Regulating tumor myeloid-derived suppressor cells by microRNAs

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Myeloid-derived suppressor cells (MDSCs) are one of the major cell components responsible for cancer immune evasion. Studying mechanisms associated with the regulation of MDSCs is becoming appreciated as another way to manipulate immune responses. MicroRNAs (miRNAs) have been recognized as substances which may interact with MDSCs, and eight miRNAs including miR-17-5p, miR-20a, miR-223, miR-21, miR-155, miR-494, miR-690 and miR-101 are of particular interest regarding MDSC accumulation and function. We have reviewed the data supporting this activity of these entities.

Keywords: microRNA; MDSC; tumor microenvironment; immunosuppression; immunotherapy


Introduction

MDSCs represent a heterogenic population of immature myeloid cells consisting of myeloid progenitors and precursors of macrophages, granulocytes and dendritic cells. Aberrant expansion and accumulation of MDSCs in tumor-bearing animals and patients has been extensively reported [1, 2]. miRNAs are evolutionarily conserved small non-coding RNAs that post-transcriptionally modulate the expression of multiple target genes and are hence implicated in a wide series of cellular and developmental processes [3-5]. In the hematopoietic system, miRNAs have emerged as important regulators of myeloid lineage development and differentiation [6, 7]. Recent studies have begun to unravel the significance of individual miRNAs in MDSC biology. Moreover, accumulating evidence establishes an emerging role of miRNAs for functional MDSC accumulation during tumor development. Here, we review mechanisms by which miRNAs regulate MDSC activation and expansion; and explore the potential tumor promoting functions of MDSCs by individual miRNAs.

MicroRNA biology

A miRNA is a small non-coding RNA molecule found in plants, animals, and some viruses, which are well conserved in eukaryotic organisms. miRNA functions in transcriptional and post-transcriptional regulation of gene expression via base-pairing with complementary sequences within mRNA molecules, usually resulting in translational repression or target degradation [3-5]. The genes of miRNA are transcribed by RNA polymerase II (Pol II) that binds to a promoter found near the DNA sequence encoding the hairpin loop of the pre-miRNA. Animal miRNAs are initially transcribed as part of one arm of an ~80 nucleotide RNA stem-loop that generates part of a several hundred nucleotides long miRNA precursor defined as primary miRNA (pri-miRNA). A single pri-miRNA contains several miRNA precursors, which are
composed of about 70 nucleotides each. The catalytic RNase III domain of Drosha will liberate hairpins from pri-miRNAs by cleaving RNA, which results in the formation of pre-miRNA. Pre-miRNA hairpins can be exported from the nucleus into cytoplasm with the help of nucleocyttoplasmic shuttle Exportin-5. Exportin-5 recognizes the 3' end of the pre-miRNA hairpin and transports it to the cytoplasm, which is energy-dependent consuming GTP. Within the cytoplasm, the pre-miRNA hairpin is cleaved by the RNase III enzyme Dicer. The imperfect miRNA:miRNA duplex is produced with the length of about 22 nucleotides. At last, one of strand from this duplex is incorporated into the RNA-induced silencing complex (RISC), where the miRNAs become functional and interact with its mRNA targets.

Recently, miRNAs are increasingly being appreciated as key regulators of the immune system. Extensive evidence demonstrates that miRNAs play crucial roles in the development, differentiation and function of different immune cells, such as B and T lymphocytes, dendritic cells and macrophages[8-11]. Furthermore, emerging data have identified an important contribution of miRNAs to the development and function of MDSCs[12], which is one of the main cellular constituents of cancer immune evasion.

Murine MDSC

In mouse, MDSCs are uniformly characterized by the expression of CD11b and Gr-1 markers but with varied subtypes[13, 14]. Murine MDSCs generally consist of two subtypes, the CD11b’Ly6G’Ly6Clow, exhibits a granulocytic phenotype, while the CD11b’Ly6GLy6Chigh displays a monocytic phenotype. CD11b’Ly6G’Ly6Clow produce high levels of reactive oxygen species (ROS) but only nominal amounts of nitric oxide (NO), indicating that ROS are the primary mediators of their suppressive functions. In contrast, CD11b’Ly6G’Ly6Chigh expresses high levels of NO and low levels of ROS, which is IFN-γ/STAT1-dependent. They can effectively suppress T-cell function in both antigen-dependent and independent manners without cell-cell contact. iNOS inhibitors are able to block the suppressive effect of CD11b’Ly6G’Ly6Chigh MDSCs. In most tumor models, CD11b’Ly6G’Ly6Chlow MDSCs are the major subset to be expanded in the peripheral lymphoid organs, while the CD11b’Ly6G’Ly6ChighMDSC population possesses more potent inhibitory activity.

