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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)

1 pt. CK-20 before manipulation (I)

Discussion: The results indicate that at least in some patients tumor cell dissemination occurs during surgery. Further data is required to determine the diagnostic sensitivity and specifity of tumor cell detection using RNA- and DNA markers, resp., and to estimate the prognostic and clinical relevance of intraoperative tumor cell dissemination.

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Human glioma cells: genetic alterations and radiation and temozolomide sensitivity

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Purpose: Determination of the genetic profile of early passage cell cultures derived from human gliomas and established glioma cell lines and correlation with their in vitro radiation- and temozolomide sensitivity.

Materials and Methods: Cell cultures of 6 patients were processed from fresh brain tumour specimens. Also the glioma cell lines Gli 6 and D 384 were used. Genetic characterisation included determination of mutational and loss of heterozygosity (LOH) status of TP 53, LOH of chromosome 10 status and Epidermal Growth Factor Receptor gene (EGFR) amplification. Irradiation of cells ranged from 0 to 6Gy (RT), or cells were treated with TMZ by incubation for 3h or 24h in 10 ZM TMZ-containing culture medium (3hTMZ; 24hTMZ). For combined treatment, cells were irradiated immediately following 3hTMZ and 24TMZ. Cell survival was determined by clonogenic assay and survival curves were generated. Surviving Fractions after 2Gy (SF2) and 4Gy (SF4) were used as radiosensitivity parameters.

Results: The genetic profile of Gli 6 shows LOH but no mutation of TP 53, complete LOH 10 but no EGFR amplification. In the genetic characterisation of VU 15 a mutation and LOH of TP 53, but no LOH 10 and EGFR amplification were diagnosed. The VU 19 cell culture showed incomplete LOH 10 but no other genetic aberrations. In the VU 24 cell culture an incomplete LOH 10 and EGFR amplification, but no TP 53 alteration was seen. The other cell cultures and

D 384 cell line showed no genetic aberrations. Surviving fractions after 3hTMZ ranged from 0.19 to 0.91 (mean: 0.65) and from 0.16 to 0.72 (mean: 0.49) after 24hTMZ. SF2/SF4 values after RT alone ranged from 0.47/0.24 to 0.90/0.54 (mean: 0.73/0.36) from 0.36/0.15 to 0.75/0.51 (mean: 0.60/0.30)after 3hTMZ RT and from 0.35/0.15 to 0.68/0.45 (mean: 0.54/0.26) after 24hTMZ RT. Two cell cutures (VU 15, VU 20) showed a reduction in cell survival after 3hTMZ to 0.19 and 0.52 respectively while the other cell cultures and established cell lines showed surviving fractions of >0.55 after 3hTMZ.

Conclusions: Glioma cells show a low radiosensitivity. Combination of RT with TMZ decreased the SF2- and SF4 values, with a trend towards synergistic effect in TMZ sensitive cells and additive effect in less TMZ sensitive cells. Alterations in cell regulatory genes are frequently found and, together with the data on radiation and TMZ sensitivity, this information might be used for individualized therapy of glioma patients.

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Helix pomatia agglutinin (HPA) binding: an independent prognostic factor in resected adenocarcinomas of the lung

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Introduction: The incidence of adenocarcinoma of the lung rises worldwide, unfortunately no significant prognostic marker beyond the classical TNM staging exists to stratify the patients for appropriate therapy. A panel of lectins (Helix pomatia agglutinin (HPA), Phaseolus vulgaris agglutinin (PHA-L), Ulex europaeus agglutinin (UEA-1), Maackia amurensis agglutinin (MAA), Sambucus nigra agglutinin (SNA-I)) with different carbohydrate specificities were tested for their prognostic relevance.

Patients and Methods: Paraffin wax sections of 93 patients (pts) with adenocarcinomas of the lung who had undergone surgery between 1990 and 1995 were investigated by lectin histochemistry. All pts were followed up systematically for a minimum of up to five years.

Results: 63 male/30 female; median age 59 years [range 27-81]; 72 pts stage I/II, 19 pts stage IIIA, 1 pts stage IIIB, 1 pts stage IV disease. The overall 5-year survival rate was 49.5%. Distant metastases or local relapse were diagnosed in 49 patients (53%). 9 tumours were classified as HPA-negative, and 83 as HPA-positive. Pts with HPA-positive tumours had a significantly poorer survival than pts with HPA-negative tumours (p=0.015, log rank test). All patients with HPA-negative tumours survived 5 years. Next to HPA also binding of PHA-L (p=0.017, log rank test) and UEA-I (p=0.022, log rank test) to adenocarcinoma cells were prognostic indicators for overall survival, whereas MAA and SNA-I binding had no prognostic significance. In multivariate Cox regressions analysis next to stage (stage II: p=0001, standard error 0.39, risk ratio 6.00; stage III/IV: p=0001, standard error 0.39, risk ratio 8.35) and gender (p=0004, standard error 0.37, risk ratio 3.65) only HPA (p=0.04, standard error 1.06, risk ratio 8.75) was an significant independent prognostic factor on survival.

Conclusion: HPA binding was the primary marker-based predictor of prognosis in our patient population and allows to stratify patients with adenocarcinomas of the lung into a low and a high risk group.

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The response of advanced pancreatic cancer (APC) to gemcitabine monochemotherapy in relation to the expression of proliferation markers, oncogenes Her-2, Bcl-2, C-myc and p53 antioncogene. A retrospective clinico-pathological study

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Background: Gemcitabine(G),a nucleoside analogue,is accepted as palliative treatment in APC. The cytotoxic effect of G is mediated by the DNA damage in proliferating cells, followed by an induction of apoptosis. The objective of this study was to determine, whether some immunohistochemical analysis may be useful in predicting G efficacy.

Material and methods: An immunohistochemical analysis of archived, formalin fixed, paraffin embedded tumor samples was performed in 25 patients(pts) with histologically confirmed measurable APC, treated with G. The expression of cell cycle markers Ki67, Cyclin A and Cyclin B1 has been scored as percentage of positive cells, while the Her-2, Bcl-2, C-myc and p53 expression has been rated as none, moderate or strong. The pts have been followed clinically and their objective response rate(ORR) and overall survival(OS) were recorded and compared to the results of immunohistochemistry. Results: There was no relation between the pts OS and the tumor proliferation reflected by the expression of the above mentioned markers. The overexpression of Her-2 and Bcl-2 oncogenes in tumor cells was a rare finding (one case each), a substantial expression of C-myc was recorded in 3/25 cases only. A strong expression of p53 antioncogene in tumor cells was noted in 8/25 cases, while no staining appeared in 3/25 cases. In 14/25 cases the p53 staining was of a moderate level ranging from 5-80% of tumour cell nuclei. If the pts from the later group were compared to those ones with tumor exhibiting none or very strong p53 expression, their average OS was longer (6.5 vs. 4.2 months, p=0.03-t-test) and the survival curves differed significantly (p=0.04 - Mantel-Cox).

Conclusions: The pts suffering from APC with moderate p53 expression (corresponding to the partial maintenance of p53 function,i.e. moderate level of staining) respond better to the G chemotherapy and have longer OS comparing to the pts with carcinoma with no staining (corresponding to p53 deletion or posttranslational modification of target epitope) as well as comparing to the pts with carcinoma with strong p53 expression (corresponding to the stabilization of non-functional p53 due to the missense mutation). These data suggest that detailed evaluation of p53 status might be of interest in the attempt to predict the pts response to the G chemotherapy of APC.

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