

## Invited Mini Review

## Highlighted STAT3 as a potential drug target for cancer therapy

Haeri Lee<sup>1</sup>, Ae Jin Jeong<sup>1</sup> & Sang-Kyu Ye<sup>1,2,3,4,\*</sup>

<sup>1</sup>Department of Pharmacology and Biomedical Sciences, Seoul National University College of Medicine, Seoul 03080, <sup>2</sup>Ischemic/Hypoxic Disease Institute, Seoul National University College of Medicine, Seoul 03080, <sup>3</sup>Neuro-Immune Information Storage Network Research Center, Seoul National University College of Medicine, Seoul 03080, <sup>4</sup>Biomedical Science Project (BK21PLUS), Seoul National University College of Medicine, Seoul 03080, Korea

**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 immunoediting (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

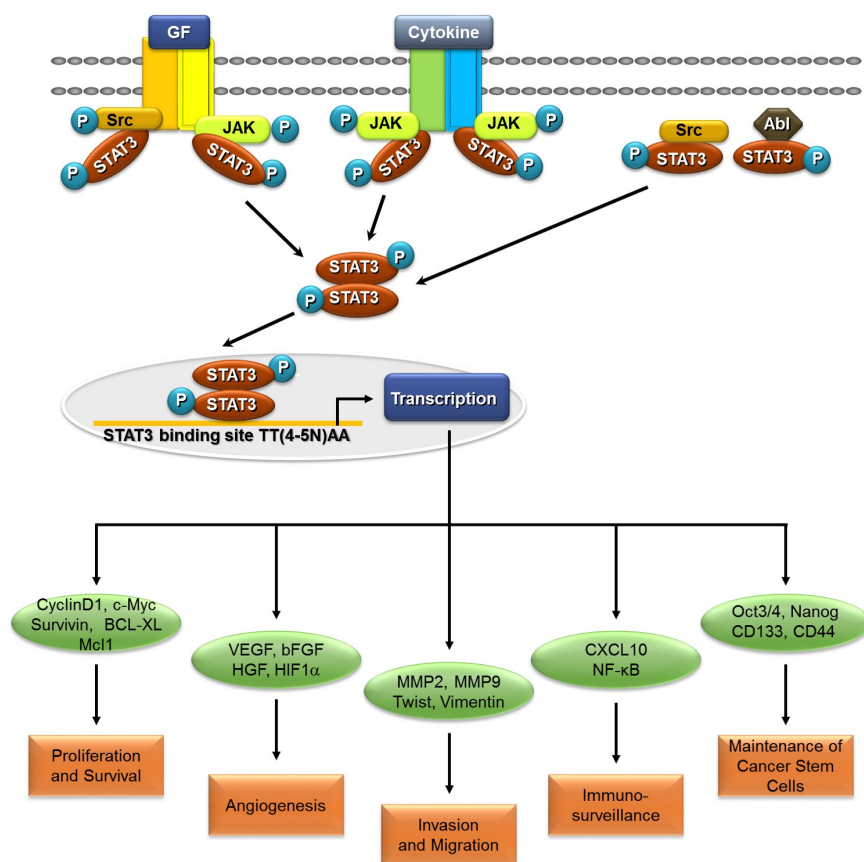
Aberrant activation of STAT3 has been involved in oncogenesis and malignant phenotypes in human cancers (10, 11). Hyperactivation of STAT3 has been reported in several types of tumors, including head-and neck, brain, breast, liver, lung, kidney, pancreas, prostate, ovary cancer, and multiple myeloma, 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).

\*Corresponding author. Tel: +82-2-740-8281; Fax: +82-2-745-7996; E-mail: sangkyu@snu.ac.kr

<https://doi.org/10.5483/BMBRep.2019.52.7.152>

Received 15 May 2019

**Keywords:** Cancer stem cells, Cancer therapy, Immune suppression, STAT3, Tumor microenvironment



**Fig. 1.** STAT3 signaling in cancer. 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 up-regulates VEGF, bFGF, HGF, and HIF1 $\alpha$ . 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. Furthermore, maintaining cancer stem cell properties, STAT3 regulates Oct3/4, Nanog, CD133, and CD44.

