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REVIEW

Signal transducer and activator of transcription proteins: regulators of myeloid-derived suppressor cell-mediated immunosuppression in cancer

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Abstract The success of cancer immunotherapy in patients depends on overcoming immunosuppressive mechanisms in addition to stimulating effective anticancer immune responses. Myeloid-derived suppressor cells (MDSCs) inhibit a spectrum of immune responses, including adaptive immune responses and innate immune responses at the tumor site. MDSCs have been targeted to overcome immunosuppression either by reducing their numbers or downregulating their immunosuppressive activities. Although signal transducer and activator of transcription (STAT) proteins are recognized as signaling and transcription factors induced by cytokines in normal cells, they also have roles in cancer and cancer-related cells, as well as MDSC differentiation and function. In in vitro and in vivo studies, including studies on humans, selective STAT3 inhibitors such as Stattic and S3I-201 have demonstrated potential in regulating MDSC-mediated immunosuppression. Thus, STAT pathways represent a promising target in cancer immunotherapy. Herein, we review the roles of STAT signaling in MDSC biology, and the clinical potential of STAT inhibitors in regulating tumor-associated immunosuppression mediated by MDSCs.

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Introduction

STAT proteins are latent cytoplasmic transcription factors that upon activation by cytokines and growth factors mediate diverse normal and pathological cellular responses in development and immunity (Darnell Jr 1997; Bromberg et al. 2000). In mammals, 7 members of the STAT family have been identified: STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6. STAT proteins contain an amino-terminal domain, a coiled-coil domain, a DNA-binding domain, an Src homology 2 (SH2) domain, and a transactivation domain. Generally, STAT proteins are phosphorylated and activated by various tyrosine kinases, including Janus kinases (JAKs), receptor tyrosine kinases (RTKs), and non-receptor tyrosine kinases (NRTKs), which promotes STAT dimerization (Levy and Darnell 2002). However, non-phosphorylated STAT proteins also exist as dimers, and both phosphorylated and non-phosphorylated STATs can activate transcription, although of different target genes (Sehgal 2008).

Herein, we focus on the role of STAT signaling in immunosuppression in the tumor microenvironment, which is mediated by several cell types including regulatory T cells (Tregs), MDSCs, and tumor-associated macrophages (Lindau et al. 2013). Owing to its diverse functions, MDSCs are one of the primary cellular mediators of immunosuppression in the tumor environment. Recent studies have demonstrated the importance of STAT signaling in MDSCs and preliminary success with selective STAT inhibitors for the regulation of MDSC function.

Hence, we believe that STAT proteins will potentially be an important target for novel cancer immunotherapies.

Roles of STATs in cancer cells

The STAT signaling pathway was originally identified as a signal transduction pathway induced by interferon-α (IFN-α) and interferon-γ (IFN-γ) in normal cells (Darnell Jr and Kerr 1994). However, STATs are also involved in oncogenic signaling, and mediate progression to malignancy (Yu and Jove 2004). Therefore, in addition to mitogen-activated protein kinase (MAPK) (Santarpia et al. 2012) and phosphatidylinositol-3-kinase (PI3 K)–AKT signal transduction pathways (Nitulescu et al. 2016), STAT signal transduction pathways represent promising targets in novel anticancer therapies.

In normal cells, STAT activation is regulated by extracellular ligand binding and it occurs transiently. In contrast, constitutively activated STAT3 is oncogenic (Azare et al. 2007). Activation of STAT3 and STAT5, which prevents apoptosis, has been reported in various human cancer cells (Yu and Jove 2004; Hassel et al. 2008; Liu et al. 2010). Constitutive activation of tyrosine kinase through genetic or epigenetic changes results in unceasing activation of downstream signaling, the STAT pathway, and plays a critical role in oncogenesis. Additionally, constitutive activation of tyrosine kinase signaling, which results in constitutive activation of downstream STAT signaling, is oncogenic (Huang et al. 2002; Levis et al. 2002). In particular, hyperactive STAT3 is frequently detected in myelomas, leukemia, lymphomas, and solid cancers (Al Zaid Siddiquee and Turkson 2008; Sansone and Bromberg 2012); hence a strategy targeting STAT3 may be a wide use approach in cancer therapy. Furthermore, constitutive activation of diverse tyrosine kinase is one of the main mechanism underlying tumor transformation, and diverse tyrosine kinase inhibitors have been developed as cancer therapeutics (Arora and Scholar 2005). Therefore, various types of cancer can be potentially treated with STAT inhibitors, which indirectly and directly target tyrosine kinase and STAT signaling, respectively.

