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Review Article

Regulation of cancer stem cell activities by tumor-associated macrophages

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Abstract: Recent studies revealed that tumor-associated macrophages play a decisive role in the regulation of tumor progression by manipulating tumor oncogenesis, angiogenesis and immune functions within tumor microenvironments. However, the role of cancer stem cells in the tumorigenic activities of tumor-associated macrophages during the course of transformation and treatment remains largely unknown. Recent studies have clarified the functional aspects of tumor-associated macrophages in the regulation of the tumorigenic activities and anticancer drug responsiveness of cancer stem cells through complex networks formed by distinct sets of cytokines, chemokines and growth factors. In this article we discuss recent advances and future perspectives regarding the molecular interplay between cancer stem cells and tumor-associated macrophages and provide future perspective about the therapeutic implication against treatment-resistant variants of cancer.

Keywords: Cancer stem cells, tumor associated macrophages, tumor microenvironments, MFG-E8, IL-6, TIM-3, M-CSF

Introduction

Genetic and epigenetic alterations in heterogeneous tumor cell populations regulate tumor initiation, progression and therapeutic responses. On the other hands, emerging evidence unveiled that heterogeneous tumor microenvironments composed of tumor cells as well as normal cells including mesenchymal stem cells, fibroblasts, endothelial cells and immune cells, have a large impact on the behavior of tumorigenic cells during the course of tumor progression [1-3]. In addition, tumor cells may modify the biological properties of stromal cells, endothelial cells and immune cells in their unique microenvironments, thereby contributing to further tumor progression and the emergence of drug-resistant tumor phenotypes [4, 5]. Thus, the interaction between tumor cells and their surrounding normal cellular components may have a determining role in the regulation of tumor initiation, progression, and responsiveness to anticancer therapeutics.

Recent advances molecular immunology and the clinical success of drugs targeting immune-regulatory circuits have emphasized the importance of tumor immune surveillance systems against nascent tumors [6-8]. On the other hand, tumor-infiltrating immune cells frequently promote tumor growth and incur invasive behavior through the coordinated activation of distinct inflammatory and angiogenic signals in the background of smoldering inflammation [9-11]. In particular, tumor-associated macrophages (TAM) have a critical role in modulating tumorigenic activities by activating oncogenic signals, angiogenesis, tissue/matrix remodeling and immune suppression [12-14].

In this review, we will describe recent advances in our understanding of the regulatory mechanisms whereby TAM impact cancer stem cell functions.

The role of tumor microenvironments in the regulation of cancer stem cell activities

Mesenchymal stem cells (MSC) are one of the critical components in tumor microenvironments. MSC enter the circulation into bloodstream from the bone marrow [15] or reside in

normal stromal tissues [16, 17]. Emergent evidence has unveiled the critical role of MSC in positively regulating the tumorigenic activities of cancer stem cells in several murine tumor models [17]. Furthermore, immunohistochemical analysis has confirmed the proximity of MSC and cancer stem cells in biopsies obtained from cancer patients, raising the possibility that MSC have an impact on the clinical course of human malignancies by modulating the cancer stem cell functions [18].

In the tumor microenvironments, MSCs have the ability to differentiate into stromal fibroblasts, which also interact with and influence tumor cells through paracrine signals and various soluble factors [19, 20]. SDF-1 produced by breast carcinoma-associated fibroblasts (but not normal fibroblasts) accelerates the growth and metastatic potential of breast cancers, which express high levels of the SDF-1 receptor CXCR4 [21, 22]. Hepatocyte growth factor (HGF) provides a co-stimulatory signal to the Wnt pathway during colon carcinogenesis [23]. Since niche activities regulated by Wnt-β-catenin cascades have a critical role in the survival and self-renewal of tissue and cancer stem cells, HGF released from stromal fibroblast may regulate cancer stem cell functions by stimulating Wnt-β-catenin pathways in a paracrine fashion [24]. Moreover, additional factors produced by stromal fibroblasts, which include NOS, PDGF, Notch ligand and Hedgehog ligands, are potential candidates to regulate cancer stem cell activities [25, 26]. In addition, the stromal signals serve as a prognostic factor in patients with breast cancer, suggesting that stromal microenvironments composed of MSC and stromal fibroblasts greatly impact the biological behavior of tumorigenic cells in actual clinical settings [27].

