

Chapter Number?

Modulation of Tumor Angiogenesis by a Host Anti-Tumor Response in Colorectal Cancer

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1. Introduction

Colorectal carcinoma (CRC) is the second most frequently occurring cancer in industrialized countries, in both men and women. The cumulative lifetime risk of developing colorectal carcinoma is about 6%, and the cancer-related five year survival rate is 62% (Smith et al., 2002). Malignant transformation of CRC occurs in a multistep process via three different pathways: the chromosomal instability pathway, the microsatellite instability pathway (Vogelstein et al., 1988) and the methylation pathway (Jass, 2002). Moreover, putative tumor-initiating cells with increased malignancy were isolated from CRC (O'Brien et al., 2007; Ricci-Vitiani et al., 2007). These cells exhibited stem cell-like characteristics; however, their role in CRC pathogenesis is still controversial (Shmelkov et al., 2008). Tumor development and metastasis require the presence of a newly formed vasculature. Tumor cells can directly promote angiogenesis but the tumor microenvironment plays also a crucial role in this process. The tumor microenvironment consists of a variety of conjunctive tissue components and cells, as well as infiltrating immune cells. It is inflammatory and undergoes constant remodelling. Immune cells are not only recruited in order to eliminate the tumor, they can also be attracted by tumor cells in order to support a tumor-promoting inflammation. In CRC, the type of immune cells infiltrating the tumor has been shown to influence tumor growth and patient survival (Galon et al., 2007; Tosolini et al., 2011). In addition, immune cells have been shown to exert antagonistic effects on tumor angiogenesis. In this chapter, we focus on the modulation of tumor angiogenesis by tumor infiltrating immune cells and on its implications in terms of diagnosis and prognosis in CRC.

2. Tumor angiogenesis and tumor vessels

Tumor growth beyond two to three millimeters in diameter and metastasis requires

angiogenesis, the formation of new blood vessels. Angiogenesis plays a crucial role in the development and progression of CRC, and this has been convincingly documented in the literature. It has been shown that microvessel density is increased in primary tumors compared to normal mucosa or adenoma tissues (Bossi et al., 1995), and this is a strong independent predictor of poor outcome (Takebayashi et al., 1996). A high microvessel density is associated with a more than threefold increased relative risk of cancer-related death from CRC (Choi et al., 1998). Moreover, the expression of vascular endothelial growth factor (VEGF), a potent angiogenesis-promoting factor, is significantly increased in all stages of colorectal carcinoma (Kumar et al., 1998). The major sources of VEGF are either the tumor cells themselves or monocytes/macrophages recruited into the tumor tissue through paracrine signalling. Intratumor expression of VEGF was also found to increase the relative risk of cancer-related death from CRC by twofold (Kang et al., 1997; Ishigami et al., 1998; Kahlenberg et al., 2003).

The recruitment and growth of tumor vessels is a critical adaptation step that has to be achieved during the development of clinically relevant solid tumors such as the CRC. This process has been termed "angiogenic switch" (Folkman, 1995) and the "induction of angiogenesis" has been included in the eight hallmarks of cancer defined by Hanahan and Weinberg (Hanahan & Weinberg, 2000; Hanahan & Weinberg, 2011). New vessels may arise through different ways in the organism under physiological and/or pathological conditions. During embryonic development angioblasts differentiate into endothelial cells in a process called vasculogenesis whereas new vessels in adults are generated through angiogenesis (Risau, 1997). The major driving molecules for angiogenic processes are VEGF, VEGF-C, angiopoietin-2, fibroblast growth factors and chemokines (Carmeliet & Jain, 2011). Active angiogenesis is achieved either by vessel sprouting, non-sprouting intussusception (splitting of existing vessels), vessel co-option (tumor cells hijack vasculature), vascular mimicry (tumor cells line vessels), luminal incorporation of bone marrow-derived endothelial progenitor cells or a recently described non-VEGF-dependent biomechanical mechanism (Risau, 1997; Kilarski et al., 2009; Carmeliet & Jain, 2011).

The role of so called "tumor stem cells" in tumor angiogenesis is currently heavily discussed. Cancer stem cells might not only have an impact on the growth and assembly of the CRC tumor cells themselves (O'Brien et al., 2007; Ricci-Vitiani et al., 2007) but also on the formation of tumor vessels (Ricci-Vitiani et al., 2010; Wang et al., 2010). The two latter studies described for the first time the differentiation of putative cancer stem cells not only into functional tumor cells but also into tumor endothelial cells. However, these findings were demonstrated for the brain tumor glioblastoma. Of note, normal neuronal stem cells are able to differentiate into endothelial cells under physiological conditions, which questions whether these findings can be also applied to non-brain tumors such as colorectal carcinoma.

