Epidemiology – Laboratory

P1

The Attitudes of Primary Care Physicians Practicing Around the Lake of Constance Towards the Screening of Common Carcinomas

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Goals: We sought to determine to what degree physicians practicing in our area agree with and act according to recommendations concerning cancer screening for common cancers.

Methods: A short questionnaire was mailed to some 4000 physicians living around the Lake of Constance in Germany (southern parts of Baden-Württemberg, Bavaria), the Principality of Liechtenstein, Austria (Vorarlberg) and Switzerland (States of St.Gallen, Graubünden, Appenzell and Thurgau). They were asked, whether and how regularly they advise their patients to undergo screening examinations (mammography, PSA testing, screening for colorectal cancer) and whether they discuss weight control.

Results: 318 evaluable questionnaires were returned: 169 from the above mentioned states of Switzerland (CH), 85 from Baden-Württemberg (BW), 48 from Vorarlberg (V) and 11 from the Principality of Liechtenstein (FL). 227 physicians work predominantly or to a significant degree in primary care. Considerable differences were noted among the attitudes of physicians according to the geographical area of their practice: whereas in V 87% of primary care physicians regulary or often recommended screening mammography to suitable patients, the same percentage was only 46% in BW, 30% in FL, 56% in Thurgau (TG), 44% in St.Gallen (SG) and even 37% and 31% in Appenzell (A) and Graubünden (GR). PSA testing was regularly or often recommended by 81% of primary care physicians in V, 61% in BW and 40% in FL. There was again significant variability in Switzerland ranging from 84% in GR and TG to 61% in A and 44% in SG. Screening for colorectal cancer was regularly or often recommended by 78% of primary care physicians in V, 71% in BW, 84% in GR, 82% in TG, 54% in SG, 39% in A and and 20% in FL. According to the physicians, the main difficulties with screening arise due to missing interest of clients (31% of physicians) but also due to insurance problems (24% of physicians).

Conclusions: There are considerable differences among primary care physicians supporting regular screening for common cancers. The differences among countries may be due to different health care systems, reimbursement rules but possibly also due to the numbers of medical practitioners.

P2

Novel O6-Methylguanine-DNA Methyltransferase Single Nucleotide Polymorphisms (SNPS) Detected Among Heathy Individuals from the Swedish Normal Population

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The DNA mismatch repair protein O6-methylguanine-DNA methyltransferase (MGMT) is involved in the cellular defence against alkylating agents. Genetic alterations in the MGMT gene may impair the cellular capacity to remove alkyl groups from the O6-posotion of guanine, raising mutation rates and as a consequence increase the risk of cancer, whereas genetic alterations leading to increased MGMT activity may contribute to intrinsic resistance to alkylating drugs. We assessed genetic variation in the 5-noncoding region and the 5 exons of the MGMT gene among 76 healthy volunteers from the Swedish population with the aid of single strand conformation polymorphism (SSCP) combined with nucleotide sequence analysis. In total eleven SNPs were detected, 5 in the 5-noncoding region, one in exon 1, two in exon 3 and three in exon5. Six of these SNPs are novel, five were located in the 5-noncoding region and one in exon 5. The registered SNP frequencies ranged from 0.7 to 36%. Not much is know on the functional significance of MGMT SNPs. Comparative studies on MGMT variant frequencies among patient populations and in vitro studies of the cloned variants are in process in order to clarify the clinical impact on the recognized genetic variability.

P3

Indomethacin Stimulates Neutrophil and Monocyte Intracellular Killing

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Retrospective and prospective studies suggest the possible protective effects of nonsteroidal antiinflammatory drugs (NSAIDs) against colorectal cancer. Indomethacin (IN) has been one of the most actively investigated NSAIDs and is believed to act mainly through mechanisms of cyclooxygenase inhibition and synthesis of prostaglandins. Using acridine orange method for phagocytosis and 2,7-dichlorofluorescin diacetate method for oxidative burst evaluation and living yeast cells as targets, we investigated effects of 10^{-5} M and 5×10^{-5} M concentrations of IN on intracellular killing (% of killed ingested yeasts, PK) and oxidative burst (% of fluorescence-positive cells, OB) of peripheral blood polymorphonuclear granulocytes (PMN) and monocytes (Mo) in vitro in 10 healthy volunteers. IN at both concentrations significantly potentiated PIVIN PK (15% vs 6%, $p = 0.004$ at 10^{-5} M; 21% vs 6%, $P < 0.001$ at 5×10^{-5} M) and Mo PK (50% vs 38%, $p = 0.002$ at 10^{-5} M; 58% vs 38%, $p = 0.001$ at 5×10^{-5} M) compared to controls. IN at lower concentration also stimulated PIVIN OB (39% vs 17%, $p = 0.004$) and Mo OB (32% vs 13%, $p = 0.004$). Its higher concentration was stimulative to PIVIN OB (23% vs 17%, $p = 0.046$), although less than the lower one, but was without effect

to Mo OB. At lower, OB stimulating IN concentration, there was high positive correlation between PK and OB of PIVIN ($r = 0.800$; $p = 0.031$) and Mo ($r = 0.954$; $p = 0.001$). It is concluded that IN stimulates PIVIN/Mo intracellular killing and that this effect is, at least at lower IN concentration, mediated by stimulation of oxygen free radicals production which could Contribute to possible anticancer actions of IN involving immune mechanisms mediated by phagocytes.

