Posters

P1

Vitamins and trace elements in the chemoprevention of elderly prostate cancer patients

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Background: The anticarcinogenic and antioxidant properties of vitamins and of trace metals have recently attracted increased attention.
Objectives: To examine the levels of antioxidant vitamins (A, C and E), selenium and malondialdehyde (MDA), and trace metals (Zn, Cu) in patients with prostate cancer and to determine the effect of a 6-months of antioxidant vitamins and minerals on prostate cancer and on biochemical markers.
Methods: 141 elderly subjects (91 prostate cancer patients and 50 healthy controls) were recruited in this study. The levels of trace elements in whole blood were determined by spectrophotometry. Serum levels of Se were determined using a fluorimetric method, while gas chromotography was used for serum levels of vitamins and MDA at baseline and after 6 months. The men were randomized to take either a placebo or a supplementation with nutritional doses of vitamin A, C and E, selenium and the trace elements zinc and copper daily for 6 months. Biochemical markers of prostate cancer risk such as prostate-specific antigen (PSA) and insulin-like growth factors (IGFs) were measured on plasma samples collected at baseline and at the end of therapy.
Results: The levels of vitamins A and E were significantly lower and MDA levels were significantly higher ($P<0.001$) in patients with prostate cancer compared to controls. Serum vitamin C was significantly lower in patients with prostate cancer when compared to controls ($P<0.01$). Moreover, Se and Zn levels were also significantly lower and Cu were higher ($P<0.001$) in patients with prostate cancer than in controls.
Conclusions: The findings suggest that the administration of vitamins A, C, and E, and Se and Zn may be beneficial in the prevention and treatment of human prostate cancer in the elderly.

P2

The potential of n-3 fatty acids in inhibiting prostate cancer in elderly men

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Background: Data from animal models suggest that n-3 fatty acids inhibit prostate cancer proliferation, whereas n-6 fatty acids promote it.
Objective: To evaluate the association between lon chain n-3 fatty acids and prostate cancer in elderly male patients.
Methods: Blood fatty acid levels were determined for 176 men diagnosed with prostate cancer and from 89 healthy age matched controls. Fatty acid levels were correlated to grade of prostatic cancer. Cases were according to their clinical aggressiveness. Non-aggressive cases were defined as those with localized tumors (stage A or B) and Gleason <7 at diagnosis. Cases were considered aggressive when they presented as advanced disease (stage Cor D) or Gleason z7 at diagnosis or subsequently developed distant metastases or died from prostate cancer.
Results: Whole blood levels of all long-chain n-3 fatty acids examined and of linoleic acid were inversely related to prostate cancer. Blood levels of linolenic and dihomo-;-linolenic acids, fatty acids resulting from the metabolism of linoleic acid, were directly associated with prostate cancer.
Conclusions and Implications: Higher blood levels of long-chain n-3 fatty acids, mainly found in sea foods, and of linoleic acid, mainly found in non-hydrogenated vegetable oils, are associated with a reduced risk of prostate cancer. Because intake of polyunsaturated fats may help prevent other common chronic diseases of the elderly, notably heart disease and diabetes these findings may have broader implication in chronic disease prevention and carcinogenesis in the elderly.

P3

Is there any association between perlecan *Bam*H1 polymorphism and renal failure or colorectal cancer?

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Perlecan (1p36.1-p35), a major HSPG in basement membranes, plays active roles in cell adhesion, proliferation, differentiation, glomerular filtration, development etc. The change of expression levels and the ability to bind different growth factors sustain the consideration of perlecan as a candidate gene for some common diseases (e.g. cancer, diabetic complications, arteriosclerosis). Reduced levels of HSPG have been also correlated with increasing metastatic risk of many tumors.
The aim of this study was to test the association of HSPG *Bam*H1 polymorphism with two common diseases in aging patients: colorectal cancer and ESRD in type II diabetes mellitus.
Clinical information and biological samples from 464 unrelated Romanian Caucasian voluntaries were collected. They are fall into four groups: CRC (patients surgical treated for CRC, n = 77), DZ2 (type II diabetes mellitus with ESRD, n=87) and HC (healthy controls, n = 300). Controls and patients were matched for sex, age and Romanian Caucasian ethnicity.
The HSPG BamH1 polymorphism was genotyped by PCR-RFLP. We found that the association between HSPG *Bam*H1 genotypes and ESRD is not significant. The same lack of association has been observed in CRC lot ($P > 0.05$). This result could represent a feature of Romanian population or a bias caused by study design.
Conclusions: These results show that the HSPG *Bam*H1 polymorphism is not an important risk factor for CRC or ESRD in Romanian patients.

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P4

The cellular response of human hepatoma cells with different expression of CYP3A4 isoenzyme to treatment with triazoloacridinone derivative C-1305

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Triazoloacridinones are potent antitumor compounds and developed at our Department. The most active derivative C-1305 was selected for extended pre-clinical studies.
Our previous studies showed that metabolic activation of C-1305 was crucial for its biological action. The aim of current studies was to examine how the expression level of enzymes responsible for drug metabolism can altered cell cycle progression and cellular response induced by C-1305. HepG2 cells and its mutant Hep3A4 (stably expressing CYP3A4) cells were selected for these studies.
C-1305 at EC50 concentration, did not induce any significant changes in the cell cycle distribution of hepatoma cells. However, gradual increase in the number of sub-G1 fraction of the cells was observed after prolonged drug treatment.
The morphological examination of HepG2 cells showed alterations of the nuclear morphology (condensed chromatin, apoptotic body-like structures), but only in limited part of cell populations. The morphological observation of Hep3A4 cells showed also the appearance of few cells with characteristic apoptotic morphology, concomitantly with much more enlarged cells with multiple micronuclei, typical for mitotic catastrophe.
Percentage of Annexin-V and PI positive cells increased gradually upon treatment and after 144 h it reached about 36% and 76% for HepG2 and Hep2A4, respectively, what indicated late stages of apoptosis and/or necrosis.
Surviving HepG2 cells, starting from 72 h, developed features of drug-induced senescence with flattened, enlarged morphology and increasing degree of SA-β-galactosidase staining. Such effect was also observed in Hep3A4 cells, but with a much lower efficiency.
The overall results suggest that C-1305 induce different cellular response (apoptosis, mitotic catastrophe, necrosis, senescence) in human hepatoma cells in dependence on the level of CYP3A4 isoenzyme.

P5

Effect of quercetin on apoptosis and telomerase activity in leukemia cell lines

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Along with being frequently consumed compounds of human diet, flavonoids need multiple mechanisms to be defined for their biological and pharmacological features. Major effects of flavonoids can occur in consequence of release of radicals. Another possible mechanism is activation of flavonoids over various enzyme systems. Cellular activities of them are unclear. For instance; along with protecting cells from oxidative stress, flavonoids can contribute apoptosis and genotoxicity of tumor cells by their prooxidant features.

In the previous studies, various effects of flavonoids such as anti-tumor, anti-oxidant and anti-inflammatory were indicated. Limited data related with being antagonist or synergistic of biochemical interactions among polyphenols found in fruits and vegetables exist. Characterization of potential interactions among these compounds can be effective in determination of the effects of nutrients contain polyphenol in prevention of cancer development.

Various hypotheses exist about having higher cancer risk reduction by consuming fruits and vegetables rich in terms of phytochemicals. Anti-cancer mechanisms, anti-oxidants, anti-imflammatory and anti-proliferative activities of flavonoids are associated with inhibition of bioactive enzymes and induction of detoxification enzymes. Furthermore, muscadine grapes also include sufficient amount of quercetin. Quercetin is a commonly used flavonoid as elagic acid. It affects cell cycle kinetics, proliferation and induction of apoptosis.

While normal cells lose their telomeric DNAs progressively, telomere length is stable in neoplastic cells. Telomerase is composed of a RNA component called hTR and, a reverse transcriptase component that a catalyses synthesis reaction is hTERT.

Although most of normal cells do not have telomerase enzyme activities, most of tumors have it. Investigations in solid and hematopoietic cancers showed that, telomerase expression would be a new target in cancer treatment and would be an important diagnostic parameter.

In our study we aimed to investigate quercetin dependent apoptosis induction, inhibition of proliferation of leukemia cells and the effects of quercetin in telomerase activity.

Cell viability, cytotoxicity and evaluation of apoptosis were performed by using Trypan blue dye exclusion, XTT and Acridine orange/Ethidium bromide dye technique assay. hTERT mRNA expression is determined by using Real-time Online RT-PCR.

As the effect of quercetin in cell viability evaluated in leukemia cell lines; CCRF-CEM, HL–60 and K562, IC50 was determined as 25 mM and a distinct decrease was determined in time and dose dependent manner in cell viability. As apoptosis evaluated, in HL- 60 and K562 cell lines, induced apoptosis correlated with dose was detected as 70% and 65% , respectively.

In the evaluation of hTERT mRNA expression, 12% and 25% decreases were detected in HL–60 and K562 cell lines respectively in IC50 doses.

P6

Corelations between apoptotic process and p53, p21 protein expression in laryngeal cancer

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Our study was determined by the fact that laryngeal cancer has a higher incidence in the last years, on the national and worldwide level, and the surgical treatment is a difficult procedure with grave consequences on the human body. This study aimed to determine whether the combined use of protein expression (p53, p21) and markers of apoptosis (bax and bcl-2) can predict prognosis in laryngeal cancer patients. We examined 29 patients with laryngeal cancer (more 65 years), according to the following criteria: no history of previous malignancies, primary squamous cell carcinoma of the larynx, no previous radiotherapy or chemotherapy, and complete surgical excision of the tumor. The nontumoral epithelial laryngeal tissues prelevated from these patients were used as the control. Therefore, in order to analyze the protein expression we used immunohistochemical staining (IHC), ELISA test and indirect immunofluorescence method. The apoptotic process was detected by using a flow-cytometric method.
Our data obtained by IHC showed that:
— in 15 patients (52%) was detected a increased p53 protein expression;
— there was a heterogenous distribution of p21 staining in tumor cells, with the proportion of positive cells ranging from 5 to 64% in the analyzed tumor;
— bax and bcl-2 protein expressions were detected in 13 patients (45%) and 5 patients expressed these proteins in the peritumoral inflammatory infiltrate; but the high levels of bcl-2 protein was expressed only in 15% of analyzed tumors, while we found an increased levels of bax expression in most 85% patients.
— none of the controls expressed of the proteins studied
— the frequency of spontaneous apoptosis was lowered in tumor tissues than in non tumor tissues, but in the case of patients which the p53 expression was high we inregistrated a increased level of the apoptotic process.
In conclusion, we found a good relationship between p53 expression and apoptosis in laryngeal tumors while p21 protein expression did not show any relationship with apoptotic process. In addition, high degree of apoptosis could be used to identify patients with poor prognosis in laryngeal cancer. Our results suggest that abnormal expression of p53, p21, bax and bcl-2 may be involved in the phases of laryngeal tumorigenesis and may be clinically relevant in patients with laryngeal cancer.

P7

Mesenchymal stem cell aging

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Adult stem cells are capable of self-renewal and multilineage differentiation, thereby maintaining homeostasis of their residing tissue throughout life. Given that aging can be envisaged as a process where tissues lose regeneration capacity and, as a consequence, decline in function, stem cells are greatly believed to play a considerably important role.
Besides in other tissues of higher organisms, mesenchymal stem cells (MSC) appear to be located in bone and bone marrow, supporting the continuous renewal and repair of variety of tissue types, as well as regulating hematopoiesis. By now it is poorly understood how MSC are involved in these processes during aging.
We set out to study the quantitative and qualitative changes this stem cell type undergoes during *in vitro* and *in vivo* aging by isolating MSC from bone of both females and males of different ages, and assessing the following properties: MSC numbers, their proliferative capacity, telomere length, differentiation potential, rate of endocytosis, and mRNA levels of several candidate aging biomarkers as revealed by array analysis.
Although MSC number remains largely the same, their proliferative capacity significantly declines with donor age. Interestingly, VCAM-1 mRNA levels were found considerably increased in elderly donors while telomere length and endocytosis rate remained unchanged. When cultivating MSC at physiologic oxygen conditions (3% O2 tension), proliferative capacity was found increased, however differentiation was by and large inhibited, while VCAM-1 mRNA levels were significantly lower. Notably, MSC are greatly responsive to pro-inflammatory cytokine such as TNFα which also upregulates VCAM-1 transcription.
We therefore conclude that MSC basic properties may be grossly altered *in vivo* by an inflammatory milieu. A continuous chronic stimulus may in due course lead to a decline in proliferation potential and the dysregulation of differentiation capacity thus contributing to a diminished regenerative vigor in older age.

P8

Determination of cytochrome P450 isoforms expressed in stomach tissue samples

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Cytochrome P450 (CYP) is a heme-containing enzyme superfamily metabolizing a wide variety of xenobiotics, including drugs and carcinogens. Over the past years there have been an increased number of researches on the relationship between CYP expression and cancer. Approximately 20 individual CYPs have been identified in human, however relatively little is known about the individual CYP forms present in stomach tissues. To understand more thoroughly the function of CYP systems in human gastrointestinal tissues and their significance in the metabolism of xenobiotics and endogenous compounds, we analysed the mRNAs for the CYPs in stomach tissue samples using reverse transcriptase-polymerase chain reaction (RT-PCR) method. The mRNA detections were made by using specific primers for CYPs -2B6, -2C, -2D6, -2E1, -3A3/4 and -3A5 and the expressions were compared to the CYP expression in liver tissue, the main organ of CYP synthesis. Among the CYP isoforms, CYP2A6 deserved a special attention because of its catalytic properties to nitrosamines. The tissue specificity of CYP expression may possibly underlie the organ specificity of chemical carcinogens and their potential roles of extrahepatic CYPs in the protection of the body against xenobiotics as well as influence in the bioavailability of therapeutic compounds.

