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REVIEW

Emerging role of tumor-associated macrophages as therapeutic targets in patients with metastatic renal cell carcinoma

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Abstract Tumor-associated macrophages (TAMs) derived from peripheral blood monocytes recruited into the renal cell carcinoma (RCC) microenvironment. In response to inflammatory stimuli, macrophages undergo M1 (classical) or M2 (alternative) activation. M1 cells produce high levels of inflammatory cytokines, such as tumor necrosis factor-α, interleukin (IL)-12, IL-23 and IL-6, while M2 cells produce anti-inflammatory cytokines, such as IL-10, thus contributing to RCC-related immune dysfunction. The presence of extensive TAM infiltration in RCC microenvironment contributes to cancer progression and metastasis by stimulating angiogenesis, tumor growth, and cellular migration and invasion. Moreover, TAMs are involved in epithelial–mesenchymal transition of RCC cancer cells and in the development of tumor resistance to targeted agents. Interestingly, macrophage autophagy seems to play an important role in RCC. Based on this scenario, TAMs represent a promising and effective target for cancer therapy in RCC. Several strategies have been proposed to suppress TAM recruitment, to deplete their number, to switch M2 TAMs into antitumor M1 phenotype and to inhibit TAM-associated molecules. In this review, we summarize current data on the essential role of TAMs in RCC angiogenesis, invasion, impaired anti-tumor immune response and development of drug resistance, thus describing the emerging TAM-centered therapies for RCC patients.

Keywords Cancer · Clinical trials · Inflammation · Innate immunity · Renal cell carcinoma · Tumor-associated macrophage

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Introduction

Renal cell carcinoma (RCC) has historically been considered highly resistant to both chemotherapy and radiation therapy, and for this reason, other therapeutic approaches have been investigated. In RCC, the ability of the immune system to recognize tumor antigens has been demonstrated, suggesting the possibility to effectively treat RCC patients with immunotherapeutic approaches. Rare cases of spontaneous tumor regression have been reported [1, 2], and diffuse tumor infiltrate consisting of T cells, natural killer cells (NK), dendritic cells (DCs), and macrophages has been observed [3, 4]. Despite being strongly infiltrated, RCC is generally not eliminated by different immune effector cells, and this immune dysfunction likely contributes to tumor evasion.

As recently reported, tumor microenvironment seems to be involved in the immune-escape mechanisms in RCC [5, 6]. Particularly, tumor-secreted factors such as CXCL8/interleukin 8, interleukin 6 (IL-6) and vascular endothelial growth factor (VEGF) play a crucial role in the intratumor alteration of DC differentiation, inducing a specific

