Expression of Angiogenic Proteins in Tumor and Stroma Affects Survival in Patients With Gastric Cancer

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Highlights

- Laser Capture Microdissection (LCM) enables precise separation of tissue.
- In 29 human gastric cancer samples, tumor and stroma were separated by LCM.
- VEGF-A, VEGF-D and SCF-1 are significantly higher expressed in the tumor.
- High stromal VEGF-A and SCF-1 are correlated with shorter overall survival.
- These cytokines could help selecting treatment options for gastric cancer patients.

Background

Gastric cancer is one of the most frequent malignancies worldwide. Angiogenic growth factors play a crucial role in mediating the crosstalk between cancer cells and the surrounding microenvironment. In addition to the expression of angiogenic proteins by the tumor cells, the crosstalk between tumor cells and the surrounding microenvironment has been under constant investigation over the last couple of years (1,2). Different expression levels of angiogenic cytokines and their transcripts were shown to correlate with survival in pancreatic, colorectal and esophageal cancer. The aim of this exploratory and translational study was to investigate how these cytokines differ between the tumor and the surrounding stroma and how this is correlated to the survival of patients with gastric cancer.

Materials and Methods

For this study 29 patients who underwent resection of gastric cancer in the years 2010 to 2016 were randomly selected from an institutional, prospectively maintained database at the Department of General, Visceral and Transplant Surgery at the University of Heidelberg. Tumor samples were taken in the operating room and immediately snap-frozen. Briefly, 16 µm sections were cut using a cryostat and stained with cresyl violet. The tumor and stromal compartments were separated using laser capture microdissection (Figure 1). Angiogenic protein expression was measured using a bead-based immunoassay and correlated with tumor stage and overall survival.

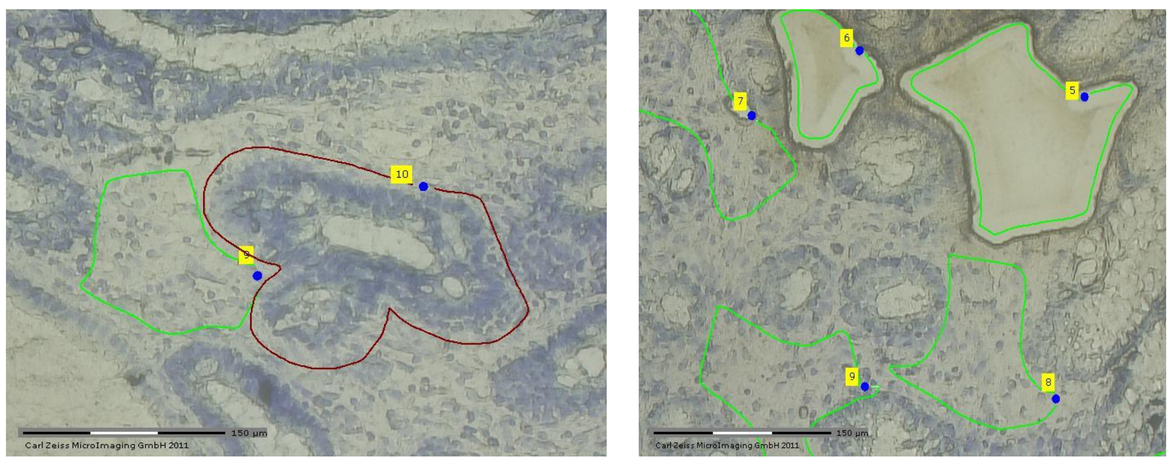


Figure 1. Representative images of gastric tumor and stroma before (left) and after (right) laser microdissection. Scale bares represent 150 µm.

Results

Comparison of the protein concentration levels between the tumor and stroma area showed significant differences for five cytokines. The concentrations of VEGF-A (p=0.003), VEGF-D (p=0.009), and SCF (p=0.04) were significantly higher in the tumor compared to the stroma while PECAM (p=0.02) and FGF (p<0.0001) concentrations were detected at a significantly higher level in the stroma compared to the tumor (Figure 2). To investigate the correlation between cytokine concentration and survival, patients were divided by the median into equal groups representing a “high“ and a “low“ population. We found that patients with a high concentration of VEGF-A in the stroma had significantly shortened overall survival (23.5 (+/- 17.6) vs. 33.6 (+/- 21.0) months; p=0.009) compared to patients with a low VEGF-A concentration. The same trend was observed for stromal VEGF-D even if it was not statistically significant. Higher concentrations in the stroma showed a trend towards a worse survival (26.8 (+/- 22.0) vs. 37.2 (+/- 19.0) months, p=0.09). The second cytokine that showed a significant correlation between high stromal concentration and shorter survival was SCF (22.2 (+/- 18.5) vs. 33.6 (+/- 21.8) months, p=0.01), (Figure 3). We did not observe any significant correlation between tumor-specific expression of angiogenic cytokines and survival.

