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The evaluation of circulating endothelial progenitor cells and related angiogenic markers as prognostic factors in soft-tissue tumors

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ABSTRACT

Introduction: Neovascularisation is a critical step in the progression of malignant tumors. Circulating endothelial progenitor cells (cEPC) have been proposed as surrogate markers of vasculogenesis in malignancies. In this project, we studied the impact of tumor-specific therapy on cEPC and associated angiogenic factors in patients with soft tissue tumors.

Materials and methods: Fifty-three patients with soft tissue tumors (25 soft tissue sarcomas, 19 GIST, 9 desmoids) and 15 healthy controls were included. Blood samples were obtained at two time points, before and 8 weeks after start of tumor-specific therapy. Peripheral blood mononuclear cells (PBMCs) were isolated. cEPCs were characterised as $CD34^{+}$, $CD133^{+}$, $CD45^{dim}$, $CD31^{+}$ and vascular endothelial growth factor 2 (VEGFR-2) positive cells. Serum concentrations of VEGF-A and angiopoetin-2 were determined by enzyme-linked immunosorbent assay.

Results: VEGF-A and Ang-2 concentrations were significantly higher in tumor patients than in healthy controls in both samples ($p < .01$). Sarcoma patients with progressive disease developed a significant increase in cEPC levels between the two blood samples compared to those with stable disease ($p = .002$). GIST patients with progressive tumor or metastatic disease showed significant increase in VEGF-A values ($p = .01$).

Discussion: The pre-treatment values of the angiogenic markers did not correlate with the clinical course of the disease. However, cEPCs levels were significantly higher in sarcoma patients with progressive disease compared to those with stable disease and should be further evaluated as early markers of disease progression in sarcoma patients. VEGF-A and angiopoetin-2 clearly play a role as mediators of the vasculogenesis contributing to tumor progression.

Introduction

Tumor vascularisation and growth depends on the proliferation of existing and sprouting of new vessels through angiogenesis or vasculogenesis. Angiogenesis describes the proliferation of existing vessels. Vasculogenesis refers to the formation of new vessels. It requires bone marrow derived circulating endothelial progenitor cells (cEPCs) being recruited from the angiogenic system to circulate to the foci of tumor progression and differentiate into mature endothelial cells to form the new vessels [1–3].

It has been postulated that cEPCs blood levels are elevated in various conditions including malignant tumors and have been proposed as a possible tumor marker [2,4,5]. Furthermore, cEPCs have been correlated with higher mitotic rates, rapid tumor growth and clinical progression in pancreatic, breast and lung cancer [5–7].

Vascular endothelial growth factor (VEGF-A) is an angiogenic factor closely related to the mobilisation of the endothelial progenitors from the bone marrow. The angiogenetic features of

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VEGF-A have been widely associated with tumor progression in different tumor types. However, the available data concerning mesenchymal tumors are scarce and the interactions with angiopoetin-2 (Ang-2), a further angiogenetic factor upregulated by the secretion of VEGF-A, have not been previously investigated in soft tissue tumors [8].

Soft tissue sarcomas and gastrointestinal stroma tumors (GIST) are highly vascularised tumors of mesenchymal origin. The incidence of malignant soft tissue sarcoma is roughly 6 cases per 100.000 population/year [9]. The standard therapy for soft tissue sarcomas comprises of a wide surgical resection as well as adjuvant irradiation [10]. Although some patients with large high-grade tumors seem to benefit from systemic treatment, adjuvant chemotherapy is not the standard treatment since randomised trials did not show an improved survival rate and the selection of patients who will benefit from chemotherapy is not possible based on current selection criteria [11]. Experimental and clinical trials demonstrate some anti-tumor efficacy especially when combined with other treatment modalities [12].

GISTs (gastrointestinal stromal tumors) are sarcomas of the gastrointestinal tract. Most of them harbour a specific driver mutation in the c-kit or PDGFR gene and can be treated with receptor tyrosine kinase inhibitors (RTKI) if the tumor is locally advanced or metastasized [13]. RTKI can provide tumor control but tumor recurrence frequently occurs after the cessation of treatment or development of therapy resistance.

