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Running Title: Targeting STAT3 for Cancer Therapy

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ABSTRACT

Signal transducer and activator of transcription 3 (STAT3), is a cytoplasmic transcription factor that regulates cell proliferation, differentiation, apoptosis, angiogenesis, inflammation and immune responses. Aberrant STAT3 activation triggers tumor progression through oncogenic gene expression in numerous human cancers, leading to promote tumor malignancy. On the contrary, STAT3 activation in immune cells cause elevation of immunosuppressive factors. Accumulating evidence suggests that the tumor microenvironment closely interacts with the STAT3 signaling pathway. So, targeting STAT3 may improve tumor progression, and anti-cancer immune response. In this review, we summarized the role of STAT3 in cancer and the tumor microenvironment, and present inhibitors of STAT3 signaling cascades.

INTRODUCTION

Hallmarks of cancer consist of sustaining cellular proliferative signals, attenuating cell death, inappropriate replication with lacking growth suppressors, inducing angiogenesis and promoting invasion and metastasis in tumorigenesis (1). Recently, the impact of the tumor microenvironment and tumor-induced immune suppression on tumor progression, has been subjected to intense investigation, and the STAT3, is a crucial mediator of tumor cell progression and tumor-associated immunosuppression.

STAT3 is integral for transducing signals from receptor and/or non-receptor tyrosine kinases activated in cancer cells, as well as transcriptional factors regulating expression of numerous gene contributing tumor progression (2). STAT3 signaling cascade is triggered by upstream kinase signals, and undergo phosphorylation, homo-dimerization, translocate in to

nuclear, and bind to DNA, leading to target gene expression involved in tumor cell proliferation, angiogenesis, metastasis, and immunoediting (3-5). So, STAT3 is a prominent target for cancer therapy.

The tumor microenvironment is composed of tumor cells and their surrounding circumstance, including hypoxic condition, blood vessels and extracellular matrix (ECM), as well as stromal cells, immune cells, and inflammatory cells (6, 7). STAT3 is a key mediator modulating tumor milieu to promote tumor progression, and is a promising target for antitumor immune response (8, 9).

Emerging evidence suggests the key role of STAT3 in cancer cells and their microenvironment. However, there are knowledge gaps remaining regarding interaction between STAT3 signaling, and the tumor microenvironment immune system. Additionally, STAT3 is a promising target for cancer treatment. Therefore, this review article summarizes recent reports related to the role of STAT3 in cancer cells, and the relationship between cancer cells and tumor microenvironment in tumor progression. Also, this review focuses on the therapeutic agents and inhibitors that specifically target STAT3.

Persistent STAT3 activation in cancer cells

Aberrant activation of STAT3 has been involved, in oncogenesis and malignant phenotypes in human cancers (10, 11). Hyperactivation of STAT3 has been reported in several types of tumors, including head-and neck, brain, breast, liver, lung, kidney, pancreas, prostate, ovary, and multiple myeloma, as well as acute myeloid leukemia (AML) (12-21). Expression levels of activated STAT3 are positively correlated with poor prognosis in these cancers. Constitutive STAT3 activation is primarily due to hyperactivation of growth factor receptor

tyrosine kinase and overexpression of stimulatory receptor-ligand interactions. Phosphorylation of tyrosine 705 residue, leads to nuclear translocation of STAT3, which allow induction of STAT3 target genes (4). As an oncogene, STAT3 is a major signal transduction pathway involved in multiple cellular processes, including proliferation, survival, angiogenesis, metastasis, invasion, and immune escape (22-24). (Fig. 1).

