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Angiogenesis in gastrointestinal stromal tumors: From bench to bedside

Angiogenesis in GIST: From bench to bedside

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Abstract

Gastrointestinal Stromal Tumors (GISTs) are rare neoplasms with an estimated incidence from 0.78 to 1-1.5 patients per 100,000. They most commonly occur in the elderly during the eighth decade of life affecting predominantly the stomach, but also the small intestine, the omentum, mesentery and rectosigmoid. The available treatments for GIST are associated with a significant rate of recurrent disease and adverse events. Thorough understanding of GIST's pathophysiology and translation of this knowledge into novel regimens or drug repurposing is essential to counter this challenge. The present review summarizes the existing evidence about the role of angiogenesis in GIST's development and progression and discusses its clinical underpinnings.

Key Words: GIST; cancer; oncology; angiogenesis; gastrointestinal oncology; stromal tumors

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Core Tip: Thorough understanding of GIST's pathophysiology and translation of this knowledge into novel regimens or drug repurposing is essential to counter this challenge. The present review summarizes the existing evidence about the role of angiogenesis in GIST's development and progression and discusses its clinical underpinnings.

INTRODUCTION

Gastrointestinal Stromal Tumors (GISTs) are rare neoplasms with an estimated incidence from 0.78 patients to 2 patients per 100,000^{[1],[2]}. Their highest prevalence is noted during the eighth decade of age, when they affect up to 3.06 individuals per 100,000^[3]. GIST typically present as subepithelial masses mainly in stomach (60%) and

small intestine (20-30%) with omentum, mesentery and rectosigmoid areas being less-frequently involved areas^[4]. According to their primary location, GISTs could clinically present as gastrointestinal hemorrhage, anemia, dyspepsia or vomiting when the upper gastrointestinal tract is involved and as bowel obstruction, frequent urination or diarrhea in implication of the lower gastrointestinal tract^[5]. The metastatic disease principally concerns the liver, omentum, and peritoneum presenting as abdominal pain or constipation while extra-intestinal metastases to lymph nodes and lungs are infrequent^[4]. The pathological diagnosis relies on the tissue's morphological and molecular characteristics. Based on their morphology, GISTs are classified into three groups according to the predominant cell type: spindle cell type (70%), epithelioid cell type (20 %) and a mixed type (10 %). CD117 comprises a transmembrane protein which is the end-product of the *c-kit* expression^[6]. The KIT (CD117) positivity in immunohistochemistry (IHC) in tissues which are morphologically consistent with GIST establishes the diagnosis in the 95% of the cases. In KIT negative cases, the discovered On GIST 1(DOG1) and CD34, which is an antigen of the myeloid progenitor cells, staining or the documentation of KIT or platelet-derived growth factor receptor(PDGFR) gene mutations are sufficient to institute a diagnosis. Seldom in pediatric and young populations, GIST formation arises in the context of succinate dehydrogenase-deficiency in conjunction with paragangliomas and pulmonary chondromas^{[7],[8]}.

The pharmacologic targeting of angiogenesis in cancer therapeutics was introduced as a groundbreaking approach. Nevertheless, the anti-vascular endothelial growth factor (VEGF) targeting alone or in conjunction with chemotherapy displayed only modest benefit in overall survival(OS) in solid tumors indicating the complexity of the mechanisms that regulate tumor angiogenesis^[9]. Thus, the necessity arose to develop a broad spectrum of anti-angiogenic treatments such as: direct VEGFR2 antagonists (ramucirumab), VEGF-Traps (aflibercept), several receptor tyrosine kinases inhibitors (RTKIs) targeting the platelet-derived growth factor receptor(PDGF-R), CD117 (c-KIT), fibroblast growth factor receptors (FGFR), epidermal growth factor receptor (EGFR),

RET, RAF kinases and the repurposing of drugs like the mammalian target of rapamycin inhibitors and lenalidomide^{[9],[10]}. In fact, anti-angiogenetic therapy has gained ground in the management of advanced, unresectable disease. Imatinib, an abl, c-KIT and PDGF-R tyrosine kinase inhibitor(TKI), constitutes the empiric treatment when the mutational status of the disease remains unknown and the first line of treatment in KIT and PDGFRA positive metastatic, inoperable GISTs. The D842V mutation in *PDGFRA* comprises a therapeutic exception and is being treated with avapritinib while *KIT* and *PDGFRA* wild type tumors are treated with sunitinib or regorafenib^[11].