Tumor vessels are structurally and functionally abnormal compared to vessels in healthy tissues (Carmeliet & Jain, 2000; Hida et al., 2008). In contrast to normal vessels, they show a deficient support provided by only few perivascular cells with loose connections to the endothelium and the vessels maintain an immature structure. The tumor vasculature is commonly disorganized and heterogenous, with excessive branching and shunts, reduced interendothelial cell contacts, reduced barrier function and uneven vessel lumen. This disturbs the blood flow in the tumors, leads to hypoxia and acidification in the tumor as well as high fluid pressure concomitant with increased resistance to the application of

systemic drugs [reviewed in (Carmeliet & Jain, 2000; Hida et al., 2008; Carmeliet & Jain, 2011)]. Tumor cells attempt to overcome this issue by the expression of more pro-angiogenic factors such as VEGF resulting in amplified formation of abnormal vessels. However, tumor hypoxia cannot be rescued by the formation of abnormal vessels (Leite de Oliveira et al., 2011).

When anti-angiogenic treatment was initially developed, tumor endothelial cells (TECs) were thought to be similar in all tumor types and, in contrast to tumor cells, genetically stable. However, subsequent studies showed that TECs are different in tumors from different organs and are actually genetically unstable. It has been suggested that this is due to the involvement of endothelial cells (ECs) from different vascular beds. In addition, tumor cells and TECs interact strongly with each other and with additional cells present in the stroma via paracrine and possibly also juxtacrine pathways. Importantly, these interactions might induce microenvironment-dependent abnormalities in TECs that could differentiate them from normal endothelial cells. Recently, studies in mice and humans showed that abnormalities observed in TECs are maintained over long periods in cell culture, and include chromosomal abnormalities (Streubel et al., 2004; Hida & Klagsbrun, 2005; Akino et al., 2009), resistance to apoptosis (Bussolati et al., 2003), increased adhesiveness for tumor cells (Bussolati et al., 2003), drug resistance (Xiong et al., 2009), abnormal angiogenic capability (Ghosh et al., 2008; Xiong et al., 2009), and pronounced growth in the absence of serum (Bussolati et al., 2003).

TECs have been isolated from numerous animal models and from a limited number of human tumors mentioned above (Bussolati et al., 2003; Streubel et al., 2004; Buckanovich et al., 2007; Xiong et al., 2009). Until recently, no viable, pure TEC cultures from human colorectal carcinomas were available, and the biological phenotype of these cells was not characterized at the functional level. We have developed the first protocol for the routine isolation of both CRC TECs and the corresponding ECs from normal colon tissue (NECs) by collagenase II-digestion followed by multiple CD31-MACS selections (Schellerer et al., 2007). It was demonstrated that the cells were of endothelial blood cell origin (CD31-, CD105-, VE-cadherin-positive; E-selectin-, VCAM-1-, ICAM-1-positive after stimulation with inflammatory cytokines; capability to form capillaries in matrigel, take up acetylated LDL and bind *ulex europaeus*; CD45-, CD68-, CK-20-, podoplanin-negative). Moreover, the isolated TECs maintained differences from NECs during long-term culture for example by decreased von Willebrand factor (vWF) levels in the isolated tumor endothelial cells as well as in the original cancer tissue biopsies compared to the corresponding normal endothelial cells and normal colon biopsy (Schellerer et al., 2007). Meanwhile, we could show that the TEC isolated from CRC differ from each other also at the transcriptome and genome level (data unpublished).

TEC-specific markers were isolated from CRC by serial analysis of gene expression after laser-microdissection of tumor vessels (St Croix et al., 2000). The identified genes were designated as tumor endothelial markers (TEMs) (St Croix et al., 2000; Nanda et al., 2004). However, out of the nine different TEMs initially described, five were not pursued in future studies and two were shown to be expressed by other cells rather than tumor endothelial cells (Lee et al., 2006; Christian et al., 2008). These results indicated that the initial samples were most likely contaminated with non-endothelial cells such as pericytes that cover the mature vessel. Up to now, no widely accepted specific marker for tumor vessel endothelial cells in the CRC or other human tumors has been identified. Accordingly, a superior

approach would be to specifically isolate pure, viable TEC cultures from CRC and then use these cells to identify TEC-specific markers.