### 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 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.

### 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-1 $\alpha$  (HIF-1 $\alpha$ ), another regulator of angiogenesis (35). During hypoxic conditions in core of cancer cells, STAT3 and HIF1 $\alpha$  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 homeobox 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

**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-1 $\alpha$		(34,35)
	HGF		(36)
	bFGF		(36)
		IL-12	(4)
		IFN $\beta$	(4)
		IFN $\gamma$	(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)
		IFN $\beta$	(4)
		IFN $\gamma$	(8)
		IL-12	(4)
		CD80	(4)
		CD86	(4)
		CCL5	(4)
		CXCL10	(4,49)

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- $\beta$  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

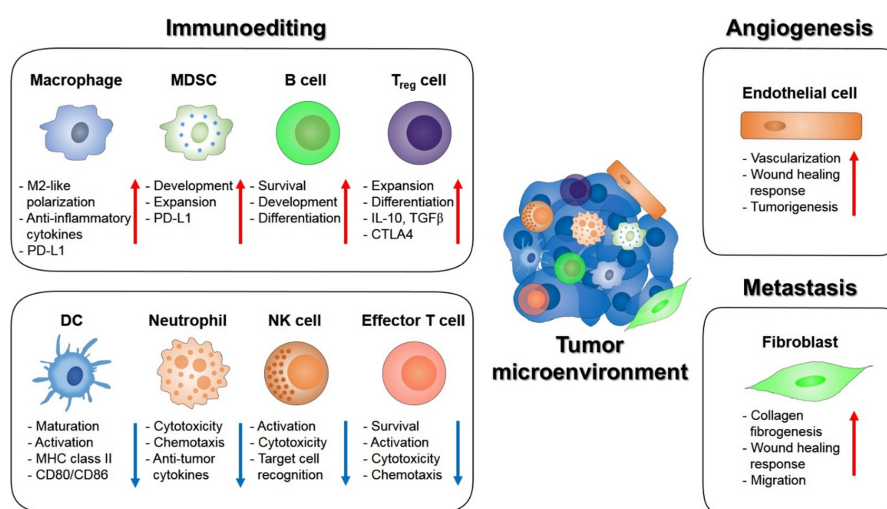
proinflammatory cytokines and/or chemokines, suggesting activation of STAT3 negatively regulates the expression of immune stimulating molecules (4). In addition, STAT3 also promotes pro-inflammatory mediators via nuclear factor kappa B (NF- $\kappa$ B) signaling pathways. IL-6/GP130/JAK signaling pathway promotes STAT3 recruitment in colon cancer cells and T cells, which upregulate IL-10 secretion (48, 49). Additionally, STAT3 downregulates 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 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<sup>+</sup> 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.



**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 proliferates MDSC population. STAT3 exerts immune tolerance in regulatory T (T<sub>reg</sub>) 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 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-1 $\alpha$ , 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 macrophages 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- $\beta$ ), 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.

### 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

**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)

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 through 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.

## ACKNOWLEDGEMENTS

This work was supported by grants from the National Research Foundation of Korea (NRF) funded by the Korea government (NRF-2014R1A2A1A11053203, NRF-2017R1A2B2006839 and NRF-2018R1A5A2025964), and the Seoul National University Hospital (SNUH) Research Fund (0320190210).

## CONFLICTS OF INTEREST

The authors have no conflicting interests.

