STAT3 inhibition, a novel approach to enhancing targeted therapy in human cancers (Review)

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Abstract. Signal transducer and activator of transcription 3 (STAT3) regulates many critical functions in human normal and malignant tissues, such as differentiation, proliferation, survival, angiogenesis and immune function. Constitutive activation of STAT3 is implicated in a wide range of human cancers. As such, STAT3 has been studied as a tumour therapeutic target. This review aimed principally to summarise the updated research on STAT3 inhibition studies and their therapeutic potential in solid tumours. Recent literature associated with STAT3 inhibition was reviewed through PubMed and Medline database, followed by critical comparison and analysis. Constitutive activation of STAT3 has been identified as abnormal and oncogenic. The pathway of STAT3 activation and signal transduction identifies 3 approaches for inhibition: modulating upstream positive or negative regulators, regulating RNA (DN-STAT3, anti-sense RNA, siRNA and microRNA) or targeting STAT3 protein at different domains. The last approach using small molecule STAT3 inhibitors has been the most examined so far with both preclinical and clinical studies. Targeting STAT3 using a specific inhibitor may be a useful cancer treatment approach, with the potential for a broad clinical impact.

Contents

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1. Introduction

Signal transducer and activator of transcription 3 (STAT3) is a transcription factor which in humans is encoded by the STAT3 gene. The protein is a latent cytoplasm transcription factor that relays signals of cytokines and growth factors from the cell membrane to the nucleus to regulate gene expression critical to normal cellular processes, including cell development, differentiation, proliferation, survival, angiogenesis and immune function (1-5). Constitutive STAT3 activation is associated with various human cancers and commonly suggests poor prognosis (6-9). Thus STAT3 has been studied as a tumour therapeutic target. Very recently a tumour suppressor role of STAT3 in a few tumours has also been reported (10,11), but this is not the focus of the present review.

In mammals, the STAT family consists of seven protein members: STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b and STAT6, which are mapped to different human chromosomal regions (12). STAT2, STAT4 and STAT6 are only activated in normal human function, while STAT1, STAT3 and STAT5 play an important role in cancer development (STAT1 as tumour suppressor, STAT3 and STAT5 as oncogenes) (13,14). STAT3 appears to be a critical regulator and is therefore the focus of this review. All of the STAT family comprise 750-850 amino acids and 6 conserved domains: the NH2-terminal, coiled-coil, DNA binding, linker, SH2, and C-terminal transactivation domains (15,16). The C-terminal transactivation domain of STAT3 plays an important role in activation through a tyrosine residue at position 705 and a serine residue at position 727 (17). The SH2 region, a well-characterized small protein module of approximately 100 amino acids (18), is responsible for the binding of STAT3 to the tyrosine-phosphorylated receptors and also the homodimerization or heterodimerizaion of two STAT monomers that is necessary for DNA binding and gene expression (19). SH2 region, NH2-terminal and DNA-binding domain became the targets for development of STAT3 inhibitors.

This review aimed principally to summarise the therapeutic potential of STAT3 inhibitors, specifically STAT3 inhibition and preclinical studies, as well as inhibiting STAT3 in clinical trials.

Table I. Constitutive STAT3 activation and oncogenesis.

