

Myeloid-derived suppressor cells in the tumor microenvironment: expect the unexpected

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Our understanding of the role of myeloid-derived suppressor cells (MDSCs) in cancer is becoming increasingly complex. In addition to their eponymous role in suppressing immune responses, they directly support tumor growth, differentiation, and metastasis in a number of ways that are only now beginning to be appreciated. It is because of this increasingly complex role that these cells may become an important factor in the treatment of human cancer. In this Review, we discuss the most pertinent and controversial issues of MDSC biology and their role in promoting cancer progression and highlight how these cells may be used in the clinic, both as prognostic factors and as therapeutic targets.

Introduction

As immunotherapy has become a viable strategy for treating different types of cancer, it has become clear that it faces serious barriers that limit its clinical efficacy. The search for the factors blunting spontaneous or therapeutic immune responses in cancer has resulted in the discovery of myeloid-derived suppressor cells (MDSCs), a group of pathologically activated immature myeloid cells with potent immunosuppressive capacity. Although the term MDSC was formally introduced in 2007 (1), cells with similar characteristics were described over 35 years ago (2). In recent years, a vast amount of information has been generated detailing the biology and clinical significance of these cells in various pathologic conditions. In this Review, we will discuss recent progress as well as the main unresolved issues associated with these cells.

Definition of MDSC

MDSCs are broadly defined as myeloid cells and are distinct from terminally differentiated mature myeloid cells (i.e., macrophages, DCs, or neutrophils). MDSCs include a small group of myeloid progenitors as well as immature mononuclear cells, which are morphologically and phenotypically similar to monocytes (M-MDSCs), and immature polymorphonuclear (PMN) cells, which are morphologically and phenotypically similar to neutrophils (PMN-MDSCs) (Figure 1). In mice, MDSCs are broadly identified as CD11b⁺Gr1⁺ cells. The two major subsets of MDSCs can be differentiated by variable expression of the Gr-1 marker (Gr-1^{hi} cells are mostly PMN-MDSCs, and Gr-1^{lo} cells are mostly M-MDSCs) (3). The Gr-1 marker is not a singular molecule, but instead is a combination of the Ly6C and Ly6G markers, and these subsets can be more accurately identified based on Ly6C and Ly6G markers (M-MDSC as CD11b⁺Ly6C^{hi}Ly6G⁻ and PMN-MDSC as CD11b⁺Ly6C^{lo}Ly6G⁺) (4, 5).

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In humans, MDSCs are identified in the mononuclear fraction. PMN-MDSCs are CD14⁺CD11b⁺CD33⁺CD15⁺ or CD66b⁺ cells and M-MDSCs are CD14⁺HLA-DR^{-/lo} cells. Populations of Lin⁻HLA-DR⁻CD33⁺ MDSCs represent a mixed group of cells enriched for myeloid progenitors. Accurate characterization of MDSCs in cancer patients requires the analysis of all three groups of cells. Several other markers have been suggested to characterize MDSCs further; however, none has emerged as a clear MDSC-specific marker. This subject has been recently reviewed (6, 7).

A topic of some controversy is the distinction of PMN-MDSCs from neutrophils. In some reports, neutrophils with immunosuppressive and protumor functions are called N2, as opposed to antitumor N1, neutrophils (8, 9). It is rather difficult to envision that very short-lived, terminally differentiated PMN cells could be effectively polarized in tumor tissues. It is more likely that N1 cells represent activated bona fide PMN cells, whereas N2 cells are in fact PMN-MDSCs. However, this question cannot be resolved without identification of markers that allow for the delineation of PMN cells and PMN-MDSCs. In mice, several parameters that could distinguish PMN-MDSCs from PMN cells have been suggested (10); however, none is sufficient, and more effort is needed to better distinguish these cells.

In humans, separation of neutrophils from PMN-MDSCs is done via density gradient, with PMN-MDSCs staying in the PBMC fraction while neutrophils are pelleted out over Ficoll (11). In healthy individuals, PMN-MDSCs are practically undetectable, and hence, few to no cells with this phenotype remain in the PBMC fraction. Although very useful in providing scientific information, the use of cell density as a clinical biomarker is limited by the fact that the density of PMN cells depends on the conditions of blood collection and storage and is prone to fluctuations that may affect the interpretation of the results (12). Development of definitive markers that will allow for one-step identification of MDSCs is necessary. A similar challenge exists for M-MDSCs; however, it is less critical in humans due to the fact that monocytes can be distinguished from M-MDSCs by their phenotype (CD14⁺HLA-DR^{hi} vs. CD14⁺HLA-DR^{-/lo}, respectively).