1. STAT3 accelerates the cellular proliferation and survival

Accumulating evidence shows that STAT3 activation, participates in cellular proliferation and survival. Persistent activation of STAT3 induces up-regulated expression of CyclnD1, c-Myc and Survivin, to accelerate cell cycle progression in renal and colon cancers (25-27). Correspond to its role in cellular proliferation, multiple studies have shown that STAT3 signaling pathway suppresses apoptosis in cancer cells. Activated STAT3 also upregulates anti-apoptotic protein such as Bcl-2 (B-cell lymphoma-2), Bcl-XL (B-cell lymphoma-2-like 1), and Mcl1(myeloid cell leukemia sequence 1) expressions to prevent apoptosis of tumor cells in multiple myeloma (28, 29) (Table 1). Inhibition of STAT3 results in decreased cell proliferation, and promotes apoptosis in various cancers including breast cancer, colorectal cancer, gastric cancer, lung cancer, and so on (30-32). According to these studies, STAT3 is a key regulator of cancer cell proliferation and survival.

2. STAT3 enhances the Angiogenesis

The formation of a new blood vessel called angiogenesis, is a fundamental step in tumor growth and metastasis.

It is well known that STAT3 induces vascular endothelial growth factor (VEGF) directly, which is the most angiogenic molecule. (33, 34). Moreover, STAT3 induces hypoxia-inducible factor-1α (HIF1α), another regulator of angiogenesis (35). During hypoxic

conditions in core of cancer cells, STAT3 and HIF1α bind to the VEGF promoter, leading to angiogenesis (36). Additionally, pro-angiogenic factors such as bFGF (basic fibroblast growth factor) and HGF (hepatocyte growth factor), also downstream target of STAT3 (37) (Table 1). Angiogenesis led to malignant cancer cell transformation. Invasion and migration are key steps for tumor growth and metastasis.

3. STAT3 contributes to promotion of Metastasis

Cancer metastasis is a complicated procedure in which cancer cells invade adjacent tissue enabling such cells to accomplish migration and invasion, known as epithelial-mesenchymal transition (EMT). According to previous studies, STAT3 activation is pivotal in regulating expression of Twist, Vimentin, Snail, HMGB1 (high-mobility group box 1), ZEB1 (zinc finger E-box binding homeobox 1), and so on (38-42). Persistent STAT3 activation, leads to upregulated expression of MMP2 (matrix metalloproteinase) (43). Moreover, STAT3 activation also regulates other matrix metalloproteinases, such as MMP9 and MMP1 (44, 45) (Table 1). Based on these studies, STAT3 activation promotes cellular invasion. Additionally, earlier studies have shown evidence that aberrant STAT3 activation is required for cell motility, and plays a key role in wound healing and migration (46, 47). Thus, inhibition of neo-angiogenic factors and/or migration factors by suppressing STAT3 signaling pathway is an attractive strategy for preventing tumor formation.

4. STAT3 induces the immune evasion

Tumor immune surveillance plays a pivotal role in identifying cancerous and/or precancerous cells, and eliminates them before they abnormally transform. Recent findings show that abnormal cells may evade the immune system, to form malignant cancers. Additionally, hyperactivated STAT3 in tumor cells and tumor-associated immune cells, could enhance tumor immune evasion, or establish immune tolerance. (Fig. 2).

Numerous mechanisms which cancer cells escape from detection, include induction of immunosuppressive cytokines such as IL-6, IL-10 and TGF-β and reduction of cancer antigens, and MHC-I and MHC-II (major histocompatibility complex) molecules for T cells (4). Several lines of evidence implicate suppression of STAT3 activation elevates release of proinflammatory cytokines and/or chemokines, suggesting activation of STAT3 negatively regulates the expression of immune stimulating molecules (4). In addition, STAT3 also promotes pro-inflammatory mediators via nuclear factor kappa B (NF-kB) signaling pathways. IL-6/GP130/JAK signaling pathway promotes STAT3 recruitment in colon cancer cells and T cells, which upregulate IL-10 secretion (48, 49). Additionally, STAT3 down regulates C-X-C motif chemokine ligand 10 (CXCL10) expression, which could enhance cytotoxicity of natural killer (NK) cells (50) (Table 1). Emerging evidence indicates that STAT3 inhibitors reduce immunosurveillance, thus upregulating anti-tumor ability of immune cells.

