Session 8. Biochemistry of tumours

Oral Presentataions

O8.1

Mast cells, matrix and tumour biology

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The three properties that characterise cancer cells are uncontrolled proliferation, the capacity to invade local tissues, and the ability to metastasize to distant sites. The importance of mast cells in local homeostasis, inflammation and angiogenesis is supported by many studies; and the association of mast cells with a variety of tumours has long been recognised. In many cancers mast cells preferentially accumulate at the tumour periphery where they have the potential to release numerous mediators that may modify the microenvironment – the tumour–host interface. The mitogenic effects of the well-characterised mast cell mediators histamine, serotonin, heparin and various cytokines/growth factors have been reported for a variety of cells *in vitro*, but not all cells; an observation probably related to the fact that different cells express different receptors, especially in relation to specific cell types and phases of the cell cycle. The major proteins of the extracellular matrix (ECM) have traditionally been considered as inert, but the physiological/enzymic processing of the ECM may result in small bioactive fragments with potential mitogenic properties. ECM is purported to exert both chemical and physical influences on the cell *via* transmembrane receptors and the cytoskeleton; with damaged or lysed matrix often stimulating a more aggressive tumour response. Matrix components such as basement membrane laminin and type IV collagen, as well as fibronectin, fibrinogen type I homotrimer collagen and hyaluronan are all reported to be mitogenic for some cell types. Many studies have shown that both genetic and epigenetic factors contribute to the autonomous replication of tumour cells. However, the importance of the microenvironmental host response, especially immunological and tumour:stromal interactions, should not be underestimated.

O8.2

Cell movement and metastases

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Formation of metastases plays a pivotal role in the development of cancer. Metastatic activity depends on acquiring by cancer cells several separate features. Increased motility is one of the most important characteristics of neoplastics cells which decide about a capacity of tumor cells to spread from the original tumour to distant locations in the organism. The active migration of tumour cells is important for local invasion, in the processes of intra- and extravasation and finally, in the invasion of target organs. We would like to concentrate on classical works in which the basic problems related to cancer cell motile behavior as different from normal cells have been formulated. The attention will be paid to the contemporary research on the role of specific cells surface proteins (integrins, cell adhesion molecules, cadherins and extracellular proteinases) involved in regulation of cellular motility and interaction of tumor cells with normal tissue. The role of cell-cell homo- and heterotypic interaction will be considered in specific steps of the course of metastases formation.

O8.3

The role of cell adhesion in organ-specific metastasis

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O8.4

On the significance of recurrent chromosome aberrations and gene fusions and other rearranged genes in soft tissue tumors

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Cancer is, at the cellular level, a genetic disease and acquired gene fusions and other gene mutations play a causal role in the initiation and progression of the neoplastic process by activating proto-oncogenes. Cytogenetic studies of soft tissue sarcomas have revealed several highly specific chromosome aberrations. These balanced, structural rearrangements, mainly translocations, are associated with distinct tumor subtypes with remarkable specificity and have been essential for identifying genes involved in tumorigenesis and tumor progression. The genetic mechanisms leading

to the creation of tumor-specific chromosome changes are unknown. There are suggestions that the nuclear position of chromosomes involved in a particular translocation correlates with their propensity to form this translocation. On the molecular level, the generation of the neoplasia-associated chromosomal rearrangements is correlated with genomic architecture in the breakpoint cluster regions, characterized by large, palindromic, low-copy repeats (LCR). The identification of recurring chromosome abnormalities can assist in the differential diagnosis and subclassification of malignant tumors and, hence, in the selection of the appropriate treatment. In addition, the particular chromosomal changes and/or gene mutations are associated with increased risk of metastases and recurrence of the disease. The presence and types of gene mutations in some tumors are important factors in prediction of response to treatment.

O8.5

Contact inhibition the disorder of signal transduction system

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Confluence or serum withdrawal may cause growth arrest of cultured non-transformed cells. Therefore, the first question that arose was what was the difference between sparsely populated and confluent C3H10T1/2 cells with and without serum-containing medium. The following proliferation-relevant end points were examined: cell-cycle distribution, Ki-67 antigen presence, the level of the von Hippel-Lindau (VHL) protein, and gene expression by using a microarray approach. In sparse/logarithmic cultures, the fraction of cells in G0/G1 phase increased from 55 to 85% following serum withdrawal. Moreover, the fraction of Ki-67 positive cells dropped from 89 to 47%. In confluent cultures, the majority of cells (80%) were in G0/G1 phase and only 25–30% were Ki-67 positive, regardless of serum presence. In both serum-deprived and contact-inhibited cultures, significant and distinct changes in gene expression were observed. Serum deprivation of sparsely cultured cells resulted in significant over-expression of several transcription factors, while confluent cells showed elevated expression of genes coding for *Wnt6*, *uPar*, *Tdag51*, *Egr1*, *Ini1a* and *Mor1*. These results indicate that contact inhibition and serum withdrawal lead to cellular quiescence through distinct genetic and molecular mechanisms. Since the contact inhibition may be associated with an inefficient transduction of the proliferative signal from adhesion molecules, perhaps due to cellular homogeneity. To verify this concept, the C3H10T1/2 fibroblasts were stable transfected with gene coding for FNIII/10 fibronectin fragment. This resulted in the differences in genes' expression between original C3H10T1/2 cells and their FNIII/10 transfectants. No significant differences in growth properties were observed in the original and in the transfected cells. The C3H10T1/2 cells and their transfectants when co-cultivated displayed more cells at confluence than the cells cultivated alone. Moreover, co-cultivated C3H10T1/2 cells and their transfectants showed elevated levels of phospho-ERK1/2 as compared to homogenous cultures. The obtained results support the hypothesis that cellular homogeneity is responsible for density dependent growth inhibition.

O8.6

Proteins involved in the cell adhesion mechanism in the thyroid neoplasms

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Development of malignant tumors, in particular the transition from benign lesions to invasive, metastatic cancer, is characterized by a tumor cell's ability to overcome cell-cell adhesion and to invade surrounding tissue. During the transformation from normal cells to highly malignant tumor cells, the expression of some cell adhesion molecules is up or down-regulated. A multitude of clinical and experimental studies have revealed that expression of E-cadherin is diminished in papillary and follicular while lost in anaplastic thyroid carcinomas. It was stated that changes in the expression of proteins that are part of the E-cadherin adhesion complex i.e. catenins, impair E-cadherin mediated cell-cell adhesion. Down-regulation of α, β, γ-catenins expression in thyroid lesions lead to progression of disease with metastases to regional lymph nodes. Recently, significant negative correlation was observed between dysadherin, a novel cancer associated cell membrane glycoprotein, and E-cadherin expression. Dysadherin expression is significantly higher in undifferentiated carcinoma than in papillary and follicular carcinomas. It was evidenced that aggressiveness of several malignant tumors correlates with alterations in the expression of one or more integrins. It was revealed that all thyroid epithelial cells expressed integrin β1 and α3 subunits. Neoexpression of integrin α6β4 subunits was seen in most malignant thyroid tumors, whereas α2 subunit was exclusively found in anaplastic carcinoma.

O8.7

Migration ability of selected cancer cells in correlation with β-actin expression

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Cancer treatment often fails to be fully effective due to

variable properties of tumor cells and their response to chemotherapy. Invasive tumor cells have been extensively studied and evidenced to be morphologically and molecularly distinct from non-invasive cells. β-Actin has been considered as a potential marker of cancer cell migration ability. Our former studies have shown that there is the correlation between metastatic potential of cancer cells and β-actin expression as well as with it's subcellular localization. In order to confirm this phenomenon three different cancer cell models and their variants of different migration ability, selected on Matrigel-coated filters, were examined. There were: human colon adenocarcinoma LS180, rat hepatoma Morris 5123 and human melanoma A375. We have focused on β-actin level, its subcellular distribution and actin cytoskeleton architecture. Visible changes in the cell shape of selected variants of colon adenocarcinoma and hepatoma were accompanied by β-actin filaments reorganization and condenstation. These findings were followed by the determination of statistically significant increase in the level of β-actin expression and altered actin polymerization state. However, we did not observe these effects in the case of melanoma cells. Summarizing – our data have shown that the selection on Matrigel-coated filters is a good tool for comparison of cancer cells 'behaviour' during migration process.

O8.8

Androgen receptor and its role in female carcinogenesis

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No longer it can be said that androgens make boys as boys, and estrogens, girls as girls. It is now known that high level of estrogens in male brain in early life is necessary for male sexual imprinting, and that estrogens have a fundamental role in spermatogenesis and maintenance of bone mineralization in men. The reverse also applies. Androgens have important and varied physiological actions in women. Generally androgens act directly *via* the androgen receptor (AR) in tissues, and also have a vital role as the precursor steroids for estrogen biosynthesis in the ovaries and extragonadal sites, including bone, brain, cardiovascular and adipose tissues. The data available from studies on androgens and AR presence and expression in breast and endometrial carcinoma have provided definitive evidence of AR participation in female neoplasms. However, characterization of this process is speculative at the moment. Molecular pathology investigations involve the *AR* gene, and several interconnected growth-regulatory pathways. Apart from the alteration in *AR* gene expression the CAG repeats in exon 1 of this gene seem to be of special interest. However, the data concerning CAG repeats as susceptibility *locus* and phenotypic modifiers are conflicting. Although androgens are not routinely used in the treatment of female cancers because of unacceptable side-effects, indirect clinical evidence suggests that the AR may be an important mediator of hormonal therapy in female tumors *in vivo*.

O8.9

Cystathionine, cysteine and glutathione levels in human glioma homogenates

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We have undertaken the investigations of the level of reduced (GSH) and oxidized (GSSG) glutathione, cysteine, cystine and cystathionine in homogenates obtained from human gliomas collected intraoperatively at the Department of Neurosurgery, Collegium Medicum, Jagiellonian University. We modified the HPLC method for detection and quantitation of thiol and disulfide-containing compounds (Dominick *et al.*, *J Chrom B* 761: 1-12, 2001) to determine the concentration of GSH, GSSG, cysteine, cystine and cystathionine in a single analysis. The level of cystathionine was found to be increased in high-grade gliomas (classified as WHO III/IV and IV) in comparison to those classified as WHO II and II/III (low-grade gliomas), while the level of cysteine was lower in high-grade gliomas. Based on the above results we may conclude that in high-grade gliomas, cystathionine β-synthase, responsible for cystathionine formation, is active. Cystathionine accumulates because high-grade gliomas lack the activity of γ-cystathionase, which converts cystathionine to cysteine. In consequence, the level of cysteine is decreased and it seems that this pathway of cysteine generation is of a low importance in high-grade gliomas. Malignant cells require GSH for proliferation. In high-grade gliomas, in comparison to low-grade tumors, the increased level of GSH was accompanied by the increased ratio of GSH/GSSG.

O8.10

Relationships between expression of β-catenin and progression of human prostate carcinoma

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In prostate carcinomas positive correlation between dedifferentiation of the tumor and decline in E-cadherin ex-

pression was postulated. E-cadherin forms with α-, β-, γ-catenins, cadherin-catenin complex that is involved in maintaining normal epithelial polarity, intracellular adhesion and regulation cellular differentiation and proliferation. The replacement of E-cadherin for N-cadherin might lead to its decreased control on β-catenin and to the increase of β-catenin nuclear accessibility. This affects *c-myc* expression regulated by a diverse catalog of mitogens and down-regulated during differentiation. To investigate whether expression of β-catenin correlates with the progression of prostate cancer we analyzed relationship between its expression and expression of cyclin D1, *c-myc*, cadherins and MMPs. We examined the expression of these proteins in 15 prostate cancer tissues, 20 prostatic hyperplasia tissues, 2 normal prostate tissues and three established (LNCaP, PC-3, Du145) and two primary (CA-K, BPH-K) prostate cancer cell line at mRNA and protein level. Cell proliferation was tested for all cell lines. The differences in migration was study using Boyden chamber. β-catenin was expressed in all studied samples. The expression of cyclin D1, c-myc and activity of MMPs was not found in 2 normal prostate tissue what correlated well with cell proliferation and migration in all samples. The abnormal expression of β-catenin and overexpression of MMPs was significantly associated with progress of carcinogenesis. We suggest that the high expression of β-catenin plays a principal function in progression of human prostate cancer by up-regulation the expression of cyclin D1, c-myc and MMPs which correlates with increased cell proliferation and high migration.

