Review

The Role of Tumor-Associated Macrophages in Colorectal Carcinoma Progression

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Key Words

Tumor-associated macrophages • Colorectal carcinoma • Extracellular matrix remodeling • Tumor metabolism • Tumor angiogenesis

Abstract

Tumor-associated macrophages (TAMs) are one of the most abundant immune cells in the tumor microenvironment, and they play a pivotal role in prompting the various tumor growth. However, the role of TAMs in colorectal carcinoma (CRC) is controversial, because a few papers report that TAMs is beneficial to CRC patients. In this review, we discuss the good or bad roles of TAMs in CRC progression. Interestingly, recent studies provide strong evidence that TAMs facilitate CRC growth, but do not exert tumor suppressive activities. TAMs can stimulate CRC growth by altering extracellular matrix remodeling, tumor metabolism, angiogenesis, as well as the tumor microenvironment. Therefore, TAMs could serve as a target for CRC therapeutic treatment.

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Introduction

Colorectal carcinoma (CRC) is one of the most frequently diagnosed malignant tumors and one of the leading causes of cancer-related mortality worldwide. CRC has recently been understood to be tightly associated with chronic inflammation. CRC arises following chronic inflammation, such as in patients with inflammatory bowel diseases like ulcerative colitis or Crohn's disease [1, 2].

Tumor-associated macrophages (TAMs), essential components of the immune inflammatory response, play a critical role in tumor progression [3]. TAMs release pro-inflammatory cytokines, which contribute to the formation of a tumor inflammatory microenvironment [4]. Growing evidence suggests that TAMs disrupt tissue homeostasis, and facilitate tumor growth, progression, invasion and metastasis [5-7]. As a result, TAMs

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Table 1. The role of TAMs in CRC

TAMs Function	Model	Mechanisms	References
Beneficial	Patient	-	9-12
Harmful	Patient	-	22-24
Harmful	Patient	Microvascular bed extension	49-50
Harmful	Mice/Cell	Extracellular matrix remodeling	28
Harmful	Mice/Cell	Extracellular matrix-educated macrophage polarization	29
Harmful	Mice/Cell	Suppression of spermidine production	42
Harmful	Cell	Tumor microenvironment	51-52

infiltration into the tumor microenvironment is associated with a poor prognosis in cancer patients [6, 8]. However, some studies have reported that the presence of infiltrating macrophages is correlated with improved CRC patient survival [9-12].

This review discusses the dual role of TAMs in CRC, namely, both tumor-preventing and tumor-promoting activities (Table 1). Recently recognized effects of TAMs on extracellular matrix (ECM) remodeling, tumor metabolism, angiogenesis and the tumor microenvironment provide directed evidence that TAMs stimulate tumor growth. This information could lead to important CRC therapeutic advances through targeting TAMs.

Role of macrophages in cancer

TAMs are the major inflammatory cellular component in the tumor stroma, and are abundant in all stages of tumor progression. TAMs originate from circulating monocytes or tissue-resident macrophages and infiltrate into tumor tissue, subsequently acquiring pro-tumoral functions in response to stimuli in the tumor microenvironment [13]. TAMs can also be found near or within tumor masses. Upon activation, TAMs produce and release an array of growth factors, inflammatory mediators, and proteolytic enzymes that play a critical role in cancer progression and metastasis [14, 15].

Macrophages can be categorized into two distinct subtypes: “classically activated” M1 and “alternatively activated” M2 subtypes. M1 macrophages are key players in the elimination of pathogens and cancer, and therefore exert a strong inflammatory response that includes production of pro-inflammatory cytokines such as IL1 β , IL6, TNF α , and reactive oxygen species (ROS). In contrast, M2 macrophages are associated with the production of anti-inflammatory cytokines, such as IL-10, IL-13, and TGF- β . TAMs closely resemble M2 macrophages, and can constitute up to 80% of the tumor mass (Fig. 1) [15, 16].

Numerous clinical and experimental studies have provided substantial evidence that TAMs are highly abundant in poorly-vascularized, necrotic and hypoxic tissue; they exert a strong procarcinogenic effect in many solid tumor types and contribute to a poor clinical prognosis [17]. The main functional peculiarities of TAMs are significantly associated with the suppression of an immune reaction, angiogenesis stimulation, and ECM remodeling. By producing growth factors such as EGF, TAMs stimulate carcinoma cell proliferation [18, 19]. TAMs also produce proteolytic enzymes that digest the ECM, which encourage tumor cell dissemination from the primary tumor site, thereby contributing to metastasis. Moreover, TAMs provide a supportive niche for metastatic tumor cells at distant sites by secreting inflammatory factors, such as IL1 [4]. In addition, ROS and nitrogen intermediates generated by TAMs contribute to genetic instability of cancer cells [20], a hallmark of cancer that tremendously limits the effectiveness of anti-tumor chemotherapies.

