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Correlations Between the Density of Tryptase Positive Mast Cells (DMCT) and that of New Blood Vessels (CD105+) in Patients with Gastric Cancer

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Mast cells proteases, tryptase and chymase are directly involved in the growth and progression of solid tumors due to their important role in tumor angiogenesis. We examined the density of tryptase positive mast cells and the mean density of new blood vessels in gastric malignant tumors of patients with and without Helicobacter pylori infection, using immunohistochemical staining for tryptase (for mast cells) and CD 105 (for new vessels). Tryptase and CD 105 expression was detected in gastrectomy specimens. In this study, mast cell density correlates with angiogenesis and the growth and progression of gastric cancer. It also shows that the participation of Helicobacter pylori infection in the growth and progress of gastric neoplasia is due to an increase of peritumoral angiogenesis, with subsequent local and distant tumor spread and perivascular growth, but without perineural and nodal involvement.

Key words: gastric cancer, mast cells, tryptase, new vessel density, Helicobacter pylori infection.

INTRODUCTION

Gastric adenocarcinoma ranks fourth amongst the most prevalent cancers worldwide, being the second leading cause of cancer deaths [1]. It associates a poor prognosis due to the fact that up to three fourths of the new cases are diagnosed in late or end stages when the treatment is less effective. Gastric adenocarcinoma growth and distant metastatic spread depend upon tumor angiogenesis [2-4].

Angiogenesis is vital for the uninterrupted growth of the malignant tumors, but tumoral angiogenesis produces irregular, sinuous and fenestrated capillaries that lack pericytes, incapable of vasoconstriction, causing a turbulent flow and favoring thrombosis and bleeding. Those properties prompt local tumor spread and formation of tumor emboli [5]. Angiogenesis starts within peritumoral tissues, due to paracrine signaling via VEGF 1 and endoglin (CD105), a molecule which usually promotes the endothelial cell adhesion and differentiation. The new capillaries demonstrate intense membrane positivity for CD105, which is paler and only focally present in normal vessels [6].

Endothelial cells are quiescent, only seldom undergoing mitosis, but they become activated, with an elevated mitotic rate when are exposed to hypoxia or stimulated with small amounts of TGFβ-1. This molecule binds to endoglin, a transmembrane glycoprotein which is widely expressed on the surface of endothelial cells stimulating their proliferation and formation of novel capillaries. The solid tumors depend upon an extensive network of capillaries arising from the existing surrounding normal vasculature through angiogenesis driven by TGFβ-1 produced by the tumor cells. The presence of an abundant vascular network and elevated levels of TGFβ-1 in gastric adenocarcinoma correlates with the presence of vascular metastases and a reduced survival time. The role of endoglin in the pathogenesis of gastric adenocarcinoma has not been completely established, therefore, the correlation between the density of new blood vessels within the tumors and the amount of CD105 receptors on cellular membranes remains to be determined. Endoglin levels in some solid tumors correlate with a short survival time and the presence of distant metastases. Tumor angiogenesis is crucial for the tumor growth, invasiveness and metastatic spread; therefore, the agents that effecttively interfere with it may be useful therapeutic agents. Endoglin may be targeted directly by chemotherapy agents as a promising alternative treatment [7].

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Macrophages and mast cells communicate with fibroblasts and tumor cells and consecutively promote angiogenesis. Mast cells are both promoters and inhibitors of tumor growth, being the first to infiltrate in the early stage malignancies such as breast, melanoma and colon cancer [8-9]. They may exert either an antitumor role by secreting IL-1 and TNFα; or favor tumor growth due to angiogenesis, break-down of the intercellular matrix and immunosuppression [3, 10]. They secrete proteases, such as tryptase and chymase, which act upon extracellular matrix, proteins, and adhesion molecules. The tryptase plays a decisive role in tumor angiogenesis, acting directly upon endothelial cells; and indirectly by degrading extracellular matrix, thus favoring proliferation and migration of endothelial cells. The elevated number of mast cells within malignant tumors correlates with the effectiveness of the angiogenesis.

