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REVIEW

Circulating lymphangiogenic growth factors in gastrointestinal solid tumors, could they be of any clinical significance?

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Abstract

Metastasis is the principal cause of cancer mortality, with the lymphatic system being the first route of tumor dissemination. The glycoproteins VEGF-C and VEGF-D are members of the vascular endothelial growth factor (VEGF) family, whose role has been recently recognized as lymphatic system regulators during embryogenesis and in pathological processes such as inflammation, lymphatic system disorders and malignant tumor metastasis. They are ligands for the VEGFR-3 receptor on the membrane of the lymphatic endothelial cell, resulting in dilatation of existing lymphatic vessels as well as in vegetation of new ones (lymphangiogenesis). Their determination is feasible in the circulating blood by immunoabsorption and in the tissue specimen by immunohistochemistry and reverse transcription polymerase chain reaction (RT-PCR). Experimental and clinicopathological studies have linked the VEGF-C, VEGF-D/VEGFR3 axis to lymphatic spread as well as to the clinical outcome in several human solid tumors. The majority of these data are derived from surgical specimens and malignant cell series, rendering their clinical application questionable, due to subjectivity factors and post-treatment quantification. In an effort to overcome these drawbacks, an alternative method of immunodetection of the circulating levels of these molecules has been used in studies on gastric, esophageal and colorectal cancer. Their results denote that quantification of VEGF-C and VEGF-D in blood samples could serve as lymph node metastasis predictive biomarkers and contribute to preoperative staging of gastrointestinal malignancies.

Key words: Circulating VEGF-C and VEGF-D; Gastric; Oesophageal; Colorectal cancer; Preoperative staging; Lymph node metastasis predictive markers

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INTRODUCTION

Lymph node metastasis (LNM) is a major prognostic factor for most human solid epithelial tumors.

Although the phenomenon of lymphatic spread of tumors is well recognized for over a century, many aspects of cancer cells entrance, survival and proliferation in the lymphatic system remain unclear[1,2]. To date, the experimental findings regarding active lymphangiogenesis in human solid tumors are contradictory[3,4].

The molecular and functional mechanisms of lymphatic system regulation and cancerous involvement have only recently been recognized, mainly due to the discovery of lymphatic endothelial cells (LEC) specific markers (LYVE-1, Prox-1, podoplanin, VEGFR3) in the past decade[5,6].

THE VEGF-C, D/VEGFR-3 SYSTEM

VEGFR-3 (fms-like tyrosine kinase 4, Flt4) is one of the first LECs surface molecules to be identified. It is a member of the VEGFR family, also including VEGFR-1 (Flt-1), VEGFR-2 (KDR), and which belongs to the platelet derived growth factor receptor sub-family of receptor tyrosine kinases[7]. VEGFR-3 is present on all endothelia during

development, but in the adult its expression is restricted to LECs and certain fenestrated blood vascular ECs[8,9].

The importance of VEGFR-3 for the development of the lymphatic vasculature has been shown recently, where early onset primary lymphedema was linked to the VEGFR3 locus in distal chromosome 5q[10,11]. It also protects LECs from serum deprivation-induced apoptosis, induces their growth and migration, while one study on a corneal model showed that it could play a role in adaptive immunity[12,13]. Nevertheless, this LEC specificity seems to be lacking in cancer cell types, an observation which contributes to the difficulty of defining molecular regulation of LNM[14].

The vascular endothelial growth factor (VEGF) family of glycoproteins comprises the most crucial group of neovascularization regulators in development and disease (Table 1). Currently, it consists of 5 cytokines in mammals, VEGF, PIGF (platelet induced growth factor), VEGF-B, VEGF-C and VEGF-D, and in addition, *parapoxvirus* genome-encoded VEGF (viral VEGF, also denoted as VEGF-E) and snake venom-derived VEGF (also referred as VEGF-F)[17-19].