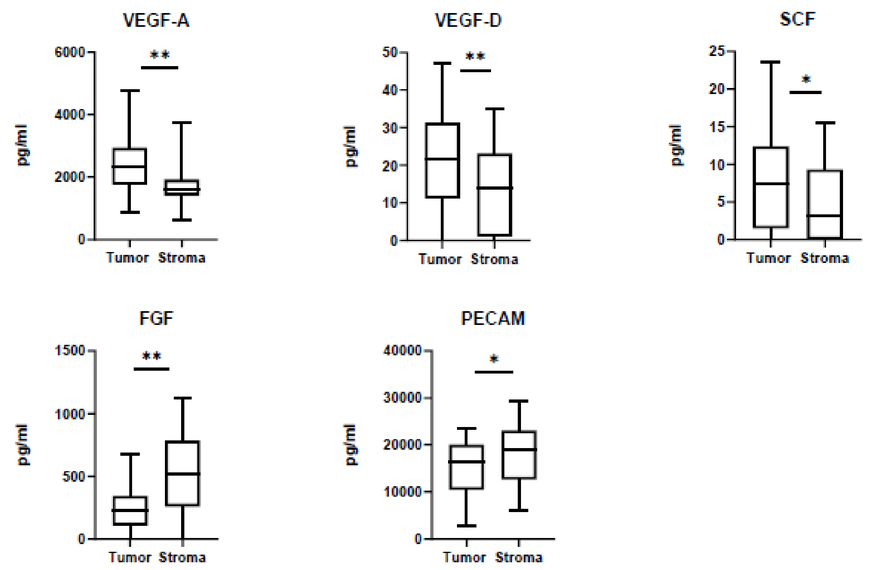
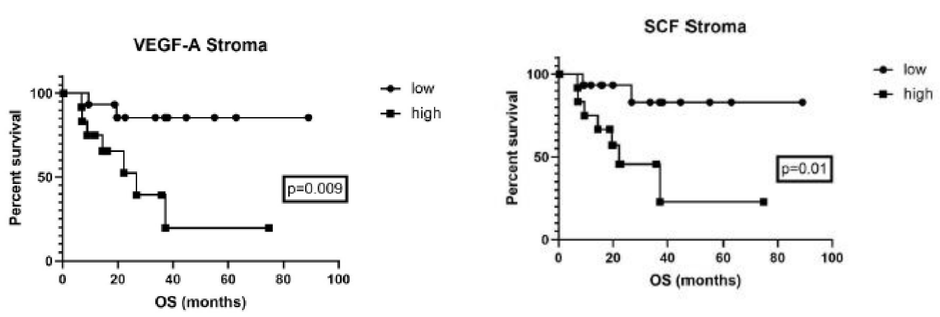


Figure 2. Box-Whisker plots showing the concentration of the indicated cytokine in the tumor and stroma compartments (p-value<0.05 is considered statistically significant).



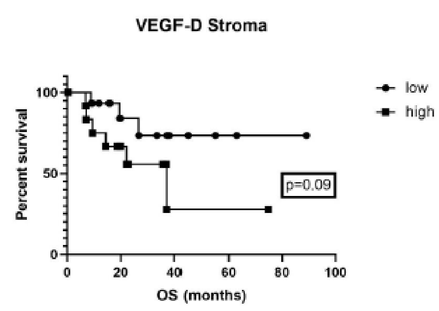


Figure 3. Overall survival (OS) in correlation with the concentration of VEGF-A, VEGF-D, and SCF in the stroma compartment of all patients (Kaplan-Meier curve,Pvalue<0.05 is considered statistically significant).

Conclusions

This translational study highlights the difference in clinical impact between tumor and stromal expression of angiogenic proteins. Correlating the concentration in tumor and stroma with clinical survival data, we found that a high concentration of VEGF-A and SCF in the stroma but not in the tumor was associated with a worse prognosis in gastric cancer patients.

Despite the interaction between tumor cells and the surrounding microenvironment has been investigated intensively in the last decades the complete crosstalk is not fully understood. Even if the total concentration of VEGF- A and SCF in the tumor compartment was significantly higher than in the microenvironment, the clinical impact of the stromal concentration seems to be more relevant.

The clinical impact of this difference as it directly relates to overall survival suggests that gastric cancer molecular profiling is complex, and more research is needed for selecting the best individual therapy options for each single patient in order to improve prognosis and survival.

(1) Lee, K., H. Hwang and K.T. Nam, Immune response and the tumor microenvironment: how they communicate to regulate gastric cancer. Gut Liver, 2014. 8(2): p. 131-9.

(2) Ishimoto, T., H. Sawayama, H. Sugihara and H. Baba, Interaction between gastric cancer stem cells and the tumor microenvironment. J Gastroenterol, 2014. 49(7): p. 1111-20.

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