Vascular Endothelial Growth Factor C Disrupts the Endothelial Lymphatic Barrier to Promote Colorectal Cancer Invasion

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BACKGROUND & AIMS: Colorectal cancer (CRC) is highly metastatic. Metastases spread directly into local tissue or invade distant organs via blood and lymphatic vessels, but the role of lymphangiogenesis in CRC progression has not been determined. Lymphangiogenesis is induced via vascular endothelial growth factor C (VEGFC) activation of its receptor, VEGFR3; high levels of VEGFC have been measured in colorectal tumors undergoing lymphangiogenesis and correlated with metastasis. We investigated VEGFC signaling and lymphatic barriers in human tumor tissues and mice with orthotopic colorectal tumors. **METHODS:** We performed immunohistochemical, immunoblot, and real-time polymerase chain reaction analyses of colorectal tumor specimens collected from patients; healthy intestinal tissues collected during operations of patients without CRC were used as controls. CT26 CRC cells were injected into the distal posterior rectum of BALB/c-nude mice. Mice were given injections of an antibody against VEGFR3 or an adenovirus encoding human VEGFC before orthotopic tumors and metastases formed. Lymph node, lung, and liver tissues were collected and evaluated by flow cytometry. We measured expression of vascular endothelial cadherin (CDH5) on lymphatic vessels in mice and in human intestinal lymphatic endothelial cells. **RESULTS:** Levels of podoplanin (a marker of lymphatic vessels), VEGFC, and VEGFR3 were increased in colorectal tumor tissues, compared with controls. Mice that expressed VEGFC from the adenoviral vector had increased lymphatic vessel density and more metastases in lymph nodes, lungs, and livers, compared with control mice. Anti-VEGR3 antibody reduced numbers of lymphatic vessels in colons and prevented metastasis. Expression of VEGFC compromised the lymphatic endothelial barrier in mice and endothelial cells, reducing expression of CDH5, increasing permeability, and increasing trans-endothelial migration by CRC cells. Opposite effects were observed in mice and cells when VEGFR3 was blocked. **CONCLUSIONS:** VEGFC signaling via VEGFR3 promotes lymphangiogenesis and metastasis by orthotopic colorectal tumors in mice and reduces lymphatic endothelial barrier integrity. Levels of VEGFC and markers of lymphatic vessels are increased in CRC tissues from patients, compared with healthy intestine. Strategies to block VEGFR3 might be developed to prevent CRC metastasis in patients.

Keywords: Colon Cancer; Mouse Model; Tumor Progression; VEGFC.

Colorectal cancer (CRC) is the second most common cause of cancer-related mortality in the Western world.[1] Although it can spread in a variety of ways, lymphatic vessel (LV) invasion and metastasis are common in CRC's early stages.[2] Previous studies have shown that vascular endothelial growth factor (VEGF) family members and their receptors (VEGFR) contribute to lymphangiogenesis and the metastatic process in CRC.[2] In fact, high levels of VEGFC and its receptor have been described to promote tumor-associated LV expansion, thus becoming one of the most efficient pathways in regulating lymphangiogenesis, during CRC dissemination.[3–5] However, functional studies regarding lymphangiogenesis and lymphatic metastasis are still lacking in CRC. The lymphatic system plays critical roles in the maintenance of fluid homeostasis, immune response, and cancer progression,[6] and to exert its biological functions it undergoes lymphangiogenesis, a process of growth and expansion of the lymphatic vessels. Lymphangiogenesis is activated by the binding of VEGFC and VEGFD to their receptor VEGFR3,[7] even if no association of VEGFD expression has been found with CRC lymphatic spread.[8] Even though VEGFC interacts with VEGFR2, which is primarily involved in the induction of angiogenesis,[9] one of the major physiological functions of this growth factor is generally accepted to be the inducer of LV growth.[10]

Tumor metastasis is responsible for most cancer-related deaths. The metastatic process can be described in several steps, which involve the transmigration of cancer cells from the primary tumor into the microvascular or the lymphatic endothelium (intravasation and/or extravasation), and subsequently, the formation of a secondary tumor at a distant targeted organ.[11] The experimental manipulation of VEGFC, together with the blocking of VEGFR3 activation, has been studied in several cancer animal models, providing functional evidences that VEGFC is able to induce tumor lymphangiogenesis, and concomitantly promote metastasis formation in various distant organs.[12–14] One of the main restrictions of tumor cell intravasation through the lymphatic system is LV integrity,[15] which is strictly related to the organization of interendothelial adherens junction

Abbreviations used in this paper: **CRC, colorectal cancer; HIBEC, human intestinal blood endothelial cell; HILEC, human intestinal lymphatic endothelial cell; LN, lymph node; LV, lymphatic vessel; MFI, mean fluorescence intensity; SAR, SAR131675; VE-Cad, vascular endothelial cadherin; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.**

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