COX-2 inhibition might be affected by contribution degree of COX-1 to intracellular PGs quantity. Therefore, the physiological roles of PGs derived from COX-1 as well as COX-2 even in many cancer cells with high expression of COX-2 require further investigation to establish COX-2 inhibition as a new modality for cancer treatment.

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Inhibition of DMBA-DNA adduct formation and modulation of TPA induced activation of AP-1 and NFkappaB transcription factors in mouse epidermis by naturally occurring plant phenols

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Mouse skin is one of the best animal models of chemical carcinogenesis which enables to study all stages of this process. Although most human skin cancers are not induced by chemicals, many events in this model could be extrapolated to humans. Moreover the biochemical changes observed in the mouse skin after application of tumor promoter 12-O-tetradecanoylphorbol acetate (TPA) are the same as those in humans after UVB radiation. Skin tumor initiator 7,12-dimethylbenz[a]anthracene (DMBA) is metabolically activated to syn- and anti-diol epoxides (DE), which form DNA adducts. The formation of dAdo adducts by DMBA diol-epoxides lead to mutation at the codon 61 of H-ras and consequently initiate tumorigenesis in mouse skin. Oncogenic H-ras can activate NFkappaB which similarly as AP-1 is considered to be a mediator of tumor promotion. In the present study we investigated the effects of topical application of plant phenols protocatechuic, chlorogenic and tannic acid on the DMBA-DNA adducts formation and the modulation of TPA induced activation of AP-1 and NFkappaB transcription factors in mouse epidermis. The application of these phenolic acids on mouse skin significantly reduced the DMBA binding to DNA. The most effective was tannic acid which almost completely inhibited the DMBADE-dAdo adduct formation. All phenols decreased the induced by TPA activation of the transcription factors AP-1 and NFkappaB by affecting their subunits expression, nuclear translocation and binding to specific sequence of DNA. Again the most efficient, particularly towards NFkappaB was tannic acid which increased the retention of IkappaBalfa in cytosol, reduced the nuclear translocation of p65 subunit and inhibited its binding to specific sequence of DNA.

In view of the important roles of the dAdo adducts activation in H-ras mutation and subsequent tumor initiation and AP-1 and NFkappaB in tumor promotion/progression the results of this study suggest that the ability of tannic acid and to lesser extent protocatechuic and chlorogenic acids to inhibit tumor development may be mediated by impairing signal transduction pathways leading to activation of AP-1 and NFkappaB.

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Experimental Therapy

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Immunotherapy with autologous dendritic cells in patients with hormone-refractory prostate carcinoma

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Currently there is no effective treatment available for metastatic prostate cancer. The enhancement of a normally weak immune response to tumor-antigens might therefore be a reasonable strategy in cancer treatment. Dendritic cells (DC) represent the most efficient antigen presenting cells, to initiate T cell responses in vitro and in vivo. For this reason autologous monocyte-derived DC, pulsed with peptides from multiple prostate antigens were used to vaccinate patients with hormone-refractory prostate cancer. Before application the DC were tested for maturation marker expression by flow cytometry and for migratory function. The DC vaccine is well tolerated and the induction of T cell responses and the course of the PSA-velocities are under investigation. The induction of an efficient immune response to over-expressed tumor antigens might be a strategy for the prevention of cancer.

P45

Selective cytotoxicity of an isolate from Cassia alata L. leaves

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In response for the continuing need for new therapeutics against cancer, leaf extracts of akapulko, Cassia alata L. were tested for their possible cytotoxic activity on five mammalian cell lines namely MCF-7, SkBr3, (both are breast cancer cells), T24 (urinary bladder cancer), Col 2 (Colon cancer) and A549 (non-small lung cancer) cell lines. The different mammalian cell lines were treated with methanol, hexane and ethyl acetate at different concentrations of 3.75, 7.5, 15, 25, 50, and 100 μg/ml. Doxorubicin, a known anticancer drug was also used to treat the cells and served as the positive control. The effects of the extracts were also tested on normal AA8 cells, from hamster ovary. The present study demonstrated that hexane (FB) caused remarkable cytotoxic effect on MCF-7, T24 and Col2 in a dose dependent manner as revealed by a low % cell survival using MTT assay and morphological investigation using light microscopy. Active FB fraction was then subjected to repeated and sequential chromatographic procedures following the bioactivity - directed fractionation and this yielded a TLC pure isolate, f6l. f6l was further evaluated using MTT, morphological and biochemical investigations and likewise showed a

remarkable selectivity to MCF-7, T24 and Col2 cells with an IC50 of 16, 17, and 17μg/ml. The isolate however showed no cytotoxicity to the normal cell line, CHO-AA8. Morphological changes like cytoplasmic membrane blebbing, detachment of cells from the substrate and neighboring cells, nuclear condensation, formation of apoptotic bodies and reduction in cell size were observed in treated cells. Observations using the DAPI, a fluorescent stain complement the morphological analysis made under the bright-field microscope. Further, terminal deoxynucleotidyl transferase-mediated d-UTP nick end labeling (TUNEL) indicated that DNA fragmentation was by apoptosis which suggest its potential as a chemotherapeutic agent. Spectral characterization of the isolate showed that f6l contained polyunsaturated aliphatic esters or carboxylic acids.