More recently, it was widely recognized that macrophages display considerable plasticity and heterogeneity in the tumor microenvironment. TAMs can shift between M1 and M2 subtypes, depending on the microenvironmental stimuli. Macrophages may acquire

Fig. 1. Macrophages differentiate into M1 and M2 subtypes in response to tumor micro-environmental stimuli.

a variety of phenotypes during different stages of tumor progression. They may present the pro-inflammatory properties of an M1-like phenotype in the initial stages of tumor growth, then convert to an M2-like phenotype when the tumor cells begin to invade and metastasize. Based on these findings, therapeutic approaches that target the switch of macrophages from an M2 to an M1 phenotype could be effective in inhibiting tumor growth [21].

Dual role of TAMs in CRC

Numerous studies have indicated that the presence of a heavy macrophage infiltration facilitates CRC growth, progression and aggressiveness, and is correlated with poor survival of CRC patients. In one study, 120 CRC patients were divided into three groups based on low, medium and high amounts of liver metastases. M1 macrophages were negatively associated with tumor metastatic ability, while M2 macrophages were positively correlated with lymphatic and liver metastases and degree of tumor differentiation. The high M2/M1 ratio was found to be the bad clinical and pathological parameters. Therefore, the amount of M2 macrophages and the M2/M1 ratio are believed to be accurate predictors for occurrence of liver metastases in CRC patients [22]. A case-control study characterized peripheral blood monocyte samples from 360 CRC patients at four European oncology centers. Their results suggest that tumor-educated circulating monocytes could be used as a biomarker in CRC diagnosis, and potentially also for follow-up and therapeutic monitoring [23]. Furthermore, the presence of TAMs in the tumor stroma was correlated with poor survival in CRC patients, but the opposite correlation was found if TAMs were at the tumor front. The relative risk of recurrence and cancer-related death in patients with high TAMs infiltration in the tumor stroma were more than double that of patients with less infiltration [24].

Mouse models have also shown that TAMs act in concert to promote cell invasion, migration and metastasis, and suppress anti-tumor immune responses [25]. In one study, infiltrating macrophages with CARD9 overexpression correlated significantly with advanced histopathologic stage and the presence of tumor metastases. CARD9-deficient mice had fewer CRC liver metastases. A potential mechanism explaining the role of CARD9 in tumor metastasis could be through CARD9-induced macrophage polarization through activation of the nuclear factor-kappa B signaling pathway [22].

Several studies have reported very different findings: the presence of infiltrated macrophages in the tumor microenvironment is associated with an improved survival in CRC patients [9-12]. Included in one study were 210 patients who had undergone surgery for CRC. The absence of TAMs was positively associated with adverse clinical characteristics, such as metastases to local lymph nodes, advanced tumor stage, tumor cell invasion into blood and lymph vessels and perineural invasion. The data confirm that macrophage density in CRC is negatively correlated to patient survival and tumor markers, such as TGF- β 1 expression [9]. To further address the clinical relevance, another study investigated M1 macrophages (iNOS⁺) and M2 macrophages (CD163⁺) in 205 CRC primary tumors. Surprisingly, heavy CD163⁺ M2 macrophage infiltration in CRC tumors was found in tumors of a lower grade and in patients with fewer lymph node metastases. A survival benefit was seen in patients with high CD163⁺ M2 macrophages, but not with iNOS⁺ macrophages [11]. In addition, in a study with 158 CRC patients with liver metastases suggested that high TAMs infiltration in primary tumors is correlated with a better clinical outcome [12].