P4

Acute Myelocytic Leukemia Stimulates Angiogenesis of Both Host and Cancer Associated Endothellal Cells

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Background: It is widely accepted that tumor metastasis and solid tumor growth beyond a certain size require a sufficient vascular supply. Recently, capillary density in the bone marrow was identified as prognostic parameter for non-solid tumors such as acute myelocytic leukemia (AML) and multiple myeloma. However, the mechanisms of vessel formation and the vascular physiology are poorly understood in hematological malignancies. We investigated the initial process of vessel formation and the role of the endothelial cell in acute leukemia. The vascular properties were studied using an established leukemia model and intravital microscopy. A green fluorescence protein (GFP) transduced endothelial cell line (H13MEC-1) was used to identify the initial process of vessel formation.

Methods: H13MEC-1 was co-implanted with a human AML cell line (M07) in the cranial window for continuous non-invasive intravital microscopy in 12 weeks old male SCID mice. Vascular parameters such as functional vascular density, velocity, leukocyte endothelial interaction (LEI) and vascular permeability were obtained using fluorescence microscopy, as described elsewhere *(Hansen-Algenstaedt et al.* Cancer Research 2000, *Yuan et al.* Cancer Research 1994).

Results: Tube formation by tumor-associated endothelial cells, the initial process of vessel formation, was observed one day after tumor cell implantation. Blood flow in newly formed vessels was detected 3 days after tumor cell implantation. New vessels were formed from endothelial cells recruited from the host Tumor cell implantation lead to an increase in the vascular permeability of pre-existing cranial vessels, in H13MEC-1 lined vessels, and in newly recruited vessels derived from host endothelium. Established tumor vessels remained hyperpermeable. Blood flow appeared heterogeneous.

Conclusions: The angiogenic process observed in this AMI-cell line is similar to that observed in solid tumors. This helps explain successful antiangiogenic therapy in AMI- and multiple myeloma patients. Furthermore, our results indicate that tumor endothelial cells and host endothelial cells are responsible for vessel formation.

P5

Antigenotoxic Effect of Polyphenolic Extracts of *Terminalia chebula* (Retz.) in Ames Assay

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The living plant may be considered as a biosynthetic laboratory or solar powered biochemical factory not only for the presence of primary metabolites but also for a multitude of secondary products of pharmaceutical significance, such as, phenols, polyphenols, triterpenoids, alkaloids, flavonoids and related compounds. Recent research has highlighted the immense importance of secondary metabolites because of their anticancer/antimutagenic properties. *Terminalia chebula* is an important medicinal tree, the fruit of which contains a large amount of polyphenols and tannins. It is one of the most active component of herbal drug Triphala (a highly efficacious cardiotonic). It is useful in treating cough, asthma, urinary disorders, constipation and heart diseases. Two different extracts of *T. chebula*, viz. chloroform and acetone extracts, were examined using two modes of experimentation, i.e., co-incubation and pre-incubation in Ames assay. Their antimutagenic effects were observed against direct-acting mutagens [sodium azide and 4-nitro-o-phenylenediamine (NPD)] and S9-dependent mutagen [2-aminofluorene (2AF)] in strains TA98 and TA100 of *Salmonella typhimurium.* Acetone and chloroform extracts showed maximum inhibition of 94.7and 56.7%, respectively, in pre-incubation mode of experimentation against 2AF at a concentration of 1×10^3 μg/0.1 ml. Pure polyphenolic compounds, i.e. gallic acid and ellagic acid, have been isolated from the acetone fraction and have been compared with the standards using various spectroscopic techniques, viz., ^{1}H-NMR, mass spectroscopy and IR. Further studies are in progress to test the bioactivity of purified fractions.

P6

Microsatellite Instability: A Predictor of Outcome in Gastric Cancer in a High-Frequent Area

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Introduction: Microsatellite instability (MSI) in gastrointestinal cancers has been proposed as an important step in the process of developing. In current study we have evaluated correlation of MSI in 8 MS region in gastric cancer tissue of the patients with a history of gastric cancer in their first-degree-relative.