STAT3 maintains the Cancer Stem Cells

Cancer stem cells (CSC) have a significant role in cancer initiation and progression. CSC have characteristics of self-renewal and capacity to generate various tumor cells, thus providing tumor heterogeneity. Additionally, CSC are responsible for cancer development, metastasis, and drug resistance (51). STAT3 plays significant role in the tumor inflammatory environment with high expression of ROS, leading to DNA damage and oncogene activation(52). This demonstrates that STAT3 activation is also involved in CSC regulation. Recent studies have shown that STAT3 activation is essential in various cancer types, including prostate, breast cancer, hepatocellular carcinoma (HCC), colorectal cancer, and glioblastoma (53-57).

STAT3 activation by IL-6 or ROS, results in upregulated self-renewal ability of

prostate CSSs (58). Additionally, glioma-associated-human mesenchymal stem cells (GA-hMSC) enhance glioma stemness through the IL-6/gp130/STAT3 pathway (59). High levels of aldehyde dehydrogenase (ALDH) activity in endometrial cancer, upregulates CSC activities through IL-6/JAK1/STAT3 signaling pathways. Inhibition of these pathways significantly reduced tumor cell growth (60).

Activated STAT3 in CSC, required co-expression of pluripotent stem cell markers, Oct3/4 and Nanog (61). These signaling pathways upregulate CSC markers such as CD44, thereby increasing CSC properties (62). Moreover, high levels of CSC marker, CD133, positively correlate with poor prognosis and tumor growth in HCC. On the contrary, inhibition of CD133 resulted in cell cycle arrest and tumor suppression, by downregulating cytokine-related genes. Treatment with sorafenib and nifuroxazide lead to inhibition of STAT3 activation, and CD133 expression (55). Recent investigation showed that VEGF promotes self-renewal capacity through VEGFR2/STAT3 signaling pathway, by upregulating Myc and Sox2 expression (63). Highly activated STAT3 correlates with decreased self-renewing and radiochemoresistant abilities, in thyroid cancer-derived $CD133^{+}$ cells (64). Due to the importance of STAT3 maintaining CSC properties such as self-renewing abilities in carcinogenesis, blocking this signaling pathway may eliminate CSCs in preventing cancer.

STAT3 in the tumor microenvironment

It is well known that tumor cells modify and adapt to their surrounding milieu. Constitutive activation of STAT3 promotes tumor growth through oncogenic signaling pathway, and interacts with tumor cells and their surrounding factors. Aberrant activation of STAT3 recruits immune cells and compromises their functions to benefit tumor cells (65). Additionally, STAT3 is a negative regulator of T helper 1 cells, suggesting inhibition of STAT3 activation,

promotes release of proinflammatory cytokines (4).

In the core of tumor tissue, hypoxic stress is generated and induces hypoxia-inducible factors. It is known that STAT3 regulates stability and activity of HIF-1α, inducing expression of cytokines, chemokine, and growth factors to improve cancer development (66, 67). Also, in response to surrounding tumor cells, stromal cells upregulate their C-X-C motif chemokine ligand 12 (CXCL12) receptors, resulting in enhancing metastatic potential in tumor cells (68). Additionally, activation of STAT3 promotes polarization of tumor-associated microphages as M2 phenotype and PD-L1 expression as well, which increase tumor progression. Inhibition of STAT3 activation shows anti-tumor activity by suppressing polarization of macrophages (69). In addition, activation of STAT3 in endothelial cells increases cell adhesion molecule expression and it is important for the tumor metastasis (70).

Tumor cells can evade immune response by regulating their immunological circumstance. Activation of STAT3 is crucial for immune escape of tumor cells, by promoting transforming growth factor-beta (TGF-β), VEGF, myeloid-derived suppressor cell (MDSC) expansion and suppressing NK cell function (71-73). Using STAT3 inhibitors has shown reduction of immunosuppressive response, therefore upregulating anti-tumor activity of immune cells. (Fig. 2)

Targeting STAT3 in cancer

Since STAT3 regulates a central role in cell proliferation, differentiation, apoptosis, angiogenesis, immune response and metastasis, STAT3 is rational strategy for development of novel cancer therapeutics (74). STAT3 inhibitors or agents can have two major strategies, in which STAT3 activation is inhibited, directly or indirectly. Direct inhibitors block the SH2

domain, DNA-binding domain, and N-terminal domain, which regulate STAT3 activation by blocking phosphorylation, dimerization, nuclear translocation, and DNA binding (75, 76). Indirect inhibitors target upstream regulators of STAT3 pathway, such as receptor-ligand binding and kinases.

