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REVIEW

Role of endoglin and VEGF family expression in colorectal cancer prognosis and anti-angiogenic therapies

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Abstract

Colorectal cancer (CRC) is one of the cancer models and most of the carcinogenic steps are presently well understood. Therefore, successful preventive measures are currently used in medical practice. However, CRC is still an important public health problem as it is the third most common cancer and the fourth most frequent cause of cancer death worldwide. Nowadays, pathologic stage is a unique and well-recognized prognostic indicator, however, more accurate indicators of the biologic behavior of CRC are expected to improve the specificity of medical treatment. Angiogenesis plays an important role in the growth and progression of cancer but its role as a prognostic factor is still controversial. Probably the most important clinical implication of tumor angiogenesis is the development of anti-angiogenic therapy. The goal of this review is to critically evaluate the role of angiogenic markers, assessed by either endoglin-related microvessel density or expression of vascular endothelial growth factor family members in the CRC setting and discuss the role of these angiogenic markers in anti-angiogenic therapies.

Key words: Angiogenesis; Colorectal cancer; Colorectal cancer treatment; Endoglin; Prognosis; Vascular endothelial growth factor

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COLORECTAL CANCER EPIDEMIOLOGY

Colorectal cancer (CRC) is the third most common cancer and the fourth most frequent cause of cancer death worldwide[1-3]. Globally, CRC incidence varies widely, with higher rates in North America, Australia and Western Europe and lower rates in developing countries [4], although, in recent years, high CRC rates have also been reported in these countries[5]. In terms of mortality, geographic disparities have also been observed[6]. In Western countries, CRC is

the second most common cause of death from malignant disease, and despite improvements in treatment mortality remains high with metastatic spread to the liver occurring in about 50% of patients[7].

European countries rank highest in the global statistics, both in terms of CRC incidence and mortality. From 1998 to 2002, the incidence of CRC in Europe for men and women was 38.5 and 24.6 (world age standardization (ASR-W)) per 100 000 inhabitants and mortality over the same period was 18.5 and 10.7 (ASR-W) per 100 000 inhabitants, respectively[8]. However, over the past twenty-five years, mortality rates among Caucasians have steadily declined[9]. Data from the World Health Organization (WHO), between 1997 and 2007 have revealed that mortality from CRC declined by around 2% per year from 19.7 to 17.4/100 000 for men (world standardized rates), and from 12.5 to 10.5/100 000 for women, and these recent decreases in CRC mortality rates in several European countries are likely due to improvement in earlier diagnosis and treatment, with a consequent higher survival[10].

CRC incidence is generally higher in men, and the risk increases with age, as the majority of cases are diagnosed in patients older than 50 years[1, 3, 8], with only 5% of cases recorded in patients younger than 40 years[1]. A large nationwide study identified CRC as one of the 10 most commonly diagnosed cancers among men and women aged 20-49 years[11]. The prevalence of advanced CRC also increases with age and is higher among men than women[12].

COLORECTAL CANCER PROGNOSIS AND DISEASE PROGRESSION

The main prognostic factors in CRC are tumor size (T), lymph node involvement (N), grade of differentiation (G) and distant disease spread (M)[1-3,9,13,14]. Other important factors include invasion of blood and/or lymphatic vessels and penetration or perforation of the bowel wall[14].

Long-term survival correlates with stage of the disease[9, 15-17], and this is the most important predictor of mortality. The five-year survival rate for localized disease is 90.4%, but only 39% of CRC is diagnosed at this early stage[9, 16]. Approximately 15-20% of patients die as a consequence of CRC in early stages compared with 40-80% in advanced stages[15]. The overall 5-year survival rate varies among studies but is approximately 60%[9, 15, 16]. Stage-specific survival rates are 96%, 87%, 55%, and 5% for TNM stage Ⅰ, Ⅱ, Ⅲ, and Ⅳ, respectively[9,17,18].

One third of the patients submitted to curative intent surgery die of local and/or distant tumoral recurrence [15]. Among the sites of metastasis, liver is the organ most frequently involved (38%-60% of cases), followed by abdominal lymph nodes (38%), lung (38%) and peritoneum (28%)[14]. Of those diagnosed with metastatic disease, less than 10% are still alive after 5 years[16]. The 5-year overall survival rates for patients in whom hepatic resection was technically feasible and who had metastasis confined to the liver was only 25%-40%[7,19,20]. Better results were reported by Abdalla *et al* and Choti *et al*, with a 5-year overall survival rate of 58% following resection[21] and a rate of 67% described by de Haas *et al*[22]. These higher survival rates likely reflect improvements in patient selection, perioperative and postoperative care, multidisciplinary treatment, and an appropriately aggressive approach to safe hepatic resection[21]. Therefore, early diagnosis is critical to improve survival rates in CRC[23] and owing to its typically slow growth, there is a large potential for reducing the burden of the disease by early detection and removal of precancerous lesions or early cancer stages[24].

On the other hand, the pathologic clinical stage is currently the single most well-established prognostic indicator, but it does not fully predict individual clinical outcome[7, 25, 26]; also, the response of clinically-identical tumors to the same treatment may be vastly different[1]. This is particularly contentious for those tumors with intermediate stage disease (Stage Ⅱ, T3-T4N0M0)[7], where one third of patients with tumor-free lymph nodes have recurrences, and therefore, adjuvant chemotherapy may be beneficial[27]. In this group, carcinoma cells are not detected in lymph nodes by conventional staging methods in 24% of patients. Surgical technique and specific pathological staining may improve staging accuracy and the appropriate selection of patients for chemotherapy[27]. Furthermore, the identification of cancer penetration or perforation is particularly important in defining CRC aggressiveness[14]. Accordingly, identification of prognostic molecular markers capable of categorizing those patients at high-risk, would be very helpful for improving treatment strategies mainly in lymph node negative patients, determining the characteristics of patients' outcome, predicting cancer dissemination and recognizing which patients might benefit most from adjuvant chemotherapy and those unlikely to benefit thus sparing them the toxicities of treatment[14, 27-29].

