Tumor-Induced Myeloid-Derived Suppressor Cells

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Abstract Myeloid-derived suppressor cells (MDSCs) represent a heterogeneous, immune-suppressive leukocyte population that develops systemically and infiltrates tumors. MDSCs can restrain the immune response through different mechanisms including essential metabolite consumption, reactive oxygen and nitrogen species production, as well as display of inhibitory surface molecules that alter T-cell trafficking and viability. Moreover, MDSCs play a role in tumor progression, acting directly on tumor cells and promoting cancer stemness, angiogenesis, stroma deposition, epithelial-to-mesenchymal transition, and metastasis formation. Many biological and pharmaceutical drugs affect MDSC expansion and functions in preclinical tumor models and patients, often reversing host immune dysfunctions and allowing a more effective tumor immunotherapy.

MDSCs as a Hallmark of Cancer Progression

Tumors are composed of heterogeneous, transformed cell populations with different morphologies and phenotypes, which are organized in a pyramidal architecture determined by self-renewal ability, differentiation grade, and tumorigenic and clonogenic potential (1). During tumor progression, cancer cells secrete tumor-derived factors (TDFs), like cytokines, chemokines, and metabolites, which promote the development of a flexible microenvironment inducing both the generation of new vessels and the modification of the immune responses (2). Tumors can escape the immune system by three main mechanisms: (i) cancer cells can veil their identity to escape recognition by immune effectors, (ii) they can directly modify antitumor immunity, or (iii) they can recruit other immune regulatory cells whose normal function is to inhibit immune reactions and prevent the unfavorable effects of uncontrolled immune stimulation (3). Probably the most pervasive and efficient strategy of “tumor escape” relies on the tumor’s ability to create a tolerant microenvironment by modification of normal hematopoiesis. In fact, cancers can induce the proliferation and differentiation of myeloid precursors into myeloid cells with immunosuppressive functions, in both the bone marrow and other hematopoietic organs such as the spleen, at the expense of additional myeloid cell subsets, such as dendritic cells (DCs) (4). Additionally, the persisting imbalance in the number and type of myeloid cells can deeply influence myeloid cell recruitment and function at the tumor site and secondary lymphoid organs. In the bone marrow, hematopoietic progenitor cells give rise to immature DCs (iDCs). To reach complete maturation, iDCs require inflammation-related stimuli because, although able to take up, process, and present antigens, they express few or none of the costimulatory molecules, such as CD80, CD86, and CD40, necessary to exert their functions (5). The higher number of iDCs found at the tumor site stems from defects in myelopoiesis rather than simply from the lack of appropriate activation signals at the tumor site. In vitro treatment of tumor-infiltrating DCs with appropriate stimuli—such as granulocyte-macrophage...
colony-stimulating factor (GM-CSF), tumor necrosis factor α (TNF-α), or CD40L—was not sufficient to induce DC maturation; this evidence supports the concept that the reduced functionality of DCs is most likely due to defects in differentiation from their iDC progenitors (6). However, iDCs are not the only myeloid cell populations modified in cancer. In postnatal life, hematopoietic stem cells present in hemopoietic compartments give rise to lymphoid and myeloid multipotent precursor cells. Other pluripotent cell types originate from the myeloid precursors: the common DCs and the immature myeloid cell precursors (IMCs). The first originates iDCs and plasmacytoid DCs, and the second is the common progenitor for macrophages, granulocytes, and monocytic-derived DCs (7). In healthy mice, IMCs rapidly differentiate into their descendant lineages; consequently, they represent a relatively low percentage of circulating myeloid cells. However, under pathological conditions, including cancer, there is a partial block in IMC differentiation, leading to the accumulation of CD11b+/Gr-1+ myeloid cells with immunosuppressive function, named myeloid-derived suppressor cells (MDSCs) (8).

About 30 years ago, Strober (9) highlighted, for the first time, the immunosuppressive properties of myeloid cells. These cells were originally defined as natural suppressor (NS) cells; they lacked common markers for lymphocytes, natural killer (NK) cells, and macrophages and showed the peculiar property of suppressing T-cell functions, affecting alloreactive immune responses in allogeneic bone marrow chimera experiments (9). NS cells were deemed to appear only transiently in some life phases, such as in the placenta during pregnancy, in fetal newborn tissues, and in the neonatal maturation of lymphoid tissues. However, they could be induced by immune system manipulation, for example, through total body irradiation or chemotherapy; during chronic inflammatory pathologies, such as graft-versus-host disease; and in cancer. Technical limitations in identification, purification, and in vitro culture conditions delayed the definition of biological properties and phenotype of NS cells. The first clear involvement of myeloid cells in lowering immune surveillance and in promoting tumor growth was provided in 1995. The administration of an antibody directed against the antigen Gr-1 (recognizing the cross-reacting molecules of lymphocyte antigen 6 complex locus C and G, i.e., Ly6C and Ly6G) to immunocompetent mice reduced the growth of a UV light-induced tumor (10). The effect of the in vivo anti-Gr-1 administration was originally attributed to the elimination of granulocytes, but successive reports described that the Gr-1+ cells were mostly CD11b+ and comprised both polymorphonuclear and mononuclear cells, including elements at different maturation stages along the myelomonocytic differentiation lineage (11, 12).

CD11b+/Gr-1+ cells have been identified in noncancerous disease settings such as sepsis (13), toxoplasmosis (14), candidiasis (15), and leishmaniasis (16), as well as during autoimmune diseases (17), stress (18), and aging (19, 20). The heterogeneity of the CD11b+/Gr-1+ cells has for many years generated some misunderstanding, somehow amplified by the use of different acronyms to define the same cell population (i.e., NS cells, immature myeloid cells, or myeloid suppressor cells) (21). In 2007, a board of investigators agreed to use the common term “MDSC,” which highlights the frequent (but not absolute) finding of immune regulatory properties coupled with enhanced myelopoiesis in tumor-bearing hosts; de facto, the term “MDSC” acknowledges the incomplete understanding of the relationship between the cell subsets originated by the tumor-driven, enhanced myelopoiesis (22). Even though MDSCs are not a uniform cell population—rather, they include several subgroups distinct in their morphology and expression of surface markers—the recent identification of specialized molecular programs that orchestrate MDSC differentiation, joined with innovative new technologies, has provided insights into understanding the complex and unique myeloid deviation leading to MDSC generation.

**DEFINITION OF MOUSE AND HUMAN MDSCs**

**Mouse MDSC Phenotype and Differentiation**

MDSC composition is flexible and peculiar for each disease scenario and often changes, following the kinetics and development of the disease. In healthy mice, CD11b+/Gr-1+ cells can be detected in sufficient numbers only in the bone marrow (about 30 to 40%), but they do not show a relevant suppressive activity ex vivo. A similar scenario is observed even when CD11b+/Gr-1+ cells are isolated from bone marrow of tumor-bearing mice (23). Suppression of T-cell function can be observed only when supraphysiologic numbers of cells are used in in vitro assays (24) or when bone marrow cells are previously cultured for a few days in the presence of GM-CSF, granulocyte CSF (G-CSF), and interleukin-6 (IL-6) cytokines (23). Indeed, bone marrow CD11b+/Gr-1+ cells contain pluripotent cells that can differentiate (23), depending on the cytokine/chemokine context, into cells able to either enhance (e.g., myeloid DCs) or restrain (MDSCs) the immune response (25, 26).
In many models, indeed, the dysfunctional immune responses of T lymphocytes in tumor-bearing mice depended almost entirely on the accumulation of MDSCs in the blood and secondary lymphoid organs. Primary tumor resection, Gr-1 depletion, pharmacological inhibition, or genetic MDSC inactivation often resulted, in fact, in a complete correction of T-cell dysfunctions; as further endorsement of their dominant role, MDSC adoptive cell transfer into vaccinated, tumor-bearing mice dramatically contracted the effectiveness of immunotherapy (27, 28). Moreover, while CD11b+/Gr-1+ cells from naïve mice adoptively transferred into congenic mice differentiated into mature CD11c-positive, major histocompatibility complex class II (MHC-II)-positive DCs and Gr-1−F4/80− macrophages within 5 days, MDSCs from tumor-bearing mice preserved their immature phenotype longer (CD11b+/Gr-1+), and the differentiation to mature macrophages was significantly impaired (29). Despite this panoply of differentiation options, for practical reasons, mouse MDSCs have been divided into two main subsets: monocytic (MO-MDSCs) and polymorphonuclear/granulocytic (PMN-MDSCs) (24).