Effects of STAT signaling in the tumor environment

STAT signaling affects not only cancer cells but also the surrounding tumor environment. Constitutive STAT3 activity induces expression of vascular endothelial growth factor (VEGF), a critical angiogenic factor, and promotes invasiveness of cancer cells (Niu et al. 2002). Similarly, STAT3 activation in cancer cells induced by an oncoprotein contributes to VEGF expression and invasiveness of cancer (Wang et al. 2010). STAT3 is also activated in tumor-associated endothelial cells and treatment with STAT3 inhibitors reduces angiogenesis directly (Lee et al. 2015). In addition, STAT5 activation influences endothelial cell migration, invasion, and tube formation (Yang and Friedl 2015). These studies suggest that STAT proteins, and in particular STAT3, are potential targets of anti-angiogenic therapy.

STAT signaling also regulates immune cell responses in the cancer environment. Ablation of STAT3 signaling in hematopoietic cells enhances antitumor responses of T cells and natural killer (NK) cells (Kortylewski et al. 2005). $CD8^+$ T cells expressing constitutively activated STAT6 exhibit defective tumor infiltration (Sasaki et al. 2008). STAT3 activation induced by tumor-derived factors inhibits myeloid cell differentiation and functional dendritic cell (DC) maturation (Nefedova et al. 2004). Constitutive activation of STAT3 in DCs results in the expansion of Tregs (Matsumura et al. 2007). These findings demonstrate the importance of STAT signaling to the antitumor functions of diverse immune cells, and underscore the broad potential of selective STAT inhibitors as immunomodulatory anticancer agents. Collectively, STAT inhibition may be effective for inhibiting the proliferation, growth, and survival of cancer cells and the invasiveness of cancer-associated vascular endothelial cells, and relieving antitumor immunosuppression.

Myeloid-derived suppressor cells (MDSCs)

MDSCs are immature immunosuppressive myeloid cells, including precursors of macrophages, dendritic cells, and granulocytes (Gabrilovich et al. 2007). MDSCs expand and accumulate during pathological conditions such as during infection, inflammation, and cancer (Gabrilovich et al. 2007; Gabrilovich and Nagaraj 2009). MDSCs were originally identified as $CD11b^+$ $Gr1^+$ cells; however, they actually comprise a heterogeneous cell population, which can be generally classified as polymorphonuclear (PMN-MDSC) or monocytic (Mo-MDSC) (Youn et al. 2008; Kim et al. 2012). In tumor-bearing mice, $CD11b^+Ly\text{-}6G^{low}Ly\text{-}6C^{high}$ cells are Mo-MDSCs, whereas $CD11b^+Ly\text{-}6G^{high}Ly\text{-}6C^{low}$ cells are PMN-MDSCs (Kim et al. 2012). In humans, PMN-MDSCs express $CD11b^+CD14^-CD33^{+\text{-}}CD15^+$ and/or $CD66b^+$, whereas Mo-MDSCs express $CD14^+HLA\text{-}DR^{low}$ (Gabrilovich et al. 2012). S100A9 has also been suggested as a functional marker of MDSCs (Zhao et al. 2012). In addition to PMN-MDSCs and Mo-MDSCs, fibrocytic MDSCs, which are distinguished from conventional fibrocytes, were reported in human umbilical cord blood cell culture systems (Zoso et al. 2014). Notably,

B7-H3^{+} MDSCs were found in the tumor sites of patients with non-small cell lung carcinoma and a murine lung cancer model, and their frequencies were correlated with poor prognosis (Zhang et al. 2015). Other novel functional markers and MDSC subpopulations have also been reported; however, the significance of these putative markers and MDSC subpopulations will require experimental validation showing the loss or gain of MDSC-mediated responses following their selective targeting.

Diverse functions of MDSCs

Eponymously named MDSCs are immune suppressor cells. They are activated by tumor-derived and host-derived factors, including proinflammatory mediators, and inhibit both innate and adaptive immune responses (Ostrand-Rosenberg and Sinha 2009; Gabrilovich et al. 2012). MDSCs mediate immunosuppression through several mechanisms. Arginase-1 (Arg-1)-dependent L-arginine depletion (Rodriguez et al. 2004) and L-cysteine depletion (Srivastava et al. 2010) mediated by MDSCs result in downregulated T cell receptor (TCR) expression and reduced T cell proliferation. MDSCs also generate reactive oxygen species (ROS) and reactive nitrogen species (RNS), which contribute to the inhibition of T cell function. Hydrogen peroxide downregulates TCRζ chain expression and cytokine production of T cells (Schmielau and Finn 2001). Nitrogen oxide (NO)-producing MDSCs impair IL-2 receptor signaling pathways in T cells and inhibit T cell proliferation (Mazzoni et al. 2002). TCR nitration induced by MDSCs affect antigen recognition (Nagaraj et al. 2007). MDSCs limit T cell migration through the downregulation of L-selectin expression in T cells (Hanson et al. 2009) and chemokine nitration (Molon et al. 2011). MDSCs also mediate regulatory T cell activation and expansion (Huang et al. 2006). MDSCs also suppress the activity of other immune cells. They dampen NK cell cytotoxicity and IFN-γ production (Hoechst et al. 2009), and decrease DC-mediated T cell responses (Hu et al. 2011).