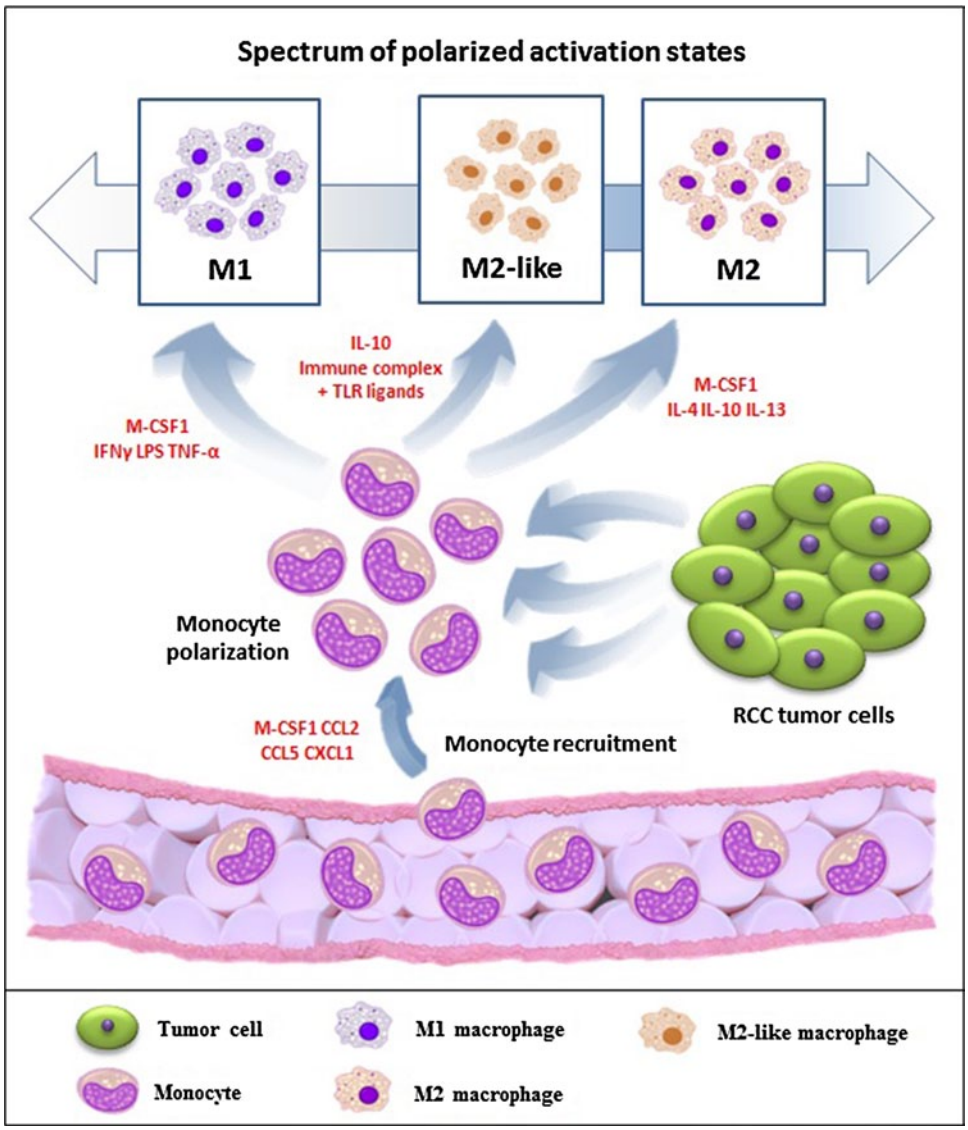

Fig. 1 Renal cell carcinoma (RCC) microenvironment induces monocyte recruitment and polarization

DC subset (ercDC), which co-expresses markers of DCs (CD209) and macrophages (CD14 and CD163). The ercDCs promote tumor cell proliferation by secreting high levels of metalloproteinase 9 (MMP-9) and by enhancing tumor-promoting tumor necrosis factor-α (TNF-α), while reducing specific chemokines, such as CXCL10 and CCL5 [5, 6].

Tumor-associated macrophages (TAMs) represent a major leukocyte population infiltrating tumors. In many but not all human cancers, a high frequency of TAMs is associated with poor prognosis [7]. TAMs constitute a quantitative and functional important subpopulation in the RCC microenvironment that is able to induce the alternative activation and differentiation of TAMs. In fact, TAMs originate from circulating blood monocytes that differentiate into macrophages following their extravasation into tissues. Tissue microenvironmental signals, such as colony-stimulating factor (CSF)-1, act as monocyte chemoattractants and induce macrophage differentiation [8]. In response to various signals, macrophages can undergo classical (M1) or alternative (M2) activation (Fig. 1), which are the extremes of a wide spectrum of polarized activation states that differ in terms of receptors, cytokine and chemokine expression and effector functions (Fig. 2). Classical, or M1, macrophages are characterized by the expression of high amounts of iNOS and TNF-α, whereas, alternatively activated, M2 macrophages typically express arginase 1 (ARG1), but not the inducible nitric oxide synthase (iNOS) [9].

In this paper, we will focus on the role of TAMs and associated molecules in RCC tumor angiogenesis, invasion and development of drug resistance, thus underlying their potential as therapeutic targets in RCC patients.

