Molecular Pathways: Targeting Tumor-Infiltrating Myeloid-Derived Suppressor Cells for Cancer Therapy

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Running Title: Tumor-Promoting Role of MDSCs

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Abstract

Tumor-infiltrating myeloid-derived suppressor cells (MDSCs) are a heterogeneous and immunosuppressive cells subset that blocks the proliferation and the activity of both T and NK cells and promote tumor vasculogenesis and progression. Recent evidence demonstrates that the recruitment of MDSCs in tumors also blocks senescence induced by chemotherapy promoting chemoresistance. Hence the need of novel therapeutic approaches that can efficiently target MDSCs recruitment and function in cancer. Among them, novel combinatorial treatments of chemotherapy and immunotherapy or treatments that induce depletion of MDSCs in peripheral sites should be taken in consideration.
Background

An increasing amount of evidences from mouse and human unveiled the significance of the tumor immune microenvironment in tumor growth and progression. Consequently, activating the immune system has emerged as a promising way to treat cancer and numerous new immune-based treatments are currently under investigation for the treatment of cancer. Recent successful phase 3 clinical trials of therapeutic cancer vaccines include the FDA-approved Sipuleucel-T prostate cancer vaccine, melanoma peptide vaccines and personalized lymphoma vaccines (1). Furthermore two immune checkpoint inhibitors (anti-PD1 and anti-CTLA4) have reignited enthusiasm for the development of immunotherapy drugs for cancer, having demonstrated high response rates and prolonged overall survival in cancer patients (2). However, tumor-induced immune suppression limits the potency of several of standard and novel therapeutic interventions. Tumor-infiltrating MDSCs are the most common mediator of immune suppression in tumors. MDSCs are a heterogeneous cell population co-expressing Gr-1 and CD11b myeloid lineage differentiation markers in mouse and either or both of the common myeloid markers CD33 or CD11b in cancer patients (3,4). MDSCs cells represent a heterogeneous population that comprises both cells of granulocytic (G-MDSCs) and monocytic (M-MDSCs) origin. Monocytic MDSCs are characterized by a HLA-DR⁻CD11b⁺CD33⁻CD14⁺ phenotype in humans (CD11b⁺Ly6G⁻/Ly6C⁺ in mice), whereas human granulocytic MDSCs are defined by a HLA-DR⁻CD11b⁺CD33⁺CD15⁺ phenotype (CD11b⁺Ly6G⁺/Ly6Clow in mice). Of note, MDSC with the phenotype of CD33+HLA-DR−/low that are lineage negative (CD15−, CD14−) have also been well described in cancer patients (3). Importantly, both the monocytic and the granulocytic subsets display an equal immune suppressive activity in tumors. Different studies have demonstrated that tumor formation promotes
the migration of MDSCs from the bone marrow to the tumors. Mechanistically, this is associated with tumor secretion of cytokines and chemokines that induce myeloid cell trafficking, proliferation and infiltration to the tumor bed. Indeed, the resection of solid tumors has been shown to decrease MDSCs frequency in the peripheral blood and to reverse T cell suppression, indicating that tumors directly impact this inflammatory cell population (5,6). In the bone marrow, MDSCs can be generated in response to cancer derived factors such as granulocyte colony stimulating factor (G-CSF), IL6, granulocyte monocyte colony stimulating factor (GM-CSF), IL1β, prostaglandin E2 (PGE2), tumor necrosis factor α (TNFα) and vascular endothelial growth factor (VEGF) and are then recruited to the tumor site by mean of chemokines belonging to the CCL and CXCL family (7).

Once recruited to tumors, MDSCs exert a significant effect on tumor progression, as the mediate immunosuppression of anti-tumor effectors cells. Through the release of arginase, reactive oxygen species and nitric oxide and secretion of immune-suppressive cytokines, MDSCs suppress tumor immunosurveillance mediated by T and NK cells (8). Indeed, antibody-mediated depletion of MDSCs was shown to restore T cell frequency and function in vivo (9). In addition, MDSCs have been shown to drive the expansion of CD4⁺CD25⁺FoxP3⁺ regulatory T cells (Tregs) localized in the tumor site and to inhibit antigen presentation from tumor-infiltrating dendritic cells, thus indirectly enhancing the immuno-tolerance towards the tumor (10).