1. SH2 domain inhibitors

The SH2 domain of STAT3 has a binding pocket to phosphorylated tyrosine (pTyr) residue, and formation of STAT3 dimerization involves pTyr interacting with the SH2 domain. Therefore, inhibiting SH2 domain of STAT3 suppresses activation of STAT3 protein. Numerous kinds of small molecule peptides have been developed as STAT3 inhibitors that directly target the SH2 domain of STAT3 by using high-throughput screening and structure-based virtual screening system. These small molecules and peptides include PY*LKTK (Y* is the phosphorylated tyrosine) (77), S3I-M2001 (78), S3I-1757 (79), curcumin-proline (80), cryptotashinone (81), STA-21 (82), Stattic (83) and S3I-201 (84) (Table 2).

2. DNA binding domain inhibitors

STAT3 has a DNA binding domain, and binds to the gene's promoter and regulates gene expression. Thus, targeting the DNA binding domain of STAT3 interrupts interaction with the promoter of target gene, thereby inhibiting activity of STAT3, and various inhibitors have been developed. These small molecules include HIC 1 (85), IS3-295 (86) and DBD-1 (87) (Table 2).

3. STAT3 upstream regulatory inhibitors

Receptor-associated and non-receptor tyrosine kinases are critical upstream regulators of STAT3 activation, so targeting these kinases has attractive potential for STAT3 activation.

KDI1, one of the receptor tyrosine kinases (RTK) inhibitor, complexes with EGFR and inhibits EGF-induced STAT3 phosphorylation (88). Another RTK inhibitor, PD153035, suppresses phosphorylation and activation of EGFR and STAT3 *in vivo*. This is reported to inhibit the growth of oral squamous cell carcinoma (89).

Additionally, STAT3 is phosphorylated by various protein kinases in the cytoplasmic region. It is well known that JAK and Src kinases are common STAT3 upstream regulators. JAK and Src kinases inhibitors have various anti-cancer effects such as inducing cancer cell apoptosis and reducing metastasis through decrease in the level of STAT3 phosphorylation (90-103). Some of these small molecule inhibitors have recently been in clinical trials for chemotherapy for various cancer treatment, and inflammatory syndromes including rheumatoid arthritis, psoriasis, and inflammatory bowel disease (IBD) (99, 104-110)

CONCLUSION

Although STAT3 expression is properly controlled in normal cells, constitutive activation of STAT3 occurs in various cancers. Aberrant activation of STAT3 provides favorable conditions for tumor metastasis involved in tumor cell proliferation, angiogenesis, invasion, and migration. In addition, induction of STAT3 signaling has a pivotal role in evasion of immune surveillance. Aberrant activation of STAT3 lead to burn out of immune cells, so, STAT3 signaling is an instigator of immune evasion in the tumor microenvironment. STAT3 signaling regulate oncogenic pathway in tumor cells, but also mediate immune evasion. Therefore, targeting STAT3 inhibits tumor progression and improves anti-tumor immune responses as well. Thus, it is a valuable therapeutic target for cancer therapy.

The tumor microenvironment consists of heterogeneous population of cancer cells and various infiltrating cells, secreted factors and extracellular matrix proteins (ECM), and their

surrounding circumstance such as blood vessels and hypoxic region. The interactions of tumor cells with their microenvironments promotes development and progression of tumor cell thought STAT3 signaling pathways, thus interrupting this signaling pathway in the tumor microenvironment is a promising target for cancer therapy.