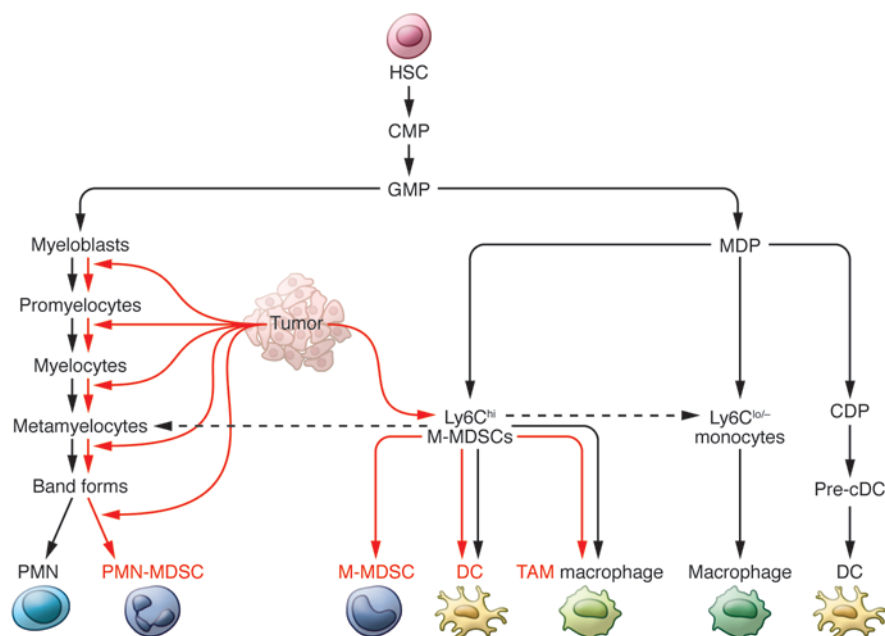


Figure 1. MDSC ontogeny. Differentiation of neutrophils and mononuclear cells in naive mice is shown by black arrows. Hematopoietic stem cells (HSC) differentiate into common myeloid progenitors (CMP), then into granulocyte-macrophage progenitors (GMP), which give rise to mature neutrophils via sequential steps of differentiation involving myeloblasts, promyelocytes, myelocytes, metamyelocytes, and band forms. Differentiation of macrophages and DCs involves macrophage/DC progenitors (MDP), DC progenitors (CDP), and pre-cDCs as well as several types of monocytes. The most prominent are Ly6C⁺ inflammatory monocytes and Ly6C^{int} patrolling monocytes. Differentiation of myeloid cells in tumor-bearing mice is shown by red arrows. Tumor-derived signals affect all steps of granulocytic and monocytic cell differentiation, causing expansion of pathologically activated PMN-MDSCs and Ly6C⁺ M-MDSCs. During tumorigenesis, these cells become more prevalent in bone marrow and spleen than in their nonsuppressive counterparts. M-MDSCs acquire the ability to differentiate to PMN-MDSCs and, at the tumor site, differentiate to TAMs and DCs. The dashed line represents pathways that are not yet firmly established.

Immunosuppressive functions of MDSCs

As evident from their name, the initial defining feature of MDSCs is their ability to suppress immune cell function. Main factors implicated in MDSC-mediated immune suppression include expression of arginase (ARG1), inducible NOS (iNOS) (3–5), TGF- β (13–15), IL-10 (16), and COX2 (17–19), sequestration of cysteine (20), decreased expression of L-selectin by T cells (21), and induction of Tregs (22), among others. In recent years, it has become clear that M-MDSCs and PMN-MDSCs utilize different mechanisms of immune suppression. M-MDSCs suppress both antigen-specific and nonspecific T cell responses and, on a per cell basis, are consistently shown to have stronger suppressive activity than PMN-MDSCs. M-MDSCs primarily utilize mechanisms associated with production of NO and cytokines (reviewed in ref. 23). In contrast, PMN-MDSCs are capable of suppressing immune responses in an antigen-specific manner only. ROS production is essential for this function. Reaction of NO with superoxide generates peroxynitrite (PNT), which directly inhibits T cells by nitrating T cell receptors (TCRs) and reducing their responsiveness to cognate antigen-MHC complexes (24). Additionally, nitration reduces binding of antigenic peptides to MHC molecules on tumor cells (25) and blocks T cell migration by nitrating T cell-specific chemokines (26).

