Angiogenesis in liver metastasis of colo-rectal carcinoma

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TABLE OF CONTENTS

1. ABSTRACT

In liver metastasis of color-rectal carcinoma, angiogenesis is supported by vascular endothelial growth factor (VEGF) and E-selectin while it is suppressed by Netrin-4 and LK-68. Sinusoidal endothelial cells and the hepatocyte-derived extracellular matrix support growth of microvessels in liver metastases of color-rectal cancers. Based on these insights, the new treatment for liver metastasis of color-rectal cancers target diverse pathways and molecules of angiogenesis, especially the VEGF pathway. Additional agents target the cyclooxygenase-2 pathway or follow regimens for inhibiting angiogenesis. In this review, we discuss angiogenesis and treatments avaiable for color-rectal liver metastasis.

2. INTRODUCTION

Color-rectal cancer (CRC) is the second most common cancer in women and the third most common in men, with 1.2 million new cases annually worldwide. CRC is responsible for approximately 9% of all cancer-related mortality (1). In the United States, approximately 143,000 patients are diagnosed with CRC each year and one-third of patients die annually from this disease (1). While the prognosis of American Joint Committee on Cancer (AJCC) (2) stages I and II CRC is good, with 5-year survival rates after treatment of 82% for stage II and 93% for stage I (3), untreated metastasis to the liver has survival rates of only 0 to 3% (4-8). The liver is the most common site of CRC metastasis. About 25% of patients present with liver

metastases at diagnosis and about 70% of patients develop liver recurrence after radical surgery for CRC (50% of patients with stage III and 20% with stage II cancer) (9). However, treatment of CRC metastases is complicated and still controversial (10,11). Thus, knowledge about CRC liver metastasis is important for prevention and therapy in order to improve patients' outcome.

Angiogenesis is the formation of new blood vessels (12) and is used by tumors to promote growth and metastases (13). After the prevascular phase of neoplastic growth, endothelial cells migrate towards the tumor and proliferate inside it, forming capillaries. The newly formed vessels pass nutrients to the tumor stroma and allow cancer cells to enter into the circulation. In this vascular phase, the neoplasia grows quickly and the risk of remote metastasis increases. In this stage, the blood supply is vital for both the tumor's potential for continued growth and metastasis, and the primary causes of cancer-related death (14). In CRC, angiogenesis is such a hallmark of tumor growth and metastasis that high levels of tumor angiogenesis are associated with advanced tumor growth, distant metastases, and an adverse prognosis (15).

Vascular endothelial growth factor (VEGF) and hypoxia-inducible factor 1 alpha (HIF-1alpha), two important regulators of angiogenesis, are reported to significantly promote CRC liver metastasis (16). VEGF secreted by tumor cells can enhance neovascularization and proliferation of vascular endothelial cells to accelerate differentiation and formation of new microvessels around the tumor tissues. This enhances tumor invasion and metastasis (17). HIF-1alpha activates the expression of the VEGF gene by binding to a hypoxia-response element in the VEGF promoter region (18,19). VEGF isoforms are associated with liver metastasis and poor prognosis in CRC (20). Therefore, it's urgently needed to understand the mechanisms of color-rectal liver metastases and tumor angiogenesis. In this review, we discuss angiogenesis in color-rectal liver metastasis and the development of antiangiogenesis therapies.

3. MECHANISM AND EFFECT OF ANGIOGENESIS ON CANCER: DOWNSTREAM

3.1. Endogenous factors

The production of angiogenic factors is controlled by intrinsic properties of the tumor cell and the host microenvironment. Among the complex and endogenous factors associated with angiogenesis, VEGF is an important inducer. In response to VEGF, vessels in the tumor transition from normal nonproliferating host vessels to tumor vessels (21). In a mouse model of experimental liver metastasis, VEGF regulates human CRC tumorigenesis (22). When VEGF targets the flk-1 receptor, the VEGF-flk-1 pathway promotes tumor angiogenesis in color-rectal liver metastasis and this is associated with bcl-2 (23). However, the precise mechanism of this process need further study.

