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REVIEW

Tumor-associated macrophages: role in cancer development and therapeutic implications

Arash Salmaninejad[1,2] · Saeed Farajzadeh Valilou[3] · Arash Soltani[2,4] · Sepideh Ahmadi[5,6] · Yousef Jafari Abarghan[2] · Rhonda J. Rosengren[7] · Amirhossein Sahebkar[8,9,10,11]

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Abstract

Background Tumor-associated macrophages (TAMs) are known to play important roles in the initiation and progression of human cancers, as well as in angiogenesis. TAMs are considered as main components of the tumor microenvironment. Targeting TAMs may serve as a therapeutic strategy for the treatment of cancer. In this review, the signaling pathways, origin, function, polarization and clinical application of TAMs are discussed. The role of TAMs in tumor initiation, progression, angiogenesis, invasion and metastasis are also emphasized. In addition, a variety of clinical and pre-clinical approaches to target TAMs are discussed.

Conclusions Clinical therapeutic approaches that show most promise include blocking the extravasation of TAMs along with using TAMs as diagnostic biomarkers for cancer progression. The targeting of TAMs in a variety of clinical settings appears to be a promising strategy for decreasing metastasis formation and for improving patient outcome.

Keywords Macrophage · TAM · Cancer · Angiogenesis · Metastasis · Targeted therapy · Immunotherapy

1 Introduction

It has been well-established now that solid tumors are composed of both malignant cells and a number of non-malignant hematopoietic and mesenchymal cells [1–3]. Among these non-malignant cells, macrophages play an important role in promoting tumor neovascularization and progression. Macrophages represent a multifunctional component of innate myeloid cells that are released from bone marrow as immature monocyte precursors, circulate in the bloodstream and migrate to different tissues where they differentiate [4]. These cells can engulf invading microbes or cell debris from injured sites, and also release immunomodulatory cytokines that activate the adaptive immune system [5]. The broad degree of heterogeneity of macrophages enables these cells to adapt or alter their phenotype to match the microenvironment in which they reside.

✉ Amirhossein Sahebkar
sahebkara@mums.ac.ir; amir_saheb2000@yahoo.com

[1] Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

[2] Medical Genetics Research Center, Student Research Committee, Department of Medical Genetics, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

[3] Medical Genetics Network (MeGeNe), Universal Scientific Education and Research Network (USERN), Tehran, Iran

[4] Department of Clinical Biochemistry, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

[5] Student Research Committee, Department of Biotechnology, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[6] Cellular and Molecular Biology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[7] Department of Pharmacology and Toxicology, University of Otago, Dunedin, New Zealand

[8] Biotechnology Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran

[9] Neurogenic Inflammation Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

[10] School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran

[11] Department of Medical Biotechnology, School of Medicine, Mashhad University of Medical Sciences, P.O. box: 91779-48564, Mashhad, Iran

A classification mirroring the Th1/Th2 division of T-lymphocytes has been introduced [6]. The ability of macrophages to adapt to a large variety of biological stimuli by rapidly changing their phenotype and function has resulted in the understanding that a classification of macrophages into M1 versus M2 is a simplification of the in vivo situation [7]. Another classification of macrophages that has been suggested is into pro-inflammatory or classically activated macrophages and alternatively activated macrophages [6, 8]. M1 macrophages are stimulated by Th1 mediators such as interferon-γ (IFN-γ), tumor necrosis factor-alpha (TNF-α) and lipopolysaccharide (LPS) [8]. The main cytokine is IFN-γ. Receptors using Janus kinase Jak1 and Jak2 adaptors can activate signal transducer and activator of transcription 1 (STAT1) and interferon regulatory factors [8]. For the regulation of signaling pathways, IFN-γ and STAT1 recruit toll-like receptors (TLRs), inflammatory factors, tissue-destructive cytokines, anti-inflammatory cytokines and cytokines that activate opposing STATs. These signaling pathways disclose insight into how IFN-γ regulates macrophage activation, inflammation, tissue remodeling, and helper and regulatory T cell differentiation [9]. Some specific genes regulated by IFN-γ include those encoding the cytokine receptors CSF2RB, IL15 receptor alpha (RA), IL2RA and IL6R, cell activation markers (CD36, CD38, CD69, and CD97), as well as a number of cell adhesion molecules (intercellular adhesion molecule 1(ICAM1), integrin alpha L (ITGAL), ITGA4, ITGbeta-7 (B7), mucin 1 (MUC1), and ST6 beta-galactosamide alpha-2,6-sialyl transferase 1 (SIAT1)) [10]. According to the M1/M2 paradigm, IFN-γ is combined with LPS, and the gene expression profile of this mode is different from the LPS or IFN-γ profiles alone [11, 12]. The M1 and M2-associated gene expression and function profiles are listed in Tables 1 and 2.

M1 macrophages secrete pro-inflammatory cytokines such as IL-12 and IL-23, present antigens by their MHC-II complex molecules and enhance the differentiation of naïve $CD4^+$ T-cells into Th1 effector cells and Th17 cells. They also remove intracellular bacteria and viruses [6, 13, 14]. By contrast, M2 activators are classified primarily based on their capacity to antagonize prototypical inflammatory responses and markers. However, as M1 activators, their origin, function, receptors and signaling pathways differ. So far, five main M2 stimuli have been identified: firstly interleukin 4 (IL-4), which is produced by Th2 cells, basophils, eosinophils, or macrophages themselves and is recognized by three different receptor pairs. To form these receptor pairs, IL-4Rα1 can either pair with the common gamma chain (γc), allowing IL-4 binding, or with IL-13Rα1 enabling IL-4 or IL-13 binding. After binding, the receptors can activate JAK1 and JAK3. Activation of JAK leads to STAT6 activation and translocation, after which IL-4 induces macrophage fusion and a decrease in phagocytosis [15]. Interleukin 13 (IL-13) is another stimulator that has a signature similar to IL-4, but they do not completely overlap [16]. Other stimulators of M2 macrophages are glucocorticoids, IL-10 and macrophage colony-stimulating factor (M-CSF). The M-CSF receptor is a tyrosine kinase transmembrane receptor. When M-CSF binds to its receptor, this may lead to several specific modifications, including dimerization, autophosphorylation, activation of extracellular signal-regulated kinases (ERK), activation of phosphatidylinositol 3-kinase, activation of phospholipase C and, eventually, Sp1 transcription factor nuclear localization. Ultimately, M-CSF causes a series of transcriptional responses that comprise transient gene clusters with overrepresentation of cell cycle genes (e.g. cyclins A2, B1, D1 and E1) and downregulation of human leukocyte antigen [17] members and stable gene clusters, including TLR7 and the complement C1QA/B/C subunits. Activated M2 stimulates CD^+ Th2 cells and regulatory T cell (Treg) differentiation. M2 cells have also been implicated in homeostatic processes and to be critical in angiogenesis, tissue remodeling, anti-inflammatory processes and wound healing [18, 19]. Several investigators have attempted to classify them into different sub-groups, to better reflect the situation in vivo. Among them, Mantovani and colleagues sub-classified the M2 population into M2a, M2b and M2c, where M2a was stimulated by the representative Th2 cytokines IL-4 and IL-13, M2b by immune complexes along with TLR or the IL-1 receptor antagonist and M2c by glucocorticoids and IL-10 [7].