MATERIAL AND METHODS

This is a retrospective case-control study including 15 specimens of total gastrectomy from patients with gastric cancer and no neo-adjuvant oncological therapy and a control group of 15 patients with similar sex and age, that underwent gastrectomy for non-neoplastic conditions. World Health Organization classification (2010) was used for tumor microscopic grading. There were 15 male and 15 female patients, with ages ranging from 30 to 70 years. The tumors were divided into two histological subgroups: well-differentiated type, including papillary and tubular adenocarcinoma, and poorly differentiated type, including signet ring cell carcinomas and mucinous adenocarcinoma. The tissue samples were fixed in formalin and embedded in paraffin according to standard procedures and cut into 4 μm-thick sections which were further mounted on glass slides. Gastric carcinoma tumors were studied. The inclusion criteria for the case group were histologically proven stage T3/T4 malignancies with regional lymph node metastases. The study design was approved by the Ethics Commission of the "Colentina" University Hospital, Bucharest; and written consent was obtained from all patients participating in this study.

Mean density of mast cells (DMC) and mean density of vessels (DMV) were evaluated using a three-layer biotin-avidin-peroxidase system. From each gastrectomy specimen, formalin-fixed and paraffin-embedded samples of gastric wall from the most distant resection margin, from 5 cm lateral to the tumor, from the lateral and deep invasion front and from the tumor were cut into 4 μm thick serial sections. Those were deparaffined and further microwaved at 500 W for 10 min, for antigen retrieval and the endogenous peroxidase activity was blocked with 3% hydrogen peroxide solution. Adjacent slides were incubated with monoclonal antibody anti-CD105, diluted 1:50, for 1 h at room temperature and with monoclonal antibody anti-tryptase diluted 1:100 for 1 h at room temperature. The bound antibody was visualized using biotinylated secondary antibody, avidin-biotin peroxidase complex. All sections were counterstained with Giemsa stain for the identification of Helicobacter pylori infection, and the primary antibody was omitted in negative controls.

DMC and DMV mean values ± 1 SD were evaluated for each tissue sample and in all series of sections. The correlations between those indexes and the clinic-pathological features listed in Table 1 were analyzed using student t, Pearson, Spearman, ANOVA tests. In all analyses, $p < 0.05$ was considered significant. All statistical analysis was performed with the SPSS statistical software package (SPSS, Inc., Chicago, IL, USA).

RESULTS

The analyzed parameters of the patients enrolled in tumor group are normally distributed as shown in Table 1. Some of the histological findings are presented in Figures 1-7.