P9

Phenotyping of melanoma cells: expression of ligands for activating receptors involved in NK cell-mediated cytotoxicity

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NK cell-mediated lysis of tumor cells is a complex process involving multiple interactions between NK receptors and their ligands on target cells. Although several NK activating receptors have been identified, NKG2D represents a unique receptor since its ligands are molecules frequently over-expressed by tumour transformed cells. In humans, the NKG2D receptor can recognizes MHC class I-related A and B antigens (MICA and MICB) and UL16-binding proteins (ULBPs). NKG2D ligands (NKG2DL) can be found up-regulated upon tumour transformation or infection and can activate NK cells. Our results showed that MICA/B molecules are expressed on 78% of melanoma cell lines whereas ULBP expression is found only in 25% of melanoma cell lines analysed. Detailed analysis showed that 85% of melanoma cell lines expressed at least one ligand for NKG2D. The high expression on melanoma cell lines of ligands for NKG2D suggests that NKG2D-NKG2DL interaction may represent an important mechanism in NK cell recognition of melanoma cells. Analysis of NKG2D-mediated cytotoxicity was done by using an NK cell line, NKL. NKG2D-NKG2DL interaction triggered NK cell cytotoxicity against several melanoma cells that was abrogated by the addition of anti-NKG2D, anti-MICA/B or anti-ULBPs. Although these results support the role of NKG2D in the immune surveillance against melanoma, the expression of MICA/B or ULBPs on melanoma cells did not always correlate with their susceptibility to NK cell-mediated lysis. Thus, even in the absence of inhibitory signals, some NKG2DL+ melanoma cell lines remained resistant to NK killing suggesting that other activating receptor-ligand interactions may be also required.

P10

Correlation of effector function with phenotype and cell division after *in vitro* differentiation of naïve MART-1 specific CD8+ T cells

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Adoptive transfer of antigen-specific CD8+ T cells may represent an effective strategy for immunotherapy of tumors such as melanoma, but is limited by the number and functionality of *in vitro* expanded T cells. Here, we document that although MART-1-specific CD8+ T cells from different donors initially possessed a naïve phenotype, after antigen-induced *in vitro* expansion two distinct phenotypes correlating with cell proliferation rate emerged in the different donors. Those cultures achieving fewer cumulative population doublings (CPD) displayed an effector (CD45RA+CCR7-) phenotype and were cytotoxic. In contrast, cultures reaching higher CPD were non-cytotoxic CD45RA- CCR7- phenotype T cells. Thus, the generation of larger numbers of MART-1-specific CD8+ T cells correlates negatively with the acquisition of a CD45RA+CCR7- phenotype and cytotoxic capacity. A better understanding of the differentiation pathways of cytotoxic T cells to obtain optimally efficient cells for adoptive transfer will allow the development of new immunotherapy protocols.

P11

In vitro reactivity of peripheral blood mononuclear cell from subjects with different genetic background to amyloid β (Aβ 42) peptide: implication for cancer immunotherapy

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Alzheimer's disease (AD) is a progressive and degenerative disorder of CNS that results in the loss of cognitive functions. The pathological hallmarks of AD are senile plaques and neurofibrillary tangles (NFT) in the brain, accompanied by neuronal and synaptic loss. The senile plaques are extracellular deposits in the brain parenchyma, mainly consisting of a 42 amino-acid peptide named amyloid β (Aβ42) (1–3). Recent studies have focused the attention in immunotherapy approach as possible solution to clear the neurotoxic amyloid beta peptide from the brain that leads to cognitive decline(4). Active immunization with the amyloid beta peptide of patients with AD has shown promising results, demonstrating slower decline of cognitive functions over a year period. However 6% of immunized patients developed meningoencephalitis that forced the cessation of clinical trial. Post-mortem analysis of brain section, revealed decreased amyloid plaques in neocortex regions associated with activated microglia and T cell infiltrates in CNS (5, 6).

The aim of the present study is to analyze the *in vitro* effects of amyloid peptide on PBMCs of young and old subjects, Alzheimer's disease patients or subjects with immunological risk phenotypes (IRP) and with different genetic background on genes implicated in the immune response (7). For this study we used a recombinant amyloid peptide (rAβ42) which has been recently expressed and purified (8). The study of the effects of this peptide on just mentioned subjects will be interesting to evaluate if there is a correlation between biological effects and genetic background, age, gender, presences/absences of Alzheimer. Eventually positive data, could give suggestions about the subjects to insert on trials to study the effects of vaccination. Moreover due the well known link between cancer and inflammation this approach can be useful in immunotherapy of cancer.

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P12

Differetiated use of endometrium ablation in patients with the endometrial hyperplasias

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Introduction: At present time the rate of the endometrial hyperplasias (EH) origin has increased sufficiently. Simultaneously the change of age proportion is registered towards the rejuvenation of the given pathology. Today the EH treatment is carried out taking into account the patient's age, her reproductive intentions, and also presence or absence of contraindications to the hormonal therapy prescription. There are two types of the EH treatment: conservative (hormonal therapy) and surgical (endometrium ablation, polyp resection, supravaginal amputation of the uterus with or without appendages). The conception about priority of conservative method of EH treatment in patients of reproductive and perimenopausal ages is present in modern medicine. However apart from the great number of contraindications to the hormonal therapy prescription conservative treatment is ineffective in many cases (35–50%) leading to such undesirable effects as psychological trauma and socioenvironmental inadaptability of person. Besides that the negative economic effect of the prolonged hormonal therapy is important too. In this connection we think that elaboration of the differentiated approach to the EH treatment in women of reproductive and perimenopausal age is very important.

Materials and Methods: We have studied the effectiveness of the endometrium hysteroscopic ablation in the reproductive and perimenopausal age patients with the EH taking into consideration nitric oxide (NO) activity in the uterine mucosa before the treatment. Endometrium ablation was carried out to 25 patients with the EH. All patients were divided into 2 subgroups depending on the presence (II-nd subgroup) or absence (I-st subgroup) of the disease recidivation (uterine bleeding) during one year of observation.

Results: The middle age of the I-st subgroup patients was 34.4±4.1, and in the II-nd subgroup this index was 35.3±4.8. NO activity in the I-st subgroup patients' endometrium was traced in 7 (41.2%) women and absent in 10 (58.8%) patients. In II-nd group patients the initial activity of NO in the endometrium was weak in 6 (75.0%) women and moderate in 2 (25.0%) patients. Therefore endosurgical endometrium ablation is sufficiently effective in the patients with initial traced or absence of NO activity in endometrium. At the same time the given method of treatment was complicated with recidivation like a uterine bleeding in the weak or moderate NO activity in endometrium.

Conclusion: NO activity in the endometrial tissue can serve as a criterion of treatment method choosing in reproductive and perimenopausal age women with the EH. In the absence or traced NO activity in the uterine mucosa the method of choice is hysteroscopic endometrium ablation that is highly effective. In the presence of weak and moderate NO activity and in the absence of contraindications the conservative (hormonal) therapy should be prescribed to the patients with EH.

P13

Identification of the new genes-markers of the endometrial cancer on the grounds of DNA ISSA-regions polymorphism

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The endometrial cancer is the most common invasive malignant tumor of the female genital tract in the industrial countries now. From 1999 till 2005 the endometrial cancer incidence rate have increased from 22.2 till 26.4 per 100 000 population in Ukraine. In accordance with WHO prognosis the given pathology incidence rate will exceed the breast cancer incidence rate in the female population. So the problem of an early diagnosis of the endometrial carcinoma that leads to more effective treatment of the given pathology is very actual at present time.

We have investigated the endometrial cancerous tissue received with the help of endometrial biopsy of 30 patients and compare them with endometrial tissue of 20 healthy women.

The new DNA parts (both expressing and surrounding sequences) that possibly participate in the endometrial carcinoma development (or they are markers for these) detection and identification was carried out with the help of sequencing of the polymorphic DNA ISSR-fragments that differ DNA specimens from endometrial cancer tissue and healthy tissue. The homology was established for sequenced polymorphic sequences with genes SIRT5, RANBP9, CD83, RAI2, PLK3 that can promote cancer genesis with own functions but don't discussed in literature as to the endometrial carcinoma development.

So our research was help to discover some genes that directly or indirectly take part in the endometrial carcinoma development and such data haven't reported in scientific literature yet.

P14

Protein oxidation and xanthine oxidase activity in hepatitis C virus-related hepatocellular carcinoma: role in tumor apoptosis and cytoproliferation

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Background: Hepatitis C virus (HCV) infection causes a state of chronic oxidative stress, which may contribute to hepatocarcinogenesis. The formation of reactive oxygen species (ROS) in the liver can promote oxidative damage of intracellular proteins, and thereby influence a diverse array of cellular processes. The present work was designed to study the changes in protein oxidation and xanthine oxidase (XO) activity, a primary source of ROS, in cirrhotic patients with hepatocellular carcinoma (HCC) in relation to the rates of tumor apoptosis and cell proliferation. **Methods**: The plasma levels of carbonyl proteins and advanced oxidation protein products (AOPP), as markers of protein oxidation, and plasma XO activity were determined in 50 patients with HCV-related cirrhosis (30 with histologically-proven HCC of different grades and stages and 20 without HCC) and 20 healthy subjects. Core liver biopsies of hepatic tumors were examnied for the rate of apoptosis using the terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) method and for mutant p53 expression by immunohistochemistry. The proliferative activity of HCC was quantitatively assessed using proliferating cell nuclear antigen (PCNA) labeling. **Results**: The plasma levels of carbonyl proteins and AOPP and plasma XO activity were significantly higher in cirrhotic patients with and without HCC than in healthy subjects and in patients with than in those without HCC ($P<0.0001$). The apoptotic rate was always less than the proliferative activity in HCC tissues and their ratio tended to decrease with higher tumor grade, size and stage and in p53-positive tumors ($P<0.001$). The markers of protein oxidation [but not XO activity] in HCC patients showed positive correlations with the proliferative activity and inverse correlations with the ratio between the apoptotic rate and the proliferative activity ($P<0.05$).

Conclusions: These findings suggest that enhanced protein oxidation and XO activity seems to play a role in the development and progression of HCV-related HCC with imbalance between rates of apoptosis and cytoproliferation. Bettter understanding of these processes might lead to the development of improved therapeutic approaches for the prevention and treatment of HCC.

P15

The diagnosis in angiosarcoma and kaposis sarcoma histopathological and immunohistochemical study

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Summary: Angiosarcoma is one of the rare sarcomas which occurs on the head and neck, especially on the scalp and face of elderly men. Kaposi's sarcoma is a mesenchymal tumor characterised by the proliferation of spindle-shape cells, neoangiogenesis, inflammation with fibrosis and hyperemia. It represents the most common tumor associated with HIV-infection.
This study investigates the histopathological and immunohistochemical features in different cases of Kaposi's sarcoma and angiosarcoma which are representative for medical practice.
Material and Methods: Ten cases of angiosarcoma and twenty cases of Kaposi's sarcoma from all histological stages (patch, plaque and nodular) were studied. The surgical excision pieces had been fixed in buffered 10% formalin, paraffin embedded and stained by Hematoxylin-Eosin for the histophatological examination. Antibodies used for immunohistochemical stainig were CD34, CD31, vimentin, cytokeratin, CK19, CD68.
Results: Histopathologically, angiosarcoma is characterized by multiple vascular spaces of different size lined by endothelial atypical cells with voluminous and hyperchromatic nuclei. Lymphocytic infiltrate is present. In nodular and tumoral stages of Kaposi's sarcoma, nuclear atypia as well as atypical mitoses, irregular vessels surrounded by tumoral cells, erythrocyte extravasation and the presence of siderophages may be encountered. The tendency of desposing the cells in a directional streaming pattern can be mentioned usually. Immunohistochemical stainig reveals that angiosarcomas express vimentin, CD34 and cytokeratin. The Kaposi's sarcoma lesions showed a diffuse positivity for vimentin; CD34 and CD31 was positive in vascular structures and cytokeratin was negative. Antibodies anti CD31 are one of the most specific endothelial markers.
Conclusion: The diagnosis of such tumors can be confirmed by immunohistochemical staining. In correlation with anatomo-clinical findings, this method permits a good differential diagnosis and rules out a large number of lesions with a completely different specific treatment.

P16

Metabolism and aging: oxidative stress and chronological lifespan in *Saccharomyces cerevisiae* strains with single deletions in glycogen metabolism

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Chronological aging in yeast represents an interesting model because of its resemblance with aging in mammalian, post-mitotic tissues, that show low proliferation but conserved metabolic activity. The survival in the stationary phase in yeast is strongly associated with oxidative-stress resistance. In the present study hypo- and hyper-glycogenic phenotypes of *S. cerevisiae* strains with deletions (Euroscarf, Yeast Deletion Project) of glucidic-metabolism enzymes were selected, a comparison of their chronological lifespans was achieved, and the following assays were performed in the emerged candidates: stress sensitivity, ROS levels, and apoptosis markers during aging. Among the strains that accumulated greater amounts of glycogen, the deletion of glycogen phosphorylase, gph1Δ (59 *vs* 29 μg glycogen/10^8 cel. in wt. $P<0.05$), was the unique in showing a shortened lifespan, stress intolerance, and higher levels of ROS during its survival. The transcription of SOD1 and 2 were analyzed in gph1Δ, the transcript levels being 4- and 3-fold lower than in wt at the end of the stationary phase (8 and 5 *vs* 31 and 16 UA in wt, respectively. $P<0.05$), and during aging. Hypo-glycogenic deletions as the one of glycogen synthase, gsy2Δ (8 μg/10^8 cel. $P<0.05$), demonstrated a little longevity advantage but similar stress tolerance, ROS and RNA levels of SOD1/2 compared with the wt. Low-copy-plasmid-mediated overexpression of SOD1 and SOD2 together rescued gph1Δ from its accelerated aging and stressed phenotype.
The incapability to degrade glycogen (deletion of GPH1), produced a strain with rapid-aging what would be attributed, at least in part, to the impoverished stress resistance associated to the decreased transcript levels of both SOD in this mutant. It remains to further clarify the putative dialogue between glycogen availability and the negative regulation of these genes in the aging process.