All the above mentioned drugs achieve, at least partially, their cytotoxicity disrupting signaling pathways which are implicated in angiogenesis, as it would be further analyzed below. This suggests that angiogenesis might be of paramount importance for the carcinogenesis process in GISTs and an attempt to summarize all the pre-clinical and clinical data would be of great value.

THE ROLE OF ANGIOGENESIS IN GIST'S DEVELOPMENT AND PROGRESSION

The molecular mechanisms of angiogenesis in GISTs - Preclinical data

The regulation of angiogenesis is necessary for cancer cells initially to cope with their increased metabolic needs and in the process to promote their metastatic potential. Its significance was firstly recognized by J. Folkman *et al*, which stated that the magnified rate of neovascularization compared with wound healing and inflammation as a result of an interplay between tumor cells and endothelial cells was a prerequisite in order to achieve tumor growth^[12]. Presently, it is widely known that the angiogenic process is being coordinated by the balance of several angiogenesis inducers and inhibitors in tumor's microenvironment. The dominance of the pro-angiogenetic factors, a phenomenon called "angiogenic switch"^[13], triggers the angiogenesis and could result either as result of the consequent hypoxia from the increased tumor proliferation or by

the immune cell infiltration^[14]. The primary induction phase with the undeveloped vessels paves the way for the remodeling phase when the blood vessel generation is sustained^[15]. Several models of angiogenesis have been described explaining partially the poor outcomes of the selective angiogenic blockage as certain tumors can utilize alternative modes of angiogenesis^[14]. Their analytical presentation has been done elsewhere^{[14],[16],[17]} and goes beyond the scope of this review but a brief presentation in Table 1 would be helpful.

Angiogenetic Mechanism

Function

Implicated Signaling/ Pathways

Sprouting angiogenesis

Vessel formation from a parental one as a sprout outgrowth

VEGF, Dll4/notch pathways and neuropilins

Intussusceptive Angiogenesis

Splitting of a parental vessel into two newly formed

VEGF, PDGF pathways and erythropoietin

Vasculogenesis/ Endothelial progenitor cells

Vessel formation from endothelial progenitor cells differentiating into mature endothelial cells

VEGF pathway, chemokines

Vasculogenic mimicry

Vessel-like formations without endothelial cells

Hepatocyte growth factor receptor (HGFR)

Trans-differentiation of cancer stem cells (CSCs)

CSC give rise to endothelial cells

Tie-2, TGF- β , CXCL12/CXCR4

Table 1 The basic mechanisms of angiogenesis. PDGF-R: platelet-derived growth factor receptor, VEGF: vascular endothelial growth factor, HGFR: hepatocyte growth factor receptor, TGF- β : Transforming growth factor beta, CSCs: cancer stem cells, CXCL12: c-x-c motif chemokine ligand 12, CXCR4: c-x-c motif chemokine receptor 4

Xenograft studies in mice constitute an invaluable source of evidence about the angiogenetic mechanisms in GISTs. Our fundamental conceptualization about the orchestration of the angiogenetic process descended from Giner F. *et al* They utilized an intensely CD117, DOG1 and CD34-positive GIST with continual Ki-67 expression in about 15% of the tumor's mass. The neovascularization experiments demonstrated the propagation of the induction phase during the first 96 h after implantation which proceeded by the remodeling phase. The induction phase was guided by the VEGF, VEGFC, PDGFA, PDGFB gene expression in conformity with their receptors. In more detail, the IHC data indicate that the VEGF ligand and the VEGFR2, VEGFR3 were positive at day4 after the xenografting. As regards the chemokine expression, CXCL9, CXCL10, GRO and their receptors CXCR3, CXCR2 were stained in tumor cells and stroma soon after the implantation with a slight staining predominance of the chemokine receptors. These effects are possibly orchestrated by HIF1 α and the CXCL12/CXCR4 axis, which are constantly expressed^[18].