VEGF-C and VEGF-D subtypes have been identified as the ligands for the lymphatic endothelial receptor VEGFR3, as well as for the blood vessels endothelial receptor VEGFR2, while VEGF-C also binds the LEC surface molecule NRP-2[20-23].

Among the other family members, they share a central VEGF homology domain, but they differ because of the distinct presence of long N- and C-terminal propeptides. VEGF-C and VEGF-D are secreted as precursor proteins, which are cleaved to their VEGFR-specific active forms through a two-step proteolytic procedure[24-27]. The extent of the proteolytic process defines its receptor affinity and presumably its biological activity, although this connection is only partially understood. Both VEGF-C and VEGF-D are able to induce proliferation and migration of lymphatic endothelial cells *in vitro*[23].

The correlation of VEGF-C, D/VEGFR3 axis to the lymphatic spread of tumors is documented in several experimental models and clinicopathological studies in a variety of human malignancies.

The findings derived from malignant cell line models provide the most "direct" evidence for the implication of lymphangiogenic growth factors in tumor lymphatic spread[28-32]. However, the observed correlation does not explain the underlying mechanisms, nor does it clarify the role of active tumor induced lymphangiogenesis in cancer metastasis. Nevertheless, and more importantly, the inhibition of the ligand-receptor axis raised interest in anti-lymphangiogenic targeting research, a potentially promising novel field of cancer treatment[33-35].

Taking into account the rationale of VEGF-C and VEGF-D involvement in lymphatic system regulation, researchers throughout the world studied the expression of these growth factors in human tumors and their possible connection to metastatic potential[36,37]. The methodology used for "quantitative" determination was either immunohistochemistry (IHC), or RT-PCR to detect mRNA and subsequently the level of gene expression. These studies include a wide range of solid tumors (gastrointestinal, breast, genitourinary, melanoma, thyroid, head and neck), in an attempt to relate VEGF-C and VEGF-D expression to clinicopathological parameters (lymph node involvement, lymphatic and vascular invasion, clinical outcome).

Table 1 Mammalian vascular endothelial growth factor family of ligands

	Receptor	Chromosomal location	Angiogenesis	Lymphangiogenesis
VEGF	VEGFR1, VEGFR2, NRP1	6p23.1	+	Conflicting data[1]
VEGF-B	VEGFR1, NRP1	11q13	Conflicting data	-
PIGF	VEGFR1 NRP1, NRP2	14q24	Modest	Not determined
VEGF-C	VEGFR2 VEGFR3 NRP2	4q34	Modest	+
VEGF-D	VEGFR2 VEGFR3	Xp22.31	Modest	+

[1]Indirect lymphangiogenic effect, by recruiting VEGF-C and VEGF-D producing macrophages[15]. Lymphangiogenesis *via* alternative, VEGFR3-independe pathway[16].

The majority of such studies confirmed a positive correlation between growth factor expression and adverse oncological features. Yet, the results are not always consistent[38-41].

Conflicting results could be attributed to methodological considerations[42,43]: (1) Immunohistochemical quantification is a somehow subjective observer-dependant modality. Terms like "overexpression" are not always well defined, as they are based on variable scoring systems; (2) RT-PCR does not discriminate the location of mRNA expression among cancer cells, adjacent normal epithelium and stromal cells in a tissue specimen, nor does it necessarily reflect the actual protein level[44].

Van der Auwera *et al*[45] proposed a composite method for lymphangiogenesis quantification in solid tumors, in order to establish standardization of immunohistochemical assessment.

CIRCULATING VEGF-C AND VEGF-D IN HUMAN SOLID TUMORS

An alternative method of VEGF-C and VEGF-D quantification is indirect enzyme-linked immunoadsorption assay (ELISA), which measures the protein levels in peripheral circulation samples. This approach has been applied in selected studies during the last 5 years (Table 2).