Effect of TAMs-induced ECM remodeling on CRC growth

The ECM is composed mainly of collagen, glycoproteins, and proteoglycans, and forms a physical and biochemical framework that regulates tissue homeostasis, organ development, inflammation and disease. Tumors are characterized by high levels of proteolytic degradation of physical barriers between cells. Dysregulated deposition, remodeling and degradation of the ECM can be caused by aberrantly expressed or modified structural proteins due to specific proteolytic and protein cross-linking enzymes. The ECM provides the critical tumor cellular ecosystem, which drives tumor cell growth, invasion, survival, as well as metastasis. Furthermore, a well-recognized ECM alteration in cancer is collagen deposition in tumor tissue. Collagen is the most abundant ECM component that constitutes up to about 30% of the total ECM protein network and 90% of the ECM in humans, and provides the tensile strength and structural integrity of human tissues and organs [26]. It has been reported that abnormal collagen deposition and metabolism results in increased fibrosis, and enhanced tumor development and invasion. During tumor progression, matrix metalloproteinase (MMPs) are crucial for cleaving collagen fibers and for degrading and organizing the ECM that ultimately facilitates collective tumor cell migration [26]. Genes encoding tumor-associated ECM components have been shown to tightly correlate with clinical outcomes. An increased expression of ECM remodeling-mediating genes may be used to evaluate the prognosis in patients with breast, lung and gastric cancers [26]. An increased secretion of hyaluronan by fibroblasts in the tumor microenvironment is observed in patients with pancreatic cancer [27].

A recent study demonstrated that TAMs promote tumor development, invasion, and dissemination by facilitating ECM remodeling in a CRC model. Ly6C^{hi} monocytes infiltrated into CRC tumors in a CCR2-dependent manner and matured into TAMs [28]. Therefore, CCR2^{-/-} mice served as a suitable model for studying TAMs-mediated remodeling of the ECM in CRC. Through a liquid chromatography-mass spectrometry approach, the proteomic analysis from CCR2^{-/-} and WT colorectal tumors revealed a distinct ECM signature in TAMs composed of an ensemble of matricellular proteins and remodeling enzymes that provided a critical role in the induction of tumor growth and ECM buildup. It was found that 46 ECM-related proteins were differentially expressed in the TAMs from the CCR2^{-/-} and WT colorectal tumors, including the core matrisome proteins collagen types I α 1, I α 2, IV α 1, the proteoglycans heparan sulfate proteoglycan 2, lumican, prolargin, TGF β -induced protein, and the ECM-associated modulator procollagen-lysine 2-oxoglutarate 5-dioxygenase 3. To study TAMs-mediated remodeling of the ECM composition, decellularized three-dimensional ECM fragments extracted from CCR2^{-/-} and WT colorectal tumors were used. It was found that the ECM from TAMs-positive tumors were tumorigenic, but not ECM from TAMs-deficient tumors. TAMs were found to fully assemble at the invasive margins of colorectal tumors and significantly contributed to the construction of a collagenous ECM at an early CRC

developmental stage. Further genomic and proteomic investigation revealed a matrix-related gene signature coding for different matrix proteins, as well as several proteolytic matrix enzymes involved in matrix synthesis and assembly. The proteins related to collagenous ECM components that were overexpressed in TAMs were involved in tumor collagen maturation (the $\alpha 1$ chains of collagen I, the three α chains of collagen VI, the collagenase MMP14, the enzyme P4HA1, the glycoprotein PCO LCE). Interestingly, TAMs also expressed ECM covalent cross-linking enzymes, such as TGM2 and F13A1, the complement C1q complex, and THBS1. To explore TAMs-related mechanisms contributing to tumor development, collagens XIV and I were extracted from cancer-associated fibroblasts (CAFs) in TAMs-deficient tumors. It was found that CAFs from TAMs-deficient tumors down-regulated the gene expression of collagen types I and XIV. This provides an additional mechanism by which TAMs may indirectly contribute to collagenous matrix remodeling through CAFs. Differences were only observed at the gene expression level and not the protein level, likely because of the scarcity of CAFs. It was also found that there was an increase in TAMs-defined ECM proteins (the abundance of collagen types I, VI, and XIV) in CRC patients, which confirm a clinical relevance for TAMs-induced ECM remodeling.

Another study investigated the impact of human colorectal tumor ECM on macrophage polarization, and showed that tumor ECM-educated macrophages develop into M2 macrophages in the tumor microenvironment [29]. In this study, paired tissue derived from CRC patients were decellularized to the native ECM, where native tissue characteristics (architecture and mechanical properties) and major ECM components (fibronectin, laminin, collagens type I and IV, and hyaluronic acid) are preserved. It was found that macrophages that differentiated within patient tumor matrices up-regulated anti-inflammatory markers (IL-10, TGF- β and CCL18) and down-regulated pro-inflammatory markers (IL-6 and TNF). MMP1, a matrix metalloproteinase attributed to M2-like polarization of TAMs, was increased in tumor matrices. These findings suggest that tumor-derived decellularized matrices significantly induce an anti-inflammatory M2-like macrophage polarization. Moreover, a close association between a more advanced tumor stage and high expression of chemokine CCL18 at the invasive front of human CRC, the region with the densest infiltration of macrophages, was observed. More importantly, tumor-derived ECM-educated macrophages efficiently stimulated CRC cell invasion via the CCL18 chemokine.

