

## VEGF EXPRESSION AND MICROVESSEL DENSITY IN GASTRIC CARCINOMAS

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### Summary

The angiogenesis is one of the most important factors in tumor development and dissemination. We aimed to evaluate the angiogenesis and its involvement in gastric carcinomas natural history, based on immunohistochemical expression of vascular endothelial growth factor and tumor microvessel counting compared with the most known prognostic factors. We selected 95 cases of gastric carcinomas from patients investigated in gastroenterology and surgery clinics and diagnosed in pathology laboratory of Emergency Hospital Craiova between 2005 and 2009. The immunohistochemical study was performed using the LSAB /HRP (DAKO LSAB 2) technique. VEGF was expressed more frequently in intestinal type tumors, related to tumor differentiation and progression. The highly angiogenic tumors were associated with poor prognostic parameters (blood vessels and lymphatic invasion), and lymph node and distant metastases. Tumor microvessel density (MVD) varied according to histological type and differentiation, and also with tumor progression and dissemination.

**Key words:** gastric cancer, angiogenesis, prognostic factors, VEGF, MVD

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### Introduction

Tumor angiogenesis has been proven to be one of the most important processes involved in tumor growth and metastases (Folkman, 1992; Risau, 1997). This process depends on the production of angiogenic factors by host and/or tumor cells and consists in developing of new blood vessels from the preexisting network of capillaries (Folkman, 1997).

The vascular endothelial growth factor (VEGF) is considered as the most potent and specific promoter of tumor angiogenesis that is involved in tumor development as vascular permeability factor, and/or as endothelial growth factor, being a selective endothelial cell mitogen and angiogenic agent (Hicklin and Ellis, 2005; Senger *et al.*, 1993).

The angiogenic process can be quantified by both immuno-histochemical assessment of VEGF expression in tumor cells and measurement of microvessel

density (MVD) in tumor mass, based on CD31 immunostaining.

### Material and Methods

Our study included 95 cases of gastric carcinomas diagnosed, staged and evaluated for morphological parameters using histopathological standard technique and subject to further processing by immunohistochemical techniques for VEGF and CD31 antibody, using LSAB /HRP (Labeled Streptavidin Biotin 2 /System Horseradish Peroxidase) (DAKO LSAB 2) technique.

VEGF expression was evaluated as index of positivity (IP) based on quantitative score (P) and intensity score (I). The quantitative score was classified in for categories depending on percentage of positive tumor cells: 1(<25%); 2(25-50%); 3(50%-75%); 4(>75%) whereas the intensity score (I) was classified in three categories depending on staining intensity: 1(weak); 2(moderate); 3(strong). The IP

value, calculated as  $IP = PxI$ , was in the range 1-12 setting four classes of tumors:  $IP=0$ (negative staining);  $IP=1-4$ (low immuno-positivity)(+);  $IP=5-8$ (moderate immunopositivity)(++);  $IP=9-12$ (high immunopositivity)(+++). Tumor cell immunoreactivity for VEGF was mainly in cytoplasm (supra nuclear, frequent in differentiated tumors or diffuse, in particularly in poorly differentiated tumors) and only occasionally membranar; any staining of stromal elements – endothelial, fibroblastic, or other stromal cells, was excluded in this assessment.

MVD (Microvessel density) was evaluated based on CD31 immunostaining of thin-walled blood vessels or isolated endothelial cells in tumor mass, using the “hot spot” morphometry method.

VEGF clone VG1 and CD 31 clone JC 70A (DakoCytomation, Denmark) were diluted 1:50 in PBS followed by 20 minutes tissues pre-treatment with heat inducing epitope retrieval (MW) in DakoCytomation target retrieval solution high pH and 30 minutes incubation at room temperature with primary antibody; the visualization was realized with Dako EnVision+HRP; negative control was DakoCytomation Mouse IgG, diluted in the same concentration as primary antibody.

The correlation between morphological parameters, VEGF immunostaining and MVD were statistically analyzed by calculating the correlation coefficients ( $p$ ) and a  $p$  value below 0.05 was considered statistical significant.

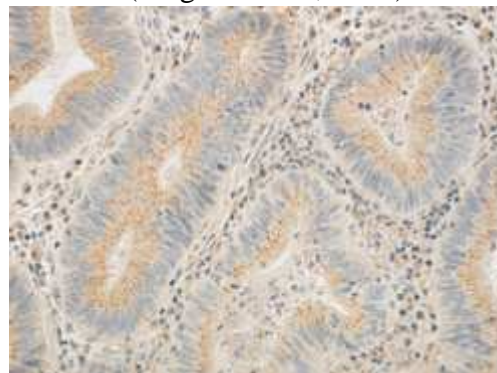
## Results and Discussions

The 95 studied gastric carcinomas included 76 intestinal type tumors – 14 well-differentiated, 25 moderate-differentiated and 37 poorly differentiated – and 19 diffuse type tumors; majority were serosal invasive (T3, 34 cases) followed by T2 (29 cases), T4 (22 cases) and T1 tumors (10 cases); vascular invasion were associated in 25 cases and lymphatic invasions in 40 cases; lymphnode