## REFERENCES

1. Hanahan D and Weinberg Robert A (2011) Hallmarks of Cancer: The Next Generation. *Cell* 144, 646-674
2. Yu H and Jove R (2004) The STATs of cancer — new molecular targets come of age. *Nat Rev Cancer* 4, 97-105
3. Chen J, Wang J, Lin L et al (2012) Inhibition of STAT3 Signaling Pathway by Nitidine Chloride Suppressed the Angiogenesis and Growth of Human Gastric Cancer. *Mol Cancer Ther* 11, 277-287
4. Wang T, Niu G, Kortylewski M et al (2004) Regulation of the innate and adaptive immune responses by Stat-3 signaling in tumor cells. *Nat Med* 10, 48-54
5. Huynh J, Chand A, Gough D and Ernst M (2019) Therapeutically exploiting STAT3 activity in cancer — using tissue repair as a road map. *Nat Rev Cancer* 19, 82-96
6. Joyce JA and Fearon DT (2015) T cell exclusion, immune privilege, and the tumor microenvironment. *Science* 348, 74-80
7. Spill F, Reynolds DS, Kamm RD and Zaman MH (2016) Impact of the physical microenvironment on tumor progression and metastasis. *Curr Opin Biotechnol* 40, 41-48
8. Herrmann A, Kortylewski M, Kujawski M et al (2010) Targeting Stat3 in the Myeloid Compartment Drastically Improves the *in vivo* Antitumor Functions of Adoptively Transferred T Cells. *Cancer Res* 70, 7455-7464
9. Kortylewski M and Yu H (2008) Role of Stat3 in suppressing anti-tumor immunity. *Curr Opin Immunol* 20, 228-233
10. Frank DA (2007) STAT3 as a central mediator of neoplastic cellular transformation. *Cancer Lett* 251, 199-210
11. Roeser JC, Leach SD and McAllister F (2015) Emerging strategies for cancer immunoprevention. *Oncogene* 34, 6029
12. Sonnenblick A, Shriki A, Galun E et al (2012) Tissue microarray-based study of patients with lymph node-positive breast cancer shows tyrosine phosphorylation of signal transducer and activator of transcription 3 (tyrosine705-STAT3) is a marker of good prognosis. *Clin Transl Oncol* 14, 232-236
13. Schaefer LK, Ren Z, Fuller GN and Schaefer TS (2002) Constitutive activation of Stat3 $\alpha$  in brain tumors: localization to tumor endothelial cells and activation by the endothelial tyrosine kinase receptor (VEGFR-2). *Oncogene* 21, 2058-2065
14. Geiger JL, Grandis JR and Bauman JE (2016) The STAT3 pathway as a therapeutic target in head and neck cancer: barriers and innovations. *Oral Oncology* 56, 84-92
15. Li S, Priceman SJ, Xin H et al (2013) Icaritin Inhibits JAK/STAT3 Signaling and Growth of Renal Cell Carcinoma. *PLoS One* 8, e81657
16. He G and Karin M (2010) NF- $\kappa$ B and STAT3 — key players in liver inflammation and cancer. *Cell Res* 21, 159
17. Saini U, Naidu S, ElNaggar AC et al (2016) Elevated STAT3 expression in ovarian cancer ascites promotes invasion and metastasis: a potential therapeutic target. *Oncogene* 36, 168
18. Bar-Natan M, Nelson EA, Xiang M and Frank DA (2012) STAT signaling in the pathogenesis and treatment of myeloid malignancies. *JAK-STAT* 1, 55-64
19. Fukuda A, Wang Sam C, Morris John P et al (2011) Stat3 and MMP7 Contribute to Pancreatic Ductal Adenocarcinoma Initiation and Progression. *Cancer Cell* 19, 441-455
20. Redell MS, Ruiz MJ, Alonzo TA, Gerbing RB and Tweardy DJ (2011) Stat3 signaling in acute myeloid leukemia: ligand-dependent and -independent activation and induction of apoptosis by a novel small-molecule Stat3 inhibitor. *Blood* 117, 5701-5709
21. Alas S and Bonavida B (2003) Inhibition of Constitutive STAT3 Activity Sensitizes Resistant Non-Hodgkin's Lymphoma and Multiple Myeloma to Chemotherapeutic Drug-mediated Apoptosis. *Clin Cancer Res* 9, 316-326
22. Yu H, Pardoll D and Jove R (2009) STATs in cancer inflammation and immunity: a leading role for STAT3. *Nat Rev Cancer* 9, 798
23. Bromberg J and Darnell JE (2000) The role of STATs in