The quantification of circulating cytokines has the advantage of being more objective approach, which lacks the drawback of interobserver variability. Moreover, as a preoperatively practicable modality, it exhibits a potential application as a readily available LNM marker and subsequently surgical decision making tool, particularly in cases such as: (1) Early malignant lesions, which bear a small, yet substantial risk of lymphatic dissemination and which, otherwise, could be treated with minimally invasive techniques; (2) Cancers which necessitate accurate preoperative staging, in order to employ stage-specific neoadjuvant therapy; (3) Cancers whose treatment approach relies on the presence or extent of lymph nodes metastasis.

Table 2 Studies on circulating VEGF-C/D in human solid malignancies (clinicopathological association)

Tumor	Marker	Sample	Cases (*n*)	LNM	Prognostic impact	Ref.
Gastric cancer	VEGF-C	Serum	80	$P = 0.001$	$P = 0.001$	67
Esophageal cancer	VEGF-C	Serum	70	$P = 0.022$	ND	102
Esophageal cancer	VEGF-C	Serum	73	(+)[1]	ND	103
Colorectal cancer	VEGF-D	Plasma	59	(-)	ND	38
Colorectal cancer	VEGF-C	Plasma	41	(+)[2]	ND	144
Colorectal cancer	VEGF-C	Plasma	120	(-)	ND	145
	VEGF-D			(-)		
Colorectal cancer	VEGF-C	Serum	66	(+)	ND	146
Breast cancer	VEGF-D	Plasma	51	(-)	ND	46
Breast cancer	VEGF-C	Plasma	122	(-)	(-)	47
Breast cancer[3]	VEGF-D	Plasma	142			48
Nsc[4] lung cancer	VEGF-C	Serum	92	$P = 0.0260$	ND	49
Nsc lung cancer	VEGF-C	Serum	78	$P = 0.0004$	ND	50
Nsc lung cancer	VEGF-C	Serum	116	$P = 0.0007$	ND	51
Cervical cancer	VEGF-C	Serum	78	$P = 0.0001$	$P = 0.0112$	52
Cervical cancer	VEGF-C	Serum	205	(+)[2]	(+)[2]	53
Prostate cancer	VEGF-D	Plasma	30	$P = 0.0043$	ND	54
HNSCC[5]	VEGF-C	Plasma	46	(-)	(-)	55

ND: Not determined; [1]Indirect result; [2]None statistically significant; [3]Post-treatment study; [4]Non small cell; [5]Head and Neck Squamous Cell Carcinoma.

Circulating lymphangiogenic growth factors have been investigated in malignant tumors whose lymph node status detection is crucial in terms of treatment planning, including cancers of the gastrointestinal tract.

Gastric cancer

Gastric cancer remains a leading cause of cancer mortality worldwide, despite its declining incidence in the West in the last decades, and lymph node metastasis is the most powerful prognostic factor in R0 resected cases.

Clinicopathological studies mainly from Japan, where gastric cancer is the most common malignancy, have correlated mRNA and immunohistochemical expression of VEGF-C and VEGF-D in gastric tumour cells with lymphatic invasion and lymph node metastasis[56-60]. Their quantitative expression has been reported as a prognostic factor[56,59], while the experimental blocking of the VEGFR-3 signalling pathway is under investigation[61]. Nikiteas *et al*[62] showed that VEGF is also implicated in lymphatic spread of gastric cancers, a finding reproduced in a Japanese population[63].

Studies on early gastric adenocarcinoma (EGC) have been carried out as well. Kabashima *et al*[64] found using immunohistochemistry that the incidence of positive expression of VEGF-C in lymphatic invasion-positive EGC (36%) was significantly higher than that in lymphatic invasion-negative EGC (14%). The incidence of positive expression of VEGF-C in nodes (+) or venous invasion-positive EGC tended to be higher than that in nodes (-) or venous invasion-negative EGC. Ishikawa *et al*[65] studied the expression of VEGF-C and VEGF-D in resection specimens related to tumors differentiation, concluding that in EGC of histologically undifferentiated type with negative expression of VEGF-C and -D, limited surgery might be safely applied because the possibility of nodal metastasis is very low. Onogawa *et al*[66] investigated whether expression of VEGF-C and/or VEGF-D correlates with clinicopathological features of submucosally invasive gastric carcinoma. VEGF-C immunoreactivity was associated with histological type, lymphatic invasion, lymph node metastasis, and microvessel density, while no association was identified between VEGF-D immunoreactivity and clinicopathological variables. Those studies suggest that the detection of VEGF-C and VEGF-D could play a role as an additional element of EGC local excision criteria.