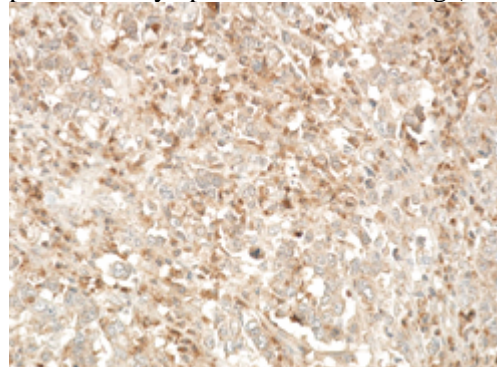
involvement was found in 47 cases and distant metastases in 17 cases.

VEGF was expressed in 72.63% of the 95 gastric carcinomas, more frequently intestinal than diffuse type tumors (73.68% vs. 68.42%) related with histological grade ( $p < 0.01$ ). The poorly differentiated tumors were most frequently characterized by VEGF over expression ( $IP+++$ , 54.05%) while well differentiated tumors were in majority VEGF negative (57.14%)(Figure 1-2). Referring to diffuse type carcinomas, these tumors were equally VEGF intense positive and negative (31.57%) (Table 1).

These results are consistent with those of similar studies that rapport variable frequency between 50.3% and 77.8% of VEGF expression in gastric tumors, in comparison to diffuse type of cancer, the intestinal type being more dependent on angiogenesis (Park *et al.*, 2005; Yanislav *et al.*, 2007; Yasuhiko, 2010). In their study, Jang and Kim are also reporting the VEGF positivity significantly higher in moderately and poorly differentiated gastric carcinoma than in well differentiated gastric carcinomas (Jang and Kim, 1997).



**Figure 1.** Well-differentiated carcinoma, VEGF supra nuclear cytoplasm immunostaining ( $IP++$ )



**Figure 2.** Poorly-differentiated carcinoma, VEGF diffuse cytoplasm immunostaining ( $IP+++$ )

**Table 1.** VEGF expression in gastric carcinomas referred to morphological parameters.

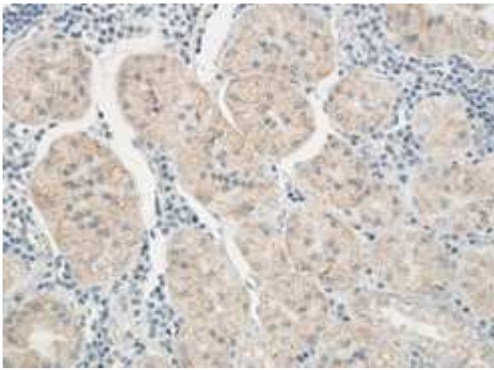
Morphologic parameters	Cases	VEGF Immunopositivity index (IP)			
		0	+	++	+++
	95	26	12	22	35
Histological type		$P>0.05$			
Intestinal type	76	20	10	17	29
Diffuse type	19	6	2	5	6
Grading		$P<0.01$			
Well-differentiated	14	8	1	5	0
Moderate-differentiated	25	6	5	5	9
Poorly-differentiated	37	6	4	7	20
Tumor progression		$P<0.05$			
pT1	10	7	1	2	0
pT2	29	14	3	7	5
pT3	34	5	6	7	16
pT4	22	0	2	6	14
Blood vessel invasion		$P<0.001$			
absent	70	26	10	16	18
present	25	0	2	6	17
Lymphatic vessel invasion		$P<0.001$			
absent	55	26	11	12	6
present	40	0	1	10	29
Lymphnode metastases		$P<0.001$			
absent	47	26	9	8	4
present	48	0	3	14	31
Distant metastases		$P<0.001$			
absent	78	26	12	20	20
present	17	0	0	2	15

VEGF, with an important role in tumor angiogenesis dynamic, is involved in the tumor advancement and the lymph node metastasis, and his over expression appear to be related to clinical staging, lymph node and distant metastasis (Liu *et al.*, 2001; Sheng *et al.*, 2005).

According to these data, in our study, VEGF immunoreactivity was related with tumor progression ( $p<0.05$ ), majority of T4 stage tumors being high angiogenic (63.63%).

The immunostaining pattern also correlated with the prognostic parameters ( $p<0.001$ ). VEGF over expression was found in 68% of tumors with vascular invasion and in 72.5% of those with lymphatic invasion (Yanislav *et al.*, 2007; Yasuhiko, 2010).