- transcriptional control and their impact on cellular function. *Oncogene* 19, 2468-2473
24. Kim BH, Yi EH and Ye SK (2016) Signal transducer and activator of transcription 3 as a therapeutic target for cancer and the tumor microenvironment. *Arch Pharm Res* 39, 1085-1099
  25. Horiguchi A, Oya M, Marumo K and Murai M (2002) STAT3, but not ERKs, mediates the IL-6-induced proliferation of renal cancer cells, ACHN and 769P. *Kidney Int* 61, 926-938
  26. Lin L, Liu A, Peng Z et al (2011) STAT3 Is Necessary for Proliferation and Survival in Colon Cancer-Initiating Cells. *Cancer Res* 71, 7226-7237
  27. Corvinus FM, Orth C, Moriggl R et al (2005) Persistent STAT3 Activation in Colon Cancer Is Associated with Enhanced Cell Proliferation and Tumor Growth. *Neoplasia* 7, 545-555
  28. Catlett-Falcone R, Landowski TH, Oshiro MM et al (1999) Constitutive Activation of Stat3 Signaling Confers Resistance to Apoptosis in Human U266 Myeloma Cells. *Immunity* 10, 105-115
  29. Epling-Burnette PK, Liu JH, Catlett-Falcone R et al (2001) Inhibition of STAT3 signaling leads to apoptosis of leukemic large granular lymphocytes and decreased Mcl-1 expression. *J Clin Invest* 107, 351-362
  30. Chen H, Yang Z, Ding C et al (2013) Discovery of O-Alkylamino-Tethered Niclosamide Derivatives as Potent and Orally Bioavailable Anticancer Agents. *ACS Med Chem Lett* 4, 180-185
  31. Kanai M, Konda Y, Nakajima T et al (2003) Differentiation-inducing factor-1 (DIF-1) inhibits STAT3 activity involved in gastric cancer cell proliferation via MEK-ERK-dependent pathway. *Oncogene* 22, 548-554
  32. Pancotti F, Roncuzzi L, Maggiolini M and Gasperi-Campani A (2012) Caveolin-1 silencing arrests the proliferation of metastatic lung cancer cells through the inhibition of STAT3 signaling. *Cell Signal* 24, 1390-1397
  33. Wei D, Le X, Zheng L et al (2003) Stat3 activation regulates the expression of vascular endothelial growth factor and human pancreatic cancer angiogenesis and metastasis. *Oncogene* 22, 319-329
  34. Kujawski M, Kortylewski M, Lee H, Herrmann A, Kay H and Yu H (2008) Stat3 mediates myeloid cell-dependent tumor angiogenesis in mice. *J Clin Invest* 118, 3367-3377
  35. Xu Q, Briggs J, Park S et al (2005) Targeting Stat3 blocks both HIF-1 and VEGF expression induced by multiple oncogenic growth signaling pathways. *Oncogene* 24, 5552-5560
  36. Jung JE, Lee HG, Cho IH et al (2005) STAT3 is a potential modulator of HIF-1-mediated VEGF expression in human renal carcinoma cells. *FASEB J* 19, 1296-1298
  37. Wojcik EJ, Sharifpoor S, Miller NA et al (2006) A novel activating function of c-Src and Stat3 on HGF transcription in mammary carcinoma cells. *Oncogene* 25, 2773-2784
  38. Cheng GZ, Zhang W, Sun M et al (2008) Twist Is Transcriptionally Induced by Activation of STAT3 and Mediates STAT3 Oncogenic Function. *J Biol Chem* 283, 14665-14673