O8.11

Metastatic capacity stimulated by cancer cells' cultivation in non-adhesive conditions

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The ability to survive out of specific niche or without adhesion to the extracellular matrix is characteristic for metastatic tumor cells. These cells were found to develop special mechanisms that allow them to avoid anoikis, survive in circulation and colonize distant organs. The aim of our study was to determine how the prolonged cultivation in non-adhesive conditions influences cancer cells' properties especially expression of specific genes and *in vivo* characteristics. Mouse sarcoma L1 cell line was used throughout our study. Cells were cultivated on plates coated with polyHema during 14 days and in consequence several clones were selected for further analysis. The selected clones had higher capacity of lung colonies formation in BALB/c mice than the control cells (>17 *vs* 3 colonies, $P < 0.05$). Several of them displayed expression of TrkB protein that was shown to be involved in anoikis' suppression and metastasis' induction. The expression analysis performed with cDNA array and RT-PCR have revealed higher expression of genes encoding proteins involved in regulation of cell invasiveness (Tmsb4x, Emmprin, Vim), antiapoptotic proteins (mIAP3, Dad1) or stress response proteins (Hsp84, Gstm, Gstp). The prolonged cancer cells' cultivation in non-adhesive conditions stimulated the selection of cells that had higher metastatic capacity and expressed specific factors involved in anoikis' resistance and invasiveness.

O8.12

Cellular memory: molecular mechanisms and practical examples

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The system of cellular memory is based on epigenetic mechanisms enabling establishment and mitotic transmission of the characteristic local and/or global profile of the accessibility of DNA in chromatin. The key elements of this system: DNA methylation, post translational modifications of histones and ATP-dependent remodeling of nucleosomes are all subject to changes that can either directly induce cancers or enhance the probability of their occurrence. Examples will be discussed that illustrate the possible links of the defects in mechanisms of chromatin modulation with cancers.

O8.13

Epigenomics – a novel approach in studies of tumor biology

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The combination of DNA microarray technology and bioinformatics delivers an experimental tool that allows surveying expression of genetic information on a genome-wide scale at the level of single genes — for the field termed functional genomics. However, the expression of genetic information is regulated by a number of factors that cannot be directly targeted by standard gene expression profiling. The genetic material of eukaryotic cells is packed into chromatin which provides the compaction and organization of DNA for replication, repair and recombination processes, and is the major epigenetic factor determining the expression of genetic information. Genomic DNA can be methylated and this modification

modulates interactions with proteins which change the functional status of genes. Both chromatin structure and transcriptional activity are affected by the processes of replication, recombination and repair. Modified DNA microarray technology could be applied to genome-wide study of epigenetic factors and processes that modulate the expression of genetic information. This new approach termed epigenomics becomes an emerging tool in studies of molecular biology of cancer.

O8.14

MicroRNA – biogenesis, function and dysfunction in cancer

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MicroRNAs (miRNAs) are a class of small endogenous RNAs known to regulate post-transcriptionally gene expression in multicellular organisms in a sequence-specific manner. These tiny RNAs are processed from larger precursors, first in cell nucleus by ribonuclease Drosha and then in cytoplasm by ribonuclease Dicer into small duplexes. Only the active miRNA strand of these duplexes associates with RNA-induced silencing complex (miRISC) and targets mRNAs for translational repression or cleavage. Hundreds of miRNAs are thought to regulate thousands of mRNAs which are involved in various biological processes including development, differentiation, growth and metabolism. In this presentation the miRNA pathway will be reviewed with emphasis on its links with human cancer, and potential applications of miRNAs in diagnostics and therapy of cancer will be discussed.

O8.15

Cancer gene expression profile – basic and clinical implications

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Gene expression profiling enables both looking for multigene classsifiers as potent diagnostic tools in oncology and discovering new subclasses of cancer histotypes, which may fit better to cancer clinics, therapy and outcome. In this way microarray-based analysis may outperform traditional histopathology, paralelly supplying information about the mechanisms of neoplastic transformation and cancer progression. Thyroid cancer is an excellent model for DNA microarray-based analysis of cancer gene expression profile as the majority of tumors are well differentiated ones and originate either from various epithelial lineages (follicular versus C cells) or diverge from a common cell (papillary versus follicular cancer). This creates a field for comparisons, widened by an easy accessibility of benign lesions. Thus, some general rules will be illustrated by examples from our own research related to thyroid cancer. Until now we have analyzed over 120 thyroid tissue samples by high density oligonucleotide DNA microarrays (U133 GeneChip, Affymetrix), some 80 tumors and 40 benign lesions. These results will be presented in comparison to other datasets available in the public domain to address the questions related mainly to papillary thyroid ca (the most frequent form of thyroid cancer). Sources of variance in gene expression profile, its relation to the most important clinical and pathological prognostic features of cancer disease as well as gene expression signature for diagnostic purposes will be addressed. Simultaneously, data on gene expression profile of other cancer types (among them laryngeal, lung and pancreatic cancrer) will also be discussed. In the gene expression profile of papillary thyroid cancer, obtained by validation of published thyroid gene lists on our dataset, genes controlling extracellular matrix and cell-matrix interactions prevail, followed by the signal transduction genes or genes participating in the remodelling of cytoskeleton related to the aberrant signal transduction. The comparison indicates a good reproducibility of gene signatures obtained in various approaches based on microarrays or related techniques and creates a basis for specification of similarities between various thyroid cancer histotypes. By means of a Support Vector Machine-based method developed at the Silesian University of Technology (Recurrent Feature Replacement) multigene classifiers were selected to support thyroid cancer diagnosis. The analysis was widened by bootstrap-based techniques which enabled to specify confidence intervals for molecular diagnosis of thyroid cancer as well as to rank genes for their diagnostic value and to rank samples for the level of difficulties at their molecular evaluation. The validation step by multiple real-time PCR analyses will be also discussed and illustrated, both in the context of diagnostic decisions as well in the context of analysis of tumor biology. The latter will be shown on the example of genes related to hypoxia in thyroid cancer.

O8.16

Expression of cytokeratin 19 in thyroid benign and malignant neoplasms

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The cytokeratins are family of intermediate filament proteins that expression varies with epithelial cell type, extent of differentiation and development of the tissue. During

the transformation of normal cells into malignant cells, the cytokeratin patterns are usually maintained, and this property has enabled cytokeratins to be applied as tumor markers. One of the most frequent difficulties in thyroid pathology is differentiating adenomas from carcinomas, especially follicular carcinoma. It has been suggested that cytokeratin 19 might be useful in the classification of thyroid lesions. The aim of this study was analysis of cytokeratin 19 expression in benign and malignant thyroid neoplasms. The studies were performed on 36 specimens of follicular adenomas, 8 – follicular carcinomas, 61 – papillary carcinomas, 4 – anaplastic carcinomas and 4 – medullary carcinomas. Thirty seven cases of non-neoplastic specimens were used as a control. The presence of cytokeratin 19 in homogenate, post-nuclear or cytosolic fractions was analyzed by Western blot and enzyme-linked immunosorbent assay (ELISA). Cytokeratin 19 expression was found in 11% of non-neoplastic specimens, 22% – follicular adenomas, 66% – papillary carcinomas and 25% in case of follicular, anaplastic and medullary carcinomas. ELISA method used for semi-quantitative analysis of cytokeratin 19 expression confirmed much higher level of this protein in the case of papillary carcinomas in comparison with non-neoplastic specimens , adenomas and the others carcinomas. Our results suggest that strong cytokeratin 19 expression is a characteristic feature of papillary carcinoma and can be helpful in the differential diagnosis from the others thyroid benign and malignant neoplasms.

O8.17

Drg1 expression is down-regulated in colon cancer and correlates with tumor lymph node metastasis

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Colorectal carcinoma is the second most deadly cancer in Poland and the prognosis of colon cancer is directly correlated with the extent of tumour invasion and metastases. Molecules involved in cancer metastasis might be markers for early detection of metastasis. Many studies showed that differentiation-related gene 1 (*Drg1*) also known as N-myc down regulated gene 1 (*NDRG1*) is involved in cellular growth, differentiation and tumorigenesis, but the function of this gene remains unclear. It was reported that *Drg1* was down regulated in tumor cell lines, breast, prostate, renal and colon cancer. However, some studies show that *Drg1* mRNA and protein levels gradually increase during colorectal cancerogenesis. This study was performed to evaluate *Drg1* expression in colon cancer and neighbouring normal mucosa. Expression of *Drg1* gene was examined by multiplex PCR with *GAPD* gene as a housekeeping control gene. Obtained data were analyzed by t-paired test and Pearson's chi-square test. Performed examination showed that *Drg1* was expressed in all analyzed tissues, and its mRNA level appear to be decreased in cancer tissues comparing to adjacent mucosa ($P < 0.05$). However, some cases had elevated expression of *Drg1* or unchanged. There were no correlations between *Drg1* expression and clinical factors such as gender, age, distant metastasis, tumor grade, other genes expression such as *TSP-1*, *ssp-1*, and amplification of *c-myc* and *c-erb-B2*. Interestingly, patients with tumor lymph node metastasis had often lower expression *Drg1* ($P = 0.036$). Moreover, there was statistically important correlation between TNM stage and down-regulation of *Drg1* gene ($P = 0.009$).

O8.18

Genomic instability in the *RAD51* and *BRCA2* regions in breast cancer

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Breast cancer is the most prevalent cancer type in women. A wide variety of cellular pathways alterations may confer and increase the risk for breast cancer. Among them DNA damage response seems to be of great importance. *BRCA2* tumor suppressor gene is essential to maintain genome integrity. This is mediated *via* regulation of *RAD51* during homologous recombination. In this study loss of heterozygosity (LOH) and microsatellite instability (MSI) in the *RAD51* and *BRCA2* regions and their association with breast cancer were evaluated for polymorphic markers D15S118, D15S214, D15S1006 and D13S260, D13S290, respectively. The LOH and MSI analyses at the *RAD51* and *BRCA2 loci* were performed using DNA isolated from 36 primary breast cancer and matched blood samples. Fluorescent label PCR products were analyzed in ABI PRISM 37 DNA sequencer. The fluorescent signals from different size alleles were recorded and analyzed using GeneScan version 3.1.2 and Genotyper version 2.5 software. Genomic deletion detected by allelic loss varied according to the *locus* tested, and ranged from 29% to 46% of informative cases for *RAD51* region and from 38% to 43% of informative cases for *BRCA2* region. Microsatellite instability was noticed for microsatellite markers D15S118, D15S214, D15S1006 in 23%, 24%, 14% and for microsatellite markers D13S260, D13S290 in 14%, 19% of cases that were informative. 14% of breast cancer cases displayed LOH and 11% of cases displayed LOH and/or MSI for at least one microsatellite marker at *RAD51 locus* exclusively. On the other hand 42% and 61% of cases

manifested LOH and LOH and/or MSI, respectively for at least one microsatellite marker simultaneously in *RAD51* and *BRCA2* regions. LOH in the *RAD51* region similarly as in the *BRCA2* region appeared to correlate with steroid receptors status. Obtained results indicate that alteration in *RAD51* region may contribute both to the disturbances of DNA repair and *BRCA2* penetrance and thus enhance the risk of breast cancer development.

O8.19

Flow cytometry analysis of integrin expressions in uveal and cutaneous melanoma cells

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Changes in integryn expression have been reported during the malignant progression of many tumors and much evidence exists implicating their involvement in melanoma metastasis. Cell adhesion molecules belonging to the integryn family have been implicated in melanoma progression. In our study human cutaneous melanoma cells representing radial growth phase (RGP) and uveal primary melanoma cells were used. The purpose of our research was to establish the expression of the α_2, α_3, α_4, α_5 and α_v integrins on the surface of uveal (ESTDAB 127, ESTDAB 128) and cutaneous (ESTDAB 014, ESTDAB 037) melanoma cells. These cell lines were provided thanks to participation in ESTDAB-NAS 5FP of EC "Quality of life". Levels of integrins expression were assessed by flow cytometry with the use of appropriate FITC-labeled secondary antibodies. Our results indicated that all melanoma cells showed the expression of α_2, α_3, α_4, α_5 and α_v integins, but with completely different intensity. The highest expression of all tested integrins was on the ESTDAB 037 cell. This was correlated with the strongest adhesion of these cells to all investigated ECM proteins. The uveal melanoma cells, not showing adhesion to laminin, collagen and fibronectin, possess also very low expression of all investigated integrins. Both cutaneous and uveal melanoma cells showed very low expression of α_v integryn, which is consistent with the previous data, because in radial growth phase $\alpha_v\beta_3$ integrin expression was absent or low. The up-regulation of the $\alpha_v\beta_3$ integrin in VGP melanoma correlates with a tumorogenic phenotype.