Recently, the divergent role of MDSCs in inflammation (Chang et al. 2013) has attracted a lot of attention. Although MDSCs are generally immunosuppressive cells, they are also involved in immune-surveillance as immune effector cells under specific conditions, like acute inflammation (Cuenca et al. 2011). MDSCs, which accumulate in ascites of ovarian carcinoma-bearing mice, are capable of priming cytotoxic T lymphocytes (CTLs) and mediating antitumor immunity (Tomihara et al. 2010). Furthermore, activated natural killer T (NKT) cells convert MDSCs into immunogenic antigen presenting cells (APCs); these MDSCs have the potential to be used as a cell-based vaccine (Ko et al. 2009a; Lee et al. 2012). In addition, injection of attenuated *Salmonella* into mice induced the conversion of MDSCs into TNF-α producing immune effector cells (Hong et al. 2013b).

Gene expression in MDSCs

Although MDSCs are recognized as major cells of immunosuppression in the tumor environment, the nature and characteristics of MDSC subpopulations and the regulatory factors that modulate their differentiation and functions have not been fully elucidated. Potential therapeutic interventions targeting MDSCs may be based on factors related to their functional markers, accumulation/recruitment to tumor sites, or immunosuppressive activities. These factors have been analyzed in various studies using the following technologies: cDNA microarrays (Kim et al. 2012; Ko et al. 2014), microRNA (miR) microarrays (Hegde et al. 2013; Li et al. 2014), transcriptomics analysis (Fridlender et al. 2012), flow cytometry (Movahedi et al. 2008), or proteomics analysis (Boutte et al. 2011).

Various MDSC phenotypic markers have been identified, the majority of which do not depend on the tumor model used; however, some are still controversial and may be variable in different tumor models (Movahedi et al. 2008; Ko et al. 2009a; Talmadge and Gabrilovich 2013). MDSCs express low levels of CD86 and MHC class II molecules, and are therefore poor antigen presenting cells (Ko et al. 2009a); however, they express significant levels of molecules that suppress T cells, including CD80 (Mencacci et al. 2002) and B7-H1 (Liu et al. 2008). The adhesion molecules, CD11a, CD162, and CD54, are expressed on the surface of both Mo-MDSCs and PMN-MDSCs (Movahedi et al. 2008). IL-4 receptor α (IL-4Rα/CD124), which is reported to mediate the immunosuppressive activities of MDSCs (Kohanbash et al. 2013), is also found on the surface of both MDSC subtypes in mice and humans (Mandruzzato et al. 2009). However, F4/80 and CD115 are Mo-MDSC-specific surface markers (Movahedi et al. 2008), and CD115 defines a specific immunosuppressive MDSC subpopulation (Huang et al. 2006).

As shown by miR microarray analysis, miR-155 and miR-21 are upregulated in MDSCs and are associated with the expansion of functional MDSCs (Li et al. 2014). Expression of miR-494, which targets phosphatase and tensin homolog (PTEN) and AKT signaling pathways, plays a critical role in the accumulation and activity of MDSCs (Liu et al. 2012). In contrast, downregulation of miR-223 in MDSCs is correlated with the differentiation of MDSCs from bone marrow cells (Liu et al. 2011). Overexpression of Ki67, which promotes cell proliferation, in

MDSCs facilitates their expansion in tumor-bearing hosts (Kim et al. 2012; Hegde et al. 2013).

The establishment of an immunosuppressive tumor environment depends not only on generating sufficient numbers of MDSCs, but also on recruiting MDSCs to the tumor site. Lack of 5-lipoxygenase, which generates leukotriene B4 and cysteinyl leukotrienes, in MDSCs is associated with their limited recruitment to primary tumor sites and lower expression of Arg-1 (Cheon et al. 2011).

As shown by comparative proteomics analysis, various proteins associated with lipid metabolism, platelet activation, angiogenesis, and tumor invasion are elevated in MDSCs from metastatic tumor models compared to MDSCs from non-metastatic tumor models (Boutte et al. 2011). Proteins that are associated with metastasis and whose expression are regulated by the cAMP-responsive element-binding protein (CREB) transcription factor are also upregulated in MDSCs from malignant tumor-bearing mice compared to in MDSCs from non-metastatic tumor-bearing mice. Consistent with these findings from proteomics analysis, CREB mRNA is upregulated in PMN-MDSCs (Youn et al. 2012). CREB induces the expression of another transcription factor, CCAAT-enhancer-binding protein β (C/EBPβ), which controls downstream M2 macrophage-specific target gene expression (Ruffell et al. 2009). The expression and activity of proteins involved in immunosuppression, including Arg-1 and inducible nitric oxide synthases (iNOS), are decreased in C/EBPβ-deficient MDSCs, suggesting that C/EBPβ is a critical regulator of MDSC function (Marigo et al. 2010).