Molecular markers may improve clinicopathologic staging and provide a basis to guide novel therapeutic strategies which target specific tumor-associated molecules according to individual tumor biology[1, 2, 7, 14], however, so far, no ideal molecular marker has been found to predict disease progression[29].

HIGHLIGHTS OF THE ANGIOGENESIS PHENOMENON

Angiogenesis plays a key role in tumorigenesis and metastatic processes[1, 28, 30]. It consists of the formation of new blood vessels from the endothelium of pre-existing vasculature[2, 30]. Sprouting from existing blood vessels is the principal process of angiogenesis and involves proliferation of activated endothelial cells, migration of endothelial cells to reach remote targets, assembly of endothelial cells into new capillary tubes, followed by synthesis of a new basement membrane and maturation of vessels with formation of a vascular lumen[30]. However, recruitment and *in situ* differentiation of bone marrow-derived endothelial

progenitor cells are also involved[30].

Tumor angiogenesis is essential to allow neoplastic mass development favoring access to the blood components, and also strengthening the vascular routes in the metastatic process[25, 31-33]. Neovascularization as a whole promotes tumor growth by supplying nutrients, oxygen and releasing growth factors that promote tumor cell proliferation[25, 30, 34-36]. Hypoxia in solid tumors occurs at a distance of ≥ 70 μm from functional blood vessels and it is generally accepted that tumors do not exceed a volume of 1-2 mm^3 without induction of angiogenesis[36]. Intratumoral vasculature density is believed to be associated directly with cancer cell entrance into the systemic blood circulation, with the ability of cancer cells to invade locally normal anatomic structures, and the establishment of blood-borne metastases in distant organs[32, 37]. Regulation of tumor angiogenesis is the result of a complex balance between many stimulatory and inhibitory factors, which are secreted by both tumor cells and host-infiltrating cells as well as by tumoral stroma-cells activity[2, 30, 34]. Malignant neoplastic cells promote angiogenesis by secreting growth factors such as vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF) and platelet-derived growth factor (PDGF), among others that stimulate endothelial migration and proliferation[2,25,31,33,37,38].

The role of angiogenesis as a prognostic factor, however, is still controversial[13, 39]. Weidner *et al* first reported a direct correlation between the incidence of metastasis and the number and density of blood vessels in invasive breast cancers. Similar studies have endorsed this correlation in gastrointestinal cancers[33] and in a variety of malignancies[2, 7, 13, 25, 35, 37]. An association between increased angiogenesis and an increased incidence of metastases and a subsequent decrease in survival curve rates was observed for the vast majority of solid tumors[2, 7, 3, 25, 35, 37].

Several studies revealed high angiogenic activity in CRC, which was more likely correlated with aggressive histopathological features that included parietal invasion, tumor stage, grade of tumor differentiation, metastatic potential and poor patient survival[1, 13, 32]. Tanigawa[35] *et al* confirmed this premise, although a significant variation in patient populations and techniques was used, which can explain, in part, the inverse relationship between tumor vascularity and patient survival observed by these authors. Gurzu[13] *et al* added that augmented angiogenesis in CRC was higher in early-stages of tumoral proliferation but was not a progressively increasing process, having rather an oscillating character.

However, other studies revealed that angiogenesis does not provide any significant information[13, 28, 30]. These controversial statements may be credited to the lack of standardization of the different methods of counting tumoral blood vessels and to the different cut-offs used to define relevant parameters to consolidate the results and, lastly, to the different antibodies used to highlight the blood vasculature[13, 28, 30].

Despite the debates, assessment of tumor angiogenesis may be particularly useful in prognostic classification of patients with apparent early cancer by conventional tumor staging, some of which may still develop early recurrence or metastasis (despite being staged as having early cancers by conventional parameters such as tumor size)[30].

De Vita[37] *et al* observed that highly angiogenic tumors were associated with the presence of lymph node invasion . Nevertheless, a higher percentage of patients with node-positive colon cancer than those without will experience recurrence and might benefit from anti-angiogenic adjuvant therapy. Thus, angiogenesis can be used to identify a subset of patients at high risk for recurrence regardless of their lymph node involvement[35].

There is evidence that blood vessel density is also important in predicting cancer response to chemotherapy or radiotherapy[20]. Angiogenic tumors have a more aggressive phenotype and the degree of intra-tumoral microvessels is significantly predictive of poor response to platinum-based chemotherapy in terms of complete response, as seen in two studies, one in squamous cell carcinoma patients[40] and the other in patients with epithelial ovarian cancers[41]. In addition, Takagi[42] *et al* observed that blood vessel density was a valid predictor of the effects of intra-arterial targeted carboplatin chemotherapy and concurrent radiotherapy for treating human oral and oropharyngeal squamous cell carcinomas. Zhang[43] *et al*, trying to identify reliable predictive factors for local control of hypopharyngeal cancer (HPC) treated by radiotherapy, observed that microvessel density (MVD) in biopsy specimens was closely correlated with local control of HPC treated by radiotherapy. In one study of 28 patients with advanced gastric cancer treated by paclitaxel and carboplatin, tumors with medium MVD showed a significantly higher response rate compared with those with either a high or low MVD[44]. Long course of radiotherapy significantly decreased angiogenesis in rectal cancer tissue. MVD have been found to be a favorable marker for tumor behavior during radiotherapy and a predictor of overall survival after a long course of radiotherapy. Further investigations are now needed to determine the changes in angiogenesis during a shorter course of radiotherapy[1]. However, the most important clinical implication of tumor angiogenesis is probably the development of anti-angiogenic therapy, targeting tumor vessels instead of cancer cells[30].

