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

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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

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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

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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

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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

tumors. Overexpression of members of the Fibroblast growth Factor family (Int2/Fgf3 and Kgf/Fgf7) caused Wnt-pathway like mammary tumors. This result suggests cooperativity between the Wnt and Fgf pathways, which is also supported by virus insertion analyses in these genetically engineered mice. We conclude that genotypes of transgenic mammary tumors are correlated to their histological phenotypes, and that analysis of a tumor's histomorphology can reveal the signaling pathway that induced the tumor. Our observations suggest that the principle of pathway pathology can be applied to tumors of other organs and to the human disease. This work was supported by the DAAD (A.R., individual grant), the State of California, BCRP JB-0014, and RO1CA89140 from NCI.

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A phase I clinical trial of an oral formulation of the histone deacetylase inhibitor suberoylanilide hydroxamic acid (SAHA)

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SAHA is a potent inhibitor of histone deacetylase activity. A phase I study of SAHA administered by daily intravenous infusion has shown that SAHA can be given at doses that cause an accumulation of acetylated histones in peripheral blood mononuclear (PBM) cells and in tumors, without intolerable adverse effects. A clinical trial of oral SAHA was initiated to define the maximal tolerated dose and the pharmacokinetic profile and oral bioavailability of oral SAHA in patients with refractory solid tumors (group A), lymphomas (group B) and leukemias (group C). All patients were required to have adequate hepatic, renal and hematologic function with the exception of lymphoma and leukemia patients for whom a platelet count >25,000 and a neutrophil count >500 were required. All patients provided informed consent. The dose of oral SAHA was independently escalated in each group of patients with planned doses levels of 200 mg daily, 400 mg daily, 400 mg q12, 800 mg q12 and 1200 mg q12. Pharmacokinetic studies were performed on day 1 (identical dose given intravenously), day 8 (oral dose fasting), day 9 (oral dose non-fasting) and day 29 (oral fasting). Western blot analyses of histones isolated from PBM cells obtained pre- and post-SAHA dosing were performed. Twenty-five patients (A=17, B=6, C= 2) have been entered into 3 dose levels. Myelosuppression and fatigue were dose limiting toxicities at 400 mg q12 for solid tumor and lymphoma patients. Mean SAHA oral bioavailability among patients receiving the 200 mg and 400 mg doses was 56% and 48% respectively. A dose proportional increase in AUC and Cmax was observed when comparing the 200 mg and 400 mg dose levels. A prolonged duration of acetylated histone accumulation was observed following oral SAHA administration compared to the same dose administered intravenously. Reduction in measurable disease has been observed in refractory papillary thyroid cancer (1 patient), squamous cell carcinoma of the larynx (1 patient), renal cell carcinoma (1 patient) and B-cell lymphoma (1 patient). The preliminary results of this Phase I study of oral SAHA demonstrate that this formulation is readily bioavailable and results in prolongation of acetylated histone accumulation in PBM cells relative to an identical dose administered intravenously. This study is currently ongoing with the goal of identifying the optimum dose and schedule for oral SAHA administration. Support: CA 096228-01, CaPCURE, Aton Pharma.

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The forced reexpression of the keratin 18 gene in human breast cancer cells results in redifferentiation and a dramatic drop in malignancy