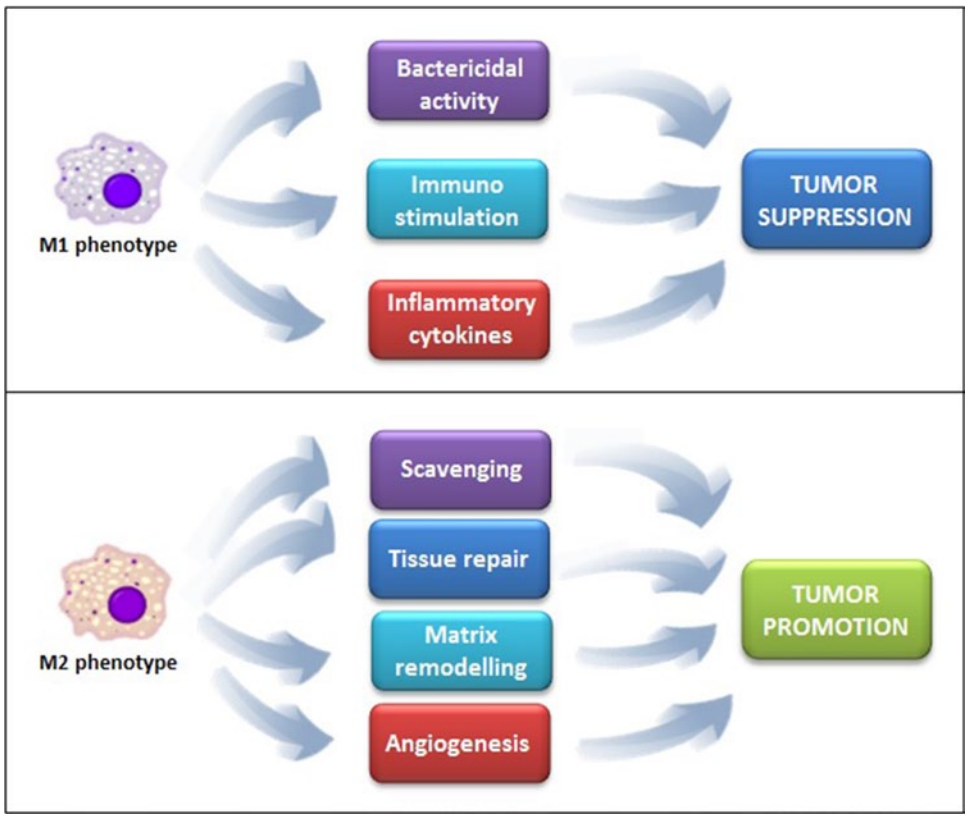

Fig. 2 Different functions exerted by M1 and M2 macrophage phenotypes

Role of TAMs in RCC tumor angiogenesis and invasion

TAMs are a key component of the RCC tumor microenvironment and orchestrate various aspects of cancer, such as tumor cell growth, invasion, metastasis, angiogenesis and immunoregulation. In RCC, the number of TAMs significantly correlates with tumor microvessel density and VEGF level [10]. Accordingly, the presence of E- and P-selectin-positive RCC tumor microvessels correlates with the amount of TAMs, and the expression of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) on neoplastic epithelia is associated with an increased density of macrophages and a minor degree of tumor differentiation [11].

TAMs significantly contribute to tumor angiogenesis by producing a wide array of growth factors such as platelet-derived growth factor (PDGF) and transforming growth factors β (TGF-β) [12, 13], and consequently stimulating several crucial signaling pathways, including the VEGF/VEGFR-1 pathway. Li and colleagues reported that VEGF in clear cell RCC is mainly produced by tumor stromal cells instead of the tumor cells themselves. The critical role of TAMs in the regulation of angiogenesis in RCC is suggested also by the evidence that knockdown of VEGFR-1 expression significantly attenuates macrophage tumor infiltration and inhibits the expression of monocyte chemoattractant protein-1 (MCP-1). Therefore, a reduction in VEGF production and tumor microvessel density has been reported [14].

The TAM production of placental growth factor (PIGF) that is a homolog of VEGF and is able to bind to VEGFR1 may also contribute to stimulate tumor angiogenesis [15] and may in part explain the resistance to VEGFR-targeted therapies [16]. Moreover, in RCC, TAMs and microvessels express simultaneously gastrin-relasing peptide (GRP) and its receptor suggesting the existence of an autocrine or paracrine loop within the tumor that regulates TAM recruitment, tumor growth and neoangiogenesis [17].

TAMs can adapt to the hypoxia status that characterizes RCC, resulting in an enhanced expression of pro-angiogenic genes. Indeed, hypoxia promotes the activation of the hypoxia inducible factor-1 (HIF-1) and hypoxia inducible factor-2 (HIF-2), which induce the expression of various protumoral genes in TAMs, such as VEGF and IL-8, thus significantly supporting angiogenesis, tumor growth and invasion [18].

Role of TAMs and associated molecules in RCC growth and metastasis

Macrophages are dynamic and heterogeneous cells. This is due to different mechanisms governing their differentiation, tissue distribution and responsiveness to stimuli.

Mosser and Edwards proposed to classify macrophages on the basis of their functions in three distinct subgroups: classically activated macrophages involved in host defense, wound healing macrophages involved in tissue repair and remodeling, and regulatory macrophages that play a role in immunoregulation [19]. Based on the evidence that wound healing and regulatory macrophages are basically variations of the M2 state, Mantovani et al. suggested the presence of three macrophage polarization states: M1 (=classical activation), M2 (=alternative activation) and M2-like (that incorporate all the other variations of M2 state) [20]. M1 macrophages stimulate cell-mediated responses via the production of pro-inflammatory cytokines IL-1, IL-6, IL-12, IL-23, TNF-α and high levels of IL-1 receptor type I (IL-1RI). On the other hand, M2 macrophages stimulate humoral responses, tissue remodeling and angiogenesis through the production of anti-inflammatory cytokines (IL-10 and TGF-β) and high levels of decoys that antagonize IL-1, such as IL1RII and IL-1 receptor antagonist [21]. M2 macrophages include at least three subsets: M2a induced by IL-4 or IL-13; M2b induced by immune complexes and agonists of TLRs or IL-1Rs; and M2c induced by IL-10 and glucocorticoid hormones [22].

M1 and M2 macrophages are also distinct for their chemokine expression profiles [23]. Indeed, M1 macrophages express inflammatory chemokines such as CXCL9 and CXCL10, whereas M2 macrophages express non-inflammatory chemokines CCL17, CCL18, CCL22 and CCL24 [20]. Furthermore, M1 and M2 macrophage phenotypes also show distinct metabolic features relating to glucose, amino acid, lipid and iron metabolism [24]. Notably, several differences have been shown between mouse and human polarized macrophages, such as the repertoire of surface receptors and arginine metabolism [25].

