P9

Molecular Characterization of JC Virus Strains Detected in Human Brain Tumors and Their Interaction with Individual Genetic Factors

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The JC virus, a neurotropic member of the polyomavirus family, commonly establishes an asymptomatical latent infection in the kidney of up to 80% of the adult population. The virus, as the result of a targeted lysis of oligodendrocytes, is the established etiologic agent of the Progressive Multifocal Leukoencephalopathy (PML), the fatal demyelinating disease, which usually affects individuals with cell-mediated immunity defects. Recent mounting evidences indicate that JCV has the capability to induce tumor of neural and glial origin in animal models and several clinical reports suggest the association between JCV and human cancers, more notably brain tumors. Tumorigenity of JCV is most likely induced by the viral highly conserved early gene product T-antigen (LT), which has the capability to bind and inactivate several tumor suppressor proteins, in particular p53.

To further verify the possible involvement of JCV in human brain tumors, the viral DNA was searched using a nested PCR designed to amplify LT coding region in brain tumor tissue, peripheral blood cells and cerebrospinal fluid (CSF) collected from 30 histologically different cases of brain tumors.

Viral sequences were amplified in eight of fourteen glioblastomas (57.1%), in two of seven meningiomas (28.6%), in one of three astrocytomas (33.3%). Moreover JCV genotype distribution has been studied by nucleotide sequencing of VP1 region. Only JCV genotype 1 has been detected, and in particular the subtype *a* was found in four tumor tissues and one CSF, and the subtype *b* in three tumor tissues and one CSF. TCR nucleotide sequencing revealed the presence of one archetypal derived (type II) and five *Mad-4* TCR rearrangements.

Moreover familial episodes of brain tumors suggest an inheritance predisposition. The genetic implication in brain cancer aetiology has been evaluated comparing the patients-HLA distribution with those of asymptomatic controls from the same population and local area. Molecular HLA, A B, C, DQB1 and DRB1 typing have been performed by SSP method in 24 out of the 30 enrolled patients and 46 controls. Evidence of a possible association with all forms of brain tumor was observed for HLA A2 and A3 alleles. In particular HLA A2 frequency was 33% in patients and 16.3% in controls ($p < 0.05$); moreover HLA A3 showed 22.9% frequency of distribution in patients versus 5.4% in controls ($p < 0.01$), suggesting a possible genetic predisposition to develop brain tumor in subjects with HLA A2 and/or A3 alleles.

Since distribution of HLA alleles resulted similar in both JCV positive and negative patients and JCV frequency was remarkable in glioblastoma, the data obtained support the possibility that JCV virus, more notably genotype 1, *Mad-4* strain, could play a crucial role in pushing a genetic neoplastic predisposition towards glioblastoma, the most frequent and aggressive of primary brain tumors.

Bio- and Chemoprevention

P10

Chemopreventive Properties of Some Dietary Food Components Found in Traditional Central European Cuisines

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The results of epidemiological studies carried out in 1960s correlating the incidence of cancer of the large intestine with meat intake in 23 countries suggested that diets of certain Central European states might contain components lowering the risk of this cancer. Surprisingly, to these countries belonged Poland and Germany, whose traditional cuisines are rich in fat and meat but low in vegetables, hence from current perspective would be expected to increase cancer risk. Therefore, we decided to seek in diets of these countries a common ingredient, which could account for tumor preventive properties. The major vegetable traditionally eaten in Poland and Germany in substantial amounts is white cabbage, especially its fermented by lactic bacteria produce – sauerkraut, available all year round. There are many reasons to believe that sauerkraut may indeed be a health promoting food component. It contains antioxidative vitamins and polyphenolic substances, compounds modulating activity of II phase enzymes, as well as lactic bacteria and fibre neutralising mutagenic activity of foodborne carcinogens.