Even though the exact molecular mechanisms underlying MDSC accumulation remain unknown to date, current evidence suggests that MDSC accumulation can be separated into two processes governed by different signal transduction pathways, which is called the two-signal model[15]. One signal is predominantly responsible for MDSC differentiation and the second one for activating MDSCs to convert them into immune suppressive phenotypes. It is identified that the first process is induced by various cytokines and growth factors produced by tumors such as GM-CSF, M-CSF, G-CSF, IL-6, VEGF etc., and signals primarily via STAT3 and STAT5 to block differentiation of mature myeloid cells and drive proliferation of immature myeloid cells. On the other hand, MDSC activation requires a second signal, which is manifested in the up-regulation of arginase, NO, and various immune suppressive cytokines. The second signal is activated by pro-inflammatory molecules, such as IFN-γ, IL-1β, IL-13, TLR ligands mainly through the STAT1, PI3K-Akt and NF-κB transcription factors.

miR-17-5p and miR-20a

Recent studies have shown that the expression of STAT3 can be modulated by both miR-17-5p and miR-20a[16]. Under these conditions, transfection of miR-17-5p or miR-20a remarkably reduced the production of ROS and H2O2, which are regulated by STAT3. Expression of both miR-17-5p and miR-20a was down-regulated by tumor-associated factors. In MDSCs, ectopic expression of either miR-17-5p or miR-20a decreased their ability to suppress antigen-specific CD4+ and CD8+ T cell responses[16]. Importantly, both miR-17-5p and miR-20a promoted the STAT3-mediated suppressive function of MDSCs in vivo. Notably, only granulocytic MDSC-mediated suppression was regulated by miR-17-5p and miR-20a via targeting of STAT3. Monocytic MDSCs were not significantly affected[16]. These results suggest that, by inhibiting STAT3 expression, miR-17-5p and miR-20a may serve as important immune regulators and might be an option for improving current immunotherapy strategies. Nevertheless, miR-17-5p and miR-20a need to be evaluated in a clinical setting (human patients) to determine if they are effective in modulating human MDSCs and subsequently if manipulation of miR-17-5p and miR-20a could help overcome the immune tolerance mediated by MDSCs.

miR-223

miR-223 is a highly conserved miRNA and largely expressed in myeloid cells[17]. Earlier studies have shown that miR-223 is expressed specifically in cells of the granulocytic lineage and its expression changes during maturation. Moreover, miR-223 negatively regulates progenitor proliferation and granulocyte differentiation and activation[18]. Interestingly, miR-223 can suppress differentiation of tumor-induced CD11b’Gr1’ MDSCs from bone marrow (BM)[19]. Decreased expression of miR-223 was observed in tumor-associated MDSCs, including mononuclear and polymorphonuclear subsets, compared with...
CD11b+Gr1+ cells from disease-free mice \[19\]. They also confirmed that the expression of both pri-miR-223 and mature miR-223 is regulated by tumor-associated factors. miR-223 can remarkably inhibit differentiation of BMs into CD11b+Gr1+MDSCs by targeting myocyte enhancer factor 2C (MEF2C). Considering that miR-223 and the target molecule MEF2C are conserved between mouse and human, upregulation of miR-223 within hematopoietic system by tumor-associated factors may result in expansion of CD11b+Gr1+MDSCs in cancer patients.