The proliferation and immunosuppressive functions of MDSCs are regulated by several factors. The calcium binding protein, S100A9, contributes to the proliferation of MDSCs and suppression of T cell responses (Cheng et al. 2008). FK506 binding protein 51 (FKBP51), a member of the immunophilin protein family, is overexpressed in both Mo-MDSCs and PMN-MDSCs, and FKBP51 deficiency in MDSCs attenuates MDSC immunosuppressive activity and reduces Arg-1, iNOS, and NADPH oxidase 2 (NOX2) expression (Kim et al. 2012). Complement factor C5a also contributes to immune suppression via regulation of MDSCs (Markiewski et al. 2008). C5a receptor-deficient MDSCs exhibit defective ROS/RNS production and migration to tumor sites.

Role of STATs in MDSCs

Tumor-derived and host-derived soluble factors affect MDSCs, in terms of expansion, accumulation and suppressive function (Gabrilovich et al. 2012). Inflammation induces MDSC generation by inflammatory mediators, such as IL-1β (Bunt et al. 2006) and PGE2 (Rodriguez et al. 2005). IL-1β induces the production of several cytokines, which can also affect accumulation and immunosuppressive function of MDSCs. In IL-1 receptor-deficient mice, IL-6 partially compensated the proinflammatory function of IL-1β in MDSC induction (Bunt et al. 2007). IL-17 was required for MDSC-mediated immune suppression, and IL-17 receptor-deficient mice exhibited low levels of MDSCs in the tumor sites. Moreover, immune regulation by these MSDCs was defective (He et al. 2010). Proinflammatory S100A8/A9 induced MDSC accumulation and promoted MDSC migration (Sinha et al. 2008). IFN-γ produced by T cells induced MDSCs, which suppressed $CD8^+$ T cells (Gallina et al. 2006).

The STAT pathway was originally discovered through the study of IFN-α and IFN-γ-mediated signaling (Darnell Jr and Kerr 1994). IL-6 is one of the most well-known activators of STAT3 (Zhong et al. 1994), and various cytokines, including G-CSF, IL-10, and IL-2, activate other STAT signaling pathways (Yu et al. 2014). Th2 cytokines, IL-4, and IL-13, activate STAT signaling pathways in myeloid cells, which regulate target gene expression (Bhattacharjee et al. 2013). Therefore, it is no wonder that aforementioned MDSC modulating factors are involved in STAT signaling. By activating STAT signaling, these tumor-derived and host-derived soluble ligands modulate MDSC proliferation, survival, and immunosuppressive function (Fig. 1).

As shown in Fig. 1, VEGF, GM-CSF, G-CSF, and IL-6 are involved in MDSC generation. Tumor-derived VEGF, which activates STAT3 and upregulates ROS production in MDSCs, induces MDSCs (Jayaraman et al. 2012). G-CSF contributes to MDSC generation in a STAT3-dependent manner, which is negatively regulated by suppressor of cytokine signaling 3 (SOCS3) (Yu et al. 2015). Another myelopoietic growth factor, GM-CSF activates STAT5 and downregulates interferon regulatory factor-8 (IRF-8) transcription, and induces MDSC accumulation (Waight et al. 2013). IL-6 induces STAT3 phosphorylation, and IL-6 treatment of human PBMCs increases the percentage of MDSCs and upregulates Arg-1 expression in these MDSCs (Chen et al. 2014).

Flt3 ligand (Flt3L), which activates STAT3 signaling, results in the expansion of MDSCs with immunosuppressive functions (Rosborough et al. 2014). Several cytokines also activate these functions. STAT1-activating IFN-γ, STAT3-activating IL-10, and STAT6-activating IL-13 regulate gene expression in MDSCs (Ma et al. 2011). IL-4Rα, which is an important marker of MDSCs, is involved in binding IL-4 and IL-13. IL-4 and IL-13 binding can lead to similar or distinct effects depending on cell type (Kruse et al. 2002; Hallett et al. 2012). In human monocytes, IL-13 binding to its receptor results in JAK2 and TYK2 activation, whereas IL-4 activates JAK1 (Bhattacharjee et al. 2013).