The angiogenetic process in GIST has been further delineated and several regulatory molecules have been identified. CCL2 represents a chemokine expressed by the tumor cells to attract CCR2-expressing endothelial progenitor cells from the circulation as documented in HER-2/neu-driven breast cancer^[19]. On the other hand, the VEGF-induced NF- κ B upregulation is frequently utilized to attract inflammatory cell into tumor to stimulate the angiogenesis^[20]. The bromodomain and extraterminal domain (BET) family mediates immunity regulating several signaling pathways^[21]. In GISTs, the BRD4 upregulation enhanced the migratory and invasion processes regulating angiogenesis through the NF- κ B/CCL2 signaling pathway. The BRD4-expressing cells

attract tumor-associated macrophages (TAMs) *via* the expression of CCL2 potentiating the tumor's microvessel density and secrete various pro-angiogenic molecules such as VEGFA, LOX and MMP9^{[22],[23]}. Towards the same direction, mutations of the protein phosphatase 2, regulatory subunit A, alpha (PPP2R1A) affect the carcinogenesis process^{[24],[25]}. In GISTs, mutations in *PPP2R1A* gene are found in nearly 20% of the cases and correlate with a more aggressive tumor phenotype. They result in increased growth rate *via* enhancing phosphorylation of c-kit, Akt1/2, ERK1/2 and WNK1. The latter seems to mediate the regulation of the angiogenic process^{[26],[27]}. A further analysis of the specific mechanisms would be of great value and it should be applied.

Furthermore, while the contribution of epigenetic mechanisms in the GIST progression is well established, its impact in the angiogenic mechanisms could be further delineated. Several gaps in our understanding that remain unaddressed by the subdivisions according to the driver gene mutation status could be further elucidated by the tumor's epigenetic landscape. The alterations in the tumor's methylation profile are associated with a more aggressive phenotype^[28] and the methylation status of the CD133 could reshape the management of the disease and it would be presented below in more depth^[29]. The KDM4 family members (KDM4A-D) reshaping the structure of chromatin are implicated in the pathogenesis of a wide variety of cancers^[30]. In GIST, the upregulation of KDM4D potentiates the angiogenesis *in vivo*, as indicated by the overexpression of CD31 in IHC. These effects are mediated by the HIF1 β /VEGFA pathway in the presence of demethylation in the promoters of the *H3K9me3* and *H3K36me3* genes^[31].

Finally, it is worth mentioning that several multi-TKIs exert their anti-tumor efficacy at least partially by the inhibition of angiogenesis. Cabozantinib exerts its activity inhibiting the receptor tyrosine kinases MET, VEGFR2, Flt-3, c-Kit and RET^{[32],[33]} while sorafenib inhibits the signaling of VEGFR-1, VEGFR-2, VEGFR-3, PDGFR- β , Flt-3, c-Kit and the Raf serine/threonine kinases^[34]. Both diminish the tumor's microvascular density (MVD) as evidenced by CD31 IHC^{[35],[36]}.

The association between imaging data and angiogenesis in GISTs

There have been several classification systems to stratify the malignant potential of GISTs such as: the ¹ National Institutes of Health (NIH) consensus criteria (Fletcher's criteria), the Armed Forces Institute of Pathology (AFIP) criteria (Miettinen's criteria) or the International Union against Cancer (UICC) TNM classification. Their main drawback constitute the inability to validate the tumor's aggressiveness without surgical resection and detailed pathologic examination of the entire tumor to estimate the mitotic count^{[37],[38]}. Although taking into consideration the current therapeutic trends, the management of the advanced, unresectable disease is unequivocal, there are margins for improvement in the management of primary localized disease, especially in small-sized tumors. It could not be emphasized enough that even small GISTs could develop malignant behavior. Thereat, it could provide us a wealth of valuable predictive and prognostic information an attempt to incorporate imaging data about the vascularization of the tumor such as the vessels' irregularity or the blood perfusion^[39].

The above mentioned gap was attempted to be filled by a landmark study by Iannicelli E. *et al* The computed tomography (CT) constitutes the fundamental imaging modality in patients presenting with the clinical manifestations of GIST. Reviewing past literature, several studies have documented that aim to associate certain imaging features with pathologic parameters^{[40],[41]}. Iannicelli E. *et al* presented that GISTs with irregular margins tended to have superior mitotic rate than tumor with regular margins. Furthermore, a heterogenous pattern of contrast enhancement (CE), the angiogenesis and necrosis correlated with an increased tumor size and a more aggressive clinical behavior. It worth mentioning that the intensity of contrast enhancement although it represents a novel mark of biologic activity, was not correlated with neither the number of mitoses nor the tumor's risk stratification^[42]. The above comprise an indirect link between tumor's margins and mitotic rate, which is essential in order to stratify before surgery the clinical behavior of the tumor and highlight the importance of angiogenesis in disease progression. The latter could also be deduced by dynamic PET (dPET) analysis. Strauss L *et al* reported an association between the rate in which the F-18-fluorodeoxyglucose (FDG) diffused into the tumor

with the expression of VEGF-A^[43]. The main limitation of CT comprises its low sensitivity as regards the imaging of vascularity in small sized tumors^[39]. This divergence could be addressed by the endoscopic ultrasound (EUS) technology.