The first activity tested was antioxidative potency of sauerkraut and some other foods of plant origin, traditionally found in Central European diets, evaluated by two methods: ABTS assay enabling the assessment of overall radical scavenging potency and comet assay that allows to detect DNA damage, hence also its prevention, at a single cell level. The food products studied were purchased in a local grocery shop and belonged to frequently consumed items. We found that some of the foods tested displayed free radical scavenging activity comparable to green tea used as a positive control. Particularly effective in this regard were beet root concentrate, sauerkraut juice and fermented cucumbers. Interestingly, the fermentation by lactic bacteria enhanced antioxidative activity of vegetables studied. Additionally, comet assay showed that sauerkraut juice protected cultured cells against oxidative DNA damage in a dose dependent manner. These studies are currently continued and any new worthwhile results will be presented.

P11

Tea Consumption and Cancer Prevention

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To investigate whether tea consumption have etiological associations with ovarian cancer, a case-control study conducted in China during 1999-2000. Cases were 254 patients with histologically confirmed epithelial ovary cancer. The 652 controls comprised 340 hospital visitors, 261 non-neoplasm hospital outpatients, and 51 women recruited from the community. Information on the frequency, type, and duration of tea consumption was collected based on a verified questionnaire through personal interview. The risks of ovarian cancer for the tea consumption assessed by adjusted odds ratios (OR) based on multivariate logistic regression analysis, accounting for confounding demographic, lifestyle, familial factors and hormonal status, family ovarian cancer history and total energy intake. The ovarian cancer risk declined with increasing frequency and duratin of tea consumption. The adjusted ORs for those drinking tea daily and those drinking tea 30 years were 0.41 and 0.25 respectively! compared to never/monthly tea-drinkers with significant dose response relationship. When different types of tea were taken into account, only daily drinking of green tea showed significant inverse association (OR was 0.57). We concluded that increasing frequency and duration of tea drinking, especially green tea drinking, was associated with reduced risk of ovarian cancer, which has been supported by numerous in vitro and in vivo experiments.

P12

Chemoprevention 13Y Avemar, a Wheat Germ Extract Measured 13Y In Vitro Cytotoxicity, Cell Proliferation and Activation Studies on Human Lymphocytes and Different Cell Cultures

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The main purpose of the adjuvant application of Avemar® (a fermented wheat germ extract) in the treatment of tumor patients is to reduce the side-effects of cytostatics used in the chemotherapy. We studied the in vitro effects of Avemar® in different experiments: (1) flow cytometric measurements of apoptosis and cell proliferation in different groups of occupationally exposed donors in addition to the cytogenetic methods used in our "multiple end point genotoxicological monitoring system", (2) flow cytometric measurements of the proportion of lymphocyte subpopulations and cell activation in unstimulated or phytohemaagglutinin stimulated immunocompetent cells treated in vitro with tioguanine and fluorouracil, (1) changes in [lie antitumor activity of cytostatics in the presence of Avemar® with the help of the MTT test in Vero and HepG2 cell lines. Avemar® alone had no effect on apoptosis and cell proliferation and (also in combination with cytostatics) had no influence on the slight increase of the apoptotic cell fraction and the decrease in cell proliferation caused by cytostatics. The proportion of lymphocyte subpopulations was not altered by in vitro treatment of Avemar® and/or cytostatics, Avemar® treatment alone did dot activate lymphocytes while when Avemar® treatment was combined with cytostatics, in the case of T lymphocytes cell activation was decreased in comparison to those caused by cytostatics alone. Avemar® in low doses (<4000 μg/ml, also in combination with cytostatics) was not cytotoxic and Avemar® treatment reduced the cytotoxic effects of cytostatics in a varying measure depending on the type of cytostatics. On the bases of our results, data suggest that cytotoxic side-effects of chemotherapy on normal cells can be reduced with the simultaneous Avemar® treatment of cancer patients.

P13

Antimetastatic and Antitumor Effect of Chemically Modified Glucans

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Perspective drug with antitumor activity soluble 1,3-β-D-glucan is internalized after binding to a specific β-glucan receptor on macrophages (Mph) and reduced internalization of glucan is possibly responsible for the down-regulation of Il-11 and TNF-α secretion. The biological activity of 1,3-β-D-glucans was suggested to significantly affect by their chemical properties, such as side chains, molecular weight.

