BCL-2* or *C-RAF* lose their ability to undergo apoptotic death being resistant to chemotherapeutic agents and/or physiologic mediators of cell death (e.g. TNF-α). Both anti-*C-RAF*, and anti-*BCL-2* oligonucleotides were tested as chemosensitizers in cancer therapy.

The aim of the study was to test the effects of simultaneous / combined use of antisense oligonucleotides (ASOs) targeting the *BCL-2* and *C-RAF* transcripts on the cancer cell cultures *in vitro* exposed to etoposide. *BCL-2* and *C-RAF* ASOs with phosphorothioate backbones were designed according to the general rules within the coding mRNA sequences. For *BCL-2* a consensus sequence targeting both α-, and β- isoform was designed. Cells were transfected with *BCL-2* or *C-RAF* ASOs or with combination of both daily for 5 h, either for 1 or 3 days. 3-day transfection was followed by a single treatment with 20 μM etoposide for 5 h. The following cancer cell lines were tested: A549, HeLa, and T24.

Sequence-specific decrease in BCL-2 and C-RAF mRNA levels were confirmed by Real-Time RT-PC. After 1-day treatment mRNA levels decreased by 9%-42% of the normal expression in cells treated with 50-1200 nM ASOs. One day treatment also confirmed induction of cell death in all transfected cultures in a concentration-dependent manner (even at the lowest 50 nM concentrations) as was revealed by MTT assay and by microscopic analysis of cell morphology (Hoechst 33258 staining). To sustain high intracellular level of ASOs directed against *BCL-2/C-RAF* we used repeated (3-day period) ASOs transfection. Results showed that both ASOs effectively stimulated cell death and potentiated etoposide-induced cytotoxicity. We also confirmed that the cytotoxic effect was at least partially mediated by apoptosis induction which was evidenced by microscopic analysis Those effects depended on ASOs concentrations. Significant differences were evidenced in the response of certain cancer types (models) analyzed in that study. The strongest resistance to ASOs treatment was observed in HeLa cultures while the most promising results were obtained in A549 (lung cancer) cells. This observation suggests that lower concentrations may be used for this type of cancer to obtain high efficiency of enhancement in etoposide-induced cell death. Comparison of the control ASO (*scrambled* sequence) with sequence-specific anti-*BCL-2* or anti-*C-RAF* ASOs indicates that at higher concentrations of ASOs (~ 1 μM) a significant proportion of the cytotoxic effects came from general oligonucleotide and/or lipofectamine toxicity. Nevertheless, simultaneous transfection with both ASOs targeting *BCL-2* and *C-RAF* transcripts allowed us to use lower concentrations to obtain maximal results. These conclusions suggest that maximal attainable effects were gained and the rest of cell pools were resistant to etoposide and/or gene silencing by ASOs. Simultaneous use of two ASOs in 3-day treatment allows us to lower the concentration needed to obtain the same treatment results compared with single use of each ASO thus enabling to diminish sequence-unsepcific toxicitiy, although the combined treatment does not lead to higher efficiency overall.

The study was supported by the funds of Medical University of Silesia (KNW-1-117/09, KNW-2-198/08).

33. TNFα-INDCUCED ACTIVATION OF NFκB SIGNALING AFFECTS REGULATION OF P53-DEPENDENT PATHWAY IN UV-IRRADIATED HCT116 CELLS

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Signaling pathways that depend on p53 or NFκB transcription factors are essential components of cellular responses to stress. In general, p53 is involved in either activation of cell cycle arrest or induction of apoptosis, while NFκB exerts mostly anti-apoptotic functions. Both regulatory pathways apparently interfere with each other yet molecular details of such interactions remains to be elucidated. A mathematical model describing functional interactions between p53- and NFκB-dependent pathways has been recently published by Puszyński & Lipniacki, that suggested different output depending on the time sequence of each pathway activation.

