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REVIEW ARTICLE

Dynamic Biomarkers of Response to Antiangiogenic Therapies in Colorectal Cancer: A Review

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Abstract: ***Background*:** Identification of clinical and molecular biomarkers to predict dynamic response or monitor in real-time the efficacy of antiangiogenic therapy represents a major point in the treatment of patients with advanced colorectal cancer. Several studies have been conduced to identify some predictive biomarkers to select patients who will benefit from bevacizumab, the most widely used antiangiogenic monoclonal antibody.

***Conclusion*:** After a decade since the introduction of bevacizumab, no effective predictive biomarkers are available in routine clinical practice. In this review, we summarized the potential candidate dynamic biomarkers that may play a role in this setting.

Keywords: Colorectal cancer, bevacizumab, biomarkers, angiogenesis, chemotherapy, metastatic disease.

1. INTRODUCTION

Colorectal cancer is the second most common cancer in women (614,000 cases) and the third most common cancer in men (746,000 cases) worldwide, with 694,000 deaths every year [1]. New biological therapies based on angiogenesis blockade were approved over the last year for the treatment of patients with metastatic disease.

Bevacizumab was the first molecular-targeted antiangiogenic therapy approved by the European Medicines Agency (EMA) and the U.S. Food and Drug Administration (FDA). An increased expression of Vascular Endothelial Growth Factor A (VEGFA) has been found in most human cancers examined, including colon cancer [2, 3]. This monoclonal antibody binds to the circulating VEGFA which inhibits tumor angiogenesis [4, 5]. In advanced colorectal cancer, bevacizumab has been shown to increase the overall survival (OS), the progression-free survival (PFS) and the response rate (RR) in a first-line treatment associated with 5-fluorouracil/leucovorin/irinotecan (IFL) and in combination with 5-fluorouracil/leucovorin, or capecitabine alone [6]. Bevacizumab has also been shown to improve the PFS in combination with fluoropyrimidines plus oxaliplatin in the first-line treatment [7]. Furthermore, the triplet drug combination FOLFOXIRI plus bevacizumab presents one of the longest survivals reported up to date [8]. Therefore, bevacizumab added to standard chemotherapy is recommended as a first-line treatment for patients with potentially resectable and unresectable metastatic disease [9, 10].

By other part, bevacizumab has been shown to prolong marginally survival among patients with metastatic colorectal cancer at a relatively high cost [11]. In fact, bevacizumab appear to not be a cost-effective treatment and is deemed to be of low value at their current cost. Using biomarkers to treat only the patients likely to get a significant survival benefit would also improve safety, quality of life and cost-effectiveness.

To date, no validated biomarkers have been established for antiangiogenic-based colorectal cancer treatment [12-14]. The aim of this report is to describe the most promising biomarkers to evaluate the antiangiogenic effect of antiangiogenic therapy in this setting.

2. CLINICAL PREDICTORS

2.1. Blood Pressure

Hypertension has been found in patients treated with anti-VEGF therapies. Several randomized trials have shown that bevacizumab improves both progression-free survival (PFS) and overall survival (OS). In all these studies hypertension was observed to be a

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common side effect associated with bevacizumab. Hypertension has been suggested to predict treatment efficacy in patients with CRC [15, 16]. Osterlund *et al.* [17] described a study to evaluate the association between treatment-related hypertension, outcome and safety following treatment with bevacizumab-congaining chemotherapy. The study evidenced that early hypertension was predictive for an improved OS. Other studies showed an association between VEGF genotype and the development of clinically significant hypertension. Thus, hypertension is thought to be a possible clinical predictor of response.

3. IMAGING PREDICTORS

3.1. Ultrasound Imaging

A recent study in murine colon cancer models using three-dimensional dynamic contrast-enhanced ultrasound imaging [18] provides information on the variability of tumor response to antiangiogenic therapy and may be further developed as biomarker for predicting treatment outcomes.

3.2. MRI

Response and time progression could be evaluated with surrogate biomarkers in bevacizumab-containing regimen of advanced colorectal cancer patients. Pharmacokinetic parameters obtained *via* dynamic contrast-enhanced magnetic resonance imaging (MRI) could be a good option to explore [19].