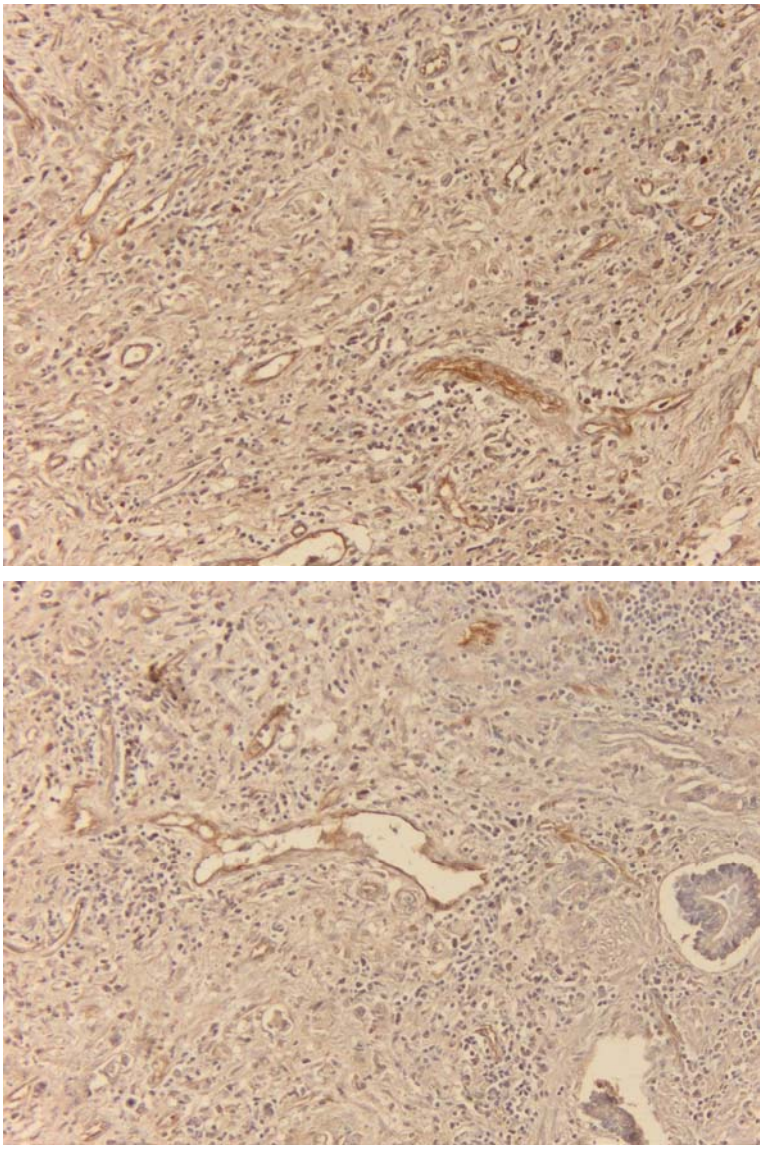
Correlations between DMC and DMV in the tumor group

In tumor group, the mean mast cell density within the tumor (intestinal type adenocarcinoma – photos 1, 2, 3; diffuse type carcionama photo 4) and within lateral and deep invasion front (photos 5, 6 and 7, 8), DMCin is strongly correlated ($r = 0.44$; $p = 0.07$) with mean mast cell density outside the tumor, DMCout (evaluated in gastric mucosa situated at 5 cm from the tumor and at the most distant resection margin). Thus, in gastric cancer, there is a uniform rise in DMC within the gastric wall.

On the other hand, DMCin correlates well ($r = 0.44$; $p=0.01$) with mean density of new vessels (CD105 positive) in the gastric wall distant from the tumor, namely DMVout; but not with the one within the tumor DMVin ($r = 0.21$; $p = 0.12$). Those findings show a different effect of mast cell on the angiogenesis process: mast cells enhance formation of new vessels in the gastric wall distant from the tumor, but they have also an alleged antiangiogenic intratumoral activity. Thus, there are fewer new vessels within the tumor than in the rest of the gastric wall.

Table 1
Pearson correlations between density of mast cells tryptaso-positive and density of neo-vessels in tumoral and non-tumoral areas – statistical results of the research

Correlations					
		DMCout	DMCin	DMVout	DMVin
DMCout	Pearson Correlation	1	.445**	.280	.280
	Sig. (1-tailed)		.007	.067	.067
	N	30	30	30	30
DMCin	Pearson Correlation	.445**	1	.641**	.218
	Sig. (1-tailed)	.007		.000	.124
	N	30	30	30	30
DMVout	Pearson Correlation	.280	.641**	1	.274
	Sig. (1-tailed)	.067	.000		.071
	N	30	30	30	30
DMVin	Pearson Correlation	.280	.218	.274	1
	Sig. (1-tailed)	.067	.124	.071	
	N	30	30	30	30
**. Correlation is significant at the 0.01 level (1-tailed).					



Figures 1, 2. Angiogenesis intratumoral and peritumoral gastric adenocarcinoma marked by immunostaining of the newly formed vessels with CD105 (endoglin) and counterstain with Giemsa.