  39. Banerjee K and Resat H (2016) Constitutive activation of STAT3 in breast cancer cells: A review. *Int J Cancer* 138, 2570-2578
  40. Wendt MK, Balanis N, Carlin CR and Schiemann WP (2014) STAT3 and epithelial-mesenchymal transitions in carcinomas. *JAK-STAT* 3, e28975-e28975
  41. Chen M, Liu Y, Varley P et al (2015) High-Mobility Group Box 1 Promotes Hepatocellular Carcinoma Progression through miR-21-Mediated Matrix Metalloproteinase Activity. *Cancer Res* 75, 1645-1656
  42. Xiong H, Hong J, Du W et al (2012) Roles of STAT3 and ZEB1 proteins in E-cadherin down-regulation and human colorectal cancer epithelial-mesenchymal transition. *J Biol Chem* 287, 5819-5832
  43. Kalluri R (2003) Basement membranes: structure, assembly and role in tumour angiogenesis. *Nat Rev Cancer* 3, 422-433
  44. Dechow TN, Pedranzini L, Leitch A et al (2004) Requirement of matrix metalloproteinase-9 for the transformation of human mammary epithelial cells by Stat3-C. *Proc Natl Acad Sci U S A* 101, 10602-10607
  45. Itoh M, Murata T, Suzuki T et al (2006) Requirement of STAT3 activation for maximal collagenase-1 (MMP-1) induction by epidermal growth factor and malignant characteristics in T24 bladder cancer cells. *Oncogene* 25, 1195-1204
  46. Sano S, Itami S, Takeda K et al (1999) Keratinocyte-specific ablation of Stat3 exhibits impaired skin remodeling, but does not affect skin morphogenesis. *EMBO J* 18, 4657-4668
  47. Silver DL, Naora H, Liu J, Cheng W and Montell DJ (2004) Activated signal transducer and activator of transcription (STAT) 3: localization in focal adhesions and function in ovarian cancer cell motility. *Cancer Res* 64, 3550-3558
  48. Herbeuval J-P, Lelievre E, Lambert C, Dy M and Genin C (2004) Recruitment of STAT3 for Production of IL-10 by Colon Carcinoma Cells Induced by Macrophage-Derived IL-6. *J Immunol* 172, 4630-4636
  49. Stumhofer JS, Silver JS, Laurence A et al (2007) Interleukins 27 and 6 induce STAT3-mediated T cell production of interleukin 10. *Nat Immunol* 8, 1363
  50. Saudemont A, Jouy N, Hetuin D and Quesnel B (2005) NK cells that are activated by CXCL10 can kill dormant tumor cells that resist CTL-mediated lysis and can express B7-H1 that stimulates T cells. *Blood* 105, 2428-2435
  51. Thakur R, Trivedi R, Rastogi N, Singh M and Mishra DP (2015) Inhibition of STAT3, FAK and Src mediated signaling reduces cancer stem cell load, tumorigenic potential and metastasis in breast cancer. *Sci Rep* 5, 10194
  52. Blaylock R (2015) Cancer microenvironment, inflammation and cancer stem cells: A hypothesis for a paradigm change and new targets in cancer control. *Surg Neurol Int* 6, 92
  53. Liu X, He Z, Li C-H, Huang G, Ding C and Liu H (2012) Correlation analysis of JAK-STAT pathway components on prognosis of patients with prostate cancer. *Pathol Oncol Res* 18, 17-23

