supplemented with various combinations of low glucose and glutamine concentrations at different pH-values. Cell growth was determined by cell count, while metabolism was monitored by the activities of pyruvate kinase, lactate dehydrogenase and mitochondrial dehydrogenases (XTT-assay). Moreover, a test system based on electronic sensor chips was used to monitor in parallel and in real-time the rate of extracellular acidification and oxygen consumption. Sensor chip measurements showed that a low serum concentration along with an acidic pH immediately reduced cellular acidification and respiratory activities, demonstrating the high plasticity of tumor cell metabolism. This metabolic reduction correlated with reduced cell growth. Cell growth was further attenuated when levels of glutamine and glucose fell below 0.5 and 1 mM, respectively; however, the specific activity (per cell) of XXT-conversion to formazan increased, indicating an inverse relationship between growth inhibition and the activity of mitochondrial dehydrogenases. On the other hand, the optimal concentration of glucose was highly dependent on the medium pH. When testing combinations of the metabolites at different concentrations, 0.1 mM glutamine with 2.5 mM glucose produced an extraordinary increase in formazan formation and pyruvate kinase activity, a key enzyme of the glycolytic pathway. In contrast, neither the activity of lactate dehydrogenase, of which pyruvate is a substrate, nor cell number showed a similar increase. Progressive doubling of the concentrations of this glucose and glutamine combination increased the cell number only by a factor of 1.3 during a 5-day incubation. Therefore, these parameters of energy metabolism do not correlate with tumor cell growth. This could mean that at low nutrient levels, cell proliferation is attenuated to ensure energy metabolism required for cell survival.

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Fra2 is an antagonist of p53

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Repression of oncogene-induced apoptosis plays a key role in tumor development and progression. Through a phenotype screen in mammalian cells we have isolated Fra2 as a gene that confers protection from oncogene-induced apoptosis. Fra2 (Fos-related antigen 2) is member of the Fos family of transcription factors that, together with Jun proteins, participate in the formation of the AP-1 complex. Alterations of the AP-1 complex have been reported for a variety of cancers. Nevertheless, the specific role of Fra2 in this context has been only marginally investigated.

Here we provide evidence that Fra2 is an antagonist of the p53 pathway. Ectopic expression of Fra2 promotes the bypass of p53-dependent apoptosis and growth arrest of mouse embryo fibroblasts. Ectopic expression of Fra2 is accompanied by increased degradation of p53, which only in part depends on MDM2.

These data, together with the finding of elevated levels of Fra2 in a significant fraction of breast and colorectal cancers, support a role for Fra2 as important modulator of p53 in human cancer.

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The association of expression level of PRL-3 mRNA and liver metastasis in primary colorectal cancer

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Liver metastasis occurs in 40-60% of all colorectal cancer patients, and main cause of deaths in colorectal cancer is liver metastasis. Studying the molecular basis of liver metastasis and identifying metastasis specific markers would provide critical information for the diagnosis and treatment of colorectal cancer. PRL-3 is a newly identified metastasis-related gene, which codes a 22 KDa non classical protein tyrosine phosphatase. In this study, we examined the relationship between PRL-3 expression and liver metastasis in colorectal carcinoma. Our purposes of the study are to asses PRL-3 mRNA expression in the primary colorectal cancers by quantitative analysis, investigate the correlation between clinicopathological features and PRL-3 expression and verify the utility of PRL-3 in predicting liver metastases in patients with colorectal cancer. From January of 2004 to December of 2006, the data of 86 patients who underwent surgical resection for colorectal cancer was collected. Resected specimens were cooled by liquid nitrogen and preserved at -80°C. RNA was extracted by usual manner from the preserved tissue. Real time RT-PCR using Light Cycler instrument (Roche Molecular Systems, Alameda, CA) was performed for quantitative analysis of PRL-3 mRNA. Retrospective analysis of correlation between PRL-3 mRNA expression and clinicopathologic factors (Gender, age, stage, cell differentiation, lympho-vascular invasion, and neural invasion). In our study, the association among PRL-3 mRNA expression and liver metastasis, and lympho-vascular invasion showed statistically significant correlation. High expression of PRL-3 was closely associated with extent of lymph node metastasis and tumor stage. These results suggest that high PRL-3 expression may participate in the liver metastasis of colorectal carcinoma. A close association between PRL-3 mRNA expression and liver metastasis of colorectal cancer suggests that PRL-3 assessment can be used to predict liver metastasis of patients with colorectal cancer. PRL-3 might be a novel molecular marker and a potential therapeutic target for colorectal cancer liver metastasis.

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Anti-metastatic therapeutic modalities based on the aged cancer patient as a model - a suggestion

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Background: Metastasis is the most appalling aspect of cancer, responsible for the large majority of cancer- related deaths. However, practically almost no effective anti- metastatic treatment is available.

An apparently surprising phenomenon is the fact that while tumor incidence is known to increase with age, tumor growth and metastatic development often proceed at a slower rate in many cancers of elderly patients.

The question is what in the old organism reduces the aggressiveness of tumors. We reasoned that some information might be learned from this phenomenon by trying to identify the factors in the aged organism which are responsible for the reduced progression of tumors. Based on these mechanisms, we can then attempt to design anti- metastatic treatment modalities.

Materials and Methods: We compared in two experimental tumors, B16 melanoma and AKR lymphoma, in which we have shown a differential tumor growth in young and old mice (1), the degree of apoptosis (according to Apoptag staining and DNA flow cytometry), angiogenesis (microvessel density) and macrophage content in tumors from mice of different ages.

Results: We demonstrated that mechanisms responsible for the reduced tumor progression in the aged can be: increased apoptotic cell death, decreased angiogenesis and modification in immune response. The differences between tumors from young and those from old animals for the three properties were very marked: about 2 and 3 fold increase in apoptosis, according to cell morphology, in B16 melanoma and AKR lymphoma, respectively, 2 and 6 fold decrease in angiogenesis in B16 melanoma and AKR lymphoma, respectively, and about 15 fold increase in macrophage content in AKR lymphoma and a more modest increase, difficult to assess quantitatively, in B16 melanoma.

Conclusions: We suggest that drugs enforcing these mechanisms, namely inducers of apoptosis, anti- angiogenic drugs and immuno-modulators, might act as anti- metastatic drugs.

Moreover, anti- metastatic treatments based on the mechanisms responsible for the reduced tumor progression in the aged might be biologically more relevant and possibly less toxic than chemotherapeutic drugs which act against one and only cell property, tumor cell proliferation, a characteristic not necessarily relevant to the metastatic phenotype.

We can view the aging organism as a model of reduced metastatic spread. Studying the metastasis- inhibitory mechanisms of the aged may suggest new, so desperately needed, possibilities to treat metastatic disease, in old as well as in young patients. Studying the ways an elderly patient deals with metastatic spread could conduce to the discovery of treatment modalities which might be more relevant to physiological factors and possibly less offensive to the host.

Reference
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Elevated apoptosis in tumors of aged as compared to those of young mice is more pronounced in primary than in metastatic tumors of AKR lymphoma

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Background: While incidence of neoplasia is known to increase with age, tumor growth and metastatic spread proceed, paradoxically, at a slower rate in aged as compared to young patients. Although not a general feature, this intriguing phenomenon is observed in many human and experimental tumors. We have shown this particular behavior in the AKR lymphoma and B16 melanoma.

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,