The relatively weak suppressive activity of PMN-MDSCs has raised the question of whether these cells play a role in immune

suppression. A recent study made the claim that PMN-MDSCs were not involved (27), based on the fact that myeloid leukemia cell-1 (MCL-1) deletion, which results in deletion of granulocytes, including PMN-MDSCs, did not affect tumor incidence in mice. However, deletion of PMN-MDSCs in these mice was associated with a substantial increase in M-MDSCs, which could effectively compensate for any loss of PMN-MDSCs. Additionally, the impact of M-MDSC deletion on tumor incidence was not explored, nor were immune correlates investigated. In contrast to this report, a number of studies have demonstrated improvement of immune responses and antitumor immunity following depletion of PMN-MDSCs (but not M-MDSCs) with a Ly6G-specific antibody (28–30). It is also important to note that, although M-MDSCs are more suppressive on a per cell basis, PMN-MDSCs are generally much more prevalent in cancer (4). Thus, the role of PMN-MDSCs in tumor-associated immune suppression requires further elucidation.

The large number of different immunosuppressive mechanisms described for MDSCs does not mean that these mechanisms are simultaneously operational. The prevalence of a particular immunosuppressive mechanism depends on the type of MDSCs that expanded in response to disease as well as on the stage of the disease

and the site where the suppression is occurring. It is likely that at any given time there is a dominant suppressive mechanism used by MDSCs and that this mechanism could change throughout the progression of the disease. This suggests that evaluation of the role of MDSCs in cancer probably cannot be based only on one or two factors produced by these cells, but should include analysis of their functional activity. It also suggests that, for therapeutic purposes, targeting of one mechanism may not be effective unless this mechanism has been determined to be dominant in the type of cancer being treated.

MDSCs in the regulation of tumor development and progression

The ability of MDSCs to support tumor growth and metastases can be broadly divided into four functions: (a) protection of tumor cells from immune-mediated killing, (b) remodeling of the tumor microenvironment, (c) establishment of a pre-metastatic niche, and (d) interaction with tumor cells to induce “stemness” and facilitate the epithelial-to-mesenchymal transition (EMT) (Figure 2).

The mechanism of immune privilege recently was shown to be important in the increased permissiveness for metastasis seen in pregnancy. Specifically, decreased NK cell functionality in pregnant mice was dependent on MDSC accumulation. This accumu-