In summary, the described results indicate that the induction and maintenance of tumor angiogenesis is an important feature in CRC growth and progression and that the interaction of TECs with tumor cells and other stromal cells changes the TEC phenotype. Furthermore, pure viable TEC cultures isolated from CRC might be a valuable tool, allowing functional analysis of the TEC phenotype in CRC and the identification of TEC-specific markers. Pure CRC-derived TEC cultures will shed light on the manifold interactions between tumor and endothelial cells and their impact on the pathogenesis and prognosis of this tumor. This understanding will lead to improved anti-angiogenic treatment strategies in the CRC.

3. Host anti-tumor response and angiogenesis in colorectal cancer

3.1 Tumor infiltrating immune cells and angiogenesis in CRC

In CRC, tumor progression is tightly associated with and partly promoted by the tumor microenvironment. The tumor microenvironment consists of extracellular matrix, the vasculature and tumor-infiltrating cells. Infiltrating cells are recruited through inflammation and chemoattractants produced by the tumor cells or by cells of the stroma. Tumor infiltrating cells comprise cancer-associated fibroblasts (CAFs), endothelial cells, platelets, mesenchymal stem cells and various types of immune cells. Initial studies addressing the prognostic role of intratumoral immune cells infiltrates in colorectal cancer were partly contradictory. Some studies supported a protective role of inflammatory infiltrates (Jass, 1986; Harrison et al., 1994; Ropponen et al., 1997; Naito et al., 1998; Leo et al., 2000; Guidoboni et al., 2001; Galon et al., 2006) but other reports did not (Roncucci et al., 1996; Nielsen et al., 1999).

It is now clear that the type, the subtype and the localization of the infiltrating immune cells determine their effects on the tumor cells and the tumor microenvironment. Both the innate and the adaptive immune responses are involved in this process. For instance, the infiltration of cytotoxic T cells and type I helper T cells (Th1 cells) in CRC correlates with a prolonged disease-free survival, whereas the presence of infiltrating Th17 cells is of poor prognosis (Galon et al., 2006). In the same way, polarization of tumor-associated macrophages towards either M1 or M2 subpopulation results in anti-tumorigenic (M1) or pro-tumorigenic (M2) effects (Mantovani & Sica, 2010). Some forms of inflammatory infiltrates participate to the anti-tumor immune response while other immune cells are actively recruited by the tumor to exploit their pro-angiogenic and pro-metastatic effects (Balkwill & Mantovani, 2001; Coussens & Werb, 2001).

In addition, there is a growing body of evidence that tumor infiltrating immune cells can modulate tumor angiogenesis in cancer and particularly in CRC as summarized in table 1 and discussed in more detail below.

Pro-angiogenic	Anti-angiogenic
Tumor-associated macrophages (M2)	Lymphocytes (Th1)
TIE-2 expressing monocytes	NK cells
Mast cells	NKT cells
Neutrophils	Dendritic cells
MDSCs	
Immature DCs	
Th17 lymphocytes	
Immature dendritic cells	

Table 1: pro-angiogenic or anti-angiogenic features of tumor infiltrating immune cells.

3.1.1 Tumor-associated macrophages

The recruitment of tumor-associated macrophages (TAMs) is mediated by various factors such as colony-stimulating factor-1 (CSF-1), which is produced by colon carcinoma cells, or the chemokines CCL2, CCL3, CCL4 and CCL5 (Sica et al., 2008a; Sica et al., 2008b). Tumors are predominantly infiltrated by TAMs with M2 polarization and high TAM infiltration in CRC is associated with a poor prognosis (Bacman et al., 2007). TAMs express pro-angiogenic factors including VEGF, basic fibroblast growth factor (bFGF), TNF- α , IL-8, IL-1 β or platelet derived growth factor- β (PDGF- β) (Figure 1) (Barbera-Guillem et al., 2002; Sica et al., 2008a; Sica et al., 2008b). In addition, TAMs secrete matrix metalloproteases (MMP-7, MMP12) which participate to tumor angiogenesis by remodelling the extracellular matrix (Peddareddigari et al., 2010).

3.1.2 TIE-2 expressing monocytes

TIE-2 expressing monocytes (TEMs) represent a subset of monocytes differing from the classical inflammatory monocytes (De Palma et al., 2005). The number of TEMs is increased in the blood of cancer patients and the tumor stroma of various types of cancers including CRC (De Palma et al., 2007; Venneri et al., 2007). TIE-2 is an angiopoietin receptor which is normally found at the surface of endothelial cells or haematopoietic stem cells. TEM recruitment in tumors is mediated by the chemokines CCL3, CCL5 and CCL8, and the expression of angiopoietin-2 by tumor cells or tumor endothelial cells (De Palma & Naldini, 2009; De Palma & Naldini, 2011). TEMs have been shown to promote tumor angiogenesis and tumor growth in tumor mouse models (De Palma & Naldini, 2011).