P17

The cAMP dependent kinase mediates glucose-deprivation-induced apoptosis in hepatic cells

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Glycolysis and apoptosis are highly conserved and finely regulated multi-step processes, which are crucial for cell homeostasis. Both pathways connect at different levels, although the links are not fully clarified. Hepatocytes have a key role in glucose metabolism. The cAMP dependent kinase (PKA) mediates the modulation of diverse hepatocyte functions, being its specificity ensured by compartmentalization by its anchoring proteins (AKAPs). Our aim was to study if glucose deprivation induces apoptosis in primary-cultured or immortalised hepatic cells, and if PKA is involved in this pathway. Rat hepatocytes or the hepatocarcinome-derived cells HepG2 were cultured for 6 h in the presence (C) or absence (Glc0) of glucose, with or without the PKA inhibitor H89. Cell viability and the activation of apoptosis was analysed. Cell viability as assessed by Tripan Blue exclusion and lactate dehydrogenase release to the medium did not differ between groups. Glucose withdrawal induced caspase 3 activation in primary cultured hepatocytes (8.6 ± 1.8 *vs* 12.0 ± 2.2 pmol product/mg protein/min, C and Glc0, respectively, $P<0.05$), but not in HepG2. This effect was prevented by the incubation with H89 (7.6 ± 4.7 *vs* 7.0 ± 2.7 pmol product/mg protein/min, C+H89 and Glc0+H89, respectively). Apoptotic phenotype in glucose-deprived hepatocytes was confirmed by nuclear DAPI staining.
Our results indicate that 6 h-glucose deprivation induces apoptosis in normal hepatocytes but not in HepG2 cells. It would be interesting to analyze the basis of the lack of this apoptotic response in the transformed hepatic cells. We found that PKA mediates this pathway in normal hepatocytes. It has been characterised a complex assembled by the AKAP WAVE-1 in hepatic mitochondrias pulling together glucokinase, PKA, the protein phosphatase PP1, and the proapoptotic protein BAD. Further studies are necessary to elucidate if this complex has a role in the integration of glucose metabolism and apoptosis.

P18

Cellular localization of the proto-oncogenic p53 inhibitor AGR2 protein in cancer

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Proteomic technologies verified AGR-2 as a protein family over-expressed in human cancers, including breast, prostate and oesophagus cancers, with the ability to inhibit the tumour suppressor protein p53. AGR2 gene is a hormone responsive gene with an unexpected induction by the anti-cancer drug tamoxifen highlighting the pro-oncogenic role of this protein.
Anterior Gradient-2 encodes one protein that gives two forms·the full length and the mature one. We have analyzed the mechanism of regulation and function of the AGR protein family. Localization studies of AGR2 were performed using fluorescence microscopy in order to determine in which compartment the protein functions. Although full-length AGR2 localizes to the ER and the Golgi compartment, the mature AGR2 protein requires the C-terminal KTEL sequence for strong nuclear localization. Deletion of the KTEL, potential ER retention, sequence do not alter the localization of the wt full length form in a big extent but has a strong effect on the localization shift of the mature one. Subcellular fractionation data verified the difference in the localization patterns of the wt forms and their mutants. Moreover, the localization of the protein and each of the mutants differs significantly in various cell lines, suggesting a multi-potent role of the protein when it comes to activation pathways and localization patterns within the cell. Yeast two hybrid analysis has identified potential nuclear binding proteins for AGR2 and we present data showing models of how the AGR2 family might function as drug-resistance survival factor in cancer as well as a p53 inhibitor.

P19

Reversible stop of the development fat dormouse's in experiment as a possible key to the decision of a problem of ageing of the human

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Unique feature of biology fat dormouse (*Myoxus glis*) — the stop of age changes previous hibernation — is found out. Animals can be in such condition vaguely long (the reversible 16-months arrest of development of young fat dormice). The certain sets of factors of an environment (variability of a light mode, cooling of an organism) possess property to interrupt the given condition, and at long influence variable a light mode ability of an organism to stop age changes completely is lost.
Received in experiment the reversible stop of development can be interpreted as hypothalamic diapause during which the quantity of receptors in cells hypothalamus remains constant, that will well be coordinated with some positions elevation hypothesis of ageing by V. Dilman. Such diapause should be caused by change of a expression level one or several genes in cells of hypothalamus. The obtained results may serve the evidence of the possibility of the mammals' biologic age "freezing". Not looking at that fat dormouse is not the widespread laboratory object, studying of the unique mechanism of an arrest of development at this kind could become a basis of a technique operated reversible stops of age changes at animals and the human.

P20

Silencing of the expression of an antiapoptotic protein in cancer treatment — preclinical data

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Insensitivity to apoptotic stimuli is one of most problems in cancer treatment. Inhibition of activity of proteins that belongs to IAP (Inhibitor of Apoptosis Protein) family is one of solutions of this problem. There are many papers about inhibiting or silencing survivin – the most studied member of IAP family. Expression of this protein is cell-cycle-dependend, but there is no evidence that expression of survivin causes carcinogenesis. In our studies we aimed to break blockade of apoptosis in cancer cell lines by decreasing protein level of the others IAP family members, involved in inhibition of caspases 3, 7, and 9.
We assumed that decreasing a level of this protein should sensitize cells to apoptotic stimuli and even could induce apoptosis without any additional agents. We had designed fifteen siRNA sequences against one of those genes and we have tested them using number of human cancer cell lines (A549 — lung cancer model, JIMT 1, MCF7, MCF7bcl2 [MCF7 with bcl2 overexpression], MDA-MB-231 and MDA-MB-231bcl2 [MDA-MB-231 with bcl2 overexpression] — breast cancer models and PC3 — prostate cancer model). In our screenings the first cut-off was defined by inhibition of proliferation, which was measured by MTT assay. Measurements were performed 24h, 48h and 72h after transfection.
In almost all cell lines tested the best silencing was observed 48h after transfection. The best silencing was achieved with sequence No. 7 — the number of viable cells was only 10% of the control. We observed induction of apoptosis by siRNA molecule alone as well as the increase of sensitivity to apoptotic stimuli (by Oxaliplatin and VP16). The decrease in mRNA and protein level after treatment with siRNA was in correlation with inhibition of proliferation.
Among fifteen sequences designed we have chosen few which will be studied *in vivo*. Basing on our results we find IAP members as potent targets for anticancer therapy. It is known that efficient and specific siRNA delivery system is needed and we are working on to find it.

P21

Gene expression alterations in prostate cancer cells due to crosstalk with osteoblasts in the process of bone metastasis

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Prostate cancer preferentially metastasizes to bone and osteoblast-derived factors specifically support its progression. At early stages of bone metastasis, crosstalk of prostate cancer cells and osteoblasts through soluble molecules results in a decrease of cancer cell proliferation, accompanied by altered adhesive properties and increased expression of bone-specific genes, or osteomimicry. The mechanism by which these changes, which presumably facilitate prostate cancer colonization of the bone, are incurred, remains unclear.

Osteoblasts synthesize a wide variety of biologically active factors, which contribute to the unique bone microenvironment. Fibroblast growth factor-2 (FGF2) and interleukin-6 (IL6) are two important proteins which can influence osteoblastic differentiation and function, and have also been linked to prostate cancer progression. In an *in vitro* model of bone metastasis, we find that osteoblasts secrete high levels of FGF2 and IL6, whereas prostate cancer cells produce these proteins in low or undetectable amounts.

This study investigates the ability of FGF2 and IL6 to induce gene expression changes in prostate cancer cells, with a focus on osteomimicry and osteolysis. Known downstream target genes of FGF2 and IL6 signaling become upregulated in prostate cancer cells due to crosstalk with osteoblasts or exposure to osteoblast-conditioned medium. These include osteomimetic molecules such as bone morphogenetic protein-2 (BMP2) and osteopontin (OPN), as well as factors involved in regulating osteolysis: transforming factor beta-1 (TGFβ1), receptor activator of nuclear factor-kappa B ligand (RANKL) and osteoprotegerin (OPG). The mechanism and significance of these alterations in gene expression is currently under investigation.

P22

Analysis of anti-tumor immune responses induced with 12-aa constrained peptide mimics of GD2 ganglioside in mouse models

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Children with high risk neuroblastoma have poor outcome despite multimodal therapy. This stresses the need for new therapies to control minimal residual disease. Over-expression of GD2 ganglioside (GD2) on neuroblastoma cells opens the possibility to use the antigen as target for immune attack. However, if such active immunotherapies are to be successful, they have to overcome self-tolerance to GD2 and its poor immunogenicity in humans.

We used phage display technology and anti-GD2 mouse monoclonal antibody 14G2a to identify and characterize five peptide mimics of GD2.

In a BALB/c mouse model we tested the five peptides conjugated to KLH protein carrier in adjuvant setting for induction of GD2-specific humoral immune responses. We showed that sera samples from the peptide-KLH immunized animals contained antibodies recognizing the ganglioside. In a complement dependent cytotoxicity assay on IMR-32 cells we also measured higher effector functions of pooled sera samples form animals immunized with 3 peptide vaccines as compared to control samples.

Finally, to evaluate anti-tumor efficacy of the selected peptides we used a mouse neuroblastoma model, based on A/J mouse strain and syngenic NXS2 neuroblastoma cell line. In preliminary experiments, we analyzed protective anti-neuroblastoma effect of 2 peptide-KLH vaccines. The neuroblastoma growth was induced 1 week after the last immunization by i.v. injection of 1.5×10^5 of NXS2 cells. Animals (control non-immunized group, KLH-immunized group and peptide–KLH immunized groups) were screened for the presence of the metastases in liver, ovaries, lungs, lymph nodes and peritoneum. We had also analyzed sera samples to find immune correlation of the anti-neuroblastoma protection induced with GD2 mimotopes. As compared to control non-immunized animals, the mice that had received peptide-KLH vaccines showed prolonged life and significantly reduced number of metastases. Further research is planned to investigate and optimize the observed anti-neuroblastoma effect of the GD2 mimotopes.

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P23

The relation between estrogen and mitochondrial genom in hereditary breast cancer

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Background: Both environmental factors and genetics have an impact on the risk of BC. Although the effects of environmental factors may vary with age, it has been assumed generally that the penetrance of BRCA mutation is constant throughout life. Approximately 90% of breast cancers are sporadic while the remaining 10% are inheritable. Mutations of BRCA1 and BRCA2 genes are associated with a greatly increased risk for development of hereditary breast cancer (HBC). After gender, age is the strongest known predictive risk factor for breast cancer and the number of breast cancer cases rises dramatically with age. In other hand, mitochondria do not only produce less ATP, but they also increase the production of reactive oxygen species (ROS) as byproducts of aerobic metabolism in the human aging. In this article, we will develop a model for predisposition to HBC incorporating the ER, BRCA mutation and mtDNA4977 mutation.
Material and methods: Patient samples were drawn from four medical centers in Iran. We retrieved 203 formalin-fixed, paraffin-embedded tissue blocks from women with breast cancer diagnosed, the age of 25–80 years for the years 2004 and 2006. All cases were reviewed using a special questionnaire, which allowed taking into account the presence or absence family history of breast cancer and also other pathology information. Verification of every cancer reported in a relative was sought through pathology reports, hospital records. Multiplex PCR was used to detect the simultaneous detection of three common mutations. For each BRCA mutation, three primers (one common, one specific for the mutant, and one specific for the wild-type allele) were used.
Results: Seventeen mutations were detected by multiplex PCR from 204 patients. The proportion of cases with one of three BRCA mutations (5382insC) was approximately 9% familial breast cancer. Among HBC selected in our study 75% and 69% were negative for ER and PR respectively, and 63% were positive for TP53. We detected the mtDNA4977 deletion in 56% of the peripheral blood samples with ER (negative).
Conclusions: In the current study, the results demonstrated that certain BRCA mutation exhibit HBC risk associations that vary considerably with age. Compared with non-HBC cases, the expression of ER was lower at young ages and the relative risk of the expression of ER was decreased by age in HBC.However, our results shows the BRCA1 inhibits the transcriptional activity of ER and that tumor-associated mutants of BRCA1 failed to inhibit ER activity or showed quantitatively reduced inhibition. In other hand, germ-line point BRCA mutations transmitted from ancestors accelerate the somatic oxygen damages and mutations in mtDNA leading to phenotypic expression of premature aging and breast cancer.

P24

Cell arrays-approach to high-throughput cell line screening on immunocytochemistry base assessed by new software colgraph

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Associated cell blocks – cell arrays – are new approach to the standardisation of immunohistochemical methods (IHC). They are suitable for proteomic research and are ideal tool for extensive studies carried on cell lines – we can screen effects of new therapeutics and test antibodies and their dilution for IHC. Great advantages of this method are low material usage, huge volume of highly reproducible data assessed by computer analysis with defined colour intensity levels and possibility of comparing 23 different specimens of cell lines in one experiment.
Primarily, cell suspensions are fixed in formalin, embedded in agarose and than in paraffin to form blocks. Subsequently, cylinders (1 mm in diameter) are punctuated from the each block, and placed in distinct positions in cell array. Complete array is than sliced by microtom and thawed to SuperFrost+ glass. Cell line arrays can be used mainly for immunocytochemistry testing and DNA in situ hybridisation techniques or drug effects screening.
The purpose of our cell array development was to overcome the problems of classical immunocytochemistry on formalin-fixed paraffin-embedded material and to improve the testing of newly developed antibodies.
By this methodical approach, we analysed the expression of p53 and MDM2 proteins with antibody DO-1 and 2A9, respectively, in breast cancer cell lines using newly designed software for automatic assessing IHC-ColorGraphicAnalysis (COLGRAPH). We quantified p53 expression and determined p53 status in cell lines on the basis of comparing histoscores of proteins p53 and MDM2. We successfully pointed up a distribution of wild type and mutant p53 cell lines according to homogeneity of p53 expression and to positivity of p53 IHC reaction.
We developed a new methodological approach to IHC that is full of flexibility, because array can be designed according to acquired attributes like cell origin, protein expression, studied treatment etc. In the combination with new software ColorGraphicAnalysis (COLGRAPH), this method gives us a powerful tool for extensive sample screening in studies. On the basis of positive results meant above, our method is going to have rich possibilities of application in research and diagnostics fields in future.

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P25

Determination and signification of E6 gene promoter in human papillomavirus 16 in cervical intraepithelial neoplasia and cervix carcinoma

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Human papillomaviruses (HPVs) are small non-enveloped dsDNA viruses with circular genome. High-risk tribes of HPVs frequently infect cervical mucosa of women and demonstrably give rise to cervix intraepithelial neoplasia (CIN) and carcinoma. HPV 16 and 18 belong to the most risk HPV tribes. Early viral proteins E6 and E7 are able in cooperation to transform cells of infected epithelium by functional blocking tumour suppressor proteins p53 and pRb. E6 in cooperation with human ubiquitin-ligase marks p53 for degradation, while E7 facilitates phosphorylation of pRb and loosens S-phase transcriptional factor E2F. With respect to a high number of infected women and to the relative low number of cervix carcinoma, we suppose an existence of inactivating epigenetic mechanism (CpG methylation in E6 gene promoter — it means inactivation) in control of CIN and carcinoma rise.