Cancer type	Constitutively activated STAT3	Mediators (or regulators)	Down-stream regulation and target genes	(Refs.)
Breast cancer	Human cell lines	EGF/EGFR, Src, or JAK	Cell cycle progression	(61,108,109)
Endometrial and cervical cancer	Human cell lines		Anti-apoptois by caspase-3 Genes: Bcl-X_L, survivin and Mcl-1	(49)
Lung cancer	Human cell lines	EGF/EGFR, IL-6 and HGF, followed by Src, or mutant EGFR or CXCL12/CXCR4-JAK2	Anti-apoptosis prolifreration	(110-113)
Multiple myeloma	Human cell lines	IL-6/JAK	Anti-apoptosis Gene: Bcl-X_L cyclin D1 and p-STAT3: mutually exclusive events	(69,114)
Ovarian cancer	Human cell lines		Genes: Bcl-X_L, cyclin D1	(47)
Sarcomas	Human cell lines		Proliferation and anti-apoptosis by caspases	(115)
Head and neck cancer	Human HNSCC cell lines, *in vivo* mouse models, primary cultures of oral keratinocytes	IL-6/gp130, TGF-α or EGFR	Proliferation, tumour growth, anti-apoptosis, and cell cycle progression (G_2-M phase accumulation) Genes: cyclin D1, Bcl-2 and Bcl-X_L	(42,43,45, 46,116-118)
Prostate cancer	Human cell lines, rat cell lines, *in vivo* mouse models	JAK1 or JAK2	Proliferation, tumour growth, anti-apoptosis by caspase-3	(25,119-122)
Melanoma	Mouse cell lines, human cell lines, *in vivo* mouse models	Src, but not EGFR or JAK	Proliferation and anti-apoptosis Genes: Bcl-X_L, Mcl-1	(94,95)
Hepatocellular carcinoma	Human celll lines, *in vivo* mouse models	TGF-α, or dysfunctional TGF-β signaling	Proliferation Genes: cyclin A, c-jun, c-fos, and c-myc	(103,123)
Pancreatic cancer	Human cell lines, *in vivo* mouse models		Tumour growth, angiogenesis and metastasis Gene: VEGF	(53)

2. Biology of STAT3

Normal function of the STAT3 pathways. The STAT3 signalling pathways regulate the gene expression of proliferation, survival, migration and invasion, as well as angiogenesis (2,20) in human. Furthermore, STAT3 is essential at early stages of embryo development (21) and modulates embryonic stem cell differentiation such as TH17 helper T cells (22). In benign cells, the signalling by STAT3 is under tight regulation so that the signal is transient in accordance with physiological responses.

Activation of STAT family and associated pathways. The process of STAT activation commences with the Janus kinases (JAKs) which bind to and are phosphorylated by cytokine or growth factor receptors in response to external signals such as interleukin-6 (IL-6), interferon-α, tumour necrosis factor (TNF),

epidermal growth factor (EGF), platelet-derived growth factor (PDGF), transforming growth factor-alpha (TGF-α) and TGF-β1 (17,23-25). Subsequently, phosphorylated JAKs in turn cause multiple phosphorylations of tyrosine residues within the cytoplasmic domain of the cytokine receptor (26-28). Monomeric unphosphorylated STAT is recruited to these activated receptors through an interaction between the STAT SH2 domain and phosphotyrosine docking sites on the receptors. JAKs then phosporylate the critical tyrosine on C-terminal domain of STAT, which causes dissociation of STAT from the receptor, this then leads to dimerization of two STAT monomers through reciprocal interaction (2,29,30). Dimeric STAT complexes translocate to the nucleus where they bind to specific DNA response elements in the promoters of target genes (31).

The non-receptor tyrosine kinases such as Src can activate the STAT signalling pathway in the absence of receptor engagement (32-34). In some cases, JAKs as intermediaries are simultaneously involved in activation of STAT by non-receptor tyrosine kinases (35).

Cross-talk with other signalling. In a hepatocellular carcinoma cell (HCC) study, JAK/STAT activated by interferon-α inhibited MAPK signalling by cross-talking with MEK/ERK pathway (36). Our results in sarcoma suggest this may be due to IFNα induced alteration of the balance between STAT1 and STAT3/STAT5 favouring inhibition of proliferation and subsequent apoptosis (37). Cross-talking among the signalling pathways is complicated and requires further investigations. Increased understanding of cross-talking may provide the rationale for combination therapy using STAT3 inhibitor and other drugs.

Constitutive activation of STAT3 and oncogenesis. Constitutive activation of STAT3 has been reported at high frequency in large numbers of malignant cell lines, *in vivo* animal experiments and human tumours (35,38,39). Table I includes a broad range of pre-clinical studies, which show that (activated) phosphorylated-STAT3 (pSTAT3) is a common characteristic of many cancers. Constitutive activation of STAT3 involves multiple signalling pathways in both a cell- and tissue-specific manner, making single upstream pathway treatment difficult. So far, there has been no STAT3 gene mutation detected in any cancer. Persistent activation of STAT3 has been attributed to dysregulation of upstream tyrosine kinases and negative regulators in the STAT3 signalling pathway (20,40).

