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Molecular markers to predict outcome to antiangiogenic therapies in colorectal cancer: Current evidence and future perspectives

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ABSTRACT

Angiogenesis is a universal requirement for the growth of solid tumours beyond the limits of oxygen diffusion from the existing vasculature. The expression and function of proangiogenic and antiangiogenic factors are altered in solid malignancies to drive net neoangiogenesis. Vascular endothelial growth factor (VEGF) has been confirmed in several clinical trials as an important therapeutic target in colorectal cancer (CRC) treatment. However, given that the efficacy of antiangiogenic agents appears to be limited to a subset of patients, the identification of who will obtain the greater benefit from this therapy or suffer from specific toxicities and when or for how long they should be administered in the treatment algorithm are major open questions for clinicians and challenges for present and future research. Current evidence indicates some predictive value for particular circulating measures, such as an increase in VEGF, a decrease in vascular endothelial growth factor receptor 2 (VEGFR-2) or circulating endothelial cells, tissue biomarkers, microvessel density, KRAS and BRAF gene mutations or polymorphisms affecting components of the VEGF pathway. Many questions relating to these and other surrogate biomarkers, however, remain unanswered and their clinical usefulness has yet to be proven. This review will focus on the present status of knowledge and future perspectives for developing molecular tools to foresee and monitor antiangiogenic therapy activity in CRC patients.

Introduction

Angiogenesis, the process of new blood vessel formation from endothelial precursors, is a complex process regulated by numerous endogenous factors that stimulate or inhibit neovascularization of both healthy and pathological tissues. Gaining access to the host vascular system and the generation of a tumour blood supply are rate-limiting steps for the growth and progression of solid malignancies beyond the limits of oxygen diffusion from the existing vasculature, metastatic spreading and disease aggressiveness.[1,2] Early in tumourigenesis, the so-called "angiogenic switch", the induction of tumour vasculature or switch to an angiogenic phenotype, is activated by hypoxia, activated oncogenes and/or metabolic stress. The previously closely maintained physiological balance that keeps adult vasculature in a relatively quiescent state is then tipped in favour of angiogenesis through the expression of proangiogenic growth factors.[3]

Tumour cell expression of many of the angiogenic factors in colorectal cancer (CRC) is regulated by hypoxia through the transcription factor hypoxia-inducible factor (HIF). As the tumour cells proliferate, oxygen becomes depleted, and a hypoxic microenvironment develops within the tumour. HIF is degraded in the presence of oxygen, and therefore, low oxygen levels lead to increased levels of HIF and ultimately HIF activation and transcription of target genes.[4] One of the major pathways involved in this process is the vascular endothelial growth factor (VEGF) family of proteins and its receptors. The VEGF family includes VEGF-A (usually referred to as VEGF), VEGF-B, VEGF-C, VEGF-D, VEGF-E and a structurally related molecule, the placental growth factor (PlGF). Three high-affinity VEGF tyrosine kinase receptors have been identified: VEGF receptor 1 [VEGFR-1, also known as fms-like tyrosine kinase (FLT-1)], VEGFR-2 [also known as FLT-2 or kinase insert domain receptor (KDR)] and VEGFR-3 (FLT-4).[5,6] When VEGF is secreted from tumour or stromal cells, it interacts with both VEGFR-1 and VEGFR-2, located on vascular endothelial cells and bone marrow-derived cells. VEGFR-2 is believed to mediate the majority of the angiogenic effects of VEGF-A, whereas the role of VEGFR-1 is more complex and not fully understood. In addition, VEGFR-2 has been the principal target of antiangiogenic

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therapies, although additional studies have underlined the importance of signalling through VEGFR-1.[7] VEGF-B and PlGF have high affinity for only VEGFR-1, whereas VEGF-C and VEGF-D bind both VEGFR-2 and VEGFR-3 to regulate angiogenesis and have been implicated in lymphangiogenesis.[8,9] The binding of VEGF to these receptors initiates a cascade of signalling pathways which plays a crucial role in normal and pathologic angiogenesis because it induces the proliferation of endothelial cells, increases vascular permeability, and promotes the extravasation of proteins from tumour vessels, contributing to the formation of the fibrin matrix through which stromal cells invade. Some of the known signaling cascades include the phospholipase Cγ (PLCγ), protein kinase C (PKC), Raf kinase-mitogen-activated protein kinase (Raf-MAPK), phosphatidylinositol 3-kinase (PI3K)-AKT-mammalian target of rapamycin (mTOR) pathways, and Src tyrosine kinases.[10,11] Finally, there is clear evidence that VEGF-independent angiogenesis is mediated through additional pathways that include basic fibroblast growth factor (bFGF) family members, interleukin 8 (IL-8), interleukin 1β (IL-1β) (Src kinases), epidermal growth factor (EGF), and insulin like growth factor 1 (IGF-1).[4,12]