O8.20

DNA hypermethylation of DNA repair genes in neurological tumors

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DNA methylation is the main and best characterized epigenetic event. In human genome methylation occurs on cytosines of CpG dinucleotides that occur in promoter region of many genes and leads to their transcriptional silencing. During tumorigenesis two main epigenetic phenomena are observed: global hypomethylation of DNA with activation of silenced elements and hypermethylation of suppressor genes, which inactivates their expression. Silencing of specific genes influence every aspect of malignant transformation: promotion of cell proliferation, cell cycle perturbation, apoptosis and response for medical treatment. In this study we focus on DNA hypermethylation of promoters of three genes involved in DNA repair system: *MGMT*, that encode enzyme that directly remove covalent adducts from guanine and *MLH1*, *MSH2* encoding two important elements of mismatch repair system. MGMT and MMR play a significant role in medical treatment of neurological tumors with alkylating agents. Hypermethylation of MGMT is favourable prognostic factor and defects in MMR system decrease response for teraphy. In our study we intend to assess whether hypermethylation of *hMLH* or *MSH2* decrease response of *glioblastoma* patients with hypermethylated *MGMT* for treatment with temozolomide. So far we determined frequency of promoter hypermethylation of *MGMT*, *MLH1* and *MSH2* in DNA samples derived from stereotatic biopsy or craniotomy from 22 patients, using nested MSP (methylation specific PCR). We observed promoter hypermethylation at *MGMT, MLH1* and *MSH2* in 10, 5 and 8 patients, respectively.

O8.21

Estrogen and tamoxifen epigenetically increase of *CXCR4* and *CXCL12* expression in estrogen positive human endometrial Ishikawa cancer cells

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Endometrial cancer is the seventh worldwide common malignant disease that morbidity frequency differs among world regions. In developing countries the rare endometrial cancer incidence is associated with lower exposure for common risk factors Unopposed estrogen exposure, hyperestrogenic risk factors and a history of breast cancer with long term treatment of tamoxifen (Tam) increase risk of endometrial cancer development. CXCL12 chemokine binds to CXCR4 receptor that belongs to the G-protein-coupled-receptors family. This activates a variety of

intracellular signal transduction pathways and effector molecules, which regulate cell survival, adhesion, migration, proliferation and angiogenesis. Using real-time quantitative PCR, Western blotting and flow cytometry analysis, we investigated the effect of 17β-estradiol (E2) and Tam on *CXCL12* and *CXCR4* expression in oestrogen receptor positive (ER^+) and negative (ER^-) Ishikawa endometrial cancer cell lines. We observed that E2 and Tam increase in transcript and protein biosynthesis of *CXCR4* and *CXCL12* in ER^+ Ishikawa endometrial cancer cells. Employing bisulfite sequencing we found that up-regulation of expression was associated with demethylation of promoters CpG island of *CXCR4* and *CXCL12*. However, E2 and Tam did not cause increase of *CXCR4* and *CXCL12* expression in ER^- Ishikawa endometrial cancer cells. We also found that E2 and Tam did not result in demethylation of promoters CpG island in ER^- Ishikawa endometrial cancer cells. Our results suggest that E2 and Tam through their ability for gene-transcription regulation, change the cellular milieu that maintains the hypermethylated stage of *CXCR4* and *CXCL12* genes. E2 and Tam induced *CXCL12* and *CXCR4* expression may contribute to increase of survival, proliferation and metastases of endometrial cancer.

O8.22

Cytochrome P450 genes expression profile in cervical cancer

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Cytochromes P450 (CYP) are a family of genes encoding proteins catalyzing biotransformations of many endobiotics: cholesterol, steroides and lipides (arachidonic acid, prostacyclines, tromboxanes) and xenobiotics such as drugs and environmental pollutions. Humans possess of 57 sequenced *CYP* genes and 58 pseudogenes (www: drnelson.com). *CYP 1* to *3* code first of all enzymes participating in metabolism of exogenic substances (80% of accessible drugs on market), but also endogenic steroides and all other only the endogenic substances. CYP are specific for substrates and their distribution in organism is also tissue specific. The aim of this work was estimated the expression profile of cytochrome P450 genes in cervical cancer using oligonucleotide microarray (Affymetrix) method. On base of information obtained from literature were qualified the panel of 90 genes and pseudogenes of cytochrome P450 family, which were used to identify their transcripts on examined microarray. Obtained results were normalized and then clusterized by euclideans distance of programme Cluster 3.0. Genes differentiating control cells from cells of cervical cancer were obtained basing on analysis of linear regression function. In the first stage of hierarchical cluster was confirmed the homogenity of control samples. Next stages were grouping the cervical cancer in support of *CYP* genes expression profile. Comparison of *CYP* genes expression in cervical cancer with controls allows to separate the transcripts, which differentiate these two groups. The *CYP4B1* and *CYP24A1* are considerable downregulated in cervical cancer.

O8.23

Overexpression of heme oxygenase in murine melanoma: increased proliferation and viability of tumor cells, decreased survival of mice

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Heme oxygenase-1 (HO-1), a cytoprotective, proangiogenic and anti-inflammatory enzyme, can be induced in tumors in response to therapies providing benefits to cancer cells. We investigated effects of HO-1 overexpression on murine melanoma B16(F10). Cells stably transfected with HO-1 cDNA (B16-HO-1) showed increased proliferation and were more resistant to oxidative stress generated by H_2O_2 (25–800 (μM, 4 h) than wild type line (B16-WT). Additionally, they demonstrated stronger angiogenic potential as determined by induction of endothelial cell divisions and capillary formations. HO-1 overexpression in tumors shortened significantly ($P = 0.017$) survival of mice after subcutaneous injection of cancer cells (38 and 22 days for B16-WT and B16-HO-1, respectively). It resulted also in development of more packed tumors, with higher number of melanoma cells, reduced inflammatory edemas and decreased leukocyte infiltration. Mice injected with B16-HO-1 had lower levels of tumor necrosis factor, and higher concentrations of its soluble receptor TNF-RI in serum, whereas tumors overexpressing HO-1 displayed augmented vascularization and stronger production of vascular endothelial growth factor. Finally, B16-HO-1 cells injected intravenously formed more and bigger metastases in the lungs. Microarray analysis of B16-WT and B16-HO-1 transcripromes indicated that important mediators resposible for the

observed effects can be among others hyaluronidase-1, thymosin-β4, epidermal growth factor, fibroblast growth factor receptor-1, metallothionein-1X, and glutathione S-transferase-A1, which were upregulated in the HO-1 overexpressing cells. Thus, overexpression of HO-1 increased viability, proliferation, and angiogenic potential of B16(F10) cells, augmented metastasis, and decreased survival of tumor-bearing mice. This suggests that induction of HO-1 may be detrimental in therapy of melanoma.

O8.24

Marker genes commonly used to detect circulating cancer cells are expressed in activated lymphocytes

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Background: High sensitivity, RT-PCR-based tests used for circulating tumour cell (CTC) detection in cancer patients often show low specificity, partly due to the expression of the molecular markers in non-cancerous cells. Therefore validating molecular markers against an adequate control is a critical issue. Malignancies are often associated with inflammation, and inflammation has been shown to influence mRNA expression in lymphatic cells. Thus, we addressed the question whether so-called cancer-specific molecular markers are also expressed in stimulated peripheral blood mononuclear cells (PBMNC). Materials and Methods: Normal, unstimulated and mitogen-stimulated PBMNC were examined by RT-PCR for the expression of marker genes commonly used to detect CTC, squamous-cell carcinoma antigen (SCCA), epidermal growth factor receptor (EGFR), mammaglobin (hMAM), small breast epithelial mucin (SBEM) and carbonic anhydrase 9 (CA9). Results: Normal PBMNC were RT-PCR negative for SCCA, EGFR, hMAM, SBEM and CA9, while mitogen-stimulated PBMNC, in both early and late phases of stimulation, were positive for SCCA, EGFR, hMAM, and SBEM. Conclusions: Expression of some target mRNAs commonly used to detect CTC is inducible in lymphatic cells. Thus, molecular markers for CTC detection should be validated not against normal peripheral blood, but against activated lymphoid cells, such as *in vitro* stimulated PBMNC.

O8.25

New targets and strategies in anticancer treatment

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The development of anticancer chemotherapy reflects the progress in our basic knowledge in cancer research and especially in better understanding of the role of immediate and systemic environment in the course of the disease, especially in its progressive stage, when in addition to local aggressive growth the metastases are present. The initial program of chemotherapy, named "empirical program", during which hundreds thousands of new chemicals and plant extracts have been examined for their potential antitumor effects in various screening experimental animal tumor models. This program was followed by "disease-oriented program", which aim was to select new agents with antitumor tissue or organ specific specificity. The *in vitro* propagated cancer cell lines originated from different mostly human tumors were widely applied. Out of enormous number of agents examined, more than 40 antitumor drugs are being widely used in the treatment of cancer patients with different results. However, the main limitation of anticancer chemotherapy is lack of antitumor specificity, induction of drug resistance and undesired general toxicity. The actual program, named rational or rationalized one, can be defined as "research for molecular target assessment" is reflecting the achievements in the present understanding of tumor biology thanks to the application of modern molecular techniques to these studies. Actually, the modern trends are to search for specific agents directed towards or against newly defined molecular targets – molecules known as important for the chain of events responsible for tumor progression and metastatic potential. In addition to cancer cells as the target for therapy, the cellular and humoral components of cancer immediate environment are being considered as the potential targets, especially immune and endothelial cells, as well as other stromal components. This approach resulted in various attempts to apply immunotherapy and antiangiogenesis treatment. The target molecules of special interest for searching of the new antitumor agents with expected specific activity, important for inhibition of tumor growth and progression are these involved in: (i) expression of oncogenes, tumor suppression genes, (ii) inter- and intracellular signal transmission, (iii) control of cell cycle, (iv) regulation of apoptosis, (v) telomerase activity, (vi) growth factors and their receptors expression and function, (vii) adhesion molecules, their excretion and function, (viii) proteolitic enzymes, their production and function, ECM's components, (ix) neoangiogenesis. Out of these new, called target-oriented, or tailor-made "intelligent drugs" some appeared to be not only specific but efficient in their antitumor effectiveness and will be briefly described during the lecture.

O8.26

Perspectives of tumor immunotherapy

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Different approaches used in tumor therapy include application of:
- selected cytokines demonstrating antitumor activity,
- monoclonal antibodies directed against tumor antigens,
- cytotoxic T lymphocytes,
- cancer vaccines.

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

O8.27

Dendritic cell-based vaccination against tumor antigens for melanoma and lymphoma patients

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Twenty two patients with stage III melanoma with negative prognostic factors after therapeutic lymphadenectomy were vaccinated with dendritic cells (DC) pulsed with HLA-A2-binding peptides derived from tyrosinase, Melan-A/MART-1 and gp-100 or/and HLA-A1-binding peptides derived from MAGE-1 and MAGE-3. Five to 17 cycles (median: 11) of intradermal and subcutaneous injections were performed. Cutaneous delayed-type hypersensitivity (DTH) to melanoma peptides was induced in 12/22 patients. Peptide-specific interferon-γ producing $CD8^+$ cells were found in 13/19 patients. Patients were followed-up for 8 to 44 months (median: 27 months). Seven patients remained disease free, 7 achieved CR or PR after surgery and/or Chth of in-transit or/and distant metastases, 1 patient has SD after Rth, 4 are alive with metastatic disease, and 3 died due to melanoma progression. Seven patients with indolent B cell lymphomas were vaccinated with irradiated DC-lymphoma hybrids and DCs pulsed with tumor lysate. Autologous and/or allogeneic DC were electrofused with autologous lymphoma cells. Four patients had not achieved CR after standard treatment prior to vaccination, and the vaccination had to be discontinued because of disease progression (after 2, 2, 6, and 5 cycles). At present, three patients (MCL-2, FL-1) vaccinated during remission following prior standard chemotherapy are symptomless with persistent bone marrow (BM) involvement, disease free with residual BM involvement, or disease free (14, 16, and 8 months after starting the immunization, respectively, and after 8, 9, and 7 cycles of vaccination performed so far, respectively). DTH to DC-lymphoma hybrids was demonstrated in three patients, and interferon-γ producing $CD8^+$ cells specific for autologous lymphoma in one patient. No adverse reactions to vaccinations were observed.