ENDOGLIN AND ASSESSMENT OF MICROVESSEL DENSITY AS ANGIOGENIC MARKERS

Microvessel density (MVD) assessment is the most common technique used to quantify intratumoral and peritumoral angiogenesis in cancer[2, 7, 28, 30, 39]. It was first developed by Weidner *et al* in 1991 who used pan-endothelial immunohistochemical staining of blood microvessels, mainly with Factor VIII related antigen (F. VIII Ag or von Willebrand's factor), CD31 or CD34, and rarely CD105[2].

Measurement of angiogenesis is complicated by the

fact that it is a dynamic process. Intra-tumoral microvessels can be identified by immunostaining of endothelial cells by two categories of human endothelial cell-specific antibodies: the pan-endothelial cell markers and specific antibodies that bind selectively to proliferating endothelium[44, 45]. CD31 is utilized as the pan-endothelial marker of choice; it is characterized by equal intensity of staining for small and large vessels. The disadvantages associated with staining for CD31 antigen include co-staining of inflammatory cells. The selective antibodies, such as endoglin, distinguish quantitatively between tumor neovascularization and pre-existing vessels with no or poor staining of lymphatics and normal quiescent blood vessels[46]. Most studies revealed that high MVD predicts occurrence of metastatic disease[2, 7, 13, 25, 32, 35, 37], and although tumor angiogenesis is unlikely to be the only factor responsible, it provides large numbers of leaking blood vessels for vascular invasion[25].

Endoglin (CD105) is a receptor for the TGF-β1 molecule that is up-regulated in tumor angiogenesis [13, 25, 29]. Its secretion is induced by hypoxia[29] and, as it is present mainly in new vessels, it is very useful in the assessment of newly formed vessels in malignant neoplasms[13, 25, 29]. It is also currently accepted as a potential target for anti-angiogenic therapy, especially in cancer patients at risk of developing metastases[29]. The endoglin antibody binds preferentially to the activated endothelial cells that participate in tumor angiogenesis, however, endoglin expression is weak/or negative in vascular endothelium of normal tissues; accordingly, it is a more specific and sensitive marker of tumor angiogenesis than the others commonly used such as pan-endothelial markers[25, 29]. Intra-tumoral MVD determined by immunohistochemical staining for endoglin has been reported to be an indicator of poor prognosis in many types of solid neoplasia such as breast carcinoma, cervical cancer, endometrial carcinoma, gastric carcinoma, melanoma, some testicular tumors, non-small cell lung cancer, prostate cancer, renal cell carcinoma and squamous cell carcinoma[29].

In CRC, many reports indicate that endoglin assessed immunohistochemically correlates not only with MVD, but also with survival curves, and it has also been identified as a valuable parameter for predicting increased risk of developing metastatic disease[25, 29,42]. Yan[47] *et al* reported that MVD was higher in CRC patients with metastases than in those without and observed that the specificity and sensitivity of MVD in predicting metastatization in CRC was 66.22% and 51.72%, respectively. In other studies, the presence of endoglin also had a prognostic meaning, showing a positive correlation with the presence of angio-lymphatic invasion, lymph node metastases, tumor stage and hepatic metastases, reinforcing the premise that endoglin might be considered for further therapeutic trials as anti-angiogenic therapy[25, 29].

Endoglin is not only expressed on the cell surface but its soluble form can also be detected in the blood[29, 48]. Myśliwiec[29] *et al* demonstrated an apparent continuous endoglin rise in plasma from patients with metastatic colorectal cancer, and Li[48] *et al* reported that circulating endoglin levels positively correlated with CRC Dukes' stage and survival; patients with a high MVD, above the median 3.10 × 250, showed the worst prognosis. Takahashi[49] *et al* observed that increased serum endoglin was associated with metastasis in patients with solid tumors including colorectal and breast carcinomas; and, in CRC patients, the difference in endoglin levels between the metastasis-negative patients and the metastasis-positive patients was statistically significant. Conversely, it was recently demonstrated that assessment of endoglin in plasma is not a useful maker of CRC, but might be helpful in selecting patients with metastatic diseas[29].

VASCULAR ENDOTHELIAL GROWTH FACTOR FAMILY AND CRC

Quantification of angiogenic factors in solid malignant tumors provides an alternative to MVD evaluation in assessing tumor angiogenic activity[28, 30]. Numerous studies have demonstrated that tumor overexpression of vascular endothelial growth factor (VEGF) correlates with high tumor MVD and is associated with advanced tumor stage or tumor invasiveness in various common human cancers[30, 37, 50, 51] and, its overexpression in colon cancer tissue indicates poor prognosis[51]; although paradoxically, some data showed that MVD might have a significant prognostic value in colon cancer tissue, whilst VEGF has not[52].

VEGF is the most widely studied angiogenic factor; it increases vascular permeability and is the most potent, direct acting, angiogenic protein known[28, 29, 36, 37, 52]. Normally, VEGF is weakly expressed in a wide variety of human and animal tissues; however, high levels of VEGF expression can be detected at sites where physiologic angiogenesis is required, such as fetal tissue or placenta, or in the vast majority of human tumors and other diseases i.e., chronic inflammatory disorders, diabetes mellitus, and ischemic heart disease[37]. Furthermore, both VEGF and its receptors are expressed at high levels in metastatic human colon carcinomas and in tumor-associated endothelial cells, respectively[37]. Consequently, VEGF is recognized as a prominent angiogenic factor in colon carcinoma and the assessment of VEGF expression may be useful for predicting metastasis from CRC[37]. In fact, VEGF expression was found to be higher in patients with metastatic tumors than in those with non-metastatic tumors[37,38], and high levels of VEGF expression were associated with advanced cancer stage and related with unfavorable prognosis[51-53].

De Vita *et al* [37] reported that preoperative serum VEGF levels might be useful for predicting the outcome of colon cancer patients following surgery. After surgery, VEGF levels tend to decrease compared with preoperative concentrations[30, 37]. Conversely, elevated VEGF levels after surgery may indicate significant residual disease, even

if it is not evident macroscopically[37].