3.1.3 Mast cells

Mast cells are myeloid-derived cells which contain numerous granules rich in histamine and heparin. They are resident in tissues and represent key effectors of allergic reactions. Mast cells can also infiltrate tumors where they localize in the vicinity of blood vessels (Maltby et al., 2009). A high mast cell infiltration is usually associated with increased tumor growth,

invasion and vascularisation. It has been shown that low mast cell numbers in CRC samples correlate with a better patient survival and hypovascularization (Gulubova & Vlaykova, 2009). Mast cells are able to produce numerous pro-angiogenic factors such as VEGF, bFGF, angiopoietin-1, TNF- α , heparin, histamine or various proteases (Maltby et al., 2009). It has been suggested that mast cell infiltration triggers the “angiogenic switch” during tumor growth: mast cells might be involved in angiogenesis at early stages of tumor growth, while at late stages largely the tumor cells control growth and angiogenesis in a mast cell-independent manner (Coussens et al., 1999).

3.1.4 Neutrophils

Infiltrates of neutrophils have been observed in various cancers including CRC (Roncucci et al., 2008; Tazzyman et al., 2009). In addition, neutrophils are involved in the pathogenesis of inflammatory bowel disease (Roessner et al., 2008). The recruitment of neutrophils is mediated by the chemokines CXCL1 and CXCL8 (Eck et al., 2003). Neutrophils stimulate tumor angiogenesis by releasing proteins including VEGF, CXCL1, CXCL8 or MMP9. The latter induces the release of VEGF from the extracellular matrix by cleavage of heparan sulfates (Hawinkels et al., 2008; Tazzyman et al., 2009).

3.1.5 Tumor infiltrating lymphocytes

Recent studies have highlighted the prognostic importance of tumor infiltrating lymphocytes (TILs) in colorectal carcinoma (Galon et al., 2006; Katz et al., 2009). The type, density and localization of T-cells in colorectal tumors have been found to be a better predictor of patient survival than the classical histopathological staging (Galon et al., 2006). T-cells can be divided in different subtypes. Naïve CD4⁺ T-cells differentiate in T helper (Th) cells of type 1 (Th1) in the presence of IL-12 or of type 2 (Th2) in the presence of IL-4 (Zhou et al., 2009). Th1 and Th2 cells inhibit each other. The presence of a Th1 adaptive immune response in CRC correlates with a better survival and an anti-angiogenic phenotype (Galon et al., 2006; Naschberger et al., 2008). Th1 cells facilitate the recruitment and the action of CD8⁺ cytotoxic T cells (Zhang et al., 2009). In CRC, CD8⁺ infiltrating T cells are the cell type most strongly associated with an improved survival (Galon et al., 2006). Th1 cells and CD8⁺ T-cells produce IL-12 and IFN- γ , both anti-angiogenic cytokines (Figure 1) (Zhu & Paul, 2010; Briesemeister et al., 2011). IL-12 promotes the production of IFN- γ by CD8⁺ T-cells and reduces the production of pro-angiogenic proteases such as MMP-9 by endothelial cells (Tartour et al., 2011). IFN- γ induces the production of angiostatic chemokines (CXCL9 and CXCL10) by endothelial cells and blocks the production of both VEGF and bFGF (Tartour et al., 2011).

Besides Th1 and Th2 cells, two other populations of T-cells have been shown to be involved in cancer, namely the regulatory T-cells (Treg) and the Th17 cells. In CRC, the infiltration of Treg, as well as of Th2 cells, seems to have no influence on patient survival (Tosolini et al., 2011). However, a direct association was found between the presence of a Th17 response and a worse prognosis (Tosolini et al., 2011). Th17 cells differentiate from naïve CD4⁺ T-cells upon exposure to IL-6 or TGF- β , and produce IL-17, IL-17F and IL-22 (Zhou et al., 2009). IL-17 promotes angiogenesis by inducing the production of angiogenic growth factors and chemokines by tumor cells and fibroblasts (Figure 1). Furthermore, IL-17 exerts a direct

effect on endothelial cells, increasing migration and tube formation. Finally, IL-17 can indirectly promote angiogenesis by recruiting neutrophils to the tumor site (Tartour et al., 2011).