In tumor-bearing mice, MO-MDSCs (Gr-1lox/−/CD11b+Ly6C+Ly6G−) are highly immunosuppressive and exert their effect largely in an antigen-nonspecific manner, whereas PMN-MDSCs (Gr-1hi/CD11b+Ly6CloLy6G+) are moderately immunosuppressive and promote T-cell tolerance via antigen-specific mechanisms. MO-MDSCs are side scatter low (SSClo), while PMN-MDSCs are SSChi. The same phenotypes in tumor-free mice define inflammatory monocytes and polymorphonuclear neutrophil neutrophil, respectively, both lacking the immunosuppressive activity (30). MO-MDSCs usually express higher levels of F4/80 (macrophage marker), CD115 (c-Fms, the receptor for macrophage colony-stimulating factor, and CCR2 (receptor for monocyte chemotactic protein, also known as CCL2), although these markers are not uniformly expressed by MDSCs induced by all tumors. Moreover, MO-MDSCs but not PMN-MDSCs mature in vitro and acquire F4/80 and CD11c expression when cultured with GM-CSF (24, 31, 32). In addition, the expression of CD49d marker was associated with MO-MDSC immunosuppressive function: the CD49d+ MDSC cell subset strongly inhibited antigen-specific T-cell proliferation in a nitric oxide (NO)-dependent fashion (33).

In many tumor models, as well as in cancer patients, PMN-MDSCs are the predominant subset, representing 70 to 80% of the tumor-induced MDSCs, compared to 20 to 30% of the cells reflecting the monocytic lineage (34). The two subpopulations also differ in the effector pathways used to suppress T-cell activation. MO-MDSCs suppress CD8+ T-lymphocyte proliferation mainly through activation of inducible NO synthase (iNOS) and arginase 1 (ARG1) enzymes and through the production of reactive nitrogen species (RNS) (24, 32). PMN-MDSCs, instead, can express some levels of ARG1 but suppress CD8+ T cells mainly through the release of reactive oxygen species (ROS) (32). A schematic summary of the main mouse and human MDSC markers is presented in Table 1. The main MDSC subsets are not two completely distinct and fully differentiated myeloid populations but rather differentiation states along a common lineage (35). A relevant fraction of MO-MDSCs, in tumor-bearing but not tumor-free mice, acquires phenotypic, morphological, and functional features of PMN-MDSCs by a mechanism that involves the epigenetic downregulation of the retinoblastoma protein (Rb1) by histone deacetylases (35). Thus, MO-MDSCs not only have the capacity to strongly downmodulate antitumor immunity but also serve as “precursors” that maintain the PMN-MDSC pool. Indeed, mouse MO-MDSCs proliferate faster than either PMN-MDSCs or the normal monocytic counterpart, can form colonies in agar, and generate a wide range of myeloid cells when either adoptively transferred to tumor-bearing hosts or exposed to TDFs, as well as the GM-CSF and IL-6 cytokines in vitro (28). The interplay between PMN-MDSCs and MO-MDSCs might also require reciprocal influence among the cytokines that each subset secretes: PMN-MDSCs produce high levels of gamma interferon (IFN-γ) and discrete levels of IL-13, whereas MO-MDSCs secrete low levels of both IL-12 and IL-10 (36).

### Table 1: Surface and molecular markers of mouse and human MDSCs

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| Human MO-MDSCs  | CD11b+  | Human PMN-MDSCs | CD11b+  |
|                 | CD33+   |                 | CD33+   |
|                 | CD14+   |                 | CD14+   |
|                 | CD15+   |                 | CD15+   |
|                 | HLA-DR+ |                 | HLA-DR+ |
|                 | CD124+  |                 | CD124+  |
cytokines. When primed by interaction with activated T lymphocytes, MDSCs produce both IL-13 and IFN-γ, which are utilized in an autocrine manner to enhance the production and activity of both ARG1 and iNOS enzymes; in this loop, IFN-γ is required for the upregulation of IL-4Ra, which mediates IL-13 signaling and promotes the survival of MO-MDSCs (36). Thus, the elevated production of IFN-γ by PMN-MDSCs may serve to maintain MO-MDSCs’ suppressive activity and prevent their apoptotic death (37).

The plasticity of MDSCs depends on the ability of myeloid cells to lose lineage identity in response to specific microenvironmental signals (38). Furthermore, TDFs induce and promote tumor-infiltrating MDSC differentiation into other myeloid cell subsets, such as tumor-associated macrophages (TAMs) and tumor-associated neutrophils (TANs). However, reports on this topic are still scattered and often controversial, and some of the discrepancies could be clarified by the fact that often the expression of markers for the myeloid lineage does not univocally define these cells (39). The current view of macrophages depicts two extremes: M1 “classically” activated macrophages (MHC-II(ICD80/CD86*NO5*IIL-12*IIL-6*IIL-1*IIL-10(I)) respond to IFN-γ by releasing proinflammatory cytokines such as IL-12 and IL-23 and are involved in Th1 cell-mediated responses; M2 “alternatively” activated macrophages (MHC-II(IICD11c*CD206*CD163*IIL-10*IFN-γ)) react to IL-4/IL-13 and are involved in Th2-type responses, fibrosis, and tissue repair (40). This clear-cut dichotomy was recently revised toward a continuum of activation states with partially overlapping phenotypes and functions, ranging over host defense, wound healing, and immune regulation by taking into consideration three main factors: the source of macrophages, the activating molecules, and the specific set of markers that define macrophage activation (41). Tumor-infiltrating MDSCs (defined as CD11b+Gr-1*F4/80*CCR2*CX3CR1+) expressed macrophage markers of both M1 and M2 polarization (42). The expression of CCR2 receptor can highlight a key role for CCL2 chemokine in MDSC recruitment from bone marrow and secondary hematopoietic organs (like the spleen), to tumor mass, where they can differentiate into macrophages. Ly6C*Ly6G*F4/80 CX3CR1*CCR2*CD62L+ monocyte precursors, which are phenotypically related to MO-MDSCs, are able to replenish nonproliferating TAM populations (43). The MDSC conversion to immunosuppressive TAMs is mediated mainly by CSF-1 (44), but also by molecular pathways associated with the hypoxia inducible factor-1α (HIF1α), as discussed below, or through a hypoxia-independent stabilization of HIF1α by the lactic acid produced via the Warburg effect in cancer metabolic state (45). Neutrophils, like all other leukocytes, are also able to migrate to tissues from the blood under the influence of specific chemokines (i.e., Chemokine C-X-C motif ligand (CXCL)-1, CXCL-2, and CXCL-6), cytokines (i.e., TNF-α), and adhesion molecules located on their own surface (i.e., CD11b) and on the surface of endothelial cells (i.e., selectins, intercellular adhesion molecule-1, and platelet endothelial cell adhesion molecule-1) (46). When they traffic to tumors, they are often indicated as TANs. TANs sustain not only tumor growth but also metastatic tumor cell spread through the secretion of cytokines, such as transforming growth factor β (TGF-β) (47). PMN-MDSCs and naïve neutrophils (NNs) are functionally and phenotypically different. PMN-MDSCs, but not neutrophils, are immunosuppressive (48) and they express higher levels of CD115 and CD244 and lower levels of CXC-chemokine receptor 1 (CXCR1) and CXCR2 (49). Moreover, transcriptomic analysis comparing TANs with either PMN-MDSCs from mesothelioma-bearing mice or NNs revealed that the gene profiles of NNs and PMN-MDSCs are more closely related to each other than to TANs, suggesting that TANs are not simply “tissue-based MDSCs” but are a distinct population of neutrophils (50). However, a simple analysis of surface marker expression may not be sufficient to distinguish NNs from PMN-MDSCs and inflammatory monocytes from MO-MDSCs. Substantial help on this topic can be provided by the functional and molecular characterization of these myeloid subsets. For example, PMN-MDSCs produce higher levels of ROS, ARG1, and myeloperoxidase than NNs (32, 48), whereas MO-MDSCs can be distinguished from inflammatory monocytes because they upregulate iNOS, ARG1, IL-10, and TGF-β (36).