Here we aimed to verify experimentally effects of NFκB pathway on activation of p53-dependent genes. Colon carcinoma HCT116 cell line was used, in two congenic variants either containing or lacking transcriptionaly competent p53. Cells were incubated with TNFα cytokine to induce NFκB, and/or treated with ultraviolet radiation to induce p53 pathway; both factors were used in several different combinations. Activation of NFκB and p53 pathways was monitored by Western-blotting, while levels of expression of selected genes were assessed by semi-quantitative RT-PCR or quantitative real-time Q-RT-PCR. We have observed that activation of p53-dependent genes was enhanced in cells exposed to TNFα cytokine after UV irradiation. In contrast, exposure to TNFα before irradiation reduced UV-induced activation of p53-dependent genes.

This work was supported by the Ministry of Science and Higher Education, Grant PBZ-MNiI-2/1/2005 and N514-411936.

34. COMPARATIVE STUDY OF DIFFERENT DIAGNOSTIC TECHNIQUES (AI, MLPA, FISH) IN BREAST CANCER PATIENTS WITH 1q25.3 ALTERATIONS

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Breast cancer is the most frequently diagnosed malignancy among women and is the leading cause of cancer related death worldwide. Molecular genetic studies have revealed many subgroups of breast cancer within which the genomic alterations affecting chromosome arm 1q are considered to be an early event in breast carcinogenesis and are correlated with good prognosis for the patients. We have found a high percentage of concordance between Allelic Imbalance (AI) and Multiplex Ligation-dependent Probe Amplification (MLPA) assay pointing towards gain of the 1q25.3 region that includes Regulators of G protein Signaling (RGS) genes in breast cancer (1).

Our main objective was to compare the sensitivity and specificity of AI and MLPA with other cytogenetic method (FISH) in breast cancer patients with or without previously confirmed alterations within 1q25.3.

FISH was performed on formalin-fixed paraffin-embedded tumour material in order to verify previous findings and assess the level of genetic alterations of 1q25.3 in breast cancer. The 1q25.3 test and 1p32 reference probes labeled with different fluorochromes were utilized for analysis.

A total of 70 nuclei from each breast cancer case were examined and scored for the percentage of 1q25.3 alterations. The non-tumourigenic nuclei obtained from healthy individuals served as adequate cut-off for the 1q25.3-specific changes. The overall FISH results are consistent with results obtained from previous analysis in majority of analysed cases. Futhermore, FISH resolved the level of 1q25.3 alterations in few cases that were uncertain by AI and MLPA analysis.

This study shows that both AI and MLPA assays are able to map regions of copy number changes in cancer genome and are in concordance with molecular cytogenetics. This study shows that these three techniques complement each other and are able to map regions of copy number changes in the cancer genome and proved to be an efficient tool for diagnosis of 1q25.3 alterations in breast cancer.

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35. D-METHIONINE IN NOISE INDUCED HEARING LOSS

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Both ROS and reactive nitrogen species can attack a wide range of biological targets, leading to oxidative cellular injury. ROS have been shown to play a toxic role in the cochlea both *in vitro* and in *vivo,* such as noise-induced cochlear injury in animal studies. D-Methionine, a sulfur containing amino acid, functioning as an ROS and/or reactive nitrogen species scavenger, may thus prevent noise-induced cochlear dysfunction.

C57BL/6 mice were used for this study. The animals were divided for three group: I – non-exposed control, II – exposed to noise, III – exposed to noise and given injections with D-Met i.p. D-methionine was administrated 1 h before and 1 h after noise exposure. One additional dose each was given on days 1, 2 and 3 after noise exposure. The control and II groups received equivalent volume of saline ate the same intervals as the D-Met.

In result, D-methionine treatment significantly decreased threshold shift 1, 7 and 14 days after noise exposure. A significantly lower growth in LPO level was observed after day 1 and day 14 after noise exposure in the mice group exposed to noise and treated with D-Met in comparison with the mice group exposed to noise, only. In the mice group exposed to noise and treated with D-methionine a significant decreased was observed in catalase activity after day 14 and SOD activity after day 1 noise exposure as compared to the group exposed to noise alone.

In summary, the administration of D-methionine seems to attenuate the noise-induced changes. It results in decreased hearing threshold shift and influence on oxidative stress process.