Endothelial cells may impact biological behaviors of cancer stem cells in tumor microenvironment by direct interaction with tumor cells as well as by their role in blood vessel formation. Endothelial cells constitute an important component of normal hematopoietic and neuronal stem cell niches, but tumor vasculature is different from normal vasculature, raising the possibility that tumor microenvironments produce defined factors that modulate the genetic and epigenetic profiles of endothelial cells. Indeed, more than 1,000 genes are differentially expressed comparing tumor *vs* normal endothelial cells, including FGF receptors, MMPs and NF-κB-regulated transcripts [28, 29]. In addition, cytokines produced by endothelial cells, which includes HGF, VEGF, PDGF and PIGF stimulate the self-renewal and survival of adjacent cancer stem cells [30, 31]. Interestingly, recent reports demonstrate that glioblastoma stem cells can trans-differentiate into endothelial cells to generate their own vasculature, thereby providing blood supply to adjacent tumor cells and further accelerating tumor progression [32, 33].

Together, the complex networks created by cancer stem cells and the surrounding normal cellular components contribute to the proliferation as well as the invasive activities of tumors in murine models as well as cancer patients (**Figure 1**).

The role of TAM in tumor progression and anticancer drug resistance

The immune system provides both inhibitory and stimulatory effectors in tumor initiation, promotion and metastasis, and the balance of these effects may be determined by different tumor microenvironments. Thus, the distinct modes of interplay between tumorigenic cells and host immunity may profoundly influence both the biological behaviors of tumors during the course of tumorigenicity and their responsiveness to anticancer modalities [6-11]. In particular, emerging evidences have unveiled the molecular mechanisms by which myeloid cells such as macrophages and myeloid-derived suppressor cells (MDSC), interact with tumor microenvironments to further accelerate tumor progression [12, 34].

Tumor-associated macrophages are characterized by distinct phenotypic polarization referred as “M1 and M2” subsets [14, 35]. The M1-polarized macrophages manifest high levels of proinflammatory cytokines, high production of reactive nitrogen and oxygen intermediates, and promote Th1 responses, which contributes to tumoricidal activity and antitumor immunity. On the other hands, M2 macrophages serve as the main players facilitating parasite containment, tissue remodeling and immune tolerance, which may be linked with tumor progression [36, 37]. The M1 polarization in macrophages is mainly regulated by distinct transcriptional networks consisting of IRF-1/5, Stat-1/4 and NF-κB, whereas M2 polarization is regulated through other transcription factors such as IRF-4, Stat-