Interestingly, MDSCs may also influence other key events in tumorigenesis, such as angiogenesis and metastasis formation. When MDSCs isolated from murine tumors are co-injected with mouse cancer cells into mice, the growth rate and blood vessel density of tumors is significantly higher than in controls (11,12). Furthermore,
tumor angiogenesis is significantly lower in tumor-bearing mice treated with neutralizing anti-BV8, which reduce the numbers of infiltrating MDSC (13). In addition, MDSCs have been shown to be directly implicated in the promotion of tumor metastases by participating in the formation of premetastatic niches (14). Finally a recent paper demonstrates that MDSCs are implicated in senescence evasion in two different mouse models of oncogene-induced senescence. Tumor-infiltrating MDSCs promote also evasion of senescence induced by chemotherapy thereby conferring treatment resistance. Intriguingly senescence evasion by MDSCs is promoted through the secretion in the tumor microenvironment of IL1RA which is capable to block IL1 signalling, needed for the execution of senescence in Pten null K-RasG12v tumors (15).

**Signaling Pathways**

In recent years data have emerged that link several signaling pathways with MDSC trafficking, expansion and suppressive activity (16). Signal transducer and activator of transcription 3 (Stat3) plays a crucial role in both MDSCs suppressive activity and proliferation. MDSCs isolated from tumor-bearing mice showed increased levels of JAK2 and STAT3 activation when compared with myeloid progenitors from naive mice (17). Notably, the exposure of MDSCs to conditioned media collected from tumor cells, resulted in STAT3 activation and cell expansion in vitro (18). Additionally, in vivo inhibition of Stat3, by means of the multi-targeted tyrosine kinase inhibitor Sunitinib and the JAK2/STAT3 inhibitor Cucurbitacin B was shown to block MDSC expansion in tumor-bearing mice (19, 20). Interestingly, new insights into the downstream of the STAT3 signalling pathway showed that STAT3 controls the modulation of the CCAAT-enhancer-binding protein beta (C/EBPβ), a transcription factor known to drive MDSCs differentiation from myeloid progenitors.
Recent findings also provided evidences that the immunoregulatory activity of tumor-induced MDSCs relies on the C/EBPβ transcription factor and adoptive transfer of tumor antigen-specific CD8⁺ T lymphocytes, in mice lacking C/EBPβ in the myeloid compartment, resulted in reduced tumor growth in both OVA-expressing EG-7 tumors and MCA203 fibrosarcomas (22). Moreover additional members the STAT family are implicated in the modulation of MDSCs activation. On this regard, Stat1, Stat5 and Stat 6 have been shown to mediate arginase and INOS1 production and to be therefore implicated in the immune-suppressive function of MDSCs. In addition, evidences indicate that Nf-κB acting downstream MyD88, plays a pivotal role in MDSCs activation and functionality and Myd88⁻/⁻ MDSCs showed considerably reduced ability to suppress T cell activity and release immune-regulatory cytokines compared to the wild type counterpart, both in vitro and in vivo (23). Also prostaglandins and COX2 exerts a regulatory role on the functionality of MDSCs (24). Indeed, in vivo administration of the Cox2 inhibitor significantly reduced MDSCs accumulation in a model of lung carcinoma. MDSC-mediated promotion of tumor progression was dependent on PGE2 in this model. In addition, BALB/c mice deficient for the prostaglandin E2 receptor and injected with 4T1 breast carcinoma cells had delayed tumor growth and reduced tumor-infiltration of MDSCs. Accordingly, administration of a COX2 inhibitor in 4T1 tumor-bearing mice delayed tumor progression and reduced MDSCs accumulation to the tumor site (16).