36. TGFβ AUXILIARY RECEPTORS IN ENDOMETRIAL CANCER

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Transforming growth factor β (TGFβ) signalling pathway regulates such divergent processes as cell differentiation and proliferation, apoptosis, embryogenesis, angiogenesis, immunological response. It has been shown that TGFβ plays an important role during neoplastic transformation, tumour progression and metastasis. Up till now, three TGFβ auxiliary receptors that differ in TGFβ isoforms binding specificity have been identified: betaglycan (TGFβ receptor type III), endoglin (CD105 antigen) and CD109 antigen. These molecules do not posses enzymatic activity, however they modulate presentation of TGFβ ligands to canonical TGFβ receptors type I and II.

In this study, betaglycan, endoglin and CD109 expression was compared with TGFβ1, TGFβ2 and TGFβRII status in endometrial cancer and normal tissue. Quantitative PCR method was employed to asses mRNA levels, whereas ELISA assay was performed in order to measure protein expression.

The results have shown betaglycan, endoglin and CD109 mRNA down-regulation and protein level up-regulation in tumour cases. Observed alterations in TGFβ auxiliary receptors expression were found to correlate with changes in expression level of other studied members of TGFβ signalling pathway. Equivocal expression of TGFβ accessory receptors at mRNA and protein level suggests complexity of TGFβ signalling regulation.

37. ANTI-PROLIFERATIVE PROPERTIES OF OLIGO(3-HYDROXY-BUTANOATE) CONJUGATES WITH IBUPROFEN

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Ibuprofen [2-(4-isobutylphenyl)propanoic acid] is a non-steroidal anti-inflammatory drug (NSAID) used widely in rheumatoid arthritis, osteoarthritis and a number of other painful conditions. Besides its positive effects on inflammation and pain, ibuprofen could also present potential use for cancer prevention of colorectal cancer and cancer treatment of disease such as cardiovascular disease[1,2].

The therapeutic use of non-steroidal anti-inflammatory drugs (NSAIDs) is often restricted by the necessity to deliver the drug to specific sites of target organ or tissue. The use of this kind of drugs is also limited by their irritant side effects on the gastro-enteric mucus. Many drug-polymer delivery systems have been used offering improved efficacy, reduced toxicity and better patient compliance compared to conventional oral dosage forms of pure drugs. Looking for biodegradable and biocompatible non-toxic polymers for drug delivery systems we performed the synthesis of non-toxic biocompatible poly(3-hydroxybutyrate) [3-4].

This communication shows that anionic ring-opening polymerization of (R,S)-β-butyrolactone initiated with an alkali metal salt of (S)-(+)-2-(4-isobutylphenyl) propionic acid (ibuprofen) may constitute a convenient conjugation methods of ibuprofen with biodegradable OHB. Furthermore using the MTT cell proliferation assay we demonstrated that ibuprofen conjugated with OHB exhibited significantly increased, as compared to free ibuprofen, potential to inhibit proliferation of HT-29 and HCT 116 colon cancer cells. Although the mechanism of antiproliferative activity of ibuprofen-OHB conjugates (Ibu-OHB) has to be established, we suggest that partially it can be related to more effective cellular uptake of the conjugate than the free drug. This assumption is based on the observation of much more efficient accumulation of a marker compound - OHB conjugated with fluorescein, in contrast to fluorescein sodium salt, which entered cells inefficiently.

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Addendum

38. WO SUBTYPES OF SEROUS OVARIAN CANCER WITH DIFFERENT SURVIVAL

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Ovarian cancer is characterized by asymptomatic development until its advanced stages. Cancer cells spread by migration inside the peritoneal cavity rather than via lymph- or bloodstream. As the disease is diagnosed late, the standard treatment option is surgery and adjuvant chemotherapy. The aim of our study is to better understand the molecular mechanisms of ovarian cancer tumorigenicity and chemotherapy resistance. The long range aim is to select potential prognostic or predictive molecular markers and/or potential new therapeutic targets for serous ovarian cancer.