Other studies have shown that VEGF is also a useful marker for prognosis by significantly correlating with angio-lymphatic invasion, lymph node status and depth of invasion, notwithstanding it was not an independent prognostic factor[25, 29].

Although numerous publications dealing with the measurement of circulating VEGF for diagnostic and therapeutic monitoring have been published, the relationship between the production of tissue VEGF and its concentration in blood is still unclear[31]. Some of the controversies regarding the clinical value of VEGF serum level measurement are related to the well-known fact that circulating VEGF is largely found in platelets, and as a consequence an open debate is ongoing to clarify if VEGF serum levels truly reflect tumor expression of VEGF or whether there are other potential sources of circulating VEGF, such as blood cells[30]. Cressey[31] *et al* noted that the cell-associated isoform (VEGF189), but not the soluble isoforms (VEGF121 and VEGF165) appear to play an important role in tumor progression. In addition, Serum VEGF protein levels are a prognostic parameter for progression-free and overall survival in CRC. Patients with high soluble VEGF levels might have a more aggressive disease, and the improved outcome observed in their series might be a reflection of the disease biology[54,55].

The effect of VEGF depends not only on tumor cell expression of VEGF, but also on the VEGF receptors in the endothelial cells[30]. The ligands of the VEGF family include VEGF-A, VEGF-B, VEGF-C, VEGF-D and VEGF-E; and the receptors are VEGFR-1, R-2 and R-3[56].

VEGF-A is commonly overexpressed by a wide variety of human tumors, and this overexpression has been correlated with progression, invasion and metastasis, MVD, and poorer survival and prognosis[56]. In CRC, VEGF-A is the ligand of the VEGF family most abundantly expressed[29]. VEGF-A promotes angiogenesis through enhancement of permeability, activation, survival, migration, invasion, and proliferation of endothelial cells[57]. VEGF-A and VEGF-B play a role in early tumor development at the stage of adenoma formation[7, 58].

Myśliwiec[29] *et al* found a strong positive association with VEGF-A plasma concentrations assessed postoperatively and the presence of distant metastases. Zlobec[59] *et al* also correlated high VEGF expression with response to preoperative radiotherapy in patients with rectal tumors.

VEGF-C and -D are glycoproteins structurally similar and sharing areas of sequence homology with VEGF-A. In CRC, augmented VEGF-C expression has been found to correlate with lymphatic invasion and lymph node metastasis[60]. Elevated levels of serum VEGF-C have been found in patients with breast cancer, lung cancer and cervical cancer and it appears to be an independent marker for early diagnosis of cancer metastasis. Moreover, increased VEGF-C mRNA expression in tumor tissues correlates positively with lymphatic metastasis and poor prognosis[61]. A correlation between VEGF-D expression levels in the primary tumor and lymph node metastasis is still disputable, with controversial data reported[62].

Another important fact is that through the development of anti-angiogenic therapy, CRC prognosis is improving[30, 63-65]. Median survival of patients with metastatic CRC (mCRC) treated with best supportive care is approximately 6 mo. Palliative chemotherapy considerably improves treatment outcome, with fluorouracil (FU) plus irinotecan and/or oxaliplatin extending median overall survival to approximately 20 mo[66]. Thus, in the past decade, the median overall survival of patients with mCRC has increased from 12 mo to approximately 20 mo, mainly due to the development of new combinations with standard chemotherapy[67]. Currently, anti-angiogenic treatment can prolong the survival time by some months, however, the results are not reproducible for all cases[13]. There have been clinical trials which show as many as 94% of invasive carcinomas and 88% of *in situ* carcinomas having a complete response[68]. Unfortunately, there are no tumor characteristics or molecular markers at present that help to identify patients who are likely to benefit from anti-angiogenic treatment[69].

Bevacizumab (BV) is a monoclonal antibody against VEGF with anti-angiogenic properties, and several clinical trials supported the use of BV in the first-line treatment of mCRC[70]. BV is typically used in combination with other chemotherapeutic agents such as oxaliplatin, irinotecan, leucovorin, and 5-fluorouracil (5-FU) for treatment of patients with mCRC[70, 71]. In addition to its direct anti-angiogenic effects, BV may also improve the delivery of chemotherapy by changing tumor vasculature and decreasing the elevated interstitial pressure in tumors[69]. When combined with standard chemotherapy regimens, it has been associated with significant improvements, compared with chemotherapy alone, in the efficacy end points of overall survival, progression-free survival, and response rates in patients with mCRC and for some facilitates secondary resections[72]. Jubb[73] *et al* demonstrated that in patients with mCRC, the addition of BV to irinotecan, 5-FU/leucovorin (IFL) improves survival regardless of the level of VEGF expression, or MVD. In a review by Tappenden[74]*et al*, the addition of BV to IFL resulted in a statistically significant increase in median overall survival (OS) of 4.7 mo, and in a median progression-free survival (PFS) of 4.4 mo. An overall tumor response rate of 44.8% was reported for BV plus IFL compared with 34.8% for IFL plus placebo within one study. In a pivotal, placebo-controlled, phase III trial in patients with mCRC (Genentech Study 2107), the addition of BV to IFL resulted in a significantly longer survival time (20.3 *vs* 15.6 mo) and progression-free survival time (10.6 *vs* 6.2 mo) than with IFL plus placebo[73, 75-78]. In a placebo-controlled, phase II trial (Genentech Study 2192), adding BV to 5-FU plus LV resulted in a significantly longer progression-free survival time than with 5-FU and LV plus placebo in