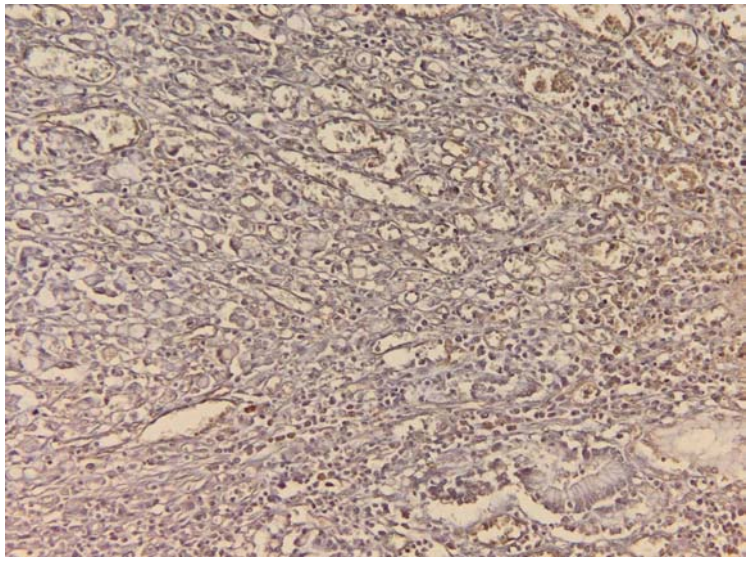


Figure 3. Intratumoral angiogenesis in gastric carcinoma cell signet ring – immunohistochemical marker CD105, counterstain with Giemsa; 20 ×.