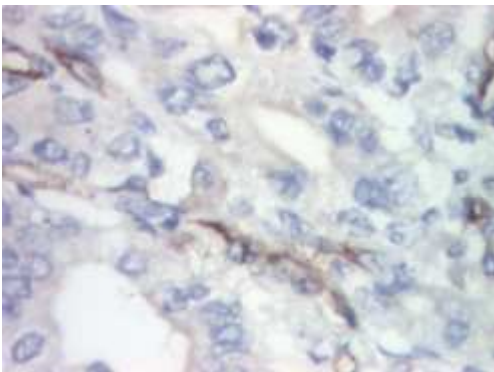
The same high level of VEGF expression was found in relation with lymphnode and distant metastases ( $p<0.001$ ) (Figure 3), lymphatic (64.58%) and haematogenous (88.23%) spread being more frequent in intense angiogenic tumors.



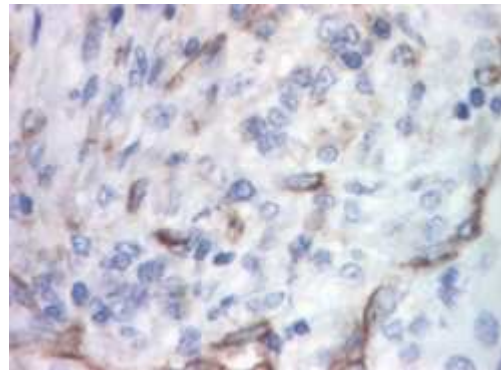
**Figure 3.** VEGF expression in lymphnode metastasis

This direct relationship between VEGF expression and metastatic potential of gastric carcinoma is supported in several other studies (Maehara *et al.*, 2000).

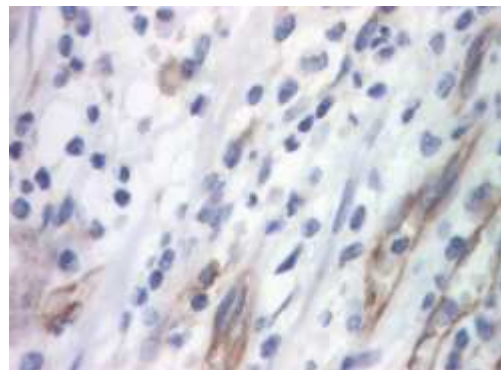
MVD was in 5-31 /hpf range, depending on histological type and grade, with a average of 19.38 /hpf for intestinal type and 16.48 /hpf for diffuse type tumors (Figure 4-6).



**Figure 4.** Well-differentiated carcinoma, MVD



**Figure 5.** Poorly-differentiated carcinoma, MVD



**Figure 6.** Diffuse type carcinoma, MVD

The highest average values of MVD were found gastric carcinomas of intestinal type low-differentiated tumors (22.01 /hpf) (Table 2), known to be much more dependent on angiogenesis phenomenon than diffuse type carcinomas the latter tumors showing an obviously lower DMV value (Takahashi *et al.*, 1996; Erenoglu *et al.*, 2000).

**Table 2.** Microvessel density in gastric carcinomas

Microvessel density					
Gastric carcinomas	Well-differentiated	Moderate-differentiated	Poorly-differentiated	Diffuse type	MVD
Tumor progression					
pT1	9	10.66	-	8.33	9.33
pT2	12.77	13.33	15.38	11.66	13.26
pT3		18.44	21.69	15	18.37
pT4	-	20.72	26.85	22.41	23.32
MVD	11.26+/-2.76	17.58 +/-3.87	22.01+/-5.34	16.48+/-5.33	-
19.38 +/- 5.78					
Prognostic parameters					
Blood vessel invasion		21	25.90	23	23.3
Lymphatic vessel invasion		20.33	25.05	18.2	21.34
Lymphnode metastases	12.44	18.78	22.01	18.5	17.93
Distant metastases	-	21.66	28.75	24.5	24.97

The intensity of tumor angiogenesis increased parallel to tumor stage, reaching the highest average value in tumors with peritoneal involvement (pT4) (23.32/ hpf) and supporting the hypothesis that angiogenesis is essential for growth and tumor progression (Takahashi *et al.*, 1996).

A direct relationship between angiogenesis and tumor stage is found in similar studies that also confirm the increased tendency to vascular space invasion and lymphnode and distant metastasis in tumors with elevated MVD, exceeding the average value corresponding to the histological type and degree of differentiation (Table 2) (Tao *et al.*, 1998; Zhao *et al.*, 2006).

The ability of malignant gastric tumor to induce the formation of new blood vessels essential to tumor growth, progression and metastasis, is dependent on growth factors, including VEGF, the main modulator of angiogenesis, secreted by tumor cells and cellular elements of the associated inflammatory response and whose expression, related with histological type, differentiation and tumor stage was found in tumors with high levels of DMV (Veikkola *et al.*, 2000; Tsujitani *et al.*, 2004).