54. Wei W, Twardy DJ, Zhang M et al (2014) STAT3 Signaling Is Activated Preferentially in Tumor-Initiating Cells in Claudin-Low Models of Human Breast Cancer. *Stem Cells* 32, 2571-2582
55. Won C, Kim B-H, Yi EH et al (2015) Signal transducer and activator of transcription 3-mediated CD133 up-regulation contributes to promotion of hepatocellular carcinoma. *Hepatology* 62, 1160-1173
56. Zhang X, Hu F, Li G et al (2018) Human colorectal cancer-derived mesenchymal stem cells promote colorectal cancer progression through IL-6/JAK2/STAT3 signaling. *Cell Death Dis* 9, 25
57. Haftchenary S, Luchman HA, Jouk AO et al (2013) Potent Targeting of the STAT3 Protein in Brain Cancer Stem Cells: A Promising Route for Treating Glioblastoma. *ACS Med Chem Lett* 4, 1102-1107
58. Qu Y, Oyan AM, Liu R et al (2013) Generation of Prostate Tumor-Initiating Cells Is Associated with Elevation of Reactive Oxygen Species and IL-6/STAT3 Signaling. *Cancer Res* 73, 7090-7100
59. Hossain A, Gumin J, Gao F et al (2015) Mesenchymal Stem Cells Isolated From Human Gliomas Increase Proliferation and Maintain Stemness of Glioma Stem Cells Through the IL-6/gp130/STAT3 Pathway. *Stem Cells* 33, 2400-2415
60. van der Zee M, Sacchetti A, Cansoy M et al (2015) IL6/JAK1/STAT3 Signaling Blockade in Endometrial Cancer Affects the ALDH<sup>hi</sup>/CD126<sup>+</sup> Stem-like Component and Reduces Tumor Burden. *Cancer Res* 75, 3608-3622
61. Gibbs CP, Kukekov VG, Reith JD et al (2005) Stem-like cells in bone sarcomas: implications for tumorigenesis. *Neoplasia (New York, N.Y.)* 7, 967-976
62. Marotta LLC, Almendro V, Marusyk A et al (2011) The JAK2/STAT3 signaling pathway is required for growth of CD44<sup>+</sup>CD24<sup>-</sup> stem cell-like breast cancer cells in human tumors. *J Clin Invest* 121, 2723-2735
63. Zhao D, Pan C, Sun J et al (2014) VEGF drives cancer-initiating stem cells through VEGFR-2/Stat3 signaling to upregulate Myc and Sox2. *Oncogene* 34, 3107
64. Tseng L-M, Huang P-I, Chen Y-R et al (2012) Targeting Signal Transducer and Activator of Transcription 3 Pathway by Cucurbitacin I Diminishes Self-Renewing and Radiochemoresistant Abilities in Thyroid Cancer-Derived CD133<sup>+</sup> Cells. *J Pharmacol Exp Ther* 341, 410-423
65. Groner B, Lucks P and Borghouts C (2008) The function of Stat3 in tumor cells and their microenvironment. *Semin Cell Dev Biol* 19, 341-350
66. Jung JE, Kim HS, Lee CS et al (2008) STAT3 inhibits the degradation of HIF-1 $\alpha$  by pVHL-mediated ubiquitination. *Exp Mol Med* 40, 479
67. Samavati L, Rastogi R, Du W, Hüttemann M, Fite A and Franchi L (2009) STAT3 tyrosine phosphorylation is critical for interleukin 1 beta and interleukin-6 production in response to lipopolysaccharide and live bacteria. *Mol Immunol* 46, 1867-1877