The goal: to study the effectiveness of Mph stimulators with different branches and terminal groups (interaction with glucan or/and fucose receptors) during antitumor treatment with special attention to cysteine proteinases (CP) and CP inhibitors – cystatin C and Stefin A as markers of efficacy of antitumor therapy.

Methods: CBA and CBA/C57Bl mice with LS lymphosarcoma or Lewis lung adenocarcinoma were treated by cyclophosphane (CPA, 150 mg/kg, i.p., single) at 10th day and/or CMG and/or ChitoCMG (Institute of Chemistry SAS, Slovakia, 25 mg/kg, i.p. single, one day before or 3 days after, or simultaneously with CPA) and/or U (Nowicky Pharma, Austria, 0.5 mg per mouse i. p., single) as Mph stimulators. Cysteine proteinases (cathepsins B and L) activity was measured against fluorogenic substrates with specific inhibitor (CA-074 for cathepsin B); cystatin C and Stefin A concentration was determined by ELISA kits (KRKA, Slovenia).

Results: Significant antimetastatic effect (to lung) and retardation of tumor growth and as a consequence prolongation of life-span effect were shown in the murine tumors with help of combination of CPA and Mph stimulator CMG or U treatment. CMG (but not ChitoCMG) alone reduced the number of metastases to lung in Lewis lung adenocarcinoma In mice with Lewis lung adenocarcinoma treatment by CPA + ChitoCMG had no positive (antimetastatic and antitumor) effect. In untreated groups CP activity in tumor tissue was increased and low concentration of CP inhibitors was noted. CPA or CPA+U treatment of LS lymphosarcoma increased extracellular CP inhibitor cystatin C in tumor tissue (also in spleen and serum) as compare to untreated mice. *Intracellular* inhibitor of

CP Stefin A concentration was low in tumor of untreated and treated mice. The level of *extracellular* CP inhibitor cystatin C is important marker of antitumor treatment efficacy as compare to *intracellular* inhibitor level of Stefin A. CPA + U or U treatment of LS lymphosarcoma increased cystatin C concentration in tumor tissue and in less degree – in liver.

Conclusion: The efficiency of chemotherapy by CPA of both murine tumors studied was increased by CMG, but not by ChitoCMG in the same doses and mode of administration. Our data suggest that specific interaction of CMG and ChitoCMG with different Mph receptors may be important in antitumor and antimetastatic activity of water-soluble glucans.

P14

Induction Chemotherapy Followed by Concomitant Chemoradiation Therapy in Advanced Head and Neck Cancer: A Phase II Study with Organ-Sparing Purposes Evaluating Feasibility, Effectiveness and Toxicity Including a Chemoprevention Study

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The purpose of the study was to assess response rate, clinical outcome, organ/function preservation and toxicity in head and neck cancer patients treated with induction chemotherapy followed by concomitant chemoradiotherapy and, when necessary, limited surgery. A secondary endpoint of the study was to evaluate the effectiveness of a chemoprevention against a second malignancy. The study design was a phase II non randomized trial in hospitalized patients setting. The treatment plan consisted of 3 cycles of induction chemotherapy with cisplatin, fluorouracil (5-FU), l-leucovorin and interferon α2b (PFL-IFN) followed by 7 cycles of 5-FU, hydroxyurea and concomitant radiation for 5 days (FHX) for a total radiation dose of 70 Gy. 13 cis-retinoic acid was added to treatment regimen for chemoprevention and a systematic prophylaxis of mucositis was administered to all patients during FHX. Conservative surgical resection was reserved to patients with no optimal response (PR $\geq$70%), whereas radical surgery was performed as salvage treatment. Twenty-six patients were treated at one institution: more than 90% had stage IV disease and only 19.2% had laryngeal cancer. Eigthy-one percent of patients had performance status 0 and 23.1% of patients had >5% weight loss at treatment start. Nineteen patients were analyzed for response to PFL-IFN: 3/19 (15.8%) patients achieved a CR and 7/19 (36.8%) achieved a PR for an ORR of 52.6%. FHX was administered on protocol to 12 patients: 6 patients (50%) had CR, one patient (8.3%) had PR for an ORR of 58.3%, 2 patients (16.7%) had SD and 3 patients (25%) had PD. At the completion of FHX, no patient underwent local therapy according to treatment plan. At a median follow-up time of 13.5 months (range 1-28$^+$) at June 2001, among 26 patients enrolled 12 (46.1%) were still alive and 9 (75%) of them were progression-free. The median duration of response was 9 months (range 0-25$^+$), the median progression-free survival was 10.5 months (range 0-28$^+$), the median overall survival time was 9 months (range 1-22). The toxicity was significant and consisted mainly of mucositis and, to a lesser extent, neutropenia/thrombocytopenia. The results of the chemoprevention study will be available at the proper time. In the present study, the low serum levels of leptin and the high serum levels of proinflammatory cytokines in advanced stage cancer patients were confirmed. In conclusion, this sequential induction chemotherapy and chemoradiotherapy program has been found moderately active and significantly toxic; moreover, it is to be taken into consideration the long overall treatment duration. For these reasons, this regimen could not be recommended for a phase III randomized study.