Effect of TAMs metabolism on CRC growth

Tumor metabolism plays an essential role in sustaining tumor growth and metastasis [30]. Tumor cells often utilize amino acids and fatty acids as substrates to generate metabolic products for cellular energetic maintenance and tumor microenvironment buildup. There is growing evidence that the metabolic profile of immune cells, and especially of macrophages, shape their activation state and function [31-33]. The metabolic profile of M1 macrophages is defined by aerobic glycolysis, flux through the pentose phosphate pathway, fatty acid synthesis, and a truncated TCA cycle, contributing in a distinct manner to their pro-inflammatory phenotype. This highlights the link between M1 macrophage metabolism and pro-inflammatory functionality. The metabolic profile of M2 macrophages is characterized by activation of the fatty acid oxidation pathway, oxidative phosphorylation, decreased glycolysis, and activation of the pentose phosphate pathway. In contrast to M1 macrophages, M2 macrophages show different energy metabolism. While M1 macrophages mainly produce ATP through glycolysis, M2 macrophages preferentially obtain their energy through the oxidative TCA cycle coupled to oxidative phosphorylation. During the metabolic process used by M2 macrophages, triacylglycerol-rich lipoproteins, such as LDL and VLDL, are important fatty acid sources. Besides differences in energy metabolism, M2 macrophages have an opposing arginine metabolism compared to M1 macrophages, which contributes to their functional polarization [34].

TAMs often have numerous cytoplasmic lipid droplets [35]. However, it remains unknown that the accumulated lipids are how to catabolism and TAMs metabolism is

how to reprogram to adapt the tumor invasion. For the first time, it was shown that TAMs metabolism prompts CRC tumor growth via a novel pathway and that the lipolytic factor ABHD5 suppresses spermidine production in a SRM-dependent manner. A gene-microarray analysis was used to examine lipid-related gene-expression. Among the catabolic enzymes of glycerolipids [36], only AB-hydrolase containing 5 (ABHD5) expression was found to be increased in CRC TAMs. ABHD5 is a lipolytic co-activator of adipose triglyceride lipase, which is involved in lipolysis of triglycerides into free fatty acids and diglycerides [37], and it also supports macrophage mitochondrial function as well as helps sustain the M2 phenotype [38-40]. Polyamine spermidine, a lipolytic factor, has been reported to have a strong association with various cancers [41]. A study revealed that macrophages expressing ABHD5 potentiate CRC growth through the suppression of polyamines spermidine production. These findings provide direct evidence that ABHD5 stands at the crossroads of polyamine synthesis and lipid catabolism in TAMs. Furthermore, *in vivo* and *in vitro* gene knockdown experiments verified that ABHD5 attenuate spermidine production in a spermidine synthase (SRM)-dependent manner in macrophages. To identify the possible binding sites between ABHD5 and SRM, a series of mutated sequences harboring different regions of the mouse SRM promoter (PV2-PV5) were constructed. Several potential elements for C/EBP ϵ , Elk-1 and STAT5A (–605B to –600) were found to be high scores, and may be involved in ABHD5 deficiency-induced SRM transcription. Reporter-gene assay and chromatin immunoprecipitation assay experiments confirmed that only the core sequences of the C/EBP ϵ -binding element (5'-GAGCAA-3') were essential for ABHD5 suppression of SRM promoter activity. The binding sites were locating at –605 to –600 bps in the C/EBP ϵ protein, whereas the binding sites for Elk-1 and STAT5A were non-functional. To explore the potential mechanism of ABHD5-mediated suppression of C/EBP ϵ -mediated SRM expression in macrophages, an antioxidant treatment was used to investigate ABHD5 deficiency-stimulated ROS production. Interestingly, ABHD5-mediated suppression of spermidine production was found with antioxidant treatment, leading to inhibition of CRC growth through the ROS-C/EBP ϵ -SRM-spermidine pathway in macrophages. ABHD5-mediated mitochondrial function may be another critical molecular mechanism in macrophages since mitochondrial dysfunction can cause ROS accumulation [42].