68. Gao H, Priebe W, Glod J and Banerjee D (2009) Activation of Signal Transducers and Activators of Transcription 3 and Focal Adhesion Kinase by Stromal Cell-Derived Factor 1 Is Required for Migration of Human Mesenchymal Stem Cells in Response to Tumor Cell-Conditioned Medium. *Stem Cells* 27, 857-865
69. Fujiwara Y, Takeya M and Komohara Y (2014) A novel strategy for inducing the antitumor effects of triterpenoid compounds: blocking the protumoral functions of tumor-associated macrophages via STAT3 inhibition. *Biomed Res Int* 2014, 348539
70. Kim KJ, Kwon SH, Yun JH et al (2017) STAT3 activation in endothelial cells is important for tumor metastasis via increased cell adhesion molecule expression. *Oncogene* 36, 5445
71. Kujawski M, Kortylewski M, Lee H, Herrmann A, Kay H and Yu H (2008) Stat3 mediates myeloid cell-dependent tumor angiogenesis in mice. *J Clin Invest* 118, 3367-3377
72. Wu L, Du H, Li Y, Qu P and Yan C (2011) Signal transducer and activator of transcription 3 (Stat3C) promotes myeloid-derived suppressor cell expansion and immune suppression during lung tumorigenesis. *Am J Pathol* 179, 2131-2141
73. Sun X, Sui Q, Zhang C, Tian Z and Zhang J (2013) Targeting Blockage of STAT3 in Hepatocellular Carcinoma Cells Augments NK Cell Functions via Reverse Hepatocellular Carcinoma-Induced Immune Suppression. *Mol Cancer Ther* 12, 2885-2896
74. Haura EB, Turkson J and Jove R (2005) Mechanisms of Disease: insights into the emerging role of signal transducers and activators of transcription in cancer. *Nat Clin Pract Oncol* 2, 315-324
75. Debnath B, Xu S and Neamati N (2012) Small Molecule Inhibitors of Signal Transducer and Activator of Transcription 3 (Stat3) Protein. *J Med Chem* 55, 6645-6668
76. Jinxia D, Fedora G and Nouri N (2007) Small Molecule Inhibitors of Stat3 Signaling Pathway. *Curr Cancer Drug Targets* 7, 91-107
77. Turkson J, Ryan D, Kim JS et al (2001) Phosphotyrosyl Peptides Block Stat3-mediated DNA Binding Activity, Gene Regulation, and Cell Transformation. *J Biol Chem* 276, 45443-45455
78. Siddiquee KAZ, Gunning PT, Glenn M et al (2007) An Oxazole-Based Small-Molecule Stat3 Inhibitor Modulates Stat3 Stability and Processing and Induces Antitumor Cell Effects. *ACS Chem Biol* 2, 787-798
79. Zhang X, Sun Y, Pireddu R et al (2013) A Novel Inhibitor of STAT3 Homodimerization Selectively Suppresses STAT3 Activity and Malignant Transformation. *Cancer Res* 73, 1922-1933
80. Kumar A and Bora U (2012) Molecular docking studies on inhibition of Stat3 dimerization by curcumin natural derivatives and its conjugates with amino acids. *Bioinformation* 8, 988-993
81. Shin D-S, Kim H-N, Shin KD et al (2009) Cryptotanshinone Inhibits Constitutive Signal Transducer and Activator of Transcription 3 Function through Blocking the Dimerization in DU145 Prostate Cancer Cells. *Cancer Res* 69, 193-202
82. Song H, Wang R, Wang S and Lin J (2005) A low-molecular-weight compound discovered through virtual database screening inhibits Stat3 function in



