Highlighted STAT3 as a potential drug target for cancer therapy

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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. [BMB Reports 2019; 52(7): 415-423]

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 immuneediting (3-5).

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. 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 cancer, 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).
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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 CyclinD1, 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 Aclt (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.

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 (HIF-1α), 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).

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 homebox 1), and so on (38-42). Persistent STAT3 activation, leads to upregulated expression of MMP2 (matrix metalloproteinase 2) (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 aggressiveness.

**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-κB) 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 downregulates CX-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 immune evasion, thus upregulating anti-tumor ability of immune cells.

**STAT3 maintains the cancer stem cells**

Cancer stem cells (CSCs) have a significant role in cancer initiation and progression. CSCs have characteristics of self-renewal and capacity to generate various tumor cells, thus providing tumor heterogeneity. Additionally, CSCs 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 CSCs 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 CSCs (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 CSCs 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 increased 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.

### 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)                |       |
|                     | IFNγ             | (4)                |       |
|                     | IFNα             | (8)                |       |
|                     | CXCL10           | (4)                |       |
| Metastasis          | MMP2             | (42)               |       |
|                     | MMP9             | (43)               |       |
|                     | MMP1             | (44)               |       |
|                     | TIMP1            | (37)               |       |
|                     | Vimentin         | (38)               |       |
|                     | HMGB1            | (40)               |       |
|                     | ZEB1             | (41)               |       |
| Immune escape       | IL-6             | (4, 47)            |       |
|                     | IL-10            | (47)               |       |
|                     | IFNβ             | (4)                |       |
|                     | IFNγ             | (8)                |       |
|                     | IL-12            | (4)                |       |
|                     | CD80             | (4)                |       |
|                     | CD86             | (4)                |       |
|                     | CCL5             | (4)                |       |
|                     | CXCL10           | (4, 49)            |       |

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Fig. 2. The role of STAT3 signaling in the tumor microenvironment. 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) cells suppresses maturation, activation and antigen presentation which promotes immune tolerance. STAT3 activation in neutrophil, NK cells and effector T cells also has immunosuppressive effects. STAT3 signaling in macrophage favors M2-like polarization and increases PD-L1 expression while STAT3 activation promotes M2 polarization. STAT3 exerts immune tolerance in regulatory T (Treg) cells by enhancing CTLA4 expression and tumor immunity in B cells by promoting survival, proliferation and development. STAT3 effect on endothelial cells to promote tumor vascularization. STAT3 in tumor associated fibroblast also enhances tumor progression. Collectively, STAT3 signaling is a key regulator of hallmark of cancers.

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 therefore 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-β (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 a 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.

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 (Table 2) (84).

**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 (Table 2) (87).

**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, migration, and invasion. In addition, induction of STAT3 signaling has a pivotal role in evasion of immune surveillance. Aberrant activation of STAT3 leads to burn out of immune cells, so, STAT3 signaling is an

| 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 | Prostate cancer | (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) |
| Dusatinib     | 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) |
| Sophoraffilavanone G | Src and JAK inhibitor | Breast, prostate, lymphoma, human multiple myeloma, large cell lung cancer, colorectal carcinoma | (101) |
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 (ECM) proteins, 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 have no conflicting interests.

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