The role of TAMs on tumor angiogenesis CRC growth

A few studies have demonstrated that the amount of TAMs in a tumor are closely linked to the number of blood vessels in human cancers [43-46]. Tumor growth is characterized by hypoxia, therefore macrophages are recruited to hypoxic areas of the tumor. Hypoxia-inducible factor-1 α (HIF-1 α), expressed in TAMs and other cells, regulates the transcription of genes associated with angiogenesis in hypoxic sites in a HIF-1 α -dependent manner. TAMs secrete vascular endothelial growth factor, basic fibroblast growth factor, platelet-derived growth factor, TNF- α , IL-1 β , thymidine phosphorylase, MMPs, and other molecules, enhancing the formation of angiogenesis that ultimately provides the nutrition for tumor growth [47]. It has been found that TAMs are closely associated with the tumor vasculature in orthotopic and transgenic tumor models [47, 48].

In a published study, tumors from 44 CRC patients were studied to investigate macrophages infiltration and microvessel density. Macrophage infiltration and vascular density were found to be correlated with clinical stage and lymph node metastases. TAMs infiltration induced pro-inflammatory mediators such as IL-1 and IL-6, which increased the production of angiogenesis-related growth factors [49]. Another study demonstrated the same tendency. In this study, it was found that there was a significant correlation between macrophage infiltration and microvascular density in a series of tumors from 76 CRC patients. Therefore, TAMs could be used as a marker for angiogenesis-mediated CRC [50].

Fig. 2. Dual role of TAMs in CRC. 1) Only several prior studies support the idea of TAMs exhibiting tumor suppressive abilities, while most recent studies support the notion that TAMs contribute to tumor promotion. 2) Data showing TAMs tumor suppressive abilities are from a limited number of CRC patients and little molecular evidence is exhibited.

TAMs-mediated effects on the tumor microenvironment and CRC growth

Recently, several studies have indicated that TAMs are involved in CRC development and progression via tumor microenvironment modulation. One study confirmed that TAMs-driven oxidative stress, the pro-oxidant enzyme NADPH oxidase in macrophages, was significantly reduced. TAMs contributed to the development of colon carcinoma in an oxidative stress-dependent manner that potentiated the redox status and the angiogenic capacity of the tumor microenvironment [51]. It was found that after co-culturing TAMs with SW480 colon cancer cells, MMP-9 expression was increased, and the epithelial-mesenchymal transition proteins E-cadherin, β -catenin, vimentin, and snail were induced in SW480 cells. These findings showed that TAMs promote colon cancer cell invasion via the induction of epithelial-mesenchymal transition and MMP-9 expression [52].

CD8⁺ T cells in the tumor microenvironment can trigger an immune response and limit cancer growth and metastasis, which are features linked with favorable outcomes in patients with various cancer types. One study revealed the close relationship between macrophages and CD8⁺ T cells, where a low ratio of macrophages to CD8⁺ T cells predicted breast cancer patient survival, indicating a major role for macrophages in suppressing T cell activity against tumors [53]. In CRC tumors, CD8⁺ T cells were also reported to benefit patient outcome, serving as a useful prognostic marker [54]. Previous research indicated that the presence of TAMs within CRC was associated with the presence of T cells, a positive correlation with numbers of infiltrating regulatory T cells, and an inverse correlation with numbers of circulating T cells [55]. Further studies are required to better understand how macrophages within CRC tumors sustain a robust CD8⁺ T cell-mediated anti-tumoral immune response.

Conclusion

Macrophages are crucial components of mammalian tissues, in which they perform a variety of supportive functions including their classical functions as antimicrobial phagocytes. However, the molecular mechanism of how macrophage tissue specificity and their origin affect their tumor promoting/suppressive functions remains unclear. It is known that macrophages have high plasticity, therefore, the biological functions of macrophages likely differs based on organ/tissue specificity.

TAMs generally have been shown to be immune suppressive, which contributes to tumor development. However, some studies have reported that the presence of infiltrating TAMs may be advantageous to CRC patient survival. These studies have only provided data

correlating CRC patient survival and presence of TAMs, without any molecular evidence. Recently, strong evidence was found to elucidate the molecular mechanism of TAMs-mediated CRC growth promotion, primarily via modulation of ECM remodeling, tumor metabolism, angiogenesis, and the tumor microenvironment (Fig. 2). Thus, our review has emphasized the molecular mechanisms showing TAMs to facilitate CRC growth, which could potentially lead to important CRC therapeutic advances via therapeutically targeting TAMs.

Collectively, strong evidence was provided that demonstrate that TAMs facilitate CRC tumor progression, growth and aggressiveness. It is tempting to speculate that the diverse roles of TAMs might be influenced by the different stages of CRC progression. Further studies are required to understand how the macrophage phenotype changes at different stages of CRC tumorigenesis and development.

Disclosure Statement

The authors report no conflicts of interest in this work.

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