Myeloid cell plasticity creates particular situations in which unexpected cell development can take place. For example, mitogen-activated protein kinase kinase 6 (MKK6)–p38 mitogen-activated protein kinase (MAPK) activation in human peripheral blood- or CD34-derived neutrophils starts a molecular program that culminates in the transdifferentiation to monocytes. This process requires proteasomal degradation of CCAAT/enhancer-binding protein α (CEBPα) and is, at least in part, mediated by c-Jun induction and phosphorylation (51). Accordingly, Ly6G*F4/80+ neutrophils adoptively transferred to mice 4 h post-induction of thioglycollate peritonitis progressively acquired a monocyte phenotype characterized by progressive upregulation of F4/80 marker and downregulation of Ly6G (51). Moreover, a peculiar MDSC fraction (CD33*CD15*CD66b*IIL-
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4Rα*CD11b*HLA-DR−) that displayed classic fibrocyte markers, such as α smooth muscle actin and collagen I/V, possessed a potent tolerogenic activity by suppressing T-cell proliferation and promoting regulatory T cell (Treg) expansion primarily via indoleamine deoxygenase (52, 53). Moreover, CCR2*Ly6C++CD11b+ MDSCs are able to differentiate into fibrocytes by the activation of the transcriptional factor Kruppel-like factor 4 in tumor metastasis (54). As an example of the “ectopic” differentiation in cancer, GM-CSF secreted by tumor cells can induce the conversion of CD11bhiCD27hi NK cells into Ly6C+Ly6G+ MDSCs (55).

**Definition of Human MDSCs and Use as Novel Biomarkers in Oncology**

Human MDSCs can be found in the blood of patients with solid tumors; many if not all patients have elevated levels of MDSCs that correlate with clinical cancer stage and metastatic tumor burden. There is a long list of solid tumors in which MDSCs have been identified: breast cancer; non-small-cell lung cancer; colon and colorectal carcinoma; pancreatic adenocarcinoma; prostate cancer; sarcoma; carcinoid; gall bladder, adrenocortical, thyroid, and hepatocellular carcinoma; and melanoma (56); head and neck squamous carcinoma (57); renal cell carcinoma (RCC) (58); gastrointestinal cancer and esophageal cancer (59); bladder cancer (60); and urothelial tract cancer (49). MDSCs have also been detected in the blood of patients with hematologic malignancies, including multiple myeloma and non-Hodgkin’s lymphoma (61). However, a substantial heterogeneity of expression and levels of cell surface markers in human MDSCs was found within different studies and tumor types. Initially, human MDSCs were defined as tumor-infiltrating and blood-circulating CD34+ cells that were amplified by tumor-released GM-CSF and able to suppress immune functions (62). Nowadays, CD34 is no longer considered a universal marker of immunosuppressive myeloid cells in humans. The most immature MDSCs are positive for the common myeloid marker CD33 and negative for HLA-DR and other lineage-specific markers of differentiated lymphocytes and NK cells and hence defined as Lin−/− (Lin− cocktail usually contains antibodies to CD3, CD14, CD16, CD19, CD20, and CD56). Lin−HLA-DR−CD34−CD11b+ MDSCs share features and granule content in common with promyelocytes (even though normal promyelocytes are not immune suppressive) (63) and are increased in the blood of patients with different tumors including glioblastoma and breast, colon, lung, and kidney cancer (61, 64). This immature fraction might be indicative of the overall tumor burden, and the increased circulating levels correlate with worse prognosis and radiographic progression in breast and colorectal cancer patients (56).

Although there are disputes in unanimously defining human MDSC subsets and also for the lack of a human ortholog for the mouse Gr-1 marker, it is possible to divide the more differentiated MDSCs into two main subsets: PMN-MDSCs (CD11b*CD14*CD15+HLA-DR−) and MO-MDSCs (CD11b*CD14*IL-4Rα*CD15+HLA-DR−) (65). PMN-MDSCs are negative for costimulatory molecules (CD80, CD86, and CD83) and very cryosensitive (66); thus, functional studies can be performed only on fresh samples. PBMCs collected from RCC patients were contaminated with PMN-MDSCs able to suppress the proliferation of T cells stimulated with CD3/CD28 antibodies by ARG1-dependent, CD3ζ-chain downregulation (67). Circulating MO-MDSCs (CD11b−Lin−CD33+ HLA-DR−CD14+) and Tregs are increased in metastatic prostate cancer patients compared to healthy donors and negatively correlate with patient survival. Moreover, MDSCs isolated from prostate cancer patients’ blood possessed immune-suppressive capabilities on activated T cells, probably through upregulation of iNOS (68). Circulating MDSCs might thus represent a predictive biomarker, and there is an ongoing effort to relate MDSC subsets in blood with tumor progression, serological concentration of immune regulatory molecules, and clinical outcome. In this context, a positive association between higher MDSC (HLA-DR−CD14+CD11b+CD33+) and IL-13 and IL-6 cytokine levels in blood and increased CD163+ TAM infiltration can be detected in esophageal cancer; accordingly, IFN-γ and IL-12 serum concentrations were reduced in this cancer patient group (69). A similar correlation was described in pancreatic adenocarcinoma cases. Indeed, patients with stable disease showed significantly lower MDSC levels in blood than those undergoing progression. Accordingly, MDSC systemic accumulation correlated with higher serum concentrations of mediators associated with MDSC proliferation and acquisition of an immune-suppressive phenotype, such as angiogenic factors (fibroblast growth factor-2 and vascular endothelial growth factor [VEGF]), interleukins (IL-4, IL-8, IL-17), chemokines (CCL5), and platelet-derived growth factor (70). Similarly, CD11b−Lin−CD33+ HLA-DR−CD14+ MO-MDSCs endowed with T-cell-suppressive properties accumulate early in the blood of melanoma patients and are associated with higher serum IL-8 concentrations (71). MO-MDSC levels are inversely correlated with the presence of either NY-ESO-1- or Melan-A-specific T cells and with clinical outcome (72). Recently, phase I/II clinical trials showed that vaccines
based on tumor-associated peptides could prolong survival in patients with RCC and colorectal cancer who showed signs of a multipeptide-specific immunization. Moreover, positive and negative predictors of clinical responses could be found in the blood among leukocyte subsets (Tregs and MDSCs) and serum proteins (chemo-

responses could be found in the blood among leukocyte subsets (Tregs and MDSCs) and serum proteins (chemo-kines and apolipoproteins) (28, 64). In these studies, a panel of antibodies was developed to identify six MDSC phenotypes in a single multicolor staining. Levels of all MDSC subsets except one were significantly increased in the blood of patients with RCC, suggesting a global myelopoiesis alteration, as seen in mice. However, in a retrospective analysis, only two MDSC phenotypes were negatively associated with survival: CD14+HLA-DR−/lo and CD11b+CD14−CD15+. Interestingly, in RCC and colorectal cancer patients, the prevaccination serum levels of the chemokine CCL2 inversely correlated with the clinical response to the cancer vaccine in subjects responding to multiple peptides present in the vaccine formulation. Indeed, in addition to its known chemotactic activity, CCL2, together with IL-6, can have a double function to induce antiapoptotic effects on CD11b+ cells isolated from human peripheral blood by the enforced expression of c-FLIPL (cellular FLICE [FADD-like IL-1β-converting enzyme]-inhibitory protein large), and drive in vitro differentiation to M2-polarized macrophages, as assessed by the overexpression of CD206 (mannose receptor) marker (73). Mouse models also allowed unveiling of a tolerogenic role for this chemokine. CCL2 released by mesenchymal and myeloid cells in the spleen of tumor-bearing mice, in fact, was essential for the generation of a tolerogenic environment in the marginal zone of spleen. In this specific compartment of the white pulp, peculiar Ly6C+ monocytic cells (i.e., MO-MDSCs) attracted by the chemokine cross-presented tumor antigen to CD8+ T cells, inhibiting their activity, and likely formed a pool of precursors for other MDSC subsets (28). Taken together, the existing data on human MDSCs indicate that these cells share many of the functional properties found in mice.