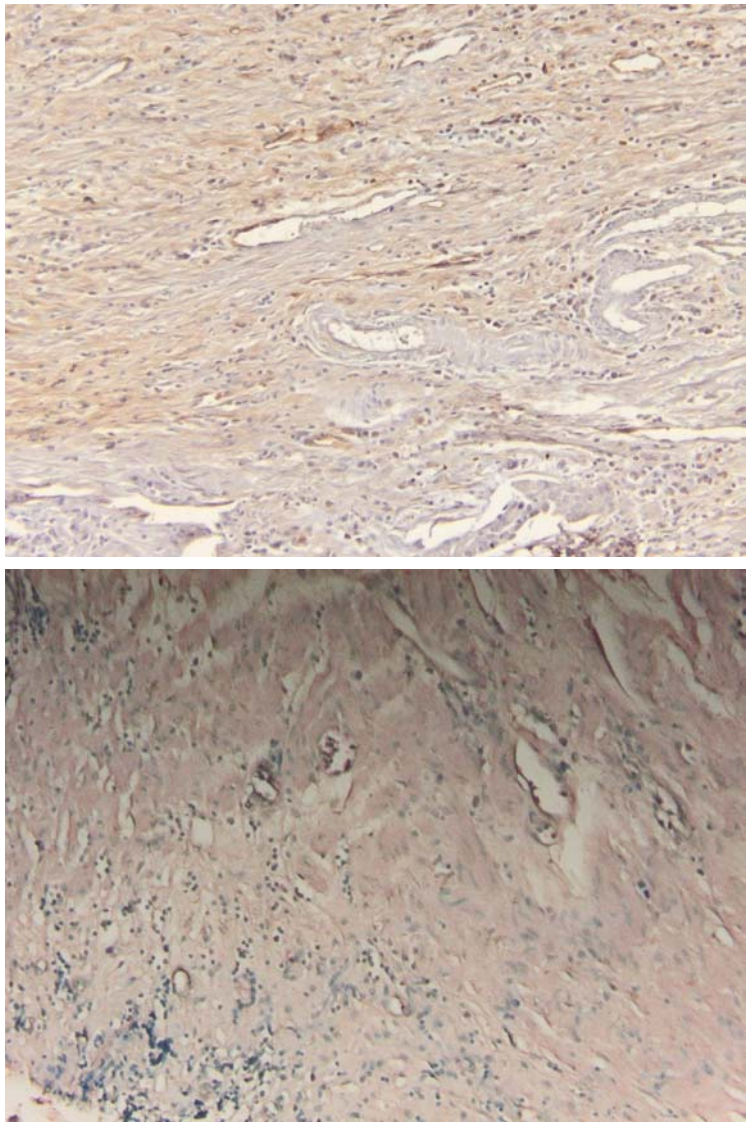
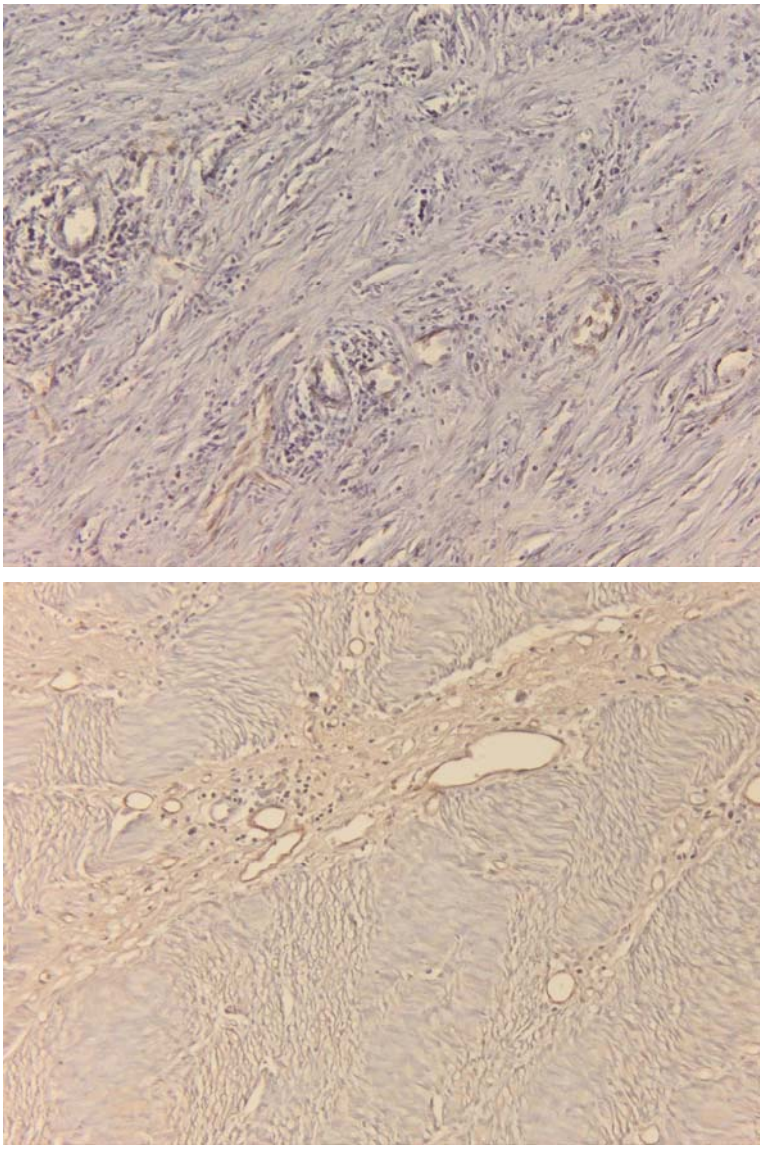


Figure 4, 5. Newly formed vessels in the distal limit of surgical resection of the tumor, marked with CD105 (endoglin) intense, discontinuous.



Figures 6, 7. Neoformation vessels CD105 positive in gastric wall at the level of depth tumoral front.

Correlations between tumor type and DMC and DMV

DMCin correlates well (r = 0.32; p = 0.04) with tumor type, the greatest number of mast cells being identified within recurring gastric tumors, with smaller numbers being found in adenocarcinoma, the diffuse type ranking first, over the intestinal one. DMCin was greater in gastric GIST tumors than in lymphomas. The smaller amount of mast cells was identified in gastric metastases of other malignancies and in gastric spread of a neighboring tumor.

