to hypermethylation of its promoter region. Both 5-AZA-CdR and TSA alone showed growth suppression in both cell lines. RA-resistant SqCC/Y1 cells did not express RAR-beta by treatment with 1 microM atRA alone. Also, 5-AZA-CdR alone did not activate the expression of RAR-beta. However, the combination of atRA and 5-AZA-CdR appeared to increase RAR-beta expression in SqCC/Y1 cells. In SqCC/Y1 cell, flow cytometric analysis indicated that TSA augumented atRA-induced cell cycle changes. Inhibition of methylation and deacetylation may reverse sensitivity of atRA by reactivation RAR-beta in RA-resistant cell line.

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COX-2 inhibition may not be related with growth inhibition and cell cycle phase-specific apoptosis by celecoxib in human NSCLC cells *in vitro*

H. Kuh, J. Kang, K. Choi. *Catholic Research Institutes of Medical Science, Seoul, Republic of Korea*

Background: Cyclooxygenase-2 (COX-2) is an inducible enzyme which produce prostanoids by various stimuli. Overexpression of COX-2 in many tumor types supported its association with tumor progression, which has been a target for chemoprevention and chemomodulation. Celecoxib, a specific COX-2 inhibitor, originally developed for antiinflammatory agent showed anticancer activity. We studied conc and time dependency of COX-2 inhibition, growth inhibition, and cell cycle arrest induced by celecoxib in A549 COX-2 overexpressing human NSCLC cells.
Methods: Inhibition of COX-2 activity and COX-2 expression were measured using an Enzyme Immunoassay (EIA) for PGE2 and Western blot, respectively. Growth inhibition and cell cycle distribution were determined by SRB assay and flow cytometry, respectively. Relationship between cell cycle arrest and apoptosis induction was studied using TUNEL/DNA-content two parameter flow cytometry.
Results: Inhibition of COX-2 activity was conc- and exposure-time dependent. COX-2 inhibition at 0.1μM increased with increasing exposure time i.e., 20% at 6hr to 60% at 24hr. IC_{50} and IC_{80} for 24hr exposure were approx. 0.1 and 1μM, respectively. Growth inhibitory effect was also showed conc and time dependency. Cytotoxic IC_{50} after 6hr exposure was 110 μM and decreased to 20 μM after 72hr exposure. These conc were about 600 fold higher than those of COX-2 inhibition. At 50 μM ($IC_{80,72hr}$) G1 phase block and apoptosis was induced after 24hr and the apoptotic cell population appeared from G1 phase. No significant apoptosis was shown at 20 μM ($IC_{50,72hr}$). The level of COX-2 expression was not altered when treated up to 20 μM.
Conclusion: In human NSCLC cells, the inhibitory conc of COX-2 activity and cell growth were more than 600 fold different, suggesting that these two effects may not have direct causal relationship. Growth inhibition and apoptosis induced by celecoxib are associated with G1 phase arrest, which may be important in designing of combination regimen of celecoxib. Changes in expression level of COX-2 and other factors at higher conc are under investigation to elucidate the mechanism of growth inhibition by celecoxib in human NSCLC cells.

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Regulation and function of Cyclooxygenase-2 (COX-2) in ovarian carcinoma cells