**MDSCs AND IMMUNE DYSFUNCTIONS**

MDSCs’ ability to suppress T-cell activity is considered a hallmark of tumor progression (74). As discussed, MDSCs exert their immunosuppressive effect in both an antigen-specific and -nonspecific manner, depending on their localization and the specific characteristics of the tumor. MDSCs can restrain the immune response through different mechanisms including essential metabolite (l-arginine, l-cysteine, and l-tryptophan) consumption and/or their metabolic conversion to active by-products, ROS and RNS production, as well as display of inhibitory surface molecules that alter T-cell trafficking and viability. All these mechanisms can operate singularly or in combination. Moreover, these mechanisms can be either direct or indirect, in this latter case involving the generation or the expansion of other regulatory populations such as CD4+CD25+ Tregs (Fig. 1).

**MDSC Immunosuppressive Mechanisms**

**Related to the Depletion of Essential Metabolites**

T-cell proliferation and fitness rely on the availability of l-cysteine. Cells can import cysteine, the oxidized form of cysteine, from exogenous sources either through the x_c transporters or from the conversion of methionine by cystathionase. However, T cells lack both enzymes, so the only way to collect cysteine is during the immunologic synapse occurring between antigen-presenting cell (APC) and T cell. During this process, APCs release in the extracellular space, through the ASC transporter, reduced cysteine, which can be taken up by T cells. For this reason, media used for in vitro T-cell culture are supplied with a reducing agent (β2-mercaptoethanol) (75). MDSCs lack ASC transporter and cystathionase and do not export cysteine, thus reducing the availability of both oxidized and reduced amino acid. Cysteine/cysteine extracellular deprivation limits as well the supplying of cysteine to T cells because APCs can obtain it only from the modification of methionine (75). Another amino acid involved in T-cell function and immune regulation is tryptophan. The two isoenzymes responsible for its catabolism, indoleamine 2,3-dioxygenase 1 (IDO1) and IDO2, catalyze the degradation of the amino acid along the kynurenine pathway. l-Tryptophan starvation activates GCN2 kinase, which in turn inhibits CD8+ T-cell proliferation by causing cell cycle arrest and inducing anergy, and directs CD4+ T-cell differentiation toward the Treg phenotype by Foxp3 upregulation (76). The kynurenines produced by the reaction have similar immune-modulatory properties on CD4+ T cells and can reprogram DCs toward a tolerogenic function by binding the aryl hydrocarbon receptor (77). IDO1 is an enzyme expressed by tumor cells and specific leukocyte subsets, such as TAMs, plasmacytoid DCs, and MDSCs (78), involved in regulation of local inflammation that contribute to tumor-acquired immune tolerance. Accordingly, its expression inversely correlates with T-cell trafficking and clinical outcome in many tumors and is associated with higher Treg tumor presence and disease progression (79). l-Arginine represents the common
FIGURE 1 MDSCs suppress the immune response by four main mechanisms. (1) MDSCs deplete essential metabolites for T lymphocyte fitness, such as L-cysteine, L-tryptophan (by the activation of IDO1), and L-arginine (by the activation of both ARG1 and NOS2), inducing the T-cell proliferation arrest. T-cell proliferation block is exacerbated by MDSC-released TGF-β. L-Arginine depletion by ARG1 activity also induces the translational repression of the CD3ζ chain, which prevents T cells from responding to various stimuli. NO production inhibits T cells by interfering with the signaling cascade downstream of the IL-2 receptor. (2) High arginase activity in combination with increased NO production by the MDSCs not only results in more pronounced T-cell apoptosis but also leads to an increased production of ROS and RNS, such as the free radical peroxynitrite (ONOO−), by the MDSCs. This process requires collaboration with NOX2 enzyme, which contributes to large amounts of ROS, such as H2O2, which then affect T-cell fitness by downregulating CD3ζ chain expression and reducing cytokine secretion. RNS can act on α and β TCR chains, preventing TCR signaling and promoting dissociation of CD3ζ chain from the complex. (3) MDSCs interfere with T-cell migration and viability. MDSCs express the metalloproteinase ADAM17, able to cut the integrin CD62L on the T-cell membrane. RNS also modify leukocyte trafficking, promoting homing of immune-suppressive subsets other than T cells by tyrosine nitration of selective chemokines (like CCL2) or their receptors. MDSCs expressing PD-L1 can induce T-cell apoptosis by engaging PD-1. Moreover, NO produced by MDSCs has a direct proapoptotic role mediated by the accumulation of p53 and signaling by Fas, TNF receptor family members, and caspase-independent pathways. Finally, the MDSC-derived TGF-β can promote NK-cell inhibition. (4) MDSCs drive the differentiation of specific subsets into regulatory cells: by TGF-β release, MDSCs promote the clonal expansion of antigen-specific natural (n) Treg cells and drive the conversion of naive CD4+ T cells into induced (i) Treg cells. MDSCs skew macrophages toward an M2 phenotype by release of IL-10. For abbreviations and more details, see the text.
substrate for two enzymes: iNOS, which generates NO; and ARG1, which converts L-arginine to urea and L-ornithine. MDSCs express high levels of both ARG1 and iNOS, and a direct role for both of these enzymes in the inhibition of T-cell function is well established (80). The production of NO could inhibit, in an antigen-independent way, the T-cell signaling cascade downstream of the IL-2 receptor by different mechanisms, involving the blockade of phosphorylation and activation of JAK3 and STAT5 transcription factor, inhibition of MHC-II gene expression, and induction of T-cell apoptosis (81). Moreover, a direct proapoptotic effect has also been observed in T cells exposed to high concentrations of NO, likely mediated by the accumulation of the tumor suppressor protein p53, signaling by CD95 (also known as Fas) and TNF receptor family members, or signaling through caspase-independent pathways (82).

ARG1 activity causes the depletion of L-arginine and translational blockade of the ζ chain of CD3, which prevents T cells from responding to various stimuli (83). Moreover, L-arginine starvation blocks protein translation through the accumulation of empty aminoacyl-tRNAs, which in turn activate GCN2 kinase and phosphorylate the translation initiation factor eIF2α. The phosphorylation increases the affinity of eIF2α for eIF2β, which can no longer exchange GDP with GTP, thus interfering with protein synthesis (84). Finally, L-arginine consumption blocks the lymphocyte cell cycle in the G0-G1 phase because of the imbalance between cdk6 (increased) and the reduced levels of cyclin D3 and cdk4 (85). ARG1 is a player in T-cell immune dysfunction since its inhibition with the selective antagonist Nω-hydroxy-L-arginine affects, in a dose-dependent manner, tumor growth of a mouse lung carcinoma (86).