One study on circulating VEGF-C in gastric cancer has been reported so far. Wang *et al*[67] investigated whether serum VEGF-C and immunohistochemicaly determined VEGF-C expression and lymphatic vessel density (LVD) in tumor tissues are related to lymph node metastasis and prognosis in gastric cancer. LVD was determined based on brown staining of endothelial cells with podoplanin under a 200-fold light microscopic field.

The sVEGF-C level was significantly ($P = 0.000$) higher in patients with gastric cancer (595.9 ± 201.0 ng/L) than in healthy donors (360.0 ± 97.4 ng/L). With a cut-off value for sVEGF-C of 367.5 ng/L, the sensitivity and specificity for diagnosis of gastric cancer patients was 85% and 80%, respectively ($P = 0.000$). VEGF-C positive expression was significantly ($P = 0.001$) higher in gastric cancer tissue (50/80) than in normal gastric tissue (4/20). There was significantly ($P = 0.000$) more LVD in the experimental group (10.7 ± 3.1/200 HP) than in control subjects (4.9 ± 1.3/200 HP). The sVEGF-C level was significantly ($P = 0.000$) higher in VEGF-C positive patients (675.4 ± 153.9 ng/L) than in negative patients (463.5 ± 200.4 ng/L). There was a positive correlation between sVEGF-C and LVD ($r = 0.728$, $P = 0.000$). LVD in VEGF-C positive and negative groups was 12.2 ± 2.8/200 HP and 8.3 ± 2.0/200 HP, respectively ($P = 0.000$).

With respect to clinicopathological correlations, sVEGF-C was significantly ($P = 0.001$) higher in differentiation degree G3 group, LNM (+) group, M (+) group and pTNM Ⅲ-Ⅳ group. With a 3 year follow up, the mean survival of patients with high (> 595.9 ng/L) sVEGF-C and low (< 595.9 ng/L) sVEGF-C was 29.1 ± 13.3 mo and 44.0 ± 4.6 mo, respectively ($P = 0.001$).

Clinical relevance: It is well known that a notable discrepancy exists between Japan and USA-Europe regarding gastric cancer, with respect to staging, surgical management and outcome[68-70]. The extent of lymph node dissection (D1 *vs* D2) is considered a major factor of curative outcome according to the Japanese. The reluctance of the Western surgical community to uniformly adopt this approach is supported with evidence derived by two large prospective randomized controlled studies conducted in Europe in the 1990's[71,72]. Both trials concluded that D2 dissection is followed by significantly higher morbidity and mortality, without an overall proven survival benefit. However, the same investigators revised their long term results[73-75] and acknowledged that the complications should be largely attributed to modifiable technical aspects, such as splenectomy and distal pancreatectomy and to limited experience, and most importantly, there is evidence that D2 dissection could be beneficial for a subgroup of patients, basically

those with stage Ⅱ and Ⅲa according to TNM. Similar conclusions are derived from non-randomized and retrospective studies from selected centers[76-80].

Taking into account the suggestion of stage-specific benefit, the next challenge would be to identify the candidates for extended lymph nodes dissection. Several modalities for preoperative staging have been studied; but, the results on nodal detection are insufficient to dictate an individualized surgical approach.

