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Cytosolic Vascular Endothelial Growth Factor (VEGF) in Primary Breast Cancer – Correlation with Classical Risk Factors and Clinical Follow Up

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Introduction: The prognostic value of VEGF protein, known to stimulate endothelial growth and angiogenesis, was evaluated in node negative and node positive invasive ductale and lobular breast cancer. Elevated VEGF levels were correlated with established prognostic factors and clinical outcome.
Patients and methods: Cytosols of primary breast cancer tissue could be obtained of 173 patients. Patients were treated either with breast conserving surgery or modified radical mastectomy. All patients underwent axillary dissection. Systemic treatment was performed dependent on nodal status. Nitrogen stored frozen breast cancer tissue was taken to isolate cancer cell cytosols. The cytosolic VEGF leves were measured by a commercial ELISA-kit for human VEGF 121 antibody (Quantikine, R&D Systems, Oxon, UK).
Results:VEGF levels of 116 invasive ductal and 57 invasive lobular carcinomas were measured. 80/173 patients were nodal negative. Median age of all patients was 56 years. Median follow up was 28 month.
The average VEGF value of invasive ductal carcinomas was 266pg/mg proteine (median 57 pg/mg), of lobular carcinomas 99pg/mg (median 115pg/mg) proteine, respectively. This difference was significant ($p< 0,05$; student T-test). The elevated VEGF level was correlated with grading, negative hormone receptor status and histology. No correlation could be shown between VEGF and nodal status and the number of positive lymphnodes. The univariate analysis showed a significant longer disease free survival for VEGF negative carcinomas ($p<0,02$). This was best seen in lobular carciomas ($p<0,009$). The multivariate analysis however showed no prognostic influence due to elevated VEGF levels compared to grading and nodal status.
Conclusion: Elevated cytosolicVEGF levels showed no prognostic influence compared to classical risk factors in breast cancer. However high VEGF levels could demonstrate high proliferative activity in malignant tumours. Therefore it could be used as a predictive factor for specific antiangionetic therapy.

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Metronomic Anti-angiogenic Combination Therapy

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Malignant tumor progression necessitates the acquisition of an angiogenic phenotype manifested by closely orchestrated interactions between the tumor cells, stromal cells and the tumor-associated endo- thelial cells. With respect to drug resistance, the target of an anti-angiogenic would be a genetically stable, albeit activated, endothelial cell, which is less likely to develop drug resistance. We have demon-strated in the past, that using a low-dose, 'metronomic' administration of chemotherapy preferentially targets tumor vasculature, and can be enhanced by specific anti-angiogenic agent, such as a monoclonal anti-body against directed the type II receptor for VEGF. Before any clinical trials can be approached, the combination manifesting the highest therapeutic index needs to be established. To address this question, we established chemo-resistant tumor xenografts and treated them with the exact drug to which they were resistant. Continuous low-dose "metronomic" regimens with Taxol, vinblastine, cyclophosphamide, adriamycin and cisplatinum were compared. Monotherapy with vinblastine and taxol, retarded tumor growth, but cisplatinum and adriamycin alone in fact induced the growth. All tested agents, when used in combination with a monoclonal antibody against VEGFR2 (DC101), produced tumor regressions over and above those that would be produced by addition. Clear patterns emerged with regard to the therapeutic index of the different combinations. Adriamycin/DC101 combination, produced a significant regression of tumor growth, but did so with significantly more host toxicity than the Vinblastine/ DC101 synergistic combination. We propose a combination of a tubulin inhibitor and VEGFR2 inhibitor may be the optimal combination for this therapy.

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Enhanced Antitumor Activity of Anti-VEGF Antibody HuMV833 in Combination with Gemcitabine in Human Pancreatic and Renal Tumor Xenografts.

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We have previously shown synergistic antitumor activity of the anti-VEGF mAb HuMV833 in combination with gemcitabine in human pancreatic tumor xenografts, dependent on their VEGF levels. The present study was aimed to confirm these results in a high VEGF expressing and massively vascularized human renal cancer model. We further investigated whether changes in the administration schedule could improve the activity of gemcitabine/HuMV833 against *the pancreas carcinoma PAXF 546 and the RXF 944XL renal tumor,* transplanted subcutaneously into nude mice. Gemcitabine 300 mg/kg was given i.v. on days 1,8,15 and HuMV833 i.p. at 100 µg/mouse every 4 days (days 1,5,9, etc.). These schedules were also used in the combination starting simultaneously or sequentially with a one-day interval. Gemcitabine alone caused growth inhibition in the renal cancer RXF 944LX (T/C = 19%), HuMV833 alone was inactive (T/C 63%). The combination, however, was synergistic (T/C 4%) and led to regressions. Sequential administration of both compounds showed similar results (T/C 4-8%). A comparable pattern was seen in the pancreas carcinoma PAXF 546. Our data suggest that gemcitabine can enhance the effects of anti-angiogenic therapies. Furthermore, the outcome of this study supports clinical trials in pancreatic and renal cell cancers with HuMV833/gemcitabine combinations and indicates that *the* latter could be beneficial for patients with high levels of tumor VEGF.

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Cytotoxic Activity of Recombinant bFGF-rViscumin Fusion Proteins

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For the first time a fusion protein containing the rViscumin A-chain (rMLA), representing the toxic component of rViscumin (mistletoe lectin), is described. As a specific targeting domain the mitogen basic fibroblast growth factor (bFGF) was fused to the 5´-end of rViscumin A-chain. The bFGF-rViscuminA construct was expressed in *E. coli*, purified and functionally characterized. bFGF-rViscuminA is cytotoxic for mouse B16 melanoma cells expressing the FGF receptor with an IC_{50}-value of approximately 1 nM. rViscuminA shows no significant effect on the viability of the B16 cells up to a concentration of 141 nM. In order to assess the modulating function of rViscuminB during cellular processing, bFGF-rViscuminA was associated with the rViscuminB-chain in an *in vitro* folding procedure. The IC_{50}-value of bFGF-rViscumin to B16 cells in presence of lactose - to block rViscuminB carbohydrate binding activity - was 134 pM. Thus, it was possible to enhance the efficacy of a rViscumin A-chain mitotoxin through addition of the B-chain by a factor of 8. We conclude that rViscumin fusion proteins may be generally applicable for the receptor-specific inactivation of target cells and point out their potential in anti-cancer drug development.