Activated STAT3 regulates many genes whose expression is required in aspects of cancer initiation, development and progression, including uncontrollable proliferation, anti-apoptosis, invasion, angiogenesis and immune surveillance evasion. STAT3 signalling has been implicated in the up-regulation of cell proliferation by cylin D2 and c-Myc (20,34,41-43). In addition, pSTAT3 contributes to malignancy by preventing apoptosis, allowing accumulation of long-lived tumour cells and mediating chemoresistance via increased expression of anti-apoptotic genes such as Bcl-2 family member Bcl-X_L and Mcl-2 as well as survivin (43-49). Recently, miRNA-21 has been proved to be STAT3 target gene, which functions as an inhibitor of apoptosis in multiple myeloma and Sézary cells (50,51). Increased VEGF expression in cultured cell lines, animal models and patient cancer specimens, as well as tumour angiogenesis *in vivo* is also induced via STAT3 pathway in diverse human cancers from head, neck, breast, pancreas, cervix as well as melanoma (27,52-54). Over-expression of matrix metalloproteinase-2 (MMP-2) in melanoma and MMP-9 in breast cancer is attributed to elevated STAT3 activity (55,56). STAT3, which may also be activated in tumour-infiltrating immune cells, can inhibit tumour immune function by promoting the expression of immune suppressive factors and inhibiting the product of pro-inflammatory mediators (39,57-60).

Over-expression of pSTAT3 occurs in a wide range of tumours (25,61,62), suggesting STAT3 may be both a therapeutic target and a clinical prognosticator. Table II outlines clinical studies of pSTAT3 expression in various human tumours. There was a significant correlation between higher pSTAT3 and worse outcomes of tumours, such as breast cancers exhibiting invasiveness, head and neck as well as gastric cancers with nodal metastasis and/or in late clinical stage, as well as prostate cancers displaying high Gleason score (25,43,63,64).

The role of STAT3 as a resistance pathway to targeted therapy. As a class the targeted therapies that inhibit specific biologic pathways represent a novel therapeutic strategy either as single agents or in combination with conventional chemotherapeutics in treating a variety of malignancies. However, patient response has been less than expected and early development of resistance has been a major issue (65). The identification of novel alternative signalling pathways represents one resistance mechanism. For example one recent study demonstrated that EGFR and IGFR signal through JAK/STAT pathway, in addition to the two classical pathways of ras-raf-MEK-ERK and PI3K-Akt (66). Indeed STAT3 is a common alternative pathway for many growth promoting factors effectively bypassing a single tyrosine kinase inhibitor acting at an early point in the receptor activation pathway. For example, owing to the high level of IL-6 in NSCLC, EGFR inhibitor was ineffective due to ongoing STAT3 activation (13). STAT3 is a more downstream point of convergence in many ligand/receptor pathways (such as growth factor and cytokine receptors) and non-receptor tyrosine kinase pathways (such as Src) and consequently cross-talk among these signalling pathways may contribute to resistance to EGFR inhibitors. In addition STAT3 acts as a nuclear transcription factor (41) upregulating genes of cell survival and proliferation. Its inhibition represents a promising target for improving targeted cancer treatment in a number of preclinical studies. Furthermore, considering its late position in activation pathways, its inhibition may be less likely to be overcome through an alternative pathway.

3. STAT3 inhibition and preclinical studies

Since STAT3 is involved in regulating fundamental biological processes and pSTAT3 contributes to malignant transformation and progression, targeting STAT3 signalling appears to be a novel approach to preventing and treating cancers. Several strategies are being developed to target the STAT3 signalling pathway.

