Understanding the mechanisms of this interesting phenomenon is of importance in itself and even more so in view of the possibility that these mechanisms may eventually suggest modalities for age- adjusted anti-tumoral therapy. We have shown that one such mechanism is increased tumor cell apoptosis in the old animals (1).

In the present study we attempted to verify whether the aging microenvironment affects differently primary and metastatic tumors of the AKR lymphoma.

Materials and Methods: We compared the tendency to apoptosis of primary and metastatic AKR lymphoma cells from young and aged AKR/J mice, according to various cellular (Apoptag staining, DNA flow cytometry) and molecular (ladder type DNA fragmentation, Bcl-2, Fas receptor and caspase expression) characteristics of apoptotic cells.

Results: We found that tumor cell apoptosis was increased in tumors of old as compared to those of young mice in both primary and metastatic growths of the lymphoma. However, the age- related induced apoptosis was more pronounced in primary than in metastatic tumors.

Conclusions: It appears that the apoptosis- inducing effect of the aging microenvironment depends on the tendency to apoptosis of the tumor. We have previously shown that primary tumors of AKR lymphoma are more prone to apoptosis than those of metastatic tumors (2). It is therefore expected that inducing tumor cell apoptosis as a therapeutic modality in the old (1) can be more effective at early stages of tumor development than at late ones.

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Expression of platelet-derived growth factor (PDGF)-B and PDGF-receptor β is associated with lymphatic metastasis in gastric carcinoma

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Background & Aims: Lymphatic metastasis is closely related to clinical outcome in patients with gastric carcinoma. Recent research regarding lymphangiogenesis focused on two members of the vascular endothelial growth factor (VEGF) family, VEGF-C and VEGF-D. However, recent studies have revealed that platelet-derived growth factor (PDGF) also plays a direct role in promoting lymphangiogenesis and metastatic spread to lymph nodes in murine fibrosarcoma. The purpose of this study was to examine the relation between PDGF and PDGF receptor (PDFGF-R) expression and lymphatic metastasis in human gastric carcinoma. Methods: We examined the expression of PDGF-B and PDGF-Rβ in five human gastric carcinoma cell lines (TMK-1, MKN-1, MKN-28, MKN-45, and KKLS) and in 38 surgical specimens of gastric carcinoma by real-time quantitative PCR, ELISA, and western blotting. Immunofluorescence was performed to examine PDGF-B and PDGF-Rβ expression in surgical specimens and in human gastric carcinoma cells (TMK-1) implanted orthotopic ally in nude mice. Results: PDGF-B and PDGF-Rβ mRNA expression was significantly higher in patients with lymph node metastasis than in those without (P=0.03 and P<0.001, respectively) and was also significantly higher in diffuse-type carcinoma than in intestinal-type carcinoma (P=0.02 and P=0.01, respectively). In most surgical specimens, tumor cells expressed PDGF-B, but PDGF-Rβ was expressed predominantly by stromal cells. Under culture conditions, expression of PDGF-B mRNA was found in all of the gastric cell lines except KKLS. Two of the five gastric carcinoma cell lines (KKLS and MKN-1) expressed low PDGF-Rβ mRNA levels. In orthotopic TMK-1 tumors, cancer cells expressed PDGF-B but not PDGF-Rβ. PDGF-Rβ was expressed by stromal cells, including lymphatic endothelial cells. Conclusions: These data indicate that PDGF-B secreted by tumor cells and PDGF-Rβ expressed by tumor-associated stromal cells are associated with lymphatic metastasis in gastric carcinoma.

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Stem cell factor expression at perinecrotic tumor sites is associated with a high microvessel density and endothelial cell KIT expression in human cancer

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Background: A few studies suggest that KIT receptor tyrosine kinase may be involved with tumor angiogenesis. We investigated association of KIT and stem cell factor (SCF) expression with tumor angiogenesis in human cancer.

Materials and methods: KIT and SCF expression was assessed from 248 human tumors consisting of 15 different histological types of cancer using immunohistochemistry. The results were correlated with tumor microvessel density counted from tissue sections stained with an anti-CD31 antibody.

Results: In general, SCF expression was elevated in perinecrotic tumor regions. SCF expression at perinecrotic tumor sites was associated with a high tumor microvessel density (P=. 0004) and with marked KIT expression in tumor endothelial cells (P=. 0005). Endothelial cell KIT expression was most prominent in glioblastoma (58%), testicular teratocarcinoma (33%), renal cell carcinoma (29%), and melanoma (20%).

Conclusions: The results lend further support to the hypothesis that SCF and KIT are important players in tumor angiogenesis. Perinectotic tumor tissue SCF expression is associated with a high microvessel density. Inhibition of SCF/KIT signalling might be a target for anti-angiogenic therapies.

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Snail is overexpressed in human lung cancer and tumor associated stroma

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Purpose: The acquisition of migratory and invasive capabilities by tumor cells recapitulates a developmental process known as epithelial-mesenchymal transition (EMT).A hallmark during this process is the loss of E-cadherin.Snail is considered to be one of the transcription facors responsible for E-cadherin repression.Here, we evaluate the role of E-cadherin and Snail expression in lung cancer and tumor associated stroma.