Imaging techniques are the most widely used, relying on morphologic criteria[81-85]. Abdominal CT scan is the most popular method, with poor specificity on detecting N status, as the sole factor is nodal size, and without capability for accurate number detection. Endoscopic ultrasound is considered more valuable for evaluating primary tumors; yet it is not proven superior to CT regarding nodal involvement evaluation. Positron emission tomography scan is helpful in detecting occult distant metastasis; but, has no role in regional nodal staging. Besides, modern imaging techniques bear a considerable cost and entail operator-dependant variables.

Invasive staging methods, such as laparoscopy, peritoneal cytology and intraoperative ultrasonography are certainly not "preoperative" and studies on sentinel lymph node biopsy using radiographic mapping seem promising in gastric cancer staging; yet, the latter necessitates trained personnel and special equipment and is not widely applicable[86,87]. Maruyama and co-workers developed a computerized database program to calculate the probability of individual lymph node station involvement, a model with limited clinical impact[88,89].

The connection of VEGF-C and VEGF-D to lymphatic spread, as previously shown in clinicopathological studies, provides the rationale that preoperative quantification of these cytokines could yield additional information regarding lymph nodes involvement in gastric cancer patients.

Wang *et al*[67] study converges as to that point. This study indicates that preoperative serum VEGF-C level might be a useful biomarker for the presence of LNM in patients with gastric cancer and a prognostic parameter to identify patients with poor outcome. Nonetheless, the researchers provide no evidence with respect to the extent of lymph node dissection they employed, and so no correlation can be made regarding prognostic significance of VEGF-C and extent of surgery.

Esophageal cancer

Cancer of the esophagus is a human malignancy with unfavorable prognosis, regardless histological type and despite the induction of multimodality approach in the treatment of this formidable disease. The prevalence of adenocarcinoma of the distal esophagus in particularly, is reported to increase in Western populations[90] and due to its location, bilateral lymph node metastasis to thoracic and abdominal cavity occurs with adverse prognostic aftermath.

Clinicopathological and experimental studies on VEGF-C/D expression in squamous cell (escc)[41,91-94] as well in adenocarcinoma (ac)[94-96] specimens have been published. Studies on escc resulted on a relatively consistent correlation of growth factors expression to tumor progression and lymphatic spread, while evidence for their role in ac is contradictory. Interestingly, all studies on adenocarcinoma come from the West.

It is worth noting that there is evidence which correlate lymphangiogenic growth factors to malignant potential of early or precancerous lesions. Auvinen *et al*[97] showed immunohistochemically that VEGF-C expression increases in Barett's epithelium as it progresses through dysplasia to adenocarcinoma and that VEGFR3 parallels this increase. Additionally, tumor-induced lymphatics were detected which could provide the route for systemic cancer dissemination. Ishikawa *et al*[98] examined the expression of VEGF-C and -D in 26 esophageal carcinoma cases and 11 dysplasia cases using IHC and found that active production of VEGF-C and -D was observed, not only in esophageal carcinomas, but also in some dysplastic lesions and in none of the normal mucosa specimens, raising the possibility that VEGF-C and -D might play positive roles in the early stage of esophageal carcinogenesis. Matsumoto *et al*[99] examined VEGF-C expression and tumor microvessel density of the primary tumors in escc and analyzed relationships between VEGF-C expression and clinicopathological findings, including lymph node micrometastasis (LMM), in 87 submucosal esccs. The findings indicate that in escc with submucosal invasion, VEGF-C overexpression of the primary tumor is a strong high risk factor for lymph node metastasis, including LMM.