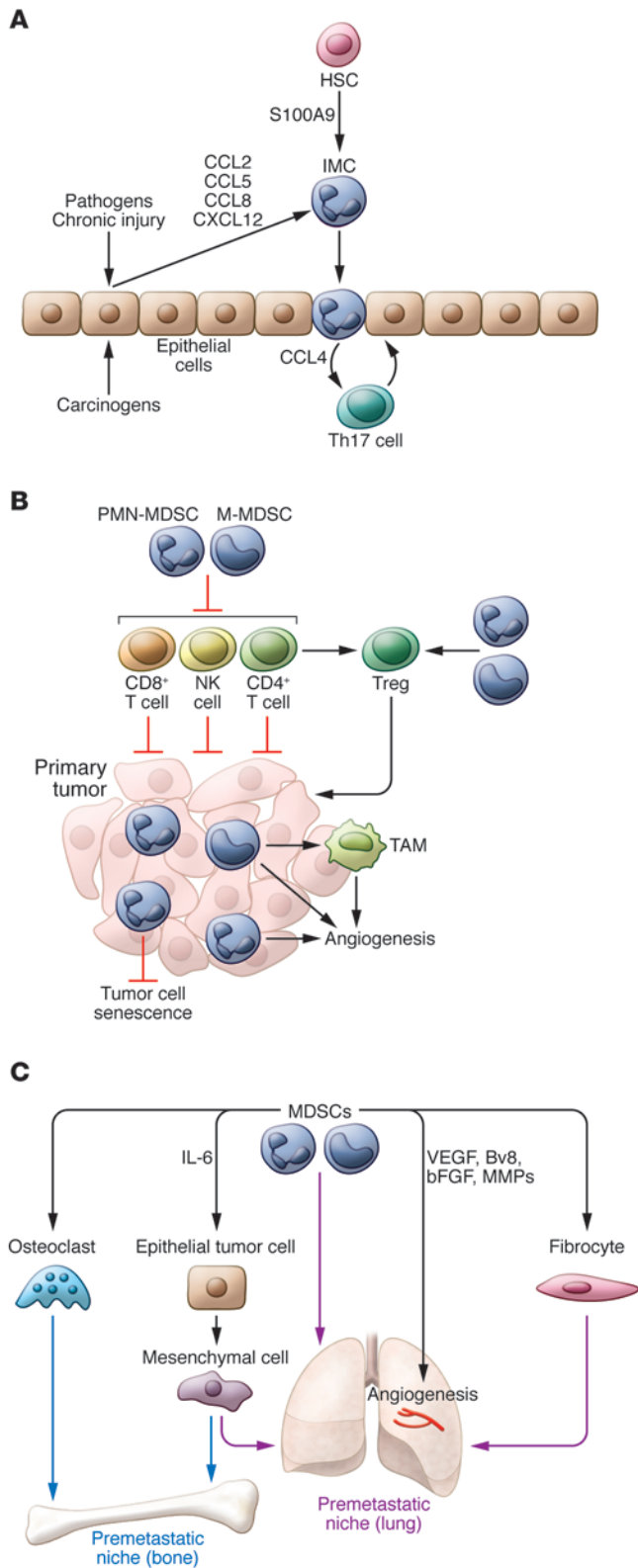


Figure 2. Potential role of immature myeloid cells and MDSCs in the regulation of tumor development and progression. (A) Immature myeloid cells (IMCs) with the typical phenotype of MDSCs are produced in response to inflammatory stimuli. However, these cells often lack immunosuppressive activity. They contribute to tumorigenesis by recruiting proinflammatory CD4⁺ T cells that promote epithelial cell proliferation. (B) Tumor development is associated with the expansion of cells with acquired immunosuppressive activity (MDSCs). These cells also promote tumor cell invasion and angiogenesis and neutralize tumor cell senescence. (C) In metastatic tumors, MDSCs, in addition to promoting tumor cell invasion and angiogenesis, can support EMT and differentiation of osteoclasts supporting bone resorption.

MDSCs are capable of supporting tumor growth through remodeling of the tumor microenvironment (33, 34). They have been shown to produce VEGF, bFGF, VEGF analogue Bv8, and MMP9, all essential mediators of neoangiogenesis and tissue invasion at the tumor site (35–37). Expression of these mediators has been linked to MDSC-mediated metastasis and is independent of their immunosuppressive capacity (38).

The concept of the premetastatic niche refers to the situation wherein bone marrow–derived cells prepare distant organs for the arrival of tumor cells (39, 40). Mouse models have shown that MDSCs appear in the lungs as early as two weeks prior to the appearance of metastases. The presence of these cells correlated with decreased immune function in the lungs. Importantly, myeloid-specific deletion of *Mmp9* essentially eliminated metastasis, suggesting that the role of MDSCs in tissue remodeling, and not just their ability to suppress immune responses, was indispensable for their metastasis-promoting function (41). Tumor-derived hypoxia-induced lysyl oxidase, VEGF, S100A8/A9, IL-6, and IL-10 (via activation of the S1PR1/STAT3 axis in the lungs) have all been implicated in the recruitment and activation of MDSCs in the lungs and the establishment of the premetastatic niche (42–44).

The contribution of myeloid cells to EMT has only recently begun to emerge. Coculture of MDSCs with tumor cells induced a stem-like phenotype in tumor cells and increased their ability to metastasize in vivo (45). In the ret model of spontaneous mouse melanoma, CD11b⁺Gr1⁺ cells were shown to infiltrate primary tumors in a mechanism that was dependent on CXCL5. Depletion of these cells resulted in smaller, less nodal tumors and a drastic reduction in metastasis. In vitro coculture experiments showed that these cells induced a stem-like phenotype in tumor cells and that inhibition of the TGF-β, EGF, and/or HGF pathways could reverse this effect (46). Studies using the 4T1 model of transplantable breast cancer implicated MDSC-derived IL-6 in increased tumor cell stemness (47). Because IL-6 and HGF have been shown to upregulate each other’s receptors (48) and IL-6R/EGFR cross-talk has previously been implicated in EMT (49), it is possible that MDSC induction of EMT is mediated primarily through IL-6; however, more studies are needed to confirm this assumption.

Bona fide MDSCs are not produced in healthy individuals. Even in the early stages of cancer, cells with an MDSC-like phenotype may not have immunosuppressive activity (50). Recent evidence has linked accumulation of immature myeloid cells with an MDSC-like phenotype during chronic inflammation with the early stages of tumor development. Exposure of mice to cigarette smoke caused accumulation of these cells in lung and spleen

lation was shown to be an essential part of pregnancy-associated metastasis, as no difference in the metastatic potential of NK cell-resistant tumor cell lines was observed during pregnancy (31). Additionally, an increase in the immunosuppressive potential of MDSCs was also shown to correlate with lymph node metastasis in breast cancer patients (32).