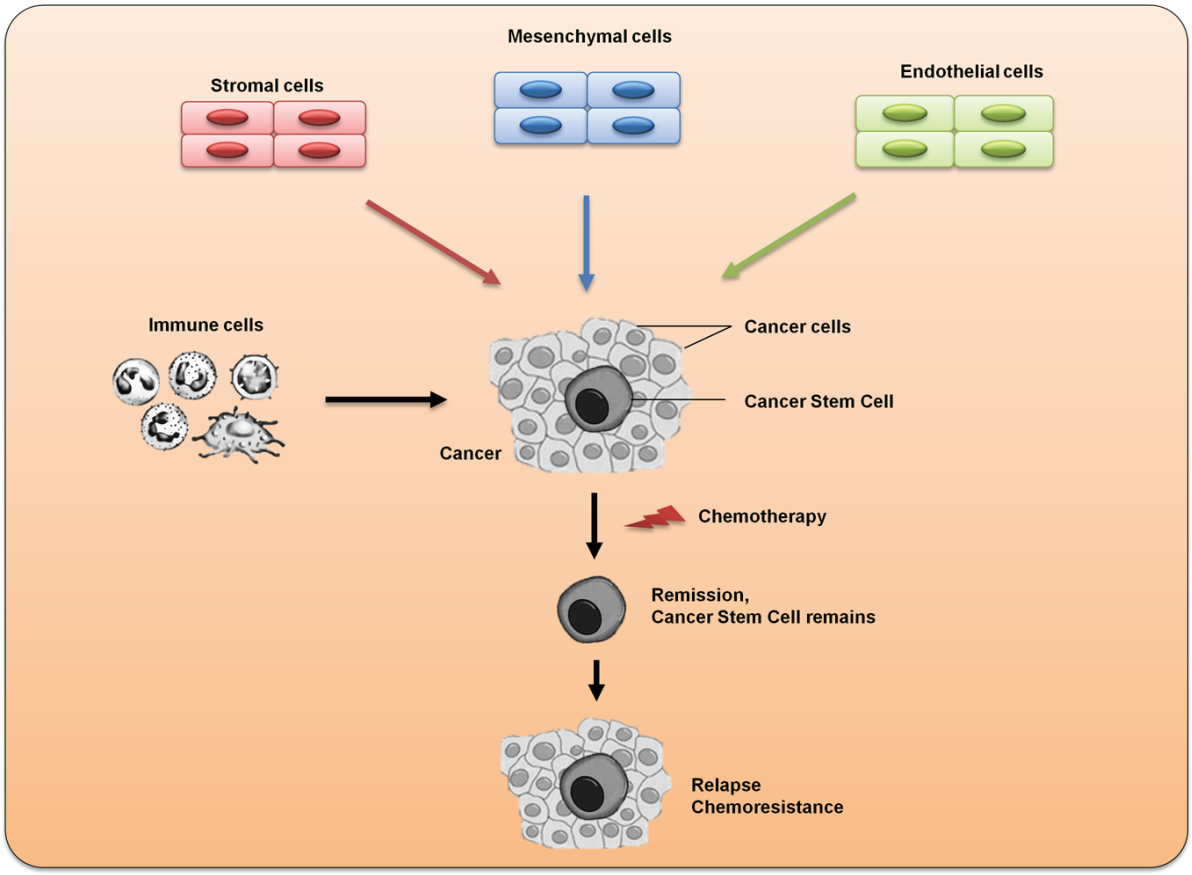

Figure 1. The complex networks created by cancer stem cells and their tumor microenvironments contribute to the tumorigenic and invasive activities of tumors.

3/6, PPAR-γ, KLF-4 and histone demethylase such as Jmjd3 [38-42]. In several murine models of carcinogenesis, tumor progression is frequently associated with a phenotypic switch from M1 to M2 in TAM [43]. Furthermore, M1-polarized macrophages mediate elimination of senescent hepatocytes, which drive subsequent carcinogenesis [44]. It is therefore possible that classically activated M1 macrophages contribute to the tumor elimination and equilibrium phases during tumor progression *via* T cell-mediated mechanisms [6].

However, there is a growing appreciation that TAM are composed of several different populations that bear sufficient plasticity and flexibility to enable them to cause dynamic changes between the M1 and M2 phenotypes depending on the different tumor microenvironments [46, 47]. Furthermore, recent genetic profiling and phenotypic analyses have also revealed that tumor cells, through distinct sets of signaling molecules, transcription factors, and epigenetic modifiers, manipulate tumor-infiltrating myeloid cells to differentiate them into peculiar subsets with tumor-promoting capacities [48, 49]. In this regard, tumor microenvironments characterized by smoldering inflammation and/or modulated by anticancer drug-mediated stress responses may serve as driving forces to alter the genetic and phenotypic profiles of tumor cells, thus promoting tumorigenic activities of myeloid cells in an autocrine and paracrine fashion.

Recent comprehensive analysis revealed that the numbers and activities of tumor-associated macrophages may influence the prognosis of patients with Hodgikin's lymphoma and breast cancer [49, 50]. Furthermore, treatment with M-CSF kinase inhibitors had significant antitumor activity against patients-derived primary tumors arising in immunodeficient mice when com-