We studied 99 ovarian cancer specimens by DNA microarray technique. Unsupervised analysis revealed the two subtypes of serous ovarian cancers that differ significantly by gene expression pattern. This division did not overlap with the differences defined by most of the clinical or molecular features (FIGO, grade, etc.), however it appeared to be associated with the overall survival time (OS). The majority of genes that are differentially expressed in the two subtypes of serous cancer code for proteins engaged in cell adhesion and communication, development, metabolism, and immune response. We selected 6 genes to be analyzed by real time RT-PCR and all were positively validated. Expression values obtained by quantitative PCR were used to build a 6-gene predictor of the OS by Cox regression method. This model was able to predict successfully the subtype of a tumor and survival time of the patients (ROC 0.92). We conclude that among ovarian cancer samples of serous histology two molecular subtypes are present that differ by their biological properties and are associated with different survival of the patients. Our current project is aimed to evaluate the role of selected genes differentially expressed in the two subtypes, in particular, their impact on cancer cell tumorigenicity and sensitivity to chemotherapy.

This work was partially supported from the grant 1/0-PBZ-MNiI-2/1/2005 from Ministry of Science and Higher Education.

39. EXPRESSION OF GENES CONNECTED WITH GLYCOLYSIS PROCESS IN COLORECTAL ADENOCARCINOMA

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Glycolysis is the oldest evolutionary process which can raise energy (in the form of NADH and ATP) from glucose, fructose, galactose, mannose and glycerol as a result of enzymatic degradation of these compounds to pyruvate. This process may have occurred both in aerobic and anaerobic condition, what gives great opportunities the cells, in which began running process or a malignancy. It is known that glycolysis is the primary source of energy for cancer cells. The Warburg effect (a reduction in respiration and growth of glycolysis), and hypoxia are commonly present in human cancer cells.

The aim of this study was to evaluate expression of genes closely connected with glycolysis process in the cells of colorectal adenocarcinoma, comparing the expression of these genes in segments which found to be macroscopically intact with segments changed by cancer obtained from patients in different clinical stage of disease.

The total RNA extracted from segments of normal colon and adenocarcinoma using TRIzol reagent (Invitrogen). Then subjected to RNA purification, and about 8 micrograms of total RNA was used to synthesize double-stranded cDNA, then synthesized cRNA labeled biotin, fragmentated and hybridizated with microarrays test3 and HGU 133A (Affymetrix), and labeled with streptavidin-phycoerythrin complex (according to the manufacturer's protocol). The fluorescence intensity of the transcripts were analyzed using a microarray scanner GeneArray Scanner G2500A (Agilent). Quantity and quality of total RNA, cDNA and cRNA received in subsequent degrees of transciptome determining, assessed spectrophotometrically and by agarose electrophoresis. After reading the fluorescence signal from the microarray for 22,283 mRNAs, before proceeding to further analysis of results, performed the statistical classification of microarray using Affymetrix's internal programs and other statistical programs, then the final were normalizing data using RMAExpress (Robust Multichip Analysis) based on the logarithmic function ($\log_2$). From obtained transcripts identified 49 genes closely connected with glycolysis process and subjected them to analysis which allowing comparison of results between the studied groups: control and adenocarcinoma with different clinical stage.

The differentiating genes of these groups are determined by the analysis of linear regression using the Statistica 7.1. and the SAM (Significance Analisys of Microarrays) programs.

From obtained comparative analysis the expression of genes connected with glycolysis process in control samples and samples varying of stage of disease that PKM2 (PYRUVATE Kinase 2) and ENO2 (Enolase 2) in the fourth, LDH (Lactate dehydrogenase) only in the second and ALDOB (Fructose-1 ,6-bisphosphate aldolase) in both the second and first clinical stage of disease (CS) were overexpressed. But for PFKL (Phosphofructokinase; IICS) and ENO1 (Enolase 1, II and IV CS) and HK2 (Hexokinase-2, III and IVCS) related to the control were significantly silenced.