The presence of TAMs and high serum levels of these cytokines represents poor prognostic factors in RCC patients [26]. Accordingly, Yanase et al. reported that treatment with IL-1β, TNF-α or IL-6 increases in vitro the invasiveness of RCC cells. These effects are inhibited in the presence of an anti-VCAM-1 monoclonal antibody (mAb) [27].

A retrospective analysis by Dannenmann and colleagues has shown that clear cell RCC can progressively attract macrophages and promote their skewing into immunosuppressive M2 TAMs. The analysis of TAM-related transcripts reveals that the M2 but not M1 phenotype is associated with reduced survival and advanced tumor stage [28].

Furthermore, Komohara et al. have investigated the role of an anti-inflammatory macrophage phenotype M2 in clear cell RCC patients using CD163 and CD204 as markers. The number of CD163(+) cells was significantly associated with age, sex, nuclear grade and TNM classification. In addition, in vitro direct co-culture of RCC cells with macrophages led to stronger activation of signal transducers and activators of transcription-3 (STAT3) in RCC cancer cells. Interestingly, STAT3 activation was suppressed by down-regulating the membrane-type macrophage colony-stimulating factor (mM-CSF), thus suggesting for a potential contribute of mM-CSF to cancer cell activation [4].

Macrophages are the major producers of TNF-α and interestingly are also highly responsive to TNF-α. Indeed, TNF-α induces the activation of the MAPK cascade in a c-Raf-1 and Raf-B-independent fashion [29]. In addition, low doses of TNF-α, produced by RCC cancer cells and stromal cells, promote tumor growth and metastasis [30, 31]. Notably, IL-4 inhibits TNF-α-induced proliferation of RCC [32]. In addition, Ho et al. have shown that TNF-α induces epithelial–mesenchymal transition (EMT) and promotes tumorigenicity of RCC by repressing E-cadherin, up-regulating vimentin, and enhancing MMP9 expression and invasion. TNF-α-mediated tumor promotion of RCC is associated with TNF-α-induced inhibition of glycogen synthase kinase-3β (GSK-3β) activity through serine-9 phosphorylation mediated by the phosphatidylinositol 3-kinase/protein kinase B (PI3 K/Akt) pathway [33].

Sarcomatoid RCC often has an aggressive clinical course characterized by rapid disease progression. The sarcomatoid conversion of clear cell RCC can be associated with the process of EMT. In this context, TGF-β1 seems to play a major role during the sarcomatoid transdifferentiation of clear cell RCC [34].

Moreover, Kominsky and colleagues have demonstrated that TGF-β promotes the establishment of RCC bone metastasis [35]. TGF-β1 stimulation of RCC bone metastasis cells resulted in the initiation of tumor-promoting paracrine interactions between tumor cells and the bone microenvironment, thus promoting tumor growth and subsequent osteolysis in vivo. In addition, an extensive crosstalk between the Notch and TGF-β signaling pathways in clear cell RCC that is associated with the aggressiveness of this disease has been reported [36].

IL-6 has been implicated in the osteoclastic bone resorption and hypercalcemia associated with metastatic RCC [37]. The results published by Fitzgerald et al. have shown that enhanced levels of IL-6 and IL-8 result in RCC cell invasion and that activation of AMP-activated protein kinase (AMPK) reduces the expression of the NADPH oxidase isoform Nox4, IL-6 and IL-8 production and RCC cell invasion [38]. Furthermore, IL-6-induced proliferation of RCC cells is mediated by increased DNA binding activity of STAT3 and, to a lesser extent, of STAT1 [39]. Recently, Porta et al. have reported that progression in RCC patients is preceded by a significant increase in pro-angiogenic cytokines other than VEGF, such as IL-6, bFGF and HGF [40].

Role of TAMs and associated molecules in RCC-related immune dysfunction

TAMs isolated from RCC tumors mediate their immunosuppressive activity by a number of mechanisms, including the secretion of inhibitory cytokines, such as IL-1β, TNF-α, TGF-β and IL-6 [41], the generation of reactive oxygen species and the promotion of Treg development. Furthermore, TAMs increase the production of IL-10 by T cells and enhance the expression of the co-inhibitory molecules programmed death 1 (PD-1) and T-cell immunoglobulin mucin 3 (TIM-3) [28].

Moreover, RCC TAMs secrete IL-10, and this release can be prevented by inhibition of lipoxygenase activity in accordance with their high levels of 15-lipoxygenase-2 (15-LOX2) expression [42]. Furthermore, RCC TAMs can induce the pivotal T regulatory cell transcription factor forkhead box P3 (FOXP3) and the inhibitory cytotoxic T-lymphocyte antigen 4 (CTLA-4) coreceptor in a 15-LOX2 independent manner [42]. Taken together, these data suggest that RCC TAMs contribute to RCC-related inflammation, immunosuppression and malignant progression by activating the 15-LOX2-dependent pathway.

