mTOR Signaling and the development of MDSCs

Accepted 16th January, 2017

ABSTRACT

MDSCs (myeloid derived suppressive cells, MDSCs) are a heterogeneous population of immature myeloid cells, including two subpopulations namely M-MDSC and G-MDSC according to the expression of surface molecule Ly6C and Ly6G of Gr-1 subtype respectively. Cytokines like GM-CSF, G-CSF, VEGF, TGF-β and IL-6 from cancer or chronic inflammation can induce the development of MDSCs by activating Jak/STAT signaling pathway, especially Jak2/STAT3. PI3K/Akt/mTORC1 cascade plays an important role in differentiation and activity of immune cell such as T cells and dendritic cells by affecting metabolism. However, the effects of mTORC1 signaling pathway on the development of MDSCs draw little attention. In this review, the differentiation and subsets of MDSCs and positive or negative regulation of mTORC1 signaling on the development of MDSCs will be discussed. But the exact downstream of mTORC1 signaling pathway in MDSCs is not clear.

Keywords: MDSCs, STAT3, mTOR, Rapamycin.

INTRODUCTION

As we all know, immunization therapy is a promising tool to treat cancer. However, there is a big challenge during the therapeutic process, tumor-induced immune tolerance, which makes this method suffer a poor effect. Sepsis is a systemic inflammatory response syndrome and disease process is characterized by a shift from early pro-inflammatory response to a late anti-inflammatory. Sepsis has a high mortality rate in late phase because of immunosuppression and recurrent infection (Murphey et al, 2004). The mechanism of immune tolerance in tumor and immune suppression in sepsis attracts researchers' great interest and the suppressive myeloid cells were subsequently put forward. The suppressive myeloid cells in cancer patients were described late and these suppressive myeloid cells contribute to the tumor-induced immune tolerance and promote the development of tumor (Bronte and Zanovello, 2005). Delano et al found immature myeloid cells characterized by common surface marker Gr-1 and CD11b in sepsis model, and he also confirmed that suppressive functions of immature myeloid cells contribute to the immune defects in sepsis mice (Delano et al, 2007). Gabrilovich et al named these suppressive myeloid cells as myeloid-derived suppressor cells (MDSCs) and pointed that MDSCs are a complex population composed by precursors of dendritic cells, macrophages and granulocytes and restrict the proliferation and function of T cells (Gabrilovich et al, 2007; Gabrilovich and Nagaraj, 2009). Many reports have focused on the origin of MDSCs. These studies pointed that many kinds of cytokines (GM-CSF, G-CSF, IL-6, TGF-β, VEGF) block the normal differentiation process of multiple hematopoietic lineages, inhibit the maturation of myeloid cells, and induce MDSCs (Gabrilovich and Nagaraj, 2009; Serafini et al, 2004; Bunt et al, 2007). It has been clarified that Jak-STAT3 (tyrosine 705) signaling pathway mostly contributes to MDSCs development from expansion and activation to polarization. Recently, mTORC1 has emerged as a regulator of the differentiation of MDSCs. However, mTORC1 has contradictory effects on the development of MDSCs in different backgrounds and the molecular mechanism is not understood.
The differentiation and subsets of MDSCs

In healthy individual, bone marrow hematopoietic stem cells (HSCs) differentiate into common myeloid precursor cells, and subsequently immature myeloid cells which migrate into peripheral lymphatic organs and differentiate into mature dendritic cells, macrophages and granulocytes. However, cytokines from diverse pathologic conditions like cancer, transplantation rejection, or microorganism infection will interfere with the development of myeloid precursors which fail to polarize into mature myeloid cells and functionally antigen-presenting cells (APC) and finally differentiate into MDSCs producing immune suppressive factors such as inducible nitric oxide synthase (iNOS) or arginase I (ARG1) which initiating the release of nitric oxide (NO) and reactive oxygen species (ROS) (Gabrilovich, 2004; Bronte and Zanovello, 2005). Although the members of MDSCs are complex, they consistently express membrane surface proteins CD11b and Gr-1 in mice and Movahedi et al divided them into different subpopulations according to their clear morphologic, molecular and functional differences (Movahedi et al, 2008). In his research, CD11b+Gr-1+MDSCs are grouped into two major subsets based on differential expression of Ly6C. Ly6C+ subset are similar to inflammatory monocytes and represents mononuclear cells (MO-MDSCs). Ly6C− subset resemble immature neutrophils and represents polymorphonuclear cells (G-MDSCs). Recent studies have demonstrated that both two MDSCs subsets, M (monocytic)-MDSCs and G (granulocytic)-MDSCs, are easier to polarize from a classically activated phenotype M1 to an alternatively activated M2 (F4/80+/CD206+) in tumor-bearing mice (Luo et al, 2006; Yang et al, 2013). M2 and M1 state have contrary functions. M2 MDSCs benefit tumor growth mainly by increasing the production of arginase and immunosuppressive cytokines, whereas M1 counterparts suppress tumor growth through the enhancement of inflammatory response.[Fridlender et al, 2012]. In sepsis mice, CD11b+Gr-1+ cells recovered from early septic mice and late septic mice possess the opposite functions. The Gr-1+CD11b+ cells from sepsis mice on day 3 were pro-inflammatory, but those on day 12 were anti-inflammatory (Brudecki et al, 2012). In conclusion, the subsets of MDSCs are defined either according to their surface marker or according to their functions.