3.1.6 Myeloid-derived suppressor cells

Myeloid-derived suppressor cells (MDSCs) are immature myeloid cells including progenitors of macrophages, granulocytes and DCs. The number of MDSCs has been shown to be increased in the blood of CRC patients (Mandruzzato et al., 2009). MDSCs are immunosuppressive and in particular inhibit T-cells (Condamine & Gabrilovich, 2011). In addition, they modulate the action of NK cells and induce Treg cells. MDSCs exert their functions through up-regulation of NO, arginase or ROS (Gabrilovich & Nagaraj, 2009). In mouse models, MDSCs have been shown to promote angiogenesis, tumor cell invasion and metastasis (Youn & Gabrilovich, 2010). MDSCs are very heterogenic but one can distinguish two different subtypes: the granulocytic (G)-MDSCs and the monocytic (M)-MDSCs (Youn & Gabrilovich, 2010). G-MDSCs are found in the spleen or in peripheral lymphoid organs, use primarily ROS for immune suppression, require cell-cell contact with T cells and are dependent on antigen-specific interactions (Youn & Gabrilovich, 2010). M-MDSCs are found in tumors, use primarily iNOS, arginase and cytokines for immune suppression, their action does not require direct cell-cell contact. M-MDSCs exert a non-specific suppression and are more potent (Youn & Gabrilovich, 2010). M-MDSCs are able to differentiate towards TAMs under hypoxic conditions (Corzo et al., 2010). Some MDSCs express endothelial markers such as CD31 or VEGFR2 and are able to incorporate into the tumor endothelium (Figure 1) (Yang et al., 2004).

3.1.7 Dendritic cells

Dendritic cells (DCs) are bone-marrow derived cells and represent the most important antigen-presenting cells (Salama & Platell, 2008). In CRC, DCs localize at the invasive margin of the tumor and in lymph nodes (Ambe et al., 1989; Suzuki et al., 2002). The presence of high number of DCs in CRC correlates with a better prognosis, in particular when DCs infiltrate the intra-epithelial compartment of the tumor (Dadabayev et al., 2004; Sandel et al., 2005). Mature DCs are able to produce IL-12 which induces the polarization of immune cells towards the Th1 anti-tumorigenic and anti-angiogenic phenotype. Tumors are in addition able to recruit immature DCs (iDCs) which have been shown in ovarian cancer to secrete pro-angiogenic factors and to be capable of incorporating in newly formed vessels (Figure 1) (Curiel et al., 2004).

3.1.8 NK and NKT cells

NK cells are lymphocytes from the innate immune system which are able to recognize tumor cells as target. The immune infiltration of NK cells represents a positive prognostic marker in various solid tumors including CRC (Coca et al., 1997). They represent together with CD8+ T cells the most likely effectors of the anti-tumor immunity. NK exert their anti-tumorigenic effects notably through the production of IFN- γ and participate therefore in the anti-angiogenic immune response (Levy et al., 2011).

NKT cells are a small population of T cells which also exhibit NK cells markers. They have the property to modulate immune responses and to link the innate and the adaptive immune responses. NKT cells are able to recognize lipid antigens that are not recognized by other T cells subsets (Terabe & Berzofsky, 2008). Two subtypes of NKT cells have been described. The most frequent type of NKT cells, called type I, has a very restricted T-cell receptor (TCR) repertoire and expresses the invariant V α 24J α 18 TCR. On the contrary, the type II NKT cells express different TCRs (Terabe & Berzofsky, 2008). NKT type I cells exert anti-tumor effects through IFN- γ but independently of perforin (van der Vliet et al., 2008). In addition, they activate DCs to produce IL-12. In colorectal carcinoma, a high infiltration of type I NKT cells, which are V α 24 positive, correlates with a better overall and disease-free survival (Tachibana et al., 2005). Through their production of IFN- γ and their activation of DCs, type I NKT cells participate in the Th1 anti-angiogenic immune response in CRC (Figure 1). While type I NKT cells enhance anti-tumor immunity, mouse models showed that type II NKT cells repress it (Terabe & Berzofsky, 2008).