Aim of our study was to determine the occurrence of HPV 16 and 18 in patients with CIN or cervix carcinoma and healthy or asymptomatic women and to analyse the methylation status of E6 gene promoter of HPV 16. The presence of viral DNA was detected by PCR with new designed highly specific primers; in HPV 16 positive samples the methylation of E6 gene promoter was proved by PCR using DNA digested by specific restriction endonuclease McrBC.

We analysed 51 cervix smears from healthy women (histological negative smears), where HPV 16 and 18 positivity was determined 57%. HPV 16 and 18 were present in 87% of 118 patients with CIN III and in 83% of 41 cervix carcinoma samples.

The methylated state was detected in 73% of negative smears compared to 35% in CIN III group; likewise unmethylated status was detected in 65% of CIN III and 58% of carcinoma compared to 27% in negative smears group.

We detected that the frequency of HPV 16 in our samples is higher compared to published data. This result confirms high sensitivity and specificity of newly introduced PCR detection system. We found important differences in distribution of methylated and unmethylated states among negative smears, CIN III and carcinoma. These results imply the importance of epigenetic state of E6 gene promoter in cervix pathogenesis. This method should be applicable in routine diagnostics for assessment individual risk of patients infected by HPV 16.

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P26

Innate immunity of blood leukocytes in senile diseases

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Background: In our previous studies an deficiency of innate immunity was found in elderly (>60) healthy and leukemia patients, Now, the innate immunity of carcinoma and Alzheimer disease (AD) patients (age>60) was compared with control group in aspect of:
— resistance of leukocytes *ex vivo* to viral infection,
— secretion of cytokines (TNFα, IFNs, IL-10, IL-12).
Methods: The resistance of leukocytes to VSV infection was designated just after isolation. Titer of VSV 0–1 log TCID50 indicate complete resistance, titer 2–3 log for partial resistance, > 4 log indicate for very low or lack of resistance. Cytokines were determined by microplate ELISA test.
Results: Results of experiments showed that leukocytes of the three groups are very sensitive to VSV infection, but in particular cytokines production some differences were shown. The leukocytes of cancer group (n = 15) produced more TNFα than the control (n = 10) and AD group (n = 35), less IFNs and IL-10, and not et all IL-12. The leukocytes of AD patients produce high level of early IL-12 and IL-10 (spontaneous and VSV-induced) and less than control TNFα and IFNs.
Conclusion: The sensitivity of leukocytes in these three groups is caused by deficiency of innate antiviral immunity. In the development of innate immunity different panel of cytokines produced by leukocytes of patients with senile diseases: TNFα in cancer and IL-12 in AD.

P27

Short term effect of metformin against oxidative stress induced DNA damage in lymphocytes from healthy aged subjects

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Metformin(1-(diaminomethylidene)-3,3-dimethyl-guanidine) is an anti-hyperglycemic agent which has also antioxidant effects. Although the origin of the antioxidant activity of metformin is not clearly understood, it might result from direct effect on reactive oxygen species or indirect action on superoxide anions which are produced by hyperglycemia. However, the ability of metformin to modulate DNA damage which is produced by oxidative stress is not known. For this reason, we examined the short time effect of metformin (50 μM, 2 h) on the DNA damage of cumene hydroperoxide (CumOOH)-induced lymphocytes from aged (n=l0) and young control (n=l0) groups. In this study, DNA damage which is caused by oxidative stress produced by CumOOH (l mM) was detected with Comet Assay and ELISA technique. According to our results, significant increase in apoptotic DNA fragmentation and DNA strand breaks (Comet assay tail factor %) was detected before and after CumOOH induction in lymphocytes of healthy elderly, when compared with healthy young control. Metformin was significantly decreased CumOOH-induced apoptotic DNA fragmentation and DNA strand breaks in lymphocytes from aged subjects, although it did not produce a long term effect. These results indicate that the *in vitro* short term effect of metformin is able to protect against the prooxidant stimulus induced-DNA damage in lymphocytes from elderly subjects.

P28

Mortalin-based distinction of normal and immortal human cells

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Mortalin is a stress chaperone that is differentially distributed in normal and cancer human cells. By using nanoparticles (quantum dots), a highly sensitive and photostable alternative to the conventional organic dyes, we demonstrate that mortalin staining pattern changes when senescence is induced in cancer cells by low doses of a variety of drugs. To develop a more specific mortalin-based induced-senescence detection kit, we generated a large variety of anti-mortalin monoclonal antibodies. We found that some of the anti-mortalin antibodies have unique cell-internalizing property. By using these unique cell-internalizing antibodies as nano-carrier for optic molecules (FITC and quantum dots) and DNA, we found that these antibodies display cancer cells specific internalizing property and can be used for (i) cancer diagnostics and (ii) therapeutics involving cancer cell specific gene delivery.

P29

Use of quantum dots for *in vivo* imaging, cell tracking and cancer diagnostics

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Quantum dots (QDs) are fluorescent nanoparticles that emit higher and wide range of fluorescence than the conventional organic probes and are photostable. Since their first use, as an alternative to organic fluorescent dyes, they have made their way in molecular analysis, bio-imaging, diagnostics and therapeutics. Toxicity of QDs, their internalization in living cells, optical resolution and stability have been the issues of concern, and have led to rapid modifications and developments of a variety of QDs with modified surface properties.

We conjugated the QDs with an internalizing antibody and found that the QD-antibody conjugate efficiently gets internalized into the cancer cells. We investigated the fate of internalized QDs inside the cells and found that they were visible even after multiple cell cycles *in vitro* and *in vivo*. The illuminating cells (i-Cells) thus generated by internalized QDs underwent normal cell divisions, without affecting normal functions of the cells, *in vitro* and *in vivo*. We demonstrate that the internalized QDs are non-toxic and provide a sensitive tool for cancer diagnostics.

P30

Evaluation of the blood antioxidant defense status in Polish centenarians

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Background: There is substantial evidence that free radical reactions are implicated in aging and pathogenesis of age-related diseases. The efficiency of antioxidant mechanisms seems to be crucial for maintenance of proper body functions. In view of a complexity and a co-operative nature of the blood antioxidant defense system, there is still a need to find out markers suitable for assessment of the total antioxidant status.

Objective: To develop a predictive model that translates individual enzymatic and non-enzymatic blood antioxidants measures into an index of global defense.

Methods: Fasting blood samples from 156 centenarians (24 male and 132 female) and 66 elderly 65-y old (23 male and 43 female) apparently healthy individuals were assayed for RBC activities of antioxidant enzymes (superoxide dismutase-SOD, catalase-CAT, glutathione peroxidase-GPx, glutathione reductase-GR) and plasma concentrations of α- and γ-tocopherols, retinol and uric acid. Total antioxidant potential index (POTAOX) was calculated as a sum of standardized values (mean=0; SD=1) of both enzymatic and non-enzymatic antioxidants.

Results: As compared to the group of elderly subjects, the POTAOX index adopted lower values in centenarians and tended to decrease with a decline in their cognitive status as assessed in the Mini Mental State Examination (MMSE) test. Female centenarians were characterized as having higher values of POTAOX index than males, mainly due to higher activities of antioxidant enzymes (SOD, CAT, GPx), whereas uric acid was found to contribute the most to the blood antioxidant system of male centenarians. Supplementation of the oldest old individuals with vitamin and mineral micronutrients positively affected the efficiency of antioxidant defense as evidenced by changes in their POTAOX index values.

Conclusions: The reported model represent an useful tool for determination of individual blood antioxidant status.

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P31

Cell–cell fusion of the glioblastoma T98g cell line

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Cell–cell fusion is a phenomenon that affects precisely defined cells during different stages of the organism development. Since fusion plays also a significant role in tissue remodeling, as well as in tumor progression, it is necessary to understand better this process. The knowledge concerning fusion comes mainly form studies employing viral systems. This approach, besides many benefits, is artificial and moreover the spontaneous fusion may be regulated distinctly, especially in case of cancer cells. In the present study we describe the unique ability of glioblastoma multiforme T98g cell line to perform spontaneous fusion. Our results show that these highly poliploid and anuploid cells fuse together what lead to formation of multinuclear cells. Since many tumor cells are anupolid the fusion might be the process that helps to survive cells without appropriate pattern of chromosomes. Simultaneously, glioblastoma T98g appears to be a good model to study cell–cell fusion.

P32

Cross talk between reactive oxygen species producing NADPH oxidase and mitochondria

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Recent studies have suggested that NADPH oxidase (Nox) enzymes are not only present in phagocytic cells but also in various non-phagocytic cells. For example, Nox1 is primarily present in normal colonic epithelium, vascular smooth muscles, uterus and prostate. The Nox family of proteins contains seven other members: Nox1-5, Duox1, and Duox2. These flavoproteins catalyze the NADPH-dependent reduction of oxygen to superoxide and other related reactive oxygen species which are involved in intracellular signaling. Nox enzymes are the second major source of ROS in cells besides oxidative phosphorylation in mitochondria. Based on these observations, we tested the hypothesis that mitochondria control Nox1 redox signaling, and that the loss of this control contributes to tumorigenesis. Our study revealed that mitochondria indeed control Nox1 expression. Confocal microscopy studies revealed that Nox1 localizes in the perinuclear mitochondria and to a lesser extend in the cell membranes. Secondly, inactivation of mitochondrial genes led to down regulation of Nox1, and the transfer of wild type mitochondrial genes restored Nox1 expression to a level comparable to that in parental cell line. Thirdly, exposure of cells to the mitochondrial inhibitors antimycin and rotenone as well as uncoupler FCCP caused upregulation of Nox1. In addition, our histochemistry studies revealed that Nox1 was highly expressed in breast (86%) and ovarian (71%) tumors. Additionally our *in vitro* studies revealed that over expression of Nox1 in MDA MB 435 cells resulted in increase of colony formation in soft agar which is an indicator of increased tumorigenicity of the cells. More interestingly, Nox1 over expressing cells showed oncogene induced senescence phenotype. Further investigation is underway to identify the genetic mechanisms of mitochondrial regulation of Nox1 and the role in tumorigenesis.

P33

The polymorphisms in estrogen, glucocorticoid and leptin receptor genes associated with the altered risk of diabetes mellitus and cardiovascular events, may play a role in longevity promotion

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Background: All hormones exert their biological functions by interactions with their receptors (HRs). Even subtle changes in the sequence of the gene encoding HR might result in the damage of gene activity or alter the sequence and function of the encoded receptor. Disturbed action of HRs involved in the regulation of glucose and fatty acids homeostasis can lead to the development of cardiovascular disease, diabetes mellitus, stroke and many other disorders affecting human longevity.
Aim of the study: The purpose of our study was to look for the potential association of the selected single nucleotide polymorphisms (SNPs) in the genes encoding estrogen α (ESR1), glucocorticoid (GR) and leptin (LEPR) receptors with ageing.
Material and Methods: 147 Polish Centenarians and 412 young controls (aged 18–45) were genotyped for the following polymorphisms: T-397C, A-351G in the ESR1, ER22/23EK, N363S, BclI in the GR and K109R, Q223R, K656N in the LEPR by the PCR-RFLP method.
Results: Analysis of the K109R SNP in the LEPR revealed that the "KK" variant was significantly less frequent in Centenarians compared to controls (42.5% *vs* 52.7%, $P = 0.034$, OR = 1.51). For the BclI C/G polymorphism located in the GR we observed a trend for the higher frequency of the "GG" genotype in Centenarians, compared to the young individuals (18.9% *vs* 13%, $P = 0.089$, OR=1.56). Analysis of the of remaining SNPs revealed no significant differences in alleles' and genotypes' frequencies between the studied groups ($P > 0.05$). The investigated polymorphisms remained in a strong linkage disequilibrium that suggested that they may construct haplotypes. However haplotypes constructed by ESR1, GR and LEPR polymorphisms were equally distributed in the both studied groups ($P > 0.05$).
Conclusions: The results of the present work suggest that certain polymorphisms in genes encoding hormonal receptors regulating glucose and lipids homeostasis (K109R in LEPR which influence glucose tolerance and BclI in GR which influence BMI value) may play a role in natural ageing. However further studies performed in larger groups are required to put this data into perspective.

P34

The association between GSTP1, MDR1, and MTHFR polymorphisms and the recurrent status of breast cancer patients treated with 5-fluorouracil, epirubicin, and cyclophosphamide (FEC) adjuvant chemotherapy

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In the present study, multiple genetic polymorphisms, including Glutathione S-transferase P1 (GSTP1), multidrug resistance 1 (MDR1), 5,10- methylenetetrahydrofolate reductase (MTHFR) and thymidylate synthetase (TS) tandem repeats, were analyzed in breast cancer patients by using combinations of gene polymorphisms to predict the clinical outcome of breast cancer patients receiving 5-fluorouracil, epirubicin, and cyclophosphamide (FEC) adjuvant chemotherapy. Genomic DNA was isolated from the peripheral blood samples of 192 breast cancer patients who underwent operation. The genotypes were determined by means of a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Patients' characteristics and prognosis of postoperative relapse were investigated and analyzed with GSTP1, MDR1, MTHFR and TS genotype groups.
TS tandem repeats showed no significant association with postoperative recurrence between each genotype when considered individually ($P = 0.598$). However, there was a significant correlation between postoperative recurrence in patients with gene polymorphisms of both MDR1 3435CC and MTHFR 677CC (OR: 2.609, $P = 0.013$) and patients with additional GSTP1 313AG gene polymorphism. The GSTP1, MDR1, and MTHFR genotypes could be potential prognostic factors in breast cancer patients receiving FEC adjuvant chemotherapy, where gene-gene interactions among the GSTP1, MDR1, and MTHFR genotypes may occur.

