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Pretreatment vascular endothelial growth factor (VEGF) and matrix metalloproteinase-9 (MMP-9) serum levels in patients with metastatic non-small cell lung cancer (NSCLC)☆

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Summary
Purpose: In the present study, we investigated the prognostic value of vascular endothelial growth factor (VEGF) and matrix metalloproteinase (MMP)-9 serum levels in patients with metastatic non-small cell lung cancer (NSCLC).
Patients and methods: From September 1999 to June 2001, pretreatment serum levels of VEGF and MMP-9 were analysed in 194 patients of a randomized phase III trial with enzyme-linked immunoassays.
Results: Patients with a VEGF serum level higher than the median serum level (10,995 pg/ml) had a significantly shorter overall survival than those with a lower serum level ($P = 0.04$). The MMP-9 serum level did not correlate with survival. In a multivariate Cox regression analysis, only the pretreatment serum level of VEGF, the Karnofsky performance status, and the presence of bone metastases were identified as independent prognostic factors.
Conclusions: The pretreatment VEGF serum level was identified as independent prognostic factor in this study and may help to assess individual risk and treatment profiles in patients with metastatic NSCLC.

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1. Introduction

In Western Europe and in the United States, lung cancer is the most frequent cause of death among malignant diseases. More than one million new cases of lung cancer occur worldwide every year [1]. Non-small cell lung cancer (NSCLC) accounts for more than 75% of pulmonary carcinomas. Treatment of patients with NSCLC is a particular challenge in oncology, because more than one third of patients have distant metastases at diagnosis [2], allowing only palliative treatment. For patients with metastatic NSCLC, only a few prognostic factors, like performance status and gender [3,4], exist. The aim of the current research is to find new molecular prognostic factors to assess individual risk profiles as well as to identify new targets for anticancer treatment strategies.

Angiogenesis represents the formation of new blood vessels from existing vasculature. Neovascularisation is a requirement for growth of solid tumours beyond 1—2 mm in diameter [5,6]. The angiogenic process is a balance between stimulatory and inhibitory factors. The pro-angiogenic stimuli may be released by the tumour, stromal and inflammatory cells, by the extracellular matrix, or by the endothelial cells themselves. Tumour cells secrete or induce the release of growth factors which stimulate migration and proliferation of endothelial cells. Furthermore, these factors may be involved in capillary morphogenesis or release of proteolytic enzymes [7—9].

Vascular endothelial growth factor (VEGF) and matrix metalloproteinase-9 (MMP-9) are two of the most potent factors involved in angiogenesis.

VEGF is the most potent and specific growth factor (e.g. proliferation and migration) for endothelial cells and also increases vascular permeability. Until now, six different mRNA spliceforms of VEGF (VEGF 121, 145, 165, 183, 189, 206) have been discovered [10]. Of these, VEGF 121 and 165 are soluble, whereas the other isoforms are almost exclusively tissue-associated [10]. All isoforms of VEGF are capable of binding to the receptor tyrosine kinases Flt-1 and KDR/Flk-1 [11,12]. High levels of expression of VEGF are found in many solid tumour types [13—15].

The matrix metalloproteinases (MMPs) belong to a family of zinc-dependent neutral endopeptidases. Under physiological conditions, they are capable of degrading extracellular matrix and basement membrane components. Increased MMP activity has been implicated in tumour invasion and formation of metastases. Although more than 20 members of the MMP family have been described [16], the gelatinases MMP-9, formerly known as gelatinase B or 92 kDa type IV collagenase, and MMP-2 have been detected most consistently in malignant tissues and were associated with tumour aggressiveness and metastatic potential [17,18].

The aim of the present study was to analyse the association of pretreatment serum levels of VEGF and MMP-9 with clinicopathologic parameters and outcome, and to evaluate their prognostic relevance in patients with metastatic NSCLC.

2. Patients and methods

2.1. Patient selection

Twenty milliliters venous blood was taken before chemotherapy from 200 non-fasting patients, which was subsequently spun at 3400 rpm for 7 min at 4 °C. The supernatant was transfered into microtubes and stored at −70 °C until use.

Serum samples were analysed for VEGF and MMP-9 with the Human VEGF Immunoassay Quantikine™ (R&D Systems Inc., Minneapolis, MN, USA) and Human MMP-9 (total) Immunoassay Quantikine™ (R&D Systems Inc., Minneapolis, MN, USA). The principle of these assays employs a quantitative sandwich enzyme immunoassay technique. The minimum detectable concentration of VEGF is less than 9.0 pg/ml and of MMP-9 less than 0.156 ng/ml. Each serum sample was analysed twice. VEGF and MMP-9 concentrations were calculated using Delta SOFT 3 computer software (Bio Metallics Inc., Princeton, NJ, USA).

The 200 patients were a non-selected subset of 300 patients who were treated in a randomized phase III trial comparing gemcitabine plus vinorelbine (GV) and gemcitabine plus vinorelbine plus cisplatin (GVP) [19]. All patients gave written informed consent prior to entering this study. The study was approved by the local ethic committee and by the protocol committee of the German Cancer Society.

Response was determined in comparison to baseline assessment after each two cycles of treatment according to the criteria of the WHO for the indicator lesions. In the event of response or stable disease, response assessment was repeated 4 weeks later in order to confirm response or stable disease, respectively.

2.2. Treatment schedule

All patients received gemcitabine 1000 mg/m^2 and vinorelbine 25 mg/m^2 on days 1 and 8 every 3 weeks. Gemcitabine was given as a 30-min infusion followed 1 h later by vinorelbine (15-min infusion).