Association between bevacizumab-related chemotherapy regimens and serum vascular endothelial growth factor-A level in patients with metastatic colorectal cancer

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Abstrac: Colorectal cancer is the third most common cancer and the third leading cause of cancer-related death. Bevacizumab improves survival for metastatic colorectal cancer patients with chemotherapy, but no proven predictive markers exist. The aim was to investigate the possible predictive value of vascular endothelial growth factor (VEGF)-A levels in this setting. Pre-treatment serum samples and response evaluations were available from 20 patients. All patients received bevacizumab and chemotherapy comprising fluorouracil and leucovorin or capecitabine combined with either oxaliplatin (FOLFOX or XELOX) or irinotecan (FOLFIRI or IFL). The expression serum VEGF-A levels were analyzed by an ELISA. Response was evaluated according to RECIST version 1.1, and group comparisons were made using the independent sample test. The serum VEGF-A levels were 373.93±29.86pg/ml and 294.22±35.53pg/ml in responders and non-responders (p=0.101). There was no significant difference between them. No correlation between the efficacy of bevacizumab-related chemotherapy and serum VEGF-A levels was observed. In this correlative evaluation, pretreatment serum VEGF-A levels were not predictive for bevacizumab-based treatment benefit.

1. Introduction

Colorectal cancer (CRC) is the third most common cancer and the second leading cause of cancer-related death [1]. One million people are diagnosed with CRC worldwide each year [2], and about half of them will ultimately develop metastatic disease and become candidates for palliative therapy. Metastatic colorectal cancer (mCRC) is not curable, except for a small number of patients with isolated liver metastasis, and the median overall survival usually does not exceed 2 years [3,4].The addition of targeted agents to standard chemotherapy regimens provides a modest improvement in survival. Although many biological markers have been evaluated for their ability to predict efficacy in advanced disease, these studies have no consistent conclusion.

Angiogenesis is a biological event of critical importance in tumor growth and progression. One of the main growth factors is Vascular endothelial growth factor (VEGF) involved in vessel formation [5,6]. There are six subtypes of VEGF that have been reported, VEGF-A, VEGF-B, VEGF-C, VEGF-D, virus VEGF-E and placental VEGF (PIGF) [7]. VEGF-A, which exists in humans in multiple isoforms [8], is a proangiogenic ligand that is upregulated in a considerable proportion of primary malignancies [9]. Tumor expression levels of VEGF-A have been correlated with vascularization, pathologic stage, metastasis, and poor outcome in patients with metastatic colorectal cancer (mCRC), metastatic renal cell carcinoma (mRCC), and non–small cell lung cancer [7–14]. Furthermore, VEGF-A levels are elevated in a proportion of patients with carcinomas, and several reports have suggested an association between VEGF-A levels and patient outcomes [15].

In recent years, bevacizumab, the first antiangiogenesis therapy to be approved for use in mCRC, is a humanized monoclonal antibody that binds to VEGF-A [16]. Phase III studies have shown that bevacizumab associated with 5-fluorouracil-based chemotherapy significantly improves progression-free survival (PFS) and overall survival (OS) in patients with mCRC and advanced nonsquamous NSCLC [17–19]. Treatment containing bevacizumab in previously untreated mRCC has also been associated with significant improvements in PFS compared with immunotherapy alone [20, 21]. Though efficacy of this treatment regimen has been proved [22, 23], several challenges still remain to be worked out. Objective response can only be demonstrated in half of the patients.In addition, treatment with anti-angiogenetic therapy is associated with rare but potentially life-threatening adverse events, and the continuousunselective use of these drugs constitutes an economic burden to the health care system. There is consequently an obvious need for predictive markers with respect to efficacy, and a better selection of patients is a prerequisite for a more effective treatment of patients with mCRC.

Some studies have focused on identifying biomarkers for prediction of bevacizumab efficacy. The most obvious protein biomarker to select for testing bevacizumab efficacy is VEGF-A, however those studies have not found changes in VEGF-A concentrations during therapy to have consistent predictive value [24]. In contrast, correlations between outcomes with bevacizumab and ICAM, IL8 and

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VEGF in NSCLC [25] have been demonstrated. Overall, there still has been considerable debate over the role of VEGF levels as a predictive marker for the use of bevacizumab.