DMCout (number of mast cells at the most distant resection margin from the tumor) is negatively correlated (r = -0.45; p = 0.002) with tumor stage (pT) in the presence of Helicobacter pylori infection. DMVin is well correlated (r = 0.55; p = 0.002) with the presence of Helicobacter pylori infection in the tumor group; whilst DMVin correlates well (r = 0.48; p = 0.003) with the tumor stage, in the presence of Helicobacter pylori infection. On the other hand, in the absence of the bacterial infection, there is a negative correlation between the above mentioned parameters. The participation of Helicobacter pylori infection in the growth and progression of gastric carcinoma is supported as well, by the correlation (r = 0.48; p = 0.03) between the tumor stage and DMVout (density of new vessels in the most distant resection margin from the tumor) in Helicobacter pylori positive patients.

Regardless of the state of Helicobacter pylori infection, DMVout correlates well (r = 0.55; p = 0.001) with the presence of nodal metastases in gastric cancer. In contrast, DMVin correlates (r =

0.52; p = 0.002) with the presence of nodal metastases only in advanced gastric tumors (at least pT2b) and only in cases with Helicobacter pylori infection.

DISCUSSION

Mean density of mast cells was increased in tumoral specimens, in equal measure within the tumor (DMCin) and in non-tumoral wall (DMCout). These results are demonstrating that tumor cells attract mast cells in gastric wall, as a response to tumoral activity, but they do not influence the distribution of mast cells in the gastric wall. Multiple studies identified this feature and also obtained significant correlations of the number of mast cells with depth of invasion, presence of nodal metastasis and perivascular and perineural invasion. Also, a high number of mast cells within the gastric wall was correlated with a poor outcome and a reduced survival in gastric tumors. These actions of mast cells are considered to be the result of mast cell degranulation and consecutive release of histamine, heparin and other molecules that favor angiogenesis (by increasing endothelial cell migration, proliferation and differentiation, and also by promoting adhesion between tumor cells and endothelial cells).

These observations support the idea that mast cells are a negative prognosis factor in gastric tumors, tumoral angiogenesis being the most important pathway that they stimulate [3].

Therefore, we consider that the significant correlation between DMCin and DMVout found in this study is consistent with the the participation of mast cells in the angiogenesis of solid tumors of the digestive system [2, 5]. But the lack of correlation between DMCin and DMVin, demonstrated by this study, may be seen as a sequel of previous studies which have analyzed the mean mast cell density and the mean new vessels density in the gastric wall as a hole, and not on separate, specific areas and depending upon the presence or the absence of Helicobacter pylori infection. Our finding that there are fewer new vessels within the advanced gastric tumors (at least IIB) than in the rest of the stomach wall, correlated with their recognized pro-angiogenic role in the growth and spread of the early stage tumors, as our previous work has demonstrated [3, 11], suggests that probably other components of the complex tumoral microenvironment, as well as the rapid growth of advanced tumors, inhibit angiogenesis and limit the density of tumoral new vessels. Low density of tumoral vessels, in contrast with the increased angiogenesis in the rest of the gastric wall is, most probably, another indicator of tumoral aggressiveness, since the tumoral growth is so rapid that exceeds the angiogenesis.

The significant correlation between DMVout and DMCin found in this study is consistent with other studies demonstrating the participation of intratumoral mast cells in the formation of new vessels in the gastric wall distant from the tumor [2, 5]. This association has been reported in different sites (for breast cancer [12] and colorectal cancer – [13]) implying that mast cells chemotaxis and accumulation in tumoral environment is independent of the type of cancer and influenced by the presence of malignant transformation. Also, in all studies, mast cells were correlated with increased angiogenesis, higher histological stage and poor outcome. CD105 is a sensible marker for new vessels, thus we can consider that DMV is a value for vascular structures resulted from tumoral-induced angiogenesis. Correlation with mean density of mast cells includes gastric tumors in the spectrum of tumors that enhance angiogenesis via mast cell effects, supporting local growth and distant spread.