(34); however, these cells became immunosuppressive MDSCs only after the development of lung cancer. Nevertheless, their depletion increased survival (34). In a model of skin carcinogenesis, accumulation of immature myeloid cells without suppressive function in the skin of mice strongly promoted tumor formation. That effect was caused by CCL4-mediated recruitment of IL-17-producing CD4⁺ T cells (51). CD11b⁺Gr1⁺ cells were also recently shown to oppose cellular senescence and promote a more aggressive disease in a mouse model of spontaneous prostate cancer by antagonizing IL-1 α -mediated senescence (52). Thus, cells with an MDSC-like phenotype play a significant role in tumor development and progression via mechanisms that are not necessarily related to their ability to suppress tumor-specific immune responses (Figure 2).

Mechanisms of accumulation and activation of MDSCs in cancer

It is likely that, in addition to the common mechanisms regulating both expansion of immature myeloid cells and acquisition of suppressive function, there are distinct mechanisms that mediate these two effects. We previously suggested that accumulation of bona fide MDSCs requires two sets of signals (53). The first of these signals supports the expansion of immature myeloid cells and is mediated largely by granulocyte-macrophage CSF (GM-CSF), macrophage CSF (M-CSF), granulocyte CSF (G-CSF), and other growth factors produced by tumor cells and tumor stroma (54). Although expression of these factors is sufficient to expand cells with an MDSC-like phenotype, they do not imbue them with the suppressive phenotype that defines this cell type (55), which requires a second signal. Many different proinflammatory cytokines (IL-6, IL-1 β , TNF- α , IL-13/4, IFN- γ , etc.) have been suggested as candidates; however, the effect of these cytokines has been observed mostly during extended culture of progenitor cells in vitro. Elimination through genetic knockout or in vivo blockade of one or several of these mediators has shown limited effect on the ability of MDSCs to suppress T cell responses (23). Additionally, models of chronic inflammation caused by smoking or skin irritation showed that these conditions were not sufficient to generate bona fide MDSCs (34, 51). Thus, the mechanism controlling the conversion of expanded immature myeloid cells to MDSCs is complex and requires further investigation.

Members of the STAT transcription factor family (STAT3, STAT5, and STAT6) have long been considered to be critical factors in the regulation of MDSC expansion and activity (56–59). In recent years, more information has emerged regarding the mechanism of MDSC regulation by STAT3. STAT3 can regulate arginase production by binding directly to the *ARG1* promoter (60). The effect of STAT3 on MDSC accumulation and suppressive function was found to be mediated by C/EBP β (55) and IFN regulatory factor-8 (IRF8) (61, 62). Pharmacological inhibition of STAT3 in MDSCs favored their conversion toward mature DCs, in part through inhibition of PKC β II (63).

HIF-1 α has emerged as another important regulator of MDSC function and differentiation. At the tumor site, HIF-1 α facilitates the differentiation of MDSCs to tumor-associated macrophages (TAMs) and promotes the suppressive activity of MDSCs via upregulation of iNOS and arginase and downregulation of compo-

nents of the NADPH oxidase complex (64). A recent report demonstrated that HIF-1 α bound to the promoter of programmed cell death ligand-1 (PD-L1) and upregulated its expression on myeloid cells (65). Further studies will be necessary to determine whether this mechanism represents a function of MDSCs or is indicative of their switch to TAMs. The canonical Wnt pathway has been shown to antagonize MDSC differentiation and support the differentiation of mature DCs (66, 67). A recent pathway analysis comparing MDSCs, both in the periphery and at the tumor site, with normal immature myeloid cells has provided a comprehensive look at the number of factors enriched in MDSCs in different physiological settings (68). It represents the first step toward a comprehensive understanding of how MDSC function is controlled.