## Conclusion

VEGF was expressed more frequently in intestinal type tumors, related to differentiation grade and tumor stage. The highly angiogenic tumors were associated with poor prognostic parameters (blood vessels and lymphatic invasion), and lymph node and distant metastases. Tumor microvessel density (MVD) varied according to histological type and differentiation, and also with tumor progression and dissemination. Angiogenesis, evaluated as VEGF expression and MVD, is related to tumor progression and metastasis and can be considered a prognostic indicator for gastric carcinoma.

## References

- Erenge, C, Akin, ML, Uluku, H, Tezcan, L, Yikirim, S, Bakin, A. Angiogenesis predicts poor prognosis in gastric carcinoma. *Dig. Surg.*, 17, 6, 581-586, 2000
- Folkman, J. Angiogenesis and angiogenesis inhibitor: an overview. *EXS*, 79:1-8, 1997
- Folkman, J, Shing, Y. Angiogenesis. *J. Biol. Chem.*, 267, 10931-10934, 1992
- Hicklin, DJ, Ellis, LM. Role of the vascular endothelial growth factor pathway in tumor growth and angiogenesis. *J. Clin. Oncol.*, 23, 1011-1027, 2005
- Jang, TJ, Kim, JR. Immunohistochemical Study of the Vascular Endothelial Growth Factor in Gastric Carcinoma. *The Korean Journal of Pathology*, 31, 5, 401-409, 1997
- Liu, DH, Zhang, XY, Fan, DM, Huang, YX, Zhang, JS, Huang, WQ, Zhang, YQ, Huang, QS, Ma, W.Y, Chai, YB, Jin, M. Expression of vascular endothelial growth factor and its role in oncogenesis of human gastric carcinoma. *World J. Gastroenterol.*, 7, 500-50, 2001
- Machua, Y, Kabashima, A, Koga, T, Tokunaga, E, Takeuchi, H, Kakeji, Y, Sugimachi, K. Vascular invasion and potential for tumor angiogenesis and metastasis in gastric carcinoma. *Surgery*, 128, 408-416, 2000
- Pak, GS, Jo, YE, Kim, HS, Choi, SK, Rew, JS, Pak, CS, Kim, SJ. Expression of PIEN and its correlation with angiogenesis in gastric carcinoma. *Korean J. Gastroenterol.*, 46, 3, 196-203, 2005
- Risau, W. Mechanisms of angiogenesis. *Nature*, 386, 671-674, 1997
- Senger, DR, Van De Water, L, Brown, LF, Nagy, JJ, Yeo, KT, Yeo, TK. Vascular permeability factor in tumor biology. *Cancer Metastasis Rev.*, 12, 3, 303-324, 1993
- Sheng, W, Wu, BY, You, WD. Influence of VEGF and Ki-67 expression on biological behavior of gastric cancer. *World Chin J. Digestol.*, 13, 6, 716-719, 2005
- Takahashi, Y, Cleary, KR, Mai, M, Kitachi, Y, Bucana, CD, Ellis, LM. Significance of Vessel Count and Vascular Endothelial Growth Factor and Its Receptor (KDR) in Intestinal-type Gastric Cancer. *Clin. Cancer Res.*, 2, 1679-1684, 1996
- Tao, H, Lin, Y, Yin, H, Wang, R. Prognostic value of tumor vascularity in gastric carcinoma. *Zhonghua Wai Ke Za Zhi*, 36, 5, 307-9, 1998
- Tsujitani, S, Saito, H, Maeta, Y, Yamaguchi, K. Neovascularization in Patients with Gastric Carcinoma in Relation to the Expression of Vascular Endothelial Growth Factor and Thymidine Phosphorylase. *Anticancer Research*, 24, 1853-1859, 2004
- Veikkola, T, Karkkainen, M, Claesson-Welsh, L, Alitalo, K. Regulation of angiogenesis via vascular endothelial growth factor receptors. *Cancer Res.*, 60, 203-212, 2000
- Yanai, K, Hiroyuki, U, Satou, I, Toshiaki, I, Tatsuyuki, K, Sugihara, K. Prognostic Significance of VEGF Expression in Correlation With COX-2, Microvessel Density, and Clinicopathological Characteristics in Human Gastric Carcinoma. *Annals of Surgical Oncology*, 14, 2738-2747, 2007
- Yasuhiko, K. Angiogenesis and Lymphangiogenesis of Gastric Cancer. *Review Article. Journal of Oncology*, 2010
- Zhao, HC, Qin, R, Chen, XX, Sheng, X, Wu, JF, Wang, DB, Chen, GH. Microvessel density is a prognostic maker of human gastric cancer. *World J. Gastroenterol.*, 12, 47, 7598-603, 2006