194 POSTER

Adhesion and invasion of follicular thyroid cancer is regulated by vascular endothelial growth factor (VEGF) *in vitro*

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Tumor progression and metastasis are angiogenic dependent

Methods: We studied the effects of VEGF on invasion and adhesion of 3 metastatic follicular thyroid cancer cell lines including a primary (FTC 133) and two metastatic cell lines (FTC236: lymph node-; FTC238: lung metastasis) from the same patient. We tested *invasion* through an 8 μm pore membrane coated with Matrigel by the MTT assay and analyzed *adhesion* of FTC to major components of the extracellular matrix (ECM) (collagen I + IV, fibronectin, laminin, Matrigel).

Results: Compared to the primary tumor, both metastases had a greater basal invasion and a smaller basal adhesion. VEGF (1–100 ng/ml) dose-dependently stimulated invasion of all FTC, but the effect was greatest in the primary tumor. At 50 ng/ml invasion of FTC133 was enhanced by 35% (FTC236: 24%; FTC238: 19%; $p < 0.01$). Basal adhesion of FTC133 was enhanced by 25%, by 34% in collagen IV and by 31% in fibronectin ($p < 0.01$). Again, both metastases were less sensitive to the modulating effects of VEGF.

Conclusions: These data suggest that vascular endothelial growth factor stimulates invasion of follicular thyroid cancer cells through and adhesion to the extracellular matrix.

195 POSTER

Detection of circulating vascular endothelial growth factor (VEGF), matrix metalloproteinase-3 (MMP-3) and -9 (MMP-9) in gastrointestinal cancer

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Purpose: VEGF, MMP-3 and MMP-9 overexpressed in cancer tissues are implicated in tumor-associated angiogenesis and invasion. We measured plasma levels of those circulating molecules to clarify the clinical significance in gastrointestinal cancers.

Methods: Plasma samples were collected from 20 healthy controls, 12 patients with benign tumors (colon adenoma; 10, gastric adenoma; 2), 14 advanced cancer patients without metastasis (gastric; 9, colorectal; 5), and 40 cancer patients with metastasis (gastric; 25, colorectal; 15). Plasma levels of VEGF, MMP-3 and MMP-9 were determined by ELISA (R&D systems).

Results: Plasma VEGF and MMP-9 levels increased in accordance with the disease progression (healthy controls; VEGF 26 ± 8.2 pg/ml, benign tumors; VEGF 53 ± 33, MMP-9 22 ± 6.8 ng/ml, non-metastatic cancers; VEGF 41 ± 29, MMP-9 26 ± 8.2, and metastatic cancers; VEGF 148 ± 101, MMP-9 44 ± 32). But such relation was not noticed in MMP-3. When the discrimination level of VEGF was set at over 100 pg/ml, the sensitivity and specificity on detecting metastatic patients were 53% (21/40) and 95% (21/22), respectively. These were superior than those of MMP-3 and MMP-9. Plasma VEGF levels were correlated with MMP-9 ($r = 0.35$, $p = 0.010$) and CEA ($r = 0.40$, $p = 0.0049$) levels, but not with MMP-3 ($P = 0.588$) and CA19-9 ($P = 0.16$) levels.

Conclusion: Circulating VEGF in gastrointestinal cancers was suggested to be an useful diagnostic marker for tumor progression, especially metastasis.

196 POSTER

Determination of changes of tissue-specific genes expression in human brain tumors

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Purpose: Characterization of genes which expression is activated or repressed in human brain tumors, should provide new data for understanding of the mechanisms of tumors arising and progression. New genetic markers can be used in tumor diagnostics. The isolation of genes, differentially expressed in human glial tumors, is the objective of this study.

Methods: Differential hybridization of organized human fetal brain cDNA library (provided as high density arrays of clones, by H. Lehrach, ICRF, London) with cDNA probes synthesized on the mRNAs of normal human fetal brain, human meningioma and human anaplastic astrocytoma. The cDNAs which gave evident increase or decrease of the hybridization signal with tumor-specific probes were amplified and characterized by restriction-hybridization analysis and sequencing.

Results: The comparison of Southern hybridization patterns of DNA from isolated clones with normal human brain cDNA, astrocytoma cDNA and glioblastoma cDNA probes showed a visible decreasing of unknown mRNAs H2345, C063, 0073, and J2448 content in both tumors. The increasing mRNA content was observed for unknown clones C1134, H1352, L1033, for mRNA encoding valosin containing protein involved in signal transduction pathway and J1041 mRNA homologous to mouse TSC-22 mRNA.

Conclusion: Differential hybridization of robotically spotted high density arrays of human brain cDNA clones with cDNA probes synthesized on the mRNAs from different tumors showed characteristic pictures, specific for given tumor. The nucleotide sequences of isolated genes are under investigation.