Recently, ER stress was implicated in MDSC-mediated suppression. The ER stress response is an evolutionarily conserved process designed to protect cells from stress signals, including starvation, viral infections, changes in oxygen tension, etc. Transmission of ER stress from the tumor cells to macrophages and DCs has been shown to skew them toward promotion rather than suppression of tumor growth (69, 70). A recent study demonstrated that, compared with their counterparts from healthy animals, MDSCs from tumor-bearing mice and cancer patients showed increases in DNA damage-inducible transcript 3 (CHOP), spliced X-box binding protein 1 (XBP1), and Bip (71). These molecules are downstream effectors of the major pathways of the ER stress response (72, 73). *Chop* deletion in mice resulted in a decrease in tumor growth. Tumor MDSCs from *Chop* KO mice exhibited greater survival than wild-type MDSCs and were shifted toward stimulation, rather than suppression, of T cell responses. Decreased IL-6 production by MDSCs was observed and exogenous IL-6 could “rescue” the function of *Chop* KO MDSCs (74). However, the importance of CHOP in the immunosuppressive phenotype of MDSCs requires further characterization, since there is a lack of consensus among different groups on its role in tumor progression (75). Of particular interest is the differentiation of the role of CHOP in immunosuppression at the tumor site versus in the periphery. A recent report showed that ER stress inducer thapsigargin increased the suppressive capacity of MDSCs at the tumor site, but not in the periphery (76); however, this study evaluated the total population of CD11b⁺ cells, allowing for the interpretation that the effect of ER stress may not be MDSC specific.

Recent additions to the list of inflammatory mediators controlling MDSC function include high mobility group box 1 (HMGB1) and PPAR γ . HMGB1 promotes the development of MDSCs and contributes to their ability to suppress antigen-driven activation of T cells (77). Overexpression of PPAR γ resulted in expansion of PMN-MDSCs with immunosuppressive activity. This was associated with activation of STAT3, NF- κ B, Erk1/2, and p38 (78).

The fate of MDSCs in the tumor-bearing host

It is known that cells with an M-MDSC phenotype can differentiate into macrophages and DCs (79–81). However, the role of this differentiation in tumor progression has only recently been expounded upon. At the tumor site, adoptively transferred MDSCs primarily differentiated into immunosuppressive TAMs through

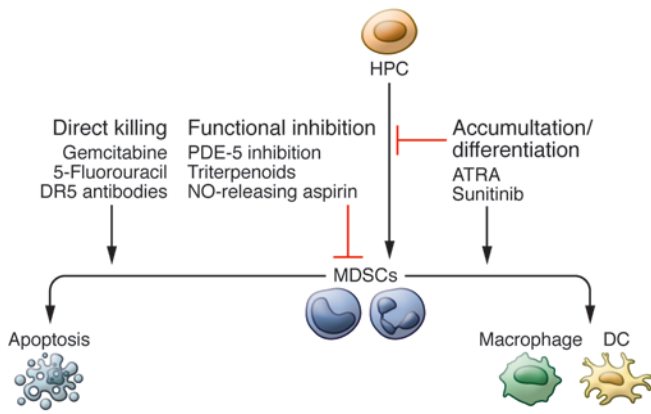


Figure 3. Therapeutic strategies targeting MDSCs. MDSCs can be targeted in one of three broadly defined ways. First, they can be directly killed. Low-dose gemcitabine, 5-fluorouracil, and TRAIL receptor ligation have shown efficacy in doing so, both clinically and preclinically. The second category of therapeutic is functional inhibition of MDSC-suppressive machinery. PDE-5 inhibitors, such as tadalafil, as well as NO-releasing aspirin and synthetic triterpenoids act effectively in this manner, in part by reducing the expression of ROS, reactive nitrogen species, and arginase, all central to the ability of MDSCs to inhibit immune responses. Finally, myelopoiesis can be skewed such that MDSC accumulation is inhibited and/or MDSCs are forcefully differentiated into more terminally differentiated, immunostimulatory myeloid cells such as DCs and macrophages. Sunitinib and ATRA have shown efficacy in these two functions, respectively.

a mechanism that was shown to be dependent on HIF-1 α . In the periphery, MDSCs transitioned equally to macrophages and DCs (64). However, a recent study suggested that MDSCs are capable of differentiating into CD11c^{lo}CD11b^{hi} regulatory DCs both at the tumor site and in the periphery (82). Discrepancies between this and the previous study may be due to the use of an experimental lung metastatic model in the latter study, which reduced the level of hypoxia at the tumor site and likely reduced HIF-1 α expression. Another study showed that MDSCs from mice bearing bone metastases were capable of differentiating into osteoclasts in an NO-dependent manner (83). These osteoclasts are capable of bone resorption and are involved in the bone remodeling necessary for the colonization of bone metastases. In the lung, MDSCs have been shown to differentiate into fibrocytes and myofibroblasts, which support the establishment of lung metastases. This transition was dependent on binding of the transcription factor Kruppel-like factor 4 (KLF4) to the fibroblast-specific protein 1 (*Fsp1*) promoter (84, 85). In fact, some studies have shown that fibrocytes represent a unique subset of MDSCs that differentiate from M-MDSCs in the presence of IL-4. These cells can suppress immune responses through expression of indoleamine oxidase (IDO) and expansion of Tregs (86, 87).