In addition to VEGF, E-selectin, which mediates the initial step of leucocyte adhesion to activated vascular

endothelium, is increased in the plasma of patients with color-rectal liver metastases. This finding reflects the increased neovascularization of metastases (24). Endothelin 1, another tumor growth stimulator and angiogenesis factor, is observed in endothelial cells, tumor cells and myofibroblasts of color-rectal liver metastases, and is increased in the plasma of patients with color-rectal liver metastases (25). The increased levels of E-selectin and endothelin 1 in peripheral blood and the upregulated expression of endothelin 1 in local metastases suggest that E-selectin mainly participates angiogenesis in local metastases, while endothelin 1 is involved in both local and systemic metastases.

3.2. Microenvironment

The host microenvironment is also a factor in metastasis. Sinusoidal endothelial cells are found lining tumor vessels in color-rectal liver metastases (26). Both sinusoidal endothelial cells and the hepatocyte-derived extracellular matrix provide a microenvironment for microvessel growth. Liver sinusoidal endothelial cells are approximately 50% of the nonparenchymal hepatic cells, which are important in hepatic microcirculation. The hepatocyte-derived extracellular matrix is associated with angiogenesis through inducing growth factors and receptors that regulate the proliferation of metastatic CRC to the liver (27). Primary tumors are associated with vascularization of liver metastases. When primary tumors are resected, the vascularization of liver metastases increases (28) as a compensatory response of metastatic tumor growth.

3.3. Suppressive factors

Although endogenous factors and the host microenvironment promote angiogenesis, other factors suppress angiogenesis. Netrin-4 and its receptors are present in endothelial cells and in vascular smooth muscle cells. Netrin-4 functions as an endothelial guidance molecule during angiogenesis (29). However, in color-rectal liver metastases, netrin-4 overexpression decreases primary CRC and tumor recurrence and metastasis after surgical resection via anti-angiogenic effects (30). In addition, fatty liver suppression of neovascularization is observed in color-rectal liver metastasis (31). A truncated kringle domain of human apolipoprotein(a), termed rhLK68, expressed in color-rectal liver metastases suppresses angiogenesis-dependent progression of prevascular micrometastases to macroscopic tumors (32), which suggests that LK-68 may be a promising candidate to treat hepatic metastasis of color-rectal cancer.

4. ANTIANGIOGENESIS THERAPY

Angiogenesis is an important target for therapeutic intervention in color-rectal liver metastasis. In a mouse model of color-rectal liver metastases, several agents such as angiocidin inhibitory peptides, the alphavbeta3 integrin antagonist S247, and a combination of dasatinib and oxaliplatin decrease tumor burden or increase survival by targeting angiogenesis (33-35). The styrene maleic acid neocarzinostatin inhibits metastatic growth by changing the microvascular architecture of color-rectal liver metastases (36). Angiogenesis provides a strategy for

antiangiogenesis therapy. VEGF and cyclooxygenase-2 pathways are the focus of antiangiogenesis therapy and research is in progress on angiogenesis inhibitors.

4.1. Antiangiogenesis by the VEGF pathway

The VEGF pathway offers a number of important targets for cancer therapies. The mammalian VEGF signaling pathway includes five glycoproteins from the VEGF family, three receptors and two co-receptors. The receptors for various VEGF ligands are tyrosine kinases (TKs) and are found primarily on vascular endothelial cells (37-39).