Two studies on serum VEGF-C as biological marker in escc have been reported, both from the same department[101,102]. Krzystek-Korpacka *et al*[100] examined serum concentrations of VEGF-C in 70 patients with escc and 47 healthy individuals. However, only 23 patients were subjected to surgery, due to advanced disease of the remainder, which were staged using endoscopy, imaging modalities and laparoscopy. Median serum VEGF-C level (sVEGF-C) in escc patients was significantly elevated in comparison to controls (17.40 ng/mL *vs* 10.57 ng/mL, $P < 0.001$). Serum VEGF-C was significantly elevated when metastatic lymph nodes were present, as median sVEGF-C was 21.78 ng/mL in N0 *vs* 15.77 ng/mL in N1 cases ($P = 0.022$). The authors also examined the dependence of sVEGF-C and combined TN status of the examined cancers and found no stage-specific correlation, presumably due to a small sample of patients. The optimal cut-off value for application of sVEGF-C as a marker of the disease presence was calculated 14.57 ng/mL (mean ± SD), whereas 16.24 ng/mL (mean ± 1.5 SD) for detection of metastatic lymph nodes. The accuracy of sVEGF-C determination as a disease marker was 83.7% while 64.4% as a lymph node involvement marker. Moreover, in an effort to address the issue of tumor induced secretion, the authors correlated WBC and PLT count to TNM stage and concluded that WBCs parallel sVEGF-C levels, rather than contribute to their elevation.

The same team enrolled the former group of patients and controls in a study on circulating levels of midkine (sMK), a cytokine whose secretion found to be an escc marker and prognostic factor in Japanese populations[102,103]. Statistically higher sMK levels were found in cancer patients than in controls (1373 pg/mL *vs* 130 pg/mL) and in cases with lymph nodes metastasis (775 pg/mL in N0 *vs*

1893 pg/mL in N1). The utility of sMK as a LNM marker was calculated to have a 91.2% sensitivity and a 77.8% specificity. The best cut-off values calculated were 563 pg/mL for determination of the presence of disease and 937 pg/mL for evaluation of LNM. Correlations of sMK and TNM stage have been implied as well. Serum midkine levels correlated significantly with serum VEGF-C levels in N1 ($P = 0.008$) and combined N + M ($P = 0.001$) cases.

Clinical relevance: Surgical resection offers the only realistic chance for cure in patients with esophageal cancer and accurate preoperative staging is of outmost importance when surgery with curative intent is contemplated.

Esophagectomy procedures are associated with high morbidity and mortality, especially when performed in low volume centers[104,105], and even if successful, they negatively impact quality of life over a considerable period of time[106], so it is imperative to identify resectable cases among the patients. Lymph nodes involvement is a major determinant of resectability, as LNM beyond regional lymph nodes as defined by the American Joint Commission in Cancer[107] precludes surgical treatment, with a debatable exception of celiac axis involvement[108].

Despite the importance of R0 resection, prognosis of esophageal cancer remains bleak, and a multimodality strategy has been introduced currently, in an effort to improve curative outcome. This approach includes the combination of surgery, chemotherapy and radiotherapy. Nevertheless, much controversy exists regarding the appropriate combination of these modalities and the determination of patient categories which will mostly benefit from multimodality treatment[109-112]. Although hard evidence is lacking, the trend is to treat patients with locally advanced esophageal cancer (stage Ⅲ, T3-4, N1) with neoadjuvant chemoradiotherapy followed by surgery, a strategy which necessitates accurate preoperative staging.

Imaging techniques are again the mainstay of staging, including CT, EUS, EUS-FNA, FDG-PET and CT-PET, with variable sensitivity, specificity, and feasibility[113-117]. Moreover, the value of case volume has been reported to influence preoperative staging accuracy[118]. The fact that only a subgroup of patients within the same pathological stage benefit from neoadjuvant therapy raised the need to identify the cases with biological favourable tumors, so as to avoid unnecessary toxicity without concomitant survival benefit[109,119]. Imaging modalities in this setting are not sufficient, as they fall short of discriminating viable tumor from necrotic or scar tissue and to date there is no universally accepted morphological means of monitoring the response to neoadjuvant chemoradiotherapy[120-122].

The most innovative alternative is the identification of genetic and molecular markers of response to neoadjuvant therapy[123-125], including gene expression, genomic polymorphism, growth factors receptors, angiogenic factors, cell cycle regulators and apoptotic factors. Experimental studies provide promising data to incorporate such markers in multimodality and targeted treatment.

