

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 XTT-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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480

Poster

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.

481

Poster

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 assess 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.

482

Poster

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 immunomodulators, 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

Donin et al., Cancer Invest. 15: 416,1997

483

Poster

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.