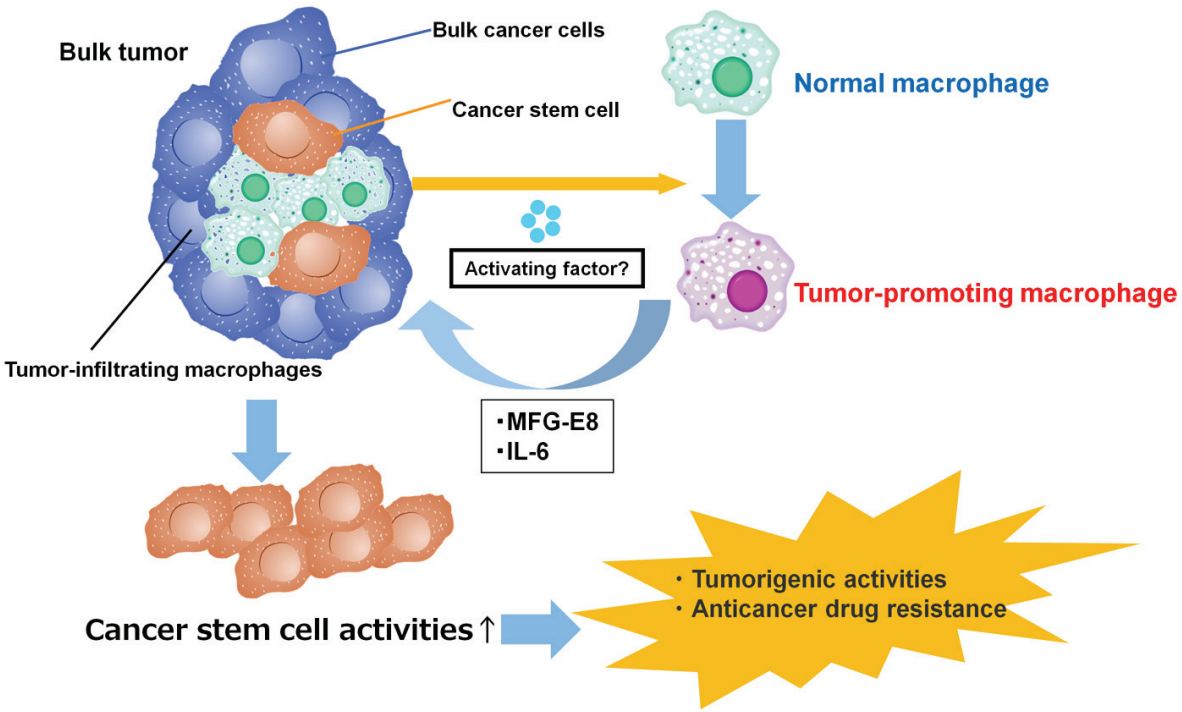

Figure 2. TAM serves as the major components of niche microenvironments regulating cancer stem cell functions. MFG-E8 and IL-6 predominantly produced from TAM in a cancer stem cell-specific fashion trigger tumorigenesis and anticancer drug resistance in cancer stem cells through the coordinated activation of the Stat3 and Hedgehog pathways. The interplay between cancer stem cells and TAM accelerates tumor progression and drug resistance through autocrine positive-feedback mechanisms.

bined with conventional cytotoxic chemotherapy [50].

These findings suggest that different tumor microenvironments may have a distinct impact on the ability of TAM on tumor growth and therapeutic responses to chemotherapy. The identification of the various cellular and molecular pathways and their downstream factors that participate in the interaction between tumorigenic cells and tumor-infiltrating myeloid cells in various human cancers will translate our understanding of cancer-related inflammation to meaningful therapeutic advances.

The interplay between cancer stem cells and TAM in the regulation of tumorigenicity and anticancer drug responses

Recent studies have clarified the importance of TAM as major contributors in the regulation of both self-renewal and anticancer drug responses of cancer stem cells through distinct networks of cytokines, chemokines and growth factors. In these processes, TAM interact with and promote the tumorigenicity of cancer stem cells *via* production of milk-fat globule-epidermal growth factor–VIII (MFG-E8) and IL-6 through coordinated activation of the STAT3 and sonic hedgehog pathways [51]. Interestingly, cancer stem cells are the major subset promoting the production of MFG-E8 and IL-6 from macrophages, implying that mediators specifically regulated by cancer stem cells render macrophages with the ability to facilitate the production of tumorigenic factors such as MFG-E8 and IL-6. In this sense, TAM might serve as a component of the "immunological niche", by which cancer stem cell activities are maintained and amplified within tumor microenvironments (**Figure 2**).

