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胃癌组织中血管内皮生长因子 -C 及其受体 -3 的表达与意义

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摘 要： 【目的】 探讨血管内皮生长因子 -C (vascular endothelial growth factor-C, VEGF-C) 其受体 -3 (vascular endothelial growth factor receptor-3, VEGFR-3) 与胃癌临床病理因素的关系。 【方法】应用免疫组织化学 (SP)方法测定胃癌及胃良性病变中 VEGF-C 与 VEGFR-3 表达，计数 (200 × 视野下淋巴管数密度 (number density of lymph vessel, $^{/200\times}n_{DLV}$)。【结果】VEGF-C 阳性率胃癌组 60.00%，胃良性病变组 15.00% ($P=0.000$)；淋巴结转移组 68.97%，无淋巴结转移组 36.36% ($P=0.008$)；低分化组 72.73%，高 + 中分化组 44.44% ($P=0.010$)；伴有远处转移组 100%，不伴有远处转移组 53.62% ($P=0.02$)；pTNM 分期 Ⅰ + Ⅱ 组 43.75%，Ⅲ + Ⅳ 组 70.83% ($P=0.015$)。 淋巴管数密度 (显微镜下每 200 × 视野)：胃癌组为 (5.800 ± 2.318 9)，胃良性病变组为 (2.3800 ± 0.462 9) ($P=0.000$)；淋巴结转移组为 (6.948 3 ± 1.583 1)，无淋巴结转移组为 (2.772 7 ± 0.428 9) ($P=0.000$)；低分化组为 (7.681 8 ± 0.982 9)，高 + 中分化组为 (3.500 ± 1.028 2) ($P=0.000$)；pTNM 分期，Ⅰ + Ⅱ 组为 (4.291 7 ± 1.688 0)，pTNM 分期，Ⅲ + Ⅳ 组为 (8.062 5 ± 0.759 4) ($P=0.000$)。VEGF-C 与淋巴管数密度相关 ($P=0.002$)。 【结论】VEGF-C 刺激淋巴管生成，有利于胃癌淋巴道与远处转移。

关键词： 胃肿瘤；血管内皮生长因子 -C；血管内皮生长因子受体 -3
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Expression and Significance of Vascular Endothelial Growth Factor(VEGF)-C and VEGF Receptor-3 in Gastric Carcinoma

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Abstract: 【Objective】To investigate the relationship between expression of vascular endothelial growth factor(VEGF)-C and VEGF receptor-3(VEGFR-3) and pathological features of gastric carcinoma(GC). 【Methods】Using immunohistochemical staining to examine the expression of VEGF-C and VEGFR-3 in 80 GC cases and 20 gastric benign disease (GBD) cases and to calculate the number density of lymph vessel (DLV)for a field of vision by /200 × under microscopy, $^{/200\times}n_{DLV}$). 【Results】The positive expression rates of VEGF-C were 60.00% and 15.00% in GC and GBD, respectively ($P=0.000$). The positive expression rates of VEGF-C in GC with lymph node metastasis and GC without lymph node metastasis were 68.97% and 36.36%, respectively ($P=0.008$). 72.73% cases in poorly differentiated type group presented VEGF-C positive, higher than that in differential type group (44.44%, $P=0.010$). The positive expression rates of VEGF-C in GC accompanied with distant metastasis was 100%, higher than that in GC whihout distant metastasis(53.62%, $P=0.02$). The positive expression rates of VEGF-C in pTNM Ⅰ + Ⅱ group and Ⅲ + Ⅳ group were 43.75% and 70.83%, respectively ($P=0.015$). The DLVs were (5.800 ± 2.318 9) and (2.380 0 ± 0.462 9)in GC and GBD, respectively ($P=0.000$). The DLVs in GC with lymph node metastasis and GC without lymph node metastasis were (6.948 3 ± 2.583 1) and (2.772 7 ± 0.428 9), respectively ($P=0.000$). The DLVs were (7.681 8 ± 0.982 9) and (3.500 ± 1.028 2) in poorly differentiated type

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and well differentiated type group, respectively ($P = 0.000$). The DLVs in pTNM Ⅰ + Ⅱ group and Ⅲ + Ⅳ group were (4.291 7 ± 1.688 0) and (8.062 5 ± 0.759 4), respectively ($P = 0.000$). were VEGF-C was associated with DLV ($P = 0.0002$). 【conclusion】VEGF-C stimulates lymphangiogenesis and favors the lymphangial and distant metastasis of GC.

