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Angiogenic and growth factors in gastric cancer

Susanne Blank, MD,[a,*] Catrin Deck, MD,[a] Lena Dreikhausen, MD,[a] Wilko Weichert, MD,[b] Natalia Giese, PhD,[a] Christine Falk, PhD,[c] Thomas Schmidt, MD, PhD,[a,1] and Katja Ott, MD[a,1]

[a] Department of Surgery, University Hospital of Heidelberg, Heidelberg, Germany
[b] Institute of Pathology, University of Heidelberg, Heidelberg, Germany
[c] Institute of Transplant Immunology, IFB-Tx, Hannover Medical School, Hannover, Germany

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ABSTRACT

Background: Antiangiogenic treatment is at the horizon in the palliative treatment of gastric cancer (GC), but data on proangiogenic biomarkers are still limited. The aim of this study was to analyze five proteins with a function in tumor angiogenesis: vascular endothelial growth factor (VEGF), angiopoietin-2 (Ang-2), follistatin, leptin, and platelet endothelial cell adhesion molecule 1 (CD31) in peripheral blood and corresponding tumor tissue.

Material and methods: From 2008–2010, tumor tissue ($n = 76$) and corresponding preoperative serum ($n = 69$) of patients with localized GC were collected; 45 had perioperative chemotherapy. Protein serum or tumor lysate levels of these factors were measured by an angiogenesis multiplex immunoassay and correlated with response and survival.

Results: Serum Ang-2 had prognostic relevance in the whole study population ($P = 0.027$). In subgroup analysis, serum VEGF and Ang-2 had prognostic relevance in primarily resected patients ($P = 0.028$; $P = 0.048$) but no association was found in neoadjuvantly treated patients. Follistatin concentration in the tumor tissue was associated with prognosis in all patients ($P = 0.019$). Tumor VEGF concentrations were correlated with histopathologic response ($P = 0.011$), with patients showing >50% remaining tumor having higher VEGF concentrations. The tissue Ang-2/VEGF ratio was significantly correlated with both clinical and histopathologic response ($P = 0.029$, $P = 0.009$). Additionally, the level of leptin in the tissue was associated with clinical response: nonresponding patients had higher leptin levels than those of responding patients ($P = 0.032$).

Conclusions: Our results show the importance of angiogenetic factors in serum and tumor tissue in GC for prognosis and treatment response. Further trials in larger patient populations are warranted for a further evaluation of proangiogenetic factors as biomarkers in gastrointestinal cancer.

1. Introduction

Angiogenesis plays a fundamental role in tumor growth and is regulated by proangiogenetic and antiangiogenetic factors [1]. Tumor angiogenesis is involved in malignant transformation [2], the invasion of tumor cells into the circulation, and the switch of dormant tumor cells to metastatic lesions [3]. Over the last two decades, angiogenesis research has developed from a

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* *Corresponding author*. Department of Surgery, University of Heidelberg, Im Neuenheimer Feld 110, Heidelberg 69120, Germany. Tel.: +49 62215635758; fax: +496221565405.
E-mail address: susanne.blank@med.uni-heidelberg.de (S. Blank).

[1] Contributed equally.

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preclinical stage to clinical applications in tumor patients, also in gastric cancer (GC). The addition of bevacizumab, which inhibits vascular endothelial growth factor A (VEGF-A, from here on termed VEGF), to cisplatinum-containing chemotherapy has shown some efficacy in metastatic GC [4]. However, the combination of bevacizumab with chemotherapy as first-line therapy in a randomized controlled trial (Avastin in Gastric Cancer [AVAGAST]) failed to increase overall survival [5]. Recently ramucirumab, a monoclonal antibody directed against vascular endothelial growth factor receptor 2, was shown to be the first antiangiogenetic drug that prolongs survival in patients with advanced GC or adenocarcinoma of the esophagogastric junction (AEG) who had failed or progressed after first-line therapy [6]. The AVAGAST trial was accompanied by a large prospective biomarker analysis including tumor tissue and serum. In the group of patients additionally treated with bevacizumab, both high serum VEGF-A and low tumor neuropilin-1 expression showed a trend toward improved survival; however, no final conclusion could be drawn from these studies [7]. Another targeted drug studied in combination with chemotherapy in GC is AMG 386, a protein that inhibits angiogenesis by neutralizing the interaction of angiopoietin (Ang)-1 and 2 with the Tie2 receptor [8]. AMG 386 showed promising antitumor activity and no interactions with the chemotherapy applied in phase I studies in advanced solid tumors [9]. In a randomized phase II study in advanced GC, addition of AMG 386 to chemotherapy did not lead to increased survival or overall response rate compared with placebo [10]. These inconsistent findings highlight the complexity of tumor angiogenesis and the importance of determining tumor or patient characteristics when antiangiogenic treatment could be beneficial.

