

mean value increased to 14,10 pg/ml (SD: 14,10) ($p=0.007$). Moreover, IL-6 decreased to 10,78 pg/ml (SD: 12,38) after 48 hours ($p=0.263$) and persisted stable (14,97 pg/ml; SD: 14,86) after 1 week ($p=0.362$). IL-8 levels did not show any statistically significant modification from the basal values after P administration. Statistical analysis showed a significant negative correlation between VEGF values and gamma-IFN values ($p=0.016$) and a significant positive correlation between VEGF and IL-8 ($p=0.040$).

Conclusions: Our data show that P has a powerful antiangiogenic effect mediated by a statistically significant increase in VEGF levels persisting also after one week from the administration of the drug.

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POSTER DISCUSSION

The expression of vascular endothelial growth factor(VEGF) is a highly significant prognostic factor in stage IB carcinoma of the cervix

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Purpose: The aim of this study was to clarify the role of VEGF expression as an independent prognostic factor and to identify the patients at high risk for poor prognosis in stage IB cervical cancer.

Methods: A total of 118 patients with stage IB cervical cancer who had radical hysterectomy and pelvic lymph node dissection were included in the study. All known high risk factors of the patients were pathologically confirmed from the surgical specimen. Of the 118 patients, 88 patients were treated with postoperative radiotherapy and/or chemotherapy. VEGF expression was examined using immunohistochemistry in formalin-fixed, paraffin-embedded specimens of post-hysterectomy surgical materials. A semiquantitative analysis was made using a scoring system of 0, 1, 2, and 3 for increasing intensity of stain. We classified the patients with scores from 0 to 2 as low staining intensity and the patients with a score of 3 as high staining intensity.

Results: Of the 118 patients, 35 patients (30%) showed high staining intensity (3) of VEGF. Strong correlations were found between the high staining intensity of VEGF and both deep stromal invasion ($p=0.009$) and the positive pelvic node ($p=0.024$). The 5-year overall and disease-free survival rates for all 118 patients were 94.6% and 92%. The 5-year overall ($p=0.01$) and disease-free survival ($p=0.0014$) rates were 97.1% and 98.6% for low intensity (0, 1, and 2) of VEGF and 90.7% and 81.7% for high intensity of VEGF, respectively. Pelvic and distant failures for low versus high intensity of VEGF were 1.2% versus 17.1%, ($p=0.003$) and 0% versus 14.3% ($p=0.002$), respectively. In a Cox multivariate analysis of survival, the high staining intensity of VEGF ($p=0.008$) and the positive pelvic node ($p=0.013$) were significant prognostic factors for overall survival. The high staining intensity of VEGF ($p=0.013$), vascular invasion ($p=0.02$), and bulky mass ($p=0.034$) demonstrated as significant prognostic indicators for disease free survival.

Conclusion: These results showed that the intensity of VEGF expression was a highly significant predictor for pelvic and distant failure and the most significant prognostic factor of overall and disease free survival for the patients with stage IB cervix cancer treated with radical surgery. We strongly suggest that the immunohistochemistry for VEGF expression be performed in a routine clinical setting in order to identify patients at high risk for poor prognosis in cervical cancer.

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POSTER DISCUSSION

Inhibition of neuroblastoma-induced angiogenesis by fenretinide

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Purpose: Retinoids participate in the control of cell proliferation, differentiation and foetal development. The synthetic retinoid fenretinide (HPR) inhibits carcinogenesis in various animal models and it has been suggested that retinoids are effective inhibitors of angiogenesis.

Methods: The effects of HPR on certain endothelial cell (EC) functions were investigated in vitro, and in vivo, by using the chorioallantoic membrane (CAM) assay.

Results: HPR inhibited VEGF- and FGF-2-induced EC proliferation without affecting endothelial motility; moreover, it inhibited growth factors-induced angiogenesis in the CAM assay. A significant anti-angiogenic potential of HPR has been observed also in neuroblastoma (NB) biopsies induced angiogenesis in vivo. We previously demonstrated that supernatants derived from NB cell lines stimulated EC proliferation. Here, we show that this effect is abolished when NB cells were incubated in the presence of HPR. VEGF- and FGF-2 specific ELISA assays, performed on both NB-cells derived conditioned medium and cellular extracts, indicated no effect of HPR on the level of these angiogenic cytokines. Moreover, RT-PCR analysis of VEGF and FGF-2 gene expression confirmed the above lack of effect. HPR was also able to significantly repress the spontaneous growth of EC, requiring at least 48-72 h of treatment with HPR, following by a progressive accumulation of cells in G1 at subsequent time points. Finally, immunohistochemistry experiments performed in the CAM assay demonstrated that endothelial staining of both VEGF receptor-2 and FGF-2 receptor-2 was reduced after implantation of HPR-loaded sponges, as compared to control CAM's.

Conclusion: These data suggest that HPR exerts its antiangiogenic activity through both a direct effect on EC proliferative activity and an inhibitory effect on the responsivity of the EC to the proliferative stimuli mediated by angiogenic growth factors.

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POSTER DISCUSSION

Pancreatic tumor growth is regulated by the balance of positive and negative modulators of angiogenesis

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There is increasing evidence for the implication of tumor-derived angiogenic and anti-angiogenic factors in tumor growth in vivo. In this study we examined how changes in the balance between these factors regulate the growth of a tumor in a pancreatic cancer model in vivo. The pancreatic cancer cell line Hs-776T (HS-W) displays slow growing tumors and we have isolated a natural occurring variant (HS-R), which grew tumors more rapidly. In vitro, HS-W and HS-R produce low amounts of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) but only HS-W cells produce endostatin. In order to examine the effect of over-expression of angiogenic factors on pancreatic tumor growth in vivo, we have transfected HS-W cells with human VEGF165 cDNA. Upon injection of VEGF overexpressing cells into immune deficient mice, rapidly growing tumors were observed. In contrast, mock transfected cells, HS-PCR, formed small tumors similar to parental cells. Tumors of VEGF-transfected clones were highly vascularized with many dilated blood vessels, lined by a single layer of CD31-positive endothelial cells. PCNA staining showed high proliferation index in VEGF producing tumors and conversely, apoptosis, as determined by TUNEL staining, was higher in HS-W, moderate in HS-R and low in VEGF producing tumors. Collectively, our study confirms that tumor growth is dependent on its ability to increase the angiogenic stimulus or to reduce the amounts of endogenous antiangiogenic factors.

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POSTER DISCUSSION

Increased angiogenesis in bone metastases of patients with metastatic breast cancer

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Introduction: In a great proportion of patients with metastatic breast cancer, the disease metastasizes to the bone causing pain, hemopoietic insufficiency and a reduction in the quality of life. However, the mechanisms that contribute to bone metastasis are poorly understood. Therefore, the aim of this study is to clarify the mechanisms of tumor-endothelial-cell-interaction and to evaluate the role of angiogenesis for the development of bone metastases in patients with breast cancer.

Methods: Specimens from patients with metastatic breast cancer and non-malignant bone tissue were studied. Paraffin embedded sections were stained with hematoxylin and eosin and examined for the presence or absence of malignant tissue. Furthermore, an indirect immunoperoxidase staining with an monoclonal antibody against the CD31 epitope was used to identify vascular endothelium. Microvessel density (MVD) was counted in hot spots as well as in representative areas of the adherent section.

Results: To date 27 bone metastases and 26 non-malignant bone tissue specimens have been examined. The number of microvessels in bone