O8.28

Catalytic nucleic acids in brain tumor therapy

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Primary brain tumors are among the most lethal of all cancers, largely as a result of their lack of responsiveness to current therapy. Patients with high-grade gliomas have adverse prognoses following standard treatment. Glioblastoma multiforme (GBM) accounts for approximately 12–15% of intracranial neoplasms. The GBM remains refractory to therapy because of tumor heterogeneity, local invasion, and nonuniform vascular permeability to drugs. Patients with GBM have the median survival of approximately 8–10 months, and for those cases where tumor recurs, the average time of tumor progression after therapy is only 8 weeks. A combination of different treatment modes as surgery and chemo- or/and radiotherapy extend survival only for a short time, if any. Recent results suggest the tenascin-C (TN-C) expression may be correlated with the grade of malignancy of human brain tumors and its presence may play a role in shortening of the patients survival. In the tumor tissue, TN-C occurs mostly in the extracellular matrix of the fibrotic stoma of highly malignant neoplasms including carcinomas of the colon and breast, fibrosarcomas, lung, melanomas, squamous cell carcinomas, bladder tumor, prostatic adeno-

carcinoma and along the tumor border. Significantly higher levels of TN-C in homogenates of GBM than in normal brain have been observed. The discovery that TN-C presents a dominant epitope in glioblastoma prompted us to investigate the potential of RNA interference (RNAi) to block the TN-C expression and its effect on the growth of human brain malignancies. The high level of expression of TN-C in human gliomas and astrocytomas correlates with a higher tumor grade and angiogenesis. RNA interference (RNAi) is a biological process that controls gene silencing in all living cells and offers a new tool with a great potential to inhibit a tumor development. This is a well recognized mechanism in which a short sequence of double-stranded RNA (dsRNA) specifically down-regulates the eukaryotic expression of an associated gene. Introduction of dsRNA into the cell triggers the RNAi response resulting in the specific suppression of the target gene expression. The mediators of the sequence-specific mRNA degradation process are 21–25 nucleotide interfering RNAs (siRNAs) generated from long dsRNAs by the DICER ribonuclease cleavage. These siRNAs are then incorporated into RNA-induced silencing complex (RISC), which degrades homologous mRNA and inhibits targeting of a gene translation into protein. It was selected 11 GMB patients with poor prognosis for an interference RNA treatment, which followed a brain resection. ATN-RNA, a double stranded RNA with nucleotide sequence homologous to tenascin-C mRNA, was administered directly into the 2–5 sites located in the area of neoplastic brain infiltration which can not be removed surgically. For the first time RNA interference technology was applied, to suppress human brain tumors (glioblastoma multiforme, astrocytoma) through inhibition of the synthesis of tenascin-C [1].

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O8.29

Normalization of tumor blood vessels: role in cancer therapy

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Abnormal, slowed-down blood flow and permeability of tumor blood vessels, together with increased intratumoral pressure, make drug access to neoplastic cells virtually restricted. Additionally, in the proximity of such vessels, weakly oxygenated tumor regions develop. As a result, cancer cells in these hypoxic regions become increasingly refractory to ionizing radiation. Certain antiangiogenic drugs can transiently "normalize" tumor blood vessels. Tumor vasculature become less permeable and tortuous, with improved blood flow and resulting better oxygenation of cancer cells present in the vicinity of such vessels. Existing data show that the so-called "normalization window" i.e. time period during which tumor blood vessels resemble normal vessels may be an important factor of tumor therapeutic strategies. During this period of time inflow of drugs into tumor mass improves; better oxygenation culminates in increased sensitivity of cells to ionizing radiation. When properly combined with chemo- and/or radiotherapy antiangiogenic therapy results in highly synergistic effect and markedly improves the therapeutic outcome.

O8.30

The inhibition of metabolic pathway as a new approach to cancer chemotherapy

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So far, systemic therapy with cytotoxic drugs is one of the most effective treatments of cancer. However, side effects of cytostatics resulting from toxicity to normal tissue are a main drawback of chemotherapy. Other problems are rapid clearance of drugs from the circulation and poor distribution to the target tumors. The research on the new chemotherapeutic drugs for cancer is going to the completely new phase. This new approach is based on the knowledge about the molecular mechanisms of cancerogenesis, mainly on the knowledge about changes in metabolic pathways caused by deregulation of cell homeostasis. Many different inhibitors of metabolic pathways are now under the study. Some of them are already in the clinical trials. The classical example of this new generation drugs is Trastuzumab and Gleevec. Trastuzumab is a monoclonal antibody against the EGFR2 receptor (HER2/NEU). Overexpression of HER2 leads to non-ligand permanent activation of HER2 receptor. This of course leads to permanent mitotic activation of cells. Trastuzumab decreases HER 2 concentration and stops the mitotic signal transduction. Gleevec is a low molecular weight molecule which inhibits the TK activity of PDGF receptor family. The mechanism of action of this compound is based on irreversible binding to the ATP place in the activity center of ATPase activity of PDGF receptor.

O8.31

Advanced Therapy Medicinal Products

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Advanced Therapy Medicinal Products (AThMP) are based on manufacturing processes focused on: (i) gene

therapy medicinal products (human and xenogeneic), (ii) somatic cell therapy medicinal products (human and xenogeneic), and recently recognized (iii) tissue engineering medicinal products. Number of AThMP are currently in clinical trials and are expected to be on the market shortly. It is expected that AThMP will significantly affect public health, quality of life of patients and will generate changes in medical practice. AThMP scientifically, legally and economically share a number of common features: (i) they are based on highly innovative manufacturing processes; (ii) scientific and regulatory competence for evaluation of these products is rare; (iii) they require monitoring of donor-recipient pathway, long term patients follow up, and detailed safety monitoring after licensing; (iv) they are developed by small and medium size innovative biotech companies, highly specialized divisions of "big pharma" hospitals or cell banks. Accordingly, they undergo very fast modifications and changes. Regulatory authorities (such as EMEA) aim to increase the safe availability of AthMP for patients by intensifying research and development, and approvals of clinical trials. Specific aims are: (i) providing the high level of health care; (ii) harmonizing marketing by legislation especially licensing and further monitoring after granting the license; (iii) fostering competitiveness of European biotech companies; (iv) providing overall legal security for evolution of science and thechnology.

O8.32

Differential scanning calorimetry of nuclei as a manner for study of anticancer drug effect on B-CLL leukemic cells

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B-cell chronic lymphocytic leukemia (B-CLL) represents a neoplastic disorder caused primarily by defective apoptosis, as opposed to increased cell proliferation. In this hetero-geneous disease, the mechanism responsible for the wide range of clinical responses are studied, however, not yet fully understood. Chemotherapeutic treatment of malignancies, including B-CLL, is based on the use of cytotoxic drugs acting on intracellular targets. It was demonstrated that differential scanning calorimetry (DSC) can be use for estimation of anti-cancer drug effect on nuclei from human cells. Our purpose was to study the potential to turn on *in vitro* programmed death by combination of purine analogs, i.e., cladribine (2-chloro-2-deoxyadenosine; 2-CdA; C) and fludarabine (9-β-D-arabinosyl-2-fluoroadenine phosphate; F) with alkylating agent – mafosfamide (CM; FM). In performed experiments we have used DSC as well as cytometric and immunoblott techniques to provide evidence of apoptosis induction of mononuclear cells isolated from peripheral blood of B-CLL patients incubated for 48 hrs without and with CM and FM combinations. The results obtained by calorimetric technique of leukemic cells revealed their different sensitivity to both drug combinations in comparison with untreated cells. In the melting profiles of nuclear preparations from B-CLL cells exposed to both drug combinations the decrease of endotherm at 95 ± 3°C was observed. These changes were accompanied by the decrease of viability of leukemic cell samples treated with CM and FM combinations compared with untreated cells. The exposure B-CLL cells to both purine analogs combination produced some changes in extent of subG1 fraction and the level of apoptotic cells. We have also noticed the changes in expression of proteins involved in apoptotic death realization. Our preliminary data indicate that DSC technique could be useful for monitoring or even selection of drug-treatment efficacy of patients with B-CLL.

O8.33

Cytochrome P450 izoenzymes involved in metabolism of antitumor 9-amino-1-nitroacridine derivatives, C-857, C-1748

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Cytochromes P450 belong to a family of heme proteins present in mammalian cells as well as in plants and prokaryotes. Polimorphism phenomena and influence of the enviromental factors are responsible for individual levels of P450 izoenzymes that are able to perform metabolic activation or detoxification of a new drug in human organism. Therefore, the identification of human liver enzymes responsible for metabolism of therapeutic agents allows to predict their pharmacokinetic behaviour and to plan the optimal therapeutic schedule. Compounds C-857 and C-1748 belong to a new set of antitumor 9-amino-1-nitroacridine derivatives developed in the laboratory headed by Professor Konopa. One of them, C-1748 which expressed lower toxicity in animals than other 1-nitroacridines was selected to I phase of clinical trials. In the present work the studies on the metabolic transformations of these agents in the presence of human and liver microsomes of indiwidual CYP overexpressions and with selected *E. coli* recombinant P450 izoenzymes have been described. We have searched for P450 isoenzymes

responsible for metabolism of C-857 and C-1748 as well as identificated of products formed after incubation with selected CYPs. Chemical structures of metabolites were proposed by comparison of their MS spectra with those obtained earlier in model enzymatic system. The obtained results demonstrated that recombinant CYP3A4 was one of the most involved in metabolic transformation of both compounds studied. However, C-1748 was metabolized by CYP1A2 and its metabolite structures differed from those of C-857.

O8.34

HIF-1 induction attenuates interleukin-8 synthesis in human endothelial cells

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Background: Hypoxia induces expression of numerous angiogenic mediators, the result of stabilization of hypoxia inducible factor-1α (HIF-1α). Activity of HIF-1 is further enhanced by p300 protein binding. Degradation of HIF-1α in normoxia is dependent on the hydroxylation of its proline residues by specific oxygen-, α-ketoglutarate- and iron-dependent prolyl hydroxylases (PHDs). Here we report an unexpected finding demonstrating that inhibition of PHDs and induction of HIF-1 attenuates the synthesis of interleukin 8 (IL-8). Methods and Results: HIF-1α was induced in human microvascular endothelial cells (HMEC-1) by hypoxia (1% oxygen) or by treatment with dimethyloxalylglycine (DMOG), an α-ketoglutarate analogue. Interestingly, hypoxia and DMOG concentration-dependently (250–1000 μM) attenuated IL-8 mRNA and protein synthesis, as demonstrated by real-time RT-PCR and ELISA. Reporter luciferase gene assay showed that DMOG significantly attenuated the activity of NF-κB, AP-1 and IL-8 promoter. On the other hand, chetomin, inhibitor of p300 binding, reversed the inhibitory effect of DMOG on IL-8 synthesis. Reversely, the mRNA expression, protein synthesis and promoter activity of vascular endothelial growth factor (VEGF) was potently enhanced when HIF-1 was activated. Addition of chetomin resulted in potent diminishment of DMOG-induced VEGF synthesis. Conclusions: Induction of HIF-1 by hypoxia or DMOG affects in the opposite way the expression of VEGF and IL-8, upregulating the former and downregulating the latter. IL-8 expression may be dependent on HIF-1-mediated attenuation of NF-κB and AP-1. Thus, complex regulation of proangiogenic genes expression occurs after induction of HIF-1, what may be of significance for the effect of anti-angiogenic therapies, including anti-cancer trials.

Supported by PBZ-KBN-107/P04/2004

O8.35

Cytotoxicity, DNA strand breakage and topoisomerase inhibition of novel Pt-berenil compounds

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Modification of platinum-based compounds is believed to be a promising approach for the development of non-cross-resistant analogs of cisplatin. We have recently synthesized of several $[Pt_2L_4(berenil)_2]Cl_4$ complexes where L is piperazine (1), 4-picoline (2), 3-picoline (3) or isopropylamine (4). Berenil preferentially recognizes and binds to AT-rich DNA sequences and it is also strong catalytic inhibitor of DNA topoisomerase II (topo II). We hypothesized that the Pt(II) complexes of berenil would lock this topo II inhibitor into a conformation that would more strongly inhibit the enzyme. Additionally, it was hypothesized that the complexes would cross-link or form a coordinate covalent adduct with either DNA, topo II protein or the topo II-DNA enzyme intermediate complex and stabilize the cleavable complex. The results of our study showed that Pt-berenil compounds had cell growth and topo II inhibitory activity much more than that of the uncomplexed berenil and cis-platin from which they were derived. These results, as well as the strand breakage data, indicate that in contrast to cisplatin, 1–4 induce the formation of topo II-DNA complexes. Because Pt-berenil compounds contain the labile dichloro structure found in cisplatin responsible for producing intrastrand crosslinks with DNA these agents were compared with cisplatin in a DNA cross-linking assay. The DNA binding properties associated with this new class of antitumor agents suggest that they may display an activity profile different from that of cisplatin and related analogues.