**MDSC Immunosuppressive Mechanisms Related to ROS and RNS Production**

High arginase activity in combination with increased NO production by MDSCs also leads to increased production of ROS and RNS, such as the free radical peroxynitrite (ONOO\(^-\)), by the MDSCs. These species have multiple inhibitory effects on T cells. ROS and RNS are radical compounds with high reactivity for macromolecules such as DNA, lipids, and proteins. By modifying their tertiary and quaternary structure, oxygen/nitrogen-derived posttranslational protein modification does not just induce inactivation/activation of the target but can modify signaling cascades and finely modulate biological processes, including immune responses. ROS production comprises superoxide anion (O\(_2\)) and peroxide hydrogen (H\(_2\)O\(_2\)) and relies on the activity of proteins of the NADPH oxidase (NOX) family, which includes NOX1 to -5, DUOX1, and DUOX2 (87). NOX2, the main actor for ROS production in leukocytes (such as granulocytes, macrophages, and MDSCs) and endothelial cells, is finely tuned at a transcriptional and posttranslational level: cytokines (i.e., TNF-α and IFN-γ), growth factors (VEGF), and transcription factors (STAT3) upregulate NOX2 levels, whereas specific phosphorylation patterns and subunit availability modulate its activity (88). As outlined above, ROS can also result from iNOS uncoupling reaction during deprivation of L-arginine. Tumor-infiltrating MDSCs produce large amounts of ROS (H\(_2\)O\(_2\)), which affect T-cell fitness by downregulating CD3 ζ-chain expression and reducing cytokine secretion, as observed in pancreatic cancer and melanoma (89, 90). In a physiological context, cells can limit the oxidative stress by an enzymatic detoxifying system such as superoxide dismutase and glutathione S-transferase. However, at high ROS concentration, radicals can directly react with macromolecules or combine with NO to generate more dangerous RNS, such as peroxynitrite and dinitrogen trioxide, which can nitrate/nitrosylate tyrosine, cysteine, methionine, and tryptophan in different proteins and enzymes, thus changing their biological functions (91). Under pathological conditions (i.e., tumor), RNS by inducing apoptosis and autophagy direct tumor evolution, and more importantly suppress T-cell trafficking and cytotoxic functions, contributing to shaping an immune-privileged environment that promotes tumor outgrowth. RNS can indeed alter the formation of a correct peptide-MHC complex by MHC-I or induce modification in the immune-dominant peptides. This results in unsuccessful peptide loading on MHC-I of target cells or failure in binding by CD8-TCR (T-cell receptor) lymphocyte antigen recognition complex (92). Moreover, RNS can act on α and β chains of the lymphocyte TCR, preventing signaling and promoting dissociation of the CD3 ζ chain from the complex (93). Finally, RNS also modify trafficking of leukocytes, promoting homing of immune-suppressive subsets other than T cells (further addressed below). This is in part mediated by tyrosine nitration of either chemokines (CCL2, CCL5, CCL21, and CXCL12) or receptors (CXCR4) (94).

**MDSC Immunosuppressive Mechanisms Related to T-Cell Migration Interference**

T-cell activation and effector functions require trafficking to lymph nodes and tumor sites. Both of these processes can be modified by MDSCs. L-Selectin (CD62L) is
a homing receptor for T cells and is critical for directing naïve T cells to lymph nodes. Peripheral blood T cells in tumor-bearing mice present a cleaved CD62L marker on their surface due to the activity of the metalloproteinase ADAM17 (disintegrin and metalloproteinase domain-containing protein 17) expressed on the MDSC surface (95). MDSC and cytotoxic T lymphocyte (CTL) trafficking to tumor is regulated by the CCL2/CCR2 axis. Intratumoral production of RNS induced nitration/nitrosylation of CCL2 in different human and mouse cancers. As a result, modified CCL2 could no longer attract tumor-specific CTLs but could still recruit myeloid cells to the tumor. Notably, this novel mechanism of tumor escape could be pharmacologically targeted. In vivo administration of a novel drug ([3-(aminocarbonyl)furoxan-4-yl]methyl salicylate; AT38) that blocks intratumoral RNS production induced a robust T-cell infiltration within the tumor and enabled transferred CTLs to reject solid tumors (96). In addition to regulating T-cell trafficking, MDSCs also decrease the number and inhibit the function of mouse and human NK cells, mostly through membrane contact-dependent mechanisms (97).

**MDSC Immunosuppressive Mechanisms Related to Treg Induction and M2 Macrophage Reprogramming**

MDSCs produce large amounts of TGF-β and IL-10 in the tumor microenvironment. TGF-β exerts direct anti-proliferative effects on T cells, arresting their cell cycle typically in the G1 phase by inducing the expression of the cell cycle inhibitors p27Kip1 and p21Cip1 (98) or by inhibiting IL-2 secretion (99). Importantly, TGF-β was shown to inhibit the differentiation of CD4+ T cells into Th1 or Th2 cells by suppressing the expression of T-bet and GATA-3 master regulators of Th1 and Th2 conversion, respectively (100). TGF-β-producing MDSCs also promote the clonal expansion of antigen-specific natural Treg cells and induce the conversion of naive CD4+ T cells into induced Treg cells. The mechanisms are not completely understood, but may involve cell-to-cell contact (including CD40-CD40L interactions) and the production of soluble factors in combination with released TGF-β, such as IFN-γ and IL-10 (101). Human CD14+HLA-DRlo/cD15–/cCD11c– MDSCs promote the transdifferentiation of Th17 cells into Foxp3+ induced Treg cells by producing TGF-β and retinoic acid (102). Moreover, through an IL-10- and cell contact-dependent mechanism, MDSCs skew macrophages toward an M2 phenotype by decreasing macrophage production of IL-12 (103). The downregulation of IL-12 is further exacerbated by the macrophages themselves, since macrophages promote the production of IL-10 by MDSCs, creating a self-maintaining, negative loop.

**MDSC-INDUCED MECHANISMS OF TUMOR PROMOTION**

MDSC activity is not simply directed to building up an immune-suppressive environment that keeps T cells at bay and protects tumors from the effector arm of the immune system, but includes mechanisms that sustain and promote tumor growth as well as metastatic spreading. These actions can be classified as direct tumor-promoting activities and include the control of cancer stemness, angiogenesis and stroma deposition, epithelial-to-mesenchymal transition (EMT), and metastasis formation.

**Cancer Stemness**

Normal cells undergo a limited number of divisions before reaching a proliferative block characterized by absence of response to growth factors, modification in morphology, and metabolism. This process, known as senescence, protects cells from immortalization and is triggered by telomere shortening, DNA damage, and upregulation of the CDKN2 locus (involved in cell cycle regulation). Molecularly, senescence follows oncogene-sustained proliferation, depletion of suppressor genes (Pten and Rb), or activation of the p53 tumor suppressor (104). Senescence is a characteristic of normal tissues and premalignant tumor cells; for example, while observed in pancreatic intraductal neoplasia and lung adenomas, it is absent in their advanced, malignant stages (105, 106). Myeloid cells finely tune tumor senescence by promoting cellular stemness. In two different spontaneous, senescence-inducing tumor models (conditional oncosuppressor PTEN+/− prostate adenocarcinoma and oncogene-mediated Ki-rasG12V lung adenocarcinoma), at tumor onset, neoplastic cells showed senescence phenotype, a condition reversed by MDSC action (107). Interestingly, MDSC interferes with the senescence-associated secretory phenotype by releasing IL-1RA (IL-1 receptor antagonist), which interrupts the IL-1α–IL-1R axis and activates a reprogramming activity in tumor cells. Accordingly, impairment in MDSC tumor trafficking (i.e., by CXCL1 and -2/CXCR2 targeting) enhanced chemotherapy-induced cell senescence in PTEN+/− mice. Another mechanism of MDSC-dependent tumor senescence inhibition was described in human ovarian carcinoma (108) and is based on targeting of C-terminal binding protein 2 (CtBP2) on tumor cells.
CtBP2 is a transcription corepressor recruiting histone deacetylases, methylases, and demethylases on target genes, which in turn remodel chromatin condensation and gene expression. CtBP2 modulates the expression of genes involved in sphere formation in ovarian primary tumor cells. MDSCs break this equilibrium and promote cancer stemness and metastasis through induction of microRNA101 in tumor cells. The microRNA101 targeting CtBP2 mRNA induces the expression of genes involved in sphere formation such as OCT3/4, SOX2, and NANOG. Accordingly, dense MDSC infiltrate, high microRNA101 expression, and low CtBP2 levels correlated in ovarian cancer patients with a worse clinical outcome (108). In pancreatic tumors, MO-MDSCs induced the proliferation of aldehyde dehydrogenase-1-positive cancer stem cells and promoted the acquisition of mesenchymal properties. The process is triggered and tuned in an autocrine loop by tumor cells, which induce recruitment of MO- and PMN-MDSCs to the tumor microenvironment and activate their immune-suppressive program through the STAT3 signaling pathway. A similar effect was observed with human CD14+ HLA-DR– MDSCs from pancreatic ductal adenocarcinoma (PDAC) patients (109).