- breast cancer cells. *Proc Natl Acad Sci U S A* 102, 4700-4705
83. Schust J, Sperl B, Hollis A, Mayer TU and Berg T (2006) Stattic: A Small-Molecule Inhibitor of STAT3 Activation and Dimerization. *Chem Biol* 13, 1235-1242
  84. Fletcher S, Page BDG, Zhang X et al (2011) Antagonism of the Stat3-Stat3 Protein Dimer with Salicylic Acid Based Small Molecules. *Chem Med Chem* 6, 1459-1470
  85. Lin Y-M, Wang C-M, Jeng J-C, Leprince D and Shih H-M (2013) HIC1 interacts with and modulates the activity of STAT3. *Cell Cycle* 12, 2266-2276
  86. Turkson J, Zhang S, Palmer J et al (2004) Inhibition of constitutive signal transducer and activator of transcription 3 activation by novel platinum complexes with potent antitumor activity. *Mol Cancer Ther* 3, 1533-1542
  87. Nagel-Wolfrum K, Buerger C, Wittig I, Butz K, Hoppe-Seyler F and Groner B (2004) The Interaction of Specific Peptide Aptamers With the DNA Binding Domain and the Dimerization Domain of the Transcription Factor Stat3 Inhibits Transactivation and Induces Apoptosis in Tumor Cells. *Mol Cancer Res* 2, 170-182
  88. Buerger C, Nagel-Wolfrum K, Kunz C et al (2003) Sequence-specific Peptide Aptamers, Interacting with the Intracellular Domain of the Epidermal Growth Factor Receptor, Interfere with Stat3 Activation and Inhibit the Growth of Tumor Cells. *J Biol Chem* 278, 37610-37621
  89. Ge H, Liu H, Fu Z and Sun Z (2012) Therapeutic and Preventive Effects of an Epidermal Growth Factor Receptor Inhibitor on Oral Squamous Cell Carcinoma. *J Int Med Res* 40, 455-466
  90. Huang C, Cao J, Huang KJ et al (2006) Inhibition of STAT3 activity with AG490 decreases the invasion of human pancreatic cancer cells in vitro. *Cancer Sci* 97, 1417-1423
  91. Ferrajoli A, Faderl S, Van Q et al (2007) WP1066 Disrupts Janus Kinase-2 and Induces Caspase-Dependent Apoptosis in Acute Myelogenous Leukemia Cells. *Cancer Res* 67, 11291-11299
  92. Pardanani A, Hood J, Lasho T et al (2007) TG101209, a small molecule JAK2-selective kinase inhibitor potently inhibits myeloproliferative disorder-associated JAK2V617F and MPLW515L/K mutations. *Leukemia* 21, 1658
  93. Scuto A, Krejci P, Popplewell L et al (2010) The novel JAK inhibitor AZD1480 blocks STAT3 and FGFR3 signaling, resulting in suppression of human myeloma cell growth and survival. *Leukemia* 25, 538
  94. Yan S, Li Z and Thiele CJ (2013) Inhibition of STAT3 with orally active JAK inhibitor, AZD1480, decreases tumor growth in Neuroblastoma and Pediatric Sarcomas In vitro and In vivo. *Oncotarget* 4, 433-445
  95. Chen Z, Lee FY, Bhalla KN and Wu J (2006) Potent Inhibition of Platelet-Derived Growth Factor-Induced Responses in Vascular Smooth Muscle Cells by BMS-354825 (Dasatinib). *Mol Pharmacol* 69, 1527-1533
  96. Michels S, Trautmann M, Sievers E et al (2013) SRC Signaling Is Crucial in the Growth of Synovial Sarcoma Cells. *Cancer Res* 73, 2518-2528
  97. Chang AY and Wang M (2013) Molecular mechanisms of action and potential biomarkers of growth inhibition of dasatinib (BMS-354825) on hepatocellular carcinoma cells. *BMC Cancer* 13, 267
  98. Premkumar D, Jane E, Agostino N, Scialabba J and Pollack I (2010) Dasatinib synergizes with JSI-124 to inhibit growth and migration and induce apoptosis of malignant human glioma cells. *J Carcinog* 9, 7
  99. Oyaizu T, Fung SY, Shiozaki A et al (2012) Src tyrosine kinase inhibition prevents pulmonary ischemia-reperfusion-induced acute lung injury. *Intensive Care Med* 38, 894-905
  100. Antonarakis ES, Heath EI, Posadas EM et al (2013) A phase 2 study of KX2-391, an oral inhibitor of Src kinase and tubulin polymerization, in men with bone-metastatic castration-resistant prostate cancer. *Cancer Chemotherapy and Pharmacology* 71, 883-892
  101. Gangadhar TC, Clark JI, Karrison T and Gajewski TF (2013) Phase II study of the Src kinase inhibitor saracatinib (AZD0530) in metastatic melanoma. *Invest New Drugs* 31, 769-773
  102. Seltana A, Guezguez A, Lepage M, Basora N and Beaulieu JF (2013) Src family kinase inhibitor PP2 accelerates differentiation in human intestinal epithelial cells. *Biochem Biophys Res Commun* 430, 1195-1200
  103. Kim BH, Won C, Lee YH et al (2013) Sophoraflavanone G induces apoptosis of human cancer cells by targeting upstream signals of STATs. *Biochem Pharmacol* 86, 950-959
  104. Aittomäki S and Pesu M (2014) Therapeutic Targeting of the JAK/STAT Pathway. *Basic Clin Pharmacol Toxicol* 114, 18-23
  105. Buchert M, Burns CJ and Ernst M (2015) Targeting JAK Kinase in solid tumors: emerging opportunities and challenges. *Oncogene* 35, 939
  106. Plimack ER, Lorusso PM, McCoon P et al (2013) AZD1480: a phase I study of a novel JAK2 inhibitor in solid tumors. *Oncologist* 18, 819-820
  107. Furtek SL, Backos DS, Matheson CJ and Reigan P (2016) Strategies and Approaches of Targeting STAT3 for Cancer Treatment. *ACS Chem Biol* 11, 308-318
  108. Puls LN, Eadens M and Messersmith W (2011) Current status of SRC inhibitors in solid tumor malignancies. *Oncologist* 16, 566-578
  109. Nam S, Wen W, Schroeder A et al (2013) Dual inhibition of Janus and Src family kinases by novel indirubin derivative blocks constitutively-activated Stat3 signaling associated with apoptosis of human pancreatic cancer cells. *Mol Oncol* 7, 369-378
  110. Liu L, Gaboriaud N, Vougioukianopoulou K et al (2014) MLS-2384, a new 6-bromoindirubin derivative with dual JAK/Src kinase inhibitory activity, suppresses growth of diverse cancer cells. *Cancer Biol Ther* 15, 178-184