Cancer stem cells have unique characteristics that manipulate complex signaling cascades which regulate oncogenesis, embryogenesis and self-renewal. In turn, these amplification loop lead to oncogenic addiction, stem cell maintenance, angiogenesis, and immune modu-

lation within tumor microenvironments [52, 53]. Several oncogenic pathways, including Wnt/β-catenin, Notch, TGF-β/FOXO cascades, support self-renewal capacity and anticancer drug resistance in cancer stem cells [54, 55]. In addition, recent studies have revealed the indispensable role of the IL-6-Stat3 signal cascade in stimulating cancer stem cell activities in coordination with NF-kB-dependent inflammatory signals derived from tumor cells and their microenvironment [56, 57]. Moreover, Hedgehog signals have been identified as sentinels linking oncogenic aberration with the developmental program of normal and cancer stem cells [58]. In this regards, our findings that MFG-E8 and IL-6 derived from TAM mediate self-renewal and anticancer drug resistance through activation of Stat3 and Hedgehog signals provide additional evidences that TAM play a critical role in activating distinct signals that are crucial to the maintenance of the stem cell properties of tumor cells.

Recent studies in human leukemia and lymphoma have suggested that tumor cells express the antigen CD47, which serves as a "don't eat me" signal to tumor-associated macrophages by engaging their cognate receptor SIRT-1. Administration of a blocking antibody to CD47 induced macrophage phagocytosis of AML stem cells *in vitro* and in mouse models [59-61]. These findings provide the first evidence that macrophage phagocytosis serves as a critical mediator of tumor immunosurveillance against leukemia stem cells.

On the other hand, T cell immunoglobulin-mucin domain protein-3 (TIM-3), which is involved in apoptotic cell phagocytosis *via* recognition of phosphatidylserine, has been identified as a functional marker for dissecting acute leukemia stem cells from bulk tumor cells [62-64]. Furthermore, AML stem cells were eradicated by the administration of a TIM-3-depleting mAb [63]. Since TIM-3 expression is detectable in macrophages and dendritic cells upon stimulation with toll-like receptor ligands such as LPS, it is of great interest to examine whether TIM-3 is detected in tumor-associated myeloid cells and to determine the functional role of myeloid cell-derived TIM-3 and its phagocytic activity in the regulation of cancer stem cell functions.

In addition, MFG-E8 not only serves as a positive modulator of cancer stem cell activities, but also functions as an immunoregulatory factor within tumor microenvironments by promoting apoptotic cell phagocytosis and inducing $Foxp3^+$ infiltration into tumors [65, 66]. Moreover, TIM-4, expressed mainly on activated myeloid cells is also critically involved in the phagocytosis of apoptotic cells *via* the recognition of phosphatidylserine and the triggering of immune tolerance [67, 68]. The TAM receptor tyrosine kinase family composed of Axl, Mer-tyrosine kinase and Tyro-3, which serve as phagocytic receptors for apoptotic cells *via* recognition of Gas6, regulates innate immune responses and could be involved in the tumorigenic potentials of cancer cells [69, 70]. It is therefore likely that distinct sets of phagocytosis-associated molecules, such as CD47 / SIRT1, TIM-3, TIM-4, MFG-E8, Gas-6 *etc.* recognize distinct tumor subtypes including cancer stem cells, which arise from different backgrounds of oncogenic or epigenetic alterations and drug responsiveness. The identification and characterization of distinct sets of receptor / ligands on phagocytic macrophages may be an ideal strategy with which to investigate the interaction of cancer stem cells and TAM, and may lead to the exploration of new therapeutic targets against cancer stem cells.

Therapeutic implication for targeting the interaction of cancer stem cells and TAM

Comprehensive genetic approaches along with advances in the field of stem cell biology facilitated the identification of cancer stem cell-specific markers and multiple pathways potentially suitable for specifically targeting cancer stem cells [71, 72]. However, whether the target molecules identified from "pure" populations in cancer stem cells are actually effective against recurrent and multidrug-resistant variants of tumors remains largely uncharacterized. Thus, the development of drugs targeting the molecular networks between cancer stem cells and macrophages should provide useful tools with which to regulate cancer stem cell activities in coordination with those drugs targeting cancer stem cells and other factors derived from MSC, endothelial cells, fibroblasts and extracellular matrixes.