VEGF receptor is thought to be the predominant angiogenic factor in malignant disease (40,41). VEGF receptor overexpression is frequently observed in color-rectal carcinoma and might play a role in metastatic disease progression (42). VEGF is synthesized by both normal and malignant cells and mediates angiogenic signals through interaction with one or more of its TK receptors (43). Dysfunction of VEGF receptor-1 TK inhibits angiogenesis in lung cancer (44). In a murine model of colon cancer liver metastasis, TK inhibitors that target VEGF receptors inhibit tumor angiogenesis and growth (45). In both mice and patients with color-rectal liver metastases, the preclinical pharmacodynamics of a novel angiogenesis inhibitor PTK/ZK, which specifically targets all three VEGF receptor TKs, correlate well with clinical activity in phase I trials in comparable exposures to the drug (46). Thus, TK of VEGF receptor would act as a target in anti-angiogenesis therapy of color-rectal liver metastases.

VEGF antibody also provides a means of targeting the VEGF pathway in color-rectal liver metastases. Antibodies inhibit the angiogenic switch and liver metastasis in an orthotopic xenograft model (47). VEGF receptor-2, also known as Flk-1/domain-containing receptor (KDR), is reported to be the most important receptor in VEGF receptor (VEGFR)-stimulated tumor angiogenesis. By targeting Flk-1/KDR, IMC-1C11, a chimeric monoclonal antibody, binds specifically to the endothelial cell surface extracellular domain of KDR, blocking VEGFR-KDR interaction and preventing VEGFR activation of the intracellular tyrosine kinase pathway (48). A phase I study of IMC-1C11 provides evidence of its safety and low toxicity in patients with color-rectal liver metastases (49), which is confirmed the promising of IMC-1C11 in clinical application.

The angiogenesis inhibitor bevacizumab, which slows the growth of new blood vessels, was approved for combination use with standard chemotherapy for metastatic CRC by the U.S. Food and Drug Administration in 2004. Bevacizumab is used with standard chemotherapy treatment (as a first-line treatment) and with 5-fluorouracil-based therapy for second-line metastatic CRC (50). The sole target of bevacizumab is VEGF-A, (often referred to as VEGF) (51). In a randomized, two-arm phase III study, a combination of bevacizumab and capecitabine plus oxaliplatin (CAPOX) increased disease-free survival compared with CAPOX alone in patients with color-rectal

liver metastases undergoing radical resection or resection in combination with radiofrequency ablation (52).

In addition to novel agents, isoleucine has a novel mechanism for downregulation of angiogenesis via inhibition of VEGF. VEGF production is initiated by phosphorylation of eukaryotic initiation factor (eIF)-4E-binding protein 1 (4E-BP1) by the mammalian target of the rapamycin (mTOR) following disruption of their binding to eIf-4E. This enables eIF-4E to translate VEGF using the m7GpppN cap (53-55). Rapamycin, an immunosuppressive drug, interacts with mTOR, resulting in dephosphorylation of 4E-BP1 and impairment of VEGF production. Isoleucine prevents liver metastases of CRC by antiangiogenesis that targets the rapamycin pathway (56) (Figure 1).

TAC-101 (4-(3,5-bis(trimethylsilyl) benzamido) benzoic acid) is an orally bioavailable synthetic retinoid, an analog of vitamin A (retinol) that binds to the nuclear retinoic acid receptor-alpha (RAR-alpha), activates and RAR-alpha transcriptional activation activity. Murakami and colleagues observed that TAC-101 was effective against hepatic metastasis of colon cancer by inhibiting tumor angiogenesis (57). Later, they further found, using a rat hepatic metastatic model *in vivo* and DLD-1 human colon cancer cells *in vitro*, that TAC-101 inhibits angiogenesis in colon cancer through reduced expression of VEGF (58).

The renin angiotensin system is expressed in several cancers and regulates proliferation and angiogenesis in several pathological conditions (59,60). In a mouse model of color-rectal liver metastases, angiotensin-converting enzyme inhibitors captopril and the angiotensin II type I receptor (AT1R) antagonist irbesartan significantly reduce the volume of color-rectal liver metastases and change the tumor microvasculature (61). In a mouse model of color-rectal liver metastasis, the mechanism of this effect is AT1R activation leading to increased VEGF and angiogenesis *in vivo* (62). Similar results were observed in the same model with irbesartan treatment (63). Thus, targeting AT1R might lead to antiangiogenesis via inhibition of VEGF.