Targeting proangiogenic factors has become an effective strategy to inhibit tumour growth in preclinical studies and, more recently, a successful clinical tool in oncologic practice.[13] Antiangiogenic therapies function through the inhibition of blood vessel generation, a reduction of microvessel density (MVD), vascular volume and tumour perfusion and through the normalization and pruning of existing tumor vasculature by aberrant VEGF signalling. These agents are also reported to enhance the effects of chemotherapy (CT) through improved drug delivery of cytotoxic drugs by lowering tumour interstitial fluid pressure and by reducing the number of nonfunctional tumour blood vessels.[14] Various strategies for inhibiting VEGF have been investigated over the last decade in CRC patients. These include neutralizing antibodies to VEGF[15], low-molecular-weight VEGFR tyrosine kinase inhibitors (TKIs)[16,17] and soluble VEGF constructs (VEGF-Trap).[18] Among these antiangiogenic-targeted treatment modalities, bevacizumab, a recombinant humanized monoclonal IgG1 antibody targeting VEGF-A, has become a standard of care for treatment of metastatic CRC.[15,19–24] The addition of bevacizumab to a variety of first-line and second-line regimens improves outcomes, although these advances come at cost of treatment-related side effects, including bleeding, hypertension, bowel perforation, and thromboembolic events. Aflibercept, a fully-humanized recombinant fusion protein consisting of VEGF binding portions from the human VEGFR-1 and -2 fused to the Fc portion of human immunoglobulin G1, in combination with FOLFIRI has also conferred statistical significant survival benefit over FOLFIRI combined with placebo in patients with metastastic CRC previously treated with oxaliplatin.[17] Finally, regorafenib, an orally active inhibitor of angiogenic tyrosine kinases (including the VEGFR-1 and VEGFR-3), and other stromal and oncogenic receptor tyrosine kinases, has recently shown activity in metastatic CRC which has progressed after all standard therapies.[18] Table 1 summarizes the results of the most relevant trials with antiangiogenic therapies in metastatic CRC.

Given that not all CRC patients respond to antiangiogenic agents, the identification of markers that predict the efficacy of this class of drugs should be a primary objective since preliminary phases of clinical drug development, particularly because these agents can be toxic and are expensive. Antiangiogenic-related arterial hypertension or proteinuria[25–27] may constitute early indicators of antitumor activity and several changes in imaging parameters, such as early radiological tumor shrinkage and morphologic criteria[28,29] or dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI)[30,31] have also been proposed as potential prognostic or predictive markers. However, no definitive clinical tools are currently available to select patients more likely to benefit from VEGF pathway inhibitors nor to exclude those who are proner to suffer from specific adverse events. In order to overcome these substantial limits, retrospective analyses and translational studies have been conducted and are currently ongoing to address this major question, investigating molecular, biological and functional biomarkers. This review focus on the present knowledge about candidate biomarkers as predictors of activity and toxicity of VEGF pathway inhibitors, the challenges this emerging field presents and the future role of these markers in CRC treatment.

Circulating biomarkers for antiangiogenic therapies

The measurement of concentrations of circulating proteins is an attractive biomarker strategy, as blood is easily accessible, the assays are inexpensive, and the proteins may be readily and quantitatively measured by automated methods. In order to assess circulating biomarkers of angiogenesis that may predict outcome to antiangiogenic therapies in CRC patients, many approaches have been tested in clinical studies but, to date, none has been validated for routine use in clinical practice.

Plasma concentrations of VEGF and related pathway components

Circulating VEGF concentrations are reported to be relevant to outlook in patients with solid tumours and they have been proposed to reflect VEGF-dependent tumour angiogenesis.[32] However, baseline VEGF as a predictive and/or prognostic marker and VEGF modulation after bevacizumab therapy in CRC is still a matter of debate with contrasting results. A retrospective subset analysis of patients treated with first-line standard CT plus cediranib (AZD2171), a highly oral selective inhibitor of VEGF signalling, has explored the value of baseline levels of VEGF and soluble VEGFR-2 (sVEGFR-2) as a prognostic biomarker for outcome and a predictive biomarker of benefit with cediranib-containing treatment in the HORIZON II and III phase III studies.[33] In HORIZON II, 860 patients received FOLFOX or XELOX with cediranib 20 mg ($n = 502$) or placebo ($n = 358$).[34] In HORIZON III, 1422 patients received modified FOLFOX6 (mFOLFOX6) with cediranib 20 mg ($n = 709$) or bevacizumab ($n = 713$).[35] Cediranib plus standard CT showed improvements in PFS vs. CT alone (HORIZON II; HR, 0.84; $p = 0.012$), but not vs. bevacizumab plus CT (HORIZON III; HR, 1.10; $p = 0.119$). High baseline VEGF was associated with a worse overall outcome for PFS in HORIZON II [HR, 1.41; 95% confidence interval (95% CI), 1.21–1.65] and HORIZON III (HR, 1.20; 95% CI, 1.04–1.38), and OS in HORIZON II (HR = 1.35; 95% CI, 1.12–1.63). Baseline sVEGFR2 was not prognostic for PFS in HORIZON II (HR, 0.99; 95% CI, 0.85–1.15) or HORIZON III (HR, 0.98; 95% CI, 0.86–1.13) or OS in HORIZON II (HR, 0.92; 95% CI, 0.77–1.10). There was no evidence that baseline VEGF or sVEGFR2 predicted for PFS or OS outcome to cediranib treatment in HORIZON II or HORIZON III; however, the groups with low baseline sVEGFR2 levels were associated with a trend to an improved cediranib PFS effect in both studies. Similar results were obtained in a combined analysis of 1816 patients enrolled in four bevacizumab phase III studies in metastatic colorectal, lung, and renal cell cancers (AVF2107g, ECOG 4599, AVAIL, and AVOREN).[36] Higher baseline circulating VEGF levels were associated with shortened PFS and OS regardless of bevacizumab treatment, but they were not useful as a predictive biomarker for bevacizumab-treated patients.

Nevertheless, VEGF concentrations are dynamic and, therefore, changes related to treatment might have a greater predictive value than pretreatment values. The assessment of circulating levels of pro- and antiangiogenic factors may provide insight into the bevacizumab-related modulation of the so-called systemic "angiogenic balance".[37–40] Keskin and colleagues have assessed whether serum