Bone morphogenetic proteins (BMPs) are multi-functional growth factors that belong to the TGF-β superfamily. In the context of RCC, BMP-4, -6 and -7 are often overexpressed [43, 44], whereas BMP antagonist sclerostin domain containing 1 (SOSTDC1) is down-regulated [45]. BMP-6 has been shown to inhibit B- and T-cell proliferation [46, 47], and it regulates the proliferation and gene expression profile of macrophages [48, 49]. In addition, Lee et al. have reported that human RCC cells frequently have a loss of expression of BMP receptors [43], suggesting a paracrine role of BMP-6 in RCC [50]. BMP-6 mediates IL-10 expression in macrophages via Smad5 and STAT3, thus leading to M2 polarization of TAMs [50].

Role of macrophage autophagy in RCC

Autophagy is a catabolic process conserved in all eukaryotes that involves the delivery of unnecessary or dysfunctional cytoplasmatic elements to the lysosome or vacuole for definitive degradation and recycling [51, 52].

Autophagic process is activated in various pathological situations involving the immune system such as cancer, pathogen infections and autoimmune diseases [53]. By using human peripheral blood monocytes exposed to CSF-1 and consequently differentiated in M2-polarized macrophages, Jacquel and co-workers demonstrated that autophagy is triggered during macrophage differentiation. The stimulation of CSF-1 receptor induces characteristic autophagic features such as LC3-II increased expression and acidic vesicle accumulation [54]. They also demonstrated by inhibiting autophagy with the use of pharmacological inhibitors, siRNA approaches and using ATG7$^{-/-}$ mice as experimental model that autophagy is an essential process for a proper differentiation of monocytes into macrophages and for the acquisition of normal phagocytic functions.

Furthermore, it has been recently reported that sorafenib can suppress the activation of human macrophages by inducing autophagy. Sorafenib inhibits the surface antigen expression and the function of macrophages, and is accompanied by morphological changes characteristic of autophagy. Moreover, in this study, sorafenib was found to reduce macrophage secretion of IL-10, but not IL-6, TNF-α or TGF-β [55].

Role of TAMs in modulating RCC response to treatment

Histamine inhibits the formation and release of phagocyte-derived reactive oxygen species, and thereby protects NK and T cells against oxidative damage. Donskov et al. have investigated the potential role of histamine in improving the efficacy of IL-2 in metastatic RCC patients. Patients with high number of peripheral blood monocytes and neutrophils had very poor survival with either IL-2 alone or IL-2/histamine treatment. While the number of blood NK cells positively correlated with cytotoxicity, that of blood monocytes and neutrophils negatively correlated with cytotoxicity. Treatment with IL-2 alone resulted in a significantly higher number of circulating monocytes and intratumoral macrophages, while no changes as compared with baseline were observed in patients treated with IL-2/HDC [56].

TAMs produce large amounts of VEGF and MMP9 and may be responsible for the tumor angiogenic switch [14]. Of interest, TNF-α and MMP-9 have been proposed as potential baseline predictive serum markers for the outcome of metastatic RCC patients treated with first-line sunitinib. In this study, TNF-α and MMP-9 baseline levels were significantly increased in non-responders and significantly associated with reduced overall survival (OS) and time to progression, respectively [57].

Using an orthotopic model of RCC, Weiss et al. have observed that IL-2/α-CD40 induces IFN-γ- and NO-dependent decrease in matrix MMP expression and activity, concomitantly with increased levels of the tissue inhibitor of metalloproteinase (TIMP) 1 and E-cadherin expression within tumors. Treatment with the NO donor JS-K significantly reduces the metastatic spread. The reduced MMP9 activity implicates M1-polarized macrophages within the tumor microenvironment as critical components of therapeutic response [58].

Furthermore, TAM depletion enhances sorafenib-induced inhibition of tumor progression, angiogenesis and lung metastasis in a metastatic liver cancer mouse model [59]. Notably, sorafenib was also found to potentially reverse the immunosuppressive cytokine profile of TAMs, rendering the tumor microenvironment more conducive to an antitumor immune response [60]. Currently, no evidence is available on the effect of mTOR inhibitors on TAM polarization and activity in RCC.

Finally, an association of IL-6, IL-8, VEGF, osteopontin, E-selectin and HGF with continuous tumor shrinkage or PFS has been reported in RCC patients treated with pazopanib [61].

Relationship between RCC myeloid-derived suppressor cells and TAMs

Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of cells that expand during cancer, inflammation and infection, and that have a remarkable ability to suppress T-cell responses and to promote angiogenesis [62]. Two main subsets are described and belong to granulocytic or monocytic lineages. MDSC originate in the bone marrow from common myeloid precursors (CMP) and often differentiate into $CD11b^{+}$ $Gr1^{med}$ $F4/80^{low/-}$ $IL\text{-}4R\alpha^{+}$ cells.