In summary, the VEGF pathway is the target of several agents for color-rectal liver metastases, since it has an important role in angiogenesis of CRC metastases. These agents affect antiangiogenesis directly or indirectly by targeting VEGF/VEGFRs.

4.2. Cyclooxygenase-2 pathway

Cyclooxygenase-2 (COX-2), which is a key enzyme in the conversion of arachidonic acid to prostaglandin (PG) H, synthesizes inflammatory mediators that induce a chronic inflammatory state. This promotes tumor growth and metastasis formation in CRC (64-67). In CRC, COX-2 regulates cancer-induced angiogenesis by stimulating the release of VEGF and increasing the production of thymidine phosphorylase (68). In human color-rectal liver metastasis, COX-2 protein is expressed by cancer cells (69). Furthermore, in patients with color-rectal liver metastasis, COX-2 expression is associated with
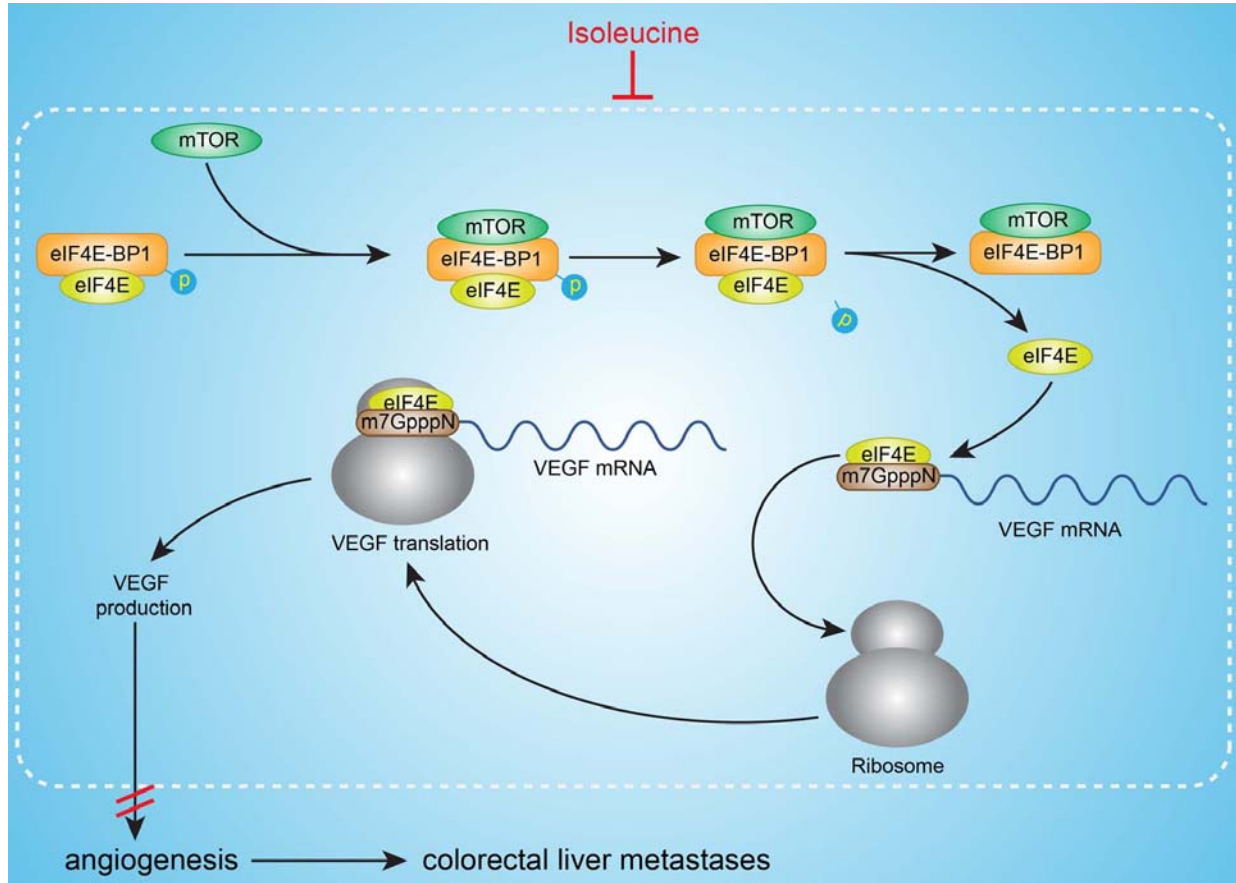
Figure 1. Isoleucine prevents liver metastases of CRC by antiangiogenesis. The mammalian target of the rapamycin (mTOR) binds to eukaryotic initiation factor (eIF) and eIF-4E-binding protein 1 (4E-BP1), and then makes eIF-4E-BP1 dephosphorylatd, following disruption of their binding to eIf-4E, enabling eIF-4E to translate mRNA of VEGF, through the m7GpppN cap, finally resulting in the initiation of VEGF production. Isoleucine prevents color-rectal liver metastases by antiangiogenesis through the target of this pathway.

