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

L. Dirix, P. Vermeulen, L. Libura, J. Libura, A. Pawinsky, E.A. De Bruijn, A. Van Oosterom. *Tumour angiogenesis and Stroma Research Group Catholic University Leuven, Belgium; University of Antwerp, Department of Oncology, Edegem, Belgium*

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

E. Barcz[1], P. Janik[2], K. Gawrychowski[2], E. Sommer[1], E. Skopińska-Różwska[1]. [1]*Immunol. Dept. Ntl. Inst. Tuberc. Lung Dis.,* [2]*Oncol. Center, Warsaw, Poland*

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

E. Sommer[1], E. Skopińska-Różewska[1], M. Filewska[1], W. Kupis[2], J. Bogdan[2], S. Miekodaj[2]. [1]*Immunol. Dept., Warsaw;* [2]*Surgery Dept. Ntl. Inst. Tuberc. Lung Dis., Warsaw, Poland*

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

A. Droll[1], R.K. Chiu[2], C. Unger[1], G.J. Dougherty[2]. [1]*Tumor Biology Center, Freiburg;* [2]*The Terry Fox Laboratory, BC Cancer Agency, Vancouver, Germany*

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

U. Massing, A. Kraft[3], B. Stier[2], M. Klose[2], C. Unger[1]. [1]*Tumor Biology Center of the University Freiburg;* [2]*Boehringer Mannheim GmbH;* [3]*Dep. of Hematology/Oncology, University of Freiburg, Germany*

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

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

I. Carreca[1], S. Cucciarrè[1], M. Tolomeo[1], L. Rausa[1], G. Diana[2], A. Vecchione[3]. [1]*Institute of Oncology, University of Palermo;* [1]*Institute of Surgery, University of Palermo;* [3]*Department of Experimental Medicine, University of Rome, Italy*

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

J. Kocik[1], K.W. Schmid[2], N. Lügering[3], B. Brandt[4], N. Senninger[1], G. Winde[1]. [1]*Department K.W. General, Surgery, University Münster;* [2]*Institute of Pathology, University Münster;* [3]*Dept. of Medicine B, University Münster;* [4]*Institute of Laboratory Medicine of the Westfälische Wilhelms, University Münster, Germany*

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

M.W. Strik, Ch. Laus, M. Duchrow, H. Schimmelpenning, R. Broll, H.-P. Bruch. *Department of Surgery, University Hospital of Lübeck, Germany*

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

L. Plasswilm[1], N. Cordes[2], A. Tannapfel[3], J. Hoeper[4]. [1]*Department of Radiation-Oncology Baser, Switzerland;* [2]*Erlangen;* [3]*Institute of Pathology, Leipzig;* [4]*Institute of Physiology, Erlangen, Germany*

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

R. Max, R.C.M. Gerritsen, P.T.G.A. Nooijen, S.L. Goodman, A. Sutter, U. Keilholz, D.J. Ruiter, R.M.W. De Waal. *Department of Pathology, University Hospital of Nijmegen, The Netherlands; Department of Internal Medicine V, University Hospital of Heidelberg; Merck KGAa, Pre Clinical Pharmaceutical Research (IMO), Darmstadt, Germany*

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.