The mechanism involved in the differentiation and function of MDSCs

Researchers have clarified that IL-6, GM-CSF, G-CSF and VEGF can activate Jak-STAT3 signaling pathway and phosphorylated STAT3 in tyrosine 705 (Y705-STAT3) enters into nucleus to promote the transcription of effector molecular (Condamine and Gabrilovich, 2011). STAT3 regulates the development of MDSCs via upregulating the expression of Bd-xL, c-myc, cyclin D1 and survivin. These proteins are of great significance in preventing cell apoptosis, promoting cell proliferation, and preventing differentiation to mature cell types (Gabrilovich and Nagaraj, 2009; Yu et al, 2009). The transcription factor CCAAT-enhancer-binding protein beta (C/EBPβ) also plays an important role in the accumulation of MDSCs. Loss of C/EBPβ decreased the percentage of CD11b+Gr-1+ cells and abrogated the immunosuppressive ability of MDSCs (Marigo et al, 2010). Since both of STAT3 and C/EBPβ are necessary for the expansion of MDSCs, it is reasonable to speculate that there is a link between these two factors. Interestingly, the research data have confirmed that STAT3 increased the protein level of C/EBPβ in myeloid progenitor cells (Zhang et al, 2010). It is probably that STAT3 promotes the translation of C/EBPβ in MDSCs.

Immunosuppression of M-MDSC and G-MDSC are achieved by different effective molecules. M-MDSCs produce ARG1 and iNOS. G-MDSC secrete ROS and ARG1. ARG1 depletes arginine which is essential for T cell proliferation (Gabrilovich and Nagaraj, 2009). iNOS produces NO which reacts with ROS and catalyzes the nitration of TCR, which consequently decreases the interaction between TCR, peptide and MHC (Nagaraj et al, 2007). S100A9 and S100A8 are also regulated by STAT3 and benefit the formation of the NADPH oxidase (Nox2) complex that contributes to the production of ROS in myeloid cells (Cheng et al, 2008).

The mTORC1 signaling pathway

mTORC1 is a serine/threonine kinase complex and is a downstream of PI3K/Akt signaling pathway. mTORC1 contains mTOR, mSt8/Gbl, PRAS40 and raptor. Insulin, growth factors, energy, stress, mitogens and amino acids activate mTORC1 signaling pathway (Figure 1 A) which then phosphorylates two well characterized downstream targets: S6K1 and 4E-BP1. S6K1 positively regulates protein synthesis, but 4E-BP1 is a negative regulators (Soliman, 2013). Both S6K1 and 4E-BP1 combine with raptor through mTOR signaling (TOS) motifs consisting of five amino acids [F(D/E)(M/F/I/L)(D/E)(I/L)] (Figure 1 B). The TOS motifs in S6K1 and 4E-BP1 are FDL/IDL and FEMDI, respectively (Figure 1 C, D) (Schalm and Blenis, 2002; Schalm et al, 2003). Yokogami et al confirmed that mTORC1 phosphorylated STAT3 in ser727 using an in vitro kinase assay (Yokogami et al, 2000). In this study, HEK293T cells were transfected with HA-tagged wild-type mTORC1 (mTOR-WT) or HA-tagged kinase-inactive mTORC1 (mTOR-NK), and then stimulated with CNTF. mTOR-WT or mTOR-NK was pulldown with anti-HA antibody. mTOR-WT but not mTOR-NK phosphorylated the STAT3 (720-731) by using kinase experiment in vitro. Dodd et al optimized Yokogami’ mTORC1 specific kinase assay (Dodd et al, 2015).

In his experiment, recombinant STAT3 with full length
Figure 1. TOS Motif.

was used as substrate and the result showed that full length recombinant STAT3 was indeed phosphorylated by mTORC1. It has been observed direct phosphorylation of STAT3 at Ser727 by mTORC1 in vitro. Since maximal activation of transcription by STAT3 requires both tyrosine and serine phosphorylation and activation of STAT3 is indispensable for the development of MDSCs (Wen et al., 1995). Phosphorylation of STAT3 at Ser727 by mTORC1 probably have important effects on MDSCs by increasing the activity of STAT3.