Key words: stomach neoplasm; vascular endothelial growth factor C; vascular endothelial growth factor receptor-3

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淋巴道转移是胃癌术后复发的重要原因之一，对肿瘤淋巴管形成予以充分研究具有重要意义。血管内皮生长因子（vascular endothelial growth factor, VEGF）家族对血管生成的作用研究较多，从属于 VEGF 家族的 VEGF-C 对淋巴管形成具有相对特异性。VEGFL 家族受体包括血管内皮生长因子受体（vascular endothelial growth factor receptor, VEGFR）-1，VEGFR-2 与 VEGFR-3，由 VEGF-C/VEGFR-3 组成旁分泌途径与肿瘤淋巴管生成关系密切，文献报道可以根据 VEGFR-3 染色计数淋巴管数密度[1]。目前，淋巴管生成与胃癌的关系尚未明确，本文目的在于测定胃癌组织 VEGF-C 与 VEGFR-3 的表达，从而探讨淋巴管生成与胃癌的关系。

1　材料与方法

1.1　材　料

80 例胃癌石蜡标本取自青岛医学院第二附属医院病理科档案室（994 – 1997），另取浅表性胃炎 14 例，萎缩性胃炎 6 例活检标本作为对照组。80 例胃癌标本蕈伞型 9 例，溃疡型 61 例，浸润型 10 例；高分化腺癌 30 例，中分化腺癌 6 例，低分化腺癌 44 例；浸润深度不超过黏膜下层者 7 例，侵及肌层者 29 例，浸及浆膜层者 44 例；淋巴结转移阳性者 58 例，阴性者 22 例；11 例有远处转移，69 例无远处转移；pTNM 分期Ⅰ期 19 例，Ⅱ期 13 例，Ⅲ期 36 例，Ⅳ期 12 例。

1.2　方　法

采用免疫组织化学（SP）法，抗 VEGF-C 多克隆抗体购自美国 Zymed 公司、抗 VEGFR-3 多克隆抗体购自美国 Santa Cruz 公司、SP kit 及 DAB kit 均由北京中山生物技术有限公司提供。操作步骤依试剂盒说明书进行。

1.3　诊断标准

VEGF-C 阳性染色为棕黄色颗粒，定位于胃癌细胞或良性病变胃腺细胞，多见于细胞浆，阳性染色细胞百分比 > 25% 者判为阳性，< 25% 者为阴性。淋巴管 VEGFR-3 阳性染色为棕黄色，位于胃癌肿瘤组织间质或良性病变的黏膜下层，多呈裂隙状，内无红细胞（图 1）。先于 100 × 视野下确定 4 个淋巴管密集区域，然后在 200 × 视野下取淋巴管平均数作为淋巴管（每 200 × 视野）数密度（number density of lymph vessel for a field of vision by /200 × under microscope, $^{/200\times}n_{DLV}$）。

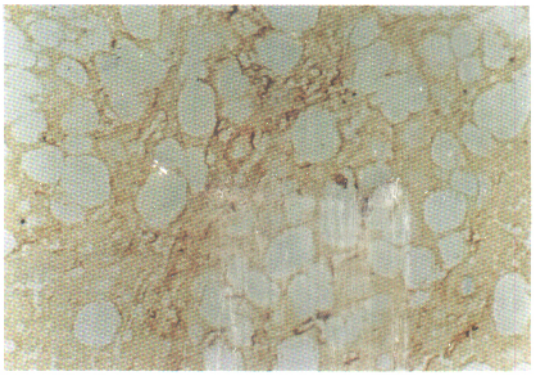

图 1　胃癌组织血管内皮生长因子受体 -3 阳性表达

Fig. 1　The positive expression of vascular endothelial growth factor receptor-3 (VEGFR-3) in gastric carcinoma (× 200)

1.4　统计方法

SPSS10.0 统计软件行 χ^2 检验与 t 检验，检验水准 $\alpha = 0.05$。

2　结　果

2.1　VEGF-C 和 n_{DLV} 在胃癌与胃良性病变中的表达比较

胃癌组织中 VEGF-C 阳性表达率及淋巴管数密度均明显高于胃良性病变，说明胃癌组织 VEGF-C 合成分泌增加，淋巴管生成增多（表 1）。

2.2　VEGF-C 和 n_{DLV} 与胃癌临床病理的关系

表 1 血管内皮生长因子 -C 和淋巴管数密度在胃癌与胃良性病变中表达比较

Table 1 Comparison of vascular endothelial growth factor-C and number density of lymph vessel between gastric carcinoma and gastric benign disease

Group	n	VEGF – C $^{+}$	VEGF – C $^{-}$	χ^2	P	$^{/200\times}n_{DLV,B}$	t	P
GC	80	48	32	12.965	0.000	5.800 0 ± 2.318 9	6.539	0.000
GBD	20	3	17			2.380 0 ± 0.462 9		

GC: gastric carcinoma; GBD: gastric benign disease; VEGF – C: vascular endothelial growth factor-C; $^{/200\times}n_{DLV,B}$: number density of lymph vessel for a field of vision by /200 × under microscope

VEGF-C 及 DLV 与胃癌大体类型、浸润深度无关。低分化,伴有淋巴结转移、远处转移以及 TNM Ⅲ + Ⅳ 期胃癌 VEGF-C 表达增强,相应淋巴管数密度增加,提示淋巴管生成促进胃癌病期进展(表 2)。

表 2 血管内皮生长因子 -C 和淋巴管数密度与胃癌临床病理间的关系

Table 2 Relationship between VEGF-C and number density of lymph vessel and pathologic features of gastric carcinoma