The classification of macrophages in this way faces two major challenges: the in vitro influences of chosen immune-related ligands on the phenotype of macrophages and in vivo evidence for distinct subsets of macrophages in disease, comparable to polarized B- and T cell responses. The main restrictions of the current view are (i) that it ignores the origin and context of the stimuli, (ii) that the M1 and M2 stimuli do not exist singly in tissues and (iii) that macrophages may not form clearly defined activation subunits nor broaden clonally [20]. Tumor-associated macrophages (TAMs) have been found to play a leading role in the development and progression of tumors, and to enhance the tumor environment to facilitate angiogenesis and metastasis. As a consequence, TAMs have been proposed as potential therapeutic targets for cancer treatment [21]. Here, we review the role of TAMs in tumor initiation, progression, angiogenesis, invasion and metastasis. Different therapeutic approaches targeting TAMs in each of these steps are also discussed.

2 Role of TAMs in tumor initiation and progression

The German pathologist Rudolph Virchow in the nineteenth century suggested a pathophysiological association between inflammation and cancer. Inflammation usually occurs in two stages, acute and chronic. Later on, it has been proposed that

Table 1 M1 and M2-associated gene expression and function panel

M1					M2				
Gene	Locus	Gene name	Function	Ref	Gene	Locus	Gene name	Function	Ref
TLR2	4q31.3	Toll like receptor 2	Beginning of inflammation and innate immune responses, tumor progression	[20]	*MSR1* (CD204, SR-A)	8p22	Macrophage scavenger receptor 1	Support of tumor growth, suppression of inflammation or inhibition of tumor progression	[21, 22]
CD16 (FCGR3A)	1q23.3	Fc fragment of IgG receptor IIIa	Expressed on macrophage, NK cell and DC	[23]	*CD163*	12p13.31	CD163 molecule	Cause macrophage switch to alternative activated phenotypes in inflammation and may take part in downregulating an inflammatory response	[24, 25]
CD32 (FCGR2A)	1q23.3	Fc fragment of IgG receptor IIa	trigger the killing machinery following binding of the Fc portion of IgG to the receptor	[26]	*CD14*	5q31.3	CD14 molecule	working with TLR4 and facilitating cellular responses to low doses of lipopolysaccharide (LPS)	[27]
CD80 (B7–1)	3q13.33	CD80 molecule	stimulation of T cell activation and tolerance	[28]	*CD23*(FCER2)	19p13.2	Fc fragment of IgE receptor II	plays a role in regulation of IgE synthesis	[29]
CD86 (B7–2 or B70)	3q13.33	CD86 molecule	stimulation of T cell activation and tolerance	[28]	*IL-1 Ra*				
TNF-α	6p21.33	Tumor necrosis factor alpha	Prevention of M2 gene expression in macrophages, proinflammatory cytokines, cancer-related inflammation, tumor promotion	[30, 31]	*IL-4 Ra*	16p12.1	Interleukin 4 receptor, alpha	plays a major role in immunoglobulin E (IgE) production; involved in STAT-6 phosphorylation and signaling, tumor inflammation	[32, 33]
IL-1β	2q14.1	Interleukin 1 beta	Pro-inflammatory cytokines, protective immunity, tumor growth	[34, 35]	*Il10*	1q32.1	Interleukin 10	Progress and survival of cancer cells, anti-inflammatory cytokine, regulation and inflammation. Reduces the immune response against tumor cells anti-inflammatory cytokines	[36]
IL6	7p15.3	Interleukin 6	Inflammatory cytokines, tumor promotion, regulators of tumor-associated inflammation and tumorigenesis	[37]	*Gpr84*	12q13.13	G protein-coupled receptor 84	Proinflammatory Receptor	[38]
IL12	–	Interleukin 12	pro-inflammatory cytokine	[39]	*Cdh1*	16q22.1	Cadherin 1	Encodes *E*-cadherin, cancer promotion by increasing proliferation, invasion, metastasis	[40]
IL23	12q13.3	Interleukin 23	Progress of Th17 cells, tumor growth promotion, Stimulates inflammation	[41]	*CCL16*	17q12	Chemokine (C-C motif) ligand 16	Immunoregulatory and inflammatory procedures, chemo attractant for monocytes and lymphocytes	[42, 43]
Csf-1	1p13.3	Colony-stimulating factor 1	Controls the differentiation, production and functions of macrophages	[44]	*CCL18*	17q12	Chemokine (C-C motif) ligand 18	Anti-inflammatory cytokines, cancer promotion, invasion metastasis	[45]
Csf-2	5q31.1	Colony-stimulating factor 2	regulates the differentiation of hematopoietic stem cell/progenitor cells into dendritic cells, granulocytes, and macrophages, promotes macrophage survival	[44]	CCL24	7q11.23	Chemokine (C-C motif) ligand 24	Anti-inflammatory cytokines, cancer promotion, invasion metastasis	[46]
CCL2	17q12			[47]	*CCR2*	3p21.31			[48]

Table 1 (continued)