Since the publication of two large randomized trials [11,12], perioperative chemotherapy is a standard of care in Europe and beyond for resectable AEG II/III and GC. The actual response rates range from 25%–50% [13] based on the regimen applied. However, an increase of the response rate, potentially by the inclusion of biologicals, would be of highest interest. The ongoing MAGIC II trial compares the addition of bevacizumab with that of perioperative chemotherapy. Safety results have already been published, showing no increased toxicity and complication rate in the bevacizumab arm in GC [14].

We examined prospectively a panel of proteins including several angiogenetic factors such as VEGF [7,15–17], Ang-2 [10,18–22], follistatin [23,24], the metabolite leptin [25–29], and the endothelial marker platelet endothelial cell adhesion molecule (PECAM)-1 (CD31) [30,31], which were determined in tumor tissue and preoperative serum. New molecular data on the analyzed angiogenetic protein panel may provide an important basis for future stratification in therapeutic trials with antiangiogenetic therapeutic regimens.

The aim of our prospective study was to test the association of the angiogenetic factors with established clinicopathologic parameters, response and prognosis of patients with GC in serum, and corresponding tumor tissue with and without preoperative treatment.

2. Patients and methods

We included 76 patients with histopathologically proven adenocarcinoma of the stomach ($n = 45$) or the gastroesophageal junction (AEG II, III) ($n = 31$) who were treated at the Surgical Department, University of Heidelberg, Germany, from 2008–2010. Written informed consent was obtained from all patients. The study protocol was approved by the Ethical Committee of the University of Heidelberg (Ethical Committee's approval: tumor tissue 301/2001 and serum 150/2002). Forty-five patients were treated with neoadjuvant chemotherapy followed by resection, 31 patients were primarily resected. Patients were treated according to the actual German S3-guidelines. Preoperative staging included a computed tomography (CT) scan and endoscopy in all patients. Clinical response was defined as a major reduction of tumor size in endoscopy and a decrease in wall thickness of >50% in CT scan [32]. The chemotherapy regimen was chosen by the treating oncologist and performed on an outpatient's basis in most of the patients. Most patients were treated with epirubicin-oxaliplatin-capecitabine-regimen but some other regimens (cisplatin-5-FU or oxaliplatin-5FU-docetaxel) were also used [33,34].

For patients with carcinoma of the esophagogastric junction, we performed a transhiatal extended gastrectomy, whereas for patients with GC a subtotal or total gastrectomy in respect of the necessary safety margins were done. A D-2 lymphadenectomy was performed in all patients [33,34].

Histopathologic work-up was done by pathologists experienced in upper gastrointestinal-cancer. TNM staging was done according to the TNM classification seventh edition [35]. We adapted all TNM stages before 2010 to the seventh edition for better comparability. Histopathologic staging includes TNM classification, R-category, and tumor regression grade (TRG). As TRG we used the Becker regression score as follows: regression grade 1a indicating complete regression (no residual tumor), 1b—subtotal regression (<10% residual tumor), 2—minor regression (10%–50% residual tumor), and 3—no regression (>50% residual tumor). Following Becker criteria, regression grades 1a and 1b are summarized as histopathologic response, regression grades 2 and 3 as nonresponse [36,37]. For analysis, we also divided patients in two groups with more or less than 50% residual tumor.

Follow-up was done on an outpatient basis in the National Center for Tumor Diseases, Heidelberg, including CT scan and endoscopy. If patients were investigated in other centers or at the local physician they contacted by phone to obtain follow-up data. Median follow-up of the surviving patients was 37.6 mo, two patients were lost to follow-up because of removal abroad (2.6%).

2.1. *Blood and tissue sampling and preparation*

Blood was collected in serum tubes the day before tumor resection. Blood samples were taken by peripheral vein puncture or from a central venous catheter. In case of blood samples from the central venous catheter, the first 5 mL was rejected to avoid dilution by blocking saline. The serum tubes were centrifugated at 2.500*g* for 10 min to extract serum. Serum was stored at −20°C before analysis. Serum was diluted in a ratio of 1:4 with a serum diluent just before analysis. Tissue sampling was done directly after tumor resection. The tissue was stored at −80°C until preparation of protein lysates. For analysis, tissue was cut in slices of 10 μm using a