The utilization of EUS has emerged during the last decades. Its ability to evade the intervention of the abdominal fat and gastrointestinal gas in conjunction with the capability of FNA biopsy render it a useful tool towards a more personalized approach in the management of GIST. In EUS the GISTs are visualized as hypoechoic masses arising from the muscularis propria or the muscularis mucosae. The presence of irregular margins, cystic areas or malignant lymph nodes (LN) herald bad prognosis^[44]. The usage of contrast media enhances further the diagnostic capacity of the EUS and promotes the tumor's vascularity as a valuable prognostic biomarker. The role of CE-EUS in the management has been extensively reviewed elsewhere^[45] and we intend to delineate the fundamentals. Sakamoto H. *et al* classified the tumor's vascularity into two subgroups according to the pattern of perfusion (homogenous or heterogeneous) and vessel appearance (regular or irregular). The homogenous perfusion with regular vessels were considered as signs of mild clinical behavior. Furthermore, they compared the diagnostic sensitivity of contrast-enhanced harmonic US (CEH-US), Power-Doppler EUS (PD-EUS) and CE-multidetector CT (CE-CT) to visualize tumor vessels. In GISTs larger than 3cm their sensitivities were 100%, 75% and 42% respectively. The differences became more emphatic in tumors less than 3cm: 100%, 25% and 0%, respectively. It was noteworthy that every malignant lesion less than 3cm in the cohort had been detected by the CEH-EUS before surgery^[39]. The above indicate that CE-US comprises a powerful tool to visualize vascularity. Taking a step further, Yamashita Y *et al* demonstrated an association between the imaging findings on CE-US and the pathologic risk stratification. In more depth, the large vessels lacked elastic tissue, indicating that neovascularization constitutes the underlying pathogenetic mechanism, and expressed VEGF^[46].

It becomes evident that the imaging findings of vascularity might be sensational and practice changing in a subset of patients with small sized tumors (<3cm) and aggressive

phenotype. A more substantial body of evidence should be collected in order to address properly those dilemmas.

Angiogenesis mediators as biomarkers in GIST – Clinical data

The development of biomarkers comprises an essential step towards the individualization of medical practice. Liquid biopsy provides a cutting-edge, non-invasive technology to access predictive information to guide the therapeutic management in a wide variety of diseases^{[47],[48],[49],[50],[51]}. It's application in GIST treatment has been started to emerge^{[52],[53]}. Reviewing subsequent and more recent literature, an extensive number of studies has been found associating molecules implicated in angiogenesis with pathologic features. Although there are several limitations in the above mentioned research, the importance of angiogenesis in GIST's malignant progression is delineated. In table 2 are summarized the most significant data.

CONCLUSION

As highlighted above, angiogenesis mediates an extensive proportion of GIST's malignant dynamics. Several signaling pathways are implicated in the regulation of angiogenesis such as: the vascular endothelial growth factors (VEGF), the fibroblast growth factor-2 (FGF2), the platelet derived growth factor (PDGF), the angiopoietins, the Eph/ephrin signaling, the Apelin/APLNR pathway, the hypoxia-inducible factors (HIFs) and several chemokines^[14]. The VEGF signaling comprises the most well-studied pathway in GIST angiogenesis.

The FGF2/R2 signaling has been extensively studied in GIST as a drug resistance mechanism. Boichuk S. *et al* demonstrated that the blockage of FGFR2 signaling could enhance the responsiveness to DNA-Topoisomerase II inhibitors^[64] while the downregulation of FGF2 signaling might stimulate the response to imatinib^[65]. It's contribution in GIST progression has been reviewed^[66] but data about potential effects in GIST vascularization process are missing. Towards the same direction, the

Eph/ephrin system has been investigated in carcinogenesis^{[67],[68]}. It would be of paramount importance an attempt to outline its contribution in GIST angiogenesis.

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