expressing no or only little $sPLA_2$. But only in patients with ovarian and gastrointestinal cancer a remarkable difference in $sPLA_2$-levels could be found between those with known metastasis and those without known metastasis. Thus, $sPLA_2$, an easy detectable protein, seems to be a marker of metastasis for defined groups of cancer patients.

204 POSTER

Cancer apoptosis: A possible novel tool to evaluate prognosis and effectiveness of therapy in breast and colon cancer

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Purpose: Cells can choose two different biological ways of death that are slightly different in morphology and biochemistry, but with a great biological difference: apoptosis or necrosis. The aim of the study is to estimate if apoptotic process may assume a remarkable role in the prognosis of cancer and effectiveness of therapy.

Method: We evaluate several specimens from breast cancer fine needle aspirations and from biopsy of colon cancer (20 breast cancer patients and 21 colon cancer). The specimens were performed during primary chemotherapy (CHT) (CNF 4 cycles b. surgery) before and after every cycle in the breast cancer group and at the beginning and stop of adjuvant therapy (FU-FA) in the colon cancer group. We correlate these data with labeling index (L.I.), histological type and grading, tumor mass and vascularization of each neoplasia. Also live, necrotic and apoptotic cells were determined morphologically by fluorescent microscopy. Cells were stained with a mixture of acridine orange and ethidium bromide (as described by Duke & Cohen) to perform microscopy observations. Flow cytometry after labeling with Hoechst 33342 and propidium was performed according to the method described by Pollack.

Results: The percentage of apoptotic cells both in colonic cancer than in breast before CHT was low (2–5%). After CHT an increase of apoptotic and necrotic cells was noted, particularly in the most vascularized areas (20–30% ca.)

Conclusion: Our data, even though further investigations and increased number of cases are necessary, support the hypothesis that apoptosis can play a role in the evaluation of the effectiveness of cancer chemotherapy and that stromal vascularization of tumor mass seems to be related to the increase of apoptosis and necrosis.

205 POSTER

Expression of soluble CD44 and splice variants V5 and V6 and its implication in tumour staging according to the TNM classification in gastric cancer

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Purpose: In gastric cancer, CD44 expression correlated with disease recurrence after curative resection and with the *Lauren* classification. This study aimed on the quantification of soluble CD44 and splice variants v5/v6 in correlation to the T and N stage of gastric cancer patients.

Material and Methods: 120 patients with different pT stages had preoperative i.v. blood examination on sCD44std, sCD44v5 and sCD44v6 using ELISA kits. Data of 118 patients were evaluable: T-staging T1: n = 14, T2: n = 54, T3: n = 40, T4: n = 10; N-staging: N0 = 33: N1 = 33. N2 = 37, Nx status in 15 patients were neglected. M.F ratio was 1:4.5, mean age of male/female patients (66.7 vs. 62 years) differed insignificantly. Control group of healthy volunteers (n = 50, mean age 61 years).

Results: sCD44std and splice variants v5 and v6 showed significant differences to the expression in healthy volunteers, and significant differences related to the T-stages in sCD44std and sCD44 v6 (rep. measures ANOVA, $p < 0.05$), but not concerning the N-stages in all sCD44 variants. Differences of the quantified expression between the splice variants were significant (rep. measures ANOVA, Tukey-Cramer test, $p < 0.05$).

Conclusions: sCD44 and splice variant v6 quantification is significantly different in healthy volunteers and gastric cancer patients. sCD44 showed sufficient correlation to the T-stage of the tumour specimens, but failed to correlate with the nodal status. Therefore, sCD44 may serve as an indirect prognostic marker in gastric cancer.

206 POSTER

Differential expression of VEGF in gastrointestinal tumors

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Purpose: Among those growth factors influencing the development of angogenesis the Vascular Endothelial Growth Factor (VEGF) has a special role because of its target cell specifity. In vitro inhibition of this growth factor proved a negative influence on tumor development. It was the aim of our study to localize those cells in a tumor and the corresponding normal tissue expressing VEGF mRNA and protein in human gastrointestinal carcinomas.

Methods: Fresh frozen sections of 13 gastrointestinal carcinomas (colon, stomach, esophagus) and the corresponding normal tissue were examined with non-radioactive in-situ-hybridisation (ISH) and immunohistochemistry. For ISH the riboprobes were generated from a 450 bpVEGF cDNA using Digoxigenin labeled nucleosidtriphosphate. For immunohistochemistry PAP-reaction with a polyclonal antibody against $VEGF_{165}$ was used.

Results: VEGF mRNA- and protein-expression was fond in all tumors and in the normal tissue, but at different levels. Besides signals were obtained in the malignant stroma cells, in the lymphocytes infiltrating the tumor stroma and in the non-neoplastic tissue. Lymphocytes infiltrating the normal tissue showed also a strong signal.

