ORIGINAL ARTICLE

SIMULTANEOUS IDENTIFICATION OF VESSELS AND MAST CELLS BY DOUBLE IMMUNOSTAINING IN GASTRIC MALIGNANT TUMORS

Puşa Nela Gaje[1], Raluca Ceauşu[1], Simona Sarb[1]

[1]Department of Microscopic Morphology Morphology/Histology, Angiogenesis Research Center Timisoara
"Victor Babes" University of Medicine and Pharmacy, Timisoara, Romania

ABSTRACT

Objective. To assess the microvessel and mast cell density in gastric tumors, and to evaluate the possible correlation between them. **Material and method.** Our study included 17 cases of gastric malignant tumors: adenocarcinomas (14 cases) and diffuse gastric carcinoma (3 cases). Five sections were stained with routine haematoxylin and eosin for histological diagnosis, and with double immunostaining (CD34/ tryptase) for vessels and mast cells, respectively. In all 17 cases we counted the mast cells and vessels in normal mucosa and in tumor area. A statistical analysis was performed with the commercially available SPSS15.0 and the relationship between MCD and MVD was evaluated. **Results.** In tumor area we found mast cells in both the peritumoral stroma and in the tumor zone. Mast cells were isolated or in small groups around tumor cells and blood vessels. The number of mast cells was significantly smaller than the number of vessels. A very high number of small blood vessels, with or without lumen were observed at the level of tumor invasion area. **Conclusions.** Correlation index demonstrated that there is no correlation between microvessel and mast cells density in gastric carcinoma. We consider that mast cells density decreases with depth of tumoral invasion.

Key words: gastric carcinoma, immunohistochemistry, mast cells, tryptase, CD34, MVD, MCD

INTRODUCTION

Mast cells are fixed connective tissue cells, located close to blood vessels, and characterized by the presence of secretory granules in the cytoplasm. During the last century, since mast cells were reported to be associated to malignant tumors, two theories concerning their role in tumor stroma were emitted. One of the theories supports the idea that mast cells have an important role in tumor growth, invasion and metastasis. The second one stipulates that mast cells have a cytotoxic effect on tumor cells via immunological mechanism [1].

Numerous data from the literature showed the presence of high numbers of mast cells in different malignant epithelial tumors such as: rectal carcinoma, lung adenocarcinoma and breast carcinoma [2, 3, and 4]. Tomita et al. observed a direct correlation between mast cells density and microvessels density, without association with VEGF expression in lung cancer. They concluded that mast cells play an important role in the intensify tumor angiogenesis [5]. Toth and collaborators used double immunostaining with antibodies against tryptase and VEGF in cases of malignant melanomas. They showed VEGF expression in peritumoral mast cells cytoplasm with positive expression for tryptase. This aspect suggested that mast cells from peritumoral stroma produced VEGF, an important proangiogenic factor, which favored tumor growth [6].

These aspects suggest that mast cells have an important role in tumor angiogenesis, and also in tumor growth, progression and metastasis [7, 8]. The role of mast cells in these processes is due to the proangiogenic factors released from their secretor granules: histamine, VEGF, FGF-2, TGF-β, TNF-α, s.o. Besides these mediators, mast cells also secrete proteases –tryptase, chymase- that are involved in the protease-mediated degradation of tumor extracellular matrix [9]. Tryptase activates metalloproteases and plasminogen activators which make extracellular matrix degradation possible- an important step of tumor angiogenesis. Together with heparin, tryptase stimulates migration and division of endothelial cells [10, 7].

It the stomach, mast cells were found next to the blood vessels of lamina propria, submucosa and muscularis layer [11]. The number of mast cells and their tinctorial affinity are related with the anatomical area of the stomach. [12]. Both the number of mast cells and blood vessels are increased in gastric carcinoma as compared with normal gastric tissue [13].

In the current study we have shown a simple immunohistochemical technique to identify both mast cells and blood vessels on the same sections, our purpose being to check if there is any relationship between mast cells and MVD in gastric cancer.

MATERIAL AND METHODS

Our study included 17 cases of gastric malignant tumors. The biopsies, taken during surgery from the pyloric antrum region of the stomach, were fixed in buffer formalin and paraffin embedded, according to the standard histological procedure. Sections from each case were stained with haematoxylin and eosin method, for the histological diagnosis. Subsequent slides were immunostained for blood vessel and mast cells identification. For the double immunostaining we used CD34, clone QBEnd10, dilution 1:25, and MCT, clone AA1, dilution 1:300, 30 minutes incubation.

