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Inverse correlation between the expression of vascular endothelial growth factor and infiltration of dendritic cells and their influence on the prognosis of non-small cell lung cancer

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Background: Vascular endothelial growth factor (VEGF), produced by many human tumors, is known to play an important role in the formation of tumor neovasculature and to be associated with poor prognosis. On the other hand, several investigators reported that VEGF inhibits dendritic cells (DCs) maturation in vitro and decreases numbers of DCs in vivo. In the present study, we analyzed the relationship between the expression of VEGF and the infiltration of DCs, and their influence on the prognosis of patients with non-small cell lung cancer (NSCLC). Methods: Immunohistochemical expression of VEGF in tumor cells, microvessel densities in tumor stroma and intra tumoral infiltration of DCs were investigated in 132 patients with surgically resected NCSLC and their influence on patientsí survival was evaluated. Results: VEGF expression was positively related to microvessel density (MVD) ($P = 0.003$), and negatively related to the degree of DCs infiltration ($P = 0.0232$). The prognosis of patients with high VEGF expression or high MVD were significantly worse than that of patients with low VEGF expression ($P<0.0001$) or low MVD ($P<0.0001$). The prognosis of patients with high DCs infiltration was significantly better than that of patients with low DCs infiltration ($P = 0.0004$). Multivariate analysis also showed that these factors are independently related to patients' prognosis. Conclusion: The present data indicates that the inhibition of DCs by VEGF is seen in clinical specimen with NSCLC, which is consistent with previous in vitro and in vivo experimented data, and that these parameters are prognostic factors of patients with NSCLC.

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Detection of circulating breast cancer cells and bone marrow micrometastases by immunomagnetic one-step RT-PCR

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Purpose: Sensitive detection of occult carcinoma cells in peripheral blood and bone marrow of patients with breast cancer may have important prognostic and therapeutic implications. PCR is a highly sensitive method for detection of neoplastic cells in hematological malignancies. However, this technique, when applied to solid tumours, is highly prone to false positives due to illegetimate transcription. By implementing immunomagnetic enrichement of carcinoma cells prior to molecular detection, the problem of false positive results can be eliminated since the number of background cells is reduced below the detection limit of illegitimate transcription. In this work we described a sensitive method for the detection of circulating epithelial cells and bone marrow micrometastases in patients with breast cancer, using a immunomagnetic one-step nested RT-PCR method.

Methods: Immunomagnetic one-step nested RT-PCR was performed in a group of 25 breast cancer patients. Serial dilutions of a positive control cell line were used for sensibility evaluation.

Results: This assay proved to be highly sensitive, with a detection limit below 1 carcinoma cell in 106 mononuclear cells.

Conclusion: The assay described is a very sensitive and specific method for detection of circulating epithelial cells and bone marrow micrometastases in patients with breast cancer. However, long-term follow-up of patients is needed, to evaluate the potential prognostic and therapeutic implications of the presence of circulating epithelial cells and medular micrometastases in breast cancer.

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Development of a novel assay for chemosensitivity testing in vitro for primary human tumours

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Purpose: Tumour chemosensitivity (TCS) assays can aid early detection of drug-resistance allowing optimal treatment to occur earlier thereby avoiding unnecessary patient toxicity. TCS assays can also help the development of new therapies. However, many patient samples are not evaluable because too few viable cells can be isolated. We aimed to develop a novel highly sensitivity assay applicable to all tumour types.

Methods & Results: Ten different exponentially growing human tumour cell lines (adherent and suspension) were seeded in quintuplicate in a variety of media in the wells of micro-titerplates from 10-10,000 cells/well, and a fluorophore added (0.1-2 μM). Fluorescence was measured at room temperature and monitored over several hours using a digital camera to record magnified images. Fluorescent viable cells were discriminated from normal cells by standard staining technology and were counted using an algorithm (CCA). This was linear up to 10,000 cells, while the total sum of fluorescent pixels (equivalent to a fluorimeter) was non-linear. The CCA-method provided a stable endpoint for ca. 2 hr in adherent cells and up to 24 hr for suspension cells if stored at 4oC. Adherent cells were resolved for cell counting by briefly treating with trypsin-EDTA. The cytotoxicity (IC50) of 18 anticancer drugs was determined by incubation of the drugs for 3-4 days at 6 different concentrations plus controls ($n\approx10$) followed by addition of the fluorophore. Assay conditions were robust, since experiments repeated at least 6 times over a 3 month period had small SD's. The mean IC50 $\pm$ 2SD provided a 95% confidence limit to compare with in-house resistant cell lines, the 60 cell line NCI database and with primary samples of chronic lymphocytic leukaemia and urological tumours. Primary human tumour cells were obtained by enzymic digestion of solid tumours or density-gradient centrifugation of leukaemic cells followed by incubation in optimised media for 3-4 days.

Conclusion: A highly sensitive fluorescent imaging assay for detecting viable human tumour cells with a wide dynamic range has been established. Up to 10 drugs in one 384-plate may be studied and the method is sufficiently robust to detect changes in resistance of just 2-fold. Comparison of IC50's with clinical response is ongoing to allow calibration of this TCS assay.

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Hematogenous tumor cell dissemination during surgery in patients with esophagus-, gastric and pancreatic cancer

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Introduction: Early generaliziation of esophagus and gastric cancer is considered to be responsible for early relapse and metastasis after surgical therapy. An evasion of tumor cells into circulation during surgery at the colon, lung and liver has been shown. Aim of this study was to investigate whether tumor cell dissemination may also be induced by resection of tumors of the esophagus, stomach and pancreas.

Methods: Tumor tissue and blood from 30 patients were analyzed. Blood samples were taken from a central venous catheter(CVK)and a tumor draining vein before and after tumor manipulation. Following tumor cell enrichment using BerEP4-coated magnetobeads, DNA and RNA were prepared and cDNA was synthesized. DNA samples were screened for k-ras mutations and p16 hypermethylation. cDNA was subjected to β-actin and CK-20 PCR.

Results: K-ras mutations or p16 hypermethylation were detected in the tumors of 10 patients. The markers (4times p16, 2times K-ras) was seen in the tumor draining vein and in the CVK in 6 and 4 cases, respectively. CK-20 was found in the blood from 9 of 22 cases studied. 8 patients had positive samples in the tumor draining vein, 5 in the CVK. Main characterisitics of the tumors and the time points of positive findings:

3 patients p16 before and after manipulation (UICC Stage II, IIIA, IV)
1 patient CK-20 before and p16 after mainpulation (IIA)
2 patients CK 20 before and after manip. (IV,x)
2 pt. CK-20 after manipulation (IA, III)
1 pt. CK-20 nd K-ras after manip. (IV)
1 pt. K-ras after manip. (II)
2 patients CK-20 after manip. (IIA, IA)