Krzystek-Korpacka *et al*[100] reported up-regulation of serum VEGF-C in esophageal squamous cell carcinoma, a finding which parallels VEGF-C expression in tissue specimens. They also correlated serum levels with the presence of lymph node metastasis and concluded that sVEGF-C up-regulation did not arise from platelets or white blood cells. Their results show that serum VEGF-C levels can be considered a biomarker of esophageal squamous cell cancer and a predictive molecular marker of lymph nodes metastasis in particular. This remark indicates a potential utility of serum lymphangiogenic growth factors in escc as a tool for early detection and LNM evaluation.

Colorectal cancer

LNM is a significant prognostic factor in colorectal cancer and a determinant of combined therapy regarding adjuvant as well as neoadjuvant treatment strategies.

Several clinicopathological studies on VEGF-C and VEGF-D tumoral expression have been reported, providing evidence that they correlate to LNM and prognosis[126-130], although findings are not always consistent[38,131]. Furodoi *et al*[131] detected VEGF-C expression at the deepest invasive site in 71 of 152 lesions (46.7%) and correlated it to histological grade, depth of invasion, lymph node metastasis, venous invasion, liver metastasis and Duke's stage. At the central portion and superficial part, there were no significant differences between VEGF-C expression and clinicopathological findings.

With respect to early lesions, Maeda *et al*[132] examined 221 endoscopically biopsied specimens from patients with T1 colorectal carcinoma prior to operation using IHC and found that VEGF-C expression was more frequently observed in tumors with nodal metastasis than in those without metastasis. Moreover, a multivariate analysis indicated that VEGF-C expression is an independent predictor of lymph node metastasis in T1 colorectal carcinoma. Kojima *et al*[133] investigated VEGF-C and VEGF expression at the invasive end of 65 T1 resected carcinomas and significantly correlated VEGF-C with the presence of LNM. Kazama *et al*[134] examined VEGF-C and VEGF-D expression in submucosal colorectal cancers and concluded that VEGF-C overexpression correlated with lymphatic involvement ($P = 0.01$) and lymph node metastasis ($P = 0.02$), but VEGF-D overexpression did not correlate significantly.

Limited studies on circulating VEGF-C, D in colorectal cancer (CRC) yielded conflicting results.

George *et al*[38] studied a sample of normal mucosa, adenomatous polyps and CRCs regarding IHC expression and RT-PCR mRNA expression of VEGF-C and VEGF-D and also plasma levels of VEGF-D. Plasma levels of VEGF-D were similar in normal controls, polyp patients, and CRC patients [median 494 (303-744) pg/mL, 416 (351-938) pg/mL, and 463 (291-745) pg/mL, respectively]. An interesting finding was an inverse balance of VEGF-C/VEGF-D mRNA expression in CRC samples, indicating that VEGF-D could act as a competitive antagonist to other family members.

Duff *et al*[135] measured plasma VEGF-C in 41 CRC patients and 31 normal controls. Median plasma levels of VEGF-C were 35.0 U/mL in colorectal cancer patients compared to 11.5 U/mL in controls ($P < 0.001$). VEGF-C levels tended to be elevated in patients with advanced disease (Dukes C and D) compared to early disease, but this was not statistically significant owing to a relatively

small number of patients in each group. Plasma levels of VEGF-C in their study may represent both partially processed and fully mature forms of the cytokine.

Nevertheless, another study by Duff *et al*[136], including 120 CRC patients, failed to show significant differences in plasma VEGF-C or VEGF-D levels between patients subgrouped by clinicopathological variables. In particular, there were no differences in median plasma VEGF-C or VEGF-D level in patients with and without lymph-node involvement (VEGF-C: 11.2 U/mL *vs* 9.9 U/mL; $P = 0.90$; VEGF-D: 335 pg/mL *vs* 316.5 pg/mL; $P = 0.68$).