C. Denkert, I. Koch, A. Fürstenberg, M. Köbel, S. Hauptmann. *Charite Hospital, Institute of Pathology, Berlin, Germany*

Cyclooxygenases are enzymes involved in production of prostaglandins and play a role in the regulation of tumor development and progression in several different tumors. We have recently shown that an increased expression of COX-2 is an independent prognostic factor for poor survival in patients with ovarian carcinomas. Based on this immunohistochemical study, we performed cell culture experiments to investigate the regulation of COX-2 in the ovarian carcinoma cell line OVCAR-3. Using RT-PCR and Western blot, we observed a strong induction of COX-2 mRNA and protein levels after treatment with interleukin-1beta. In parallel, increased levels of prostaglandin E2 were measured by ELISA. In a luciferase-assay, a basal activity of the COX-2 promoter was detected, which was increased 2-fold after treatment with interleukin-1beta. Inhibition of the p38MAPK pathway with the inhibitor SB203580 (1-10μM) reduced COX-2 protein levels as well as PGE2 levels. In contrast, inhibition of the p42/44MAPK pathway induced only a slight inhibition of COX-2 protein levels at inhibitor concentrations of 50μM. Production of PGE2 was inhibited by SB203580 at inhibitor concentrations of 2μM. We used the COX-2 inhibitor NS398 to investigate the effect of COX-2 inhibition on PGE2 levels as well as cell proliferation. While NS398 completely inhibited PGE2 production at concentrations of 1μM, cell proliferation was affected only at inhibitor concentrations of 100μM. The data indicated that the p38MAPK pathway is involved in regulation of COX-2 expression in OVCAR-3 cells and that the anti-proliferative effects of the inhibitor NS-398 are most likely mediated through a non-COX target.

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Epican forte - a specific formulation of nutrients containing lysine, proline, ascorbic acid, and epigallocatechin gallate inhibits matrix metalloproteinases activity and the invasion potential of human cancer cell lines

M.W. Roomi, S.P. Netke, V. Ivanov, M. Rath, A. Niedzwiecki. *Matthias Rath, Inc., Research Division, Santa Clara, USA*

One of the hallmarks of cancer is its ability to invade and metastasize to distal organs. Matrix metalloproteinases (MMPs) have been identified as key players in tumor invasion and metastasis. Current treatment protocols with chemotherapy and/or radiation are toxic and have the potential to destroy healthy cells. Our approach has been to develop strategies to inhibit cancer development, progression, and metastasis using naturally-occurring nutrients. Lysine and proline are the building blocks of collagen fibers that stabilize connective tissue. Vitamin C is essential for the production of collagen, and is a scavenger of free radicals that protects cells from damage. Epigallocatechin Gallate (EGCG) is a green tea extract with antioxidant and anticarcinogenic properties. It is postulated that the combination of these nutrients exerts a very potent synergistic, anticancer activity. Based on this prediction, Epican Forte (EF) was formulated by Matthias Rath, Inc. EF contains a mixture of nutrients, including lysine, proline, ascorbic acid, and EGCG. In the present study, we investigated the effect of EF on MMP expression, invasion potential, and cytotoxicity/cell proliferation in several human cancer lines of the skin (melanoma), breast (MDA-MB-231) and liver (Hep G2). We also studied the effects of EF on normal human dermal fibroblast (NHDF) and on the co-culture of melanoma and NHDF cells. MMP expression was studied by zymography, invasion through Matrigel, and cytotoxicity/cell proliferation by MTT assay. EF inhibits the expression of MMP-2 and MMP-9 in a dose-dependent fashion. The expression of MMP-2 and MMP-9 was significantly inhibited with a concentration of 100 μg/ml of EF and virtually undetectable with a concentration of 1000 μg/ml. EF used at 10 and 100 μg/ml concentrations did not significantly affect the cells viability, and at 1000 μg/ml it showed cytotoxicity at the range of 10-40 percent, depending on the cell type. The invasion of melanoma cells, MDA-MB-231 cells, and a co-culture of melanoma cells with NHDF through Matrigel was significantly reduced in a dose-dependent manner. Thus, these results demonstrate that EF is very effective for several cancer cell lines and also in co-culture. These observations reveal that EF may provide a natural therapeutic basis that makes it a valuable and promising candidate for the treatment of human cancers. Currently, experiments are in progress to evaluate the efficacy of EF in a clinical setting.