**MTORC1 regulates MDSCs subsets homeostasis by affecting glycolytic activity**

There is evidence to suggest that inflammatory responses in myeloid cells are regulated by mTORC1 pathway (Weichhart et al., 2008). Recently, it has been shown that mTORC1 also affect the differentiation of monocytes into TAM (Chen et al., 2012). Researchers put forward the reasonable speculation that mTORC1 could be involved in MDSCs development. Several recent studies have found that mTORC1 promoted the development of MDSCs. It is reported that mTORC1 influences the phenotype of MDSCs in tumor inducing a switch from M2 to M1-like MDSC. MDSCs used in this report were sorted from SIRT1KO and WT mice. Those cells were subsequently stimulated under M1 condition (LPS) and M2 condition (IL-4), respectively. Results showed that SIRT1KO MDSCs were more likely to differentiate into M1-MDSCs accompanied by increased glycolytic activities, decreased suppressive function and increased pro-inflammatory ability. SIRT1KO-MDSCs showed a stronger phosphorylation S6 ribosomal protein and HIF-1α. Upregulated glycolytic activity of SIRT1KO-MDSCs could be rescued by rapamycin treatment. Therefore, the deletion of SIRT1 promotes MDSC M1 polarization through mTORC1/HIF-1 dependent glycolytic pathway (Liu et al., 2014).

MTORC1 not only affects the phenotype switching from M2 to M1-like MDSCs but also promotes the development of M-MDSCs differentiation and immunosuppressive function in two experimental models including allo-grafs and tumors (Wu et al., 2015). In vivo, it has been showed that both rapamycin and mTORC1 deletion significantly decreased M-MDSCs induced by allo-skin grafted and tumor. In vitro, rapamycin directly inhibited M-MDSCs differentiation induced by GM-CSF. At the same time, the researchers also observed that both rapamycin treatment and loss of mTORC1 decreased glucose uptake of M-MDSC and down-regulated “genes encoding” “glycolysis-related molecules” including the transporter Glut1 and glycolytic enzymes, which confirmed that mTORC1 controlled the development of M-MDSCs through mastering cellular metabolism. Consistently, it has also reported that the
oncogenic mTOR pathway dictated MDSC accumulation through inducing the production of GM-CSF under tumor condition (Welte et al, 2016).

**Rapamycin positively regulates the expansion and function of MDSCs**

However, there are some views opposite to opinions mentioned above. Nakamura et al demonstrated that the recruitment of MDSCs to cardiac allografts was negatively regulated by mTORC1 (Nakamura et al, 2015). Treatment of rapamycin positively prolonged the survival of allograft and induced the functional MDSCs suppressing the proliferation of CD4+T cells. Recently, Zhang et al reported that mTORC1 signaling negatively influenced the recruitment of MDSCs to adipose tissue in diet-induced obese mice (Makki et al, 2014).

**Conclusion**

The cytokines of M-CSF, GM-CSF and G-CSF together with Ras/MAPK, PI3K/Akt, Jak/STAT signaling pathways are involved in the regulation of various aspects of MDSCs biology (Figure 2) (Condamine and Gabriovich, 2011). Finally, the downstream of PI3K/Akt and Jak/STAT signaling pathways regulates the expression of genes, like iNOS, IL-10 and arginase, are involved in the immune suppressive function of MDSCs (Zhang et al, 2014). STAT3 is an important transcription factor, and maximal activation of transcription of STAT3 requires both tyrosine and serine phosphorylation (Wen et al, 1995). Activation of STAT3 determines the fate of MDSCs. MTORC1 is a component of the PI3k/Akt pathway and influences the fate of MDSCs by affecting glycolytic activity. Furthermore, it has been clarified mTORC1 phosphorylates STAT3 in ser727 by kinase assay in vitro. Therefore, it is possible that mTORC1 regulates MDSCs not only by glycolytic activity but also by STAT3.

According to recent reports, mTORC1 has different effects on MDSCs in different condition backgrounds. In allo-skin grafted and tumor, mTORC1 promotes the differentiation of M-DCs induced from bone marrow cells. However, mTORC1 restricts the recruitment of MDSCs to inflammatory sites in cardiac allografts and immunological hepatic injury. This phenomenon could also be observed in innate inflammatory response cells. Rapamycin is widely used as an anti-inflammatory agent, but it can increase the production of proinflammatory cytokine IL-12 from macrophages and DCs stimulated with LPS and decrease the level of anti-inflammatory cytokine IL-10 (Zhang et al, 2014). These findings suggest that the activation or inhibition of mTORC1 in MDSCs and innate inflammatory response cells is complex and background dependent. The precise downstream of mTORC1 signaling in promoting or restricting MDSCs is not clear. Therefore, it is important to investigate link between the
mTORC1 and STAT3 in MDSCs, which will provide new insights into regulating the development of MDSCs.

Reference


Cite this article as:


Submit your manuscript at http://www.academiapublishing.org/ajb