In the mouse, MDSCs and TAMs in the mouse share several characteristics, such as the expression of the monocyte and macrophage marker CD11b. Mounting evidence suggests that, upon entering tumor tissues, MDSCs can differentiate into TAMs. This process is mediated primarily by hypoxia via HIF-1α [63]. In addition, the differentiation of MDSCs into TAMs can lead to elevated IL-10 production, inhibition of T-cell responses and promotion of angiogenesis [64]. However, the mechanism behind regulation of MDSC differentiation remains unclear [9, 64, 65]. MDSCs can oscillate between M1 and M2 phenotypes depending on the stimulation they receive. In addition, with respect to the status of polarization, some differences exist between mouse and human MDSCs [66].

The presence of MDCSs has been reported in RCC-bearing patients and mice and can account for their impaired immune responses [67]. Both monocytic and granulocytic MDSCs have been described in murine tumor models, whereas granulocytic MDSCs are the prevalent population in the blood of RCC patients [67].

Treatment with sunitinib was reported to result in significant reduction in both monocytic and granulocytic spleen MDSCs in tumor-bearing mice. This inhibition correlated with reversal of suppression on type 1 T-cell-mediated IFN-γ production [62]. Similarly, in metastatic RCC patients, treatment with sunitinib results in MDSC reduction that is associated with reversal of regulatory T-cell increase and of type 1 T-cell suppression [68]. Of interest, the development of sunitinib resistance has been found to be partially mediated by the survival of MDSCs intratumorally leading to sustained immunosuppresion and angiogenesis [69].

TAMs and associated molecules as targets for RCC cancer therapy

The role of macrophages in tumor microenvironment and the observation that the presence of TAMs is associated with advanced tumor stages and poor prognosis in RCC lead to the option of targeting these cells therapeutically. Tyrosine kinase and mTOR inhibitors have been reported to influence host immune response [70]. Recent studies suggest that medications with proven clinical benefit exert part of their action through macrophage inhibition or depletion. Zoledronic acid combined with sorafenib or other pharmacological drugs such as bevacizumab, thalidomide and linomide has been shown to inhibit macrophage infiltration and to reduce or neutralize pro-angiogenic factors [59, 71–73].

Several promising strategies have been proposed to affect TAM functions: they include suppression of TAM recruitment, TAM depletion, switch of M2 to M1 antitumor phenotype and suppression of TAM-induced tumor angiogenesis. Table 1 summarizes these promising strategies.

Decrease in TAM recruitment and accumulation

The generation of TAMs is positively regulated by several chemotactic cytokines, such as CSF-1 and CCL2. CSF-1 and its receptor CSF-1R contribute to monocyte recruitment and induction of macrophage differentiation [8], and their co-expression has been associated with RCC tumor growth [74]. Aharinejad et al. [75] have shown that CSF-1 blockade by antisense oligonucleotides suppresses tumor growth in mice xenografted with CSF-1 receptor (c-fms)-positive or CSF-1-negative human malignant embryonic or colon cancer cells. These data suggest that CSF-1 blockade could be tested in treatment for RCC patients.

TAMs isolated from human RCC produce substantial amounts of the pro-inflammatory chemokine CCL2 [42]. Daurkin et al. have shown that 15-LOX2 is involved in the regulation of CCL2 production, and may potentially represent a valuable strategy to limit the effects of CCL2 and to attenuate TAM-induced immunosuppression.

Recently, a human anti-CCL2 IgG1κ mAb, carlumab (CNTO 888), has been demonstrated to be well tolerated with evidence of transient-free CCL2 suppression and antitumor activity in a phase I study of 44 patients with solid tumors [76].

TWIST1 is a basic helix-loop-helix transcription factor expressed in newly formed mesenchymal cells.

Table 1 Emerging TAM-centered strategies

Strategies	Mechanism or targets	Effects on TAMs
CSF-1 antisense oligonucleotides [15]	CSF-1 blockade	Decrease accumulation
Carlumab (CNTO 888) [85]	Human anti-CCL2 IgG1κ mAb	Decrease TAM accumulation
Bindarit [87]	Selective inhibitor of CCL2, CCL7 and CCL8	Decrease TAM accumulation
Recombinant type I IFN-α [89]	CSF-1 blockade	Suppress TAM generation and accumulation
Trabectedin [90]	Exhibits cytotoxic activity against TAMs and reduces the production of CCL2 and IL-6	Selectively eradicate TAMs
Toll-like receptor 9 ligand CpG and anti-IL-10 antibody [93]	Revert tumor-induced immunosuppression	M2-to-M1 phenotype switch
HRG [94]	Down-regulates PDGF	M2-to-M1 phenotype switch
CT-011 [32]	Anti-PD-1 mAb	Restore antitumor immune response
IFN-γ, IL-4, IL-6, or TNF-α genes [95]	Utilize TAMs as gene delivery vector	Enhance antitumor activity
Silibinin [96]	Suppress NF-κB and STAT3 phosphorylation	Suppress TAM-induced tumor angiogenesis
WP1066 [97]	STAT3 inhibitor	Suppress the expression of Bcl-2, induces apoptosis, and inhibits VEGF secretion
Infliximab [98]	Anti-TNF-α mAb	Suppress TNF-α-induced effects
Legumain-based DNA minigene vaccine [101]	Reduce TAM density	Suppress TAMs-induced tumor angiogenesis
LCL-PLP [102]	Reduce of TAMs mediated production of pro-angiogenic factors	Suppress TAM accumulation and related tumor angiogenesis