O8.36

Inhibition of human cytochrome CYP1 enzymes by *trans*-piceatannol

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CYP1 family is comprised of CYP1A1, CYP1A2 and

CYP1B1, which are involved in bioactivation of numerous procarcinogens including polycyclic aromatic hydrocarbons, heterocyclic amines and 17β-estradiol (E2) to mutagenic and carcinogenic intermediates. Because of the postulated significant role of CYP1B1 in carcinogenicity of E2, CYP1B1 is regarded as a target enzyme for blocking tumor initiation. Selective inhibition of CYP1B1 may prevent E2-related tumor formation, on the other hand, the use of CYP1B1 inhibitors might help to overcome anticancer drug resistance. Piceatannol (*trans*-3,4,3′,5′-tetrahydroxystilbene) is a naturally occurring phytoalexin present in sugar cane, berries, peanuts, grapes and red wines. It was shown to be a product of resveratrol hydroxylation catalyzed by CYP1A2 and CYP1B1. This resveratrol analogue exerts many beneficial biological activities, acting as an antagonist of the aryl-hydrocarbon receptor, a potent tyrosine kinase inhibitor, a potent and selective inhibitor of COX-2, anti-oxidant, anti-cancer and cardioprotective agent. In the present study, the effect of piceatannol on human recombinant CYP1A1 and CYP1B1 activities was investigated and compared to the inhibitory effect of resveratrol and its methyl ethers. To assess CYP1 enzyme activities 7-ethoxyresorufin O-dethylase (EROD) activity was measured. Kinetics and mechanism of enzyme inhibition were determined by means of Lineweaver-Burk method. Piceatannol appeared to be a very potent competitive inhibitor of CYP1A1 and CYP1B1 with K_i value of 3.01 ± 0.62 μM and 0.57 ± 0.27 μM, respectively. Compared to the results of our previous studies, piceatannol is more effective inhibitor of CYP1B1 than methylated resveratrol analogues and significantly more potent inhibitor of CYP1A1 than the parent compound. This observation might be important with regard to dietary approach recommendation and potential application CYP1B1 inhibitors in cancer chemotherapy.

Posters

P8.37

Effect of *Lamiaceae* extracts on caspase-3 activity in Jurkat cells

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Plant polyphenols, due to their structural diversity, exert disparate effects on living cells, some of them are inducers, and others inhibitors of apoptosis. The aim of this study was the evaluation of selected herb extracts belonging to *Lamiaceae* family with respect to their potential proapoptotic effect on human leukemia cells. Caspase-3 was used as a marker indicating the induction of apoptotic pathway. Aqueous polyphenolic extracts were prepared from: *Thymus serpyllum* (A), *Thymus vulgaris* (B), *Majorana hortensis* (C), and *Mentha piperita* (D). Jurkat cells were cultured with 10–500 μg/ml concentrations of these extracts for 20 and 35 h, and tested with caspase-3 activity colorimetric assay. 20 and 35 h exposition of Jurkat cells on extracts B, C and D resulted in the induction of caspase-3 activity proportionately to their concentration. The highest activity increase (around 10-fold) was observed for 500 μg/ml of extracts B and D as compared with 10 μg/ml. The exception was extract A which caused the increase in caspase-3 activity at 50 μg/ml concentration in comparison with 10 μg/ml, whereas higher concentrations did not affect the activity. Therefore, extract A seems to show disparate properties as a potential proapoptotic factor in comparison with 3 other herbs. Further studies aimed at identification of purified polyphenols from A-D herbs, which would exert antiapoptotic properties, are underway.

P8.38

Novel amidine analogue of melphalan as a specific multifunctional inhibitor of growth and metabolism of human breast cancer cells

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A novel amidine analogue of melphalan (AB4) was compared to its parent drug, melphalan in respect to cytotoxicity, DNA and collagen biosynthesis in MDA-MB-231 and MCF-7 human breast cancer cells. It was found that AB4 was more active inhibitor of DNA and collagen synthesis as well more cytotoxic agent than melphalan. The topoisomerase I/II inhibition assay indicated that AB4 is a potent catalytic inhibitor of topoisomerase II. Data from the ethidium displacement assay showed that AB4 intercalated into the minor-groove at AT sequences of DNA. The greater potency of AB4 to suppress collagen synthesis was found to be accompanied by a stronger inhibition of prolidase activity and expression compared to melphalan. The phenomenon was related to the inhibition of β_1-integrin and IGF-I receptor mediated signaling caused by AB4. The expression of β_1-integrin receptor, as well as Sos-1 and phosphorylated MAPK, ERK_1 and ERK_2 but not FAK, Shc, and Grb-2 was significantly decreased in cells incubated for 24 h with 20 μM AB4 compared to the control, not treated cells, whereas in the same conditions melphalan did not evoke any changes in expression of all these signaling proteins, as shown by Western immunoblot analysis. These results indicate AB4 represent

multifunctional inhibitor of breast cancer cells growth and metabolism. Although the mechanism of antitumour action of AB4 is not yet fully elucidated, it may be due to its ability to inhibit the binding of some transcription factors to their consensus sequences in DNA, thereby preventing transcription.

P8.39

The effect of oxygen regulated protein 150 expression on the apoptosis in HeLa cells

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Correct protein folding is an important factor, for the translocation of newly synthesised proteins to specific subcellular compartments, extracellular matrix or to biological fluids. This process is regulated by a group of specific proteins, referred to as chaperones. Many stress conditions, such as oxygen or glucose deprivation, slow down the folding process and cause accumulation of unfolded/misfolded proteins in the cell. Molecular chaperones are induced in these conditions; with some named as oxygen-regulated proteins (ORPs). These bind to unfolded/misfolded proteins to facilitate correct assembly. The biosynthesis of ORPs is induced by deprivation of glucose or oxygen. The expression of ORP 150 is regulated by the concentration of glucose in the culture medium, being induced by a shortage and repressed by a presence of glucose. The cells grown for 72 h in 4.5 mg/ml glucose-containing medium demonstrated low apoptosis (3.7%) whereas in 0.5 mg/ml glucose-containing medium the apoptosis was increased to 10%. The effect of transfection on apoptosis was distinctly higher with almost 22% of apoptotic cells detected in 72 h cultures. HeLa cells grown in hypoxic conditions (despite an intensive expression of ORP 150) demonstrate higher rates of apoptosis in comparison to those cultured in normoxic conditions. Furthermore, the inhibition of ORP 150 synthesis by transfection of these cells with a specific siRNA resulted in an intensification of apoptosis, as indicated by specific markers of this process; the enhancement of poly ADP-ribose protein cleavage and the increase in Bim protein expression. We conclude from our study that the increase in ORP 150 synthesis protects the cells against the pro-apoptotic effect of glucose starvation as well as hypoxia.

P8.40

The studies of imidazoacridinone antitumor agent, C-1311, in the field of metabolic transformations with cytochrome P450 isoenzymes

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Among a large number of synthesized in our group imidazoacridinone derivatives compound C-1311 (Symadex) is currently undergoing II phase of clinical trials. Our studies on biochemical mechanism of antitumor action of imidazoacridinone showed that metabolic activation should be considered as an important preliminary step responsible for the following interactions with proteins and/or DNA in tumor cells. Therefore, studies on metabolic transformations of C-1311 with liver enzymes were carried out. The comparison of C-1311 reactivity with rat and human liver microsomes was reported earlier [1]. We showed that the rate of transformation was higher in the case of rat microsomes, whereas, the differences in the metabolite amounts were specific for each one. The attempts of identification of human cytochrome P450 isoenzymes (CYP450s) responsible for metabolic transformations C-1311 in human organism indicated that CYP2 family might be crucial. Nevertheless, the obtained results were not clear. The aim of the presented work is to search of the role of selected recombinant CYP450s in metabolism of C-1311 and also the influence of C-1311 on the activity of these isoenzymes. Although different reaction conditions have been applied any reactivity of C-1311 with CYP450s has not been observed. However, we found that this compound inhibited metabolism of 7-ethoxycoumarine and testosterone, substrates for CYP1A2 and CYP3A4, respectively. Studies on inhibition of substrate metabolism for CYP2C19 and CYP2D6 in the presence of C-1311 are being continued. The obtained here and our previous results led us to conclusion that other, than CYP450s, might be involved in hepatic metabolism of C-1311.

Refreneces:
1. Human CYP2 family of cytochrome P450 takes part in metabolism of two acridine antitumor agents, C-1311, C-1748, selected for I phase of clinical trials. Abstracts of the 40th Meeting of the Polish Biochemical Society. *Acta Bioch Polon* 52 Suppl. 1/2005, 129.

P8.41

Preoperative arginase activity in blood serum of patients with hepatocellular carcinoma – comparison with liver markers

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Hepatocellular carcinoma is one of the most common fatal

malignances cancer worldwide. The disease is extremely lethal – overall 5 – year is less than 5%, and median survival generally 4–6 months after diagnosis. Arginase (EC 3.5.3.1) catalyses the hydrolysis of arginine to urea and ornithine. The hepatic isoform plays a fundamental role in detoxification of ammonia, whereas the extrahepatic arginase is believed to supply cells with a precursor for biosynthesis of polyamines important for cells proliferation and differentiation. The aim of the study was to assess the usefulness of arginase activity determination in a diagnosis of patients with HCC, and compare it with other liver markers. The studies were performed on blood serum obtained before surgery from 50 patients (18 females and 32 males, age range 30–80 years) with hepatocellular carcinoma of various etiology. Arginase activity was determined according to Chinard., alpha-fetoprotein (AFP) level was measured by the Architect® AFP system Chemiluminescent Microparticle Immunoassay, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were assayed using Flex® reagent cartridge test. The sensitivity of arginase determination was found to be 92%, and it was much higher than that of AFP, AST and ALT (40%, 22% and 18%, respectively). The number of not detected by each marker cases (false negative) was much lower in case of arginase (only 4), than of AFP (n = 30), AST (n = 40), and ALT (n = 41). It can be concluded that the determination of serum arginase activity can be helpful in diagnosis of patients with hepatocellular carcinoma.

P8.42

The effect of resveratrol and pterostilbene on 12-*O*-tetradecanoylphorbol 13-acetate stimulated activation of AP-1 in mouse epidermis

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Resveratrol and its methoxylated derivative, pterostilbene have been reported to possess antioxidative, antimutagenic and anticarcinogenic properties in many experimental models. In our previous studies we showed that these potential chemopreventive compounds modulate the 12-*O*-tetradecanoylphorbol 13-acetate (TPA)-stimulated expression of inducible nitric oxide synthase and cyclooxygenase-2. As the one of possible mechanism of this activity, modulation of transcription factor, the activator protein-1 (AP-1) was postulated. AP-1 plays an important role as a mediator of tumor promotion. AP-1 is a heterodimer formed by c-Jun and c-Fos proteins. In the present study we evaluated the activation of AP-1 by assessing c-Jun-DNA and c-Fos-DNA binding after topical application of TPA (10 nmol per mice). Increased level of AP-1 binding to DNA was observed two hours after TPA application. Pretreatments with resveratrol and pterostilbene at a dose of 16 μmoles per mice 15 minutes before the TPA application significantly decreased the AP-1 activation. Pterostilbene was more potent inhibitor of TPA-induced AP-1 activation than resveratrol. These data suggest that the modulation of AP-1 proteins expression may be considered as one of the possible mechanisms of anti-promotional activity of naturally occurring stilbene derivatives.