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The chemopreventive activities of vitamin A, beta-carotene and all-trans and 9-cis retinoic acids during hepatocarcinogenesis in rats involve inhibition of cell proliferation but not induction of apoptosis

F.S. Moreno[1], E.M.A.V. Fonseca[1], T.P. Ong[1], R.P. Mazzantini[1], R. Heidor[1]. [1]*Faculty of Pharmaceutical Sciences, Department of Food and Experimental Nutrition, Sao Paulo, Brazil*

Although data from several epidemiological studies suggest a protective role of retinoids and carotenoids against cancer, 2 large trials (CARET and ATBC) conducted with vitamin A and/or beta-carotene have yielded negative or conflicting results. Therefore, in this study vitamin A (VA), beta-carotene (BC), all-trans and 9-cis retinoic acids (ATRA and 9CRA) chemopreventive activities were evaluated on preneoplastic lesions (PNL) induced in Wistar rats by the "resistant hepatocyte" model of hepatocarcinogenesis. Thus, animals received by gavage every other day during 8 weeks VA (1mg/100g body weight [bw]; VA group), BC (7mg/100g bw; BC group), ATRA (1mg/100g bw; ATRA group), 9CRA (1mg/100g bw; 9CRA group) or corn oil (CO) (0.25 mL/100g bw; control group). The macroscopic examination of the livers (incidence and multiplicity, respectively) showed: 100% and 44$\pm$32 (control group); 82% and 7$\pm$10 ($p<0.05$; VA group); 46% ($p<0.05$) and 4$\pm$6 ($p<0.05$; BC group); 92% and 27$\pm$31 (ATRA group); 92% and 11$\pm$15 ($p<0.05$; AT9C group). Moreover, the morphometric analysis of GST-P positive PNL (area [mm^2], % of the section area occupied by PNL and number of PNL/cm^2, respectively) revealed: 0.44$\pm$0.50, 9.7$\pm$6.3

and 166±49 (control group); 0.29±0.31 ($p<0.05$), 1.2±2.5 ($p<0.05$) and 96±43 ($p<0.05$) (VA group); 0.15±0.11 ($p<0.05$), 1.0±0.8 and 106±42 ($p<0.05$) (BC group); 0.39±0.42, 2.1±2.0 ($p<0.05$) and 107±45 ($p<0.05$) (ATRA group); 0.34±0.36, 1.7±2.8 ($p<0.05$) and 61±42 ($p<0.05$) (9CRA). In addition, the hepatic PCNA labeling indexes (%) analyzed by imunohistochemistry (normal adjacent tissue and PNL, respectively) were: 5.3±2.2 and 6.7±2.5 (control group); 1.7±0.7 ($P<0.05$) and 2.4±1.0 ($p<0.05$) (VA group); 2.3±0.8 ($p<0.05$) and 3.0±0.8 ($p<0.05$) (BC group); 3.3±0.6 ($p<0.05$) and 4.1±0.9 ($p<0.05$) (ATRA group); 2.2±0.5 ($p<0.05$) and 2.2±0.9 ($p<0.05$) (9CRA group). No significant differences were observed among the experimental groups in the hepatic apoptotic indexes (normal adjacent tissue and PNL, respectively) as determined by morphological criteria. Therefore, these data indicate that the retinoids and the carotenoid present pronounced chemopreventive activities during hepatocarcinogenesis and suggest that these protective actions could be attributed to an inhibition of cell proliferation but not to an induction of apoptosis. Financial assistance: FAPESP/CNPq/CAPES.