In addition, the targeting of TAM-derived downstream factors such as MFG-E8 and IL-6 may also be useful in repressing the emergence of chemoresistant tumors by controlling cancer

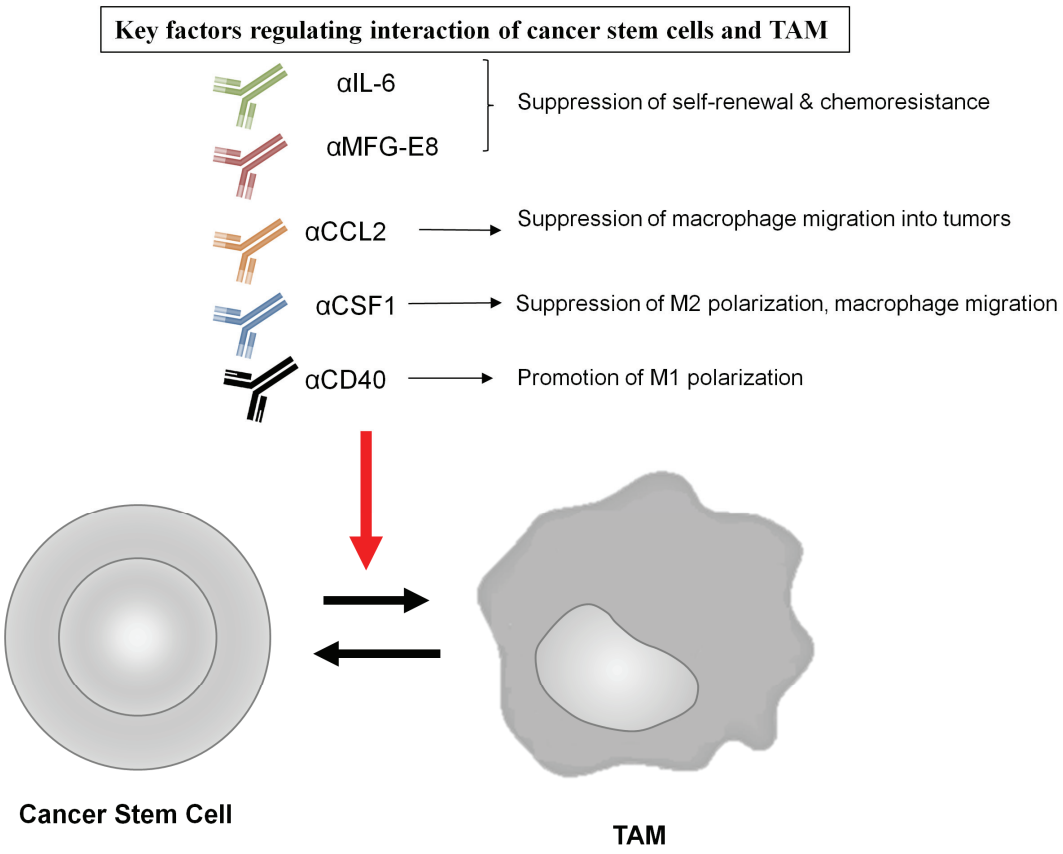

Figure 3. The strategy for targeting key mediators that influence the tumorigenic activities of TAM such as CCL2, M-CSF, CD40L and PPA has a potential to efficiently control cancer stem cell activities and might overcome the therapeutic limitations of conventional anticancer modalities.

stem cell activities [51, 73]. Moreover, therapeutic approach targeting TAM and their downstream effectors are now at the initial stages of clinical applications. Recruitment is a key determinant sustaining macrophage numbers at sites of inflammation and immunity. The CCL2/MCP-1 pathway has emerged as a new target for the prevention of myeloid cell recruitment to tumor microenvironments [74, 75]. CSF-1 receptor (c-fms) kinase inhibitors have been recently introduced into the clinical arena and exhibit antitumor and antiangiogenic activity in various murine and human tumor models [50, 76]. Furthermore, anti-CSF-1 antibodies augmented the antitumor effects of cytotoxic chemotherapy in a human breast cancer xenograft model [77].

The manipulation of macrophage polarization may serve as another strategy for controlling tumorigenicity. Consistent with this concept, CD40 agonist antibodies induce high expression of M1 markers in macrophages and augment antitumor responses to chemotherapy in pancreatic adenocarcinoma models [78]. Other therapeutic strategies that have been reported to affect macrophage polarization include PPAR-γ agonist and TLR ligands (Poly (I: C) and CpG) [79-81].

Together, these findings validate the potential applicability of targeting key mediators that influence the tumorigenic activities of TAM, and suggest that this may negatively regulate cancer stem cell activities and overcome the therapeutic limitations of conventional anticancer modalities (**Figure 3**).