Chemotherapy and MDSCs

An intriguing and novel aspect of MDSCs biology is the impact of chemotherapy on number and activity of tumor-infiltrating myeloid cells. Importantly, some chemotherapies such as Gemcitabine, Cisplatin, Paclitaxel and 5-Fluorouracil can suppress MDSCs counts and it is postulated that this may be critical to improve the
efficacy of these drugs (9,25,26). However, following anticancer treatment, the frequency of MDSCs does not decline to the level seen in tumor-free mice and healthy human subjects. Moreover tumor recurrence after treatment correlates with re-expansion of MDSCs (27). *In vivo*, treatment with 5FU was shown to selectively induce apoptotic cell death in MDSCs therefore leading to a major decrease in the number of myeloid cells in the spleens and tumor beds of tumor-bearing mice. Accordingly, Gemcitabine was able to deplete MDSCs in vivo, with no significant reduction in other cell subsets. In both cases, the selective loss of MDSCs was accompanied by an increase in the intra-tumoral trafficking of CD4, CD8 and NK cells, thus favoring immunosurveillance against the tumor. Despite these evidences, the effect of chemotherapeutic agents on MDSCs is still controversial. Indeed, recent findings reported that gemcitabine and 5-FU can activate the inflammasome pathway in MDSCs in vivo, therefore culminating in caspase-1 activation and production of interleukin (IL)-1β. The inflammasome activation induced in MDSCs in these settings, resulted in an increased IL17 secretion by CD4 T-cells that finally dampened the anticancer efficacy of the chemotherapy (28). Therefore the reduction in the number of MDSCs does not necessarily decreases their immunosuppressive function in tumors. Furthermore, other cytotoxic compounds such as Cyclophosphamide have been correlated with an increase in MDSCs number in breast cancer patients and melanoma-bearing mice and enhanced immunosuppression (29). Interestingly, MDSCs blockage have recently been shown to revert Docetaxel chemoresistance in a mouse model of prostate cancer, thus suggesting that combinatorial approaches aimed to affect MDSCs trafficking or functionality should be taken in consideration. Indeed Docetaxel-induced senescence and efficacy was increased in Pten null prostate tumors when the percentage of tumor-infiltrating CD11b+Gr-1+ myeloid cells was reduced
by treating the mice with an antagonist of CXC chemokine receptor 2 (CXCR2) (15). On this line it has been observed that prostate cancer patients having tumors infiltrated by CD33+ myeloid cells, relapsed after docetaxel treatment administered in an adjuvant setting, suggesting that MDSCs may drive chemoresistance in human prostate cancer (15).

Another recent paper reported that prostate cancer patients having an increased percentage of circulating myeloid cells and decreased number of lymphocytes experienced a worst survival after second-line chemotherapy (30). On this regard several clinical studies combining chemotherapy and immunotherapy directed against MDSCs have been initiated. Most of the treatments in use at the moment target the immunosuppressive function of the MDSCs. Among them, phase II studies that imply the administration of chemotherapeutic agents in combination with phosphodiesterase 5 (PDE-5) inhibitors or Nitro-aspirin (NO-aspirin), both able to reduce arginase and INOS 2 release from MDSCs, are currently on going in different types of cancer (7). In addition, treatment of pancreatic cancer patients receiving gemcitabine and the novel triterpenoid CDDO-Me led to significantly increased T activation, in accordance with the ability of CDDO-Me administration to decrease MDSC production of ROS and improve T cell function in tumor bearing mice (7).

**Clinical-Translational Advances**

Interestingly, it is now clear that the tumor-promoting activity of MDSCs is mainly related to their immature phenotype. As a consequence, differentiating agents are currently under investigation in the clinic. Indeed, cancer patients receiving ATRA and 25-hydroxy-vitamin D showed and increase maturation of the myeloid subsets infiltrating the tumor, associated with a potentiated immunosuppressive response (31). Alternately, agents that inhibit MDSCs trafficking to the tumor bed have been used in
Among them, antagonists for the CXCR2 receptor and for the CSF-1R are currently under investigation in cancer (Fig. 1). Another approach to block the recruitment and function of MDSCs in tumors is to use compounds that can reprogram the tumor secretome. On this respect, recent findings reported that a JAK2 inhibitor was capable to reprogram the tumor secretome decreasing the levels of myeloid-recruiting cytokines released by the tumors. This was associated to a decreased percentage of tumor infiltrating MDSCs and restoration of tumor immunosurveillance (32).