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Emodin inhibits MMPs scretion and invasion in glioblastoma cells

S. Lee[1], M. Kim[1], M. Park[2], H. Yoo[1]. [1]*National Cancer Cencer, Korea, Neuro-Oncology clinic;* [2]*Korea Cancer Center Hospital, Laboratory of Cell biology, Seoul, Korea*

Emodin (3 methyl-1, 6, 8 trihydroxyanthraquinone), is an inhibitor of the protein tyrosine kinase, has been shown to display a number of biological activities such as antiviral, antimicroviral, immunosuppressive, anti-inflammatory, and anticancer effects. Emodin was shown to suppress HER-2/neu tyrosine kinase activity in HER-2/neu overexpressing human breast and lung cancer cells and can increase the repair of UV- and cisplatin induced DNA damage in human cells. In this report, we investigated the effects and mechanisms of emodin inhibited cell invasion in human tumor cells. Cancer cell invasion requires coordinated processes, such as changes in cell-matrix adhesion, degradation of the extracellular matrix, and cell migration. We found that emodin significantly inhibited invasion of glioma cells through the modified invasion assay. Adhesion of cells to the collagen matrix was also inhibited. Moreover emodin reduced expression of MMP-2 and induced MMP-9 in various tumor cell lines(breast, cervical, prostate, glioma). Both AKT/PKB and MAP Kinase are involved in the modulation of MMP production. Our results demonstrated that emodin inhibits cell invasion by reduction of MMP expression through bolcking FAK, MAP kinase and AKT/PKB pathway and suppression of transcription factor, NF-kB and AP-1. These results suggest that emodin can contribute to the reduction of invasion in tumors. In summary, our results indicate that emodin, a tyrosine kinase inhibitor, can effectively inhibit PMA or hyaluronic acid induced MMPs activation and *in vitro* invasion of glioblastoma cells as well as other cancer cells. These results may have important chemotherapeutic implications for emodin as a anti-invasive and anti-metastatic agent

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Beta-adrenergic, AA-dependent pathways as targets for chemoprevention of pulmonary and pancreatic adenocarcinoma

H. Schuller, *University of Tennesse College of Veterinary Medic, Pathology, Knoxville, USA*

Pulmonary adenocarcinoma and pancreatic adenocarcinoma are among the leading causes of cancer deaths. Both cancers are highly resistant to existing preventive and therapeutic approaches. Our data in human cancer cell lines derived from adenocarcinomas of the lungs or pancreas with or without activating point mutations in K-ras indicated that both cancers are regulated by beta-adrenergic receptors that control the release of arachidonic acid (AA). Beta-blockers, inhibitors of cyclooxygenase-2 (COX-2) or 5-lipoxygenase (5-LOX) inhibited the growth of both cancer types irrespective of the presence of ras mutations. Preliminary data indicate cross-activation of the EGF pathway by beta- adrenegic stimulation. Bioassays in hamster models of NNK-induced pulmonary or pancreatic adenocarcinomas revealed strong chemopre- ventive effects of the beta-blocker propranolol, the COX-inhibitor aspirin, or the 5-LOX inhibitor MK886. Our data suggest blockade of beta- adrenergic receptors and the AA-cascade as promising targets for the chemomoprevention of pulmonary and pancreatic adenocarcinoma.

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The chemopreventive activities of farnesol and geraniol in rats submitted to the resistant hepatocyte model of hepatocarcinogenesis involve inhibition of cell proliferation

T.P. Ong[1], R.M. Espindola[1], R. Heidor[1], M.L.Z. Dagli[2], A. Moura[1], A. Tenuta-filho[1], F.S. Moreno[1]. [1]*Faculty of Pharmaceutical Sciences, Department of Food and Experimental Nutrition, Sao Paulo, Brazil;* [2]*Faculty of Veterinary Medicine and Zootechny, Department of Pathology, Sao Paulo, Brazil*