**miR-21 and miR-155**

A recent study indicated that miR-21 and miR-155 were closely involved in the expansion of MDSC both in monocytic and granulocytic subpopulation \[20\]. Bone marrow derived, and splenic MDSC, isolated from tumor-bearing mice were detected with high levels of miR-155 and miR-21. Furthermore, the effect of miR-155 and miR-21 on MDSC is associated with STAT3 activation by targeting SHIP-1 and phosphatase and tensin homolog (PTEN), respectively. \[20\] These data, furthermore, suggest that inhibition of STAT3 expression by depleting miR-155 and miR-21 in MDSCs could be beneficial for cancer immunotherapy.

Consistent with these results, we demonstrated that genetic ablation of miR-155 renders mice resistant to chemical carcinogenesis and the growth of multiple transplanted tumors \[21\]. We further identified a crucial cell-intrinsic role of miR-155 and its target SOCS-1 in MDSCs with a reduced ability to license the generation of CD4+Foxp3+ regulatory T cells. Moreover, miR-155 expression is required for MDSCs to facilitate tumor growth. On the other hand, we also showed evidence of defective responses of miR-155-deficient dendritic cells and antitumor T cells. As host miR-155 deficiency enhances overall antitumor immunity, our results reveal a contextual function for miR-155 in antitumor immunity, with a dominate role suggested for MDSCs in tumor growth. However, the data above on host miR155 deficiency and tumor growth differ from other reported studies \[22, 23\]. A possible reason for this discrepancy may include differences in the tumor cell lines under investigation. Different cell lines could alter the accumulation of distinct immune cell subsets in the tumor microenvironment. Therefore, the intrinsic contribution of miR-155 needs careful investigation in individual immune cell subsets, because miR-155 could be either protective or deleterious to antitumor immunity. In addition, MDSCs also promote cancer progression through non-immune mechanisms. For example, MMP-9 and VEGF produced from MDSCs contribute to tumor angiogenesis \[24-26\]. Given the decreased production of MMP-9 and VEGF from miR155-deficient MDSCs, further studies will determine whether miR155 is required for MDSC-dependent tumor angiogenesis.

**miR-494**

miR-494 is a major modulator of the cell cycle progression from gap 2 (G2) to mitosis (M). It was reported that miR-494 induced a significant arrest in G2/M in cholangiocarcinoma cells, and up-regulation of miR-494 was associated with inflammation \[27\]. Interestingly, increased expression of miR-494 was detected in MDSCs from tumor-bearing mice compared with those from tumor-free mice, and tumor-derived TGF-β was responsible for the up-regulation of miR-494 in MDSCs \[28\]. Manipulation of miR-494 affected the apoptosis and migration of MDSCs. Moreover, miR-494–induced activation of MDSCs was mediated by targeting PTEN, a major negative regulator of the PI3K/Akt signaling pathway. Thus, it appears that up-regulation of miR-494 leads to a reduction of PTEN expression, thereby resulting in an increase in Akt activity and the subsequent accumulation of functional MDSCs. Further supporting this line of study, knockdown of miR-494 inhibited primary tumor growth and metastasis in vivo with decreased MDSC accumulation, suggesting another possible new therapeutic target for cancer treatment.

**miR-690**

Δ⁹-tetrahydrocannabinol (THC) is the most widely studied cannabinoid isolated from the Cannabis sativa plant. miR-690 was found to be overexpressed in THC induced MDSCs \[29\]. Moreover, EL-4 tumor-elicited MDSCs showed increased miR-690 expression \[29\]. Both granulocytic and monocytic MDSC subsets induced by THC showed high levels of miR-690, but with attenuated expression of transcription factor CCAAT/enhancer-binding protein α (C/EBPα), indicating a reciprocal relationship between miR-690 and C/EBPα in MDSCs. Indeed, C/EBPα was identified as a potential functional target of miR-690. C/EBPα plays a critical role in the terminal differentiation of myeloid cells. Given that C/EBPα blocks cell cycle progression by interacting with E2F proteins, decreased C/EBPαexpression would lead to increased myeloid proliferation, consistent with a substantial proliferation of THC-induced MDSCs in the periphery \[29\]. Thus, these data raise a possibility that up-regulation of miR-690 and silencing of C/EBPα contribute to functional MDSC expansion, by inhibiting the terminal differentiation, which maintains the immature immunosuppressive state and aids the proliferation of MDSCs simultaneously. More evidence is needed to determine whether this single miRNA regulates both accumulation and function of MDSCs, particularly in the context of promotion of tumors, or in the supportive care.
administration of marijuana during treatment of cancer.