Finally, in a study from China[137] 66 CRC patients and 30 controls were enrolled in quantification of serum levels of VEGF-C and VEGF. Serum VEGF-C and VEGF levels were reported higher in patients with colorectal carcinoma than in healthy controls as well as in patients with lymph node metastasis than those without lymph node metastasis. Serum VEGF-C levels reached a sensitivity of 81% and a specificity of 76% with a cut-off value of 1438.0 pg/mL.

Clinical relevance: Rectal cancer is the type of large intestinal malignancy whose management is the most challenging regarding surgical resection and preoperative staging. With the introduction of total mesorectal excision (TME), optimal surgical technique is considered the most pivotal factor influencing curative outcome[138-140].

However, major rectal surgery is technically challenging, related to increased risks and can not eliminate local recurrence rates. Additionally, oncological resections may lead to debilitating functional results and substantially influence quality of life. Currently, treatment of rectal cancer should be individualized and evaluation of the extent of primary cancer is essential for planning the appropriate therapy regimen, spanning from simple local excision to complex multimodality treatments.

Transanal excision is considered an acceptable alternative to radical resection when treating intramural cancer without distant spread (T1N0M0). This approach is followed even with curative intent in some centers when confronting low-risk tumors with highly favourable features[141]. Major advantages are low morbidity and mortality rates and excellent functional outcome. On the other hand, T1 tumors are related to up to 12% risk of LNM[142,143] and to recurrence rates of 10%-25% following local excision, with a fatal result for half of these patents[144-146]. The key for these unsatisfactory results could lie in imperfect preoperative staging and unrecognizable biological behaviour.

Neoadjuvant chemoradiotherapy (CRT) has been shown to significantly reduce local recurrence rates of locally advanced rectal cancer (T3-4, N0-1) without concomitant proven benefit regarding overall survival while its effect on sphincter preservation is controversial[140,147]. Nevertheless, preoperative radiation is not without of toxicity, postoperative complications and considerable cost[148,149]. Moreover, studies evaluating treatment outcome after neoadjuvant CRT have demonstrated improved survival in responding patients compared to partial or non-responders[150,151]. These findings highlight the need for accurate preoperative staging so as not to overtreat unsuitable patients, but also for identification of biologically favorable cancers in order to predict an optimal response.

Pre-treatment clinical staging of rectal cancer is based on integration of information obtained from digital examination, endoscopy and imaging modalities. Endoluminal imaging is considered the most valuable in locoregional staging. Endorecal ultrasonography is reported to have the best accuracy in nodal staging with a mean rate of 75%, although its performance may be overestimated in the literature due to publication bias[152,153]. MRI techniques with endorectal coil is the best means for evaluating T stage and circumferential resection margin, yet LNM detection remains problematic because it relies on non specific morphological criteria[154,155].

To date, there are no clinically useful molecular predictors of response to preoperative CRT which could assist to better patient selection[156]. The clinicopathological correlations of VEGF-C and VEGF-D in colorectal cancer, including early lesions, provide evidence that these cytokines play a role in colorectal LNM. Studies on circulating levels are contradictory, yet they do not discriminate between colon and rectal cancers and as a consequence their results can not be clarified.

CONCLUSION

The role of lymphangiogenic growth factors VEGF-C and VEGF-D in malignant tumors metastasis is a novel field of cancer research. The results of current studies on their tumoral expression are strongly indicative of an active involvement of these cytokines to lymphatic spread, although the experimental and clinicopathological findings are not always consistent. Determination of circulating levels in preoperative blood samples might be a useful marker of advanced disease and a predictive factor of lymph node metastasis in gastric, esophageal and colorectal cancer, providing an additional tool in pre-treatment planning. Available studies are currently scant, with limited sample size and inadequate to conclude more than a presumption of a potential application of VEGF-C and VEGF-D in diagnostic and therapeutic regimens. However, modern research on understanding the mechanisms of lymphangiogenesis in human solid tumors is intensive and further studies on circulating growth factors are both desirable and justifiable in order to refine their role as nodal status biomarkers in gastrointestinal malignancies.

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