197 POSTER

Cyotokines and surrogate markers in HIV-related opportunistic malignancies

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Purpose: To compare the levels of IL6, TNF-α, ferritin, C-reactive protein (Crp), β2-microglobulin (B2m), and CD4+ lymphocyte counts in HIV-infected patients with Kaposi's sarcoma (KS) and opportunistic infections (OI) vs. HIV patients with non-Hodgkin's lymphoma (NHL) and OI.

Methods: Eight patients were enrolled: 3 NHL (B cell, 2 low and 1 intermediate grade, stage IV, chemotreated) plus OI (2 extrapulmonary TB and 1 CMV infection), 5 KS (end-stage, viscerocutaneous, chemotreated) plus OI (4 CMV + mycobacteriosis, 1 CMV + CNS toxoplasmosis). All patients were severely ill and died shortly thereafter.

Results:

N.V.	IL6 (3.0–6.5 pg/ml)	TNF-α (3.0–20 pg/ml)	Ferritin (15–250 ng/ml)	Crp (<1 mg/dl)	B2m (0.9–3.0 mg/l)	CD4+ (504–1224/ /mmc	Mean age (years)
KS + OI	60.4	55.4	2351	0.9	4.5	25	38.8
NHL + OI	57.6	78	1404	1.6	4.5	152	44.3

Conclusion: Although preliminary, our data seem to underscore that: the combination of KS + OI appears to be lethal quite later than the simultaneous occurrence of NHL + OI: patients belonging in the former group died with lower CD4 cell counts and higher levels of ferritin, both roughly consistent with an "older" infection and with a more impaired immune function. IL6 and B2m behaved quite similarly in the two groups, while TNF-α and Crp showed only minor differences. This study is going on to confirm these preliminary findings.

198 POSTER

Detection of tumor progression in NSCLC stage IIIB/IV patients by serial measurement of CYFRA 21-1, TPA-M, TPS, and CEA

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Purpose: The most common application of tumor markers in lung cancer is disease monitoring. In a prospective study we have investigated the value of serially assessed tumor markers CEA and cytokeratin fragments CYFRA 21-1, TPA-M, and TPS to detect tumor progression in NSCLC stage IIIb/IV patients.

Methods: Tumormarker concentrations were measured using commercially available enzyme immuno assays (CEA: EIA Roche, Basel; CYFRA 21-1: EIA Boehringer, Mannheim; TPA-M: IRMA, Sangtec Medical, Bromma, Sweden; TPS: IRMA, Beki, Bromma, Sweden). Clinical response to therapy was evaluated according to standard criteria of the WHO. For the assessment of response to therapy by changing in the marker levels the difference between 2 consecutive levels must exceed 30%. This value is based on the formula: $\text{Diff} = 2\sqrt{2} \times V_K$ (V_K: interassay coefficient of variation, i.e. <10%)

Results: Tumor progression according to WHO criteria was recorded in 15 patients monitored by CYFRA 21-1 (TPA-M: 22, TPS: 21, CEA: 14). For CYFRA 21-1 67% (TPA-M: 55%, TPS: 67%, CEA: 43%) of the evaluations by rising marker levels were concordant with the clinical assessment. Most

discordant results can be explained by insufficient increase in the marker levels or by a substantial lead-time.

Conclusion: Increasing marker levels in NSCLC stage IIIb/IV patients contribute to the clinical decision making at least in a way that these patients may no longer be treated by ineffective and toxic drugs.

199 POSTER

Comparison of the in vitro cytotoxicity of the antitumour antibiotics bleomycin and mitomycin on human colorectal cancer cells and endothelial cells

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Purpose: To investigate the absolute and relative cytotoxic effects of two antitumour antibiotics on human endothelial cells. Information of cytotoxicity on both proliferating and non-proliferating EC might be important in their toxic and antitumour effects. For both BLM and MMC cytotoxicity against EC is considered to be involved in pulmonary toxic reations of these compounds.

Methods: Cell cultures of the human colorectal cancer line DLD-1, the immortalised endothelial cell line HMEC and fresh harvested umbilical vein EC's were exposed to different concentrations (0.01 to 120 μg/ml) of bleomycin and mitomycin. Cytotoxicity was analysed with Alamar blue technique. Cytotoxic studies were performed against confluent and against proliferating cells. Different durations of incubation 2 h vs 18 hs were studies.

Results: Both BLM and MMC had a cytoxic effect against proliferating EC. BLM exhibited no such effect against confluent EC in the concentration range studied. The cytotoxic effect increased with increased duration of exposure, both for BLM and MMC.

Conclusion: Both MMC and BLM exert a cytotoxic effect against proliferating EC. This effect is dependent on both drug concentration and exposure duration. These results show that at concentrations obtained in patients endothelial cell toxicity might contribute to both the antitumour effect and the different toxic events.

200 POSTER

The influence of theobromine on angiogenic activity of human ovarian cancer cells

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Introduction: Angiogenesis plays the crucial role in growth of solid tumor and metastasis formation. The aim of present study was to evaluate if theobromine – adenosine receptor antagonist and phosphodiesterase inhibitor – shows antiangiogenic activity in human ovarian cancer cells.