Human MDSC

In humans, MDSCs are defined as Lin HLA DR CD33<sup>+</sup> or CD11b<sup>+</sup>CD14<sup>+</sup>CD33<sup>+</sup> populations, which do not express the marker Gr1 as in the mouse [13, 30-32]. In human peripheral blood, it has been proposed that monocytic MDSCs tend to be CD14<sup>+</sup>, while the granulocytic MDSCs are CD15<sup>+</sup>. Clinical correlation studies in breast, colorectal, pancreatic, esophageal, and gastric cancer patients have demonstrated that increased MDSC levels could be used as an important independent prognostic factor for survival [31]. MDSCs have been extensively studied in tumor-bearing mice, while the characteristics of human MDSC have not been well established, especially in the case of tumors in situ.

miR-101

It has been recently reported that tumor-associated MDSCs promote human ovarian cancer stemness [33]. MDSCs were isolated from the microenvironment of primary ovarian tumors for phenotypic, functional, mechanistic, and clinical studies. MDSCs facilitated tumor sphere formation and enhanced the expression of multiple stem cell core gene transcripts. Mechanistically, MDSCs induced miR-101 expression in ovarian cancer cells and promoted cancer stemness by targeting co-repressor CtBP2. Thus, this elegant study demonstrates that MDSCs function as an environmental extrinsic signal, directly target cancer stem cells to shape tumor phenotype via a single miRNA, i.e. miR-101 [33]. Additionally, miR-101 inhibition blocked MDSC-induced cancer sphere formation, while miR-101 over-expression stimulated cancer sphere formation with elevated expression of multiple stem cell core genes and genes associated with epithelial to mesenchymal transition, thereby increasing tumor incidence and liver metastasis. In addition, high levels of microRNA101 were associated with reduced ovarian cancer overall survival. Determining the presence in tumors of the combination of CtBP2 and MDSCs clearly improved prognostic stratification of ovarian cancer overall survival [33]. The data suggest that targeting miR-101 could block the cross-talk between host MDSCs and cancer (stem) cells to augment therapeutic efficacy and reduce therapy resistance.

Concluding remarks

It is becoming clear that development of MDSC-targeted strategies for effective immunotherapy is a high priority [34-36], and might be the next major breakthrough for the immunotherapy of cancer. Despite the fact that overall phenotype and function of MDSCs are now widely appreciated, the molecular basis governing the accumulation of these cells remain poorly defined. One of the major challenges is the complexity of tumor-derived factors which have been shown to regulate functional MDSC accumulation through independent or overlapping signaling pathways. As miRNAs are endowed with the unique ability to concurrently target multiple effectors in different pathways, it is reasonable to infer that manipulation of miRNA by targeting the point of convergence may be the more effective therapeutic strategy. However, more considerations warrant investigation. First, MDSC differentiation and/or activation in vivo is likely to be modulated by the concerted action of more than one miRNA. It remains to be defined how a combination of deregulated miRNAs acts together on key molecular pathways of MDSCs to control cancer progression. Second, most of our knowledge of miRNA biology comes from murine studies. It shows the limited clinical applications considering the different phenotypic and functional features of MDSCs in human cancers. The significance of miRNAs for MDSC expansion and function, both in mouse models and humans, and the relative contributions of cell-endogenous and cell-exogenous miRNAs in vivo, are yet poorly understood and hence require further attention. Finally, pharmacological strategies for targeting miRNA for cancer therapy is faced with a number of difficulties including establishing safe, efficient and site-specific delivery methods for the miRNA or their inhibitor [37-39]. Despite these challenges, improvement of miRNA-based therapeutic strategies by targeting MDSCs, would not only integrate our understanding of MDSC biology, but also reveal novel mechanisms for cancer growth and progression.

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Conflicting interests

The authors have declared that no competing interests exist.

References

2. Gabrilovich DI, Nagaraj S. Myeloid-derived suppressor cells as
37. Garzon R, Marcucci G, Croce CM. Targeting microRNAs in...