angiogenic factors such as VEGF-A and thymidine phosphorylase in primary tumors and liver metastases (70). In CRC mice, ibuprofen decreases both tumor growth and liver metastatic potential partly by modulating tumor angiogenesis (71). In patients undergoing liver resection surgery for CRC metastatic disease, the selective cyclooxygenase-2 inhibitor rofecoxib negatively regulates angiogenesis (72). Thus, both studies on animal and human shows the effect of selective COX-2 inhibitor in the therapy of color-rectal liver metastases. The selection of this agent may reduce side effect in therapy compared with other non-selective agents.
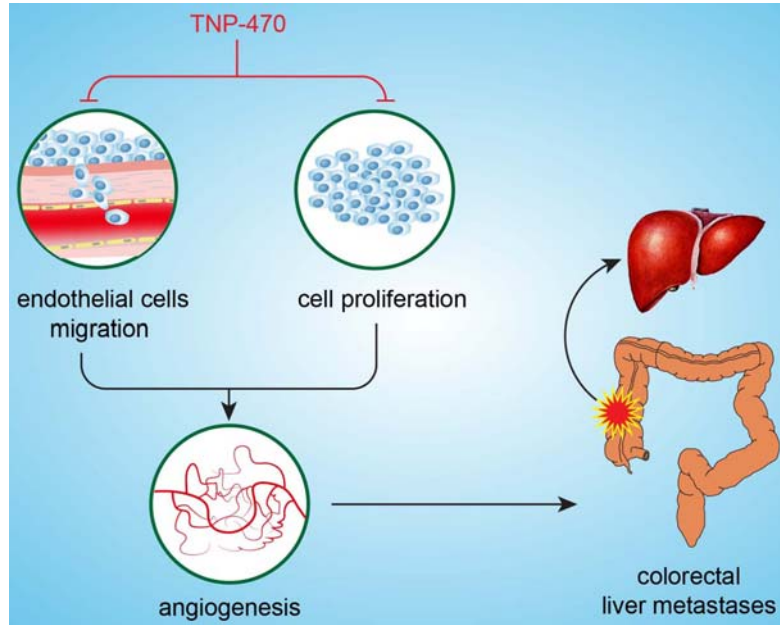
4.3. Angiogenesis inhibitors

TNP-470 inhibits angiogenesis *in vitro* and *in vivo*. TNP-470 is a synthetic fumagillin analog that interferes with angiogenesis through specific inhibition of endothelial cell proliferation and migration (73,74). In a syngeneic rat CRC model, TNP-470 inhibits liver metastasis growth (75). A similar effect of TNP-470 was reported for spontaneous liver metastasis of CRC in a rabbit model (76). Compared with mitomycin C, TNP-40 has a better effect on the growth and liver metastasis of xenotransplanted human CRC (77). *In vitro* and *in vivo*

experiments using a human colon cancer cell line show that TNP-470 inhibits angiogenesis of color-rectal liver metastases by inhibiting the proliferation of human umbilical vein endothelial cells and their migration (78) (Figure 2).