Table 1 The main results of CD105 and VEGF studies

Study	*n*	High levels of CD105 were associated with	High levels of VEGF were associated with
Barozzi *et al*[7]	101	M1	M1
Saad *et al*[25]	150	M1, N1 and angiolymphatic invasion	N1, angiolymphatic and depth of invasion
De Vita *et al* [37]	81	NE	NCs (serum levels)
Cascinu *et al*[38]	121	NE	RR
Myśliwiec *et al*[29]	48	M1	Colorectal cancer patients (plasma levels)
Li *et al*[48]	111	Dukes' stages and survival	NE
Takahashi *et al*[49]	34	M1	NE
Liang *et al*[51]	114	NE	N1, TNM staging and poor prognosis
Zheng *et al*[52]	97	NE	Poorly differentiated adenocarcinoma
Cressey *et al* [31]	76	NE	TNM
Cao *et al*[53]	71	NE	N1, M1, TNM, and OS
Miyazaki *et al*[58]	127	NE	RR, DF, OS (plasma levels)

DF: Disease-free; M1: Positive distant metastasis; N1: Positive lymph node metastasis; NCs: Non-curative surgery; NE: Not evaluated; OS: Overall survival; RR: Recurrence rate

patients with mCRC who were unsuitable candidates for first-line therapy with irinotecan (9.2 *vs* 5.5 mo). There was also a trend towards a longer survival time in patients receiving 5-FU, LV, and BV (16.6 *vs* 12.9 mo)[77]. BV was also tested in mCRC combined with an oxaliplatin-based regimen in the second-line setting. In this randomized phase Ⅲ trial (E3200), patients with previously treated CRC were randomized into 3 arms: FOLFOX4 plus BV, FOLFOX4 and BV only. Results showed superior survival and progression-free survival in the FOLFOX4 plus BV arm. In this study, BV was equally effective with the oxaliplatin-based regimen[78].

BV ultimately achieved FDA approval in 2004 as a first-line treatment for mCRC in combination with chemotherapy, based on its statistically and clinically meaningful benefits on progression-free survival and OS and has since garnered additional approval[79]. BV is the most used VEGF inhibitor with clear proof of efficacy in CRC, however, optimal use of this agent at various stages of the disease is still under investigation. Additionally, there are numerous other angiogenic agents targeting VEGF and other pro-angiogenic systems in clinical development[80]. These novel targeted agents inhibit the VEGF pathway by targeting the VEGF ligand, its receptors or by blocking downstream signaling pathway components. Anti-angiogenic agents include antibodies, small molecule tyrosine kinase (TK) inhibitors, antisense oligonucleotides and aptamers[81].

Table 1 summarized the main results of CD105 and VEGF studies.

CONCLUSION

Despite major advances, in terms of knowledge and treatment of CRC in recent years, the single most well-documented prognostic marker of pathologic stage remains the gold standard for disease stage at diagnosis. Angiogenesis plays an important role in the growth and progression of cancer but its role as a prognostic factor is still controversial. Most studies report that endoglin and vascular endothelial growth factor family expression are indicators of poor prognosis in CRC patients. Beyond these controversies, the ultimate clinical implication of tumor angiogenesis is the development of anti-angiogenic therapy, targeting tumor vasculature.

REFERENCES

1 **Svagzdys S**, Lesauskaite V, Pavalkis D, Nedzelskiene I, Pranys D, Tamelis A. Microvessel density as new prognostic marker after radiotherapy in rectal cancer. *BMC Cancer* 2009; **9**: 95
2 **Des Guetz G**, Uzzan B, Nicolas P, Cucherat M, Morere JF, Benamouzig R, Breau JL, Perret GY. Microvessel density and VEGF expression are prognostic factors in colorectal cancer. Meta-analysis of the literature. *Br J Cancer* 2006; **94**: 1823-1832
3 **Brenner H**, Hoffmeister M, Haug U. Should colorectal cancer screening start at the same age in European countries? Contributions from descriptive epidemiology. *Br J Cancer* 2008; **99**: 532-535
4 **Aljebreen AM**. Clinico-pathological patterns of colorectal cancer in Saudi Arabia: younger with an advanced stage presentation. *Saudi J Gastroenterol* 2007; **13**: 84-87
5 **Center MM**, Jemal A, Smith RA, Ward E. Worldwide variations in colorectal cancer. CA Cancer J Clin 2009; **59**: 366-378
6 **Henry KA,** Niu X, Boscoe FP. Geographic disparities in colorectal cancer survival. *Int J Health Geogr* 2009; **8**: 48
7 **Barozzi C,** Ravaioli M, D'Errico A, Grazi GL, Poggioli G, Cavrini G, Mazziotti A, Grigioni WF. Relevance of biologic markers in colorectal carcinoma: a comparative study of a broad panel. *Cancer* 2002; **94**: 647-657
8 **Zavoral M**, Suchanek S, Zavada F, Dusek L, Muzik J, Seifert B, Fric P. Colorectal cancer screening in Europe. *World J Gastroenterol* 2009; **15**: 5907-5915
9 **Alexander DD**, Waterbor J, Hughes T, Funkhouser E, Grizzle W, Manne U. African-American and Caucasian disparities in colorectal cancer mortality and survival by data source: an epidemiologic review. *Cancer Biomark* 2007; **3**: 301-313
10 **Bosetti C**, Levi F, Rosato V, Bertuccio P, Lucchini F, Negri E, La Vecchia C. Recent trends in colorectal cancer mortality in Europe. *Int J Cancer* 2011; **129**: 180-191