Methods: Patients expired of gastric cancer with a positive family history of gastric cancer in the first-degree relative were included in the study. Paraffin-blocked tissues were obtained from pathology department. DNA was extracted and reserved in 4oC. MSI was checked based on 8 MS markers including BAT25, BAT26, BAT40, D2S123, D5S346, D13S170, D17S250 and TP53.

Results: 20 patients were enrolled in the study. Low-level MSI was detected in 9 (45%) and high-level MSI in 3(15%). 8 patients (40%) was MSS. Mean survival from the time of diagnosis was 11 (4-15) months in the low-level group and 5 (2-7) months in the low-level group. Mean survival of low-level MSI patients was significantly higher than high-level MSI group ($P < 0.05$). Interestingly, the shortest survival time (2 month) was detected in a patient with 2 first-degree relatives involved by gastric cancer.

Conclusion: This study shows that MSI could be one of the predictors of outcome in patents with gastric cancer and a positive family history of the same cancer. More confirmation needs a large sample-sized study which we are proposing.

P7

Alterations of E-Cadherin, β-Catenin and FHIT in Gastric Cancer

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The E-cadherin-catenin complex plays a crucial role in epithelial cell-cell adhesion and in the maintenance of tissue architecture. Perturbation in the expression or function of this complex results in loss of intercellular adhesion, with possible consequent cell transformation and tumour progression. The FHIT gene is a putative tumour suppressor gene, abnormalities of which may be involved in the gastric carcinogenesis. This study was to analyze the genetic defects of E-cadherin, β-catenin and Fhit in gastric cancer.

We studied the alterations of E-cadherin, β-catenin and Fhit in a set of 50 primary gastric tumours by using loss of heterozygosity (LOH) analysis, gene mutation screening, detection of aberrant transcripts and immunohistochemistry (IHC).

A high frequency of LOH was detected at 16q22.1 containing the E-cadherin locus (75%) and within the FHIT gene (84%). Three cases (6%) showed the identical missense mutation, A592T, in the E-cadherin gene. We found that 7 tumours (18%) had aberrant E-cadherin mRNA in addition to the normal mRNA. Also 34 of 39 (87%) tumours exhibited low FHIT expression or aberrant FHIT mRNA. Reduced expression of E-cadherin, β-catenin and Fhit was identified at the frequency of 42%, 28% and 78%, respectively. Specially, 11 tumours (22%) exhibited positive cytoplasmic staining for β-catenin by IHC.

Our results support the hypothesis that alterations of E-cadherin, β-catenin and Fhit play a role in the gastric tumorigenesis.

P8

N-(4-Hydroxyphenyl)Retinamide Induces Cell Death and Decreases *In Vivo* Tumorigenicity of Human Retinoblastoma Cells

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The locally invasive retinoblastoma tumor is the most common intraocular neoplasia of childhood. Retinoblastoma originates from a multipotential stem cell of the developing neural retina, due to loss-of-function of the tumor-suppressor gene RB. Neural cells lacking pRB expression or expressing a defective pRB protein, undergo apoptosis during neuronal development, neverthless, retinoblastoma cells can replicate and escape the apoptotic fate for unknown reasons. The majority of children with retinoblastoma have extensive disease at diagnosis, requiring enucleation of the eye and aggressive chemotherapy or radiotherapy, which greatly increase the risk for second tumors. Early diagnosis, and the better understanding of retinoblastoma tumor biology in response to potential therapeutic agents with low toxicity, could improve the development of novel therapeutic approaches for cure and prevention of the disease. In particular, targeting programmed cell death signaling in retinoblastoma cells has been indicated as a possible tool to develop even chemopreventive strategies. The chemopreventive synthetic retinoid N-(4-hydroxyphenyl)retinamide (4HPR) has been shown to induce growth arrest and cell death by apoptosis and/or necrosis of different tumoral cell types of neuroectodermal origin. In this work we studied the sensitivity of retinoblastoma cells to 4HPR *in vitro* on suspension and attachment cultures of Y79 cells, and *in vivo* in an ectopic model of tumor growth in nude mice. 4HPR treatment in the range 2.5-10μM induced loss of Y79 cell viability, increasing on adherent cells and in the absence of serum, which was due to cell necrosis rather than apoptosis, as shown by flow cytometric analysis. Intracellular reactive oxygen species (ROS) generation in 4HPR-treated cells was demonstrated by detection of fluorescent H_2DCFDA, while catalase rescued cell viability to control values, indicating that 4HPR-induced cell death could be mediated by oxidative stress. RT-PCR analysis showed that mRNAs for the nuclear retinoic acid receptor RARβ and the retinal differentiation marker arrestin are upregulated in 1 μM 4HPR-treated Y79 cells in the absence of serum. *In vivo* tumor growth and angiogenesis, and tumor incidence were also significantly inhibited by 4HPR in a xenograft model of retinoblastoma in nude mice suggesting a novel therapeutical application of 4HPR.

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.