initially developed to measure organ blood flow, is also useful for estimating tumor blood flow in rats.

460 POSTER

Soluble P-selectin correlates with plasma vascular endothelial growth factor (P-VEGF) levels in patients with thoracic carcinoma

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Introduction: Vascular endothelial growth factor regulates tumour angiogenesis. Debate exists as to whether P-VEGF measured in patients with cancer is derived from VEGF released from tumour cells or from in-vivo platelet activation. The aim of this study is to investigate how plasma soluble P-selectin (sP-selectin), a marker of in-vivo platelet activation, relates to P-VEGF levels in patients with thoracic cancer.

Methods: Blood was obtained from 91 patients with carcinoma of the lung (n=53) or thoracic oesophagus (n=38) and 36 controls. Seventy-three of the cancer patients had localised disease and 18 had advanced disease (mediastinal involvement or systemic dissemination). The P-VEGF (pg/ml) and plasma sP-selectin (ng/ml) levels were investigated using specific ELISA kits. The platelet count (x109/l) was measured using a Sysmex SE-9500 automated haematology analyser. Data were expressed as median, with statistical analysis by Mann-Whitney U Testing. Correlation analysis was undertaken using Spearman's Rank testing.

Results: Patients with advanced thoracic carcinoma had a significantly elevated median sP-selectin level compared to controls (60.3 vs. 40.1; $p<0.0001$). Plasma VEGF was raised in patients with advanced disease compared to controls (84.5 vs. 21.2; $p<0.0001$). There was no significant difference in P-VEGF levels between those with localised disease and the controls. The median platelet count was increased in the patients with localised disease compared to controls (258 vs. 234; $p<0.05$) and between patients with advanced carcinoma and controls (395 vs. 234; $p<0.0001$). The sP-selectin levels correlated with the P-VEGF levels in all the cancer patients ($r=0.30$; $p<0.01$) but not in the control patients. The platelet count in the cancer patients correlated with the sP-selectin and the P-VEGF levels ($r=0.44$; $p<0.0001$ and $r=0.30$; $p<0.005$ respectively). The platelet count did not correlate with sP-selectin nor P-VEGF levels in the control patients.

Conclusion: There is a correlation between sP-selectin and P-VEGF in patients with thoracic cancer and both are elevated in advanced disease. Further research should be directed in establishing if this has an independent effect on the progression of cancer.

461 POSTER

Retinoic acid induced G1 arrest in hepatocarcinoma cell lines

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Purpose: Retinoids (RA) have been known to regulate growth and differentiation of epithelial cells. Treatment of various types of cancer cells with RA resulted in growth inhibition and apoptosis. RAR beta has been suggested to play an important role in the biological functions of RA. RAR beta expression is suggested to associate with the cellular sensitivity to retinoid in cancer cells. It has been shown that RA treatment caused cell cycle arrest at G1 phase through enhanced expression of cyclin dependent kinase inhibitor p21 in leukemia cells and lung cancer cell lines. Therefore, we examined whether all-trans retinoic acid (atRA) caused to arrest a cell cycle, resulting in a growth inhibition in Korean hepatoma cell lines. We investigated the expression of proteins related to a cell cycle.

Methods: Korean hepatoma cell lines (SNU354, SNU449) were purchased from Korea Cell Line Bank. Cell lines were maintained in RPMI medium containing 10% fetal bovine serum. Cells were treated with 10 μM atRA as an indicated time period. Percent growth inhibition was calculated with cell number from atRA treated cells compared to that from control cells. After atRA treatment, cells were harvested and were lysed with RIPA buffer containing protease inhibitors. Total cell lysates were resolved on 8 - 12% SDS-PAGE gel and transferred to PVDF membrane. Blots were reacted with desired antibodies. Cell cycle arrest was analyzed using FACScan after atRA treatment.

Results: Treatment of Korean hepatoma cells with atRA resulted in cell growth inhibition and the sensitivity of cells to atRA was related to induction of RAR beta expression. atRA-treated hepatoma cells showed cell cycle arrest at G1 phase starting from 3h treatment in atRA-sensitive SNU354 but it was delayed in atRA-less sensitive SNU449 cell line. Although increased expressions of both p21 and p27 proteins were observed in atRA-treated SNU354 cells, expression of p27 was not increased in SNU449 cells after atRA treatment. Since expression of p53 was not changed by atRA in both cell lines, induction of p21 and p27 could independently occur in atRA treated cells.

Conclusion: Based on our results, we concluded that retinoic acid treatment induced expression of p21 and p27 proteins, resulting in cell cycle arrest at G1 phase and inhibition of cell growth in Korean hepatoma cell lines. We suggested that expression of p27 was more likely related to the RA sensitivity of hepatoma cells.

462 POSTER

PPARgamma expression in esophageal cancer and effect of PPARgamma ligands

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Purpose: Peroxisome proliferator-activated receptor gamma(PPAR)is a nuclear receptor that has a regulatory role in differentiation of adipocyte and its expression has also been shown in several types of cancers, such as colon cancer and breast cancer, in recent years. The present study examined the expression of the PPAR in human esophageal cancer cell lines and the effect of the PPAR ligands on cell growth of these cell lines. Methods: Expression of the PPAR in five esophageal cancer cell lines were examined by Reverse transcription-polymerase chain reaction (RT-PCR) and Western blot analysis. Effect of the PPAR ligands, troglitazone and pioglitazone, on cell growth of the cell lines was investigated in vitro. Apoptotic assay and cell cycle analysis after PPAR ligand treatment were also performed. Results: PPAR expression was detected in all cell lines tested with RT-PCR and Western blot analysis. Both of PPAR ligands inhibited cell growth of esophageal cancer cell lines in dose-dependent manner. Fow cytometry demonstrated a increase fraction of Gl phase and decrease of cells in S phase after PPAR ligand treatment, and apoptotic cells after treatment were slightly increased. Conclusions: PPAR is expressed in esophageal cancer. The cell growth of esophageal cancer cells is inhibited by PPAR ligands. Our results suggest that PPAR might be a promising target of anti-cancer treatment for esop

463 POSTER

Studying of mechanisms involved in the maintenance of hepatoma differentiation status.Role of HNF4

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Purpose of the study: We have obtained a new experimental model comprising a highly differentiated slow growing transplantable mouse hepatocarcinoma (sgHCC) and its dedifferentiated fast growing variant (fgHCC) aroused from sgHCC by rapid, possibly one-step progression. The aim of this study was comparative characterization of sgHCC and fgHCC variants and analysis of possible role of liver-specific transcription factors in the maintenance of hepatoma differentiaton status.

Materials and methods: RT-PCR, Northern blot hybridization analyses and immunohistochemical staining were used to investigate the levels of liver-specific genes expression and synthesis and localization of ECM components. Transient transfection analyses with luciferase reporter vectors were used to estimate the transcriptional activity of Hepatocyte Nuclear Factor 4 (HNF4) promoter.

Results: We have found that fgHCC differs from sgHCC by lose of cell polarity and striking decrease in cell-cell and cell-ECM adhesion. It is also characterized by complete loss or strong downregulation of the expression of HNF4, which is known to be an essential regulator of liver differentiation and can induce epithelial morphogenesis in dedifferentiated hepatomas. fgHCC variant failed to express wide number of liver-specific genes.

The expression of exogenous HNF4 in cultured fgHCC led to restoration of number of liver-specific functions. In some of HNF4 transfected clones we had observed partial transition to epithelial phenotype. In transient

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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464 POSTER

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.

466 POSTER

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.

467 POSTER

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