11 **Fairley TL**, Cardinez CJ, Martin J, Alley L, Friedman C, Edwards B, Jamison P. Colorectal cancer in U.S. adults younger than 50 years of age, 1998-2001. *Cancer* 2006; **107**: 1153-1161
12 **Brenner H**, Altenhofen L, Hoffmeister M. Sex, age, and birth cohort effects in colorectal neoplasms: a cohort analysis. *Ann Intern Med* 2010; **152**: 697-703
13 **Gurzu S**, Jung J, Azamfirei L, Mezei T, Cîmpean AM, Szentirmay Z. The angiogenesis in colorectal carcinomas with and without lymph node metastases. *Rom J Morphol Embryol* 2008; **49**: 149-152
14 **Cascinu S**, Georgoulias V, Kerr D, Maughan T, Labianca R, Ychou M. Colorectal cancer in the adjuvant setting: perspectives on treatment and the role of prognostic factors. *Ann Oncol* 2003; **14** Suppl 2: ii25-ii29
15 **Calvo HJ**, Ortega GD, Pardo RJM, López, MAJ, Cubo T. Biologia molecular del processo metastásico del cancer colorectal. *Cirugia Española* **2000**, **68** : 577-587
16 **Zafar SY**, Abernethy AP, Abbott DH, Grambow SC, Marcello JE, Herndon JE 2nd, Rowe KL, Kolimaga JT, Zullig LL, Patwardhan MB, Provenzale DT. Comorbidity, age, race and stage at diagnosis in colorectal cancer: a retrospective, parallel analysis of two health systems. *BMC Cancer* 2008; **8**: 345
17 **Ries LA**, Wingo PA, Miller DS, Howe HL, Weir HK, Rosenberg HM, Vernon SW, Cronin K, Edwards BK. The annual report to the nation on the status of cancer, 1973-1997, with a special section on colorectal cancer. *Cancer* 2000; **88**: 2398-2424
18 **Townsend MC**, Beauchamp D, Evers MB, Mattox LK. Sabiston Textbook of Surgery, 18th ed.; Saunders: Canada, 2008
19 **Liu CL**, Fan ST, Lo CM, Law WL, Ng IO, Wong J. Hepatic resection for colorectal liver metastases: prospective study. *Hong Kong Med J* 2002; **8**: 329-333
20 **Lambert LA**, Colacchio TA, Barth RJ. Interval hepatic resection of colorectal metastases improves patient selection* *Curr Surg* 2000; **57**: 504
21 **Abdalla EK**, Vauthey JN, Ellis LM, Ellis V, Pollock R, Broglio KR, Hess K, Curley SA. Recurrence and outcomes following hepatic resection, radiofrequency ablation, and combined resection/ablation for colorectal liver metastases. *Ann Surg* 2004; **239**: 818-25; discussion 825-827
22 **de Haas RJ**, Wicherts DA, Flores E, Azoulay D, Castaing D, Adam R. R1 resection by necessity for colorectal liver metastases: is it still a contraindication to surgery? *Ann Surg* 2008; **248**: 626-637
23 **Lang K**, Korn JR, Lee DW, Lines LM, Earle CC, Menzin J. Factors associated with improved survival among older colorectal cancer patients in the US: a population-based analysis. *BMC Cancer* 2009; **9**: 227
24 **Brenner H**, Hoffmeister M, Arndt V, Haug U. Gender differences in colorectal cancer: implications for age at initiation of screening. *Br J Cancer* 2007; **96**: 828-831
25 **Saad RS**, Liu YL, Nathan G, Celebrezze J, Medich D, Silverman JF. Endoglin (CD105) and vascular endothelial growth factor as prognostic markers in colorectal cancer. *Mod Pathol* 2004; **17**: 197-203
26 **Gómez-Domínguez E**, Trapero-Marugán M, del Pozo AJ, Cantero J, Gisbert JP, Maté J. The colorectal carcinoma prognosis factors. Significance of diagnosis delay. *Rev Esp Enferm Dig* 2006; **98**: 322-329
27 **Bilchik AJ**, DiNome M, Saha S, Turner RR, Wiese D, McCarter M, Hoon DS, Morton DL. Prospective multicenter trial of staging adequacy in colon cancer: preliminary results. *Arch Surg* 2006; **141**: 527-33; discussion 533-534.
28 **Graziano F**, Cascinu S. Prognostic molecular markers for planning adjuvant chemotherapy trials in Dukes' B colorectal cancer patients: how much evidence is enough? *Ann Oncol* 2003; **14**: 1026-1038