Methods: Ascitic fluid was obtained from 30 patients with ovarian cancer (FIGO 3 and 4). Full suspensions of cancer cells were grafted intradermally into Balb/c mice and subsequently treated with theobromine in 0, 24, and 72 hours thereafter. Then mice were sacrificed and newly formed blood vessels were counted (TIA – tumor induced angiogenesis test). In further studies cells (full suspensions, isolated cancer cells and TILs) were preincubated with theobromine and then used to TIA test, and in vivo culture in Balb/c mice peritoneal cavity. The concentrations of angiogenic cytokines (IL8, bFGF and VEGF) in 48 hours in vivo cultures were estimated in ELISA test and shown in pg per mg of total protein. RTPCR method was used to determine the influence of theobromine on urokinase and tissue plasminogen activators (uPA and tPA).

Results: Theobromine injected subcutaneously as well as preincubated with cancer cells showed antiangiogenic activity in TIA test. We showed statistically significant inhibition of IL-8, bFGF and VEGF production by ovarian cancer cells after preincubation with theobromine in therapeutic concentration (20 μg/ml). Lack of transcript of tPA and uPA was shown in theobromine treated cell.

Conclusions: Theobromine is a potent angiogenesis antagonist. The mechanism of its action is complex and includes inhibition of proangiogenic factors production (bFGF, IL-8, VEGF) as well as of uPA and tPA transcript.

201 POSTER

Unwanted effects of evening primrose oil on tumor angiogenesis and blood granulocyte number

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Purpose: Evening primrose oil (EPO) has been investigated as a potential additional treatment for various diseases. It contains high levels of polyunsaturated fatty acids (PUFAs). However excessive amount of PUFAs in human and animal diet have been accounted with increased incidence of tumors. The aim of the study was to estimate the influence of EPO on 1) angiogenic activity of human lung cancer cells and 2) number and activity of mice blood granulocytes.

Methods: Cells from tumors of 18 lung cancer patients were grafted intradermally into 108 Balb/c mice. For the 3 consecutive days 20 mg of EPO was applied on the sites of implantation. After 72 hours mice were sacrificed and the new vessels were counted. Paraffin oil was used as a control drug. In the other experiments 48 mice were fed for 4 weeks with 250 mg/kg day EPO.

Results: The data demonstrated that EPO enhanced neovascular response (mean number blood vessels 19.31 ± 0.61 for EPO; 15.0 ± 0.61 in control group). The number of mice blood granulocytes significantly decreased after EPO treatment but their activity increased.

Conclusions: Angiogenesis – enhancing and granulocyte lowering activities of EPO should be taken into consideration in diet of cancer patients.

202 POSTER

CD44-isoforms promote adhesion to endothelial cells through recognition of chondroitin-4-sulfate

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Purpose: The transmembrane glycoprotein CD44 has been implicated in a wide range of adhesion-dependent cellular processes. Alternative splicing events can give rise to a large number of differentially expressed CD44 isoforms. The aim of the present study was to define the involvement of such isoforms in cellular adhesion to non-activated endothelium.

Methods: The CD44-negative murine lymphoma cell line TIL-1 was transduced with the human CD44H (90 kD) and CD44R1 (130 kD) cDNA by retroviral-mediated gene transfer. Adhesion of fluorescently labelled lymphoma cells to a murine endothelial cell line (SVEC) was evaluated using a fluorescence plate reader.

Results: Lymphoma cells expressing the common CD44 isoform CD44H or the alternatively spliced isoform CD44R1 containing exons v8-v10 can both bind to immobilised and soluble hyaluronan. Cells expressing CD44R1 and to a lesser extent those expressing CD44 H but not the CD44-negative parental cell line were also able to bind the murine endothelial cell line SVEC. This adhesive interaction is mediated by recognition of chondroitin-4-sulfate on SVECs as chondroitinase pretreatment abrogates the cellular interaction. Evidence was obtained that among the proteins that can present chondroitin-sulfate to CD44 is CD44 itself.

Conclusion: We conclude that modification of CD44 on endothelial cells with chondroitin-4-sulfate plays an important role in regulating cellular adhesion to vascular endothelium.

203 POSTER

Examination of secretory phospholipase A_2 ($sPLA_2$) as a maker of metastasis

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Purpose/Methods: $sPLA_2$ is a marker of inflammatory disease. It can be induced by the proinflammatory cytokines IL-1, IL-6 and TNFα. These cytokines are also increased in patients with metastatic cancer diseases. To examine if $sPLA_2$ can serve as a common marker of metastasis too, its amount have been quantified in sera of different groups of cancer patients (breast cancer (121), ovarian cancer (31) and gastrointestinal cancer (37)) as well as in healthy individuals (172) using a commercially available immunoassay.

Results/Conclusions: Significantly increased levels of $sPLA_2$ have been measured in all groups of cancer patients compared to healthy individuals