Another intriguing aspect of MDSCs biology is their localization in tumor-bearing mice and patients with cancer, which need to be taken in consideration for future therapeutic interventions targeting these cells (Fig. 1). Indeed, extramedullar haemopoiesis (EMH) in reticuloendothelial and parenchymal tissues in cancer patients is well described (33-35), even if its role in MDSC accumulation is only partially known (36-37). Notably, when tumor cells are injected in adult non-tumor-bearing mice, having few splenic progenitors, haematopoietic progenitors mobilize from bone marrow, arrest, and accumulate in lymphoid and parenchymal organs other than in the tumors (38). On this regard, studies using 4T1 tumor-bearing mice revealed a splenic myeloid cell reservoir that primarily comprises of CD11b^+Ly-6G^bright cells (39). Circulating MDSCs arrest and accumulate in the splenic marginal zones and periarteriolar lymphatic sheaths, migrate to the red pulp and proliferate within the subcapsular red pulp (Fig. 1). The GR1^dull subset of MDSCs may have a higher proliferation rate in bone marrow (40), although both MDSC subsets can proliferate in the spleen and in tumors (39,41). Splenic MDSCs counts also correlate with tumor G-CSF transcript and tumor growth (6). Indeed has been recently demonstrated that MDSCs can suppress T cells in the spleen promoting tumor
immune suppression (42). Accordingly, splenectomy in mice can change the amount of tumor-infiltrating MDSCs, promoting tumor regression in murine models of cancer (43). Therefore MDSCs in cancer patients may affect tumorigenesis by acting in multiple sites, not only in the tumors. These data also suggest that treatments that target MDSCs should affect the recruitment of these cells in multiple sites to be effective as anti-cancer treatments. Radiotherapy on the spleen could be envisioned as a potential treatment to target splenic MDSCs in combination with compounds that can either impair the recruitment of MDSCs or promote their maturation. This approach is used in the clinic for the treatment of different haematological disorders including myeloproliferative disorders in patients with splenomegaly. However data demonstrating that such an approach would remove spleen MDSCs is lacking. Another approach that could be envisioned to target these cells is leukapheresis. This maybe combined to different chemotherapies to avoid resistance or follow chemotherapy administration to decrease relapse. Intriguingly it has been proposed that removal of MDSCs could enhance the efficacy of Sipuleucel T, a type of immunotherapy approved by FDA for the treatment of castrated resistance prostate cancer patients. These patients are already subjected to leukapheresis during the first procedure of the protocol and they could benefit from the removal of these cells (1).

Another possible entry point for cancer therapy could be to use MDSCs as vehicle to deliver different types of treatments. As discussed above, an important aspect in the biology of MDSCs is their capability to massively infiltrate almost all types of tumors, and to reach peripheral metastatic sites. Intriguingly, administration of MDSCs loaded with the VSV oncolytic virus (Vesicular Stomatitis Virus) to colon carcinoma bearing mice significantly prolonged their survival by inducing tumor response as compared with systemic viral therapy (44). Furthermore, injection of
MDSCs infected with a radioactive form of *Listeria monocytogenes* (*Listeria*<sup>at</sup>) led to complete elimination of metastasis and significant reduction of tumor growth in mice bearing pancreatic tumors (45). Importantly, both bacterial and virus infection were shown to attenuate the immune-suppressive activity of MDSCs and a subset of infected myeloid cells even acquired an immunostimulatory phenotype.

In sum, both pre-clinical and clinical evidences demonstrate that MDSCs play a prominent role in supporting tumorigenesis and that different mechanisms may be targeted to limit their tumor-promoting activity.

**References**


Figure 1. Secretion of cytokines from the tumor is associated with mobilization of both monocytic and granulocytic MDSCs from the bone marrow to the peripheral blood. In the bone marrow, MDSCs can be generated in response to cancer–secreted factors such as G-CSF, IL6, GM-CSF, IL1β, PGE2, TNFα and VEGF. Circulating MDSCs arrest in the spleen and accumulate in the splenic marginal zones and periarteriolar lymphatic sheaths, migrate to the red pulp and proliferate within the subcapsular red pulp. Finally, the release of specific chemokines belonging to the CCL and CXCL family, drives the recruitment of circulating MDSCs to the tumor and their localization in the tumor bed.
Figure 1:

Bone marrow

MDSCs

Mobilization

G-CSF
IL6
PGE2
VEGF
TNFα
IL1β

Tumor

Blood vessels

MDSs reservoir and T-cell suppression

• T- and NK-cell suppression
• Chemotherapy resistance
• Senescence bypass
• Enhanced angiogenesis

CCLs
CXCLs

Spleen

Radiotherapy?

Human

HLA-DR−
CD11b+
CD33+
CD14−

Mouse

CD11b+
Ly6G/Ly6C+

Granulocytic

Monocytic

Recruitment

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