**Angiogenesis, EMT, and Metastasis**

Unrestricted tumor growth is often followed by local hypoxia. To adapt to a hypoxic environment, tumor cells, which sense O2 levels through HIF1α, release VEGF and hence stimulate sprouting and building of new vessels to increase tissue perfusion, with the purpose of fulfilling the nutrient demand and supporting the sustained proliferation. Secreted VEGF plays an additional and crucial immune-regulating role by orchestration of peripheral expansion, trafficking of MDSCs to tumor, and acquisition of immune-suppressive properties (110). Indeed, tumor hypoxia stimulates MDSCs to upregulate both ARG1 and iNOS through hypoxia response element and NF-κB (111). In addition, NO-dependent modification of HIF1α acts as positive feedback on VEGF synthesis by amplifying the reaction, and MDSCs directly reinforce this loop by producing matrix metalloproteinase-9 (MMP-9), which increases VEGF availability (112). Even though the immunoregulatory function was not specifically tested in these experiments, recruitment of cells resembling PMN-MDSCs mediated resistance to anti-VEGF antibody-mediated therapy, suggesting that PMN-MDSCs could support new vessel growth even in the presence of VEGF antibody (113). MDSCs can also mediate resistance to the tyrosine kinase inhibitor sunitinib, an antiangiogenic agent, in both preclinical models and patients with RCC (114). The presence of circulating PMN-MDSCs that produced high levels of MMP-9, MMP-8, and IL-8 inversely correlated with the clinical response to sunitinib, suggesting that MDSCs could still promote angiogenesis by different mechanisms in sunitinib-resistant tumors. Hypoxia can also affect MDSC-dependent immune dysfunctions within the premetastatic niche (115). The injection of breast cancer-preconditioned hypoxic media promoted CCL2-mediated homing of PMN-MDSCs (Ly6C+Ly6G+) and NK cells to lungs and increased lung colonization after tumor cell injection. In this environment, NK cells lost their killing ability, thus contributing to a higher metastasis incidence (116). Another factor secreted by PMN-MDSCs during exposure to a hypoxic tumor microenvironment and involved in lung metastasis generation is Bombina variegata peptide 8 (116). Also, the proinflammatory proteins S100A8 and S100A9, potent chemoattractants for MDSCs, have been implicated in tumor and metastasis promotion by MDSCs (117); serum amyloid A3 induced by S100A8/A9 directly attracted MDSCs to premetastatic lungs, stimulated NF-kB signaling in a TLR4-dependent manner, and facilitated metastatic spreading (118).

After being recruited to the tumor and premetastatic niche and following the interaction of peculiar receptors—CXCR2 (PMN-MDSC), CCR2 (MO-MDSC), and CXCR4/RAGE (receptor for advanced glycation end products; both MDSC subsets)—with respective chemokines, MDSCs contribute to the generation of an immune-tolerant environment by releasing IL-6 (119) or triggering tumor cell migration (i.e., through TNF-α release) (120). MDSCs can assist the metastatic process also by inducing EMT of tumor cells, a condition in which cells acquire improved spreading skills. MDSCs attracted by CXCL5 chemokine induced EMT of melanoma cells by releasing hepatocyte growth factor and TGF-β in the primary tumor site, and the growth of primary tumor was significantly impaired by targeting PMN-MDSCs in this preclinical model (121). High-mobility group box-1 (HMGB1), a damage-associated molecular pattern protein released by tumor cells as well as leukocytes (i.e., macrophages and MDSCs) during stress and cell death, has been associated with tumor invasiveness, metastatic spreading, and EMT in colorectal carcinoma. It acts as a proinflammatory cytokine through binding to TLRs and RAGE, promoting MDSC trafficking and activation of EMT-inducing transcription factor Snail and NF-kB, which in turn activates MMP-7 (122). However, since metastatic cells show the morphology and phenotype of epithelial cells, it is
conceivable that premetastatic MDSCs, by releasing the proteoglycan versican, may also control the opposite transition route (mesenchymal to epithelial) in tumor cells that reach the new district, favoring their ability to seed and colonize the organ (123). Despite these data that reinforce the concept of a role for MDSCs in promoting metastatic tumor cell spread, some data are discordant and suggest the ability of MDSCs to inhibit metastasis by the production of a potent antiangiogenic matrix protein (i.e., thrombospondin-1) (124); this mechanism can open a new view on the relationship between MDSCs and the metastatic process.

Cachexia is a severe neoplastic syndrome characterized by body weight loss and deep metabolic changes that cannot be corrected just by increasing food intake. In digestive system cancers, cachexia has been correlated with high serum VEGF and peripheral MDSC levels, systemic inflammation, and MDSC-dependent immune dysfunctions (125). Preclinical studies on transplantable mammary tumors revealed that MDSCs can fuel some aspects of cachexia by inducing a hepatic acute-phase protein response that is either prevented by MDSC depletion or reproduced by MDSC adoptive transfer (126).

**FACTORS DRIVING MDSC ACCUMULATION AND ACQUISITION OF SUPPRESSIVE FUNCTIONS**

CSF-1, G-CSF, and GM-CSF are the three main regulators of proliferation and differentiation of the myeloid lineage. CSF-1 is found in many types of tumors, such as RCC (127) and about 70% of breast tumors (128), and is also implicated in macrophage trafficking. However, CSF-1R is also expressed by MO-MDSCs, and its pharmacological blockade significantly affects MO-MDSC tumor homing in melanoma and prostate tumor models (129). G-CSF induces differentiation of myeloid precursors to granulocytes and directs their recruitment to tumors (130). GM-CSF is a cytokine that can trigger myeloid cell commitment toward either an immune-stimulating (DC) or an immune-suppressive (MDSC) phenotype, depending on the strength of the stimulus and on the cytokine context. Indeed, tumors promote a myeloid cell commitment toward MDSC phenotype through release of GM-CSF and IL-6, which activate an immune-suppressive, C/EBPβ-mediated program in bone marrow-derived progenitors (23). GM-CSF, with the collaboration of IL-6, can intervene in regulating MDSC function during very early stages of tumor progression. Experimental models of autochthonous PDAC, in fact, have shown progressive waves of myelomonocytic cell recruitment after initiation of the transforming program controlled by the active Kras oncogene, with Gr-1*CD11b* cells being among the first to be recruited within the developing neoplastic lesions (131). Kras oncogene controls the accrual of myelomonocytic cells, and this step is mandatory for pancreatic intraepithelial neoplasia initiation and progression. Kras oncogene-driven inflammation at the pancreatic intraepithelial neoplasia stage critically relied on GM-CSF for both progression to PDAC and Gr-1*CD11b* cell recruitment within the pancreatic stroma. This circuit was essential to alter tumor-specific CTLs, and only the blockade of either GM-CSF production or Gr-1*CD11b* cell activity restored antitumor immunity (132). Interestingly, recruited Gr-1*CD11b* cells contribute with transformed epithelial cells to the local production of the cytokines IL-6 and IL-11, which activate STAT3. As discussed below, STAT3, in turn, induces antiapoptotic and pro-proliferative genes, fueling tumor initiation, promotion, and progression (133). GM-CSF administration was used as an adjuvant in clinical trials with inconclusive results. Indeed, both preclinical and clinical studies highlight that the cytokine concentration might be the switch regulating myeloid differentiation toward either DCs (low levels) or MDSCs (high levels) (134, 135). In the tumor microenvironment, IL-6 can be secreted by macrophages, MDSCs, monocytes, fibroblasts, and neoplastic cells. The inflammatory cytokine IL-6 drives the differentiation of CD11b*Gr-1* cells into immune-suppressive cells through the activation of STAT3. IL-6’s role in tumor progression has been confirmed in different tumors, such as breast, lung, ovarian, renal, and pancreatic, to inversely correlate with the clinical outcome (136). IL-4 and IL-13 act by inducing MDSC survival and activation through the immune-suppressive pathway by binding the IL-4Rα kinase subunit of the IL-4R (36). The signaling pathway downstream of IL-4Rα entails the recruitment, phosphorylation, and dimerization of STAT6, which regulates the expression of genes involved in the immune-suppressive program and survival of MDSCs. IL-4Rα-dependent STAT6 activation indeed induces TGF-β synthesis (137), ARG1 expression (138), and, together with STAT1 and STAT3, the release of ROS (8). Moreover, IFN-γ released by T cells is able to activate inflammatory monocytes (CD11b*IL-4Rα*) to secrete IFN-γ and IL-13, which in turn induces IL-4Rα expression and triggers in a autocrine loop the molecular processes that suppress antigen-activated CD8 T cells (36).