3.3. CT Scan

A clear relationship between response to bevacizumab and the degree of contrast enhancement in evatuation by computed tomography (CT) of colorectal liver metastasis were found. Dynamic changes in the radiological parameters after contrast injection confirmed the potential of these blood perfusion as surrogate predictors [20].

Dighe *et al.* [21] investigated the ability of perfusion CT to quantify the degree of angiogenesis in colorectal cancer patients. Perfusion parameters calculated were correlated with the measurement of Microvessel Density (MVD) obtained from immunohistochemical staining of resected surgical specimens. Perfusion CT is also able to integrate anatomical detail with the assessment of vascular physiology. Pharmacokinetic modeling after tumor enhancement achieved by contrast administration allows the physiologically based quantitative vascular parameters [22, 23]. Perfusion CT may reflect angiogenesis in colorectal cancer; however, not all studies have been able to correlate blood flow with MVD [24, 25]. Perfusion measurements have also been shown to be robust enough to be useful for therapeutic assessment in colon cancer [26].

3.4. Positron Emission Tomography

Although Fluodeoxyglucose Positron Emission Tomography (FDG PET) is a biomarker of response to targeted therapy, (*e.g.*, imatinib in gastrointestinal stromal tumors), early reductions in standardized uptake value have not been evaluated in patients with colon treated with bevacizumab-based therapy. However, it may provide the detection of early stages of response to EGFR-targeted TKIs in colon cancer [27].

4. MOLECULAR PREDICTORS

4.1. VEGF/PIGF

Circulating VEGF. Correlation between the efficacy of antiogenesis treatment and circulating VEGF levels have been explored in several cinical trials. Some phase II studies have shown that the elevated levels of VEGF have been associated with a poor prognosis, instead of being a biomarker of response [28-31]. A phase II study of bevacizumab combined with chemoradiation in rectal cancer showed no correlation between VEGF levels and the outcome of therapy [32]. However, studies in other tumor types [33, 34] showed inconsistent results.

PIGF and soluble VEGF receptors. Circulating levels of PIGF have been shown to increase in response to angi-VEGF therapies. Thus, targeting PIGF is being considered as a new approach to prevent tumor resistance. Increased levels of PIGF in plasma has been associated with an improved outome in patients with rectal cancer treated with bevacizumab. Thus, circulating levels of soluble VEGFR2 and VEGFR3 have been shown to be decreased by TKIs that directly target these receptors [35-39], however, they are unaffected by bevacizumab [34]. By other part, KDR (kinase insert domain receptor) is the human gene encoding for vascular endothelial growth factor receptor 2 (VEGFR-2), and preliminary data showed that there is a potential relationship between KDR mutation and regorafenib [40]. VEGFR-2 is also a potential biomarker of Aflibercept in the recent studies [41].

5. CIRCULATING ENDOTELIAL CELLS AND ENDOTELIAL PROGENITORS

Blood-circulating cells have been studied as potential biomarkers of angiangiogenic therapy. Willet *et al.* found that bevacizumab reduced the number of viable circulating endotelial cell progenitors in patients with rectal cancer [42]. However, other studies found no differences in circulating cell levels [43].

Zuurbier *et al.* in a recent study [44] performed a DNA microarray-based transcriptional profiling screen with primary endothelial cells (ECs) isolated from normal and tumoral colon tissues. Thirteen separate populations of tumour-associated ECs and 10 of nor-

mal ECs were isolated using fluorescence-activated cell sorting. Transcriptional profiling revealed a total of 2,610 differentially expressed genes when tumoral and normal ECs were compared. In patients treated with bevacizumab in the adjuvant setting expression of *MMP12* and Apelin (APL) mRNA was significantly higher in bevacizumab non-responders compared to responders. At the protein level, high APLN expression was correlated with poor progression-free survival in bevacizumab-treated patients.

6. ANGIOGENIC SWITCH

Kopetz *et al.* [45] evaluated changes in plasma cytokines and some angiogenic factors (CAF) in a first line phase II trial bevacizumab-containing treatment for patients with advanced colorectal cancer. Elevated interleukins (IL)-8 at baseline was associated with shorter PFS (p=0.03) Before the radiographic development of progression, several CAFs increased in comparison to baseline, including basic fibroblast growth factor, hepatocyte growth factor, placental growth factor, stromal-derived factor-1 and macrophage chemo attractant protein-3.