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Interferon alpha/beta in rat liver after partial hepatectomy as growth modulator of hepatocytes

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Interferon (IFN)-α treatment is a common therapy for chronic viral hepatitis and contributes to preventing hepatocarcinogenesis. Besides, IFN is prescribed as a prolong course after surgical removal of tumors, combined with chemotherapy and radiotherapy. Along with IFN-sensitive cancers (kidney adenocarcinoma, lung sarcoma, malignant melanoma, neuroblastomas, cancers of limphoid, endocrine and generative organs), there are more resistant ones - cancers of stomach, liver and colon. Moreover, high and low concentrations of IFN can cause different answers. Our aim was to assess the expression of IFN alpha and beta in nontransformed liver after partial hepatectomy (PHE) and laparotomy. The expression of interferon α/β was assessed at RNA level by RT-PCR and as content of the protein in biological test in 0.5, 1, 3, 6, 12 h after operations. The cell specificity of IFN α/β production was assessed in isolated hepatocytes and KC. IFN α and its mRNA is detected in the intact liver. PHE induces increase of IFN α protein and mRNA content during first 3 hours after operation with further decrease till 6–12 hours. This increase is less than maximally possible liver response to the injection of PolyI-PolyC inducer. IFN β is detected neither in intact nor in regenerating rat liver. IFN α-specific mRNA was shown to be produced by KC and not by hepatocytes. The laparotomy is characterized by sharp decrease of IFN α content to null in the liver. That is why the increased IFN α production in regenerating liver is not linked with acute phase response. Laparotomy is a model for acute phase response and possibly the same processes occur after surgical interference. We assume that IFN α and its targets are essential for hepatocytes to leave quiescent state and proceed to proliferation. Liver sensitivity to IFN treatment may be different at pre- and postoperational periods.

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In-vitro antiproliferative effect of Gonatanthus pumilus lectin on various human cancer cell lines

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Gonatanthus pumilus lectin (GPL) is known to polyclonally activate human T-cells. GPL agglutinated rabbit, rat, guinea pig and sheep erythrocytes but was unable to agglutinate human ABO blood group erythrocytes. N-acetyl-D-lactosamine and serum glycoprotein asialofetuin were found inhibitory in the hemagglutination inhibition assay. The lectin was purified by affinity chromatography using asialofetuin linked amino activated silica gel. The lectin had no requirement for divalent metal ions like Ca2+ and Mn2+ for its activity. GPL has a carbohydrate content of 4.1%. Chemical modification of GPL with pyridoxal, Diethylpyrocarbonate and Bis-dithionitrobenzoic acid did not affect its activity, suggesting the absence of arginine, histidine and cysteine respectively in or near the ligand-binding site of the lectin. Modification of tyrosine with N-acetylimidazole led to 50% inactivation of GPL. However, total inactivation was observed only upon N-bromosuccinimide modification of tryptophan residues of the lectin. In vitro antiproliferative activity of GPL was tested on seven human cancer cell lines DU145 (Prostate), PC-3 (Prostate) A549 (Lungs), HCT15 (Colon), 502713 (Colon), KB (Oral) and IMR32 (Neuroblastoma). A 50% inhibition of proliferation was observed in DU145, PC3 and KB at the lowest concentration (10 mg/ml or less) of the lectin tested. HCT15 and 502713 cell lines showed 50% inhibition at 50 mg/ml of GPL. While very less inhibitory effect of GPL was observed on the proliferation of A549 and IMR32. The inhibitory effect of GPL was not associated with toxicity to the cell lines.

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Cysteine proteases as target for anticancer therapy and tumor prevention

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Human lysosomal cysteine proteases of the papain superfamily have received increasing attention as promising novel therapeutic targets (Bromme et al., 2005). Progression of human tumors is accompanied by the increased expression and activity of cysteine, aspartyl, serine and metalloproteases. A correlation was found between tumor growth and increase of cysteine proteases in humans.

Aim: to evaluate the role of inhibitor of cysteine proteases in treatment and prevention of experimental murine tumors.

Methods: CBA mice were used; lymphosarcoma LS (106 cell/ml) was implanted into tight muscles. Cyclophospamide, in the doses of 25,30, 50 and 100 mg/kg, recombinant TNF-α