P35

Clinical and biochemical implications of impaired aging process in B-CLL lymphocytes

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B cell chronic lymphocytic leukemia (B-CLL), common disorder especially among elderly Europeans, is characterized by clonal expansion and accumulation of high numbers of CD19+CD5+ B cells in peripheral blood due to defective apoptotic mechanisms. We have demonstrated earlier that failure to execute cell death may be dependent on the disturbances in the calpain-calpastatin system's senescence in leukemic B-lymphocytes and subsequent m-calpain overexpression and hyperactivity. Therefore, we decided to determine the exact mechanisms of influence of that system's impairment on apoptosis and its potential clinical implications in B-CLL.

To verify whether abnormal increase of m-calpain may affect any of the most crucial apoptotic factors in B-CLL cells, the intracytoplasmic activities of initiatory and executory caspases -9 and -3 respectively were estimated by direct flow cytometry, while concentrations of caspase-3 and Bcl-2 (suggested to increase in and be responsible for apoptotic arrest of B-CLL cells) indirectly with Cytometric Bead Array system, without or after calpain inhibition with membrane-permeant calpain inhibitor IV (Z-Leu-Leu-Tyr-CH2F). Cytometrically assessed levels of intracellular m-calpain were related to the disease stage (using the Rai-Sawitsky staging system) and to the treatment status of patients included in the study; similar relation was investigated also for the active caspase-3 and -9 values.

We noted statistically significant correlations of the μ-calpain content with clinical staging of the disease and with chemotherapy treatment, which enables us to postulate a vital role of m-calpain as a potential prognostic and predictive factor in B-CLL.

Our findings show that calpain inhibitor IV increases the amount of both active caspase-3 and -9 in untreated patients, while simultaneously decreasing Bcl-2 quantity. However, patients undergoing chemotherapy presented grossly different dependences, which suggests a novel concept of chemotherapeutics interactions with calpain-calpastatin system in B-CLL.

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P36

Prevalence of cancer stem cells and its association with ER, PR and Erb2 in breast cancer patients

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Cancer stem cells or tumorigenic cells are a small population of tumor cells that posses classic features of stem cells such as self-renewal and proliferation. Whereas majority of cells in the tumuor have limited proliferation property and cannot self-renew. The capacity of breast tumour development has been shown to be limited to CD44+/CD24-/low epithelial tumour cells. The importance of detection of breast cancer stem cells is due to its implication on development of new therapeutic strategies.

It has been also showed that in animal model ERalpha/PR+ cells scattered through the epithelium are stem cells that self-renew through asymmetric cell division and generate patches of transit amplifying and differentiated cells.

In the recent studies, we have been investigating breast cancer tissues for the prevalence of cancer stem cells and their prognostic values. Immunohistochemistry was applied to identify the population of cancer stem cells in the paraffin embedded tissues of 300 breast cancer patients. The prevalence of cancer stem cells were then correlated with level of expression of ER, PR and Erb2, also with prognostic factors including tumour size, lymph node stage, and tumour grade.

Majority of anticancer therapies target non-tumorigenic cells in tumour, while cancer stem cells are still survive and leading to tumour recurrence. Therefore, strategies designed to target cancer stem cells in combination with current treatments may lead to more effective therapies.

P37

Naive T cells in aged mice are more susceptible to death upon TCR ligation

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With age, immune system shows poor response to vaccination and memory recall. Both the innate and the adaptive components of the immune system function suboptimally. We have compared responses of naive T cells from aged mice with young mice in terms of proliferation as well as functionality. Upon stimulation with CD3+CD28, the number of CD44low naive T cells from aged mice that actually respond by upregulation of early activation markers, proliferation and cytokine secretion is lesser. Addition of exogenous IL2 to cultures rescues these defects partially in T cells from aged mice. Also our studies on c-Rel$^{-/-}$ mice and IL2$^{-/-}$ mice, which show that these mice also exhibit aged like defects, seem to indicate that there might be a contribution of suboptimal NFκB activity to the T cell defect in aged mice. The number of T cells from aged mice that go into cycle is smaller and those that do, go through fewer cycles when compared to the T cells from young mice. Besides, upon TCR ligation, naive T cells from aged mice tend to die more, which has been analysed by flow cytometry using Annexin V staining for early death and Sytox Green staining for total death. In absence of any stimulus, the death in naive T cells from young mice is much more than in those from aged mice. We hypothesize that the ability of naive T cells from the aged to undergo lesser number of divisions and be more susceptible to death upon TCR ligation result in poorer differentiation of T cells from aged mice to CD44high-CD62Lhigh central memory (CM) phenotype resulting in marked reduction in CM to EM ratios *in vitro*. This also supports our finding that the ratio of CM to EM *ex vivo* is significantly lesser in aged mice.

P38

Naive T cells from aged mice progress poorly to the central memory stage and show increased susceptibility to death upon primary activation

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The poor T cell responses in aged animals have been largely attributed to the lack of IL-2 production upon stimulation. We find that the frequency of antigen-reactive T cells generated *in vivo* is only modestly lower early after immunisation in aged mice than in young mice, but declines further over time in aged mice. Naive-phenotype T cells from aged mice respond to CD3+CD28 ligation with a reduced frequency of CD69 induction and poor proliferation, and these differences are only modestly alleviated by exogenous IL-2. Activated naive T cells from aged mice also show poor progression from an effector-memory (EM) to a central-memory (CM) phenotype, and the CM:EM T cell ratio is lower in the peripheral lymphoid organs of aged mice than of young mice. Further, while naive T cells from aged mice are relatively resistant to neglect-induced death (NID), they undergo a Fas- and IL-2-independent form of death upon activation at a greater frequency than cells from young mice. Those naive T cells from aged mice or young mice that survive activation *in vitro*, however, show similar susceptibility to re-activation-induced death. Thus, IL-2-independent early deviations involving activation and death may contribute to poor generation of long-lived memory in aged individuals.

P39

Protection from age-dependent cognitive impairment by pharmacological stimulation of β-adrenoceptors in rats

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A key event of age-related deficit in learning and memory is the decrease in cholinergic activity of basal forebrain (BF) neurons. The decrease of trophic support by NGF for the cholinergic neuron in aging brain is associated with neuronal atrophy and appearance of neurodegenerative diseases such as Alzheimer's disease. Administering neurotrophic factors could represent a strategy for the treatment of these disorders. However, the therapeutic administration of these compounds seems to be complicated because of the side effects they produce. The pharmacological induction of endogenous NGF synthesis in the brain could be an elegant way to overcome application problems. Therefore, the present experiment was undertaken to determine the influence of prolonged pharmacological stimulation of NGF biosynthesis on learning and memory in aged rats. To address these issues we used young (4 month old) and aged (28 month old) rats and specifically profiling possible recovery from cognitive deficit through endogenous NGF replacement strategy using pharmacological stimulation of neurotrophin synthesis. Clenbuterol (β_2-adrenergic receptors agonist) was delivered in the drinking water in two different doses daily during the period of eight weeks. Animals were trained immediately after termination of NGF synthesis stimulation. The cognitive behaviour of the young and aged rats was assessed by an object re-location (OLT) and object recognition (ORT) test and in the long-lasting "Non-matching to Position Test" (NMPT) — acquisition and reversal. Pharmacological stimulation with orally delivered agonist of β-adrenoceptors, clenbuterol had no effect on the cognitive abilities in young rat. In opposite, clenbuterol-treated aged rats learned much better than the respective aged control animals, however only in NMPT test. Our data suggest that in aged rats, clenbuterol positively affects cognitive processes related to formation of associations established in recognition memory and discrimination tasks, which required higher neural integration, but is ineffective on processes involved in the spatial memory and attention. As reported previously, clenbuterol has been showed to increase NGF synthesis in the brain. Thus, we suggest that neuroprotective activity of clenbuterol was exerted by endogenous NGF induction.

P40

Functional characterization of *AATF* transcriptome in human Leukemic cells

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The study addressed to explore the transcriptional expression and regulation of *AATF* gene within various types of Leukemic cell lines, revealed that *AATF* gene was overexpressed ubiquitously in all the leukemic cell lines studied and this upregulation was accompanied by *c-myc* gene overamplification in these cells. Downregulation of *AATF* gene transcription within leukemic cells not only resulted in the downregulation of c-myc gene and *vice-versa* but also contributed to apoptosis leading to cell death. Further link between *AATF* expression and leukemic cellular apoptosis involved PI3K/Akt pathway. Based on these results we propose that *AATF* gene may be of crucial importance in maintaining the leukemic state of a cell compartment through its ability to initiate cell proliferation coupled with repression of cellular apoptosis. The regulation of this gene may also assume importance in devising various orthomolecular medicines for treatment of leukemias.

P41

Effect of Zinc supplementation on thymic output in elderly

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Background: For over 50 years Zinc has been known to affect the function of the immune system and been shown to improve immune function in Zinc deficient young individuals. Age-related changes to the immune system cause a decline in the ability of elderly individuals to combat acute infection and chronic diseases. These changes are also associated with a reduction in the responsiveness to the protective effects of vaccination.
Objectives: The current study aimed to establish an accurate measurement for thymic output in individuals older than 60 years of age. In addition to determine the contribution made by Zinc supplementation on thymic output in the elderly individuals from different population across European.
Design: Healthy elderly individuals were given oral Zn supplements of 10 mg per day for 6 weeks. Peripheral blood samples were taken before and after supplementation, and measurements of thymic output were assessed using the TREC assay in each individual.
Results: A baseline measurement of 0.59 TREC per 10^5 T cells was found in the elderly population before Zinc supplementation with a marked decrease observed above the age of 90 years. Females were found to have higher TREC levels compared to males. Additionally, significant differences were observed across different European countries. After supplementation no significant difference was observed in the level of thymic output measured. However, a trend for individuals with particularly low TREC measurements to increase following supplementation was noted in some countries.
Conclusions: Thymic output can be detected in individuals between the ages of 60–99 years, at a level of 0.59 TRECs per 10^5 T cells. Using the current Zn supplementation regime no difference in thymic activity was seen.

P42

Disturbed aging process in B-CLL lymphocytes' calpain-calpastatin system and its correlations with apoptosis

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B-CLL (B cell chronic lymphocytic leukemia), a relatively benign lymphoproliferative disorder occuring mostly among elderly people, is characterized by an accumulation of monoclonal CD19+CD5+ B cells. Defective apoptosis is suggested to contribute to B-CLL cells' accumulation and resistance to therapy. We have shown before that B-CLL cells are characterized by increased amount and activity of a cysteine protease, calpain, documented to participate in the apoptosis of other cell types. This is in contrast to decreased calpain activity noted in lymphocytes of elderly people, suggesting that in the B-CLL cells aging process is reversed. As the calpain activity is controlled by its intracellular inhibitor, calpastatin, the aims of our study were to examine the activity of the calpain-calpastatin and its influence on apoptosis of B-CLL lymphocytes.
B-CLL cells were obtained from peripheral blood of patients with established diagnosis. The calpain protein amount was detected by flow cytometry and its total activity was measured by casein zymography. The actual calpain activity in B-CLL cells was measured by Western Blotting method detecting levels of calpastatin and its calpain-cleaved fragments. The influence of calpain activity on the calpastatin level and the level of apoptosis was checked by blocking the calpain activity with Z-Leu-Leu-Tyr-CH2F. Influence of calpain inhibition on apoptosis was detected by mitochondrial depolarization and loss of membrane asymmetry.
We have found that increased calpain protein level in B-CLL cells was higher than in normal elderly, and even young individuals' B lymphocytes. Contrarily to typical calpastatin level changes occuring in aging, calpastatin in B-CLL cells not only decreases significantly, but also undergoes advanced degradation processes probably due to distinct calpain hyperactivity. The fact suggests that calpastatin changes are secondary to calpain activity. Calpain inhibition induced apoptosis in B-CLL lymphocytes, the effect was strongly time-dependent.
Our findings present a new concept of the disease, suggesting the aging processes disturbances in calpain-calpastatin system and proposing a novel attitude to cell death impairment observed in B cell chronic lymphocytic leukemia, with possible clinical implications.

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P43

Doxorubicin-induced senescence like state of HCT116 cells lead to spindle checkpoint failure and increased genomic instability

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It is believed that one of the features of cancer cells that account for their uncontrolled proliferation is loosing the ability to senesce. However, it becomes apparent that cancer cells can be forced to undergo senescence. Senescence-like growth arrest can be cancer cell response to chemio/radioteraphy observed both, *in vitro* and *in vivo*. We showed that human colon cancer HCT116cell undergo senescence-like process upon doxorubicin treatment. They cease to proliferate (Ki-67 negative), enlarged their size and increase activity of senescence associated -β-galactosidase. Induction of senescence was associated with intensive polyploidyzation of cells. Examination of cell cycle related proteins expression revealed gradual decrease of proteins involved in mitosis regulation, like cyclin B, survivin, Aurora B and significant increase in p53, p21 and cyclin D1 level. Although, this molecular analysis suggests loss of proliferation potential of dox-treated HCT116 cells, the growth arrest of the whole population was transient. After few days' culture, we observed appearance of small, proliferating cells that escaped form dox-induced senescence. Those cells have changed chromosome numbers, suggesting that on the route to senescence increased genomic instability was induced leading to aneuploid progeny. Microscopic observations of polyploidy cells having amplified centrosomes number revealed that at least some of them undergo the process of asymmetric cell division. Derivatives of parental HCT116 cells which do not express p53 proteins (HCT116 $p53^{-/-}$) did not undergo polyploidization/senescence. Eventually, after few days they died upon dox-treatment as estimated by high level of PARP cleavages and DNA content analysis. Measurement of mitotic index and DNA content of dox-treated cells cultured in the presence of nocodazole revealed spindle checkpoint failure. Thus, induction of senescence in dox-treated HCT116 cells is dependent on p53 expression and correlated with disrupted spindle checkpoint leading to increased genomic instability and in consequence resumption of cell proliferation.