Sporadic desmoid tumors do not metastasize but may develop local infiltrative growth patterns. Treatment options include surgery, irradiation, chemotherapy, antihormonal therapy as well as treatment with RTKI in selected cases.

We initiated this exploratory study to determine whether cEPCs, Ang-2 and VEGF-A blood levels are elevated in patients with highly vascularized soft tissue tumors and if the levels of those markers would correlate with the course of disease.

Materials & methods

Study design

The study was designed as an exploratory analysis. The main objective was to evaluate if clinical course and tumor load are associated with absolute values or dynamic changes in VEGF-A, Ang-2 and cEPCs during treatment. In order to determine the clinical course of disease, we performed a clinical/radiological follow-up one year after the primary recruitment.

Patients

Sixty-eight patients were enrolled in the study. These comprised of 25 patients with soft tissue sarcomas and 19 patients with GIST. Nine patients with desmoid tumors and 15 healthy individuals formed the control group.

All patients signed informed consent; the study was approved by the ethics committee of the Mannheim Medical Faculty of the University of Heidelberg (2008-248N-MA).

Patient characteristics

Sarcomas

Sarcoma patients underwent surgical treatment the day after drawing the first blood sample (Table 1). Nineteen patients received an operation for their primary tumor. In one case, further abdominal masses were discovered during surgery and were treated with intraoperative radiotherapy during the same session.

Seven patients received an operation for metastatic disease with curative intention. In one case, the tumor could not be resected and the patient received a port device for chemotherapy. This patient had further metastasis that were treated with radiotherapy. For staging distribution and histological subtypes, see Table 1.

At the time of the second blood sampling, five patients were receiving chemotherapy, one was under treatment with RTKI and one was receiving adjuvant radiotherapy. Three patients had undergone an R1 resection but were not receiving any specific tumor therapy at the time of the second blood draw. One patient died in the perioperative period before the second blood sampling.

Gastrointestinal stromal tumors

Eleven patients had a localized primary tumor and eight patients had metastatic disease. All eleven patients with primary disease were operated on the day after the first blood sampling; two of them received an additional treatment with imatinib. Of the eight patients with metastatic disease, five received surgical treatment for solitary metastasis (liver), two patients were treated with imatinib and one with sorafenib.

Six GIST patients had a low-risk GIST, two had an intermediate-risk GIST and eleven had a high-risk GIST (Table 2). For twelve patients, the primary tumor was localized in the stomach, for six other patients the small bowel, and one patient had an unknown primary presenting with metastatic disease.

Desmoid tumors

Three of the patients with desmoid tumors received surgical treatment on the day after the first blood sampling (Table 2). The other six patients did not receive an operation. Two of the patients were receiving imatinib and continued this treatment throughout the study period. In another two patients, we initiated treatment with imatinib. The latter patients had small, stable desmoid tumors that did not require tumor-specific therapy during the study period. At the time of the second blood sampling, four patients were under treatment with RTKI.

Healthy individuals

In this group, we included patients who had received an operation for non-malignant diseases such as hernias, as well as healthy colleagues from our laboratory staff.

Blood sampling

We performed two blood samplings in every patient and healthy control (9 mL EDTA-blood and 7,5 mL lithium-heparin blood both times). The first blood sample was obtained just before the beginning of therapeutic procedures and the second roughly eight weeks after the first one. Blood was obtained by intravenous insertion of a 20-gauge cannula. In order to test the variability of our laboratory findings, a second blood sample was obtained from the control group at a random point in time. Levels of circulating endothelial progenitor cells, vascular endothelial growth factor and angiopoetin-2 were measured in every patient and control in both blood samples.

Flow cytometry

All blood samples were processed within 1 h after collection. Peripheral blood mononuclear cells (PBMCs) were prepared by density gradient centrifugation with Ficoll-Hypaque (GE Healthcare Bio-Science AB, Sweden). The expression of cell-surface antigens was determined by immunofluorescence staining. Briefly, 100 μl of PBMC (containing 1 × 106 cells) were incubated with 20 μl of Fc receptor-blocking reagent (Miltenyi Biotec, Bergisch-Gladbach,

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