The aim of the correlative study was to investigate the possible predictive value of serum VEGF-A level in relation to chemotherapy plus bevacizumab as first-line treatment for patients with mCRC.

2. Methods

2.1 Patients and treatment

All of the patients had histologically confirmed colorectal cancer; advanced mCRC; age <75 years; ECOG performance status of 0 or 1; no prior use of bevacizumab; no increased risk factors for bleeding; and hypertension, if present, was controlled with a double agent.

The regimen of chemotherapy in association with bevacizumab could be FOLFIRI, FOLFOX, XELOX and IFL. Bevacizumab was administered at the dose of 5 mg/kg if combined with FOLFIRI/FOLFOX and at the dose of 7.5 mg/kg if administered with tri-weekly schedules (XELOX).

All metastatic lesions had to be re-staged every 8 weeks and evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST, version 1.1) [26]. Chemotherapy was administered until progression of disease, unacceptable toxicity, refusal by the patient and, anyway, for a maximum of 6 months. Bevacizumab was discontinued in case of progression of disease, unacceptable toxicity or refusal by the patient; otherwise, it was continued, even after the 6 months of chemotherapy, until disease progression.

2.2. Evaluation of Toxicity and Efficacy

Clinical and biochemical evaluation of toxicity was performed before each cycle and graded according to the NIH Common Terminology Criteria for Adverse Events version 3.0. Tumor response was evaluated according to RECIST criteria along with computed tomography morphologic criteria [27] and classified in complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD). For the analysis, patients with stable disease (SD), partial response (PR) or complete response (CR) after 6 months were grouped in responders; patients with a progressive disease (PD).,unmanageable toxic effects, or refusal were grouped in non-responder patients. Outcome data were available from 20 patients

2.3. Serum samples collection

Patient consent was obtained before sample collection. Serum samples were obtained before bevacizumab-based chemotherapy regimens. Serum samples were kept between 2℃ and 8℃, centrifuged at 10,000 rpm for 10 min, and then frozen at -81℃ until assayed.

The study was approved by the local ethics committee and Institutional Review Board and therefore performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

2.4. ELISA for VEGF-A

Quantitative determination of the human VEGF-A concentration in the serum samples was done by quantitative solid phase ELISA. ELISA was done using the Diaclone VEGF-A ELISA kit (Gen-Probe, Besancon, France) according to the manufacturer's instructions. For measurement of VEGF-A, serum samples were diluted 1:5 in phosphate buffered saline containing 1% bovine serum albumin. VEGF-A concentration was done according to manufacturer's instructions. Each sample was analyzed in duplicate and the mean values were used as the final concentration. The interassay coefficient of variation (CV%) was 6–9.5%, the intraassay CV% was 3–6.5%.

2.5. Statistical Analysis

Statistical analyses were performed using SPSS for Windows version 13.0 (SPSS Inc., Chicago, IL, USA). Group comparisons were made using the independent samplest test. $P < 0.05$ was set as the level of statistical significance. According to the RECIST ver.1.1, the sum of the longest dimension of all the target lesions was calculated for each patient to provide the baseline before treatment. The same measurements and calculation were done after every two courses of chemotherapy to obtain the initial assessment sum. The change ratio of target lesions was calculated as:(Assessment sum − Baseline sum) / Baseline sum.

3. Result

Among the 20 patients, 11 were classified as responders and 9 as nonresponders according to RECIST. There was no significant difference between responders and nonresponders in clinicopathological factors such as gender, age and so on. The baseline clinical characteristics of the patients are summarized in Table 1. None of the patients had a concurrent primary tumor that had yet to be resected. Four patients received an antihypertensive agent (NCI-CTC grade 2) at baseline. Serum VEGF levels of responders in mCRC patients before bevacizumab-based chemotherapy was 373.93±29.86pg/ml, and those of nonresponders were 294.22±35.53pg/ml (Figure 1). There was no significant difference between the two groups (p=0.101).