The Envision Double stain Kit followed incubation with alkaline phosphatase. The antigen-antibody reaction was visualized using DAB and Fast Red. For nuclear counterstaining we used Lillie haematoxylin. All reagents were from Dako, Glostrup, Denmark. The entire immunohistochemical procedure was performed with a DakoCytomation Autostainer (DakoCytomation, Denmark). After mounting the slides in an aqueous medium, the results were assessed with a Nikon Eclipse E600 Microscope, and the obtained images were saved as JPEG.

The double immunostaining allowed the counting of microvessels (microvessel density, MVD) and mast cells (mast cell density, MCD) on the same section and in the same microscopic fields. Counting of mast cells and blood vessels was performed according to the procedure published by Weidner et al [14], at magnification x200. Briefly, three fields from the tumor area with maximum density of both mast cells and blood vessels were counted and the average was the final result. Additionally, it was possible to evaluate the spatial distribution of mast cells and blood vessels in the tumor area.

The statistical analysis was performed with the commercially available SPSS15.0. The relationship between MCD and MVD was evaluated, and $p<0.05$ was considered as significant.

The local research ethics committee approved the protocol of the study, and informed consent was obtained from all subjects, according to the World Medical Association Declaration of Helsinki

RESULTS

From the 17 cases of gastric tumors, 14 were diagnosed as adenocarcinomas with both tubular pattern (8 cases) and papillary pattern (6 cases). Only three cases were classified as intestinal type of diffuse gastric carcinoma. In gastric adenocarcinoma the tumor cells were arranged as tubes (cases with tubular pattern) or as papillary projections in the papillary type. In the diffuse gastric carcinoma type, the tumor cells were arranged in islands separated by small amounts of connective stroma. Atypical mitosis and nuclear pleomorphism were found. The mucosa lesions were accompanied by large areas of fibrosis in the submucosa and muscularis layer.

Tryptase-positive mast cells were identified in all specimens. Mast cells were present in both normal gastric mucosa and in the tumor areas. In areas of normal mucosa mast cells were observed in the lamina propria, located in close proximity to the blood vessels and gastric glands (figure 5). Mast cells were found not only in gastric mucosa, but also in the submucosa and muscularis layers. In adenocarcinoma with tubular pattern, in the surrounding areas of normal gastric mucosa, numerous partially degranulated mast cells were present. Around each gastric gland 2-3 partially degranulated mast cells were found. In tumoral area, we founded mast cells in both peritumoral (fig.2) and intratumoral stroma (fig.1). Mast cells were found isolated or arranged in small groups around tumor cells and blood vessels (fig.4). The number of mast cells was significantly smaller than the number of vessels with variable morphology (fig.3). A very high number of small vessels, with or without lumen, were observed at the level of tumor invasion zone in normal tissue. In both peritumor and intratumoral area we found degranulate mast cells.

In the diffuse gastric adenocarcinoma cases we found partially degranulated mast cells in the intratumoral areas.

In all cases included in this study the mast cells density was significantly higher in the peritumoral areas compared with intratumoral areas. In these areas the mast cells were grouped in the vicinity of vessels. In the peritumoral areas we identified a high number of degranulated and partially degranulated mast cells. The average number of mast cells for the diffuse gastric carcinoma cases was 54 per field, and 19 per field for adenocarcinomas. The number of mast cells was significantly lower than the number of blood vessels. Numerous vessels were noticed in the tumor invasion area. These vessels presented variable morphology and dimensions: small- with or without lumen, or large-sinuous, with lumen, branched and anastomosed. In the tumor area the blood vessels presented a great variety, irregular architecture and variable lumen size. Basically, in the tumor area we identified all vessel types described in the literature for other tumor entities.

The average number of mast cells and blood vessels in the tumor area in patients with gastric carcinoma was significantly higher than that in normal gastric tissue. The found correlation index was p=0,783. The obtained exit value showed the absence of a significant correlation between MCD and MVD.

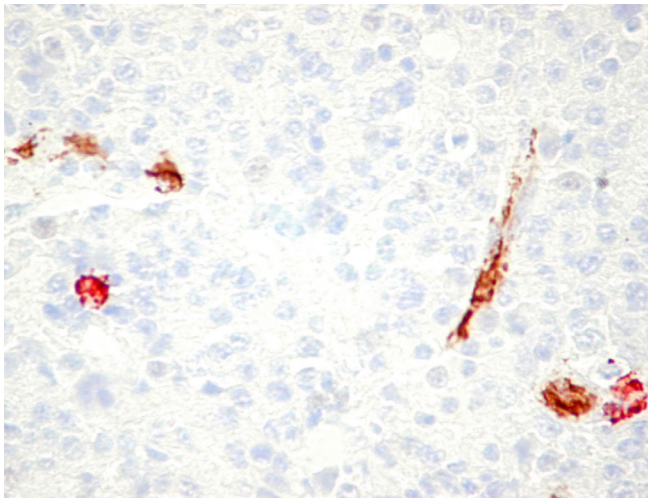

Figure 1

Intratumoral mast cells in diffuse gastric carcinoma,; double immunostaining CD 34/ mast cell tryptase, ob. 40X