29 **Myśliwiec P**, Pawlak K, Kukliński A, Kedra B. Combined perioperative plasma endoglin and VEGF--a assessment in colorectal cancer patients. *Folia Histochem Cytobiol* 2009; **47**: 231-236
30 **Pang RW**, Poon RT. Clinical implications of angiogenesis in cancers. *Vasc Health Risk Manag* 2006; **2**: 97-108
31 **Cressey R**, Wattananupong O, Lertprasertsuke N, Vinitketkumnuen U. Alteration of protein expression pattern of vascular endothelial growth factor (VEGF) from soluble to cell-associated isoform during tumourigenesis. *BMC Cancer* 2005; **5**: 128
32 **Giatromanolaki A**, Stathopoulos GP, Tsiompanou E, Papadimitriou C, Georgoulias V, Gatter KC, Harris AL, Koukourakis MI. Combined role of tumor angiogenesis, bcl-2, and p53 expression in the prognosis of patients with colorectal carcinoma. *Cancer* 1999; **86**: 1421-1430
33 **Kitadai Y**. Angiogenesis and lymphangiogenesis of gastric cancer. *J Oncol* 2010; **2010**: 468725
34 **Kwon KA**, Kim SH, Oh SY, Lee S, Han JY, Kim KH, Goh RY, Choi HJ, Park KJ, Roh MS, Kim HJ, Kwon HC, Lee JH. Clinical significance of preoperative serum vascular endothelial growth factor, interleukin-6, and C-reactive protein level in colorectal cancer. *BMC Cancer* 2010; **10**: 203
35 **Tanigawa N**, Amaya H, Matsumura M, Lu C, Kitaoka A, Matsuyama K, Muraoka R. Tumor angiogenesis and mode of metastasis in patients with colorectal cancer. *Cancer Res* 1997; **57**: 1043-1046
36 **Mosch B**, Reissenweber B, Neuber C, Pietzsch J. Eph receptors and ephrin ligands: important players in angiogenesis and tumor angiogenesis. *J Oncol* 2010; **2010**: 135285
37 **De Vita F**, Orditura M, Lieto E, Infusino S, Morgillo F, Martinelli E, Castellano P, Romano C, Ciardiello F, Catalano G, Pignatelli C, Galizia G. Elevated perioperative serum vascular endothelial growth factor levels in patients with colon carcinoma. *Cancer* 2004; 100: 270-278
38 **Cascinu S**, Staccioli MP, Gasparini G, Giordani P, Catalano V, Ghiselli R, Rossi C, Baldelli AM, Graziano F, Saba V, Muretto P, Catalano G. Expression of vascular endothelial growth factor can predict event-free survival in stage II colon cancer. *Clin Cancer Res* 2000; **6**: 2803-2807
39 **Rodrigo JP**, Cabanillas R, Chiara MD, García Pedrero J, Astudillo A, Suárez Nieto C. [Prognostic significance of angiogenesis in surgically treated supraglottic squamous cell carcinomas of the larynx]. *Acta Otorrinolaringol Esp* 2009; **60**: 272-277
40 **Gasparini G**, Bevilacqua P, Bonoldi E, Testolin A, Galassi A, Verderio P, Boracchi P, Guglielmi RB, Pezzella F. Predictive and prognostic markers in a series of patients with head and neck squamous cell invasive carcinoma treated with concurrent chemoradiation therapy. *Clin Cancer Res* 1995; **11**:1375-1383
41 **Hollingsworth HC**, Kohn EC, Steinberg SM, Rothenberg ML, Merino MJ. Tumor angiogenesis in advanced stage ovarian carcinoma. *Am J Pathol* 1995; **147**: 33-41
42 **Takagi S**, Inenaga R, Oya R, Nakamura S, Ikemura K. Blood vessel density correlates with the effects of targeted intra-arterial carboplatin infusion with concurrent radiotherapy for squamous cell carcinomas of the oral cavity and oropharynx. *Br J Cancer* 2006; **94**: 1580-1585
43 **Zhang SC**, Miyamoto S, Kamijo T, Hayashi R, Hasebe T, Ishii G, Fukayama M, Ochiai A. Intratumor microvessel density in biopsy specimens predicts local response of hypopharyngeal cancer to radiotherapy. *Jpn J Clin Oncol* 2003; **33**: 613-619
44 **Hasan J**, Byers R, Jayson GC. Intra-tumoural microvessel density in human solid tumours. *Br J Cancer* 2002; **86**: 1566-1577
45 **Poon RT,** Fan ST, Wong J. Clinical significance of angiogenesis in gastrointestinal cancers: a target for novel prognostic and therapeutic approaches. *Ann Surg* 2003; **238**: 9-28
46 **Romani AA**, Borghetti AF, Del Rio P, Sianesi M, Soliani P.