The angiogenesis inhibitor FR-118487 has anti-angiogenesis effects in synchronous liver metastases. About 25% of patients who undergo resection for CRC have liver metastases identified either preoperatively or during laparotomy, i.e., synchronous liver metastases (79-81). In synchronous liver metastases of CRC, angiogenesis mainly involves the epidermal growth factor receptor pathway (82). In a rabbit CRC model with spontaneous liver metastases, FR-118487 suppresses liver metastases from CRC by inhibiting angiogenesis in the postoperative period (83,84). These findings suggest that FR-118487 might inhibit angiogenesis via the epidermal growth factor receptor pathway in synchronous liver metastases of CRC. However, the precise mechanism needs further study.

Endostatin is an endogenous inhibitor of angiogenesis, and might interfere with the pro-angiogenic

Figure 2. TNP-470 inhibit angiogenesis of color-rectal liver metastases. TNP-470 inhibits the proliferation of human umbilical vein endothelial cells and their migration, resulting in the inhibition of angiogenesis in color-rectal liver metastases.

action of VEGF (85). In CRC patients with liver metastases, endogenous endostatin is associated with VEGF levels (86). The increased level of endogenous endostatin is a response to elevated VEGF levels. The rh-endostatin YH-16 inhibits color-rectal liver metastases in a mouse model (87). YH-16 inhibits liver metastasis of CRC by inhibiting the growth of vascular endothelial cells. YH-16 combined with 5-FU has an additive effect on inhibition of color-rectal liver metastasis (88). Furthermore, 5-FU in combination with the small peptide ATN-161 reduces liver metastasis and improves survival in a mouse CRC model by targeting integrin alpha5beta1 function. Integrin alpha5beta1 is expressed on activated endothelial cells and is critical for tumor angiogenesis (89).

5. CONCLUSION

In color-rectal liver metastases, complex factors affect angiogenesis, including angiogenic factors and microenvironments. The production of angiogenic factors is controlled by intrinsic properties of the tumor cell and the host microenvironment. The VEGF pathway is crucial for angiogenesis during the growth of primary tumors and liver metastases of CRC. Thus, several antiangiogenic agents target the VEGF pathway. In addition, the cyclooxygenase-2 pathway is an effective target for antiangiogenic therapy. However, precise mechanisms of these agents, such as which intracellular signaling pathways are invovled into them, are still not very clearly. The importance of angiogenesis in color-rectal liver metastasis and an increased understanding of its mechanism have led to the development of a number of antiangiogenic agents for CRC treatment. Insight into the mechanism of angiogenesis might aid in the design of agents with improved efficacy and safety profiles and reduced risk of

resistance. Additional research is also needed on the effect of promising antiangiogenic agents for the treatment of color-rectal liver metastases.

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Abbreviations: VEGF: vascular endothelial growth factor; CRC: color-rectal cancer; AJCC: American Joint Committee on Cancer; HIF-1alpha: hypoxia-inducible factor 1 alpha; TKs: tyrosine kinases; KDR: domain-containing receptor; VEGFR: VEGF receptor; CAPOX: capecitabine plus oxaliplatin; eIF: eukaryotic initiation factor; 4E-BP1: eIF-4E-binding protein 1; RAR-alpha: retinoic acid receptor-alpha; AT1R: angiotensin II type I receptor; COX-2: cyclooxygenase-2; PG: prostaglandin

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