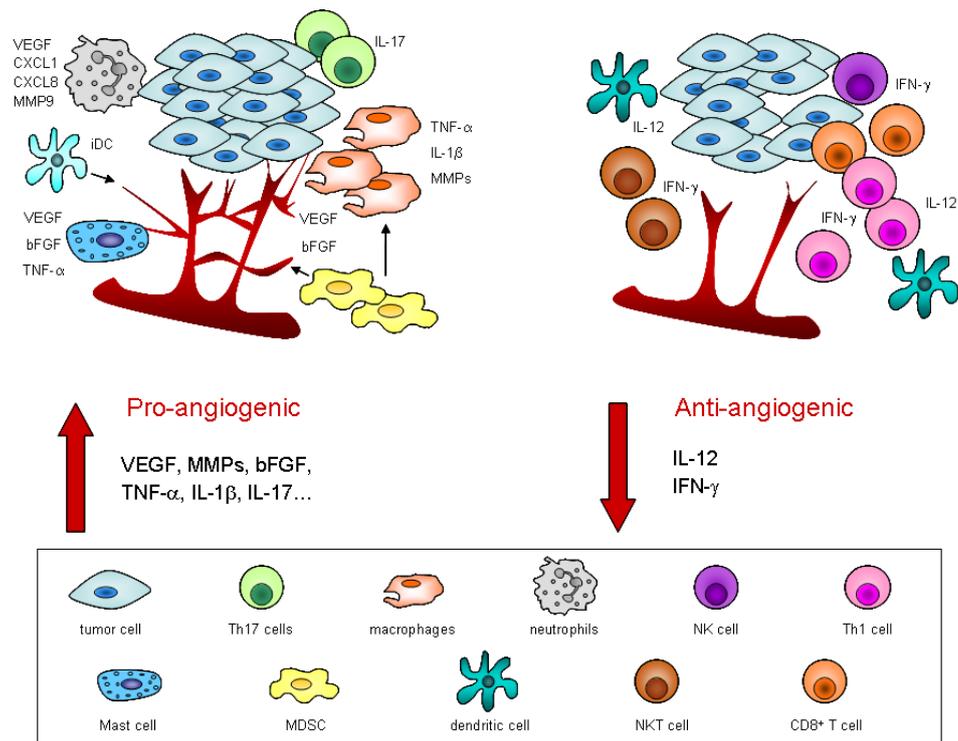


Figure 1: Tumor-infiltrating immune cells exert opposite effects on angiogenesis.

Tumor angiogenesis is promoted by the production of VEGF from the tumor cells but also from mast cells, M2 macrophages and neutrophils. In addition, macrophages and mast cells produce IL-1 β and TNF- α , which can promote a local pro-angiogenic inflammation through the further recruitment of macrophages *in vivo*, even if their direct action on endothelial cells *in vitro* is anti-angiogenic. Neutrophils and macrophages produce MMPs, inducing a matrix

remodeling necessary for angiogenesis. Th17 cells directly promote angiogenesis through the secretion of IL-17, which enhance the recruitment of neutrophils. Immature MDSCs can differentiate towards M2 macrophages or, like immature dendritic cells, can be incorporated into newly formed vessels. On the contrary, a Th1 dominated immune response exerts anti-angiogenic effects, mainly through the production of IFN- γ by Th1 cells, CD8⁺ T cells, NK or NKT cells. Th1 cells are activated by IL-12, notably produced by some DCs.

3.2 Markers for the interplay of angiogenesis and a host anti-tumor response in CRC

The impact of angiogenesis on colorectal tumor growth and progression described in the previous paragraphs was convincingly supported by a clinical phase III study in which an anti-VEGF antibody (bevacizumab) was added to fluorouracil-based combination chemotherapy. The combination therapy led to a statistically significant and clinically meaningful improvement in overall survival (20.3 months vs. 15.6 months for the control group) and progression-free survival among patients with metastatic CRC (Hurwitz et al., 2004). Based on these results bevacizumab was approved as the first solely anti-angiogenic drug used as anti-cancer agent by the FDA in 2004. Moreover, two additional anti-angiogenic drugs for the same molecular target have been approved for the clinics meanwhile: sunitinib and sorafenib. These drugs are both broad-spectrum receptor tyrosine kinase (RTK) inhibitors that target VEGFR1, VEGFR2, VEGFR3 or PDGFR- α/β among other RTKs (Escudier et al., 2007; Motzer et al., 2007).

However, in all of the clinical studies employing anti-angiogenic treatment for human tumors including CRC, only a fraction of the treated patients responded completely or partially to the therapy (10-49.3% maximum partial response rates) (Hurwitz et al., 2004; Demetri et al., 2006; Escudier et al., 2007; Motzer et al., 2007; Sobrero et al., 2009). Additionally, in some cases, severe side effects such as cardiovascular damage, perforation of the colon or venous thromboembolic events have been observed (Hurwitz et al., 2004; Sobrero et al., 2009). Furthermore, anti-angiogenic treatment is very expensive and puts a significant cost burden on the health system. This raises important questions: (1) which subset of patients will benefit most from these therapies? (2) How can these patients be preselected? (3) Can the side effects be decreased by patient preselection?