P44

Differential *in vitro* Inhibitory effects of some anti-cancer drugs on tumor-associated carbonic anhydrase isozymes CA-IX and CA-XII

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Hypoxia constitutes a challenging clinical problem, being common in many cancer types which are inaccessible to radio- and chemotherapy. CA IX and to a smaller extent also CA XII are highly overexpressed in hypoxic tumors that many such anti-cancer drugs were shown earlier to possess strong *in vitro* and *in vivo* cell profileration and metastacis on cancer types.
In this prospective study we have evaluated the efficacy of preoperative inhibition effects of 11 different anti-cancer drugs, namely Anzatax (Paclitaxel), Methotrexate-Teva (Amethopterin), Lastet (Etoposid), Campto (Irinotecan), Gemzar (Gemcitabin), 5-Fluorouracil / EBEWE, 5-Fluorouracil / Biocyn, Eloxatin (Oxaliplatin), Ellence (Epirubicin), Cisplatin and Carboplatin on the tumor associated carbonic anhydrase isozymes CA-IX and CA-XII.

P45

MTHFR (C677T) polymorphism and comet assay in ovary cancer

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MTHFR catalyses the reduction of 5,10-methylenetetrahydrofolate to 5-methylenetetrahydrofolate. This product is a methyl donor in the synthesis of methionine from cysteine. 5-methyltetrahydrofolate is the primary form of plasma folate. MTHFR folate metabolism has a very important role in DNA synthesis and repair. C677T polymorphism has 3 genotypes: homozygote mutant TT, heterozygote CT and homozygote CC.
Comet assay is a method used for to show DNA fragmentation in eucaryotic cells. The cells embeded into the agaroz on microscope lam and broken with alkali solution and appeared DNA seperated by electrophoresis.
The aim of this study was to determine C677T polymorphism and show DNA damage by Comet assay in patients with ovarian cancer in different grades. 50 ovarian cancer poatients and 54 healhy subjects were involved in this study. The genomic DNA was isolated from peripheral blood to determine C677T polymorphism, the DNA was amplified by PCR and products visualized under UV light after running on agarose gel electrophoresis.
If we compared the CC, CT, TT genotypes of C677T polymorphisms of ovary cancer with controls CC, CT, TT ratios are n = 18 (%36), n = 28 (%15), n = 4 (%8), respectively in ovary cancer patients, n = 19 (%35.2), n = 30 (%55.6), n = 5 (%9.2), respectively in controls. There was no statistical difference between CC, CT, TT genotypes of patients and control groupes. DNA fragmentation increased due to increasing grades of patients with ovarian cancer. This result may be due to DNA methylation deficiency.

P46

Overview how adenocarcinoma cancer cells avoid cell elimination; the mechansims of imune-resistance

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Colon adenocarcinomas posses several tactics to avoid cell death and to maintain cell viability. In particular, colon cancer cells resist death ligands-induced apoptosis by expressing antiapoptotic proteins including cFLIP. The results of my studies indicate that by direct interaction with FADD, the cFLIPL protein inhibits the signal transmission from death receptors to their cytoplasmic targets in COLO 205 cells. Colon cancer cells also stimulate own survival by inhibiting cytotoxic signals induced by interferons (IFNs). Moreover, IFN-γ increases the resistance of colon cancer cells to immune response by the activation of NF-κB. Additionally, the cytoplasmic retention of proapoptotic protein clusterin also supports viability of cancer cells. Upon suitable stimulation normal cells are featured by clusterin translocation to the nucleus with concomitant cell death. I found that proapoptotic activity of clusterin depends on calcium ions, and depletion of intracellular calcium pool causes extensive death of COLO 205 cells. The variety of antiapoptotic mechanisms in colon cancer is a great challenge for contemporary medicine. Detailed knowledge of the molecular mechanisms leading to antiapoptosis in tumor cells gives promise for more efficient and complete deletion of cancer by the use of immunotherapy.

P47

Decreased frequency and proliferative response of invariant Vα24 Vβ11 natural killer T (iNKT) cells in healthy elderly

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Invariant natural killer T (iNKT) cells represent a well-established T cell lineage characterised in humans by TCR consisting of an invariant α chain encoded by Vα24-JαQ genes, paired preferentially with a Vβ11 chain. iNKT cells also share some characteristics with NK cells, such as the expression of the NK-associated receptor CD161 in humans. The physiological role of iNKT cells has been well documented in anti-tumor immune responses. However, there are a limited number of studies on the effect of ageing on peripheral blood iNKT cells. Thus, in this work we analyse the effect of ageing on peripheral blood Vα24(+)Vβ11(+) iNKT cells by studying their frequency, phenotype and proliferative function in elderly individuals fulfilling the SENIEUR criteria of healthy ageing compared with healthy young donors. Our results demonstrated a significant decrease of the percentage of Vα24(+)Vβ11(+) iNKT cells in elderly donors. No significant differences were found in the expression of CD27, CD28, CD45RO, CD45RA(bright), CD161, CD94 and NKG2D on iNKT cells from young and elderly individuals. Proliferation of Vα24(+)Vβ11(+) iNKT cells in response to α-GalCer and IL2 was analysed by calculating the cumulative population doubling (PD) after 14 days of culture. The PD levels were lower in the elderly indicating that Vα24(+)Vβ11(+) iNKT cells from healthy elderly subjects had an impaired proliferative capacity. These results indicate that ageing associates with a significant decline in the percentage and proliferative response of peripheral blood iNKT cells. Since iNKT cells are important in various immune system responses including tumor immunosurveillance and response to infectious agents, these alterations in their number and function could contribute to the deleterious immune response in the elderly.

P48

Inhibitory effect of serotonin derivatives on short-term high glucose-induced adhesion and migration of monocytes on human aortic endothelial cells

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Acute hyperglycemic spikes could exert an influence on the onset of diabetic complications and on the development of the atherogenic profile on diabetic and non-diabetic subjects. Short-term high glucose (HG) exposure, either on the scale of hours, could enhance the monocyte adhesion and migration to the subendothelium *via* increasing expression of adhesion molecules and release of chemotactic factors, phenomena that represent the key events in the early stages of atherosclerogenesis. Despite of multiple adhesion molecules involvement and the importance of intercellular adhesion molecule-1 (ICAM-1) in monocyte-endothelial cell adhesion, the induction of vascular cell adhesion molecule-1 (VCAM-1) after 4 hours HG exposure suggests the principal role of VCAM-1 in the early stage of monocyte adhesion. Furthermore, early increased transmigration activity was mainly due to monocyte chemoattractant protein-1 (MCP-1) release, as shown by using specific monoclonal antibody against MCP-1.
Hyperglycemia-induced overproduction of superoxide seems to be the first event in the activation of all pathways involved in HG pathogenesis and complications; however, many large-scale intervention trials have failed to demonstrate the beneficial effects of antioxidant vitamins. In the present study, using human aortic endothelial cells (HAECs), we investigated the inhibitory effect of N-(p-coumaroyl)serotonin (CS) and N-feruloylserotonin (FS) on short-term HG-induced superoxide-dependent increase of VCAM-1 and MCP-1 that leads to adhesion and transmigration of monocytes. CS and FS exerted a protective effect on HG-induced overproduction of mitochondrial superoxide acting as scavengers of the superoxide radical, thus preventing the consequent activation of NF-κB, and up-regulation of VCAM-1 and MCP-1 mRNA and protein. These results suggest that CS and FS provide a strategy for therapeutic delivery in various aging disorders and complications related to reactive oxygen species.

P49

The XPD polymorphism, which plays a role in cancers development, might be associated with longevity

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Background: The molecular mechanisms underlying human ageing remain largely unknown. Segmental progeroid syndromes, such as Werner syndrome (WS) or xeroderma pigmentosa-Cockayne syndrome (XP-CS) present some features of natural ageing therefore, they could be a good reference point for research on ageing. WS and some cases of XP-CS are caused by molecular defects in the genes encoding helicases (WRN and XPD, respectively) — the enzymes unwinding genomic DNA for repair, replication and transcription. Effective DNA repair seems to be especially important as one of the basic mechanisms protecting cells from damage.
Aim of the study: The aim of the present study was to investigate the potential association of the selected, functional single nucleotide polymorphisms (SNPs) in WRN and XPD genes with natural ageing.
Material and Methods: 145 Polish Centenarians and 400 young controls (aged 18–45) were genotyped for the C1367R, L1074F and R834C polymorphisms in the WRN and K751Q, D312N, and R156R SNPs in the XPD by the PCR-RFLP method.
Results: Analysis of the XPD polymorphisms revealed that the frequency of the K751Q "CC" genotype *vs* "AC" genotype was significantly lower in Centenarians than in the control group ($P = 0.016$, OR = 0.517). Two of the investigated SNPs in the XPD: R156R and D312N were in a strong linkage disequilibrium, that suggested that they may be inherited as haplotypes. However, analysis of haplotypes did not reveal any difference between Centenarians and young controls ($P > 0.05$).
Conclusions: In this study we found that the XPD K751Q "CC" variant, which could modify the amino-acid configuration in a domain important for interactions with helicase activator p44, is less common in Polish Centenarians. Interestingly, in previous studies this genotype was associated with potential risk of some cancers, therefore it may influence length of human life.
Results of our study point to the potential contribution of the XPD variants to the pathogenesis of natural ageing, however additional functional studies are required to determine the role of this finding.

P50

Inhibitors of 5-lipoxygenase modulate the activity of mitogen activated protein kinases (MAPKs) and NF-κB in leukaemic HL-60

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The block of haematopoietic differentiation program in cells of acute promyelocytic leukaemia (HL-60) can be overcome with treatment with differentiating agent (Chou *et al.*, 2005). Side effect of this approach is the selection of resistant clones. Study of signal pathways leading to differentiation is therefore still useful tool to predict future targets of anti-leukaemic therapy.
In our previous study was evidenced that co-treatment of HL-60 cells by cytokine TNF-α and inhibitors 5-lipoxygenase (5-LOX) MK-886 and AA-861 potentiated both differentiation effect and apoptosis of single treatments. This work was focused on assessment of activity of crucial proteins involved in regulation of those processes as are kinases of MAPK family and NF-κB transcription factor.
NF-κB activation induced by TNF-α was quickly down-regulated by inhibitors of 5-LOX and this decrease in activity of known pro-survival regulator may be in a direct association with higher amount of apoptotic cells. Activation of p38 and ERK (p44/42) kinases was observed shortly (15 min) after treatment whereas Jun N-terminal kinase (JNK) activity was down-regulated. After three days the activity of kinases changed – pro-survival ERK was down-regulated and pro-apoptotic JNK and p38 were slightly increased (Nyunoya *et al.*, 2005, Su *et al.*, 2005, Ahn *et al.*, 2005). Increased frequency of apoptotic cells was therefore not surprising. We can also conclude that p38 and ERK pathways play an active role in process of differentiation and apoptosis induced by co-treatment with 5-LOX inhibitors and TNF-α.

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P51

Investigation of three founder mutations in BRCA1 and BRCA2 in Iranian breast cancer patient

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Background: Breast cancer (BC) is the most commonly diagnosed cancer in Iranian women, and is the leading cancer cause of death in this population. Mutations in the hereditary breast cancer suppressor gene BRCA1/2 account for almost half of the familial breast cancers (FBC) and the majority of the combined familial mammary and ovarian malignancies. The mutations with the highest number of registrations associated with breast cancer are 185delAG, 5382insC (in BRCA1) and 6174delT (in BRCA2). Mutation analysis of BRCA1/2 genes is helpful in the determination of developmental potential, early diagnosis and gene therapy for breast cancer. In our study, we used multiplex PCR to analyze breast cancer patients for three BRCA mutations in tissue samples using immunohistochemical features as criteria.
Material and methods: Patient samples were drawn from three medical centers in Iran. We retrieved formalin-fixed, paraffin-embedded tissue blocks from women with breast cancer diagnosed, the age of 25–80 years for the years 2004 and 2005. Eighty– fourth samples were used for multiplex PCR and immunohistochemical diagnosis. All cases were reviewed using a special questionnaire, which allowed taking into account the presence or absence family history of breast cancer and also other pathology information. CINAGEN Inc.'s DNA Extraction Kit was used to isolate blood and tissue DNA. A simple and rapid method was used to detect the simultaneous detection of 185delAG, 5382insC (in BRCA1) and 6174delT (in BRCA2). Morphological and Immunohistochemical diagnoses of breast cancer were retrieved from their hospital records.
Results: The proportions of cases for women with at least 1 first-degree relative with breast cancer were 32.1% in Iranian breast cancer pateints. One of three BRCA mutations (5382insC) was detected by multiplex PCR in 3 breast cancers samples. Comparison presences of 5382insC mutation in tumor samples with family history and without family history have shown that frequency of 5382insC mutation was higher in familial samples ($P<0.001$) relatively non-familial breast cancer samples.
Conclusions: The incidence of FBC increases with age, doubling about every 10 years until the menopause, when the rate of increase slows dramatically. The relative risk of breast cancer conferred by a first-degree relative with breast cancer was detected 2.08 (95% confidence interval [CI], 2.0–2.2) in young women (<50 years) and it is decreased by age. The findings of the present study suggest that family history and age may have an impact on the incidence of breast cancer in Iranian women. Our analysis shows testing of 5382insC mutation in breast cancer can be utilized as one of prognosis factors of FBC development risk in combination with ER, PR and TP53.

P52

Adult bone marrow — and cord blood-derived very small embryonic like stem cells

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Several lines of evidence support the hypothesis that pluripotent stem cells (PSC) are present in adult bone marrow (BM) and cord blood (CB). Further supporting this hypothesis is a report indicating the expression of typical PSC markers Oct-4 and Nanog (embryonic stem cells transcription factors) and SSEA (stage specific embryonic antigen) at the protein and/or mRNA level in BM- and CB-derived stem cells. Accordingly, these embryonic markers were demonstrated and described by our team in very small embryonic-like (VSEL) stem cells (*Leukemia*, 2006, **20**: 857–869 & *Leukemia*, 2007, **21**: 297–303) and by others in multipotent adult progenitor cells (MAPC), mesenchymal stem cells (MSC) and marrow-isolated adult multilineage inducible (MIAMI) cells. In addition to BM and CB, several groups have recently reported the presence of Oct-4+ cells in epidermis, heart, pancreas, testis, and bronchial epithelium. Since SSEA, Oct-4 and Nanog are the markers characteristic for embryonic stem cells (ESC), epiblast stem cells (EPSC) and primordial germ cells (PGC), the presence of these cells in adult tissues supports the concept that adult tissues contain some population of PSC that is deposited in embryogenesis during early gastrulation. It is hypothesized that these cells could be direct descendants of the germ lineage. In order to pass genes on to the next generations, the germ lineage creates soma and thus becomes a "mother lineage" for all somatic cell lineages present in the adult body. It is also hypothesized that, as with PGC, PSC deposited in the developing tissues undergo erasure of their somatic imprint. This mechanism of erasure will protect the developing organism from the possibility of teratoma formation. However, it also affects some of the aspects of the "true pluripotentiality" of these cells (e.g., their potential to complete blastocyst development). It is postulated that these Oct-4+ PSC play a role in steady-state conditions in tissue turnover (e.g., as a source of long-term hematopoiesis repopulating cells). Furthermore, during organ damage (e.g., heart infarct or stroke) these cells could be mobilized from the BM and perhaps other tissue-specific niches into peripheral blood, where they circulate in order to "home" to damaged organs and participate in their repair. On other hand they may be also a source of malignancies. Accordingly, if these cells i) do not erase somatic imprint, ii) go astray from the major migratory routes, iii) acquire critical mutations or iv) are mobilized at the wrong time into peripheral blood and are deposited in areas of chronic inflammation, they may contribute to the development of malignancies (e.g., teratomas, germinomas, pediatric sarcomas and other tumors, respectively) instead of playing a role in regeneration.