OXIDATIVELY DAMAGED DNA AND ACTIVITY OF THE PROTEINS OF NF-κB PATHWAY IN LIVER AND KIDNEY OF SUPEROXIDE DISMUTASE KNOCK-OUT MICE

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To counteract the deleterious effect of oxidative stress/oxidatively damaged DNA, all organisms developed several strategies. Important factors, in antioxidant defense are antioxidant enzymes like superoxide dismutases (SODs). Deficiency in various forms of SOD may result in higher production of free radicals and subsequently in oxidative stress. Moreover, the reactive oxygen species are considered as a second messengers leading to NF-κB pathway activation.

In our work the level of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG), a marker of oxidative stress, and the NF-κB pathway activity in the Cu,Zn SOD knock-out and wild type mice were examined. The level of 8-oxodG in DNA as well as the activity of the p50 (NF-κB1) and p65 (RelA) proteins were determined in liver, brain and kidney.

The level of 8-oxodG was higher in the liver and kidney of knock-out mice as compared to the wild type. We have found that activity of the p50 protein was also higher in kidneys, but surprisingly not in livers of SOD1-deficient mice, whereas p65 activity did not show any changes. Our results indicate the mice deficient in Cu,Zn-SOD may develop oxidative stress in some tissues, and ROS-induced NF-κB activation could be cell/organ type depended.

ACKNOWLEDGEMENT
This work was supported by a grant of Ministry of Science and Higher Education N301 2052 33 and in part by ECNIS (Environmental Cancer Risk, Nutrition and Individual Susceptibility) a network of excellence operating within the European Union 6th Framework Program, Priority 5: "Food Quality and Safety" (Contract No 513943).

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[1]L-lecture; P-poster

Authors index

Estrada Ludowa
"CZANTORIA"

"CZANTORIA" - TWENTY YEARS OF SINGING

In 2008, "Czantoria", the foremost artistic ensemble from Ustroń, celebrated its 20th anniversary. On the occasion of celebrating the 700th anniversary of the City of Ustroń, during the "What makes the people of Ustroń sing inside?" gala concert in 2005, "Czantoria" was bestowed the official title of Ustroń City Honorary Ensemble. During the past twenty years "Czantoria" gave over 600 concerts. Currently it has 52 singers. The repertoire is varied, from popular folk songs, especially from the Cieszyn region, through popular songs, as well as patriotic and religious music, notably carrols. The concerts have been invariably bringing to the audience moments of deep feelings, joy and artistic emotion. "Czantoria" has been performing regularly for spa and resort visitors home and abroad. It went on tours to Germany, Italy, Bulgaria, Hungary, Lithuania, Slovakia and Czech Republic. The artistic success of "Czantoria" undoubtedly goes back to immense efforts of late Marian Zyla, the founder and first artistic director of the Ensemble, as well as to its present Bandmaster and Musical Director, Wladyslaw Wilczak. Past years have witnessed dozens of singers performing for the Ensemble (including late Boleslaw Iskrzycki, Rafal Winter, Emil Fobr, Ernestyna Kisiala, Dorota Gerlic, Wladyslaw Hladki and Henryk Jankowski). Such rotation does not make things necessarily easy for musical directors to keep up the performance level but, what is encouraging, "Czantoria" enjoys a constant inflow of talented young singers bringing with them an invigorating breath of youth and energy. "Czantoria" ensemble has had the privilege of working with top artists such as Ewa Kornas-Biegas and Magdalena Chudzieczek-Cieslar (sopranos) or with somewhat younger generation of Musical Academy graduates such as Katarzyna Siwiec, Joanna Szcześniewska and Izabela Zwias (all of them conductors). In its Jubilee Year "Czantoria" is musically in top shape; thanks to the effort of the gifted conductor, high artistic level and rich repertoire are the trademarks of the Ensemble. The audience reception of the Ensemble is usually spontaneous and quite often ends up in joint feast singing. The Ensemble is proud to have an eight-volume Chronicle, kept from the very beginning by Jan Albrewczynski. Entries witness excellent reviews and reception of "Czantoria" everywhere. The Executive Board of the "Czantoria" Association for the Promotion of Folk Culture expresses its deep gratitude to the Ustroń Mayor's Office for financial support and harmonious cooperation on behalf of Cieszyn Land, our beloved Little Motherland.

Contents

Silesia.

Positive energy