Experimental procedure: 74 lung cancers were examined immuno-histochemically for the expression of Snail and E-cadherin proteins.The associations between these proteins and clinico-pathological parameters were also analysed.

Results:Positive Snail expression (nuclear) and impaired E-cadherin expression (reduced membranous/no-membranous) were found in 95,9% and 91,5%, respectively.The impaired E-cadherin expression was significantly associated with tumor grade (p<0,001) and tumor size (p=0,026).Snail expression did not correlate significantly with E-cadherin expression or other clinico-pathological parameters.Tumor associated stromal cells, including myofibroblast-like cells, lymphocytes and macrophages were positive for Snail expression in 94,6%, 87,6% and 79,7%, respectively.Snail expression in myofibroblast-like cells was significantly associated with tumor size (p=0,024) and lymph node status (p=0,042).

Conclusions:Our results demonstrate that Snail,a master regulator of EMT, is overexpressed in human lung cancer cells and tumor stromal cells in vivo but is not associated with E-cadherin down-regulation.

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Regulation of TNF-superfamily members by erythropoietin, in breast cancer

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Background: APRIL (CD256), a Tumor Necrosis Family (TNF) ligand has gained increasing interest in view of cell homeostasis. Although well described in haemopoietic malignancies, its role and regulation in solid tumors remain to be elucidated. Indeed, breast cancer promotion integrates a complex interplay between hormones and cytokines, mediated, among others, through cross-link of membrane initiated steroid signaling with growth factors. Materials and methods: We assayed 52 human breast cancer biopsies by immunohistochemistry for the expression of APRIL as well as its cognitive receptors (BCMA and TACI) and correlated our findings with clinicopathological data and the evolution of the disease. Moreover, utilizing three breast cancer cell lines (MDA-MB-231, T47D and MCF-7) with different phenotypes, we approached by RT-PCR the gene expression profile of this TNF member in breast cancer and the possible transcriptional regulation by membrane androgen and estrogen agonists. Results: APRIL immunoreactive expression was higher in non-malignant than neoplastic breast structures, in contrast to findings in other solid tumors. APRIL expression was associated with more aggressive and undifferentiated phenotypes, correlating with lymph node metastases. Moreover,

membrane steroid agonists modify APRIL transcription levels. Conclusions: Our data show, for the first time, an autocrine secretion of APRIL in breast cancer cells, strongly associated with loss of differentiation and metastatic potency, indicating the emerging role of this TNF-related cytokine in breast cancer biology. The regulatory effect of membrane agonists on APRIL gene regulation reflects another aspect of membrane steroid signaling, providing new insights in the hot link between inflammation and breast cancer promotion.

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PTEN deletion detected using dual-color FISH in esophageal squamous cell carcinoma correlates with poor prognosis

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Background: Esophageal squamous cell carcinoma (ESCC) is the most frequent histological type of cancer in this organ, being the sixth neoplasia in frequency in Brazil. ESCC is associated with poor prognosis, showing high mortality rates. It is important to know the prognostic factors that can help the choice of more appropriate surgical approach and then improve the survival of these patients. The tumor suppressor gene PTEN (phosphatase and tensin homolog deleted on chromosome 10) is a member of protein tyrosine phosphatase family that can inhibit cell proliferation, survival and growth by inactivating PI3-kinase-dependent signaling. Mutations of PTEN gene have been described in a variety of tumor types, but there are few studies in ESCC. The aim of this study is analyze the presence of PTEN deletion through Fluorescent in situ Hybridization (FISH) in cases of ESCC and its correlation with tumor prognosis.Material and Methods: 70 surgical resections of ESCC cases were performed in two Tissue Microarray (TMA) paraffin blocks spotted in duplicate. All medical records were reviewed. Dual-color FISH reactions were carried out using commercially available probe for PTEN locus (Vysis) and for centromere of chromosome 10 (Vysis). In each case, 100 non-overlapped, interphase tumor nuclei were evaluated. Hemizygous deletion of PTEN was defined as >20% of tumor nuclei with one PTEN locus signal and presence of CEP10 signals. Homozygous deletion was characterized as lack of both PTEN locus signals, but presence of CEP10 signals in >30% of tumor cells.Results: Our study showed that 41 (58,6%) of 70 cases analyzed showed PTEN deletion, being 38 (54,2%) hemizygous deletion and 3 (4,2%) homozygous deletion. It was not possible to perform the statistical analysis to verify the association between the FISH results and clinicopathological findings. According to univariate analyses, the variables gender (p=0,002), lymph nodal metastasis (p

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Pro-apoptotic effects of streptochlorin isolated from Streptomyces sp. in human leukemic U937 cells