P53

Molecular biology and physiological studies of aging rat heart

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Ischemia is a frequent phenomenon associated with aging which leads to changes in myocardium metabolism. The aim of our study was to point out how highly interconnected network of intracellular signaling reactions behaves in stress conditions imposed by 45 min ischemia followed by 60 min reperfusion of isolated rat heart of different ages and to what extent physiological parameters of cardiac contractility such as: heart rate (H.R.), coronary flow (C.F.) and left ventricle systolic pressure (L.V.S.P.) are influenced, as well as to assay ventricular cells for apoptosis. Isolated rat hearts of 6 and 37 months old have been mounted and perfused with Krebs Hanseleit buffer at 37°C in Langendorff retrograde perfusion system at a constant pressure over 60 min (ie. at 10′, 20′, 30′, 40′, 50′, 60′) intervals have been determined: H.R.,C.F. and L.V.S.P TACS Apoptotic DNA Laddering Etd. Br. kit (R&D System England) has been used to assay ventricular myocites for apoptosis. Our data have pointed out that, in old rats H.R. exhibits higher values than in young controls. C.F. is variable in time in aging rats *versus* young ones where is a slow decrease during the experiment. LVSP is net elevated in aging rats, but with fluctuations in time. DNA laddering on agarose gel electrophoresis was noted in ageing left verticular cardiomyocites.

P54

The G-11391A promoter polymorphism in the adiponectin gene that plays a role in the development of type 2 diabetes, is more frequent in Centenarians

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Background: The length of human life depends on many genetic and environmental factors. It is suggested, that single nucleotide polymorphisms (SNPs) located in the genes responsible for the development of diseases which frequency raises with age, might influence human longevity. Some age-related diseases, e.g. diabetes mellitus and cardiovascular disorders, are a result of the disturbances in lipids and carbohydrates metabolism, that is regulated by hormones such as leptin and adiponectin.
Aim of the study: The aim of the present study was to investigate if selected SNPs located in regulatory regions of genes encoding leptin (LEP) and adiponectin (ADIPOQ) may be associated with the length of life.
Material and Methods: 147 Polish Centenarians and 412 young controls (aged 18–45) were genotyped for A-11426G, G-11391A and C-11377G SNPs in the adiponectin gene promoter, and G-2548A SNP in the leptin gene promoter, using the PCR-RFLP method.
Results: We observed that the frequency of the -11391 "AA" ADIPOQ genotype was significantly higher in Centenarians than in the control group (2.01% *vs* 0.25%, $P = 0.046$, OR=8.45). This polymorphism was in a strong linkage disequilibrium with two other investigated SNPs, that suggested that they may be inherited as haplotypes. However, analysis of haplotypes formed by the three studied ADIPOQ polymorphisms did not reveal any difference between Centenarians and young controls ($P>0.05$). The genotypes of the LEP G-2548A polymorphism were also equally distributed between the both studied groups.
Conclusions: In this study we found a rare ADIPOQ -11391 "AA" variant to be more frequent in Centenarians compared to the young subjects. In some studies the "A" allele together with the ADIPOQ C-11377 variant was reported to play a protective role in type 2 diabetes development. Our results suggest that the -11391 "AA" genotype also may act as an independent factor in longevity promotion.

P55

On the principles of immune system adaptation

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To describe normal state of the immune system, a theoretical approach is considered based on the assumption about the availability of the immune system goal-seeking behavior — physiological adaptation. To characterize immune defense effectiveness, energy cost of host-pathogen interactions is estimated. To study the influence of environmental changes on the immune defense parameters, the stationary model of the immune system adaptation is suggested. The results of numerical experiments are used for the interpretation of the immunostimulation protective effect in chronic infections as well as for the explanation of infection anergy development in HIV infection. Some implications of this approach for aging research are discussed.

P56

Escape from premature senescence induced by DNA topoisomerase II inhibitors in human A549 tumor cells is dependent on ATM/ATR signaling

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Cellular senescence is one of the mechanisms which prevents the development of cancer by eliminating cells which acquired potentially deleterious DNA mutations. Recent studies show that treatment of tumor cells with anticancer drugs may lead to permanent growth arrest with phenotypic features of senescent cells. We have recently characterized effects induced in A549 cells by different DNA topoisomerase II inhibitors, including compound ICRF-187 and triazoloacridone C-1305. We showed that these cells exposed to IC80-IC90 doses of studied drugs ceased cell proliferation after 1–2 cell divisions and accumulated mostly in G2/M after drug treatment. Drug-induced growth arrest was accompanied by morphological and biochemical features of senescent cells as well as specific changes in the expression of proteins involved in DNA damage response and regulation of cell cycle pogression. Curiously, after prolonged post-incubation of drug treated cells (1–2 weeks), a small fraction of growth-arrested cells re-started proliferation. We postulated that induction of long-term growth arrest in tumor cells by topoisomerase II inhibitors may represent a new type of resistance mechanism.

In this study, we further characterized molecular mechanisms responsible for induction and maintenance of pseudo-senescence process by topoisomerase II inhibitors. In particular, we determined whether re-growth of drug-treated tumor cells can be prevented by using pharmacological modulators of DNA damage checkpoint and stress kinases. Based on our results, we conclude that senescent phenotype induced by studied topoisomerase II inhibitors becomes irreversible when the ATM/ATR pathway is inhibited during treatment of cells with anti-topoisomerase drugs. Our results may have important pharmacological implications considering that drug-induced premature senescence is believed to represent a new therapeutic approach to treat human cancers, as an alternative to induction of cell death by anticancer treatment.

P57

Menopause in Egypt: an overview from past to present

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With the rising life expectancy of women in the developing countries, the physical and emotional manifestations accompanying the menopause have become important health issues in these countries, including. Egypt Although osteoporosis was diagnosed in an ancient Egyptian female mummy dating from the XIIth dynasty (1990–1786 BC), who is estimated to have died at age 60, there is currently a paucity of data regarding the menopause in Egyptian women.

The mean age of the menopause in Egypt is 46.7 years, which is low compared to many countries, but this age has been rising recently. The incidence of menopause-associated symptoms in Egyptian women is higher than in the West, probably because of the different 'sociocultural attitudes' towards the menopause in different communities. Bone mineral density charts have been constructed for Egyptian women and show that, in general, they have a lower bone mineral density compared to their Western counterparts.

After the menopause, they suffer from osteoporosis, particularly at the femoral neck. Egyptian women do not know much about the menopause, except that the incidence of osteoporosis is increased. Their attitude towards the menopause is generally positive and about one-third of them regard the menopause as 'a normal physiological change'. Nevertheless, there exists a need for an awareness campaign in order to educate them about this important stage of their lives.

The aim of this work was to review the current health status of Egyptian menopausal women, in particular the prevalence of symptoms and signs and the various treatment modalities offered to this large group of Egyptian women, as well as their concerns and attitudes towards this important stage of their lives using data extracted from publications in refereed national and international studies

P58

Primary mitogens stimulate hepatocyte proliferation in old rodents after 70% partial hepatectomy and accelerate liver regeneration following sub-total liver resection

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The magnitude of DNA synthesis and the time of maximal DNA synthesis after two/third partial hepatectomy (PH) is greatly reduced in the liver of aged rodents when compared to young animals (1, 2), suggesting an intrinsic defect in proliferation of old hepatocytes. On the other hand, treatment of aged mice with primary mitogens caused an increase in hepatocyte proliferation similar to that seen in young mice, indicating that hepatocytes retain their proliferative capacity in the elderly, if challenged with appropriate proliferative stimuli (3). Here, we investigated whether primary mitogens could ameliorate the reduced regenerative response after 2/3 PH observed in old mice and rats. Sixteeen month-old mice given a single dose of the CAR-nuclear receptor ligand TCPOBOP shortly prior to 2/3 PH, were sacrificed after 48 h. While very few hepatocytes underwent into S phase 48 hours after surgery, pre-treatment with TCPOBOP stimulated a strong proliferative response (Labelling Index (LI) was 0.7 and 20.7%, respectively). The increased LI was associated with enhanced protein levels of cyclins D1 and A, PCNA and p107. Similar results were obtained when Wistar rats were pre-treated with the triiodothyronine (T3), a ligand of TRs. Indeed, a LI of 16% was found in the liver of rats receiving T3 prior to PH, *vs* a LI of only 2% in rats subjected to PH alone. Further experiments aimed at investigating whether primary mitogens could accelerate liver regeneration following major liver resection (90% PH) showed that T3 strongly enhances hepatocyte proliferation (LI was 27% *vs* 14% of rats subjected to PH alone). These findings suggest a therapeutic use of mitogens to alleviate the reduced hepatocyte proliferation seen in elderly and to provide a powerful stimulus for liver regeneration following major liver resection or transplantation.

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P59

Distinct apoptotic phenotypes in two U937 cell variants — a search for a novel model for a switch between apoptosis and necrosis

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U937 histocytic leukemia cell line is a widely studied model of apoptosis induced by various stimuli. We identified two variants of the cell line differing with respect to morphology of cells dying of apoptosis. In one of the variants U937M, we did not observe apoptotic body formation and disintegration of cells, while in another, U937ATCC these two phenomena were evident. Different morphology of dying U937M was independent on stimuli used, as those activating extrinsic or intrinsic pathway of apoptosis induced similar phenotype. The main goal of the presented study was to identify a mechanism responsible for defective apoptosis occuring in U937M.
We found that following apoptosis induction these cells in comparison to reference U937ATCC were characterized by faster and more pronounced decrease of mitochondrial membrane potential, more intense generation of reactive oxygen species. Additionally, pan-specific inhibitor of caspases z-VAD-fmk inhibited decrease of mitochondrial membrane potential only in the reference U937ATCC. Applying the caspase inhibitor in U937ATCC cells inhibited blebbing, formation of apoptotic bodies and cell disintegration. Applying Y27632, inhibitor of ROCK1 kinase, inhibited disintegration of U937ATCC stimulated by apoptosis inducers. In the U937M both caspase and ROCK1 inhibitors did not affect significantly morphology of dying cells. The lack of effect of caspases and ROCK1 inhibition in the U937M could indicate insufficient activation of executioner caspases needed for apoptotic bodies formation and cell disintegration. However, we found that in both cell lines inbibition of caspases by z-VAD-fmk inhibited DNA degradation to the same extent.
In the U937M following treatment with apoptotic inducers we observed increasing number of cells showing membrane permeability, while in the reference U937ATCC this phenomenon did not occure and cells disintegrated into apoptotic bodies.
Obtained results indicate that defective apoptosis observed in the U937M variant cells is characterized by biochemical features which may reflect distinct mechanism of mitochondrial function and disturbed executioner phase of apoptosis. In these cells, following inefficient apoptosis execution, necrotic pathway seems to be switched on.

P60

Inhibition of caspases enhances the cytotoxic effect of TNF in human myelomonocytic U937 cell line

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The eukaryotic cells treated with tumor necrosis factor (TNF) initiate programmed cell death, apoptosis, which is mainly mediated by caspase cascade activation. Therefore inhibition of caspases seems to be a perfect level of control in the process of apoptosis.
In our research we worked with the human myelomonocytic U937 cell line, which is resistant to TNF. Interestingly, U937 cells stimulated with combination of TNF a and broad-spectrum caspase inhibitor zVAD.fmk became sensitive to TNF, suggesting a caspase-independent mechanism of cell death. Moreover, the phenotype of cell death has changed from apoptotic into necrotic-like. Similar results were obtained after co-stimulation with TNF and caspase-8 specific inhibitor zIETD.fmk. Futher experiments with TNF and caspase-3 specific inhibitor zDEVD.fmk revealed that the lethality of U937 cells was not enhanced which rather excluded caspase-3 engagement in the phenomenon.
In addition, it was found that the cytotoxic effect of TNF and zVAD.fmk was prevented by preincubation with antioxidant butylated hydroxyanizole (BHA) what indicated the role of ROS in the mechanism of death. Flow cytometry analysis confirmed that in cells treated with combination of TNF and zVAD.fmk the level of ROS increased significantly. U937 cells incubated with another apoptotic stimulus – staurosporine and zVAD.fmk did not intensify cells cytotoxicity what indicates TNF-dependent mechanism of cell death when caspases are inhibited.
The phenotype of TNF/zVAD.fmk treated dying U937 cells suggest necrotic mode of death. Thus, the U937 cells studied, when properly manipulated, seem to be a suitable model of TNF induced apoptosis and necrosis.

