World Journal of Surgery and Surgical Research

Research Article
Published: 01 Apr, 2020

VEGF, VEGFR2 and VEGFR3 Protein Expressions in GIST Tissues

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Abstract

Gastrointestinal Stromal Tumor (GIST) is the most common mesenchymal tumors originating in gastrointestinal tract. Surgical resection and Tyrosine Kinase Inhibitors (TKIs) treatment are the standard of care for GISTs. But it's still hard for clinicians if an unresectable GIST is not sensitive to TKIs. Angiogenesis is a crucial event for tumor growth and it is regulated predominantly by Vascular Endothelial Growth Factor (VEGF) and its receptors (VEGFRs). In this paper, we perform Immunohistochemistry to analyze the VEGF, VEGFR2 and VEGFR3 protein expressions in 44 pairs of human GIST and matched control tissues. We find almost all the GIST tissue express VEGFR2 protein positively, and GIST tissues express higher levels of VEGF and VEGFR2 proteins than matched control tissues ($P<0.05$, Wilcoxon sign-rank test). This result is consistent with findings on other tumors. But the expression of VEGFR3 is very little in GIST. And there is no association between VEGF/VEGFR2 expressions and demographic variables of GIST tissues. We think the enhanced expression of VEGF, VEGFR2 in GIST is probable a universal phenomenon. But whether VEGF/VEGFR2 is an underlying therapy target for GIST need more research to analyze.

Keywords: VEGF; VEGFR2; VEGFR3; GIST

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Received Date: *26 Feb 2020*

Accepted Date: *27 Mar 2020*

Published Date: *01 Apr 2020*

Citation:

Miao H, Gang C, He-zhao Z, Hao S, Jing F, Wang ZW. VEGF, VEGFR2 and VEGFR3 Protein Expressions in GIST Tissues. World J Surg Surgical Res. 2020; 3: 1215.

Introduction

Gastrointestinal Stromal Tumor (GIST) which usually occurs in the stomach (60%) or small intestine (30%) is the most common mesenchymal tumors originating in the gastrointestinal tract. Surgical resection is the current standard of care for localized GISTs. And molecular-targeted therapy (such as tyrosine kinase inhibitors, TKIs) for GIST also has yielded remarkable outcomes. But we find TKI-resistant-GISTs are existent. It is hard for clinicians to make therapy decision if an advanced GIST, which R0 resection is not possible, is not sensitive to TKIs.

Angiogenesis is a crucial event for tumor growth and it is regulated predominantly by Vascular Endothelial Growth Factor (VEGF) and its receptors (VEGFRs). Vascular Endothelial Growth Factor (VEGF) contributes to tumor growth and metastasis directly targeting tumor cells. VEGF over-expression and high VEGF serum levels have been reported in tumors [1]. So what about GIST? In this paper we research the VEGF, VEGFR2 and VEGFR3 protein expressions on GIST tissues.

Materials and Methods

Ethics statement

All experimental procedures are approved by the Ethics Committee of the Chongqing University Cancer Hospital/Chongqing Cancer Institute/Chongqing Cancer Hospital. All the human tissues used in this research have the approval from patients.

Clinical specimens

Forty four pairs of human GIST tissues and matched non-tumor tissues (located >1 cm from tumor areas) are obtained from the Department of gastrointestinal tumor center of Chongqing University Cancer Hospital/Chongqing Cancer Institute/Chongqing Cancer Hospital. The characteristics of these tissues are included in (Table 1). All the tissues are fixed in formalin and embedded in paraffin for immunohistochemistry test, and the diagnosis of them is confirmed by pathological analysis.

Immunohistochemistry

The Streptavidin-Peroxidase (SP) method is adopted and performed. Primary antibodies specific to VEGF (ZA-0509), VEGFR2 (ZA-0287), VEGFR3 (ZA-0267) are purchased from Beijing Zhongshan Company, China. The SP Kit and DAB Kit are also obtained from Beijing Zhongshan Company, China. DAB staining is kept for 3 min consistently.

Evaluation of staining (for Immunohistochemistry)

VEGF and VEGFRs expressions are assessed by scoring the staining intensity and staining proportion. The staining intensity is recorded as negative = 0, light = 1, moderate = 2, or strong = 3. The staining proportion is recorded as 1 (≤ 25%), 2 (≤ 50%), 3 (≤ 75%), or 4 (>75%). The two values are multiplied for each slide to produce a terminal score. If the score is higher in GIST than in matched control tissues, this pair of tissues is marked with a "+" (corresponding to GIST express protein higher). The opposite condition is marked with a "-" (corresponding to GIST express protein lower). If the scores are the same, the pair is marked with a "0" (corresponding to similar protein expression in GIST and control). And terminal scores of 0-3 are defined as negative expressions; 4-12 are defined as positive expressions. The evaluation is also confirmed by pathologists.