Cubillo *et al.* [46] tested these results in another prospective phase II in whom patients with mCRC received treatment with XELIRI- or XELOX- bevacizumab. Initial treatment was followed by maintenance therapy (bevacizumab plus capecitabine) until progression. Plasma levels of angiogenic-related cytokines (HGF, PIGF, MCP-3, MM-9, Eotaxin, bFGF and IL-18) were analyzed. Maintenance treatment result in dynamic changes in plasma cytokines associated with better disease control and longer PFS.

High serum levels of Epidermal Growth Factor (EGF) and macrophage-derived chemokine and low levels of IL-10, IL-6 and IL-8 were associated with a higher likelihood of response to treatment [47]. IL-8 has also been reported to mediate angiogenesis by stimulating the endotelial cell proliferation in response to hipoxia [48], and resistance to angiangiogenic therapy has been associated with an increased secretion of IL-8 [38].

Hayashi *et al.* [49] evaluated 25 angiogenesis-related molecules for 25 CRC patients both before and during treatment in a previously reported phase II trial of FOLFIRI chemotherapy plus bevacizumab. The serum concentration of VEGFA decreased after the onset of treatment, whereas that of placental growth factor significantly increased. The results suggest that an early increase in the serum VEGFA concentration after the initial decrease is a potential predictive marker of a poor response and reactive resistance to bevacizumab plus chemotherapy.

7. IMMUNOGENICITY

7.1. Microsatellite Instability (MSI)

MMR-deficient colorectal cancer patients shows an enhanced immunogenicity and an increased number of intra-epithelial lymphocytes that may be associated with a favorable clinical outcome [50]. High Microsatellite instability (H-MSI) colorectal cancer tumors may follow a different pathway to angiogenesis. VEGF expression has been found lower among H-MSI adenocarcinomas, and this could partially explain why these group of tumors are less aggressive.

7.2. Immunogenitiсy/Cadherin

Some studies indicate that there is a significant host response associated with an improved prognosis of patients with advanced colorectal cancer treated in base to antiangiogenic drugs (*e.g.* aflibercept), suggesting that it may alter the natural history of this disease. The mobilization of immune cells, such as myeloid-derived suppressor cells (MDSCs), has been thought to contribute to drug resistance. Recent studies have shown that VEGFA signaling through VEGFR2 is involved in MDSCs´recruitment to metastases, and once within the tumor, these can mature into tumor-promoting macrophages. Other angiogenic factors, such as PIGF, directly or indirectly stimulate angiogenesis by affecting a wide range of different cell types or by attracting MDSCs and macrophages within the tumor microenvironment. PIGF also promotes inflammation and angiogénesis by interacting with an alternative pathway *via* VEGFR1 signalling [51].

Ma *et al.* described that CDH12 (Cadherin 12) promotes proliferation, migration and angiogenesis in colorectal cancer, and is expected to become a new diagnostic and prognostic marker, as well as potential new target of treatment for colorectal cancer. The authors concluded that CDH12 might act as a predictor of prognosis in patients with colon cancer [52].

Leucine-rich-alpha-2-glycoprotein 1 (LRG1) has been associated with several tumors and shown to be overexpressed in colon cancer patients, specially in more aggresive tumors. LRG1 induces the process of cell migration and invasion. It also promotes VEGFA expression in colon cancer cells and, therefore, contributes to tumor angiogenesis. HIF1 alpha can also be induced by LRG1 and is thought to be the mechanism by which LRG1 induces VEGFA expression and epithelial-to-mesenchymal transitions (EMT) [53].

CONCLUSION AND FUTURE DIRECTIONS

There is increasing evidence about the potential predictors of bevacizumab efficacy and a biological rationale to support such observations. Nonetheless, due to the lack of a randomized study design, the ab-

sence of prospective validation of data, or the inability to routinely use these markers, the findings were viewed with caution and have not been used in clinical practice. On the other hand, the addition of bevacizumab to cytotoxic chemotherapy as first- or second-line treatment is associated with an improvement in the outcome of all subgroups of mCRC patients. However, it is essential to identify biomarkers that permit the recognition of potentially responsive subjects and to spare those unlikely to benefit unnecessary toxicity.

In spite of the high number of static biomarker in the literature, the role of dynamic biomarker evaluating the angiogenic switch effect and the combination of clinical and radiological data in real time may result in future and successful research approaches.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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