From these questions it becomes obvious that valid biomarkers able to indicate different angiogenic or angiostatic tumor microenvironments, and in consequence patients who will benefit most from anti-angiogenic therapy, are urgently required. Numerous efforts have been undertaken to identify predictive and/or prognostic biomarkers and this research field is rapidly expanding. By definition a predictive biomarker is able to foretell the response of the patient to a certain treatment whereas a prognostic biomarker predicts the potential outcome of the disease independently of the applied therapy. Promising results have been reported in the last few years, however, none of the proposed markers has been accepted widely (Asghar et al., 2010; Gerger et al., 2011).

Different kinds of potential biomarkers for anti-angiogenic treatment have been reported in the literature in the past few years: serum, tissue and genetic markers. Initially, for obvious reasons VEGF tissue and serum levels were heavily investigated but surprisingly did not make it into the clinics due to the inability to predict response at the tissue level (Jubb et al., 2006) and contradictory results at the serum level (Loupakis et al., 2007; Willett et al., 2009).

Efforts have also been undertaken to investigate the impact of genetic polymorphisms of VEGF and the VEGFR-2 as potential biomarkers (Schneider et al., 2008). Many other potential biomarkers were reported in the last few years in the literature either to be measured at the tissue or serum/plasma level. Examples for these markers are tissue CD31 and PDGFR- β expression in breast cancer (Yang et al., 2008), soluble angiopoietin-2 (Goede et al., 2010), circulating endothelial cells (Ronconi et al., 2010), TNF- α , MMP-9 (Perez-Gracia et al., 2009), soluble KIT (Deprimo et al., 2009) as well as IL-8 (Kopetz et al., 2010). However, all of these potential markers require either confirmation in larger cohorts and unfortunately lack either prognostic or predictive value.

From these results it becomes clear that very likely different biomarkers will be required for the different kinds of anti-angiogenic treatments and the different kinds of cancers. In addition, as discussed in the section 3 of this review, a broad range of immune cells can infiltrate tumors and have been detected in CRC samples. These cells interact with tumor endothelial cells during their extravasation and some of them are able to modulate tumor angiogenesis (Figure 1). While tumor infiltrating macrophages, mast cells, Th17 lymphocytes and neutrophils are recognized to exert pro-angiogenic effects in CRC, Th1 lymphocytes are associated with an anti-angiogenic microenvironment. On the other end, tumor vessels can be more or less permissive for the infiltration of immune cells. Therefore, the interplay between immune cells and tumor endothelial cells represents an important issue with implications for the anti-tumor host response and angiogenesis.

3.2.1 GBP-1 as a marker for the anti-angiogenic Th1 immune response in CRC

As mentioned above, the presence of a Th1 microenvironment is associated with a significantly improved prognosis in CRC (Galon et al., 2006). A Th-1 microenvironment is characterized by increased IFN- γ expression, often combined with the increased expression of pro-inflammatory cytokines IL-1 β and TNF- α (Dayer, 2002b; Dayer, 2002a; Cui et al., 2007). The guanylate-binding protein 1 (GBP-1) has been identified as a marker of the Th1 microenvironment in CRC (Naschberger et al., 2008). GBP-1 expression is induced upon stimulation by IFN- γ but also by other pro-inflammatory cytokines such as IL-1 β and/or TNF- α (Guenzi et al., 2001; Lubeseder-Martellato et al., 2002). In CRC, GBP-1 is strongly expressed in infiltrating cells and in the vasculature and its expression correlates with expression of IFN- γ -induced genes, chemokines and immune reaction-associated genes (Naschberger et al., 2008). Among them, three anti-angiogenic chemokines known to play a role in tumors (CXCL9, CXCL10, CXCL11) could also be detected (Romagnani et al., 2004). GBP-1 expression in CRC stroma is associated with an increase of the cancer-related five-year survival rate and GBP-1 represents independent prognostic factor indicating a reduction of the relative risk of cancer-related death by half (Naschberger et al., 2008). In tumor-associated endothelial cells the presence of GBP-1 is associated with a decreased angiogenic activity (Naschberger et al., 2008; Guenzi et al., 2001; Guenzi et al., 2003). GBP-1 is presently the only marker available to specifically indicate whether endothelial cells in tissues are exposed to an angiostatic Th-1-like tumor microenvironment.

3.2.2 Modulation of lymphocytes infiltration by endothelial cells

The relationship between tumor angiogenesis and immunity is actually bidirectional. As described above, infiltrating immune cells can positively or negatively regulate angiogenesis in tumors. On the other hand, tumor endothelial cells are able to regulate extravasation of immune cells, notably through the expression of surface molecules. Among the potential molecular effectors identified, endothelin, endothelin receptor and CD137 seem to play a prominent role.