P61

Escape from senescence-like growth arrest leads to increased genomic instability of doxorubicin-treated human colon HCT116 cells

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Cancer cells induced to undergo senescence-like growth arrest *in vitro* become giant/polyploid and cease to proliferate. Polyploidy is a common feature of some tumors and can lead to aneuploidy and genomic instability. Treatment of human cancer HCT116 cells with 100 nM doxorubicin for one day and followed by culturing them in drug free medium up to 10 days induced hallmarks of cellular senescence, such as increased activity of common senescence marker SA-β-galalactosidase (SA-β-gal), cell enlargement and induction of p53 and p21 proteins. Giant SA-β-gal-positive cells were negative for proliferation marker, Ki67 proving their terminal growth arrest. Only small fraction of cells underwent cell death as evidenced by trypan blue exclusion test and propidium iodide staining. Western blot analysis of PARP, a substrate of executor caspases 3 and 7, showed that the level of its cleaved form detected in both adherent and floating cells remained low during the entire time of culture. Microscopic observation of multinucleated cells stained with anti-lamin A/C antibody proved nuclear membrane integrity in those cells. However, the giant cells were gradually replaced by small proliferating once. On the route to senescence-like growth arrest the cells underwent intensive DNA replication, as evidenced by BrdU incorporation, and became polyploid with more than 16C DNA content. DAPI staining revealed a nuclear morphology indicative of some cells undergoing aberrant mitoses. We observed chromatin condensation and improperly segregated and lagging chromosomes. Confocal microscopy of cells stained for actin, α-tubulin and DNA showed cells with multipolar spindle having up to seven metaphase poles nuclei with anaphase bridges and nuclei without chromatin condensation but with furrows and buds were also observed. The observations suggest that polyploid cells might divide asymmetrically giving aneuploid progeny and that at least some of their descendants could proliferate. Indeed, cell lines emerging from the survivors are characterized by higher variations of chromosome's number than observed in parental cells. Thus, induction of cell senescence-like growth arrest leads to increased genomic instability of HCT116 cells.

P62

Molecular factors associated with cutaneous melanoma aggressiveness

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Malignant transformation of melanocytes is a multistep process characterized by distinct histopathologic stages. Many studies in molecular pathology indicated that p53 and bcl-2 immunoreactivity can be used to asses unfavorable prognosis.

Aim: To study the expression of p53 and bcl-2 in 50 cases of cutaneous melanoma (15 cases *in situ* and 35 cases invasive tumors) as a prognostic factor.

Materials and Methods: Our study included 50 cases of cutaneous melanoma. Hematoxilin-Eosin and paraffin section immunostaining for p53 and bcl-2 were performed in all cases (IHC study was carried out on formalin-fixed, paraffin embedded tissue, using the avidin-biotin peroxidase complex).

Results: p53 expression was positive in all cases, values between 2% and 60%; bcl-2 was positive in 93% of cases, values between 4% and 72%.

Discussion and Conclusion: Our frequency detection is not related to histological type, tumor localization, size, depth and growth phase. Most lesions are p53 higher (24% cases) and this was associated with poor survival; bcl-2 expression was associated with good prognosis. It suggested that p53 and bcl-2 could be useful in predicting the biologic behaviour.

P63

Is antioxidant a „magic bullet"?

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In spite of a huge amount of publications in biological and medical journals concerning antioxidants there are a lot of unanswered questions about the role of antioxidants in the living processes. The first, what does the term "antioxidants" really imply and why there are a lot of definitions? Further, which classification of antioxidants is the best? What do the terms "antioxidants" and "radical scavengers" mean? What is the difference of lipid peroxidation in the bottle of oil and in the biological membranes as well as the difference of peroxidation in the lipids and oxidation of the proteins? Vitamin C — antioxidant or prooxidant? The role of tocopherols and glutathione in living organisms. Correlation between the antioxidant activity of compounds and their biological activity — in many cases there is more fortuity than reality. Radical scavengers and radiotherapy of cancer. A story of red wine and resveratrol. Why my grandfather took a glass of wine for night? Are antioxidants "elixirs of youth or tonics for tired sheep?" asked one of their investigator T.L.Dormandy. Besides, we all must always remember about the red-ox balance in our organism. We must be very careful to choose the correct methods for the research of antioxidants and radical scavengers to have a good and believable result. Is everything that is good for rats good for human, too? Besides the information about oxidative stress lately more and more data are about the reductive stress. And finally it is necessary to remind the words of Paracelsus (1493–1541) "All substances are poisons: there are none which is not a poison. The right dose differentiates a poison and a remedy."

P64

Molecular analysis of C677T *MTHFR* gene polimorphism in colorectal cancer patients

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The enzyme methylenetetrahydrofolate reductase (*MTHFR*) catalyses the formation of folate intermediates that are vital to methylation reactions. A polymorphic variant of *MTHFR* C677T (TT) has been linked to reduced levels of plasma folate, aberrant DNA methylation in leucocytes, and increased risk of colorectal cancer under conditions of low folate intake.

The specimens, whole blood or tumoral tissue, were collected, after informed consent, from 77 colorectal cancer patients and 65 healthy persons at Cantacuzino Hospital. DNA was extracted from peripheral blood leukocytes using the Promega Wizard kits. Genomic DNA (0.5–1.0 μg) was incubated in a total reaction volume of 10 μl containing 40 ng of both the forward and reverse primers for the C677T. 1.0 units Taq, 200 μM each deoxynucleotide triphosphate, 1.5 mM $MgCl_2$, 10 mM Tris/HCl (pH 8.3), 50 mM KCl. PCR amplification was accomplished using 35x (60 sec/94°C, 30 sec/59°C, 30 sec/72°C). The *MTHFR* C677T SNP creates a HinfI restriction enzyme recognition sequence and is thus detected by digestion of PCR amplified products with *Hinf* enzyme, as previously described. Genotypes were establish in PAGE (8%) after ethidium bromide staining.

The distribution of C677T genotypes was 46.8% CC, 42.8% CT, 10.4% TT for patients and 52.3% CC, 36.9% CT, 10.7% TT for control; alleles distribution was 68.2%C and 31.8%T for patients, and 70.8% C, 29.2% T for control.

For C677T polymorphism, distribution of genotype frequencies and allele frequencies don't differed significantly between all cancer patients and control (X2 0.435, $P = 0.5095$).

The role of the *MTHFR* gene polymorphisms should be further studied, and the distribution of other polymorphisms in this metabolic pathway should be studied in terms of cancer susceptibility.

P65

DNA topoisomerases as the targets of anti-cancer drugs

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Topoisomerases are ubiquitous enzymes that regulate the conformational changes in DNA topology by catalyzing the concerted breakage and rejoining of DNA strands during many genetic processes including DNA replication, transcription, recombination and transposition. These enzymes introduce or remove DNA superhelical turns, knot or unknot, and catenate or decatenate circular DNA molecules. Over the past years there has been an increased awarness on these enzymes as they were shown to be the cellular targets of a number of anti-cancer drugs. Our lab searched methanolic extracts of a number of *Helichrysum pamphylicum* plants and 1H-benzimidazole derivatives on mammalian DNA topoisomerase I *via in vitro* plasmid supercoil relaxation assays and showed considerable interference of these compounds on the enzyme at varying extends. A known topoisomerase I poision, Camptothecin, was used as the reference compound in the evaluation of the inhibition and the experimental results were quantitatively and differentially discussed terms of the individual contribution of the plant components or the effects of chemical substitutions in the course of inhibition. The inhibition manifested by the tested compounds is signficant as they can be a potential source for developing new anticancer drugs.

P66

Dosage of the short isoform of p53 (p44) controls stem cell pluripotency and proliferative capacity

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p44 is a naturally occurring short isoform of the tumor suppressor p53, lacking the transactivation domain located in the N-terminal region of the full-length protein. Increased dosage of p44 reduces murine lifespan and decreases stem cell proliferation during adult neurogenesis. To determine if over-expression of p44 alters the growth of stem cells during very early embryogenesis, we isolated embryonic stem cells (ESCs) from p44Tg blastocysts. We found that the proliferation and cell cycle distribution of p44Tg ESCs were normal. However, when directed to differentiate into neural stem cells (NSC) *in vitro*, we see that p44Tg ESCs grow at a faster rate than non-transgenic (NT) ESCs and retain a typical ESC morphology while the NT ESCs adapt a NSC phenotype. To determine if a reduction in p44 dosage would also affect stem cell behavior, we used homologous recombination in ESCs to reduce p44 dosage by 50% (dp44). In contrast to the NT or p44Tg ESCs, we found that dp44 ESCs exhibit severely decreased proliferation and reduced expression of the ESC markers Nanog and Oct3/4. dp44 ESCs have a drastically different cell cycle distribution compared to that of NT ESCs. Our results indicate that p44 does regulate stem cell self-renewal and pluripotency during early embryogenesis and supports the idea that "aging" may actually begin before birth, when the ability of cells to divide may be set for a lifetime.

P67

Overexpression of heat shock protein 70 causes death of mice at middle age

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The ability to moderate internal and external stress is arguably the central function regulating senescence in aging of animals. It is believed that molecular chaperones, such as heat-shock proteins, combat stress-related senescent dysfunction during aging. Induction of Hsp 70 expression through genetic manipulation extends the lifespan of *Drosophila melanogaster* and renders rat cardiac myocytes more resistant to oxidative stress (Chong *et al.,* 1998; Tatar *et al.,* 1997). It has been hypothesized that overexpression of Hsp 70 may increase age-specific survival by virtue of this protein's ability to renature, assemble and disassemble many non-heat-shock proteins, and to interact with other stress-response mechanisms such as superoxide dismutase (Wheeler *et al.,* 1995).
Mice continuously expressing high levels of Hsp 70 (Tg 70) were generated by Pagoulatos *et al.* These mice have a CBA x C57Bl/6 background and express the human Hsp70.1 gene under regulation of the human β-actin promoter (Plumier *et al.,* 1995). We carefully studied both male and female trangenic mice during their entire lifespan, monitoring body weight, movement, and lifespan, and examining gene-expression, glucose uptake, blood parameters, and tissue histology. The first striking observation was that Tg 70 mice have a significantly lower body weight compared to the wildtypes. We found no difference between Tg 70 and wildtypes in food intake, glucose uptake and movement at both early and middle ages. All Tg 70 mice died at the age of 18 months with no premature aging symptoms.
We therefore conclude that, in contrast to overexpression of Hsp 70 in *Drosophila melanogaster*, its expression in mice does not extend lifespan but leads to death at middle age.

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Age-related changes in the mouse and human lung and their relevance to lung cancer

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Lung cancer is a disease of ageing, but age-related changes in the lung are poorly understood. We are conducting an analysis of ageing mouse and human lungs at the cellular and molecular levels to determine whether there are changes that might promote the initiation and growth of tumours. Comparison of lungs from young (6 month) versus geriatric (30 month) C57Bl/6 x Balb/c F1 mice by microarray analysis revealed evidence of immune cell activation, inhibition of angiogenesis, and increased expression of DNA repair genes. The ability of lungs from young or geriatric mice to support metastatic growth was examined by injection of colon cancer cells into tail veins. Aged mice developed significantly more lung metastases, and median survival was significantly reduced in the aged cohort of mice. No differences in pulmonary blood flow, endothelial proliferation or lymphocyte profiles were detected.
Normal human lung samples from young or aged surgical patients are being collected for microarray analysis. Preliminary data indicate that the transcriptional profile associated with human lung ageing may be quite different from that observed in the mouse. The major transcriptional changes observed to date involve extracellular matrix genes, predominantly those encoding collagen proteins. Such changes have been verified by quantitative RT-PCR and by staining of histological sections.
We have also been interested in age and cancer-related changes in the ubiquitin/proteasome system (UPS), components of which are known to be perturbed in lung cancer. We have surveyed this system using pathway-specific microarrays, and have identified previously unreported changes that could be of clinical relevance. We are creating transgenic mouse models to test the oncogenic potential of UPS genes, and will be investigating their potential as clinical biomarkers of lung cancer.

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Xeroderma pigmentosum group D Lys751Gln genetic polymorphism was highly correlated with early recurrence of colorectal cancer patients in Taiwan

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We investigated distinct patterns of functional genetic polymorphisms that are involved in drug metabolic pathways and DNA repair in Taiwanese colorectal cancer (CRC) patients with or without 5-fluorouracial/oxaliplatin chemotherapy. Two hundred and one patients who underwent potentially radical curative surgery for CRC between March 2005 and June 2006 were enrolled in this study were analyzed regarding the prevalence of these genetic polymorphisms and to examine the feasibility of developing a gene predictor of clinical outcome. Six polymorphisms that are functional in metabolizing genes — GSTP1, MDR1, MTHFR, TS, XPD, and XRCC1 were assessed using a PCR-RFLP technique and DNA sequencing. In addition, correlations between patients'clinicopathological features and early recurrence were investigated. The present study showed that the unfavorable genotype from polymorphism in XPD Lys751Gln, lymph node metastasis and cancer stage were, in a statistically significantly way associated with early recurrence ($P = 0.006$, 0.011 and 0.008, respectively). Patients who carried XPD Lys751Gln Lys/Gln or Gln/Gln genotypes demonstrated a significantly greater acute recurrence risk (OR = 3.294, 95% CI, 1.272–8.532). Our results suggest that patients who possess one or two 751Gln alleles might have the risk genotypes for early recurrence of Taiwanese CRC. These findings require independent prospective confirmation.

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Stress-induced phosphoprotein (STIP1) as a potential biomarker for human ovarian cancer

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Patients with ovarian cancers are often associated with poor prognosis and limited survival rates because their cancers were not detected at earlier stages. Currently, CA125 is the most commonly used tumor marker for ovarian cancer, but it only results in 78% sensitivity, 82 to 95% specificity, and 82% positive predictive value. Therefore, more tumor markers for detecting ovarian cancer are warranted. Readily accessible blood is a convenient source of tumor markers. However, screening for new tumor markers from blood is difficult because tumor markers, usually at low concentrations in blood, are easily obscured by serum abundant common proteins. In 2004, Celis proposed that tumor markers are secreted by tumor cells first into tumor interstitial fluid (TIF) before their eventual appearance in circulating blood. As a proof-of-principle, his group identified 169 breast cancer-specific proteins in TIF using proteomics approaches. In this study, we attempted to identify cancer-specific proteins from TIF derived from human ovarian cancer using proteomics, Western blot analysis, immunohistochemistry, and enzyme-linked immunosorbent assay (ELISA). Using statistical analysis of 596 protein spots in two-dimensional gels between the TIF of ovarian cancer and normal interstitial fluid (NIF) of normal ovaries, we identified several differentially expressed proteins, including stress-induced phosphoprotein 1 (STIP1). Subsequent ELISA experiments revealed serum levels of STIP1 were significantly higher in ovarian cancer patients than in healthy controls. Our results suggest STIP1 has potential as tumor marker for human ovarian cancer.