transfection studies of cultured fgHCC HNF4 regulatory region found to be unactive. Number of possible activators of HNF4 transcription were also found to be downregulated in fgHCC.

Conclusions: these results confirms the hypothesis that HNF4 is one of the key regulators of both liver-specific gene expression and maintaining of epithelial phenotype and provide strong evidence for the existing of HNF4 upstream mechanisms responsible for tumor progression. The described system seems to be a powerful tool further exploring the mechanisms of hepatocarcinogenesis.

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Quantitative evaluation of tumour cell enrichment methods using a cytokeratin 20 lightcycler PCR assay

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Purpose: Cytokeratin 20 mRNA (CK20) detected in peripheral blood by RT-PCR was suggested by several authors to be a potential marker of colorectal tumour cell dissemination. Sensitive and quantitative detection of systemic tumour cell burden may have therapeutic and prognostic implications in the future. Our objective was to investigate the value of various sample preparations to CK20 detection sensitivity and specificity.

Methods: 5ml peripheral blood obtained from healthy individuals was spiked in triplicates with 10e3, 10e2, 10, 1, 0 cells/ml HT29 colorectal carcinoma cells. Samples were processed by (1) Density-gradient centrifugation with Ficoll-Hypaque (2) Immunmagnetic separation (IMS) with Dynal microsize beads(3) IMS with Immunocon nanosize beads and (4) no enrichment (whole blood). All experiments were repeated three times using blood from different healthy donors. After total RNA extraction, the relative CK20 ratios of the samples was determined using the LightCycler (LC) Instrument and a newly developed LC-CK20 Quantification Kit.

Results: Qualitatively, the 10e3 and 10e2 cells/ml concentrations were detected with a 100% sensitivity for all methods tested. At the 10 cells/ml concentration, only Immunocon IMS showed 100% sensitivity, while Ficoll enrichment, Dynal IMS and whole blood RNA extraction had sensitivities of 89%, 63% and 33%, respectively. One cell/ml was detected with Dynal IMS in 67% of samples, while Immunocon IMS, Ficoll enrichment and whole blood RNA extraction had sensitivities of 50%, 44%, 17%, respectively. Specificity determined from the non-spiked samples (0 cells/ml) for the Ficoll, Dynal, Immunocon and non-enriched series was 100%, 100%, 83% and 83%, respectively. Quantitatively, the relative CK20 ratio decreased with decreasing cell number in samples processed by Ficoll enrichment but with both IMS techniques the relative ratio remained nearly consistent.

Conclusion: These results suggest that density gradient centrifugation and IMS can increase the sensitivity and specificity of RT-PCR tumour cell detection. While PCR quantification of blood enriched by density gradient separation appears to give an indication of tumour cell load, quantification of blood enriched by IMS appears to indicate tumour cell identity.

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Preclinical evidence for a direct link between tumor hypoxia and cancer cachexia

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Background: Within hypoxic tumor regions anaerobic glycolysis is the sole energy source. It only yields 5% of the ATP which is normally gained by means of oxidative glucose dissimilation. We hypothesized that the increased need for glucose eventually results in cancer cachexia.

Methods: Fragments of the murine C26-B adenocarcinoma were implanted in 60 female BALB/c-mice. The mice were divided in 4 groups and assigned to: A. no treatment. B. erythropoietin (RhEPO) administration (25 units daily from day 1-11, 3 times per week from day 12). C. RhEPO and 25% oxygen. D. RhEPO and 35% oxygen. Three control groups of 4 healthy mice received the same treatment as group A, B and D. Hematocrit and hemoglobin levels, tumor volume and body weight were monitored. At day 17 the experiment was terminated and the lactate concentration was measured. The tumors were excised and weighed and for each mouse the percentage weight loss was calculated. The impact of tumor weight and the treatments on lactate concentration and weight loss was evaluated.