M1					M2				
Gene	Locus	Gene name	Function	Ref	Gene	Locus	Gene name	Function	Ref
		Chemokine (C-C motif) ligand 2	Support of tumor growth, inflammatory chemokines				Chemokine (C-C motif) receptor 2	Inflammatory chemokine receptors, receptor for MCP-1 and CCL2	
CCL5	17q12	Chemokine (C-C motif) ligand 5	Support of tumor growth, inflammatory chemokines	[47]	*CXCR1*	2q35	C-X-C motif chemokine receptor 1	Receptor for ELR^+ CXC chemokines, role in angiogenesis	[49]
CxCl9	4q21.1	C-X-C motif chemokine ligand 9	Attract Th1 lymphocyte, stimulates the immune system	[50, 51]	*Fizz1* (*RETNLB*)	3q13.13	Inflammatory zone 1 (resistin like beta)	Role in metabolism, regulating T cell differentiation,	[52]
CxCl10	4q21.1	C-X-C motif chemokine ligand 10	Attract Th1 lymphocyte, pro-inflammatory and anti-angiogenic properties	[51, 53]	*Chil3* (Ym1)	3	Chitinase-like 3 (Chil3)	Function as a lectin, involved in inflammation and allergy	Pubmed
CxCl16	17p13.2	C-X-C motif chemokine ligand 16	Tumor invasion, promotes cancer proliferation or metastasis	[54]	*MGL*	17p13.1	Macrophage galactose N-acetyl-galactosamin-e specific lectin	Has capacity for carbohydrate recognition; involved in the extravasation of immune cells into local inflammatory sites and contributes to the distribution of immature DCs in peripheral organs	[55]
CXCL19	3	C-X-C motif chemokine ligand 19	pro-inflammatory chemokines	[36]	*TGFB1*	19q13.2	Transforming growth factor beta 1	Regulates cell proliferation, differentiation and growth, prevent antitumor immune cells such as $CD8^+$ and Th1 cells, cancer development	[36]
Cox-2	1q31.1	Cyclooxygenase-2	Tumor growth promotion, suppresses tumor immunity	[56]	*Mrc1* (CD206)	10p12.33	Mannose receptor C-type 1	Bind carbohydrates and mucins existing in the tumor microenvironment, endocytosis of glycoproteins by macrophages	[57]
CCR7	17q21.2	Chemokine (C-C motif) receptor 7	Receptor for CCL19 and CCL21, prompted migration and invasion of CCR7-expressing cancer cells	[58, 59]	*ARG1*	6q23.2	Arginase 1	Hydrolyzes arginine to ornithine and urea, catalyzing polyamine production and collagen synthesis, cell proliferation	[60]
SOCS1	16p13.13	Suppressor of cytokine signaling 1	Regulates the phenotype of M1 macrophages, effects M1 and M2 macrophage polarization	[61]	*SOCS1*	16p13.13	Suppressor of cytokine signaling 1	Effect to M1 and M2 macrophage polarization, up-regulated in M2 macrophages, role in regulating the suppression of T cell proliferation	[61]
IRF1	5q31.1	Interferon regulatory factor 1	Activation of IFN genes, controls of genes in mediating antiviral, immuno-modulatory, and antiproliferative properties	[62, 63]	*PPARY*	22q13.31	Peroxisome proliferator activated receptor alpha	Counteract inflammatory M1 macrophages, actively stimulating M2 activation, affect the expression of target genes involved in cell proliferation, differentiation, immune inflammation responses	[64]
NOS2	17q11.2	Nitric oxide synthase 2, inducible, macrophage	Hydrolyzes arginine to NO and citrulline, low levels of expression of NO in progression and metastasis	[65, 66]	*S1P1R*	1p21.2	Sphingosine-1-phosphate receptor 1	Promotes lymphangiogenesis and metastasis	[67, 68]
Tnfsf15	9q32	TNF superfamily member 15	Modulation of vascular homeostasis and inflammation	[69]	*Alox15*	17p13.2	Arachidonate 15-lipoxygenase	Regulate inflammation and immunity, promotes CXCL10 secretion	[70]
STAT1	2q32.2			[71–73]	*STAT3*	17q21.2			[74]

Table 1 (continued)

M1					M2				
Gene	Locus	Gene name	Function	Ref	Gene	Locus	Gene name	Function	Ref
		Signal transducer and activator of transcription 1	Tumor promotion, regulation of macrophage functions, adaptive immune responses, stimulates iNOS and IL-12 transcription in M1 macrophage				Signal transducer and activator of transcription 3	Involved in cycle development and the regulation of apoptosis, role in hematopoiesis, differentiation and angiogenesis, regulators of proliferation and persistence of tumor cells	
STST4	2q32.2-q32.3	Signal transducer and activator of transcription 4	promote cytotoxic responses and Th1 cell differentiation,	[75]	*STAT6*	12q13.3	Signal transducer and activator of transcription 6	promotes expression of KLF4 and PPARs; promotes alternative activation, promotes M2 polarization	[76, 77]

inflammation in its chronic stage may trigger cancer initiation. About 90–95% of all neoplasms are connected to tobacco, obesity, smoke, radiation, environmental pollutants and chronic infections, all of which induce a chronic inflammatory state [22]. Macrophages are present in the inflammatory environment including the tumor microenvironment. Macrophages are involved in immune responses to tumors in a polarized manner: classical M1 macrophages produce IL-12 and promote tumor initiation, whereas M2 macrophages produce IL-10 and promote tumor progression. To define the role of macrophages in tumor progression, it is necessary to understand TAM differentiation and their tumor promoting properties. M2-like macrophages encompass the majority of TAMs with a representative M2 marker expression profile, including a mannose receptor, a low MHC class II complex, stabilin-1 and arginase-1 [14]. These markers are involved in tissue remodeling, immune regulation and angiogenic processes within the tumor microenvironment by releasing high levels of IL-10, low levels of IL-12, angiogenic factors such as vascular endothelial growth factor (VEGF), prostaglandin E2 and matrix metalloproteinase-9 (MMP9) [14].

M1-like macrophages promote tumor initiation via chronic inflammation in the tumor microenvironment that is caused by intrinsic and extrinsic signals and leads to proliferation and survival of malignant cells, angiogenesis, suppression of adaptive immunity and reduced responses to hormones and chemotherapeutic agents [23]. The intrinsic pathways include genetic alterations in oncogenes such as *RET* [24], *RAS* [25], *MYC* [26], or in tumor suppressor genes such as the von Hippel-Lindau tumor suppressor (*VHL*) [27], which contributes to the transcription of pro-inflammatory cytokines and growth factors in the genetically altered tumor cells and induces a tumor-driven inflammatory environment. The extrinsic pathway leads to chronic inflammation that increases the risk of developing cancer at a specific anatomical site, often prostate, colon or pancreas [28]. The extrinsic and intrinsic pathways co-operate in the activation of transcription factors such as NF-κB or STAT3, which promote tumor progression in the microenvironment [29–31]. NF-κB is an essential factor for the transcription of pro-inflammatory and angiogenic factors such as IL-12, TNF-α, inducible nitric oxide synthase (iNOS) [32] and cyclooxygenase-2 (COX-2), and is associated with the promotion of carcinogenesis in different tumor types [33]. Activation of STAT3 also allows tumor cells to resist apoptosis, inhibit inflammation, impede dendritic cell maturation, induce growth, and to stimulate migration, invasion and angiogenesis, culminating in an active tumor environment [34]. During early carcinogenesis, all macrophages exhibit a higher degree of similarity to M1, but in later stages the majority of tumors recruit M2-like macrophages to their microenvironment. In hepatocellular carcinoma, for example, macrophages in the early stage of development mostly express high levels of MHC-class II, which has been associated with

Table 2 Studies and clinical trials of mediators that target TAMs for tumor therapy