Statistical analysis

Standard statistical analysis is performed using the SPSS 17.0 Package. The Wilcoxon sign-rank test (for immunohistochemistry analysis), χ^2 test and Fisher's exact test (for association analysis between protein expression and clinical parameters of GIST) are used in this study. $P<0.05$ is considered to be significant.

Results

Expressions of VEGF and VEGFRs in GIST tissues-Immunohistochemistry for VEGF and VEGFRs expressions in GIST.

We find GIST tissues express higher levels of VEGF and VEGFR2 proteins than matched control tissues (Figure 1). However, the expression of VEGFR3 is little and does not differ significantly between the GIST and control tissues. VEGF and VEGFR2 proteins are found distributed in cytoplasm and cytomembrane (Figure 2 and 3).

VEGF and VEGFR2 expressions are significantly enhanced in GIST tissues than in non-tumor tissues ("*": $P<0.05$, Wilcoxon sign-rank test). For VEGFR3, the expression difference is not significant ($P>0.05$, Wilcoxon sign-rank test).

Association between protein expressions and clinical characteristics of GIST tissues.

No association is found between the VEGF and VEGFR2 expressions and the demographic variables of GIST tissues ($P>0.05$, χ^2 test and Fisher's exact test, Table 1).

Discussion

Gastrointestinal Stromal Tumor (GIST), the most major mesenchymal neoplasm of the digestive tract, is characterized by KIT or Platelet-Derived Growth Factor Receptor Alpha (PDGFRA) activating mutations, which approximately account for 80% or 10% of GISTs respectively. The GIST incidence rate is of 6 to 14 cases per million people in the United States of America and Europe, and approximately 16 to 22 cases per million people in Asia [2]. GISTs are generally positive for CD117 (c-KIT) with a characteristic morphology

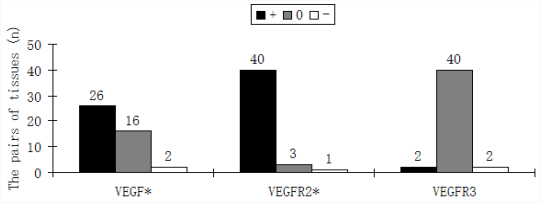

Figure 1: Comparison of VEGF and VEGFR expression in GIST and matched control tissues.
"+": expression is higher in GIST tissue than in matched non-tumor tissue.
"-": expression is lower in GIST tissue than in matched non-tumor tissue.
"0": expression is similar in GIST and in non-tumor tissue.

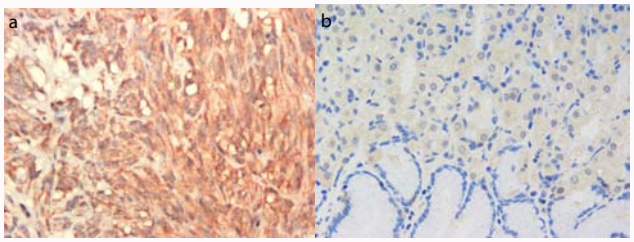

Figure 2: VEGF expressions in GIST and gastric mucosa tissue, x400.
(a) VEGF expression in GIST **(b)** VEGF expression in gastric mucosa tissue

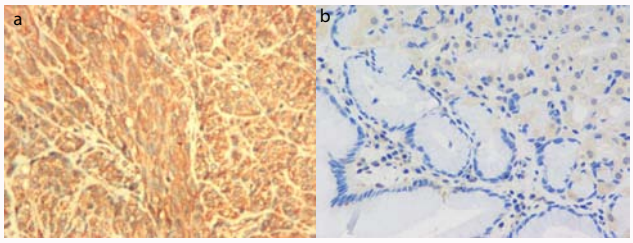

Figure 3: VEGFR2 expressions in GIST and gastric mucosa tissue, x400.
(a) VEGFR2 expression in GIST **(b)** VEGFR2 expression in gastric mucosa tissue

[3]. Traditionally, surgery was the only successful treatment approach for GISTs with a 5 year survival rate of 48 to 54 [4], while patients with unresectable or metastatic disease survived only for a median of 18 to 24 months after diagnosis with a 5 year survival rate of 5% to 10% [5].