Results: Fifty-two tumor-bearing mice were evaluated. The tumor-bearing mice had a lower food intake than their healthy controls. Significant positive correlations were found between tumor weight and lactate concentration ($p < 0.001$) and between tumor weight and % weight loss ($p < 0.001$). In the 26 mice with the largest tumors (> 1.3 grams) RhEPO displayed a significant weight loss-reducing effect and a significant negative correlation was found between hemoglobin concentration and weight loss. An oxygen-rich environment did not appear to influence weight loss.

Conclusion: Anaerobic glycolyis in a growing C26-B-tumor is related with weight loss. RhEPO-administration results in a reduction of the % weight loss; this effect is probably mediated by an increased hemoglobin concentration.

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The impact of hypoxia on plasminogen activator type-1 protein and mRNA levels in rat DS sarcoma in vitro and in vivo

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Purpose: The urokinase plasminogen activator system plays a central role in malignant progression. Tumor hypoxia and high levels of urokinase plasminogen activator (uPA), urokinase plasminogen activator receptor (uPAR) of plasminogen activator inhibitor type 1 (PAI-1), have been identified as negative prognostic factors. Hypoxia triggered upregulation of uPA or PAI-1 could therefore be one way in which hypoxia may influence malignant progression. The impact of hypoxia on the expression pattern of components of the uPA system in rat DS sarcoma was investigated in vitro and in vivo.

Methods: DS sarcoma cells were implanted onto the hind foot of SD rats. These animals were housed under(1) hypoxia [92%N2/8%O2], (2) normal room air or (3) hyperoxia [100%O2]. After 8 to 12 days, when tumors reached volumes of 1-2 ml, they were explanted and serum was collected. DS sarcoma cells were incubated in vitro for 24 h under hypoxia (< 1% O2). uPA and uPAR expression were analysed by flow cytometry and uPA activity was measured using one-phase zymography. PAI-1 protein levels in medium, serum and whole cell lysates of tumors and DS cells in vitro were examined with ELISA and PAI-1 mRNA was determined by semi-quantitative RT-PCR using b-actin as internal standard.

Results: DS sarcoma cells express uPA, uPAR and PAI-1. uPA activity is enhanced in DS-sarcomas compared to various normal tissues. The uPA activity in cell extracts of tumor or DS sarcoma cells in vitro is not influenced by the oxygenation level, but in vitro a significant increase of PAI-1 protein in culure medium as well as an upregulation of PAI-1 mRNA after hypoxia are detectable. No differences in PAI-1 mRNA or protein expression as assessed by ELISA or semi-quantitative RT-PCR were found either in sera or mRNA or protein extracts of tumors grown either under inspiratory hyperoxia or hypoxia.

Conclusion: DS sarcoma express uPA, uPAR and PAI-1 in vitro and in vivo, indicating that the tumor cell itself contributes all three components. Overall uPA activity in tumor cells is high, but not affected by hypoxia. Under in vitro conditions we could demonstrate that hypoxia is able to induce PAI-1 on mRNA and protein level in DS sarcoma cells, although differences are not detectable in vivo. Temporal and spatial heterogeneities in tumor oxygenation in vivo possibly cover this effect in vivo. PAI-1 serum levels are not a reliable marker of tumor hypoxia in this system.

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Benzo[a]pyrene increase ubiquitination of p21 protein following the stabilization of p53 and the expression of p21

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Purpose: A potent tobacco-related carcinogen, benzo(a)pyrene (B[a]P), have been found to induce a rapid accumulation of p53 gene product in human and murine cells. However, the induced p53 protein was reported to be transcriptionally inactive. In addition, we have found that the expression of wild-type p53 is not consistent with that of p21 in atypical bronchial epithelium. In the present study, the induction of p53 target gene expression after the treatment with polycyclic aromatic hydrocarbons (PAHs) such as B[a]P and 1 nitropyrene (1-NP) was investigated. Methods: B[a]P and 1-NP were exposed to four human lung cancer cells differing in their p53