	Targets	Inhibitors	Treated cells or tumors	Purpose or results	Clinical trials or Ref	Phase/Status
Angiogenesis	VEGF-A	Bevacizumab (anti-VEGF-A monoclonal antibody)	Advanced Cancers	the highest tolerable and safety combination dose of bendamustine and bevacizumab in patients with advanced cancer	NCT01152203	Phase I/completed
	VEGF	rhuMAb VEGF (Bevacizumab) in combination with chemotherapy	Breast Cancer	Evaluating the efficacy, safety, and pharmacokinetics of rhuMAb VEGF (Bevacizumab), in combination with Capecitabine chemotherapy	NCT00109239	Phase III/completed
	VEGF	carboplatin, paclitaxel, and bevacizumab	Endometrial Cancer	Patients in a complete response after 6 or 8 cycles received maintenance therapy with bevacizumab	NCT00879359	phase II/completed
	VEGF-A, VEGF-B, and PlGF	Aflibercept	solid tumors	Exerts its antiangiogenic effects through regression of tumor vasculature, remolding of vasculature, and inhibition of new tumor vessel growth.	(208)	–
	Human bispecific anti Ang2/VEGF antibody	RO5520985 (Vanucizumab) with Atezolizumab	Neoplasms		NCT01688206	Phase 1/ ongoing
	YKL-40	anti-YKL-40 neutralizing antibody (mAY)	Glioblastomas	Prevents the angiogenesis and tumor growth in glioblastoma patients.	(157)	–
	CYP4A	flavonoid FLA-16	Glioblastomas	inhibiting CYP4A in TAMs and EPCs and downregulation of TAMs and EPC-derived VEGF and TGF-β via PI3K/Akt pathway	(157, 159)	–
	CSF1/CSF1R	LY3022855, Durvalumab, Tremelimumab	Solid tumor	Evaluation of the safety of LY3022855 in combination with durvalumab or tremelimumab in patients with advanced solid tumors	NCT02718911	Phase 1a/1b/ongoing
	CSF1/CSF1R	BLZ945 alone or in combination with PDR001 (anti-PD-1 mAb)	Advanced Solid Tumors	Evaluation of safety, tolerability, pharmacokinetics, pharmacodynamics, and anti-tumor activity of BLZ945 as a single agent or in combination with PDR001	NCT02829723 (209)	Phase I/II ongoing
	CSF1/CSF1R	PLX3397/ Sirolimus	Sarcoma, Malignant Peripheral Nerve Sheath Tumors	Tolerability of treatment with PLX3397 and Sirolimus and efficacy	NCT02584647	Phase I/II ongoing
	CSF1/CSF1R	Anti-PDL1 Antibody (DURVALUMAB) Combined with CSF-1R TKI (PEXIDARTINIB)	Colorectal Cancer (CRC), Pancreatic Cancer (PDAC),	Evaluation of Safety and clinical Activity of a combined treatment associating an anti-CSF1R (PEXIDARTINIB) with an anti-PD-L1 (DURVALUMAB) in patients with advanced/metastatic colorectal or pancreatic cancers	NCT02777710	Phase I /ongoing
	CSF1/CSF1R	PLX3397	Tenosynovial Giant-Cell Tumor	Evaluation of the investigational drug PLX3397	NCT02371369	Phase III/ongoing
	EGFR					

Table 2 (continued)

Targets	Inhibitors	Treated cells or tumors	Purpose or results	Clinical trials or Ref	Phase/Status
CCL2	CNTO 888, a Human Monoclonal Antibody	Solid Tumors	well tolerated, Transient CCL2 suppression, preliminary antitumor activity,	NCT00537368 (210)	Phase1/Completed
CCL2/CCR2CCL2	anti-CCR2 monoclonal antibody MLN1202))CNTO 888, a Human Monoclonal Antibody	Bone Metastases Solid Tumors	Block the ability of tumor cells to grow and spread, well tolerated, Transient CCL2 suppression, preliminary antitumor activity,	NCT01015560NCT00537368 (210)	/Phase II completedPhase1/Completed
CCL2/CCR2CCL2/CCR2	An Anti-CCL 2 Monoclonal Antibody (CNTO 888)anti-CCR2 monoclonal antibody MLN1202))	Solid tumors Bone Metastases	Blocking the CCL2 and control tumor growth Block the ability of tumor cells to grow and spread	NCT01204996NCT01015560	Phase 1b/completed/Phase II completed
CCL2/CCR2CCL2/CCR2	PF-04136309 in combination with FOLFIRINOX An Anti-CCL 2 Monoclonal Antibody (CNTO 888)	Locally Advanced Pancreatic Adenocarcinoma Solid tumors	Inhibits CCR2 activity and prevents the mobilization of inflammatory macrophages into blood vessels Blocking the CCL2 and control tumor growth	NCT01413022 (197) NCT01204996	Phase 1b/complete Phase 1b-/completed
CCL2/CCR2CCL2/CCR2	dnCCL2-HSA chimera PF-04136309 in combination with FOLFIRINOX	Lung carcinoma cells (3LL) Locally Advanced Pancreatic Adenocarcinoma	Blocks tumor cell extravasation through inhibition of vascular permeability Inhibits CCR2 activity and prevents the mobilization of inflammatory macrophages into blood vessels	(199)NCT01413022 (197)	-Phase 1b/complete
CCL2/CCR2CCL2/CCR2	Anti-Chemokine CCL2 (Carlumab)dnCCL2-HSA chimera	Metastatic Castrate-Resistant Prostate Cancer Lung carcinoma cells (3LL)	Well-tolerated, no block the CCL2/CCR2 axis and no antitumor activity as a single agent. Blocks tumor cell extravasation through inhibition of vascular permeability	NCT00992186 (199, 211)	Phase II/ completed-
CCL2/CCR2	Anti-Chemokine CCL2 (Carlumab)	Metastatic Castrate-Resistant Prostate Cancer	Well-tolerated, did not block the CCL2/CCR2 axis and no antitumor activity as a single agent	NCT00992186 (211)	Phase II/ completed
CYP4A	HET0016 and DDMS	4 T1 breast cancer and B16F10 melanoma	Inhibits synthesis of 20-HETE and decreases pro-angiogenic factors	(158)	
CYP4A	HET0016 and DDMS	4 T1 breast cancer and B16F10 melanoma	Inhibits synthesis of 20-HETE and decreases pro-angiogenic factors	(158)	
CYP4A	HET0016 and DDMS	4 T1 breast cancer and B16F10 melanoma	Inhibits synthesis of 20-HETE and decreases pro-angiogenic factors	(158)	

high concentrations of IL-1β, IL-6, IL-12 and iNOS. In contrast, macrophages in late stages of development mainly express typical M2-associated molecules such as macrophage mannose receptor c1 (MRC1), arginase, IL-10 and TGF-β, and low levels of MHC-class II [35].

Some causative signals that are involved in the M1/M2 switch have been identified. It has been found, for example, that COX-2 innately contributes to changing TAMs from M1 to M2. Inhibition of COX-2 leads to a decreased number of M2 macrophages and causes an increase in IFN-γ level, thereby reducing the progression of intestinal tumors in patients with colon carcinoma [36]. Another regulatory pathway that has been found to be important for TAM differentiation comprises TLR signaling. Some of the extracellular matrix proteins such as versican, hyaluronan fragments or heat shock proteins, which act as ligands for TLR in the tumor environment, have been associated with tumor progression and metastasis [37]. TLR signaling pathways are dependent on both myeloid differentiation primary response gene88 (MyD88) and TIR-domain-containing adapter-inducing interferon-β (TRIF). It has been found that an imperfect activation of MyD88, but an undamaged TRIF-mediated signal transduction, increases the phosphorylation of ERK1/2. Phosphorylation of ERK1/2 leads to increased secretion of IL-10 from TAMs, causing extensive expression of interleukin-1 receptor-associated kinase M (IRAK M). IRAK M is an inhibitor of the MyD88-dependent pathway. Generally, defective TLR-MyD88/TRIF signal pathway regulation in TAMs favors an increased immunosuppressive function of these macrophages [38].

