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.

465 POSTER

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

status (wild type, deleted, mutated). Western and northern blot analysis were performed to evaluate the protein and mRNA expression of p53, p21, Mdm2, Bax with or without the presence of proteasome inhibitor, MG-132. Transient transfection and luciferase assay was performed to confirm a transcriptional activity of p53. Results: A marked induction of mRNA expressions of Mdm2, Bax and p21 was detected in wild-type p53 expressing cells after the treatment with both B[a]P and 1-NP, but not in either p53-negative or mutant cells. The induced mRNA levels of the p21 did not result in proportional p21 protein increase, indicating the possibility of post transcriptional regulation of the protein. Transcription from the wild-type p21 promoter was markedly induced by PAHs in p53 wild type cells but not in p53 deleted cells. In addition, luciferase activity was not affected by p21 promoter in which p53-binding site is truncated. By the addition of MG-132 to B[a]P treatment, both p21 and p53 protein levels were increased, however, the increase in p21 protein levels was significantly larger than the increase in p53 protein levels. On the other hand, increase in p21 protein was only modest by the addition of MG-132 to 1-NP treatment. B[a]P treatment increased the level of ubiquitinated p21. Cell cycle arrest was more obviously seen by the treatment with 1-NP than by the treatment with B[a]P. Conclusions: These results suggested that the p21 product is degraded by the ubiquitin-proteasome system induced by B[a]P. We conclude that B[a]P-induced p53 protein is transcriptionally active. However, rapid degradation of p21 protein by the ubiquitin-proteasome system may induce a blockade of p53-induced cell cycle arrest, resulting in genomic instability.

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Effectiveness of a new derivative of retinoic acid as differentiating agent on human neuroblastoma cells

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Purpose: Among the compounds that have been explored as differentiating agents, retinoic acid is one of the most potent in the regulation of proliferation and differentiation in neoplastic cells. In the present study we have explored the effect of IIF, (pat.PTC/IT99/00299) a new derivative of retinoic acid, as differentiation inducer in the human neuroblastoma cell line TS12.

Methods: Neuronal differentiation was assessed by means of morphological and cytochemical parameters, i.e. neurite outgrowth, tyrosine hydroxylase (TH) expression and acethylcholinesterase specific activity. The effect of the drug on cell growth was assessed by clonogenic assay.

Results: Treatment with IIF resulted in induction of morphological differentiation, as manifested by the appearance of neurite extension. TH expression was induced by the drug: following RT-PCR on mRNA from neuroblastoma cells, TH mRNA was detectable only in treated cultures but not in control ones. Treatment with IIF induced also a marked increase of acetylcholinesterase activity. Moreover clonogenic efficiency showed the growth inhibitory effect induced by the drug.

Conclusions: These results demonstrate the effectiveness of the new derivative of retinoic acid IIF as differentiation inducing agent on neuroblastoma cell line TS12.

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RBC CR1 in tumour patients: an implication in anaemia?

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Purpose: Tumour patients often suffer from an anaemic condition whose causes are not always completely clear. Increased levels of circulating immune complexes (cIC) are frequently present in these patients. The receptor responsible for cIC clearance is complement receptor 1 (CR1), present also on red blood cells (RBC). The aim of this study was to investigate the presence of RBC CR1 modifications in tumour subjects, as well as the possibility that they are in some way linked to the anaemic condition.

Methods: Patients (age 47-81) affected by breast, lung and colon cancer from stage 1 to 4, were studied; healthy donors, age 19-83, were used as controls. All subjects were submitted to blood withdrawal. Sera were employed for determination of cIC levels by ELISA; RBC were separated from leukocytes, and CR1 expression was evaluated at flow cytometry. The number of CR1+RBC was calculated and correlated to cIC sera levels, subject age and haematological condition.

Results: In tumour patients RBC number was decreased with respect to controls. CR1 expression was also significantly diminished, in a greater proportion than RBC number decrease; on the contrary, cIC levels were significantly increased, especially in the over-60 year old group. The over-60 control subjects also showed increased cIC levels, without CR1+RBC number modifications, and the values were comparable to those found in the under-60 patients. cIC-CR1+RBC correlation was negative in patients, indicating that the CR1 reduction accompanies the cIC increase.

Conclusion: A loss of RBC, in relation with the increased serum cIC level, is suggested as an adjunctive mechanism responsible for both the marked CR1+RBC reduction and anaemia in neoplastic patients; however, the considerable diminution of CR1+RBC (compared with the entire RBC population) also suggests an impaired CR1 production or its augmented proteolysis. CR1 decrease could be involved in the maintenance of high cIC sera levels in these patients (insufficient cIC removal), as well as in the appearance of anaemic condition consequent to the possible elimination from the circulation of cIC-carrying RBC.

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Regulation of vimentin mrna by 12-o-tetradecanoylphorbol 13-acetate (TPA) and all-transretinoic acid (RA) associated with in vitro invasive activity of hep 3b human hepatocellular carcinoma cells

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Purpose: Vimentin is a protein that assembles to form intermediate filaments, one of the major cytoskeletal structures in mammalian cells. Increased expression of vimentin is associated with increasing cancer grade, dedifferentiation, decreased cell-to-cell adhesion, motility, invasion, metastasis, drug resistance and poor prognosis in some cancers. We have reported that the vimentin mRNA was regulated by tumor promoter, TPA and differentiation agent, RA in several cancer cell lines. In this study we found that up- or down-regulation of vimentin mRNA by TPA or RA could modulate the invasive potential of human hepatoma cell line, Hep 3B in vitro.

Methods: To elucidate the role of vimentin gene expression of Hep 3B cells by TPA or RA, we evaluated the vimentin mRNA levels by Northern blot hybridization. Matrix metalloproteinases (MMP-2,-9) and urokinase plasminogen activator (uPA) activities were evaluated using substrate zymography in addition to in vitro invasion assay of Hep 3B cells.

Results: TPA (1-100 nM) treatment showed marked induction of vimentin mRNA up to 48 hrs with a dose- and time-dependent maner and then decreased its level. On the other hand, RA (0.1-10 uM) treatment showed a time-dependent gradual decrease of mRNA level. There was no change of MMP-2, MMP-9 and uPA activities in conditioned medium with concomittent treatment of TPA or RA on zymographic findings. TPA (0.1 uM) treatment significantly enhanced in vitro invasion of Hep 3B cells as much as 2 times, and RA (0.1-10 uM) inhibited the invasion with dose-dependent manner (33.4%, 71.9% respectively) ($p<0.05$).

Conclusion: These results suggest that regulation of vimentin mRNA may be related to invasiveness of Hep 3B human hepatocellular carcinoma cells by controlling cellular motility.

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Inhibition of cathepsin A activity in melanoma cell lines by lactacystin

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Purpose: In recent years considerable attention has been paid to the antitumor activity of the proteasome specific inhibitor, lactacystin. It inhibits the proteasome-mediated degradation of numerous key regulatory proteins which are involved in various cellular processes such as cell division, apoptosis, NF-kB activation, and MHC class I antigen presentation. The ability of the lactacystin to arrest cell cycle progression and induce apoptosis in various tumor cells suggests to their potential use in cancer therapy. Recently we showed, that lactacystin metabolite, b-lacton inhibited the activity