Conclusion: These results demonstrate, that VEGF expression is not restricted to a certain cell population, but is upregulated in all cell types of a malignant tumor. An unexpected result is the strong expression in lymphocytes infiltrating the tumor and the normal tissue.

207 POSTER

Effect of irradiation on microvessel density and endothelial cell proliferation in vivo

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Purpose: The in vitro effect of irradiation on the release of mitogenic and angiogenic factors has been described previously. The aim of our study was to investigate the in vivo effect of radiation on angiogenesis.

Methods: For this study fertilized eggs were used. Irradiation of the area vasculosa (A.V.) was performed with a linear accelerator on day two of incubation. The eggs received fractionated or single fraction irradiation with doses from 2 to 10 Gy. 48 hours after irradiation, the A.V. was photographed in vivo. Prints of known enlargement were evaluated for microvessel count (MC) as an indicator for the density of the blood vessels. In addition, histological sections of the area vasculosa were analyzed for endothelial cell proliferation. Proliferative activity was calculated by determining the expression of proliferating cell nuclear antigen (PCNA). All parameters were compared to untreated controls by Student's t-test.

Results: 48 hours after irradiation with 2 to 8 Gy there is a slight decrease in vascular density. After a single dose of 10 Gy or fractionated irradiation with 3 × 2 Gy and 3 × 3.3 Gy a statistical significant increase in vascular density was found. Measurements of the proliferating potential by immunostaining of proliferation associated antigens demonstrated a significantly higher PCNA index in areas with increased MC.

Conclusion: In the area vasculosa of chick embryos, angiogenesis, measured by microvessel density and endothelial cell proliferation, can be induced by irradiation.

208 POSTER

Immunohistochemical analysis of integrin $\alpha v\beta 3$ expression on tumor associated vessels of human carcinomas – Implications for anti-angiogenic treatment approaches

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Purpose: Expression of the $\alpha v\beta 3$ integrin is upregulated on sprouting endothelia. Systemic application of antibody or peptidic inhibitors of $\alpha v\beta 3$ function disrupts tumor angiogenesis and reduces growth and invasiveness of human tumors in animal models. We systematically investigated $\alpha v\beta 3$ expression on tumor-associated vessels of four different human epithelial tumors and the corresponding normal tissues.

Methods: Immunohistochemical analysis were performed on serial frozen sections using the $\alpha v\beta 3$-complex specific monoclonal antibody LM609.

Results: Variable levels of LM609 staining were found in all carcinoma lesions. A considerable number of tumor tissues (35/50) expressed $\alpha v\beta 3$ on more than 50% of their vessels. Inflammatory infiltrates and the possibly hypoxic conditions near necrotic areas of tumors were accompanied by an increased $\alpha v\beta 3$ expression. Remarkably, the vasculature in apparently normal tissue also stained for $\alpha v\beta 3$. However, the percentages of stained vessels and the staining intensity were lower than in neoplastic tissues. Besides the vascular $\alpha v\beta 3$ expression, several extravascular cell types stained positive, both in normal and tumor specimens.

Conclusion: Taken together, our findings show a considerable number of colon, pancreas, lung and breast carcinoma lesions with many $\alpha v\beta 3$-expressing vessels that could be targets for anti-$\alpha v\beta 3$ therapy.

209 POSTER

Liaison® free PSA, development of an automated chemiluminescence immunoassay for the determination of free prostate specific antigen (fPSA)

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Total PSA (tPSA) exists in human serum in two major detectable forms; PSA complexed to alpha1-antichymotrypsin (PSA-ACT) and free PSA (fPSA). Recent publications have shown that patients with benign prostate hyperplasia (BPH) tend to have higher levels of fPSA than patients with prostate cancer. The potential use of the ratio between the measured fPSA and tPSA concentration might be for the better differentiation of prostate cancer and benign disease. Liaison® fPSA is a rapid, fully automated immunoassay designed to run on the new random access Liaison® immunoassay analyzer from Byk-Sangtec Diagnostica. Liaison® fPSA is based on paramagnetic particles (Dynabeads®) for separation and a chemiluminescent label of isoluminol type with flash light kinetic. The assay is designed as a one-step two-site immunoluminometric assay using two highly specific monoclonal antibodies from Centocor (Centocor, Inc., Malvern) recognizing two different epitopes on the fPSA antigen. The monoclonal antibodies from Centocor bind only to free PSA when fPSA and ACT-PSA antigen material from Prof. Stamey, Stanford University (as reference) was used for evaluation. 100 μl sample are incubated together with 150 μl tracer antibody and the monoclonal antibody coated on the paramagnetic particle. After 10 min incubation time the paramagnetic particles are washed and unbound material is separated. Measurement of luminescence is performed for 3 s after injection of two trigger solutions. With the one-step 10 min incubation protocol, time to first result on the Liaison® analyzer is approx. 15 min. The Liaison® fPSA assay covers a clinical concentration range of 0–50 ng/ml with typical within-assay precision below 5% and sensitivity below 0.01 ng/ml respectively. The between-assay precision ranged from 4 to 8%. No hook effect is found up to 20,000 ng/ml. Samples run on the Liaison® fPSA assay show a good linearity upon dilution (±10% of the theoretical sample value). Method comparison (n = 72) of the Liaison® fPSA assay to an established method (Tandem-R Free PSA, Hybritech, Inc.) demonstrates a correlation coefficient of 0.96 and a slope of 0.96. In conclusion the Liaison® fPSA offers a rapid, reliable and precise method for the fully automated determination of fPSA.