The endothelin-endothelin receptor axis

The endothelin (ET) family comprises four members designated ET-1 to -4 (Kandalaf et al., 2009). ETs derive from precursor proteins after cleavage by membrane-bound metalloproteinases. ET-1 is the most potent ligand and the most widely expressed in endothelial cells (Kandalaf et al., 2009). In addition, ET-1 is overexpressed in many tumor cell lines and many tumors, including CRC (Kusuhara et al., 1990; Arun et al., 2004; Bagnato & Rosano, 2008). Two endothelin receptors have been identified: the endothelin A and the endothelin B receptor, respectively ET_AR and ET_BR (Kandalaf et al., 2009). In normal tissues, ET_AR and ET_BR regulate vasoconstriction and are also involved in inflammation. Both receptors exert opposite effects. In particular, ET_AR promote T-cell adhesion to endothelial cells, whereas ET_BR inhibits it. In tumor cells, concomitant up-regulation of ET-1 and ET_AR inhibits apoptosis and promotes cell proliferation, invasion and metastasis (Kedzierski & Yanagisawa, 2001; Kandalaf et al., 2009). In a study comparing the expression profiles of tumor associated endothelial cells (TECs) in ovarian cancer with or without TILs, ET_BR has been associated with the absence of TILs and short patient survival time (Buckanovich et al., 2008). Of note, in this study, GBP-1 expression in TECs correlated with the presence of TILs. The inhibition of T cells homing in tumor by ET_BR is mediated by an increase of NO synthase and NO release and by a decrease in the expression of the adhesion molecule ICAM-1. In CRC, ET-1 and ET_AR are expressed by the tumor cells, generating a stimulatory loop, while ET_BR expression in TECs is reduced as compared to normal colon blood vessels (Ali et al., 2000a; Ali et al., 2000b; Asham et al., 2001; Hoosein et al., 2007). Investigation of the expression of ET_BR in TECs in relation to TILs infiltration might provide further insights into the molecular regulation of immune cells extravasation by endothelial cells in CRC.

CD137 (TNFRSF9)

CD137 is a surface glycoprotein of the TNF- α receptor family involved in T-cell co-stimulation (Shao & Schwarz, 2011). CD137 is expressed on the surface of activated T cells, NK cells, DCs, macrophages or B cells, while its ligand, CD137L is expressed by APCs (Shao & Schwarz, 2011). CD137 is induced under hypoxia and by TNF- α , LPS or IL-1 β . CD137 is however also expressed in human tumor capillaries, notably in CRC (Broll et al., 2001; Wang et al., 2008). In tumors, CD137 is expressed on the vessel walls whereas CD137L is expressed on tumor cells (Salih et al., 2000; Broll et al., 2001). The effects of CD137 are mediated by the up-regulation of V-CAM, I-CAM and E-selectin, inducing thereby the recruitment of T

lymphocytes (Palazon et al., 2011). In addition, it has been shown that the the ligation of CD137L on lung squamous carcinoma cells with CD137 on T cells induced IFN- γ production by T cells (Salih et al., 2000). Therefore, expression of CD137 by TECs might promote the recruitment of T cells in CRC and their polarization towards the anti-tumorigenic and anti-angiogenic Th1 subtype.

4. Conclusions

In this review we tried to shed light on the current understanding of tumor angiogenesis and its modulation by a potential host anti-tumor response with a specific focus on colorectal carcinoma. Our major aim was to point out the connection of these two processes. A host anti-tumor response does not only have a direct effect on the tumor cells but also a major impact on the development and function of the tumor vasculature. Different tumor microenvironments, which can either inhibit or foster angiogenesis, are established during a specific immune response. These various microenvironments are achieved by different means: (1) immune cells such as Th1-T-cells are attracted into the tumor tissue within the context of a specific host anti-tumor response that secrete soluble mediators (e.g. IFN- γ) directly acting on tumor endothelial cells in an anti-angiogenic manner. (2) The tumor cells themselves also attract immune cells such as M2 macrophages or Th17-T-cells that might release mediators which modulate the microenvironment in a pro-angiogenic manner. (3) Endothelial cells can also modulate the stromal composition of infiltrating leukocytes which alters the soluble mediator profile to which the tumor and its vasculature are exposed. Therefore, biomarkers are required in order to characterize the specific angiogenic phenotype of each CRC patient. Moreover, these biomarkers should have prognostic and/or predictive potential for anti-angiogenic treatment and at best also give information about the presence of a host anti-tumor immune response. A potential candidate for such a biomarker might be the guanylate binding protein-1 (GBP-1).

5. References

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