Studying correlation between mast cells and tumor stage, our study identified some interesting aspects:

The negative correlation between DMCout and the tumor stage in the presence of Helicobacter pylori infection may be seen as a proof of the boost for the antitumor activity of the immune system due to the presence of the bacterial infection. Mast cells are known as promoters of inflammatory infiltrate in Helicobcater pylori infection. This inflammatory reaction of the microenvironment has not only pro-tumorigenic effects through oxidative stress, but also an immune anti-tumoral role in gastric cancer. So, in Helicobacter pylori positive patients, it seems that poor mast cell mobilization within the tumor begets a more aggressive behavior of the gastric cancer, as this author's previous work has demonstrated, as a result of an inhibition of the local immune response. Also, mast cells are involved in repair of Helicobacter pylori induced tissue damage, by limiting degradation of pericellular matrix induced by the bacteria. This mechanism can also be an explanation of this interesting observation. Subsequent studies will verify this intriguing result, looking for mechanisms to explain this correlation.

Correlation between DMVin and the tumor stage in the presence of Helicobacter pylori infection is suggestive of the tumorigenic role of this common bacterial infection, due, among other factors, to increased angiogenesis around the gastric carcinoma which favors tumor growth and spread. This finding is applying to the entire wall of the stomach, as is obvious from the correlation between the tumor stage and the DMVout.

On the other hand, the correlation between DMVout and nodal involvement in gastric carcinoma regardless of the state of Helicobacter pylori infection, which this study has described, is also an expected result, confirmed by other studies, suggesting the importance of the new vessels in the stomach wall distant from the tumor for the occurrence of nodal metastases.

Helicobacter pylori participates in nodal spread of advanced gastric cancer, as suggested by the correlation between DMVin and nodal metastases in patients with Helicobacter pylori infection. These observations sustain the importance of angiogenesis in tumoral growth and spread and offer arguments for the fact that endoglin (CD105) is a powerful target for antitumoral treatment [14]. Also, mast cell inhibiting agents should be considered as adjuvant therapy in gastric tumors, this study sustaining the fact that mast cells are pro-angiogenic and tumor stimulating factors.

CONCLUSIONS

This paper suggests the participation of Helicobacter pylori infection in the growth and progress of gastric neoplasia due to an increase of peritumoral angiogenesis stimulated by mast cell accumulation within tumor and within gastric wall, with subsequent local and distant tumor spread and perivascular growth, but without perineural and nodal involvement. There is a significant correlation between density of tryptase-positive mast cells and magnitude of angiogenesis, especially in early stage tumors, highlighting the need for efficient anti-inflammatory and anti-angiogenic therapy in the management of those patients.

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Proteazele mastocitelor, triptaza şi chimaza, sunt implicate direct în creşterea şi progresia tumorilor solide datorită rolului său important în angiogeneza tumorală. Am examinat densitatea mastocitelor triptazo-pozitive şi densitatea medie a vaselor nou formate în peretele gastric la pacienţii cu o tumoră gastrică malignă, infectaţi sau nu cu Helicobacter pylori, folosind o colorație imunohistochimică pentru tripază (pentru mastocite) şi Cd105 (pentru vasele de neo-formație). Au fost examinate expresiile imunohistochimice pentru Triptaza şi pentru CD în piesele de gastrectomie selectate. În studiul nostru, densitatea mastocitelor s-a corelat cu angiogeneza şi progresia cancerului gastric. Am observat, de asemenea, faptul că participarea infecției cu Helicobacter pylori în creşterea şi progresia neoplaziei gastrice este datorată creşterii angiogenezei peritmoral, cu o diseminare tumorală consecutivă atât local cât, şi la distanță a tumorii, precum şi cu creştere perivasculară, însă fără interesare perineurală sau limfoganglionară.

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