Another condition that affects TAM differentiation during tumor progression is a limited oxygen supply in highly expanding regions of the developing tumor. When macrophages are in a hypoxic condition, the expression of hypoxia-inducible factor-1 (HIF-1) is increased, which is closely associated with NF-κB activation [39]. The Notch signaling pathway is also involved in TAM differentiation. Notch signaling may be activated in M1 macrophages, which leads to enhanced expression of IL-12 that, in turn, limits tumor progression. In addition, the M2 response has been found to be induced by M1 inducers at the expense of M1 when Notch signaling is blocked. Macrophages deficient in canonical Notch signaling have been found to show TAM phenotypes [40, 41]. In addition to transcription factors and innate signaling pathways, chemokines are also involved in M1-M2 transition during tumor progression. CXCL12, also known as stromal cell-derived factor-1 (SDF-1), is one of the chemokines that is highly secreted from monocytes in the tumor microenvironment. This chemokine not only facilitates the attraction and migration of monocytes to the tumor site, but also leads to the differentiation of TAMs into a pro-angiogenic and immunosuppressive phenotype by up-regulating CCL1 and VEGF [41]. CXCL12 also drives TAM aggregation and survival in hypoxic tumor areas [42]. This chemokine may enhance scavenger receptor CD163 expression, thereby shaping monocyte differentiation towards an immunosuppressive and proangiogenic phenotype [41]. CXCL12 expression has been found to be significantly correlated with augmented CD163+ TAMs in tumor stroma (TS) and tumor margin (TM) in gastric cancer patients [43]. Wang et al. reported that the prodrug of the green tea polyphenol (−)-epigallocatechin-3-gallate (Pro-EGCG) may serve as an angiogenesis inhibitor in endometrial cancer. They found that Pro-EGCG decreased tumor angiogenesis in xenograft models through downregulation of CXCL12 in the host stroma, and VEGFA and HIF1α in the tumor cells, as shown by immunohistochemical staining. Down-regulation of CXCL12 in the stromal cells by Pro-EGCG treatment restricted the migration and differentiation of macrophages, thereby inhibiting infiltration of VEGFA-expressing TAMs [44].

As noted above, the presence of TAMs in the tumor environment may result in cancer promoting inflammation, which plays a central role in tumor initiation [22, 45]. Several experimental studies support the role of inflammation in cancer initiation. For instance, chronic obstructive pulmonary disease in human leads to persistent colonization of the bacterium *Haemophilus influenza* and this colonization has been associated with an increased risk of lung cancer. Concordantly, in a lung cancer mouse model, bronchial inflammation elicited by *H. influenzae* led to an increase in tumorigenesis [46], but more work needs to be conducted in lung cancer models. Mechanistically, the transcriptional factors NFκB and STAT3 work oppositely [47]. STAT3 is a transcription factor that suppresses inflammation and, therefore, absence of STAT3 in myeloid cells leads to an abundant expression of TNFα and IL-6 via macrophages, which consequently results in chronic colitis and invasive colonic adenocarcinomas [48, 49]. In addition, the inflammatory condition is controlled by NFκB, which is an essential transcription factor that triggers downstream inflammatory signaling pathways and activates TLR, which leads to the expression of inflammatory cytokines such as IL-12 and TNFα, as well as iNOS [47]. Concordantly, it has been found that impeding IκB kinase α (IKKα) in myeloid cells in a mouse model of intestinal cancer diminished inflammation and inhibited tumor progression [50]. Furthermore, macrophages produce both reactive nitrogen and oxygen species. Nitric oxide (NO) can react with peroxidases to produce nitrosoperoxycarbonate, which can lead to progression of inflammation [51]. This effect is due to the fact that the highly reactive components create a mutagenic environment that causes mutations in adjacent epithelial cells [51]. In addition, it has been found that inflammation in the tumor microenvironment may promote genetic instability within developing tumor epithelial cells [23].

The tumor immune microenvironment mainly consist of macrophages, T lymphocytes, natural killer (NK) cells,

dendritic cells, neutrophils and myeloid-derived suppressor cells (MDSCs) [52, 53]. TAMs in the tumor microenvironment express both chemokines and cytokines promoting an immunosuppressive tumor microenvironment [54, 55]. Chemokines secreted by TAMs such as CCL5, CCL22 and CCL20 recruit regulatory T (Treg) cells, whereas cytokines such as IL-10 and TGF-β induce Treg cells. In addition, TAMs may suppress the antitumor activity of tumor-infiltrating NK cells and T cells, and via MDSCs, tumor-associated dendritic cells and neutrophils that promote an immunosuppressive tumor microenvironment synergistically [55, 56]. Inhibition of T cell function may result from secretion of specific enzymes by TAMs such as NOS and arginase [57–60]. In addition, expression by TAMs of ligands such as programmed cell death protein ligand 1 (PD-L1) and B7-H1 for receptors programmed cell death protein (PD-1) and CTLA-4, may lead to the inhibition of cytotoxic functions of T-cells, NKT cells and NK cells [61]. In one study, the role of inhibitory $CD163^{+}$ monocytes or macrophages, and NK cells in diffuse large B cell lymphoma (DLBCL) and Hodgkin lymphoma [12] have been investigated. It was found that PD-1 expression in $CD3^{-}CD56^{hi}CD16^{-ve}$ NK cells was higher than in $CD3^{-}CD56^{dim}CD16^{+}$ cells, which spread in blood and tissue more markedly in cHL than in DLBCL patients. In this regard, within diseased lymph nodes TAMs have been found to express high levels of PD-L1/PD-L2. In an in vitro functional model TAM-like monocytes were found to suppress the activation of $PD\text{-}1^{hi}$ NK cells, which could be reversed by PD-1 blockade. Importantly, suppression of NK cells can occur indirectly by PD-L1/PD-L2 expressing TAMs [62].

3 Role of TAMs in tumor growth

TAMs may exhibit both tumor growth promoting and inhibiting activities [63, 64]. They may promote tumor growth not only through stimulating angiogenesis, but also through suppressing acquired immune responses. TAMs may act as gatekeepers in tumors, producing various factors for tumor invasion and metastasis (Fig. 1). The role of TAMs in the growth of various cancers, including breast cancer [65, 66], hepatocellular carcinoma (HCC) [67], colorectal cancer [68] and glioblastoma [69, 70] has been well-established. Using a spontaneous genetic model of breast cancer metastasis and orthotopic transplant experiments it has, for example, been shown that *SNAIL1,* an epidermal to mesenchymal transition inducer [71], regulates the production of GM-CSF, TNFα, IL1α and IL-6, and modulates the polarization of TAMs. This modulation ultimately leads to the growth and metastasis of primary breast tumor cells [72]. *ERK5* plays a role in determining macrophage polarity, and it has been found that in ERK5-deficient mice the growth of carcinoma grafts is halted. Targeting *ERK5* in macrophages has been found to lead to a transcriptional switch that favors proinflammatory mediators. Further molecular studies have shown that STAT3 activation via phosphorylation of Tyr705 is impaired in ERK5-deleted TAMs. So, impeding STAT3-induced gene expression via blocking ERK5 may serve as a strategy for cancer treatment through reprogramming macrophages towards an antitumor state [73].