In summary, VEGF, hematopoietic cytokines, such as G-CSF and GM-CSF, Flt3L, and other cytokines that are

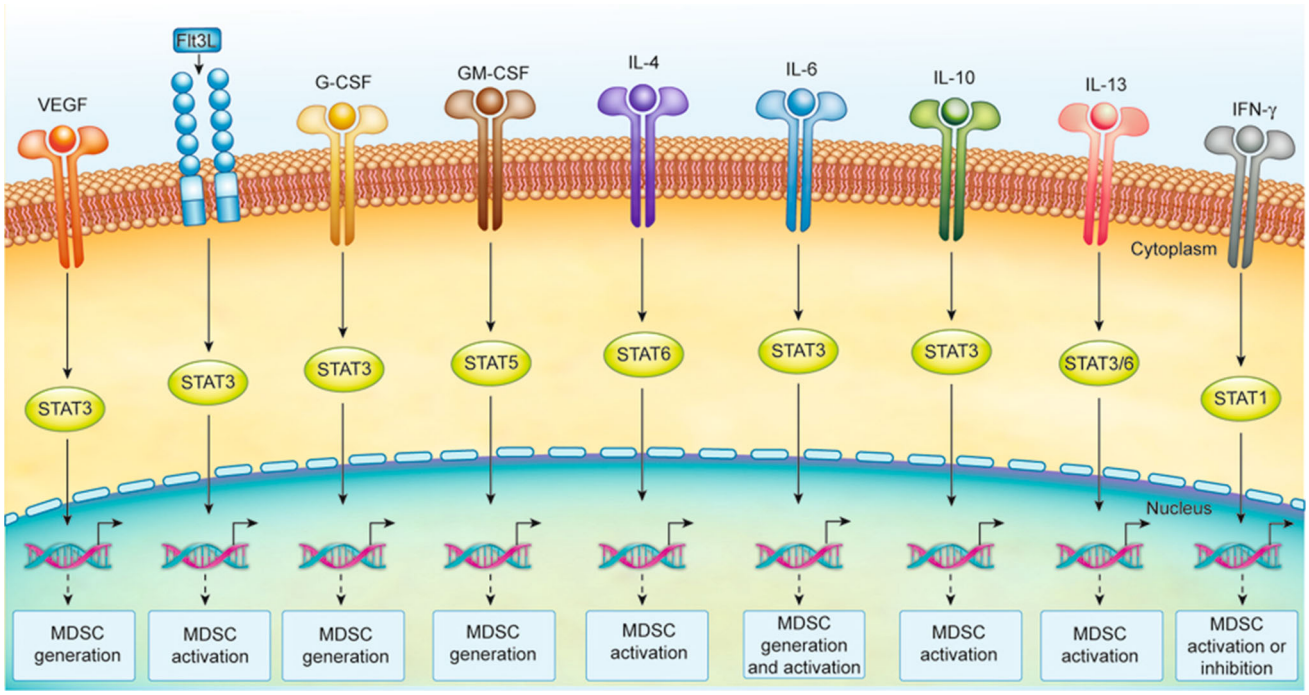

Fig. 1 Soluble ligands that activate specific STAT signaling in MDSCs. Schematic diagram shows the various soluble factors that activate specific STAT signaling pathways in MDSCs. Receptor engagement with its ligand regulates expansion, differentiation, and/or immunosuppressive functions of MDSCs

especially categorized as anti-inflammatory cytokines activate STAT signaling to regulate MDSC proliferation, survival, and activation. Thus, potential anti-cancer therapies targeting STAT signaling may directly affect both cancer cells and tumor-associated immune cells, such as MDSCs.

Regulation of MDSCs in human and mouse cancers via STAT signaling

Although there are several subpopulations of MDSCs in human cancer patients and mouse tumor models, over-activation of STAT signaling is not limited to a specific model (Table 1). In humans, STAT3 signaling is associated with MDSC differentiation and immunosuppressive functions. Immunosuppressive activity of MDSCs in melanoma patients is mediated by STAT3 (Poschke et al. 2010). In MDSCs of patients with head and neck squamous cell carcinoma (HNSCC), phosphorylated STAT3 regulates Arg-1 gene expression by binding to its promoter (Vasquez-Dunddel et al. 2013). In addition to tumor-derived growth factors and cytokines, exosomes can activate STAT3 signaling and modulate the immunosuppressive activities of MDSCs in both mouse and human cancers (Chalmin et al. 2010). Tumor-derived exosomes induce IL-6 production, and autocrine IL-6 signaling triggers STAT3 phosphorylation and activation in MDSCs.

The importance of STAT1 signaling in MDSCs is revealed by mouse knock-out models. Defective STAT1 signaling reduces the immunosuppressive activity of both Mo-MDSCs and PMN-MDSCs (Schouppe et al. 2013; Medina-Echeverz et al. 2014). Under most conditions, STAT1-deficient MDSCs suppress T cell response to a lower extent than normal MDSCs, and this can be understood in the same context with the role of STAT3 in human; however, under specific conditions, $STAT1^{-/-}$ PMN-MDSCs from tumor-bearing mice have higher immunosuppressive activity and longer lifetimes than control MDSCs (Medina-Echeverz et al. 2014).