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Streptochlorin is a small molecule produced by marine Streptomyces sp. (strain 04DH110) that is known to have anti-angiogenic and anti-cancer properties. However, the mechanism by which streptochlorin exerts is effects is not well understood. In this study, we investigated the pro-apoptotic effect of streptochlorin in human leukemic U937 cells. Streptochlorin treatment resulted in concentration- and time-dependent growth inhibition of U937 cells by inducing apoptosis, as evidenced by the formation of apoptotic bodies, DNA fragmentation and the accumulation of cells in the sub-G1 phase. The increase in apoptosis that was induced by streptochlorin was correlated with down-regulation of the anti-apoptotic Bcl-2 expression, up-regulation of pro-apoptotic Bax and FasL, a decrease in the mitochondrial membrane potential (MMP, $\Delta\psi_m$), activation of caspases and degradation of poly-(ADP-ribose) polymerase (PARP) and phospholipase C (PLC)-g1 protein. Both the cytotoxic effects and apoptotic characteristics induced by streptochlorin were significantly inhibited by z-DEVD-fmk, a caspase-3 inhibitor, which demonstrates the important role that caspase-3 played in the observed cytotoxic effects. Furthermore, Bcl-2 overexpression significantly reversed the streptochlorin-induced growth inhibitory effects via inhibition of the MMP collapse and caspases activation and effectively attenuated the apoptotic response to streptochlorin. However, the elevated levels of FasL expression induced by streptochlorin were not reduced by Bcl-2 overexpression. Taken together, these findings demonstrate that the pro-apoptotic effect of streptochlorin is mediated through activation of caspases and mitochondria in U937 cells. [This research was supported by a grant (M2007-03) from Marine Bioprocess Research Center of the Marine Bio 21 Center funded by the Ministry of Maritime Affairs & Fisheries, Republic of Korea.]

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Inhibition of invasion and induction of apoptosis by anthocyanins isolated from Vitis coignetiae Pulliat in HCT116 colon cancer cells

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Many edible plant metabolites are known to be useful as cellular anti-oxidants. Anthocyanins belong to a class of flavonoids, exhibiting anti-oxidant and anti-inflammatory actions as well as a variety of chemotherapeutic effects. However, the underlying mechanisms of its action are not completely understood in anti-cancer effects. In this study, we investigated if the anthocyanins isolated from meoru (Vitis coignetiae Pulliat) exerted anti-proliferative, anti-invasive and apoptotic effects on human colon adenocarcinomaHCT116 cells. It was found that the anthocyanins could inhibit cell growth by 50% at the concentration of 60 μg/ml for 48 h. Flow cytometric analysis showed that the anthocyanins treatment increased the sub-G1 population in a dose-dependent manner, which is closely related to modulation of Bcl-2 and inhibitor of apoptosis proteins (IAPs) family expression. Consequently, anthocyanins treatment induced the proteolytic activation of caspases and degradation of poly (ADP-ribose) polymerase (PARP). Furthermore, anthocyanins significantly inhibited the cell migration and invasion of HCT116 cells. Also, the inhibitory effects of migration and invasion by anthocyanins were associated with up-regulation of E-cadherin expression, and down-regulation of Snail, matrix metaloprotenase-2 (MMP-2), MMP-9 and claudins. Taken together, these observations indicate that the anthocyanins have anti-proliferative and anti-invasive effects, and may induce the apoptosis through the activation of mitochondrial pathway and inhibition of anti-apoptotic proteins. This study provides evidence that the anthocyanins isolated from meoru might be useful in the treatment of human cancer cells.

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Genetic instability of FADD-deficient mouse cells

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We used fibroblast cultures from the FADD-deficient mouse embryos, which were originally constructed by T.W. Mak (Toronto, Ont.) to assess directly the role of FADD, a death receptor-associated protein, in cells responses to UV radiation.

These FADD-deficient fibroblasts showed much increased growth rate compared with the wild-type homozygous cells. However, the FADD-deficient fibroblasts showed the same sensitivity to UVC radiation as the wild type homozygous fibroblasts measured by MMT test. There was no difference between repair activity of UV-induced DNA damages products in the FADD-deficient and proficient cells, either. However, delay of entering S phase after UV-irradiation was reduced in the FADD-deficient cells, indicating some abnormality in the checkpoint function of the cell cycle in these deficient cells. We further analyzed changes of p53 sequences in the FADD-deficient and wild-type cells. Genomic DNA was analyzed by allele-specific polymerase chain reaction (AS-PCR) for CC to TT mutation at codons 154-155 and 175-176 in exon 5 and for C to T mutations at codons 270 and 275 in exon 8 0f the p53 gene. The mutant-specific forward primer was used for each mutation. The reverse primers for amplification of mutations were not mutant-specific. Allele-specific PCR detection of p53 in genomic DNA were analyzed by gel electrophoresis. The results showed the high frequency of changes in the mutant-specific primer for codon 270 amplified 134-base pair product from FADD deficient cell DNASs. Although frequencies of UV-induced mutations were not different between the FADD-deficient and wild-type cells, distributions and spectrum of base-substitution mutations at the p53 were different.