Accumulation of MDSCs in tumor-bearing hosts could indicate that these cells are protected from apoptosis. Several protective mechanisms have been suggested. TNF signaling in MDSCs has been shown to be important in maintaining their survival. Upregulation of the caspase-8 inhibitor c-FLIP was a prominent feature of TNF treatment in MDSCs, and inhibition of caspase-8 rescued decreased accumulation of MDSCs in *Tnf* KO mice (38). However, a recent study suggested that c-FLIP might not necessarily affect survival of MDSCs in tumor-bearing mice, but instead

could affect MDSC development (27). No effect on the development of PMN-MDSCs in the absence of c-FLIP was shown; however, one could envision a scenario in which c-FLIP-deficient PMN-MDSCs may be phenotypically quite different from their wild-type counterparts, especially given the ability of M-MDSCs to serve as a source of PMN-MDSCs in tumor-bearing mice (88). Decreases in FasL-mediated killing have been related to increases in Bcl-xL in MDSCs relative to immature myeloid cells from tumor-free animals (89, 90). However, a recent study demonstrated that, in the periphery, MDSCs actually had a much shorter life span than their control counterparts, PMN cells and monocytes. This effect was mediated by changes in the expression of TRAIL receptors. Interestingly, MDSC cell death in the periphery directly contributed to enhanced expansion of MDSCs in bone marrow (71). These results may have substantial implications for the therapeutic targeting of MDSCs. An important distinction between this study and previous work was that it examined the survival of MDSCs directly in vivo. In vitro assays of MDSCs after isolation from tissues have inherent difficulties relating to the sensitivity of these cells to mechanical isolation, which may select for more apoptosis-resistant cells. More detailed studies are needed to establish the life span of MDSCs in the tumor-bearing host.

MDSCs as biomarker of tumor progression and responsiveness to therapy

Ample evidence supports a close association between MDSC accumulation and clinical outcome in cancer patients (91). Most solid tumors have expansion of PMN-MDSCs, with some exceptions, including melanoma, multiple myeloma, and prostate cancer. In these cancers, the M-MDSC population is more prevalent. Such preferential expansion is likely caused by variability in the factors that predominate among the different cancer types. This issue and the role MDSCs play in cancer prognosis have recently been reviewed for a number of tumor types (92). Although identification of MDSCs in the peripheral blood of cancer patients is now well established, the situation with the detection of these cells in tissues using immunohistochemistry is different. This is due to the fact that multicolor analysis of paraffin-embedded tissues, suitable for biomarker research, is very difficult. Recently, we found that double-staining of tissues with CD33 and S100A9 antibodies detected cells that phenotypically reflect the population of MDSCs and accumulate in melanoma and colon cancer patients as well as in the patients with some inflammatory conditions (51). More studies are needed to validate the use of this approach for detection of MDSCs.

In recent years, MDSCs have been implicated in resistance to anticancer therapies. Resistance to the tyrosine kinase inhibitor sunitinib was associated with increased GM-CSF levels and persistent high levels of MDSCs in the peripheral blood of patients with renal cell carcinoma. This effect correlated with elevated MMP9, MMP8, and IL-8 levels (93). Clinical response to a number of chemotherapeutics is negatively correlated with MDSC levels. In non-small cell lung cancer patients, circulating CD14⁺S100A9⁺ cells predicted a poorer response to cisplatin and other chemotherapeutics (94, 95). An earlier study showed similar findings with a population of PMN-MDSCs (CD11b⁺CD14⁺CD15⁺CD33⁺) (96). The cells in these studies were capable of suppressing CD8⁺ T cells. MDSCs

have also been associated with an inhibitory effect of chemotherapy on the immune system. Cyclophosphamide has been shown to increase MDSC numbers in both preclinical and clinical settings (97), and this was accompanied by reduced CD8⁺ T cell and NK cell activity (98) and reduced antibody responses (99).