Thus, STAT signaling pathways, in particular STAT3 signaling, are potential therapeutic targets for modulating MDSC survival, differentiation, and immunosuppressive activities. Several strategies blocking STAT signaling (Table 2) have been attempted to overcome the immunosuppressive activities of MDSCs in the tumor environment.

Targeting STAT pathways for modulating MDSCs

Strategies to overcome tumor-associated immunosuppression mediated by MDSCs can be classified based on their mechanism of action: inhibition of MDSC generation, depletion of MDSCs, differentiation of precursor cells into non-MDSC cells, inhibition of MDSC recruitment to tumor

Table 1 Roles of STAT pathways in MDSCs in various cancer models

Host organism	Type of cancer	STAT homologue	MDSC subpopulation	STAT function in MDSCs	References
Human	Advanced malignant melanoma	STAT3	$CD14^+HLA\text{-}DR^{-/low}CD80^+CD83^+DC\text{-}sign^+$	Required for suppression of T cell proliferation, IFN-γ production, and correlated with oxidative stress	(Poschke et al. 2010)
	Metastatic cancer of breast, colon, or prostate cancer	STAT3	$CD33^+HLA\text{-}DR^{-/low}$	Mediates inhibition of T cell proliferation in an TLR2/Hsp72 dependent manner	(Chalmin et al. 2010)
	Advanced lung cancers	STAT3	$CD33^+Lin^-HLA\text{-}DR^-$	Regulates differentiation of myeloid cells	(Lu et al. 2012)
	Head and neck squamous cell carcinoma (HNSCC)	STAT3	$CD14^+HLA\text{-}DR^{-/low}CD11b^+CD33^+CD34^+Arg\text{-}1^+ROS^+$	Binds to the promoter region of Arg-1 and regulates expression and activity of Arg-1, an important functional mediator in MDSCs	(Vasquez-Dunddel et al. 2013)
Mouse	EG7-OVA tumor-bearing $STAT1^{-/-}$ mice	STAT1	Splenic $CD11b^+CD115^+Ly6G^-Ly6C^{high}$ Mo-MDSCs and $CD11b^+CD115^-Ly6G^+Ly6C^{int}$ PMN-MDSCs	Contributes to suppression of T cell proliferation	(Schouppe et al. 2013)
	344SQ tumor-bearing $STAT1^{-/-}$ mice	STAT1	Splenic $CD11b^+Gr\text{-}1^{dull/int}$ Mo-MDSC	Mediates immunosuppression of T cell proliferation	(Medina-Echeverz et al. 2014)
			Splenic $CD11b^+Gr\text{-}1^{high}$ PMN-MDSCs	Downregulates anti-apoptotic molecule Bcl2a1 and immunosuppressive activity	

sites, and modulation of immunosuppressive functions of MDSCs (Chang et al. 2013). Numerous host- and tumor-derived factors, such as Flt3L, GM-CSF, and IFN-γ, activate STAT signaling in MDSCs to regulate their accumulation and/or activation.

Several classes of STAT inhibitors, including small molecules, peptides, and oligonucleotides (Furqan et al. 2013), have been reported. Small molecule inhibitors are primarily used to block STAT signaling in preclinical and clinical studies because of their availability, easy application both in vitro and in vivo, and preferable pharmacokinetic properties.

Cucurbitacins are triterpenoids isolated from members of the family, Cucurbitaceae. Several variants of cucurbitacins with improved solubility and efficacy have been developed, including cucurbitacin B and I (JSI-124), which inhibit STAT3 and JAK2 (Alghasham 2013). Treatment with cucurbitacin I induces the differentiation of immature myeloid cells into mature DCs and macrophages and relieves immunosuppression in tumor-bearing mice (Nefedova et al. 2005). Cucurbitacin I also downregulates the expression of NOX in MDSCs and reduces the production of critical ROS (Corzo et al. 2009). It has recently been reported that the oral administration of the JAK2/STAT3 signaling inhibitor, cucurbitacin B, reduces the percentage of $Lin^-HLA\text{-}DR^-CD33^+$ immature myeloid cells in patients with advanced lung cancer and improves anticancer immune responses (Lu et al. 2012). Thus, cucurbitacins can modulate MDSC-mediated immunosuppression either by reducing MDSC load or by inhibiting their activity.