Pathologic features	n	VEGF – C^{+}	VEGF – C^{-}	χ^2	P	$^{/200\times}n_{DLV,B}$	t	P
Lymph node metastasis	58	40	18	7.064	0.008 [1)]	6.948 3 ± 1.583 1	12.159 [1)]	0.000
No lymph node metastasis	22	8	14			2.772 7 ± 0.428 9		
Poor differentiation	44	32	12	6.599	0.010 [2)]	7.681 8 ± 0.982 9	18.543 [2)]	0.000
Well differentiation	36	16	20			3.500 0 ± 1.028 2		
Distant metastasis	11	11	0		0.02 [3)]	6.000 0 ± 1.183 2	1.042 1 [3)]	0.289
No distant metastasis	69	37	32			5.304 3 ± 2.102 5		
TNM Ⅰ + Ⅱ	32	14	18	5.868	0.015 [4)]	4.291 7 ± 1.688 0	11.844 [4)]	0.000
TNM Ⅲ + Ⅳ	48	34	14			8.062 5 ± 0.759 4		

VEGF – C^{+}: Positive expression of vascular endothelial growth factor-C; VEGF-C^{-}: Negative expression of vascular endothelial growth factor-C; $^{/200\times}n_{DLV,B}$: number density of lymph vessel for a field of vision by /200 × under microscopy; 1) Compared with no lymph node metastasis group; 2) Compared with other differentiated group; 3) Compared with no distant metastasis group, Fisher's test; 4) Compared with TNM Ⅲ + Ⅳ group

2.3 胃癌组织中 VEGF-C 和 n_{DLV} 的相关性

淋巴管数密度($^{/200\times}n_{DLV}$)在 VEGF-C 阳性胃癌组为 7.458 3 ± 1.202 1, 阴性组为 3.312 5 ± 0.9315 (P = 0.000), 说明 VEGF-C 与淋巴管数密度相关, VEGF-C 阳性者,淋巴管生成增加。

3 讨 论

VEGF 家族与肿瘤血管淋巴管生成关系密切,其中 VEGF-C 与淋巴管生成有关, 其基因定位于 4q34[1]。VEGF 受体包括 VEGFR-1, VEGFR-2 与 VEGFR-3, 后者特异性在淋巴管内皮细胞表达。VEGF-C 作为配体与 VEGFR-3 结合,从而促进淋巴管生长; VEGFR-2 也与 VEGF-C 结合, 促进血管生成,但需要高浓度的 VEGF-C。VEGF-C 与 VEGFR-3 促进淋巴管形成[2], 与口腔癌, 肺癌淋巴道转移有关,而且二者表达具有相关性[3-6], 表达 VEGF-C 的肿瘤细胞具有高侵袭及转移特性[7]。

本实验胃癌组织肿瘤细胞表达 VEGF-C 明显高于胃良性病变, 证实胃癌细胞具有分泌 VEGF-C 的功能, 通过自分泌方式作用于 VEGFR-3, 利于淋巴管增生; 伴有淋巴结转移组 VEGF-C 表达远远强于无淋巴结转移组, 而且与远处转移与否和 TNM 分期显著相关。Amioka 等[8]认为胃癌 VEGF-C 表达与淋巴结转移密切相关是由于新生淋巴管缺少紧密连接, 基底膜不连续, 缺少阻碍胃癌细胞进入新生淋巴管的有效屏障。文献报道可以依据 VEGFR-3 阳性染色计数淋巴管[1, 6, 9], VEGFR-3 与淋巴管生成密切相关[10, 11]。本实验胃癌组织淋巴管数密度明显高于胃良性病变,证实胃癌组织中存在淋巴管增生,而且淋巴结转移组,低分化组与中晚期胃癌淋巴管数密度较高,与文献报道基本一致[4-6, 9]。由于新生淋巴管管壁不完善, 因此淋巴管数量增加预示有较大局部与远处转移的可能性。Yonemura 等[9]报道 VEGF-C 在胃癌组织中的表达率为 55%,在正常胃黏膜为 13%, 55% 胃癌组织表达 VEGFR-3

mRNA，VEGF-C 与 VEGFR-3 呈正相关，而且淋巴管数与分化程度，淋巴结转移，淋巴管浸润有关。本实验发现 VEGF-C 阳性组淋巴管数密度明显高于对照组，与文献报道一致[9, 12]，提示肿瘤细胞产生 VEGF-C，进一步诱导淋巴管内皮细胞 VEGFR-3 表达，通过 VEGF-C/VEGFR-3 旁分泌途径，促进肿瘤细胞周围基质内淋巴管增生，为肿瘤细胞淋巴道转移提供便利条件。文献报道 VEGF-C 表达以及 DLV 与患者 5 年生存率显著相关，是预测预后的有用指标[3, 4, 12]，但也有相反报道[13, 14]，需更多资料予以证实。

总之，VEGF-C 通过与 VEGFR-3 结合而促使淋巴管增生，有利于胃癌淋巴道与远处转移。

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