4 Role of TAMs in angiogenesis

TAMs play an important role in tumor progression and invasion and act as key players in angiogenesis [74, 75]. In response to hypoxic conditions, TAMs begin to express a number of transcription factors such as *HIFs* that regulate a range of genes to promote angiogenesis, which in turn increases the invasion of tumor cells. TAMs also secrete angiogenic factors such as VEGF, FGF2, bFGF, PDGF and adrenomedullin [76], YKL-40, thymidine phosphorylase (TP), MMPs and urokinase-type plasminogen activator, all of which play important roles in tumor progression and invasion (Fig. 1) [59, 74, 77]. Badawi et al. examined the relationship between macrophage infiltration and the degree of angiogenesis in human colon carcinoma. They found that the number of infiltrating macrophages was significantly higher in malignant/invasive tumors and that their blood vessels were denser compared to those in benign polyps. Thus, a significant relationship was observed between macrophage infiltration and angiogenesis, invasion and metastasis of colon cancer cells [78]. Additionally, Tie2-expressing monocytes (TEMs), a type of TAMs that are present in both human peripheral blood and tumors, have been found to play an important role in tumor angiogenesis and growth. Studies aimed at examining human and murine endometriosis lesions, pancreatic cancer, ovarian cancer and other cancers have revealed a role for TEMs in angiogenesis [79–83]. In addition, it has been found that elimination of TEMs may lead to inhibition of angiogenesis in various tumor models. Angiopoietin-2 (Ang-2), a ligand for Tie2, is produced by angiogenic tumor vessels and serves as a chemoattractant for TEMs. Hypoxia has been found to enhance Tie2 expression in TEMs and, together with Ang-2, to down-regulate their antitumor capacity [84]. Ang is secreted by endothelial cells. According to Chen et al., there is a close relationship between tumor recurrence and Tie2 over-expression after chemotherapy. This recurrence occurs via re-regeneration of blood vessels within tumors. Hence, removal of Tie2 in myeloid cells may be used to prevent the regeneration of blood vessels and, thereby, tumor recurrence [85].

5 Therapeutic targeting of angiogenesis

Targeting TAM-induced angiogenesis serves as a potential approach for cancer treatment. VEGFA is one of the most

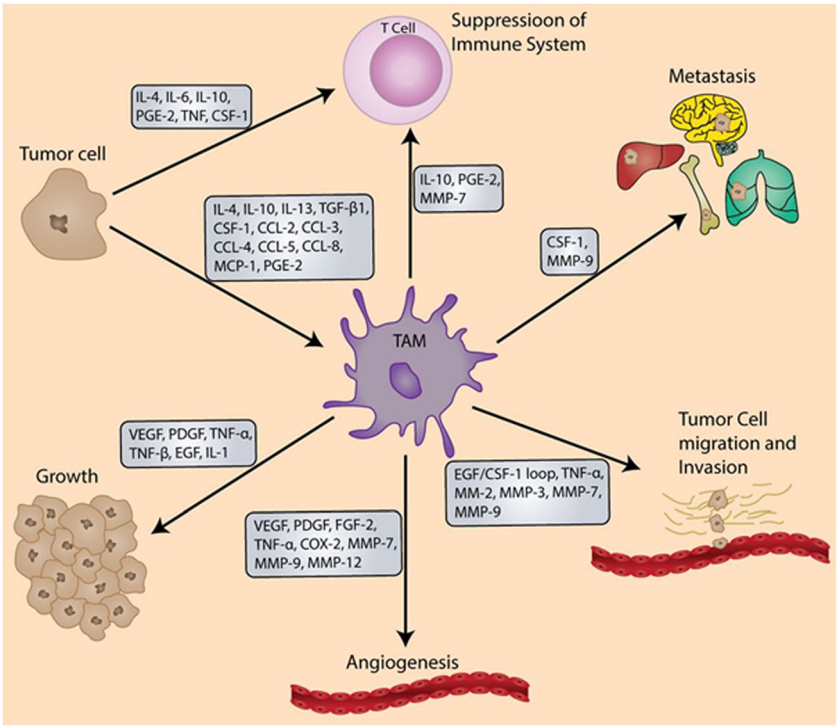

Fig. 1 Impact of tumor-associated macrophages on different aspects of tumor biology

important TAM-secreted factors that plays an important role in angiogenesis and tumor metastasis. Inhibition of the VEGF pathway is a commonly used treatment option for controlling tumors. Bevacizumab is a monoclonal antibody directed against VEGF that has been approved by the US Food and Drug Administration (FDA) [78]. It acts through binding to VEGF and, thereby, inhibiting its interaction with the VEGF receptor (VEGFR). The efficacy of bevacizumab has currently been investigated in several cancer types including ovarian, colorectal, breast, renal, non-small cell lung and cervical cancers [86–91].

The role of Ang2 as a potential target in patients with naive and bevacizumab-resistant glioblastoma was investigated by Scholz et al. [92]. Ang-2 is an angiogenic growth factor that is not expressed in normal human brain, but its expression is increased in glioblastoma patients who are resistant to bevacizumab. Blocking VEGF in murine models has led to increased expression of Ang2 in endothelial cells, thereby impeding the effect of VEGF (aflibercept). In addition, it was found that application of an anti-human Ang-2 antibody led to reduced vascular permeability, eliminated TAMs and increased the number of intra-tumoral T lymphocytes. As a result, it was found that inhibition of both the VEGF/VEGFR and Ang-2/Tie2 pathways led to the removal of TAMs and suppression of the pro-angiogenic process. Peterson et al. [93] investigated the effect of MEDI3617 (an anti-Ang2-neutralizing antibody) in combination with cediranib (a pan-VEGFR tyrosine kinase inhibitor) in orthotopic glioblastoma Gl261 and U87 models. They concluded that dual inhibition of VEGFR/Ang-2 was superior compared to inhibition of VEGFR alone, and increased the lifespan of the vessels.

YKL-40 is used as biomarker to diagnose tumor angiogenesis in renal cell carcinoma (RCC) and melanoma patients [94, 95]. It has been found that YKL-40 levels in serum and pleural fluid in patients with malignant pleural effusions are increased compared to those in patients with transudative or non-malignant exudative effusions. The importance of this biomarker in the diagnosis of malignant pleural infections has been shown [96]. Also, an increase in serum YKL-40 and IL-6 levels in patients with colorectal cancer has been reported to serve as a useful prognostic biomarker before liver resection [97]. Furthermore, Shao et al. [98] reported that an YKL-40 neutralizing antibody (mAY) and ionizing irradiation (IR) may prevent angiogenesis and tumor growth in glioblastoma patients. Specifically, it was found that mAY blocked mural cell-mediated vascular stability and angiogenesis through interfering with intercellular contact by N-cadherin, while IR only stimulated tumor cells. From their results, they also concluded that dual treatment with IR and bevacizumab in glioblastoma patients was more effective than IR alone, regardless YKL-40 expression. For patients with advanced glioblastoma, the therapeutic combination of mAY, IR and bevacizumab was found to be efficacious [98].