The risk of developing metastatic disease in colorectal cancer is related to CD105-positive vessel count. *J Surg Oncol* 2006; **93**: 446-455
47 **Yan G**, Zhou XY, Cai SJ, Zhang GH, Peng JJ, Du X. Lymphangiogenic and angiogenic microvessel density in human primary sporadic colorectal carcinoma. *World J Gastroenterol* 2008; **14**: 101-107
48 **Li C**, Gardy R, Seon BK, Duff SE, Abdalla S, Renehan A, O'Dwyer ST, Haboubi N, Kumar S. Both high intratumoral microvessel density determined using CD105 antibody and elevated plasma levels of CD105 in colorectal cancer patients correlate with poor prognosis. *Br J Cancer* 2003; **88**: 1424-1431
49 **Takahashi N**, Kawanishi-Tabata R, Haba A, Tabata M, Haruta Y, Tsai H, Seon BK. Association of serum endoglin with metastasis in patients with colorectal, breast, and other solid tumors, and suppressive effect of chemotherapy on the serum endoglin. *Clin Cancer Res* 2001; **7**: 524-532
50 **Lee SJ**, Kim JG, Sohn SK, Chae YS, Moon JH, Kim SN, Bae HI, Chung HY, Yu W. No association of vascular endothelial growth factor-A (VEGF-A) and VEGF-C expression with survival in patients with gastric cancer. *Cancer Res Treat* 2009; **41**: 218-223
51 **Liang JF**, Wang HK, Xiao H, Li N, Cheng CX, Zhao YZ, Ma YB, Gao JZ, Bai RB, Zheng HX. Relationship and prognostic significance of SPARC and VEGF protein expression in colon cancer. *J Exp Clin Cancer Res* 2010; **29**: 71
52 **Zheng S**, Han MY, Xiao ZX, Peng JP, Dong Q. Clinical significance of vascular endothelial growth factor expression and neovascularization in colorectal carcinoma. *World J Gastroenterol* 2003; **9**: 1227-1230
53 **Cao D**, Hou M, Guan YS, Jiang M, Yang Y, Gou HF. Expression of HIF-1alpha and VEGF in colorectal cancer: association with clinical outcomes and prognostic implications. *BMC Cancer* 2009; **9**: 432
54 **Paule B**, Bastien L, Deslandes E, Cussenot O, Podgorniak MP, Allory Y, Naïmi B, Porcher R, de La Taille A, Menashi S, Calvo F, Mourah S. Soluble isoforms of vascular endothelial growth factor are predictors of response to sunitinib in metastatic renal cell carcinomas. *PLoS One* 2010; **5**: e10715
55 **Cook KM**, Figg WD. Angiogenesis inhibitors: current strategies and future prospects. *CA Cancer J Clin* 2010; **60**: 222-243
56 **Duhoux FP**, Machiels JP. Antivascular therapy for epithelial ovarian cancer. *J Oncol* 2010; **2010**: 372547
57 **Hanrahan V**, Currie MJ, Gunningham SP, Morrin HR, Scott PA, Robinson BA, Fox SB. The angiogenic switch for vascular endothelial growth factor (VEGF)-A, VEGF-B, VEGF-C, and VEGF-D in the adenoma-carcinoma sequence during colorectal cancer progression. *J Pathol* 2003; **200**: 183-194
58 **Miyazaki T**, Okada N, Ishibashi K, Ogata K, Ohsawa T, Ishiguro T, Nakada H, Yokoyama M, Matsuki M, Kato H, Kuwano H, Ishida H. Clinical significance of plasma level of vascular endothelial growth factor-C in patients with colorectal cancer. *Jpn J Clin Oncol* 2008; **38**: 839-843
59 **Zlobec I**, Steele R, Nigam N, Compton CC.A predictive model of rectal tumor response to preoperative radiotherapy using classification and regression tree methods. *Clin Cancer Res* 2005; 15 : 5440-5443
60 **He M**, Cheng Y, Li W, Liu Q, Liu J, Huang J, Fu X. Vascular endothelial growth factor C promotes cervical cancer metastasis via up-regulation and activation of RhoA/ROCK-2/moesin cascade. *BMC Cancer* 2010; **10**: 170
61 **Thiele W**, Sleeman JP. Tumor-induced lymphangiogenesis: a target for cancer therapy? *J Biotechnol* 2006; **124**: 224-241
62 **Stintzing S**, Heinemann V, Moosmann N, Hiddemann W, Jung A, Kirchner T. The treatment of colorectal carcinoma with monoclonal antibodies: the importance of KRAS mutation analysis and EGFR status. *Dtsch Arztebl Int* 2009; **106**: 202-206
63 **Gille J**. Antiangiogenic cancer therapies get their act together: current developments and future prospects of growth factor- and growth factor receptor-targeted approaches. *Exp Dermatol* 2006; **15**: 175-186
64 **Sparano JA**, Gray R, Giantonio B, O'Dwyer P, Comis RL. Evaluating antiangiogenesis agents in the clinic: the Eastern Cooperative Oncology Group Portfolio of Clinical Trials. *Clin Cancer Res* 2004; **10**: 1206-1211
65 **Gruenberger B**, Tamandl D, Schueller J, Scheithauer W, Zielinski C, Herbst F, Gruenberger T. Bevacizumab, capecitabine, and oxaliplatin as neoadjuvant therapy for patients with potentially curable metastatic colorectal cancer. *J Clin Oncol* 2008; **26**: 1830-1835
66 **Ma AT**, Ma BB, Lei KI, Mo FK, Chan AT. Clinical predictors of response to cetuximab-chemotherapy in metastatic colorectal cancer. *Hong Kong Med J* 2010; **16**: 207-212
67 **Nussenbaum F**, Herman IM. Tumor angiogenesis: insights and innovations. *J Oncol* 2010; **2010**: 132641
68 **Boehm S**, Rothermundt C, Hess D, Joerger M. Antiangiogenic drugs in oncology: a focus on drug safety and the elderly - a mini-review. *Gerontology* 2010; **56**: 303-309
69 **Mahfud M**, Breitenstein S, El-Badry AM, Puhan M, Rickenbacher A, Samaras P, Pessaux P, Lopez-Ben S, Jaeck D, Figueras J, Alain-Clavien P. Impact of preoperative bevacizumab on complications after resection of colorectal liver metastases: case-matched control study. *World J Surg* 2010; **34**: 92-100
70 **Tol J**, Punt CJ. Monoclonal antibodies in the treatment of metastatic colorectal cancer: a review. *Clin Ther* 2010; 32: 437-453
71 **Shih T**, Lindley C. Bevacizumab: an angiogenesis inhibitor for the treatment of solid malignancies. *Clin Ther* 2006; **28**: 1779-1802
72 **Rougier P**, Mitry E. [Targeted biotherapy: a revolution in the management of patients with colorectal cancer?]. *Gastroenterol Clin Biol* 2009; **33**: 672-680
73 **Jubb AM**, Hurwitz HI, Bai W, Holmgren EB, Tobin P, Guerrero AS, Kabbinavar F, Holden SN, Novotny WF, Frantz GD, Hillan KJ, Koeppen H. Impact of vascular endothelial growth factor-A expression, thrombospondin-2 expression, and microvessel density on the treatment effect of bevacizumab in metastatic colorectal cancer. *J Clin Oncol* 2006; **24**: 217-227
74 **Tappenden P**, Jones R, Paisley S, Carroll C. Systematic review and economic evaluation of bevacizumab and cetuximab for the treatment of metastatic colorectal cancer. *Health Technol Assess* 2007; 11: 1-128, iii-iv
75 **Naschberger E**, Croner RS, Merkel S, Dimmler A, Tripal P, Amann KU, Kremmer E, Brueckl WM, Papadopoulos T, Hohenadl C, Hohenberger W, Stürzl M. Angiostatic immune reaction in colorectal carcinoma: Impact on survival and perspectives for antiangiogenic therapy. *Int J Cancer* 2008; **123**: 2120-2129
76 **Hurwitz H**, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, Berlin J, Baron A, Griffing S, Holmgren E, Ferrara N, Fyfe G, Rogers B, Ross R, Kabbinavar F. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med* 2004; **350**: 2335-2342
77 **Kabbinavar FF**, Wallace JF, Holmgren E, Yi J, Cella D, Yost KJ, Hurwitz HI. Health-related quality of life impact of bevacizumab when combined with irinotecan, 5-fluorouracil, and leucovorin or 5-fluorouracil and leucovorin for metastatic colorectal cancer. *Oncologist* 2008; **13**: 1021-1029
78 **Ma WW**, Hidalgo M. Exploiting novel molecular targets in gastrointestinal cancers. *World J Gastroenterol* 2007; **13**: 5845-5856
79 **Yancopoulos GD**. Clinical application of therapies targeting

VEGF. *Cell* 2010; 143: 13-16
80 **Hubbard J**, Grothey A. Antiangiogenesis agents in colorectal cancer. *Curr Opin Oncol* 2010; 22: 374-380
81 **Prat A**, Casado E, Cortés J. New approaches in angiogenic targeting for colorectal cancer. *World J Gastroenterol* 2007; 13: 5857-5866

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