4. Discussion

A plethora of predictive markers have been reported in recent years. Significant variability in methodologic assessment of markers exists, and therefore, recent reporting recommendations for predictive marker prognostic studies have been published[28,29]. Our study follows those recommendations. In this prospective study, we compared the levels of VEGF-A in responders

versus non-responders to bevacizumab therapy. VEGF-A level in responding patients is higher than non-responders', but there was no significant difference between them. Therefore our studies initially showed no predictive value for VEGF-A expression, suggesting that it was not the VEGF-A levels that determined outcome. Of course, there are

a number of limitations of our study. Firstly, we started with a small sample. Secondly, the use of RECIST is subjective. And, lastly, the complexity of angiogenesis, it is unlikely that one marker will serve a uniformly predictive function across disease types and their respective stages.

table 1. Baseline characteristics of patients (n =20)

Characteristics	Responders（n=11）	Nonresponders（n=9）
Sex: male/female	8/3	6/3
Median age	55.18	51.80
ECOG performance status		
0/1	3/8	2/7
Sites of primary tumor		
Colon/rectum	6/5	6/3
Prior resection of primary tumor	11	9
Histological appearance		
Well or moderately differentiated	8	6
Poorly differentiated	0	1
Mucinous	1	1
Unknown	2	1
Previous history/complication		
Hypertension[a]	3	1
Diabetes	1	0
Characteristics	Responders（n=11）	Nonresponders（n=9）
Sex: male/female	8/3	6/3

Evidence is emerging that VEGF-A is alternatively spliced to form proangiogenic VEGF-A165 and antiangiogenic VEGF-A165. Varey et al found that over 90% of the VEGF-A in normal colonic tissue was VEGF-Axxxb , but there was a variable upregulation of VEGF-Axxx and down regulation of VEGF-Axxxb in paired human CRC samples. In addition, cultured colonic adenoma cells expressed predominantly VEGF-Axxxb, however colonic carcinoma cells expressed predominantly VEGF-Axxx. Whereas, adenoma cells exposed to hypoxia switched their expression from predominantly VEGF-Axxxb to predominantly VEGF-Axxx. VEGF-A165b overexpression in LS174t colon cancer cells inhibited colon carcinoma growth in mouse xenograft models. Western blotting and surface plasmon resonance showed that VEGF-A165b bound to bevacizumab with similar affinity as VEGF-A165. However, although bevacizumab effectively inhibited the rapid growth of colon carcinomas expressing VEGF-A165, it did not affect the slower growth of tumours from colonic carcinoma cells expressing VEGF-A165b. Both bevacizumab and anti-VEGF-A165b-specific antibodies were cytotoxic to colonic epithelial cells, but less so to colonic carcinoma cells. These results show that the balance of antiangiogenic to proangiogenic isoforms switches to a variable extent in CRC, regulates tumour growth rates and affects the sensitivity of tumours to bevacizumab by competitive binding. Together with the identification of an autocrine cytoprotective role for VEGF-A165b in colonic epithelial cells, these results indicate that

bevacizumab treatment of human CRC may depend upon this balance of VEGF-A isoforms [30].

Although VEGF-A has been shown to be critical in CRC by inhibition studies, the expression of VEGF-A in CRC has not been investigated using tools that distinguish between the proangiogenic VEGF-Axxx isoforms and the antiangiogenic VEGF-Axxxb isoforms. The vast majority of studies containing ours'have measured total VEGF-A levels in plasma, tumours or serum using commercially available antibodies that do not distinguish between pro- and antiangiogenic isoforms, as commercial enzyme-linkedimmunosorbent assays (ELISAs) detect VEGF-Axxxb isoforms. Recently, a phase-III trial of FOLFOX4±bevacizumab assessed for VEGF165b and VEGFtotal of blinded tumor samples by immunohistochemistry and scored relative to normal tissue, and suggested that Low VEGF165b:VEGFtotal ratio may be a predictive marker for bevacizumab in mCRC, and individuals with high relative levels may not benefit [31].

5. Conclusion

Our study on the predictive value of VEGF-A demonstrate no significant relationship between the serum VEGF-A level and the treatment effect of bevacizumab. The search for predictive markers for response and benefit from antiangiogenic agents continues. One promising marker is VEGF-A$_{165}$b and provides new insight into the tumor angiogenic network. Further study with a larger patient population and longer duration is warranted to clarify its value.

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