Interestingly, IL-4Rα genetic ablation affects MDSC-dependent immune suppression in vivo (36), and targeting this receptor with aptamers triggered MDSC
apoptosis and delayed tumor progression (37). S100A8/A9 is a heterodimer produced mainly by circulating neutrophils and monocytes that is secreted following intracellular changes in Ca^{2+} levels. It promotes MDSC trafficking by binding to N-glycan-tagged plasma membrane receptors, such as RAGE. Moreover, RAGE triggering induces activation of STAT3 and NF-κB and the expression of immune-suppressive genes (117). Accordingly, S100A9 blockade decreases MDSC levels and host immune dysfunctions in tumor-bearing mice (139). Another factor that drives myeloid commitment to suppressive MDSCs is HMGB1, a structural protein located in the nucleus that, when released by necrotic cells in the extracellular space, acts as a damage-associated molecular pattern and mediator of inflammation. HMGB1 is also released by leukocytes, especially monocytes, macrophages, and DCs, as an inflammatory cytokine acting on TLRs and RAGE. In cancer, tumor-derived HMGB1 regulates MDSC levels and immune-suppressive abilities through NF-κB activation; accordingly, HMGB1 promotes differentiation of bone marrow cells toward MDSCs, contributing to suppressing antigen-activated CD4^+ and CD8^+ T cells. Finally, HMGB1 promotes IL-10 secretion in MDSCs and downregulation of CD62L on T cells (140). Osteopontin is an extracellular matrix protein produced by many cell types, such as fibroblasts, osteoblasts, osteoclasts, and bone marrow cells. Besides its role in bone remodeling, osteopontin also takes part in the immune regulatory processes, especially during cancer. Indeed, it promotes myelopoiesis and host immune suppression, and its targeting reduces immune dysfunction and tumor growth in colorectal cancer models (141). In autochthonous breast cancer models, the monocyte subset represented the highest osteopontin producer in primary tumors and lung metastases; interestingly, osteopontin genetic ablation decreased metastatic burden and altered the subset composition among MDSCs, promoting preferential PMN-MDSC development while reducing MO-MDSC-dependent immune suppression through downregulation of ARG1, IL-6, and phospho-STAT3 (142).

**SIGNaling pathways regulating MDSC functions**

Many transcription factors contribute to physiologic, steady-state hematopoiesis, but only some of them are associated with altered commitment of myeloid cells to MDSCs and regulation of their immune regulatory properties. In particular, members of the STAT family and C/EBPβ were shown to play a central role in the polarization of myeloid cell functions, as well as in tumor progression and alteration of immune responses to cancer. STAT1, -3, -5, and -6 can transmit polarizing signals to the nucleus (143), and each component of the family can play a distinct role in macrophage polarization and MDSC functions. A fundamental component of several signal transduction pathways associated with STAT is the activation of the JAK family. Receptor oligomerization, mostly induced by cytokine binding, triggers JAK activation by either auto- or transphosphorylation. Subsequently, activated JAKs phosphorylate receptors on target tyrosine residues, generating docking sites for STATs through the STAT Src homology 2 domain. Activated JAKs recruit and phosphorylate STATs, which leads to their dimerization and nuclear translocation, where they modulate the expression of target genes. STAT1 is a transcription factor that, after activation with type 1 and 2 IFNs, IL-1β, and IL-6, dimerizes and translocates to the nucleus, where it triggers the expression of genes involved in immune-suppressive properties of MDSCs. The role of STAT1 in mediating host immune dysfunction has been elucidated in STAT1-deficient mice. In this model, MDSCs were not able to inhibit T-cell activation due to defective iNOS and ARG1 upregulation (144). Accordingly, blocking T-cell-derived IFN-γ secretion also abrogated MDSC-mediated suppression, mainly via the block of iNOS upregulation (36). From a translational point of view, STAT1 activation in TAMs correlates with cancer progression in patients affected by follicular lymphoma (145). GM-CSF, the main factor mediating myeloid proliferation and survival, acts through STAT5 triggering (146). Activation of STAT5 and STAT3 by GM-CSF and G-CSF, respectively, induces downregulation of IFN-related factor-8 (IRF-8). This step is critical for the aberrant myelopoiesis since IRF-8-deficient mice develop myeloid cells phenotypically and functionally related to tumor-induced MDSCs (147). Accordingly, inhibition of STAT3 pathways with sunitinib prevents systemic MDSC accumulation and restores normal T-cell activation in tumor-bearing mice. However, sunitinib’s efficacy was limited to the periphery and dependent on the cytokine context, since the presence of GM-CSF and activation of STAT5 at the tumor site were sufficient to confer sunitinib resistance and direct differentiation of sunitinib-sensitive PMN-MDSCs toward sunitinib-resistant MO-MDSCs (148, 149). STAT6 is a downstream transcription factor for IL-4R and IL-13R, whose role in MDSC activation is suggested by different studies, as detailed above. STAT6 deficiency prevents signaling through the type 2 IL-4R, thereby inducing
enhanced immunosurveillance against primary and metastatic tumors in mice (150). The few MDSCs accumulating in STAT6−/− mice after physical injury showed impaired suppressive activity due to the reduction in ARG1 expression (151). Thus, it appears that STAT1, STAT3, and STAT6 play an important role in MDSC activation and that these STATs mediate the immune-suppressive function of MDSCs. Among the family of transcription factors, a particular importance has been attributed to STAT3. Several pathways downstream of STAT3 might be involved in the regulation of MDSC expansion and function. Following STAT3 activation, hematopoietic precursors release the proinflammatory proteins S100A8/A9, which in turn inhibit DC differentiation and promote the accumulation of MDSCs and their migration to the tumor site (117). STAT3-dependent upregulation of S100A8/A9 depends on NOX2 expression (139), which leads to the production of superoxide, one of the mechanisms by which MDSCs promote T-cell anergy and tolerance. STAT3 activation can directly promote NOX2 activation by upregulating the transcription of the p47phox and gp91phox NOX2 subunits (152). STAT3 can also play an indirect role in MDSC differentiation since it controls the expression of molecules such as acute-phase proteins, which assist MDSC mobilization, accumulation, and survival (153). In a model of polymicrobial sepsis, IL-6-activated STAT3 signaling in hepatocytes, through gp130 ligandation, resulted in the expression of serum amyloid A and chemokine CXCL1, which cooperate in promoting the accumulation of MDSCs in the spleen (153). Finally, heat shock protein 72 (Hsp72), which is present in tumor-derived exosomes, induces suppressive activity of MDSCs via STAT3 activation. Hsp72 triggered STAT3 activation in MDSCs in a TLR2- and myeloid differentiation primary-response protein 88 (MyD88)-dependent manner through the autocrine production of IL-6 (154). Interestingly, from a therapeutic point of view, in vitro STAT3 inhibition abolishes the suppressive activity of MDSCs and the multitargeted tyrosine kinase inhibitor sunitinib blocked MDSC expansion in tumor-bearing mice by STAT3 signaling interference in myeloid cells (148). In summary, STAT3 activation seems to play a dominant role in MDSC biology, affecting the cells’ function by different pathways.

The C/EBPβ is a basic leucine zipper transcription factor important for the differentiation of the myeloid lineage. Three different C/EBPβ isoforms are translated (starting from 3′ in-frame AUG) from the same mRNA: a 38-kDa liver-activating protein (LAP1 or LAP*), a 36-kDa liver-activating protein (LAP2 or LAP), and a 28-kDa liver-inhibiting protein (LIP). The upregulation of the transcriptional factor C/EBPβ induces the activation of the immune-suppressive program in myeloid progenitor cells through the activation of STAT3 (155). C/EBPβ can activate different genes by binding to their promoters, including cmyc (8), IL6 (156), and the gene encoding the common signaling β-chain receptor that regulates the signal transduction for GM-CSF, IL-3, and IL-5 cytokines (157). Thus, C/EBPβ can be considered a master regulator of MDSC biology since bone marrow cells derived from C/EBPβ-deficient mice did not have the ability to differentiate in vitro into functional MDSCs. Moreover, CD11b+ cells isolated from the spleen and the tumor of C/EBPβ-deficient mice showed an impaired immunosuppressive ability (23). C/EBPβ homologous protein (Chop) is a transcription factor induced in particular contexts, such as during endoplasmic reticulum (ER) stress. When proteins in the ER are not properly folded and cannot proceed to the Golgi apparatus for further modifications, cells activate the unfolded protein responses, which can be initiated by three different sensors: PERK (protein kinase RNA-like ER kinase), IRE1 (inositol–requiring enzyme 1), and ATF6 (activating transcription factor 6). PERK action on eIF2α induces the expression of ATF4, which in turn upregulates Chop (158). This process leads to activation of STAT3, C/EBPβ, and IL-6 release. The role of Chop was further elucidated in cancer since TDFs, through ER stress establishment, induced Chop synthesis in MDSCs and the genetic ablation of the transcription factor was able to reduce MDSC trafficking to tumor in spite of T cells, decrease their immune-suppressive properties, and critically affect tumor growth (159). Accordingly, MDSC depletion partially restored tumor growth in Chop−/− mice, suggesting a role for this transcription factor in orchestrating the immune-suppressive properties of MDSCs (159).