Imatinib, a potent inhibitor of mutated KIT, has revolutionized the clinical management of advanced, metastatic GIST. Imatinib adjuvant therapy can reduce or delay the growth of microscopic tumors after complete resection of a GIST. And Neoadjuvant imatinib therapy should be considered for localized GIST when R0 resection is not feasible or for organ function preservation.

However, some GIST patients with primary or secondary imatinib resistance do not respond to this drug. Primary resistance is defined as progression within the first 6 months of imatinib therapy, with most cases progression multifocally [6]. Second resistance is defined as progression beyond 6 months after the initiation of imatinib therapy. Secondary resistance develops mainly through acquired mutations in KIT exons 13/14 or exons 17/18. Second-line sunitinib potently inhibits KIT exon 13/14 mutants but is ineffective against exon 17 mutation. Actually, medical treatment using Tyrosine Kinase Inhibitor (TKI) alone rarely achieves a complete response in advanced GISTs as resistant tumor clones develop continuously over time after the initiation of TKI treatment.

Table 1: Positive protein expression in differential clinical characteristics of GIST N (%).

Clinical characteristics			VEGF	VEGFR2
		N	Expression (%)	Expression (%)
Gender				
	Male	22	12 (54.5)	22 (100)
	Female	22	13 (59.1)	20 (90.1)
			P>0.05	*P*>0.05
Age (y)				
	≥ 50	30	19 (63.3)	30 (100)
	<50	14	6 (42.9)	12 (85.7)
			P>0.05	*P*>0.05
Tumor size (cm)				
	≥ 5	22	14 (63.6)	21 (95.5)
	<5	22	11 (50)	21 (95.5)
			P>0.05	*P*>0.05
Mitotic index (/50HPFs)				
	≥ 5	12	8 (66.7)	12 (100)
	<5	32	17 (53.1)	30 (93.8)
			P>0.05	*P*>0.05
Primary tumor site				
	Gastric	29	16 (55.2)	27 (93.1)
	other	15	9 (60)	15 (100)
			P>0.05	*P*>0.05
Risk category				
	Low risk	21	11 (52.4)	20 (95.2)
	High risk	23	14 (60.9)	22 (95.7)
			P>0.05	*P*>0.05
Gene mutation				
	c-KIT	28	15 (53.6)	27 (96.4)
	PDGFRA	10	7 (70)	9 (90)
	other	6	3 (50)	6 (100)
			P>0.05	*P*>0.05

Angiogenesis is a crucial event for tumor growth and it is regulated predominantly by several different growth factors. Vascular Endothelial Growth Factor protein family (VEGF) and its receptors (VEGFR) are probably the most important tissue factors responsible for angioblast differentiation and tube formation. The potent role of VEGF and VEGFR in tumor angiogenesis has been widely described in the past decade. VEGF/VEGFR2 over-expression and high VEGF serum levels have been reported in majority species of cancers [1,7]. For example, VEGF plays an important role in the development of pancreatic cancer and VEGFR-2 is the most important receptor in evaluating the angiogenesis in pancreatic cancer [8]. Inhibition of intracrine VEGF signaling strongly inhibits colorectal cancer cell migration and invasion by regulating proteins involved in cell motility [9]. And Apatinib (a VEGFR2 inhibitor) inhibit VEGF-mediated cell migration and invasion in Cholangiocarcinoma (CCA) cell lines via inhibiting the VEGFR2/RAF/MEK/ERK and PI3K/AKT pathways [7]. But the VEGF/VEGFR research on GIST is little. In order to provide new insights into GIST treatment, we try to understand whether VEGF and VEGFR also be expressed significantly in GIST. In this paper we find almost all the GIST tissue express VEGFR2 protein positively! And GISTs express the VEGF and VEGFR2 proteins more significantly than matched normal tissues. This result is consistent with the findings on other tumors before. And there is no association between VEGF, VEGFR2 expressions and demographic variables of GIST. This means no matter what the Gender, Age, tumor size, mitotic index, tumor site, Gene mutation are, the VEGF and VEGFR2 expressions are always enhanced in GIST. So we think the enhanced expression of VEGF, VEGFR2 in GIST is probable a universal phenomenon. VEGF and VEGFR2 may play important roles in GIST development, but this hypothesis needs to be confirmed by more experiments. And whether VEGF/VEGFR2 is an underlying therapy target for GIST is worthy of our attention.

Acknowledgment

Decision consultation and management innovation project in Shapingba district, Chongqing, 2019, Jcd201957.

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