dose of glucoraphanin. In conclusion, broccoli sprout extracts as a source of sulforaphane either topically or in the diet protect against skin tumor formation.

P39

Antioxidant activity of flavonoids and other polyphenols isolated from Annona squamosa Linn. leaf extracts

G.A.N. Punzalan[1], G.P. Concepcion[2], R.P. Dator[3], A.R. Mazahery[4], S.D. Jacinto[5]. [1]*UP Institute of Biology, College of Science, Developmental Biology Academic Group, Quezon City, Philippines;* [2]*UP Marine Science Institute, College of Science, Marine Natural Products, Quezon City, Philippines;* [3]*UP Institute of Chemistry, Natural Products Laboratory, Quezon City, Philippines;* [4]*UP Institute of Biology, College of Science, Developmental Biology Program, Quezon City, Philippines;* [5]*UP Institute of Biology, College of Science, Developmental Biology Academic Group, Quezon City, Philippines*

Leaf extracts from Annona squamosa Linn., a ubiquitous fruit tree with recognized medicinal uses, were partitioned with chloroform and subjected to column chromatography and preparative thin-layer chromatography yielding two bands that indicate the presence of the flavonoid polyphenolic compounds. These semipure fractions exhibited the highest inhibitory activity when assayed for the ability to scavenge the diphenylpicrylhydrazyl (DPPH) free radical. This sensitive assay useful even for slow reactions, colorimetrically quantifies the removal of the DPPH free radical generated by the reaction, thus serving as a convenient in vitro monitor of potential oxidative assault on normal cells. At the same time, liquid chromatographic peaks and dereplication of mass spectral data suggest the presence of three polyphenolic compounds including a novel xanthone not yet reported for this plant. This affirms the recognition of plant phenolics' antioxidant properties and consequent antitumor role and may also provide a useful beginning for the characterization and elucidation of this class of phenolic compounds.

P40

Apoptosis induction by green tea compounds in cervical cancer cells

F. Garcia[1], J.M. Feugang[2], J. Wang[3], S.J. Cheng[4], C.P. Zou[5]. [1]*University of Arizona, College of Medicine, Obstetrics and gynecology, Tucson, United States of America;* [2]*University of Arizona, College of Medicine, Obstetrics and gynecology, Tucson, United States of America;* [3]*University of Arizona, College of Medicine, Obstetrics and gynecology, Tucson, United States of America;* [4]*University of Arizona, College of Medicine, Obstetrics and gynecology, Tucson, United States of America;* [5]*University of Arizona, College of Medicine, Obstetrics and gynecology, Tucson, United States of America*

Human Papilomavirus (HPV) infection is closely associated with the development of over 95% of cervical cancer and 50-60% of head and neck cancer and skin cancer. Clinical trials using several chemopreventive agents are underway, but early results are inconclusive. All agents used in the trials were able to inhibit the growth of cancer cells and about half of the patients responded to the treatment. However, relapse occurred after discontinuation of the drugs. Therefore, selection of non-toxic agents especially food, beverage, and natural products that can suppress HPV virus and inhibit malignant cell growth which can be used long time is vitally important in prevention of cervical cancers. We evaluated green component of EGCG and polyphenol E (poly E) on growth inhibition and apoptosis induction of cervical epithelial cells and cervical cancer cells. HPV-immortalized cervical epithelial cells, TCL-1 and HPV-positive cervical cancer cells, Me180 and HeLa were used in the study. Both green tea compound EGCG and poly E were able to inhibit cervical epithelial and cancer cell growth. Apoptosis induction by EGCG was detected in cervical cancer cells. The growth inhibition and apoptosis induction were in dose-dependent manner. Apoptosis-related genes, such as p53 and p21 were induced by EGCG in cervical cancer cells. The green tea compounds in suppression of HPV-E6 and E7 were also tested by immunohistochemistry. The results of this study provided information on potential mechanisms of green tea compounds in prevention of HPV-related cervical cancer. This information will enable us to assess the feasibility of using these agents in clinical trial setting. This study was supported by the Women's Fund for Health, Education, and Research and the National Institutes of Health, National Cancer Institute (NIH/NCI), grant NOI-CN-35158.

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Cyclooxygenase-2 (COX-2) independent tumor-killing effect of chemical COX-2 inhibitors compared to small interfering RNA of COX-2 in head and neck cancer cell lines

S.W. Park[1], M.W. Sung[2]. [1]*Seoul National University College of Medicine, Department of Tumor biology, Cancer Research Institute, Seoul, South Korea;* [2]*Seoul National University College of Medicine, Department of Otolaryngology-Head and Neck Surgery, Seoul, South Korea*

The observed over-expression of cyclooxygenase-2 (COX-2) in many types of cancer has highlighted this molecule as a potential target for therapeutic intervention. Using head and neck squamous cell carcinoma (HNSCC) cell lines, COX-2 was found to be up-regulated by many oncogenic factors and COX-2 inhibitors exhibited a good anti-tumor effect. However, little physiological change in cell viability by increased prostaglandin E2 (PGE2) was detected, contrary to cases using colon cancer cells. COX-2 inhibitors were found to have an anti-tumor effect at much higher concentrations than doses required to block COX-2 activity. From these considerations, the anti-tumor effect of chemical COX-2 inhibitors was thought to result from a COX-2-independent action at high concentrations in HNSCC cell lines. Firstly, the growth-inhibitory effect of several COX-2 inhibitors was compared with small interfering RNA (siRNA) against COX-2. Additionally, to discriminate between the mechanisms of action of inhibitors and siRNA of COX-2, the effects on intracellular signaling were tested by two inhibitory methods. In conclusion, siRNA against COX-2 was not able to inhibit the proliferation of HNSCC cell lines and its action seemed to differ from anti-tumor action of COX-2 inhibitors. On the other hand, the findings that co-inhibition of both COX-2 and COX-1 may decrease VEGF production partially in HNSCC cell lines imply that anti-cancer effect of

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.

P42

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

W. Baer-Dubowska[1], H. Szaefer, M. Cichocki. [1]*University of Medical Sciences, Department of Pharmaceutical Biochemistry, Poznan, Poland*

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

R. Maier[1], R. de Giuli[1], J. Hering[2], R. von Moos[3], C. Böhme[3], H-P. Schmid[4], T. Cerny[3], B. Ludewig[1], S. Gillessen[3]. [1]*Kantonal Hospital St. Gallen, Research Department, St. Gallen, Switzerland;* [2]*Blood bank, Red Cross, St. Gallen, Switzerland;* [3]*Kantonal Hospital St. Gallen, Oncology Department, St. Gallen, Switzerland;* [4]*Kantonal Hospital St. Gallen, Urology Department, St. Gallen, Switzerland*

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

E.I. Olarte[1], A.A. Herrera[2], I.M. Villasenor[3], S.D. Jacinto[2]. [1]*Don Mariano Marcos Memorial State University, Natural Sciences Department, Agoo, La Union, Philippines;* [2]*College of Science, Institute of Biology, Diliman, Quezon City, Philippines;* [3]*College of Science, Institute of Chemistry, Diliman, Quezon City, Philippines*

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