In myeloid cells, the TLR family plays an important role in NF-κB activation, primarily through MyD88. This is consistent with MDSC accumulation and activation during microbial and viral infections, as well as in trauma and sepsis. NF-κB, acting downstream of MyD88, is required for accumulation of MDSCs in a model of polymicrobial sepsis (13). TLR4 was shown to be directly involved in MDSC function (160), and lipopolysaccharide, in combination with IFN-γ, could promote MDSC expansion, probably by inhibiting differentiation of DCs (161). Moreover, MyD88−/− MDSCs had a substantially reduced ability to suppress T-cell activity and release cytokines compared to the wild-type counterpart both in vitro and in vivo (162).
Enzyme activity and expression (lipases, kinases, and phosphatases) may modulate myelopoiesis, contributing to tumor-promoted, MDSC-mediated host immune suppression. Phospholipase C-γ2 and Src homology 2 domain-containing inositol 5′-phosphatase-1 (SHIP-1) are two enzymes that regulate homeostasis and function of MDSCs. These genes negatively regulate MDSC biology since their ablation promotes MDSC expansion and activation in tumor-bearing mice (163, 164).

Trp53, the best-known oncosuppressor gene, found mutated in at least 50% of human tumors, is able to indirectly regulate MDSC levels. Indeed, Trp53 ablation in mice bearing melanoma tumors promoted tumor growth through expansion of a stromal network rich in fibroblast reticular-like cells, which contributed to MDSC differentiation by releasing proinflammatory cytokines/chemokines and immunosuppressive mediators, including IL-6, IL-10, CCL3, CCL21, ARG1, and iNOS (165).

In addition to transcription factors, tumors can promote altered myelopoiesis through differential expression of microRNAs (miRs). These single-stranded, noncoding RNAs tune gene expression primarily through seed-matched sites located within the 3′ untranslated regions of the target mRNA. However, they can also bind at 5′ coding sequences of the cognate mRNA, even if with less efficacy. Different miRs are either up- or downregulated during MDSC differentiation. For example, miR-21 and miR-155 upregulation is mandatory for GM-CSF- and IL-6-mediated MDSC proliferation and differentiation (166). On the contrary, miR-142-3p affects MDSC biology through binding to mRNA coding for transcription factors and receptors involved in the activation of the immune-suppressive arsenal: through canonical binding on the 3′ untranslated region of its mRNA, miR-142-3p inhibited expression of gp130, the common subunit of the IL-6 family cytokine receptor, whereas noncanonical binding to the 5′ mRNA coding sequence altered C/EBPβ isoform expression. Accordingly, stable miR-142-3p expression in bone marrow progenitors was sufficient to reprogram TAM differentiation, improving the efficacy of cancer immunotherapy (167).

CONCLUSIONS AND FUTURE PERSPECTIVE

The strong bond linking angiogenesis with immune dysfunction and promotion of invasiveness and cancer-related morbidity is inducing clinicians to reconsider approaches targeting VEGF and blood vessel generation because of the consequent induced hypoxia. In this context, MDSC targeting could interrupt the angiogenic/immune regulatory switch, increasing the chances of therapeutic intervention, as described in a preclinical model of pancreatic adenocarcinoma (168). It is also clear that MDSCs’ spectrum of protumoral actions is much broader, and MDSC targeting can open new therapeutic opportunities to control tumor progression and block metastases. Some first-generation chemotherapeutic agents, such as 5-fluorouracil (169), gemcitabine (28, 170), and docetaxel (171), as well as the combination of these drugs, such as doxorubicin plus cyclophosphamide (172), are able to control MDSC accumulation. Numerous studies in tumor-bearing mice, as well as a few clinical trials using different MDSC-targeting approaches, have shown that MDSC reduction delays tumor initiation, progression, and distal dissemination and prolongs survival of tumor-bearing hosts (Table 2). These include selective antibodies and/or aptamers (37, 113, 116, 132, 168, 173–179) against MDSC markers; molecular antagonists of essential MDSC receptors and/or molecular pathways (129, 180); molecular inhibitors of MDSC functional mechanisms used by myeloid cells to block lymphocyte reactivity and proliferation (148, 181–191); and pharmacological agents able to force MDSCs to mature into proficient APCs that can stimulate tumor-specific T cells or repolarize TAMs in proinflammatory M1 macrophages (27, 192–199).

Actually, many factors limit the characterization and analysis of MDSC subsets and thus their targeting for improving cancer immune therapy. First, MO- and PMN-MDSCs can be distinguished from monocytes and granulocytes, respectively, in terms of impairment of functional immune response and molecular signatures more than phenotypic surface markers. For example, Ly6G is a marker shared within granulocytes, PMN-MDSCs, and TAMs, and the use of a Ly6G antibody may have a positive or negative effect on tumor growth depending on the targeted myeloid subset (47). Second, MDSCs are characterized by phenotypic plasticity in that they can modify their differentiation depending on the immune context (35, 51). Platforms and tools have been implemented for identifying new surface markers that allow better discrimination of MDSC subsets and can be used for in vivo targeting (200). However, a crucial step consists in adding to the canonical surface profiling of MDSCs a molecular signature of functional markers involved in MDSC-mediated immune regulation, such as IDO, iNOS, ARG1, and programmed cell death 1 ligand 1 (PD-L1). Integration of phenotypic and functional information could help in distinguishing between suppressive and nonsuppressive MDSCs without the need for laborious in vitro assays. Moreover, gene
expression, proteomic, and metabolomic profiles will increase knowledge of MDSC biology and offer potential therapeutics for interrupting crucial switches of MDSC accumulation or suppression. Finally, another critical step for comparison of data produced in different laboratories relies on sample handling. For example, cryopreservation may alter not only MDSC phenotype but also immune-suppressive characteristics (201); thus, establishment of standard operating procedures is mandatory to avoid biased analysis. Nonetheless, despite all these technical limitations, preclinical and clinical studies indicate a pivotal role for MDSCs in immune dysfunction and promotion of cancer progression and dissemination, and support how MDSC targeting could reverse host immune dormancy, thus improving the efficacy of passive and active immunotherapies for cancer.

### TABLE 2 A synopsis of drugs targeting MDSCs

<table>
<thead>
<tr>
<th>Drug</th>
<th>Type(s) of cancer</th>
<th>Effect(s) on myeloid cells</th>
<th>Reference(s)</th>
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</thead>
<tbody>
<tr>
<td>5-Fluorouracil</td>
<td>Thymoma</td>
<td>MDSC apoptosis</td>
<td>169</td>
</tr>
<tr>
<td>Gemcitabine</td>
<td>Sarcoma, lung, and breast cancer</td>
<td>MDSC apoptosis</td>
<td>28, 170</td>
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<td>Doxorubicin-cyclophosphamide</td>
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<td>MDSC apoptosis</td>
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<td>MDSC apoptosis</td>
<td>171</td>
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<td>Colon carcinoma</td>
<td>MDSC depletion</td>
<td>173</td>
</tr>
<tr>
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*Abbreviations: ATRA, all-trans retinoic acid; HNSCC, head and neck squamous cell carcinoma; HRG, histidine-rich glycoprotein; PROK2: prokineticin 2.*
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