Cytochrome P450 4A (CYP4A) is known to play an important role in tumor angiogenesis and metastatic niche development. CYP4 enzymes can biosynthesize 20-hydroxyeicosatetraenoic acid (20-HETE), which is an important mediator of VEGF-mediated angiogenesis [99]. Blocking

CYP4A to inhibit angiogenesis in glioblastoma by the novel flavonoid FLA-16 has, therefore, been examined. It was found that inhibition of FLA-16 by CYP4A in TAMs and endothelial progenitor cells (EPCs) leads to downregulation of TAM- and EPC-derived VEGF and TGF-β via the PI3K/Akt pathway, thereby providing another mechanism for inhibiting angiogenesis and survival of glioma cells [100].

6 Role of TAMs in invasion

As the number of TAMs increases, invasion and metastasis increases [101]. Important stages of metastasis include invasion, intravasation, circulation and extravasation. In order for invasion to occur cancer cells must undergo a process called epithelial-mesenchymal transition [71] [97] [102]. A strong correlation between EMT and the beginning of the invasion phase, including loss of cell-cell binding and cell-basement membrane binding, invasive behavior and resistance to apoptosis has been found to exist [103–106]. Several studies have addressed the role of TAMs in EMT. Zhang et al. [107], for example, observed a role of TAMs and their relationship with EMT in the progression and invasion of gastric cancer by measuring the level of infiltrated TAMs and the expression of EMT markers. They concluded that TAMs may play an important role in EMT induction and in the promotion of migration and metastasis of gastric cancer cells.

7 Role of TAMs in intravasation

Intravasation is a phase through which tumor cells enter blood vessels to metastasize to distant sites [108]. It has been reported that there is a significant relationship between tumor cells and TAMs and their role in intravasation [64, 109]. This relationship has also been reported in animal models for breast cancer exploring the relationship between peripheral macrophages and the intravasation of tumor cells [110]. Through the secretion of various factors such as (epidermal growth factor) EGF, CCL18, TNF-α, cathepsin and osteonectin, TAMs are able to promote tumor cell intravasation [111]. Specifically, Gorelik et al. [112] revealed a role of macrophages in the intravasation of murine tumor cells and the subsequent development of pulmonary metastatic tumors. In a similar study, Hu et al. [113] revealed a role of TAMs in the progression and invasion of Kazakh esophageal squamous cell carcinoma (ESCC) cells. They showed that an increase in the number of TAMs, using CD163 as a marker, in the tumor stroma significantly correlated with ESCC progression and metastasis. Furthermore, they found that there was a close relationship between a high number of TAMs and an increased expression of VEGF-C, either in the tumor nests or in the tumor stroma, which ultimately led to ESCC invasion and metastasis [113].

A positive interaction between tumor cells and TAMs may lead to secretion of CSF1 by the tumor cells, which stimulates TAMs to secrete EGF. These factors play an important role in the invasion and migration of both cell types into blood vessels [114, 115]. CSF1 is a chemokine that plays an important role in regulating macrophages. A high CSF-1 level has also been found to be associated with poor prognoses in many cancers [116–118]. TAM-derived EGF leads to an increase in the invasion and movement of tumor cells by destroying the matrix and, thereby, by accelerating tumor invasion [64, 119]. The interaction between CSF1 and EGF forms a paracrine loop between tumor cells and macrophages, which subsequently has been found to result in the intravasation and migration of breast tumor cells. This observation was first made in vivo by Wyckoff et al. [119]. TNF-α is another important factor known to be involved in tumor intravasation. Wang et al. [120] found for example, using a Zebrafish model, that TNF-α and IL-6 may increase the ability of TAMs to promote tumor metastasis.

8 Therapeutic interventions to prevent intravasation

Targeting CSF1/CSF1R or EGF/EGFR has been found to inhibit the reduction of bone marrow monocyte mobilization. Thus, inhibition of CSF1-CSF1R may serve as a therapeutic tool to prevent tumor intravasation [110, 121, 122]. The effect of inhibition of CSF1R in a mouse glioblastoma model was investigated by Pyonteck et al. [123]. From their results they concluded that inhibition of CSF-1R by BLZ945 led to changes in macrophage accumulation, increased survival and blockage of tumor growth. The CSF1R inhibitor was also found to affect CSF1R expressed at the TAM level. This type of intervention is currently being investigated clinically, as a phase I/II study of BLZ945 alone or in combination with PDR001, in patients with malignant tumors (NCT02829723). Another phase I trial assessing CSF-1R inhibitor LY3022855 in combination with Durvalumab (MEDI4736) or Tremelimumab has been started in patients with advanced solid tumors (NCT02718911).

9 Role of TAMs in circulation

After tumor cells enter the bloodstream, they must survive to migrate throughout the body. These cells are called circulating tumor cells (CTCs). After tumor cells have entered the bloodstream successfully, they are faced with a new survival challenge. TAMs play an important role in the survival of CTCs in peripheral blood [124, 125]. Adams et al. [125] have shown that cancer-associated macrophage-like cells (CAMLs) can bind to CTCs in the peripheral blood. Such binding facilitates the implantation of tumor cells at distant locations.

Furthermore, the authors confirmed the presence of TAMs bound to CTCs in peripheral blood of patients with breast, pancreatic and prostate cancer. These results underscore the importance of macrophages in the circulation of cancer patients and their involvement in the development of distant metastases. It was also concluded that evaluation of the fluid phase of solid tumors is important for the detection of metastases. In a similar study, the presence of CALMs in the peripheral blood of 93% of patients with malignant breast cancer was confirmed. In 88% of patients undergoing core biopsies for the diagnosis of invasive carcinoma CAMLs were detected, compared to 26% of patients with benign breast conditions [126]. According to these results, screening for the presence of TAMs regardless of the stage of the disease is warranted [126]. In addition, it has been found that TAMs, by secreting factors such as MMPs and CXCL12, can convert solid tumor cells to CTCs. Therefore, the detection of TAM as a diagnostic biomarker for disease progression may be important.

10 Therapeutic interventions to prevent tumor cell circulation

The use of cabozantinib against circulating monocytes has shown a strong association between monocyte reprograming and therapeutic bone responsivity. As such, this observation may be used for patient selection at early stages of treatment. Based on this notion, a re-evaluation of tyrosine kinase inhibitor-based therapeutic strategies in prostate cancer has been considered for suitable patient populations based on tumor microenvironment responses [127]. In addition, inhibition of M-CSFR by PLX3397 (pexidartinib), a CSF-1R kinase inhibitor, has been found to decrease the number of TAMs and circulating monocytes effectively in mesothelioma mouse models, although the survival outcome was not favorable. The subsequent use of PLX3397 in combination with dendritic cell vaccination was found to augment survival synergistically, to decrease TAMs and to increase $CD8^+$ T cell numbers and functionalities [128]. It has also been shown that legumain, an asparaginyl endopeptidase, is highly expressed on the surface of TAMs. Specifically, legumain activates a doxorubicin-based prodrug, which selectively results in the reduction of angiogenic factors by ablation of TAMs. The use of this prodrug also inhibited CTCs and led to significant inhibition of tumor growth and metastasis formation in murine tumor models [129].