Natural occurring isoprenoids found in citric fruits and herbs essential oils have been considered a potential class of chemopreventive agents. Therefore, in this study farnesol (FR) and geraniol (GR) chemopreventive activities were evaluated on preneoplastic lesions (PNL) induced in Wistar rats by the resistant hepatocyte model of hepatocarcinogenesis (initiation with diethylnitrosamine followed by selection/promotion of initiated hepatocytes with 2-acetylaminofluorene and partial hepatectomy). Thus, the animals received by gavage during 8 consecutive weeks FR (25 mg/100g body weight [bw]; FR group), GR (25 mg/100g bw; GR group) or corn oil (CO) (0.25 mL/100g bw; control group). One hour before sacrifice the rats were injected with 5-bromo-2-deoxyuridine (BrdU). The macroscopic examination of the livers (incidence and average number of PNL, respectively) showed: 100% and 42±46 (control group); 13% and 1±3 ($p<0.05$) (FR group); 42% and 18±45 (GR group). Moreover, the morphometric analysis of GST-P positive PNL (area [mm^2], % of the section area occupied by PNL and number of PNL/cm^2, respectively) revealed the following: 0.18±0.33, 10.0±7.4 and 50±13 (control group); 0.09±0.17 ($p<0.05$), 2.8±3.6 ($p<0.05$) and 34±22 (FR group); 0.11±0.25 ($p<0.05$), 5.1±2.9 and 53±36 (GR group). In addition, the plasmatic (mg/dL) and hepatic (mg/g) total cholesterol levels evaluated by enzymatic and HPLC methods, respectively, were: 64±7 and 3.14±0.2 (control group); 55±8 ($p<0.05$) and 3.07±0.2 (FR group); 69±8 and 3.12±0.2 (GR group). Furthermore, BrdU labeling indexes (%) analysis by imunohistochemistry in the livers of the animals from the control, FR and GR groups (normal adjacent tissue and PNL, respectively) showed the following: 1.2±0.8 and 1.8±0.7 (control group); 0.4±0.7 ($P<0.05$) and 0.5±0.7 ($p<0.05$) (FR group); 0.5±0.8 and 0.6±0.8 ($p<0.05$) (GR group). Therefore, these data indicate that both isoprenoids (farnesol and geraniol) present pronounced chemopreventive activities during hepatocarcinogenesis and suggest that these protective actions could be attributed, at least in part, to their inhibitory effects on cell proliferation. Financial assistance: FAPESP(00/00918-8)/CNPq/CAPES.

Differentiation

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Pathway pathology: how to identify signaling pathways in mouse models of human breast cancer

A. Rosner[1], J. Gregg[2], R.D. Cardiff[1]. [1]*University of California, Davis, Center for Comparative Medicine;* [2]*University of California, Davis, School of Medicine, Sacramento, USA*

Human mammary cancer is frequently associated with a mutational activation of the ErbB (HER-2) signal transduction pathway. In contrast, "spontaneous" mouse mammary tumors are associated with either Wnt or Fgf signaling and do not resemble human breast cancer. Using examples of genetically engineered mice from these signaling pathways from the UCD Mutant Mouse Pathology Laboratory, we have studied the histological characteristics of mammary tumors arising in these mice. We found that the studied pathways induce tumors with unique, identifiable histomorphologies. These observations are the foundation for Pathway Pathology. Phenotypical effects of ErbB/Ras pathway activation were studied in tumors transgenic for ErbB2, mutant forms of ErbB2, PyV-mT (a viral protein substitute for ErbB2), Ras, and bigenics with both ErbB2 and another transgene. Mammary tumors caused by overexpression of these transgenes tend to resemble human Ductal Carcinoma in Situ, are solid, not metaplastic, lose myoepithelial differentiation, have scanty stroma, but frequently have an invasive growth. Examples studied for Wnt pathway activation include: Wnt-1, Wnt-10b, Adenomatous Polyposis Coli gene, Gsk-3β, Casein kinase II, and β-Catenin. The Wnt pathway mammary tumors resemble the classical, virus-induced, Type A, B and P tumors, and are more heterogeneous than the ErbB/Ras tumors. However, Wnt tumors share common histomorphologic characteristics, which allow the distinction from the ErbB/Ras tumors: organization around central ducts, presence of acinar, glandular, papillary, squamous or pilar components, retained myoepithelial differentiation, dense stroma, and expansile growth. Some genotypes predispose for spindle cell