CCL2 CSF-1 colony-stimulating factor-1, *HRG* histidine-rich glycoprotein, *IFN* interferon, *IL-4* interleukin-4, *IL-6* interleukin-6, *IL-10* interleukin-10, *LCL–PLP* prednisolone phosphate (PLP) encapsulated in long-circulating liposomes (LCLs) (LCL-PLP), *mAb* monoclonal antibody, *PD-1* programmed death-1, *PDGF* platelet-derived growth factor, *STAT3* signal transducers and activators of transcription-3, *TAM* tumor-associated macrophage, *TNF-α* tumor necrosis factor-α, *VEGF* vascular endothelial growth factor

Low-Marchelli et al. reported that TWIST1 promotes angiogenesis and tumor progression without increasing the secretion of VEGF but rather inducing the expression of the macrophage chemoattractant CCL2. Indeed, the inhibition of endogenous TWIST1 in vivo blocks macrophage recruitment and angiogenesis [77].

Bindarit is an original compound with anti-inflammatory activity due to selective inhibition of monocyte chemotactic proteins CCL2, CCL7 and CCL8 [78]. In syngeneic Balb/c mice injected under the mammary gland with murine breast cancer cells (4T1-Luc cells), bindarit treatment significantly decreases the infiltration of TAMs and MDSCs [79]. Presently, the efficacy and safety of bindarit has not been investigated in RCC patients.

Furthermore, U'Ren and colleagues have revealed that type I IFNs generated in tumors inhibit the macrophage stimulatory effects of CSF-1 and suppress the generation of TAMs [80].

Drug-mediated inhibition of TAMs

The monocytes/macrophages-selective cytotoxicity of antitumor agents represents an emerging focus in cancer research. Germano et al. have demonstrated that macrophage depletion is essential for the antitumor activity of trabectedin, a licensed and commercially available anticancer agent. They found that trabectedin is selectively cytotoxic for TAMs in four different mouse tumor models [81]. Furthermore, trabectedin impairs the production of cancer-derived CCL2 and IL-6 from cancer cells to further decrease TAM recruitment [81].

Reversal of TAM-related immunosuppression

The potential to "re-educate" TAMs may be an effective and novel therapeutic approach for cancer. Several studies have been performed testing the possibility to switch M2 TAMs to the antitumor M1 phenotype. The transcription factor NF-κB plays a crucial role in the activation of TAMs. Thus, the activation of macrophages in response to M1 stimuli, such as TLR ligands, TNF-α or IL-1β, is regulated primarily by NF-κB [22]. Moreover, the transcription of several tumor-promoting genes, such as VEGF, IL-6, TNF-α and COX2, is partly regulated by NF-κB [82]. In this regard, Hagemann et al. [83] have observed that the inhibition of IκB kinase (IKK)β, the major activator of NF-κB, reversed TAM tumor-promoting activity and promoted the switch to M1 phenotype.

Furthermore, the combined use of Toll-like receptor 9 ligand CpG and anti-IL-10 Ab has been shown to induce the switch from M2 to M1 phenotype, and this is associated with an increase in cytotoxic function [84]. Additionally, Rolny et al. [15] have reported that treatment with host-generated histidine-rich glycoprotein (HRG) results in the

down-regulation of PDGF and contributes to redirect M2 TAMs into M1 phenotype.

Concerning PD-1, it is a member of the CD28 family of receptors that includes CD28, cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), inducible costimulator (ICOS), and B and T-lymphocyte attenuator. The evidence that TAMs induce the skewing of autologous, blood-derived $CD4^+$ T cells toward a more immunosuppressive phenotype as shown by increased PD-1 expression [28], provides the rationale for targeting this pathway in RCC patients. In this regard, a phase II study is ongoing to evaluate CT-011 a humanized anti-PD-1 mAb, alone or in combination with DC/RCC fusion vaccine in RCC patients (NCT01441765). Moreover, the anti-PD-1 mAb BMS-936558 is under evaluation in a phase I study (NCT01472081) in combination with sunitinib, pazopanib, or ipilimumab and anti-CTLA-4 mAb, and in comparison with everolimus in metastatic clear cell RCC patients who have received prior anti-angiogenic therapy (NCT01668784).

Role of TAMs as functional vehicles

The use of macrophages as vehicles to deliver gene therapy in regions of tumor hypoxia is a promising approach for cancer therapy. In this regard, Nishihara and co-workers have used retroviral vectors to engineer a macrophage cell line to express IFN-γ, IL-4, IL-6 or TNF-α. They have shown increased doubling times and in vitro and in vivo tumoricidal activity by transfected macrophages against the TNF-sensitive fibrosarcoma line WEHI 164 and the TNF-alpha-resistant cell lines B16 melanoma and C1300 neuroblastoma [85].