Despite various small molecule inhibitors effectively inhibiting STAT3 signaling, further studies will be innovatively developed to improve clinical outcomes. Therefore, as this review suggests, future perspectives targeting STAT3 should focus on various combination therapies that regulate tumor cells as well as the tumor microenvironment.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

FIGURE LEGEND

Figure 1. STAT3 signaling in cancer

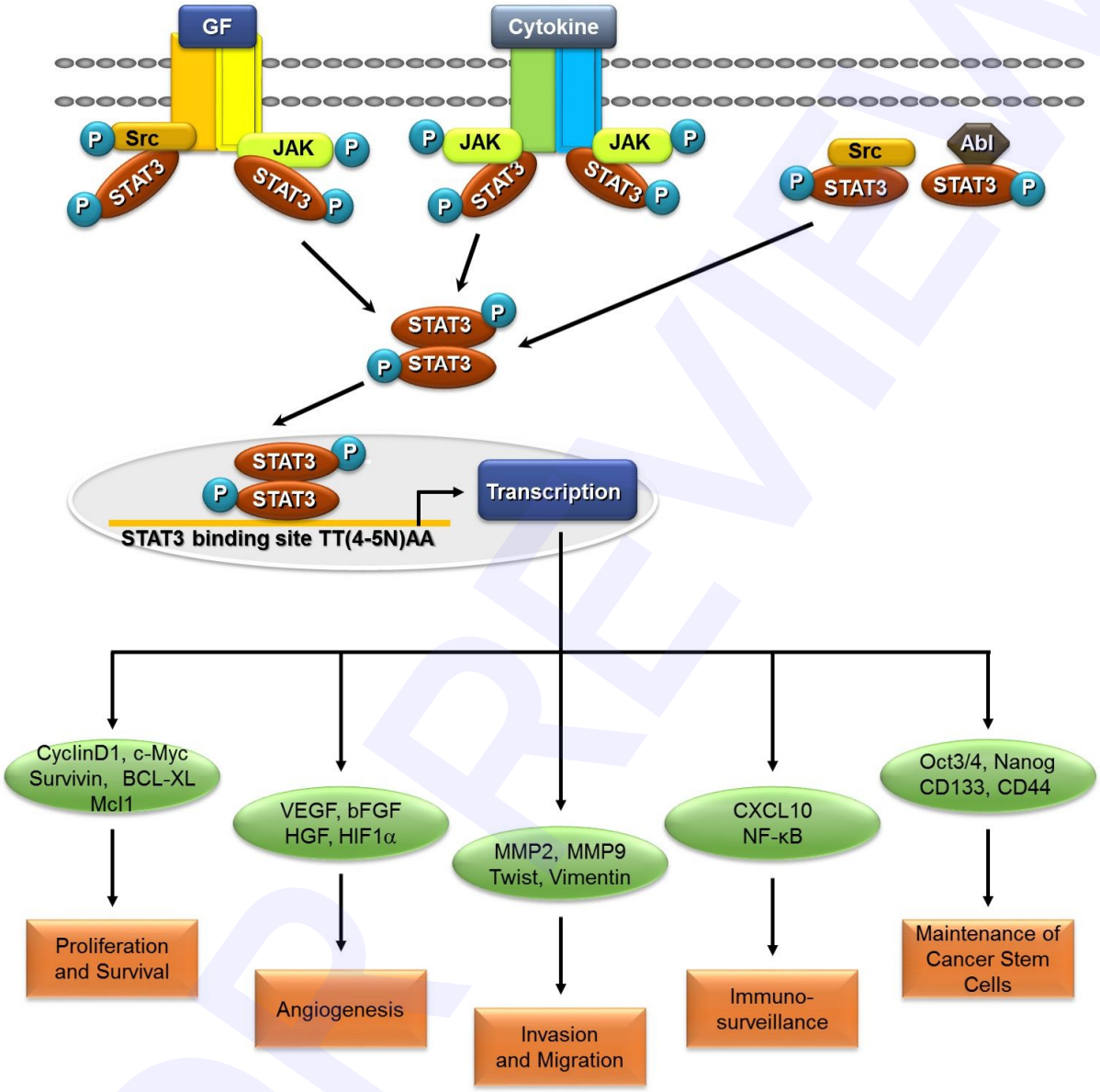

Fig 1. STAT3 signaling is activated by binding of various ligands to their cell surface receptors, leading to phosphorylation of STAT3. STAT3 also directly phosphorylated by Src and Abl, which are non-receptor tyrosine kinases. Phosphorylated STAT3 further homo-dimerized and translocated, to the nucleus. STAT3 regulate CyclinD1, c-Myc, Survivin, Bcl-XL, and Mcl1, which regulate cellular proliferation and survival. STAT3 upregulates VEGF, bFGF, HGF, and

HIF1α. Additionally, STAT3 also regulates MMP2, MMP9, Twist, and Vimentin, for invasion and migration. STAT3 activation also downregulates immune surveillance, by secretion of pro-inflammatory cytokines. Maintaining cancer stem cell properties, STAT3 regulates Oct3/4, Nanog, CD133, and CD44.