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In vitro experiments as well as clinical studies revealed that the expression of keratin 18 (K18) in breast cancer tumors is associated with a favorable prognosis and a less aggressive phenotype of the carcinoma. To prove the principle we transfected the human K18 gene into the aggressive MDA-231 cell line and isolated a permanently overexpressing clone. These cells grow in dense monolayers with epithelial morphology whereas wild type and mock transfected control are of the dedifferentiated, malignant type with cells appearing spindle shaped, motile, and only loosely attached. The K18-transfected clone is characterized by a high expression of the adhesion proteins plakoglobin, desmoplakin, desmoglein, and E-cadherin in contrast to wild type and control which are virtually devoid. In addition, keratin 8 the dimerisation partner of K18 in keratin filament formation is upregulated too. Conversely the mesenchymal filament protein vimentin, forming the intermediate filaments of the cytoskeleton in MDA-231 wild type and control, is completely down regulated in the K18 clone. The high invasiveness of the wild type in the Boyden chamber is dramatically reduced for the K18-clone. In the nude mouse no metastasis could be observed for the K18-cells whereas wt and control metastasized into lung, liver, and bone marrow. In epithelial cells the intermediate filaments of the cytoskeleton are formed by keratins and K18 is a marker of well differentiated mammary lumenal cells. The loss of K18 and its replacement by vimentin is part of a general loss of differentiation along with the malignant transformation. The forced reexpression of K18 in transfected cells obviously induces redifferentiation with a reorganisation of impaired adhesion structures. Moreover, a reorganisation of these structures in adjacent non-transfected wild type cells could be observed after cocultivation for 2 weeks. Taken together, the impressive results of the nude mouse experiments and the bystander effect on non-transfected cells seem to be good prerequisites for a successful gene therapy with a K18 delivery system.

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Transfection of follicular thyroid cancer cells with thyrotropin receptor cDNA alleviates malignant phenotype *in vitro* and *in vivo*

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Background: Thyroid stimulating hormone (TSH) is commonly seen as a thyroid specific growth factor inducing differentiation and growth of thyroid cells *in vitro*. Loss of TSH receptor expression in thyroid cancer cells is regarded as a sign of de-differentiation and made responsible for a malignant phenotype by escaping the control of differentiating growth factors. Aim: We studied the effect of TSH in the follicular thyroid cancer cell line HTC, a subclone of FTC133 cells, lacking endogenous expression of the TSH receptor (HTC), and HTC cells transfected with TSH receptor cDNA (HTC TSHr +) *in vitro* and *in vivo*.
Methods: By comparative evaluation of naive as well as HTC TSHr + cells, the effect of a functional TSH receptor was determined by its ability to alter proliferation, cell substratum adhesion, migration and invasion *in vitro*, as well as growth of xenotransplanted HTC cells *in vivo* (NCR nude mice, n=9 mice, respectively).
Results: HTC cells transfected with functional TSH receptor cDNA grew faster *in vitro* (doubling time of 1.15 days vs. 1.56 days, $p<0.05$) and TSH caused a dose dependend increase in cell number. After 5 generation times HTCtshr+ cell number had increased between 80 - 150% over HTC cells devoid of the TSH receptor ($p<0.05$). Adhesion to purified proteins of the extracellular matrix as well as migration and invasion through recostituted basement membrane were decreased in HTC TSHr+ cells, but when stimulated by TSH increased to levels comparable to, and with regard to invasion exceeding that of naive HTC cells. *In vivo* tumor latency was 11 days for HTC TSHr- xenografts and 21 days for HTC TSHr + cells. Significantly smaller final tumor volumes were registered for HTC TSHr+ cells (869 ± 427 vs. 250 ± 217 mm3, $p<0.05$).
Conclusion: This is a first demonstration of regained expression of a functional TSH-receptor in a thyroid cancer cell line to cause a decrease of *in vitro* adhesion and invasivenes of tumor cells as well as impaired *in vivo* growth, suggesting a less aggressive phenotype. However, functioning TSH-receptor enables to stimulate growth, adhesion and invasion in thyroid cancer cells *in vitro*, suggesting a key role of the TSH-receptor to affect features of the malignant pheontype *in vitro*.

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Involvement of glutathione S-transferase pi inhibitor TLK199 in myeloproliferation and myelodifferentiation

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Glutathione S-transferase pi has recently been shown to be a regulator of mitogen activated protein kinases (MAPK) and an inhibitor of c-Jun N-terminal kinase. We observed that a promyelocytic HL60 cell line resistant to TLK199, a peptidomimetic inhibitor specific for GST pi presented among other cellular alterations, a higher p42/44 MAPK activity. This cell line was resistant to PMA-induced cell growth arrest during monocyte/macrophage cytodifferentiation. This phenotype was associated with a transient activation of p42/44 MAPK (as compared to a sustained activation in wild type