P8.43

Analysis of CD69 expression on B and T lymphocytes in B-cell chronic lymphocytic leukaemia

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CD69 (type II integral membrane protein) is one of the earliest markers induced upon activation in T and B lymphocytes, NK cells, macrophages, neutrophils, and eosinofils. It acts as a signal-transducing receptor involved in cellular activation events, including proliferation and induction of specific genes. The exact role of CD69 in the pathogenesis of B-cell chronic lymphocytic leukaemia (B-CLL) is still unknown. The clinical course of B-CLL may be heterogeneous, with some patients having a long survival and never requiring treatment and others pursuing an aggressive course that demands intensive treatment. Therefore investigation of specific markers having prognostic value seems to be important. The aim of this study was to evaluate the expression of CD69 on B and T lymphocytes and to estimate the relationships between tested antigen expression and different clinical parameters. Peripheral blood (PB) and bone marrow (BM) samples were obtained from 68 newly diagnosed, previously untreated patients with B-CLL. Mononuclear cells were isolated by density gradient centrifugation on Lymphoprep. The CD69 expression was evaluated by the flow cytometry method. Results: 1) in patients treated since the moment of diagnosis significant increase in percentage of B CD69+ cells and significant decrease in T CD69+ cell percentage was observed, 2) the percentage of B CD69+ cells correlates with clinical stage of B-CLL and total tumor mass score indicating prognostic value of this parameter at the moment of diagnosis, 3) significant decrease of T cell percentage with CD69 activation marker expression in patients with higher clinical stage may be caused by progressive loss of T cell ability for activation. Evaluation of CD69 expression on leukaemic lymphocytes may be of importance in the elimination of the subgroup with unfavourable phenotype from B-CLL

patients population. In particular this group will require careful clinical observation considering possibility of fast progression and necessity of earlier initiation of therapy.

P8.44

Influence of metallothioneins on the zinc and copper distribution in brain tumours

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Background: Metallothioneins take part in the homeostasis of the ions of the metals which are necessary for the proper metabolism of the organism (zinc, copper). While isolated from the different organs of different animals they only slightly differ in the aminoacid composition from one another. The number of aminoacids is fixed in every animal group, that is 60 (or 61) aminoacids, 20 of which are the cysteine radicals what makes over 30% of the amonoacid composition. Such a large ammount of cysteine, which include the reactive sulfhydryl groups -SH determines the metallothionein's functions. The aim of this work was to determine the levels of metallothionein, zinc and copper in brain neoplastic tissues. The research was initiated to determine whether the neoplastic process changes the values metallothioneins, zinc and copper in those tissues. Material: The experimental materials were the brain neoplastic tissues resected during neurosurgical procedures. The brain tumors were divided into two groups; *astrocytoma* G-2 and G-4 (*glioblastoma multiforme*). Methods: The level of he metallothioneins was determined by the cadmium-hemoglobin affinity assay using the cadmium isotope (^{109}Cd). The value of zinc and copper were determined by means of atomic absorption spectrophotometry. Results: In our studies, the level of metallothioneins in astrocytoma G-4 patients was slightly higher than the level of these proteins in the group of G-2 patients. Correlation coefficient of the studied parameters prove an interrelation between the level of zinc and copper and the content of metallothioneins. In G-4 group, the level of zinc showed a positive relationship with the metallothionein level, whereas copper content showed an inverse relationship. Conclussions: The neoplastic process change the values metallothioneins, zinc and copper in the investigated tissues.

P8.45

HPLC–method for detection of alkaloids and its derivatives in anticancer drug Ukrain® for its using in the cancer detection, effective treatment and investigating the mechanisms of carcinogenesis

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Introduction: more than once by the independent methods shows that alkaloids (*Chelidonium majus* L.) and its derivatives specific interact with cancer cells and their constructs including the cell membrane of immunocompetent cells. This open prospects for its using in the cancer detection. However effort in this direction restrain for lack of appropriate and selective methods for detection of alkaloids and its derivatives in different materials. Therefore the purpose of this reseach was the development of the HPLC–method for detection of alkaloids and its derivatives. Material and methods: alkaloids and their derivatives were identified in anticancer drug Ukrain® and in plasma of cancer patients comparing with healthy donors which heparinized blood samples were incubated for 2.5 h at 37°C with 100 μl of Ukrain®, by means of the reverse-phase high-performance liquid chromatography on Chem Station "HP Agilent-1100" (USA). The principle of the developed HPLC-method conclude in separation of alkaloids and its derivatives in column (5 × 150 mm) thick with sorbent Zorbax SB C_{18} (5 mcm) in isocratic elution under such conditions: rate of flow 0.4–0.5 ml/min, column temperature 32–35°C, photometric (254/450 nm) and fluorescence detection (280/340 nm). Repeatability of the method 1.5%, the maximal sensitiveness 5×10^{-12} mole. Results and discussion: 1) was develope the method of the reverse-phase high-performance liquid chromatography of alkaloids and its derivatives in biological material; 2) determined concentrations of alkaloids and its derivatives in anticancer drug Ukrain®; 3) in plasma of cancer patients comparing with healthy donors the concentration of alkaloids and its derivatives with positively changed, which depend of the type of cancer. Conclusion: the results take by using this selective method maybe use in the cancer detection, effective treatment and investigating the mechanisms of carcinogenesis.

P8.46

Inhibitory effect of extracts from human colorectal cancer on arginase activity

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Arginase (E.C. 3.5.3.1) is an enzyme which hydrolyzes L-arginine to L-ornithine and urea. The presence of two distinct genes for arginase has been established. Arginase AI is a cytosolic enzyme that plays a fundamental role in urea cycle. Arginase AII is a mitochondrial isoform involved in arginine catabolism. As we have earlier shown, arginase activity was increased in tumor tissues and in blood

plasma of patients with colorectal cancer (CRC). Surprisingly, this increase was accompanied by elevated plasma arginine concentration. The aim of the study was to assess whether extracts from colorectal cancer and tumor-adjacent colon may affect arginase activity. The extracts were prepared from tissues obtained by surgery from patients with CRC (n = 22). Arginase was isolated from human kidney, and its activity was determined with and without the extracts. The results indicated that both extracts exerted an inhibitory effect on arginase activity. The effect was more pronounced by extracts from tumor-adjacent than from tumor tissues (35% and 15% of inhibition, respectively). The control extracts from human kidney and liver had no effect on arginase activity. We conclude that the inhibitory effect expressed by tumor-adjacent tissue on arginase activity can be caused either by a release of specific factor from the tumor to its surroundings or by a presence of infiltrating cancer cells which produce this factor *in situ*. The tumor may exert a local inhibitory effect on arginase activity in adjacent tissues to increase arginine supply for its own growth and development.

P8.47

The effect of microcrystalline chitosan on the pyruvate kinase M2 isoenzyme gene expression in Ehrlich ascites tumor cells

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Tumor cells demonstrate an increased glycolytic activity evoked by an increased expression of genes that encode enzymes participating in the process of glycolysis. The tumor-specific γ variant of the M_2 isoenzyme, which is stereospecifically sensitive to L-cysteine and non-sensitive to allosteric effectors (ATP and fructoso-1,6-biphosphate) has proven to be a marker of neoplastic transformation. In absence of L-cysteine, the γ variant of the M_2 pyruvate kinase (PK) isoenzyme transfers the phosphoryl group from 2-phosphoenolopyruvate (2-PEP) to ADP accompanied by ATP generation. In the presence of L-cysteine, the variant demonstrates the histone kinase activity, transferring the phosphoryl group from 2-PEP to the ε-amine radical of the H_1 histone lysine. L-cysteine evokes a change in the conformation of the bifunctional γ variant of the M_2 PK isoenzyme, from a form that displays the activity responsible for ATP synthesis to a form responsible for the H_1 histone phosphorylation. In addition to other factors, the increased glycolysis rate in tumor cells results from an increased expression of the M_2 PK isoenzyme gene. The increased negative charge of the cellular membrane of tumor cells allows for surface interactions between the membrane and highly deacetylated microcrystalline chitosan. Microcrystalline chitosan reacting with the cellular membrane of an Ehrlich ascites tumor cell inhibits the expression of the M_2 PK isoenzyme gene. A decreased activity of the M_2 isoenzyme contributes to depression of ATP synthesis and may trigger a decreased phosphorylation of the H_1 histone and, in consequence, inhibition of excessive DNA and RNA synthesis.

P8.48

Metabolic activation is probably necessary for cytotoxic activity of sesquiterpene lactones

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Sesquiterpene lactones (SLs) are the active constituents of variety of medicinal plants used in traditional medicine for the treatment of inflammatory diseases. In recent years, the anticancer property of various SLs attracted a great deal of interest and extensive research work has been carried out to characterize the anticancer activity of these compounds. Seven SLs isolated from medicinal plants: *Helenium aromaticum* – helenalin, mexicanin I, linifolin A, geigerinin and *Telekia speciosa* 6α-hydroxydihydroaromaticin, asperilin, telekin has been tested in our lab. Although in *in vitro* tests SLs showed antioxidant activity opposite effect has been observed in *in vivo* tests. It was hypothesized therefore that SLs may be metabolically activated by cellular drug metabolizing enzymes forming reactive oxygen species. Such activation could be reason of mechanism of cytotoxic activity. Anticancer activity of SLs has been therefore tested in cell culture *in vitro* model using breast cancer MDA-MB-231, ZR-75-1, MCF-7 cell lines, HepG2 (hepatocarcinoma) cell line as well as Jurkat T leukemia cells. From the tested cell lines the most sensitive were HepG2 cells (IC_{50} values ranging from 1.5 μM (helenalin) do 24 μM (telekin)) while the most resistant were MDA-MB-231 cells. (IC_{50} values above 50 μM). HepG2 cell line is known as cell line with strong expression of drug metabolizing enzymes, while expression of such enzymes in MDA-MB-231 cells is poor. These results seem to confirm theory that for cytotoxic activity of SLs tested in experiment, metabolic activation is necessary.

*both authors have first author rights.

P8.49

Cancer procoagulant (CP) from human amnion-membranes does not contain γ-carboxyglutamic acid (Gla) residues

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Background: Cancer procoagulant (CP) is an enzyme directly activating coagulation factor X *in vitro*. The expression of CP is characteristic for rapidly proliferating cells like malignant and fetal cells. Warfarin, the coumarin anticoagulant, acting as a vitamin K-antagonist, prevents post-translational formation of γ-carboxyglutamic acid (Gla) in the vitamin K-dependent carboxylation process. Some blood coagulation factors – II, VII, IX, X, PC and PS contain Gla residues. This amino acid is responsible for Ca^{2+} ions binding, protein adsorption to phospholipid membranes and protein-protein interactions. There are indirect evidences suggesting the presence of Gla residues in CP moiety. CP activity in animal tumors was depressed by warfarin while CP antigen was still present in tumor extracts. These results suggested that cancer procoagulant could be a member of vitamin K-dependent proteins. Aim: The aim of this study was to demonstrate directly the presence or the lack of γ-carboxyglutamic acid in samples of cancer procoagulant isolated from human amnion-chorion membranes. Materials and Methods: Cancer procoagulant was purified from human amnion-chorion membranes by low-pressure ion-exchange chromatographies. The presence of γ-carboxyglutamic acid was analyzed by polyacrylamide gel staining procedure with 4-diazobenzenesulfonic acid solution (DBS) and with ELISA test using monoclonal antibody against human Gla residues. In all the experiments bovine prothrombin (Gla-containing protein) was used as the positive test control. Results: The CP specific activity of the samples was between 30–55 U/mg and the total protein concentration in purified samples was about 500 μg/ml. Prothrombin – the protein which contains Gla residues – was stained red with DBS solution. Although the DBS gel-staining method is very sensitive and the presence of CP in purified preparations was high (according to their activity and protein concentration) we observed no red stained band in all analysed CP samples. The ELISA test has confirmed the lack of this amino acid in CP samples purified from human amnion-chorion membranes. Anti human Gla residues-MAb did not recognized any protein present in analysed CP preparations Conclusions: Our analysis demonstrates that cancer procoagulant derived from human amnion-chorion membranes does not contain Gla residues. Vitamin K-dependence of this enzyme is questionable.