Figure 2. The role of STAT3 signaling in the tumor microenvironment

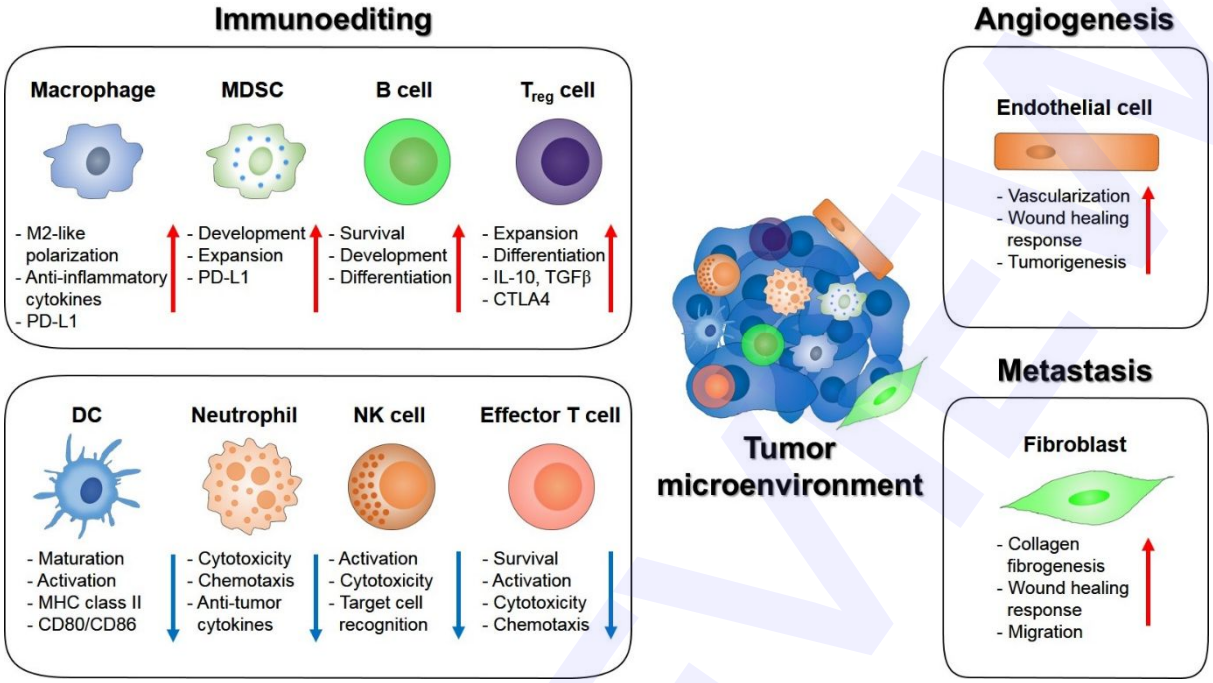

Fig 2. STAT3 signaling supports the communication between tumor cells and the tumor microenvironments. STAT3 drives immunosuppressive effects and tumor promoting effects by endothelial cells and fibroblasts. Activation of STAT3 in dendritic (DC) cell suppresses maturation, activation and antigen presentation which promotes immune tolerance. STAT3 activation in neutrophil, NK cells and effector T cell also has immunosuppressive effects. STAT3 signaling in macrophage favors M2-like polarization and increases PD-L1 expression while STAT3 activation proliferates MDSC population. STAT3 exerts immune tolerance in regulatory T (T_{reg}) cells by enhancing CTLA4 expression and tumorigenesis in B cells by promoting survival, proliferation and development. STAT3 effect on endothelial cells to promote tumor vascularization. STAT3 in tumor associated fibroblast also enhance tumor metastasis. Collectively, STAT3 signaling is a key regulator of hall mark of cancers.

Table 1. The target genes of STAT3

Function	Upregulated gene	Downregulated gene	Refs.
Proliferation	BCL-XL		[26]
	c-MYC		[23]
	Mcl1		[27]
	Survivin		[25]
	Cyclin-D1		[24]
Angiogenesis	VEGF		[32, 33,35]
	HIF-1α		[34,35]
	HGF		[36]
	bFGF		[36]
		IL-12	[4]
		IFNβ	[4]
		IFNγ	[8]
		CXCL10	[4]
Metastasis	MMP2		[42]
	MMP9		[43]
	MMP1		[44]
	TWIST1		[37]
	Vimentin		[38]
	HMGB1		[40]
	ZEB1		[41]
Immune escape	IL-6		[4, 47, 48]
	IL-10		[47]
		IFNβ	[4]
		IFNγ	[8]
		IL-12	[4]
		CD80	[4]
		CD86	[4]
		CCL5	[4]
		CXCL10	[4,49]