P15

The Influence of Melatonin Stabilized Damages Caused by Cisplatin in Bone Marrow Cell Genome During Lewis Lung Carcinoma Therapy

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The progress in present-day radio- and chemotherapy, in which outweigh DNA-damaged drugs, make prerequisite to long-term contact of oncological patient with genotoxical reagents, that can result in secondary tumors. The aim of the study was the research of antimutagenic properties of pineal hormone melatonin for the bone marrow protection from genotoxical effects of overused anticancer therapy agent cisplatin. This approach allow to work out a new strategy for genome prevention.

The methods: 1) identification of cells on apoptosis (Hoechst 33342, Sigma); 2) valueing of cells with chromosomal aberrations – CA and number of chromosomal and chromatide breaks in aberrantic cells; 3) cytotoxical analysis; 4) analysis of cytogrammmes of bone marrow and peripheral blood.

The genotoxical effect of cisplatin (CP, "Abewe",Austria; doses:1 regime-3mg/kg 6 injections every day; 2 regime-1mg/kg 9in.ev.d.)in bone marrow cells during Lewis lung carcinoma therapy in C57Bl/6 mice was characterized by induction of apoptosis at the begining of observation. A dose-dependent effect was observed for increased frequency of metaphases with CA. Chromosomal(CB) and chromatide breaks(CrB) were predominated type of CA.The level of CB exceeded spontaneuos level in 3,5-5 times.After therapy cells with stable CA were observed.Differentiation of bone marrow cells were inhibited. Myelocyte cell progenitors were indicated in peripheral blood.

During administration cisplatin with melatonin (M, Sigma, USA; 1mg/kg 9 in. ev. d., 5mg/kg 9 in. ev. d.) was demonstrated the antimutagenetic effect of this hormone. The essential falling of apoptosis and aberrations levels in bone marrow were noticed. The cytotoxical effect also reduced. After therapy apoptotic cells and cells with stable CA were not observed. On background of protection from genotoxical effect of cisplatin, melatonin shown to normalized the cells composition of bone marrow and blood. We suggest that the pineal hormone melatonin may be perspective as a protector from genotoxical effects of DNA-damages drugs with the purpose to prevent mutation in normal cells and induction of secondary tumors during chemotherapy treatment.

P16

A Phase II Clinical Trial of Anethole Dithiolethione (Sialor®, Sulfarlem®) in Smokers with Bronchial Dysplasia

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Pre-clinical studies suggested that anethole dithiolethione (ADT) may be an effective chemopreventive agent for lung cancer. This is a Phase IIb study to determine the potential effect of ADT in current and former smokers with premalignant bronchial lesions using bronchial dysplasia as the primary surrogate end-point biomarker (SEB). One hundred current and former smokers with a smoking history of ≥30 pack-years (e.g. 1 pack per day for 30 years or more) with one or more sites of bronchial dysplasia identified by fluorescence bronchoscopy directed bronchial biopsies were treated with ADT at a dose of 25 mg orally TID for six months. At least 4 bronchial biopsies were taken per subject including a minimum of two random biopsies from apparently normal areas. The same areas were re-biopsied after 6 months of study medication. Any new areas suggestive of dysplasia were also biopsied. Changes in the histopathology grade and quantitative nuclear morphometry indices were assessed before and after treatment.