Sunitinib is a multiple receptor tyrosine kinase inhibitor, which is approved for the treatment of several cancers. Its targets include platelet-derived growth factor receptor-α/β (PDGFR-α/β), VEGF receptor-1/2/3 (VEGFR-1/2/3), and c-Kit. Sunitinib inhibits STAT3 phosphorylation, which depends on p-Src but not JAK2 inhibition (Xin et al. 2009). Sunitinib treatment inhibits STAT3-regulated target gene expression, including that of VEGF, and reduces the percentage of MDSCs in the spleen and blood of tumor-bearing mice. Sunitinib treatment also reduces MDSC load in peripheral blood in renal cell carcinoma (RCC) patients, suggesting its potential to target MDSC-mediated immunosuppression (Ko et al. 2009b). The diverse effects of sunitinib likely reflect its diverse targets in tumor cells and immune cells in the tumor environment.

AG490 is a specific JAK2 inhibitor, which also blocks STAT3 activation. However, AG490 also inhibits JAK3, STAT1, STAT3, STAT-5a, STAT-5b, and other tyrosine kinases (Wang et al. 1999). Therefore, the mechanism of action of AG490 should be interpreted with caution. AG490 treatment inhibits STAT3 signaling, relieves MDSC-mediated immunosuppression of T cell responses,

Table 2 Effects of STAT3 inhibitors on MDSCs

STAT homologue	Source of MDSC	STAT inhibitor	Mechanism of MDSC inhibition	References
STAT3	Spleens of CT26 tumor-bearing mice	Cucurbitacin I (JSI-124)	Improvement of differentiation of immature myeloid cells into DCs rather than MDSCs	(Nefedova et al. 2005)
STAT3	Spleens of MC38 tumor-bearing mice	Cucurbitacin I (JSI-124)	Reduction of ROS levels by downregulation of NOX expression	(Corzo et al. 2009)
STAT3	Peripheral blood mononuclear cells (PBMCs) of patients with advanced lung cancers	Cucurbitacin B	Induction of immature myeloid cell differentiation into non-MDSC cell types	(Lu et al. 2012)
STAT3	Tumors from Renca tumor-bearing mice	Sunitinib	Downregulation of VEGF and CXCL2 in MDSCs and reduction of MDSC load	(Xin et al. 2009)
STAT3	PBMCs of patients with metastatic RCC	Sunitinib	Reduction of MDSC viability and immunosuppressive function	(Ko et al. 2009b)
STAT3	PBMCs of patients with advanced malignant melanoma	AG490	Improvement of T cell proliferation and IFN-γ production	(Poschke et al. 2010)
STAT3	Tumors and peripheral blood of HNSCC patients	Stattic	Inhibition of immunosuppressive function of and Arg-1 expression in MDSCs	(Vasquez-Dunddel et al. 2013)
STAT3	Converted normal human blood monocytes into Mo-MDSCs by co-culture with human Panc-1 cell lines	Stattic	Inhibition of MDSC-dependent increase in cancer stem cells in co-cultures	(Panni et al. 2014)
STAT3	SOCS-deficient BM cells	Stattic	Reduction of G-CSF-dependent BM cell differentiation into MDSCs	(Yu et al. 2015)
STAT3	Splenocytes from mice which were treated with recombinant human Flt3L	S3I-201	Enhancement of Flt3L-mediated MDSC expansion, but inhibition of MDSC immunosuppressive function	(Rosborough et al. 2014)
STAT3	BM–derived $CD11b^{+}Gr\text{-}1^{+}$ cells in the presence of G-CSF	FLLL32	Prevention of G-CSF-dependent downregulation of IRF-8 expression and attenuation of MDSC accumulation	(Waight et al. 2013)
STAT3	Spleens of 4T1 tumor-bearing mice	3,5,7-trihydroxy-4′-methoxy-8-(3-hydroxy-3-methylbutyl)-flavone (ICT)	Reduction in the percentage of MDSCs that differentiate into DCs and macrophages, and downregulation of NO and ROS but not arginase activity in MDSCs	(Zhou et al. 2011)
STAT5	BM–derived $CD11b^{+}Gr\text{-}1^{+}$ cells in the presence of GM-CSF	Pimozide	Prevention of GM-CSF-dependent downregulation of IRF-8 expression and inhibition of MDSC expansion	(Waight et al. 2013)

and induces the differentiation of MDSCs into immunogenic cells (Poschke et al. 2010).

Stattic, which was identified in a chemical library screen, selectively binds to the SH2 binding domain of STAT3 and inhibits the activation, dimerization and nuclear translocation of STAT3 (Schust et al. 2006). Although its STAT3 selectivity is controversial (Sansevverino et al. 2012), Stattic is widely used as a STAT3 inhibitor (Furqan et al. 2013). Stattic treatment relieves MDSC-mediated immunosuppression and inhibits differentiation into MDSCs. Phosphorylated STAT3 signaling is correlated with Arg-1 expression and Stattic treatment decreases Arg-1 expression in MDSCs. MDSC-mediated immunosuppression of T cells in cancer patients is also reduced by Stattic treatment (Vasquez-Dunddel et al. 2013). The absence of SOCS3, a negative regulator of JAK/STAT signaling, in bone marrow (BM)-derived cells results in the upregulation of STAT3 signaling. Over-activated STAT3 signaling promotes the differentiation of BM-derived cells into MDSCs, whereas Stattic treatment blocks MDSC generation induced by G-CSF (Yu et al. 2015). MDSCs induce cancer stem cells when co-cultured with human pancreatic cancer, which is also prevented by Stattic treatment (Panni et al. 2014).