P8.50

DNA methylation analysis in colorectal cancer using methyl-CpG binding domain column chromatography

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Epigenetic silencing is one of the mechanisms involved in tumorigenesis. DNA methylation, the main epigenetic phenomenon, can alter gene expression without genetic mutations. CpG islands, which sequences overlap promoters of many genes, are often hypermethylated in cancer. This modification causes significant changes in gene expression and in consequence may lead to malignant transformation. Techniques commonly used for analysis of DNA methylation are based on PCR reaction or restriction digestion and are designed for defined DNA sequences. In our study we chose technique that enable separation and analysis of DNA fragments depending on their methylation status (independently from sequence): MBD affinity column chromatography. Fragment of the rat MeCP2 protein, a methyl CpG binding domain (MBD) was clone into an expression vector. The expressed protein was than immobilized on polypropylene column with solid resin. Column was eluted with stepwise gradient of NaCl. Using control fragments of methylated *in vitro* pUC19 DNA and control DNA from AS/PWS patients with defined pattern of imprinted genes we determined the NaCl concentration that elutes methylated DNA. DNAs of 9 patients with colorectal cancer were isolated. Samples were mixed, fragmented and loaded onto affinity column. Methylated fractions were collected. Genomic subtraction with normal (non-cancerous) DNA was done to create a library of hypermethylated DNA fragments from colorectal cancer.

P8.51

Genotoxic effects of antitumor 1-nitroacridines C-857 and its novel analogue 4-methyl-1-nitroacridine C-1748

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4-Substituted 1-nitroacridines represent a new group of acridine derivatives synthesized at Gdansk University of Technology. Compared to parent 1-nitroacridines, these compounds exhibit lower toxicity and enhanced antitumor efficacy especially against colon and prostate cancers. The 4-methyl-1-nitroacridine denoted C-1748 was selected for the I phase of clinical evaluation. Earlier studies demonstrated that C-1748 similarly to its parent compound C-857 formed DNA adducts as well as induced DNA crosslinking. The level of covalent modification correlated with cytotoxic activity suggesting importance of this ability for biological effects. Current studies are

aimed at finding out genotoxicity of these acridines as an after-effect of DNA adducts formed. Two approaches were adopted to reach this goal. Firstly, comet assay has been employed for measuring DNA strand breaks and detection of DNA crosslinking. Secondly, CBMN assay (cytokinesis-block micronucleus assay) to investigate chromosomal abberations. The studies are carried out for two cell lines HT29 (colon cancer cells) and LNCaP (prostate cancer cells) for which DNA adducts formation by both acridines was demonstrated. In comet assay, C-857 and C-1748 gave rise to DNA fragmentation in a time and dose-dependent manner. However, in the case of C-857, for certain treatment regimens, the reversal of genotoxicity i.e. shortening of the comet tails, was clearly observed – the effect expected in the case of DNA crosslinking. For C-1748, this effect was never so apparent and possible explanations of this discrepancy between compounds will be discussed. CBMN assay is currently underway and the results will be presented.

P8.52

Analysis of OPN gene expression in the colorectal cancer

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Osteopontin (OPN) is a 60 kDa phosphorylated acidic glycoprotein, which is implicated in a variety of physiological and pathological events. The most important functions of OPN are stress-induced bone remodeling, maintenance or reconfiguration of tissue integrity during inflammatory processes. This protein also takes part in cell-mediated immunity and in dystofic calcification and tumor cell metastasis. It is overexpressed in several cancers for example lung, breast, colorectal, ovarian and melanoma cancer. The objective of this study was to examine the expression level of osteopontin gene (*ssp1*) in colon cancer (100 tissues) and neighbouring normal colon. We used reverse transcription and multiplex PCR with β-actin as a reference template to estimate the OPN mRNA levels. Received data were subjected to statistical analysis (chsquare test with Yates correction) for any association with the clinicopathological variables for example age, TNM stage, tumor grade, amplification of *c-myc* and *c-erb-B2* genes. The results obtained from our analysis demonstrate that OPN gene expression is presented only in cancer tissues, predominatingly in TNM III stage of colon cancer. In addition, we also observed that in this stage of tumor *c-erb-B2* and *c-myc* genes amplifiction were associated with the presence of OPN transcripts. There were no corelations with age of patients or tumor grade.

P8.53

Significance of oncogenes in breast cancer cells

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Changes in gene expression in breast cancer cells are the reason of different transformation processes and cause increase of malignancy. Oncogenes are group of genes which take important role in this processes. Changes in activity of oncogenes can be estimated successfully by modern techniques such as microarray analysis. The aim of this work was to estimate changes in oncogene expression and proliferation processes in T47D breast cancer cell line after administration of paclitaxel in comparison to control group. Materials and methods: In researches it was used T47D breast cancer cell line (ECACC No. 85012201). The cultures were incubated in a period of 72 h in 37°C temperature in 5% CO_2 atmosphere and 90% humidity of the air. Paclitaxel was administered to the cultures in a dose of 60 ng/ml (TL group) and 300 ng/ml dose (TH group). Expression of oncogenes was compared with a control group in which paclitaxel was not administered (K). Reverse transcriptase reaction in a buffer containing nucleotides ^{32}P labeled was made to produce cDNA. Received cDNA was hybridized with the matrix containing 2886 genes in a period of 18 h in a hybridization chamber. After hybridization it was scanned to count activity of oncogenes. The activities from points on matrix containing following genes were collected. The activity of oncogenes in a control breast cancer group (K) was compared with the activities in groups exposed to 60 and 300 ng/ml paclitaxel concentration (TL, and TH groups). Results: significant decrease of oncogenes expression was observed between TH and K group ($P < 0.0001$) and significant increase this activity was stated in TL in comparison to K group ($P < 0.001$). There were found significantly high correlations between the level of expression of TL and K, TH and K as well as TL and TH groups ($P < 0.001$). Conclusions: paclitaxel in higher doses inhibits activity of oncogenes in T47D line. Observed decrease of oncogenes expression in T47D line after administration paclitaxel in higher doses can be resulted from its cytotoxic effect. Increase of oncogene expression level after administration of lower doses of paclitaxel can be the reason of changes in T47D cell activity.

P8.54

Cancer stem cells from mouse sarcoma cell line – L1: preliminary results

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The latest studies have suggested that there is a population of cancer stem cells within the tumor that may regulate cancer growth and metastasis. According to the methods described in literature, specific holoclones and paraclones have been selected from mouse sarcoma (L1) cell line. Selected L1 holoclones and L1 paraclones differed morphologically from each other and from original L1 cell line. They also had distinct biological properties as analyzed by rate of growth, plating efficiency, rate of migration and doxorubicin sensitivity. The L1 paraclones migrated better in Transwell chambers and formed more colonies in lungs of BALB/c mouse as compared to holoclones as well as the original L1 cells. Selected L1 holoclones were more resistant to doxorubicin treatment similarly as it was described for stem cells. Moreover, the L1 paraclones had higher percentage of Ki-67 positive cells than L1 holoclones as detected by the flow cytometry. The gene expression analysis preformed with Atlas Gene Expression Array (Clontech) showed that L1 cells significantly differed from L1 holoclones and L1 paraclones in trascription level of several genes.

P8.55

Anticancer drug Ukrain®: mechanisms of anticancer effects and perspectives of its application in the cancer diagnostics

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Introduction: In our early studies the use of the anticancer drug Ukrain® was proven to be safe and effective, selectively accumulating in cancer tissue after a intravenous administration and controlling cancer-induced metabolic imbalance: inhibits metabolic processes in the tumor, induced the changes in certain amino acids concentrations in biological fluids and tumor tissue in animal models and cancer patients which cannot be explained with metabolic amino acid disorders in cancer. This would provide the background for the application of the drug Ukrain® in the cancer detection and effective treatment to estimate the mechanisms of anticancer effects in regard to its effects on amino acid contents. Material and methods: blood was sampled before treatment from 29 patients with different types of cancer, 3 patients with benign processes and 10 healthy donors. Heparinized blood samples were incubated for 2.5 h at 37°C with 100 μl of Ukrain®, diluted to 1:5, 1:10, 1:100 or 1:1000. Plasma samples after centrifugal separation were deproteinized with 1M $HClO_4$ while homotaurine as internal standard. Amino acids and their derivatives were identified by means of the reverse-phase high-performance liquid chromatography on Agilent 1100 system after derivatization with ortho-phthalic aldehyde. Results and discussion: in plasma of cancer patients comparing with healthy donors Ukrain® 1) affects amino acids with positively charged (His, Arg) or not charged (Tyr, Thr, Gln) R-groups; 2) decreases concentration of His and increase the concentrations of β-Ala and Tau. These changes depend on the concentration of Ukrain® and the type of cancer. Conclusion: on the basis of our studies we suggest that Ukrain's® biological actions in cancer are realized at least partly through selective interaction with amino acids, their derivatives, and plasma proteins. These data provide the background for the using Ukrain® in the cancer detection, effective treatment and investigating the mechanisms of carcinogenesis.

P8.56

Oxidative stress induced by 4-methyl-1-nitroacridine derivative C-1748 (Capridine β) in human colon cancer cells

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C-1748 (Capridine β), a 4-methyl-1-nitroacridine derivative synthesized in Gdansk University of Technology, currently is being prepared for the phase I of clinical studies. This compound was shown to exert highly potent anticancer activity against several prostate and colon human experimental tumors. We investigated C-1748-induced oxidative stress in two human colorectal cancer cell lines HCT8 and HT29, displaying high sensitivity to C-1748. Intracellular ROS generation was measured by flow cytometry following staining with DHR 123 (in mitochondria) and H_2DCFDA (in cytoplasm). HCT8 cells treated with C-1748 (EC_{90} concentration) exhibited the significant increase and maximum level of ROS generation after 3 h-exposure (DHR 123) or 4 h-exposure (H_2DCFDA) and then a high steady level up to 24 h of treatment. In the case of HT29 cells, the significant increase and maximum level of ROS generation was observed after 1 h-exposure (H_2DCFDA) or 2 h-exposure (DHR 123) and then a high steady level up to 24 h. In both cell types, C-1748 treatment did not cause any significant rapid decline of GSH levels, which means that C-1748-induced ROS generation was not mediated by a mechanism involving GSH depletion. The cytotoxic activity of C-1748 against HCT8 cells was partially reversed by 10 mM *N*-acetyl-L-cysteine (ROS scavenger) and no such effect was observed in HT29 cells.

P8.57

Colocalisation of E2HPV16 protein and

BTBD2 in C33a epithelial cell line

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BTBD2 protein was firstly identified as a topoisomerase I-interacting protein. BTBD2 protein contain BTB/POZ domain, a Kelch like region and a PHR-like region. It was shown that in human cells BTBD2 localize to punctate and elongated cytoplasmic bodies but its precise function remains poorly characterized. Using yeast two-hybrid system we previously identified BTBD2 as cellular interactor for E2 protein of human papillomavirus type 16 (HPV16). HPV16 belongs to group of small DNA tumor viruses that can cause benign proliferative lesions and cancer. The E2 HPV16 protein plays a role in recruiting viral and cellular factors for efficient regulation of transcription and replication of papillomavirus type 16. The E2 protein is a transcriptional regulator, consisting of a transactivation domain and a dimerization/DNA-binding domain, linked by a nonconserved flexible hinge region. Recently it was shown that E2 protein of HPV11 could also interact with and stimulate human topoisomerase I. Here we show results of the colocalisation studies between E2 protein and BTBD2 and their truncated forms in HPV negative epithelial cell line C33a. We have used fluorescence and confocal microscopy to substantiate preliminary findings obtained by yeast two-hybrid system.