In the site by site analysis, regression of dysplastic lesions to hyperplasia/normal was 53% in the ADT group versus 38% in the placebo group. Appearance of new dysplastic lesion or progression of pre-existing dysplastic lesions was 7% in the ADT group and 18% in the placebo group. The difference in regression and progression between the two groups was statistically significant (P=0.0003). In the person specific analysis, the regression rates were 27% in the ADT group and 11% in the placebo group. The progression rate was lower in the ADT group (32% versus 60%). The difference in regression and progression between the two groups was statistically significant (P=0.031). Adverse events were minor gastrointestinal symptoms that resolved with dose reduction or discontinuation of the medication.

Our results suggest for the first time in humans that anethole dithiolethione (Sialor®, Sulfarlem®) is a potentially efficacious chemoprevention agent for lung cancer; its therapeutic effect is based on its radical scavenger and gluthathione-inducer properties.

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Breast Cancer

P17

Human Breast Epithelial Cell (HBEC) Apoptotic Pathway Modulated by Estrogen (E)-Estrogen Receptor (ER)-Activated Genes Phosphorylation

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Breast malignant cells (HBCC) grow unrestrained, promote autonomous growth that does not respond to normal cheeks & balances for cell growth. In long term cultivation the Breast malignant cells ignore normal cell limitations, contact inhibition to stop dividing when they come In contact with each other, and lose the properties of adhesion and cell-to-cell recognition (Hakim, Arch. Geschwultforchung 47:332-350,1979) This study reports on gene expression during conversion of human apithelial cello (HBEC) into breast carcinoma cells (HBCC) (Hakim, Expt. Cell Res. 47:332-350,1979) and its relationship to the genes modulating apoptotic processes contributing to the prevention of the various stages (initiation, prevention & invasion)and killing of the tumor cells with a prescheduled malignant cell death. HBEC & HBCC cells were obtained at resection of benign (2) and post-menopausal primary(S) and metastatic (5) breast tissues. In parallel breast carcinoma: ER^+ (MCF-7, TsTd, ZR-75-1) & ER^- (MDA-MB-466) BT-20, Hs57BT) cell lines were cultured under well established conditions. cDNA was cloned from HBCC (Hakim, FASEB 2/6, 8719, 1988) was transfected into ER RBCC which contained amplified C-erb-2/Neu & mutated P 53 as described In (Hakim, Naturwissenschaften 74:593,1987, ibid Diagnostics & Clin. Testing 2:30-39,1989; J. Surg. Oncol. 40:21-31, 1989 & Naturwissenschaften 75:361, 1989). The + cello were then injected into BALB/c female nude Nu/Nu mice.

ER^+ and ER^- HBCC treated directly with anti-protein phosphatase inhibitor blocked tumor cell proliferation both in vivo and in vitro. When examined these cells showed high levels of Bcl-2, P53 and Caspase-3 activity. ER^- HBCC and ER^+ HBCC treated with estradiol proliferated and developed fatal tumors which contained amplified C-erb-2/Neu & mutated P53. ER^- HBCC cells transfected with HEBC. cDNA failed to proliferate in vivo. In-vitro culture produced cells with wild P53, Bcl-2 with moderate Caspase-3 activity, and P21 phosphate turnover. The presence of estradiol in n culture enhanced proliferation of these cells. These findings point to the role of phosphorytion mechanisms on HBCC gene expression, phosphorylation of the wP53 modulates the cell cycle, the Bcl-2 gene modulates Caspase activity and the Apoptotic response and tumor cell viability. Estradiol catalyzes gene expression through thymidine phosphorylase activation.