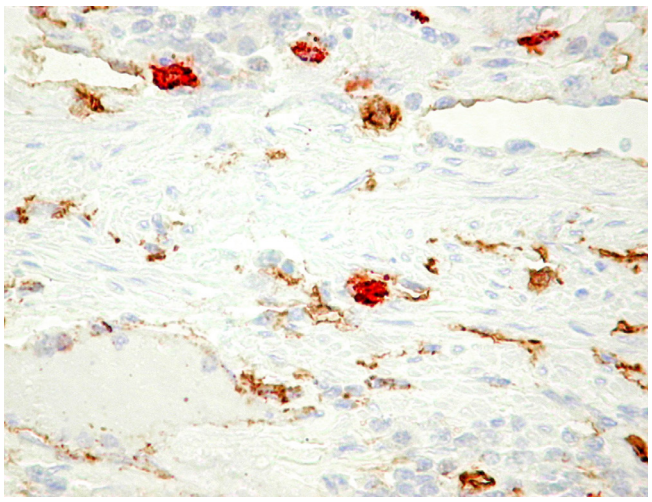

Figure 2.
Diffuse gastric carcinoma, mast cells in peritumoral stroma; double immunostaining CD 34/ mast cell tryptase, ob. 40X

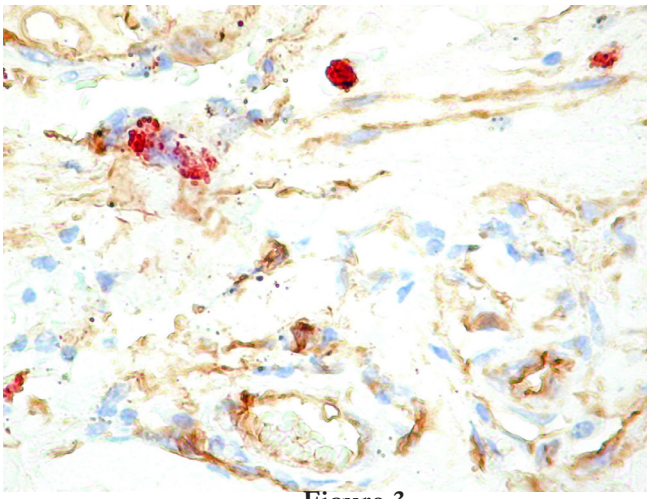

Figure 3.
Gastric adenocarcinoma with papillary pattern, mast cells in peritumoral stroma; double immunostaining CD 34/ mast cell tryptase, ob. 40X

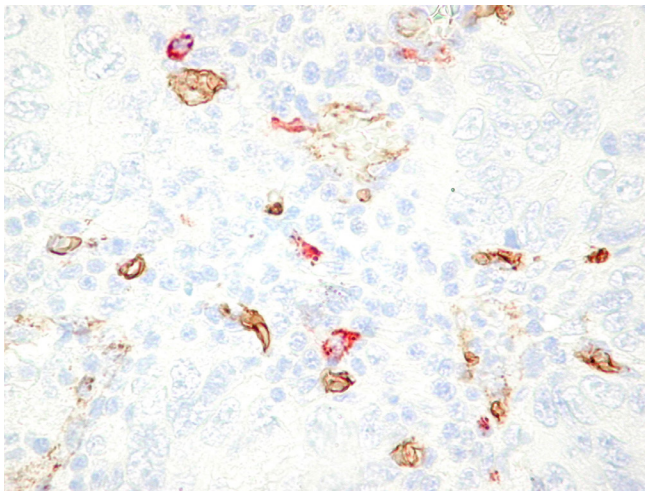

Figure 4
Gastric adenocarcinoma with papillary pattern, intratumoral mast cells; double immunostaining CD 34/ mast cell tryptase, ob. 40X

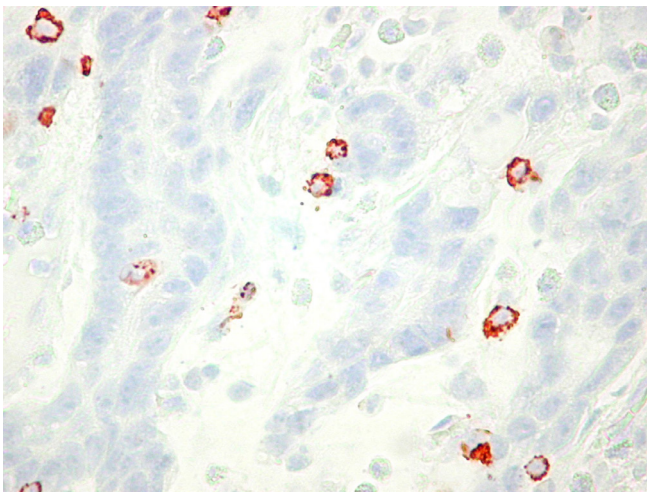

Figure 5.
Gastric adenocarcinoma with tubular pattern, mast cells in lamina propria of normal mucosa; double immunostaining CD 34/ mast cell tryptase, ob. 40X