Concluding remarks

We present the overview that TAM serve as a critical immunological niche in regulating cancer

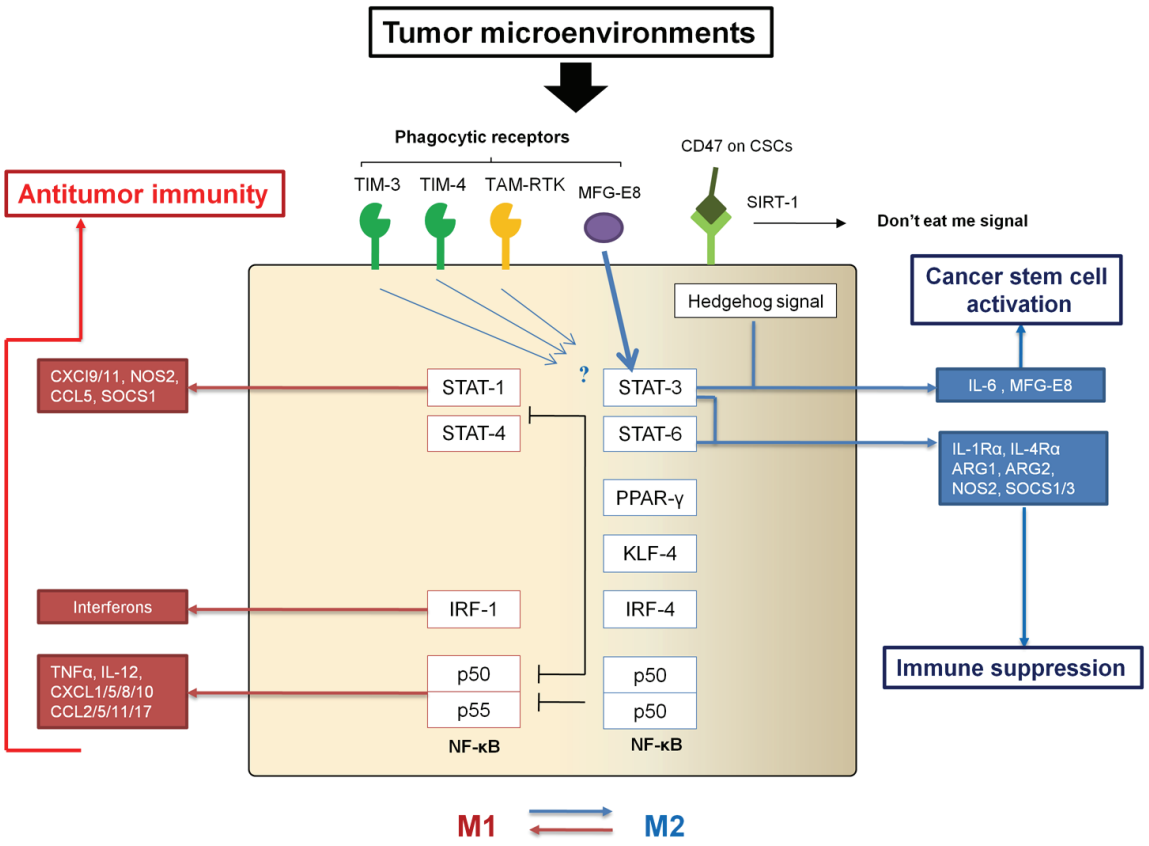

Figure 4. Tumor microenvironments regulate distinct signal cascades that are critical for determining macrophage polarization and facilitating the expression of key molecules that control interactions with cancer stem cells.

stem cell functions (**Figure 4**). Accumulating evidence reveals that the quality of tumor microenvironments may determine the direction of interplay of tumors and immune cells throughout the different stages of carcinogenesis. In addition, tumor-infiltrating immune cells other than TAM, such as MDSC, dendritic cell, granulocytes, NKT cells, B cells and $CD4^+$ T cells, also serves as positive regulators of tumor progression and metastasis, raising the possibility that various sets of immune cells interact with cancer stem cells to modulate their biological activities [10]. In this regard, comprehensive analysis of molecular intersection between intrinsic and immune-mediated pathways enriched in tumor microenvironments should provide useful insights into the regulatory mechanisms of cancer stem cell activities as well as provide new therapeutic approaches for targeting the components of immunological niche in the future.

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