Suppression of TAM-induced tumor angiogenesis

Angiogenic cytokines released by TAMs regulate angiogenesis by activating NF-κB and STAT3 transcription factors. As mentioned above, STAT3 is involved in the activation of RCC cancer cells mediated by macrophages. Thus, blockade of STAT3 signaling pathways may be considered to be potentially useful as a novel therapeutic approach for RCC. Horiguchi et al. evaluated the in vitro and in vivo efficacy of STAT3 inhibitor WP1066 in RCC cell lines and on murine xenografts. They found that WP1066 suppresses the in vitro expression of Bcl-2, induces apoptosis and inhibits the basal and hypoxia-induced expression of HIF1alpha and HIF2alpha, as well as VEGF secretion. The pathological analysis of xenografts of WP1066-treated mice showed decreased immunostaining of phosphorylated STAT3 and reduced length of CD34-positive vessels [86]. At present, the STAT 3 inhibitors ISIS 481464 (NCT01563302) and OPB-31121 (NCT00955812) are under evaluation in patients with advanced solid tumors including RCC. Similarly, silibinin, a natural polyphenolic flavonoid, has been demonstrated to suppress NF-κB p65 and STAT3 ser727 phosphorylation and to increase the expression of the endogenous angiogenesis inhibitors Ang-2 and ang-receptor tyrosine kinase (Tie-2) [87].

As mentioned above, TNF-α can be associated with in vitro invasiveness of RCC cells [27]. Maisey et al. [88] have evaluated the efficacy of anti-TNF-α mAb infliximab in patients with previously treated advanced RCC and have observed a response rate of 16 % with a further 16 % of patients with stable disease. In 2010, Larkin et al. led a phase I/II trial of sorafenib and infliximab in advanced RCC patients, without registering clinical benefits as compared to sorafenib alone. This combination was also characterized by increased toxicity, with 75 % of the patients requiring at least one dose reduction and 81 % requiring at least one dose delay of sorafenib [89]. Recently, the rLj-RGD3, a recombinant RGD (Arg-Gly-Asp)-toxin protein has been shown to inhibit the TNF-α-induced MMP-9 secretion, proliferation, migration and invasion of human RCC cells [90].

Legumain is a member of the asparaginyl endopeptidase family and is overexpressed in TAMs [101]. In 2008, Lewēn and his colleagues constructed a legumain-based DNA minigene vaccine that induced a specific CD8+ T-cell response against $Legumain^+$ TAMs and reduced tumor angiogenesis in a breast tumor model [91].

The antitumor activity of prednisolone phosphate (PLP) encapsulated in long-circulating liposomes (LCLs) (LCL–PLP) has been also evaluated. LCL–PLP is likely primarily caused by its suppressive effect on the TAM-mediated production of pro-angiogenic factors in tumors. Moreover, LCL–PLP strongly reduced the production of GM-CSF, M-CSF, G-CSF and MCP1, thus affecting TAM functions and recruitment into tumor tissues [92].

Discussion

Until a few years ago, cytokines, particularly IL-2 and IFN-α were the only available therapeutic options with promising antitumor activity in advanced RCC [93–96]. Unfortunately, patients suffered from acute toxicity, and the complete response rate was low [97, 98].

The introduction in clinical practice of several targeted agents has dramatically change the therapeutic scenario in RCC, improving the prognosis and greatly increasing the therapeutic options [99–107]. Nevertheless, tumors often develop resistance to these drugs. To date, we are unable to early select the patients who will benefit most from the treatment, lacking predictive biomarkers of response.

Macrophages are an essential component of the host defense system and have critical roles in both innate and

adaptive immune responses [108]. TAM undergo a wide spectrum of polarized activation states and have the potential both to elicit tumor and tissue destructive reactions and to promote cancer progression and metastasis by stimulating angiogenesis, tumor growth, migration and invasion in RCC. Based on these data, several TAM-centered strategies have been proposed to target these cells. Reducing the numbers and eliminating TAMs are alternatives to their re-education. However, these approaches have not been compared yet in terms of efficacy and safety for RCC patients.

Identification of the pathways responsible for the skewing of TAM functions provides the rational for macrophage-targeted therapies complementary to cytoreductive approaches,and for exploiting the prognostic role of TAMs.

In spite of the notion that TAM frequency is associated with poor prognosis in many human tumors [109], there are notable exceptions, such as in colorectal cancer [110]. The reasons of this divergence remain still unclear.

Thus, TAMs may represent a promising and effective target for RCC cancer therapy. A better dissection of the functional diversity of TAMs and further knowledge of the exact molecular mechanism of TAM-induced angiogenesis and metastasis, and of the interaction between TAMs and RCC microenvironment, may open the way to innovative therapeutic strategies for RCC patients.

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Conflict of interest We declare to have no conflict of interest.

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