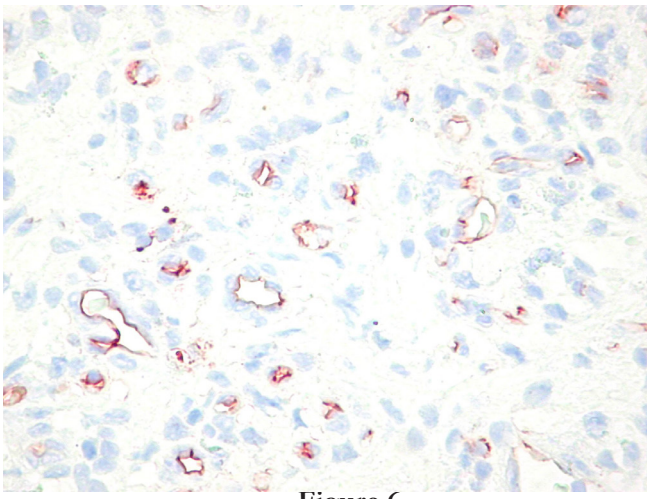

Figure 6.
Gastric adenocarcinoma with tubular pattern, intratumoral mast cells; double immunostaining CD 34/ mast cell tryptase, ob. 40X

DISCUSSIONS

Gastric cancer is an aggressive tumor, and despite significant progresses done in the field of diagnosis and treatment, the overall survival is still poor. Gastric cancer still represents a difficult problem in regard with early diagnosis, treatment, and conduct [15]. This could be due, at least in part, to the lack of data on the molecular biology of this tumor, and the missing data about the tumor stroma. This is why, in the present study, we focused the mast cells and corresponding blood vessels.

In the last decade, numerous immunohistochemical studies investigating the involvement of mast cells in tumor progression [16] and the possible correlation between mast cells proteases and tumor angiogenesis [17] were carried out. For example Ribatti and collaborators found out that there is a positive correlation between MCD, MVD and tumor grade in gastric carcinoma [8]. They found a spatial association between tryptase positive mast cells, CD31 positive blood vessels, and gastric glands. They also found that this parameter increased in

the next stages of the tumor, indicating a direct correlation between MCD, MVD and tumor stage.

Another study demonstrated that in gastric carcinoma with invasion of muscularis layer there was a correlation between mast cells, microvessels density and VEGF expression. These results suggested that both, mast cells tryptase and VEGF are important factors for tumor angiogenesis regulation [18].

In surgically treated gastric cancer patients, with both primary gastric carcinoma and regional lymph node metastase, Ammendola and collaborators [19], and Sammarco and collaborators [20], observed a significant higher density of tryptase positive mast cells in the tumor/ metastases areas. The number of mast cells correlated with vessels number, and also is higher in primary tumors in patients with lymph node metastasis and vascular invasion. These findings support the main role of tryptase as an important proangiogenic factor. Yano and collaborators correlated MVD and MCD with the prognostic, rate of metastasis in regional and from distant lymph nodes in patients with gastric carcinoma. MCD around neovessels correlated with lymph node invasion, blood and lymphatic vessels invasion. Postsurgical survival was worse atin patients with high MCD. MCD correlated with MVD, and the authors concluded that MCD can be used as prognostic marker in gastric carcinoma [21].

Joo and collaborators, found a significant correlation between MVD and depth of invasion, metastasis, rate of survival in gastric carcinoma. The rate of survival was higher in patients with smaller MVD. Also VEGF expression correlated with MVD so the authors suggested that MVD has a prognostic role in evaluating for metastasis in gastric carcinoma [22].

Despite the fact that we found a higher MVD value in the peritumoral area, our study disagrees with the above published results. We found no correlation between microvessel and mast cells density in gastric carcinoma. The different results may reside in the small number of cases included in the study, the lack of stratification of the cases, or in the large numbers of degranulated mast cells found in the peritumoral and intratumoral areas.

CONCLUSIONS

In all cases of gastric carcinoma which were examined in our study, we observed that the number of mast cells decreased at a distance from normal gastric mucosa. Also, when compared to mast cells number, the number of vessels was larger. The microvessel density was significantly higher when compared with mast cells density. The correlation index suggested that there was no correlation between microvessel and mast cells density in gastric carcinoma. We consider that mast cells density decreases with the depth of tumor invasion.

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