11 Role of TAMs in extravasation

Extravasation is the last stage of tumor cell travel in the blood circulatory system [130]. Through extravasation, tumor cells pass the blood vessel endothelium and invade target tissues [130, 131]. The extravasation of tumor cells occurs through interactions between tumor cells, endothelium and immune cells such as platelets, granulocytes/neutrophils and macrophages [132, 133]. Similar to the intravasation process, macrophages play a prominent role in the extravasation process. CCL2 is a chemokine that is secreted from tumor cells and is absorbed by TAMs. Its role in the extravasation of tumor cells has amply been shown. It has, for example, been shown that tumor-derived CCL2 can facilitate tumor metastasis through TAMs and metastasis-associated macrophages (MAMs) [134, 135]. CCL2 and its receptor CCR2 are expressed by macrophages. CCL2 stimulates the production of CCL3 from MAMs. Kitamura et al. [136] found that CCL3 secreted from MAMs and the CCL3-CCR1 axis may increase breast cancer cell implantation via MAM accumulation. In addition, they found that removal of CCL3 or CCR2 reduced the metastasis of lung cancer cells in humans and breast cancer cells in mice, and that both were associated with decreased MAM accumulation. Also, macrophages are known to produce VEGFs that play an important role in vascular permeability and facilitate the extravasation of tumor cells [137]. Therefore, prevention of extravasation may serve as a tool by which cancer metastasis may be impaired.

12 Therapeutic interventions for extravasation

Inhibition of the CCL2-CCR2 chemokine axis may be used as a mechanism to decrease tumor progression. A phase II study of PF-04136309 in combination with FOLFIRINOX in PDAC patients has indicated that this drug was safe and well tolerated by patients. PF-04136309 inhibits CCR2 activity, which decreases the infiltration of TAMs and prevents the mobilization of inflammatory macrophages into blood vessels (NCT01413022) [138]. Furthermore, inhibition of CCR2 signaling has been found to block tumor cell extravasation [139]. In another study, a protein consisting of a CCL2 mutant fused to human serum albumin (i.e., dnCCL2-HSA chimera) was constructed that prevented the absorption of inflammatory monocytes by binding to endothelial cells, thereby reducing the permeability of lung vessels and, thus, tumor seeding. This reduction in extravasation was supported by a decrease in vascular permeability in lung carcinoma samples [140].

13 Preparation of the metastatic niche

Metastatic niche refers to an environment in a secondary organ that provides the proper environment for the metastasis of a primary tumor. Primary tumor-secreted factors, such as VEGFA, TGF-β, TNF and LOX, have been found to

stimulate expression of the S100A8, S100A9 and SAA3 proteins, and to lead to extracellular matrix remodeling at metastatic sites. This remodeling provides a suitable metastatic environment before tumor cells arrive. S100A8 and S100A9 have been reported to play important roles in the establishment of pre-metastatic niches, and blocking of these factors has been found to prevent tumor cell infiltration of pre-metastatic $Mac1^+$ myeloid cells [64, 141, 142]. These TAMs represent one class of the bone marrow-derived cell (BMDC) population that contributes to the formation of metastatic niches through the promotion of tumor cell dissemination, as well as through providing an environment that supports the growth of cancer cells [143]. This was shown by Wang et al. [144], who found that colorectal cancer-derived VEGFA can stimulate TAMs to produce CXCL1 in the primary tumor. This CXCL1 subsequently recruited CXCR2-positive MDSCs and neutrophils in pre-metastatic liver tissue to form a pre-metastatic niche that ultimately promoted liver metastasis. The authors also showed that CXCR2 antagonist could prevent tumor progression and expansion. Similar results from Miyake et al. also showed that TAM- and cancer-associated fibroblast (CAF)-derived CXCL1 may play an important role in adhesion between tumor cells and stromal cells, thereby enhancing human bladder cancer growth [145].

A group of proteases secreted from TAMs, including cathepsin, MMP2, MMP7 and MMP9, has been found to be involved in ECM destruction, the migration of tumor cells and the formation of metastatic niches [63]. Another factor derived from TAMs that is involved in metastasis is CCL18. Through binding to the PITPNM3 receptor, CCL18 has been found to stimulate integrin clustering on the surface of breast cancer cells and to increase their binding to the ECM [63, 146, 147]. In addition, TAM-derived TNF-α, VEGF and TGF-β have been found to induce macrophages to produce S100A8 and serum amyloid A3, factors that recruit tumor cells and macrophages and stimulate the formation of metastatic niches [63]. CYP4A expression by TAMs and its role in the formation of pre-metastatic niches has also been reported. Chen et al. [99] showed that over-expression of CYP4A may play an important role in clinical specimens from patients with invasive breast carcinoma and melanoma. CYP4A was found to induce the production of cytokines derived from M2 macrophages, including VEGF, SDF-1 and TGF-β. These cytokines can activate the migration of $VEGFR1^+$ myeloid cells from bone marrow and fibroblasts, thereby leading to the formation of pre-metastatic niches and the promotion of metastasis [99]. Inhibition of the formation of metastatic niches may be a promising strategy to prevent tumor metastasis [148]. This could potentially be achieved by decreasing the expression of CYP4A and by inhibiting angiogenic factor production. For example, N-(4-butyl-2-methylphenyl)-N′-hydroxyformamidine has been found to inhibit the synthesis of 20-HETE and to decrease the production of pro-angiogenic factors such as VEGF [99]. Therefore, inhibition of the expression of CYP4A in CYP4A positive TAMs may be used as a method to inhibit the formation of metastatic niches and, thus, metastasis [99].

14 Conclusions and perspectives

In this review, the origin, function, polarization and signaling pathways involved in TAMs, and putative clinical applications of TAMs are discussed. The role of TAMs in tumor initiation, progression, angiogenesis, invasion and metastasis, as well as different TAM-targeting therapeutic approaches related to each step were emphasized. In addition, M1 and M2-associated gene expression profiles and their functional consequences were explained.

Since TAMs are known to play critical roles in the development and progression of human cancers, their targeting may be used as a potential therapeutic strategy. Previous and ongoing experimental, preclinical and clinical investigations have indeed shown potential. Clinical applications that appear to show most promise include blocking the extravasation of TAMs and using TAMs as diagnostic biomarkers for cancer progression. The future targeting of TAMs may well turn out to be a promising strategy for decreasing metastasis formation and for improving patient outcome.

Compliance with ethical standards

Conflict if interests None declared.

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