S3I-201, a small molecule that binds the SH2 domain of STAT3, was identified in an in silico screen of the National Cancer Institute chemical library (Siddiquee et al. 2007). S3I-201 inhibits STAT3-dependent transcription by

inhibiting STAT3 homo-dimerization. Unexpectedly, inhibition of STAT3 signaling by S3I-201 results in expansion of MDSCs that depends on Flt3L signaling (Rosborough et al. 2014). However, the immunosuppressive activity of MDSCs is blocked by S3I-201 treatment, indicating its dependence on STAT3 signaling.

In addition to cucurbitacins, several natural compounds, including cryptotanshinone and curcumin, inhibit STAT3 (Furqan et al. 2013). FLL32 is a curcumin analogue with improved pharmacokinetic parameters and potency than curcumin. FLL32 binds the SH2 domain of STAT3 and inhibits STAT3, but not STAT1, phosphorylation/activation and dimerization (Bill et al. 2012). As discussed above, both G-CSF and GM-CSF regulate the generation of MDSCs. FLL32 treatment inhibits G-CSF-induced IRF-8 downregulation, suggesting a critical role of STAT3 in mediating the effects of G-CSF signaling in these cells (Waight et al. 2013). The active ingredient of *Herba Epimedii*, 3,5,7-trihydroxy-4′-methoxy-8-(3-hydroxy-3-methylbutyl)-flavone (ICT), also reduced STAT3 phosphorylation in MDSCs (Zhou et al. 2011). Treatment with ICT resulted in conversion of MDSCs into DCs and macrophages, and the critical functional mediators, NO and ROS, were downregulated in the MDSCs.

Inhibitors of other STAT homologues were identified by several approaches. Through high-throughput cell-based screening of drugs known to be safe in humans, the antipsychotic drug, pimozide, was identified as a selective STAT5 inhibitor that does not inhibit STAT1 or NF-kB (Nelson et al. 2011). In MDSCs, IRF-8 expression is regulated by GM-CSF via the STAT5 signaling pathway, and inhibition of STAT5 signaling by pimozide increases IRF-8 expression in the presence of GM-CSF (Waight et al. 2013). These results suggest the potential of STAT inhibitors as effective immunotherapeutic anticancer agents.

Conclusion

Cancer immunotherapies can be classified as those that stimulate anticancer immune effectors or those that relieve immunosuppression. The former includes cancer vaccines that comprise stimulatory epitopes or ligands and adjuvants. However, cancer vaccines may not protect patients because of tumor-associated immunosuppressive mechanisms, and both the relief of immunosuppression and activation of immune responses are likely necessary for effective anticancer treatment. As one of the primary cellular effectors of cancer-associated immunosuppression, MDSCs represent an important cellular target of anticancer therapeutics.

STAT signaling is disrupted in various cancers. Various cancer cells are characterized by hyperactive STAT signaling, which contributes to cancer cell survival and proliferation. STAT signaling is also involved in angiogenesis and immune responses at the tumor site. STAT proteins also regulate MDSC survival, differentiation, and immunosuppressive functions. Therefore, in addition to Myd88 (Hong et al. 2013a) and NF-kB signaling (Kim et al. 2012), STAT signaling, in particular STAT3 signaling, is important to MDSC biology.

Various STAT inhibitors have been developed from natural compounds or by screening chemical and drug libraries. Due to the diverse functions of STAT proteins in the tumor environment, STAT inhibitors might be used to regulate cancer cells and the tumor microenvironment, including immune cells. Although most cancer therapies have focused on inducing cancer cell death directly, exploiting the intrinsic potential of the adaptive and innate immune system to target cancer cells provides a powerful means of indirectly removing cancer cells and preventing their recurrence.

Inhibiting STAT signaling may concomitantly decrease the viability of MDSCs and their immunosuppressive functions. In addition, STAT inhibitors have the potential to convert MDSCs into fully mature myeloid cells or non-immune suppressor cells, which may represent a better strategy than simply decreasing MDSC load. Studies on the efficacy of STAT inhibitors as anticancer immunotherapeutic agents are already under way, and together with basic research advances, fulfillment of the clinical promise of STAT inhibitors is on the horizon.

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Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

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