210 POSTER

Expression of matrix metalloproteinase 7 (MMP-7) in alimentary tracts cancer, lung cancer and breast cancer

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Purpose: Proteolytic degradation of the extracellular matrix is an important part of tumor invasion and metastasis, and matrix metalloproteinases (MMPs) which are produced and secreted in tumor tissues have been implicated. MMP-7, also known as matrilysin, pump-1 is a member of stromelysin subclass of MMPs, and MMP-7 has wide range of substate specificity. However, stromelysin 1 (MMP-3) and stromelysin 3 (MMP-11) have been shown to express only in stromal cells in cancerous tissues. In this study, we have investigated whether MMP-7 expressed in cancerous tissues and/or tumor cells.

Methods: We have investigated the expression of MMP-7 colorectal, gastric, esophageal, lung breast cancerous tissues using reverse transcription polymerase chain reaction (RT-PCR), and the immunolocation of MMP-7.

Results: The incidence of MMP-7 mRNA expression were 60% in esophageal cancerous tissues, about 50% in gastric cancer and colorectal cancer, about 40% in lung and breast cancer, respectively. In contrast, MMP-7 mRNA expression was not detected in non-cancerous tissues excluding esophageal tissues associated with dysplasia. Immunolocalizational study demonstrated that MMP-7 was restricted in tumor cells

Conclusion: These results suggest that MMP-7 may be expressed in tumor manner and play a role in tumor progression.

211 POSTER

RENCA – An animal model for the development of anti-angiogenic strategies

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Purpose: RENCA, a murine renal cell carcinoma, metastasizes similarly to human cancer. VEGF (vascular endothelial growth factor) is the most important tumor derived angiogenic growth factor. We studied the importance of the VEGF-system for the RENCA- model and its use for screening of VEGF and VEGF receptor inhibitors.

Method: RENCA-cells were injected sc, iv or ir in syngenic Balb/c mice. VEGF-expression were measured by both, Northern-blot analysis and VEGF-Elisa. Primary tumors and metastases were analyzed for vessel density and VEGF-expression by immunohistology.

Results: Supernatants of cultered RENCA-cells were able to stimulate the proliferation of endothelial cells (HUVEC). This proliferation was dependent on the secretion of VEGF. Immunohistological staining of primary tumors and metastases showed elevated vessel density in correlation with VEGF-staining.

Conclusions: We showed that the RENCA model depends on the expression of VEGF and we will use this model to establish anti-VEGF-based therapies.

212 POSTER

Are serum TPS levels important in the prognosis of lung cancer?

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Purpose: The prognostic significance of tissue polypeptide specific antigen (TPS) were investigated together with other tumor markers such as NSE and CEA in SCLC and NSCLC patients. The serum levels of these markers were also compared with those of the patients with benign pulmonary disease (BPD).

Methods: Serum samples were obtained prior to treatment in 158 patients. The study group consisted of 72 SCLC, 44 NSCLC and 42 BPD cases and 23 healthy subjects. For TPS analysis, TPS-Elisa (BEKI) kit and the method of sandwich ELISA were used. In the analysis of NSE and CEA, NSE-RIA and CEA-RIA (CIS) kits and RIA were applied.

Results: All three tumor markers were significantly higher in lung cancer cases than in patients with BPD and healthy subjects. Serum TPS levels were highest in NSCLC. TPS and others were significantly higher in disseminated SCLC than limited disease. Also, in NSCLC cases, TPS and CEA were elevated significantly related with the stage groups. Evaluating the tumor markers with performance status (PS) and survival, TPS serum levels provided the better correlation with PS and survival in cancer cases than the other markers. Survival was significantly better in cases with TPS levels below 200 U/L, NSE levels below 20 ng/ml and CEA levels below 10 ng/ml in SCLC cases. When the same values were applied to NSCLC cases, TPS and CEA exhibited significant differences in survival.

Conclusion: The significant correlations between TPS with stage, PS, survival suggest that the use of this marker together with NSE and CEA in SCLC patients and together with CEA in NSCLC patients may contribute to the clinical evaluation.