P8.58

Decrease of the somatostatin receptor subtype 3 (SSTR3) expression in gastric mucosa of patients with familiar gastric cancer and infected with *Helicobacter pylori*

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The occurrence of gastric cancer is associated with environmental conditions, including *Helicobacter pylori* infection, as a result of mutagenic changes in particular genes and disturbed expression of functionally important proteins. Recently it was published that in the gastric mucosa of patients with gastric cancer observed is a decreased density of somatostatin receptor SSTR3. The aim of this work was to answer the question whether the *SSTR3* gene expression in the gastric mucosa of patients with dyspepsia is affected by the family history of gastric cancer and the presence of *Helicobacter pylori*. The study comprised 43 patients with dyspepsia. The level of SSTR3 mRNA was determined by real time RT-PCR in 86 bioptates from the antrum and 86 from the corpus. Material from the analogous sites was collected for histopathological examination and *H. pylori* infection evaluation. The SSTR3 mRNA level was lower in the group of patients with positive family history of gastric cancer as compared to the control group, regardless of concurrent *H. pylori* infection and stomach topography. The greatest difference was observed for antrum bioptates among *H. pylori* negative patients. In *H. pylori* positive patients of both the studied groups these values were remarkably lower, regardless of stomach topography. The level of SSTR3 mRNA in the antrum bioptates of the control patients was dependent on the presence of *H. pylori* bacteria. A decrease in the density of SSTR3 in individuals with positive family history of gastric cancer may point to an inherited predisposition to the development of gastric cancer in these patients, particularly when infected with *Helicobacter pylori.*

P8.59

Identification of nuclear actin in human melanoma A375 and rat hepatoma Morris 5123 cells

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Nuclear actin has finally reached it's turn of being taken under magnifying lens to have a closer look at this what was for many years described as an artefact and cytoplasmic contamination. Nowadays, β-actin isoform of this most important eucaryotic cytoskeletal protein is believed to perform an important role in several events, that take place in the nucleus. The evidence of actin participation in transcription and its association with RNA polymerases are continuously accumulating. Nuclear actin was also proved to be one of the factors responsible for chromatin remodelling and histone modifiers recruitment. Our study focused on the isolation of nuclei from human melanoma A375 and rat hepatoma Morris 5123 cells in order to present nuclear actin organisation. Nuclei were purified with the use of Nonidet P-40 detergent which does not disrupt the nuclear envelope. Actin localization was performed by immunofluorescent staining with antibodies recognizing total actin and β-actin isoform and visualized in confocal microcope, both in whole cells and in isolated nuclei. Actin appears in the nucleus as a 'cloud' of short filaments. It does not show any specific intranuclear localization and was identified as β-actin isoform. The data have been confirmed by analysis of the nucleo-

plasm protein fraction in Western blotting. In addition the quantitative measurements of total, monomeric and filamentous actin were done by the standard DNase I inhibition assay. The assay prooved the low level of nuclear actin polymerization in hepatoma and melanoma cells.

P8.60

The significance of *MDR1* in the effectiveness of antiproliferative sitosterol action

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Sitosterols are intensively studied in the context of antiproliferative action on cancer cells. It was shown that they are associated with the cell metabolism processes including activation of the sphingomyelin cycle, cell cycle arrest and stimulation of apoptotic cell death. However, the exact mechanism of sitosterols action has not been discovered yet. To study the mechanism of antiproliferative action of sitosterols, different cell lines: MCF-7, MCF-7/ADR, HeLa WT, HeLa MDR1 were cultured and tested to elucidate if the induced *MDR1* expression is engaged in sitosterols action. The impact of sterols and their derivatives on cell proliferation was analyzed by MTT test in a time and dose dependent manner. It was shown that incubation of cells in the presence of beta-sitosterol resulted in a significant decrease in proliferation of all cell lines. Similar effect in HeLa cells was also evoked by cholesterol. However, in MCF-7 and MCF-7/ADR cells the effective cholesterol concentration was about 4 times higher. The β-sitosterol IC50 values for individual cells were respectively: MCF-7, 2 μM; MCF-7ADR, 8 μM; HeLa WT, 0.5 μM; HeLa MDR1, 4 μM. While β-sitosterol showed strong antiproliferative action on cancer cells *in vitro*, its oxy-derivative, β-epoxy-sitosterol showed much weaker contribution to the cell division. These results suggest that the antiproliferative action of β-sitosterol on cancer cells is significantly decreased in cells with induced *MDR1* expression. Thus, it is concluded that the action of β-sitosterol is related with the mechanism of multidrug resistance.

P8.61

Overexpression of Bax enhances drug sensitivity of ovarian cancer cell line-OVP-10

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Apoptosis is a major form of cell death, characterized initially by the series of stereotypic morphological changes. Often the efficacy of chemotherapeutic agents depend on their effectiveness in inducing apoptosis of tumor cells. By that reason proapoptotic gene therapy is maybe one of the method to sensitize cancer cells to a various chemotherapeutic agents. The overexpression protein involved in apoptosis, can induce cancer cells death. Overexpression of Bax enhances drug induces apoptosis in cancer cells. In our work we have decide to construct the expression vector encoding *Bax* gene and verify its presence and action following transfaction to human ovarian cells OVP-10. Ovarian cell line was exposed to adriamycin. MTT, clonogenicyty, Ac-DEVD-pNA assays and Hoechst staining were performed. Our results indicate , that transfer of proapoptotic gene *Bax* into studied cells can induces death and increase chemiosensitivity. To summarize, the expression vector encoding proapoptotic gene *Bax* seem to be potential treatment by induction of apoptosis of cancer cells. It seems the chemiotherapy may be assisted by gene transfer strategy.

P8.62

The *CCND1* polymorphism in human lung cancer

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The cyclin D1 gene (*CCND1*) is linked closely to the *bcl-1* gene on chromosome 11q13. There is a firm evidence that D-type cyclins (D1, D2 and D3) are important in cell cycle regulation and cell differentiation. Cyclin D1 is responsible for transition from the G1 to the S phase resulting phosphorylation of the retinoblastoma tumor suppressor protein pRB. It is known that increased amounts of D-type cyclins can reverse the pRB induced cell cycle arrest and can accelerate progression through G1 phase. Increased cyclin D1 expression (mRNA and/or protein) causes transformation to the malignant phenotype and has been shown in a number of primary human tumors and cell lines. A germ-line mutation in the *CCND1* gene (SNP; A870G at 242 codon) can produce abnormal, truncated cyclin D1 protein with a longer half-life. In our study we try to access whether *CCND1* has any association with histo-pathological features of lung cancer patients. The *CCND1* G/A polymorphism was detected by the PCR-RFLP method.

P8.63

The elevated expression of caveolin-1 gene (*CAV1*) in colorectal cancer

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Caveolins are the major structural proteins of caveolae. This structures mediate molecular transport, cell adhesion and signal transduction activities in the cells. The caveolin family contains three members: caveolin-1, -2 and -3. The gene of caveolin-1 is located at human chromosome 7q31.1, a region frequently deleted in a variety of human cancers. Caveolin-1 may function as a negative regulator of membrane signaling molecules. However, the functional role of caveolin-1 in malignant tumours is not clearly identified. *In vitro* studies has shown that caveolin-1 may have a role as tumor suppressor gene, but other studies have demonstrated overexpression of caveolin in colon cancer, suggesting its tumor promoting function. Our studies were devoted to explore the possible impact of the *CAV1* gene expression on colon cancer development. The expression of *CAV1* was studied in 52 normal mucosa and 109 colon cancer tissues. The level of *CAV1* expression was examined by multiplex RT-PCR with *GAPDH* mRNA as reference. The tumours were staged using the TNM staging system. None of the patients had known history of FAP or HNPCC. The predictive value of mRNA expression level for clinicopathological variables was calculated using t-paired and Pearson's chi-square tests. Performed analysis showed no correlations between caveolin-1 expression and clinical factors such as sex, age, tumour stage, nodal status, distant metastasis, other genes expression such as *TSP-1*, *ssp-1*, and amplification of *c-myc* and *c-erb-B2*. However, performed examination showed elevated expression of *CAV1* in colon cancer *vs* colon mucosa ($P = 0.039$) implying its tumor promoting function.

P8.64

Calcium response induced by stimulation of P2Y nucleotide receptors in long-term serum-deprived glioma C6 cells

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The present work is a description of the changes in the calcium response of glioma C6 cells on the stimulation by extracellular nucleotides occurring during long-term (96 h) serum starvation. Three nucleotide receptors have been studied: P2Y1, P2Y2 and P2Y12. Two of them, P2Y1 and P2Y2 directly stimulate calcium response. The protein level of the P2Y2 receptor does not change during the serum-starvation, while P2Y1 protein level dramatically falls. Observed changes of the calcium response are directly correlated with the receptor protein level as well as with amount of calcium present in the intracellular calcium stores. Calcium response evoked by P2Y1 receptor is potentiated by activity of P2Y12 dependent signaling pathways; however the later receptor is not responsible for calcium influx. The calcium influx is enhanced by partial depletion of calcium stores in starvation process and depends on capacitative calcium entrance mechanism.

P8.65

Modeling of interactions between NFκB and p53 signaling pathways

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

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

P8.66

The relationship between *TSP-1* gene expression and clinical features of patients with colorectal cancer

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Thrombospondin-1 (TSP-1) is a 450 kDa trimeric glycoprotein, which is produced by variety of cell types. TSP-1 has got a lot of biological activities in cell adhesion, migration, proliferation and angiogenesis. This protein also plays role in platelet aggregation and clot formation. In addition TSP-1 might take part in tumor progression. TSP-1 has been showed to be potential inhibitor of angiogenesis in tumor growth. The aim of this study was to investigate the expression level of *TSP-1* gene in 109 colon cancer tissues and adjacent normal colon tissues. The *TSP-1* mRNA level was examined by multiplex PCR with β-actin as a reference template. Obtained results were analyzed using ch-square test (with Yates correction) for any association with the clinicopathological variables: age, sex, tumor stage, nodal status, distance metastasis, *ssp1* gene expression, *K-ras-2* gene mutation and amplification of *c-myc* and *c-erb-B2* genes. The total survival rate was estimated using the Kapla-Maier method. Our studies showed that *TSP-1* gene is more often expressed in G3 grade tumors and it is also connected with the absence of the *c-myc* gene amplification in colon cancer. In addition, patiens with cancers in TNM I and TNM II stages, which also demonstrated the presence of *TSP-1* transcripts, showed the tendency to shorter survival. In conclusion, the *TSP-1* gene might be independent predictive factor for survival of patients with colon cancer.

P8.67

Inhibition of DNA topoisomerases I and II, and growth inhibition of breast cancer MCF-7 cells by ouabain, digoxin and proscillaridin A

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Cardiac glycosides are commonly used drugs to increase contractile force in patients with cardiac disorders. Epidemiological studies had shown that digitalis has also anti-cancer effects. Three cardiac glycosides (ouabain, digoxin, proscillaridin A) were examined for cytotoxicity in MCF-7 breast cancer cell cultures and for inhibition of topoisomerase I and II. Viability of MCF-7 cells was measured by the method of Carmichael. There is little difference between the potency of digoxin and ouabain for 24 h of incubation (IC_{50} 100 nM ± 2 and 130 ± 2 nM, respectively). In contrast, proscillaridin A showed a high level of cytotoxic potency, IC_{50} 30 ± 2 nM. To analyze if the inhibition in cell viability was due to decreased cell proliferation, we measured DNA synthesis. The concentrations of digoxin, ouabain and proscillaridin A needed to inhibit [^{3}H]thymidine incorporation into DNA by 50% were found to be 99 ± 2 nM, 124 ± 2 nM, and 30 ± 2 nM, respectively. To test whether cytotoxic properties were related to DNA-binding and topoisomerase action, these glycosides were evaluated in a cell-free system. While both digoxin and ouabain inhibited topoisomerase II at nanomolar concentrations (100 nM), neither agent inhibited topoisomerase I catalytic activity even at concentrations as high as 100 μM. On the other hand, proscillaridin A was a potent poison of topoisomerase I and II at nanomolar drug concentrations (30 nM and 100 nM, respectively). These studies suggest that the stabilization of DNA-topoisomerase II complexes is closely linked to the mechanism of digoxin, ouabain and proscillaridin A cytotoxicity.

P8.68

Cytotoxicity of chemically modified derivatives of ouabain, digoxin and proscillaridin A

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The active implementation of cardiac steroids into cancer therapy has been hampered by their concomitant cardiotoxic action. In this study, the cytotoxic action of derivatives of ouabain (1), digoxin (2) and proscillaridin A (3) that did not contain lactone in position 17β on malignant breast cancer MCF-7 cells and on fibroblasts was examined. Exposure of MCF-7 cells to the drugs revealed that the antiproliferative response is time- as well as dose-dependent. In terms of reduction in cell viability, the compounds rank in the order derivative of 3 > 2 > 1 (for 24 h of incubation IC_{50} value of 95 ± 2 nM, 130 nM ± 2, and 150 ± 2 nM, respectively). In contrast, cytotoxicity of these derivatives of cardiac glycosides in fibroblasts was significantly weaker and was almost equal (IC_{50} value for 1–3 was very similar, i.e. 400 ± 10 nM). To test whether the inhibition in cell viability was due to decreased cell proliferation, we measured DNA synthesis in presence of these compounds. The concentrations of 1–3 needed to inhibit [^{3}H]thymidine incorporation into DNA by 50% (IC_{50}) for 24 h of incubation in MCF-7 cells were found to be 150 ± 2 nM, 180 ± 2 nM, and 100 ± 2 nM, respectively. The IC_{50} value of 1–3 needed to inhibit DNA synthesis in fibroblasts were distinctly higher, i.e. 380 ± 10 nM. These data suggest that these derivatives of cardiac glycosides are promising candidates for the treatment of cancer.