Table 2. Small molecule inhibitors directly/indirectly targeting STAT3

Inhibitor Name	Mechanism of Action	Cancer Type	Refs.
PY*LKTK	SH2 domain inhibitor	NIH 3T3/v-Src fibroblasts	[75]
S3I-M2001	SH2 domain inhibitor	Breast cancer	[76]
S3I-1757	SH2 domain inhibitor	Breast and lung cancer	[77]
Curcumin-proline	SH2 domain inhibitor		[78]
Cryptotashinone	SH2 domain inhibitor	Prostate cancer	[79]
STA-21	SH2 domain inhibitor	Breast cancer	[80]
Stattic	SH2 domain inhibitor	Breast cancer	[81]
S3I-201	SH2 domain inhibitor	Breast cancer, prostate cancer, acute myeloid leukemia and human multiple myeloma	[82]
HIC 1	DNA binding domain inhibitor	Breast cancer	[83]
IS3-295	DNA binding domain inhibitor	Colon cancer	[84]
DBD-1	DNA binding domain inhibitor	Melanoma	[85]
KDI1	RTK inhibitor	Vulval and breast cancer	[86]
PD153035	RTK inhibitor	Oral squamous carcinoma	[87]
AG490	JAK kinase inhibitor	Pancreatic cancer	[88]
WP1066	JAK kinase inhibitor	Acute myelogenous leukemia	[89]
TG101209	JAK2 kinase inhibitor	Acute myeloid leukemia	[90]
AZD1480	JAK kinase inhibitor	Myeloma, Neuroblastoma and Pediatric sarcomas	[91, 92]
Dasatinib	Src and PDGF inhibitor	Synovial sarcoma, hepatocellular carcinoma, glioma, prostate cancer	[93]
PP2	Src inhibitor	Intestinal epithelial cell	[97, 100]
KX2-391	Src inhibitor	Prostate cancer	[98]
AZD0530	Src inhibitor	Melanoma	[99]
MLS-2384	Src and JAK inhibitor	Prostate, breast, skin, ovarian, lung, and liver cancer	[108]
Sophoraflavanone G	Src and JAK inhibitor	Breast, prostate, lymphoma, human multiple myeloma, large cell lung cancer, colorectal carcinoma	[101]

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