Recent studies suggest that MDSCs are an important factor regulating the response to immune therapies. The efficacy of ipilimumab, a monoclonal antibody against CTLA-4, was inversely correlated with the presence of M-MDSCs in melanoma patients prior to treatment (100, 101). Among patients receiving adoptive transfer of cytokine-induced killer cells, those with low MDSC numbers were significantly more likely to survive in the long term (102). Similar results were found in patients treated with high-dose IL-2 (103). In a cancer-preventative setting, the presence of MDSCs in patients with advanced colonic adenomas was inversely correlated with responsiveness to a vaccination against the tumor antigen mucin 1 (MUC1) (104). These studies highlight MDSCs as a valuable biomarker and suggest that targeting MDSCs may be an attractive strategy for improving the efficacy of concurrent therapies.

Strategies to therapeutically target MDSCs

The fact that MDSCs play an important role in the regulation of tumor growth has stimulated the search for a way to therapeutically target these cells. Several strategies are currently being tested in clinic (Figure 3). These strategies can broadly be placed into three categories: (a) elimination of MDSCs, (b) deactivation of MDSCs, and (c) skewing of myelopoiesis away from the accumulation of MDSCs. An extensive review of MDSC targeting has recently been published (105). Here, we will give a brief overview of this exciting field and highlight some new studies.

MDSCs can be eliminated with relatively low doses of chemotherapy. In murine models, gemcitabine has been shown to deplete MDSCs without deleterious effects on T cells, resulting in decreased tumor growth and prolonged survival (106, 107) as well as enhanced responsiveness to immune therapy (108). Cisplatin and 5-fluorouracil have been shown to preferentially deplete MDSCs compared with other immune cells (109, 110), which resulted in increased CD8⁺ T cell responses (111). Several trials that examine the ability of low-dose chemotherapy to augment vaccine-induced immune responses are now underway. Recently, it was shown that selective upregulation of TRAIL receptor DR5 on mouse MDSCs and downregulation of decoy receptors DCR1 and DCR2 in human MDSCs make TRAIL receptor targeting a viable and specific method of MDSC depletion. This has been shown to inhibit tumor growth in mice in a CD8⁺ T cell-dependent manner (71). Newly engineered peptibodies consisting of S100A9-derived peptides conjugated to antibody Fc fragments have shown promise in eliminating MDSCs in mouse models (112); however, the specificity of this targeting and its applicability to human cancer needs to be established.

MDSCs can be functionally inactivated by targeting their suppressive machinery. ROS and NO are essential components of MDSC-suppressive machinery. Nf-E2-related factor 2 (NRF2)

is a transcription factor that plays an important role in protecting cells against free radical damage. NRF2 modulates the expression of several antioxidant enzymes that scavenge ROS and NO, and upregulation of NRF2 by a synthetic triterpenoid has been shown to reduce the production of ROS by MDSCs and attenuate their suppressive activity *ex vivo* (113). Both arginase and iNOS expression have been shown to be downregulated in response to phosphodiesterase-5 (PDE-5) inhibition (114). A recent clinical report indicated that head and neck cancer patients treated with the PDE-5 inhibitor tadalafil had fewer circulating MDSCs, lower iNOS and arginase expression in these cells, and a greater number of spontaneously generated tumor-specific T cells (115). This study was published alongside another showing that tadalafil treatment decreased Tregs and increased tumor antigen-specific CD8⁺ T cells in addition to reducing MDSC numbers (116). Finally, inhibition of COX-2 decreased the production of immunosuppressive prostaglandin E2, and nitroaspirin has been shown to downregulate NO production (117, 118).

In addition to targeting suppressive functions, myelopoiesis can be diverted away from generating MDSCs for therapeutic benefit. All-trans-retinoic acid (ATRA) has been shown to differentiate MDSCs into DCs and improve their immunostimulatory capacity (119, 120). Treatment of renal cell carcinoma patients with ATRA substantially decreased the presence of MDSCs in peripheral blood (121). A recent study demonstrated that in lung cancer patients, p53 vaccine-generated immune responses were improved if patients received a short course of ATRA (122). STAT3 targeting can affect MDSC expansion and differentiation and lead to differentiation of MDSCs into immunogenic DCs (81, 123). Additionally, sunitinib, which inhibits STAT3 as well as VEGF, c-kit, and M-CSF signaling, has been shown to reduce MDSC levels in renal cell carcinoma patients and may provide an avenue for improving antitumor immunity in these patients (124).

Summary

The field of MDSC research is experiencing a renaissance. As more information regarding the clinical significance and the intriguing biology of these cells has become available, the critical role they play in the regulation of immune responses and tumor progression has become more apparent. These cells represent a unique model for studying pathological activation of myeloid cells and may serve as both a powerful biomarker for improved patient selection and as a therapeutic target that could potentially enhance not only anti-cancer immune therapeutics, but also most anticancer therapies.

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