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.

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## 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.

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## 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.

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## 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.

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## 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.

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## 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.