Modulating upstream positive or negative regulators. Inhibiting the upstream signals or enhancing negative regulators of STAT3 signalling pathway is one possible strategy.

i) Inhibition of STAT3 signalling via targeting the activation of cytokine and growth factor receptors with monoclonal antibodies or receptor antagonists: for example, the use of

Table II. Constitutive STAT3 activation in clinical studies using human tumour specimens.

Tumour type	Expression of constitutively activated STAT3	Correlation with clinical prognosis and parameters	(Refs.)
Breast cancer	15 of 16 (94%) invasive cancers, 1 of 8 (12.5%) *in situ* carcinomas	Advanced breast cancer	(64)
	18 of 23 (78%) breast tumour specimens		(61)
	57% primary breast cancer specimens		(56)
Head and neck cancer	10.6-fold higher in tumours and 8.8-fold higher in normal mucosa from cancer patients compared with normal mucosa from non-cancer patients	Early event in head and neck carcinogenesis	(45)
	74 of 90 (82%) tumour specimens 1 of 8 (12.5%) premalignant lesions	High expression in early tumour stage, subsequent degradation in late stage	(62)
	19 of 51 (37%) SCCHN tumour specimens with strong expression	Existence of nodal metastasis, late clinical stage and poor prognosis	(43)
	7 of 10 (70%) poorly differentiated tumours	STAT1 vs STAT3 balance affects the pathogenesis of tumours	(42)
Prostate cancer (PC)	44 of 45 (98%) tumour specimens, 13 of 45 (29%) adjacent nonjuror tissues	High gleason score	(25)
	All 24 tumours and adjacent non-tumour tissues		(124)
	All 15 tumour specimens	Severity of the malignancy	(122)
Gastric cancer	26 of 53 (49%) tumour specimens	Lymph node metastasis and overall survival	(63)
Melanoma	A majority of tumour specimens examined		(48)
Endometrial and cervical cancer	24 of 115 (21%) endometrial cancer specimens, 25 of 104 (24%) cervical cancer specimens	Activation in early stage and throughout all stages	(49)
Hepatocellular carcinoma	All 9 HCC tissues		(103)
Lung cancer	95 of 176 (54%) NSCLC specimens	Limited smoking history and adenocarcinoma	(111)
	56 of 92 (61%) NSCLC	Smaller tumours	(125)
	46 of 92 (50%) adenocarcinomas specimens		(112)
	All 10 SCLC specimens		(113)
Colorectal cancer	131 of 724 (18%): hight-level pSTAT3 244 of 724 (34%): low-level pSTAT3	Adverse clinical outcome (higher mortality, peritumoural lymphocytic reaction)	(9)
Sarcomas	21 of 113 (19%) osteosarcoma, 27 of 64 (42%) rhabdomyosarcoma, 22 of 151 (15%) of other soft-tissue sarcoma		(115)
	28 of 82 (34.1%) soft tissue sarcoma	Tumour grade, location, plane and necrosis	(126)
Multiple myeloma (MM)	All 24 bone marrow specimens of MM		(69)
	23 of 48 (48%) tissue specimens of MM		(114)

Table III. Preclinical treatment of cancer by interfering STAT3 mRNA.

Treatment type	Methods	Results	(Refs.)
Dominant - negative mutant STAT3	*In vitro*: SCCHN, melanoma, sarcoma, mammary carcinoma, prostate cancer, breast cancer, endometrial and cervical cancer, non-small cell lung cancer cell lines	Cell death ↑, apoptosis ↑, cell proliferation ↓, VEGF ↓, cell cycle arrest, cyclin D1↓, Bcl-2 ↓, Bcl-X_L↓	(27,43,49,54,61,94, 95,110,115-117, 119,122,127)
	In vivo: mouse melanoma and human prostate cancer xenografts in mice	Tumourigenicity↓, tumour growth ↓, tumour regression↑, apoptosis ↑	
Antisense	*In vitro*: SCCHN, melanoma, mammary carcinoma, prostate cancer, non-small cell lung cancer, breast cancer cell lines	Apoptosis ↑, cell proliferation ↓, VEGF ↓, survivin ↓	(25,45,110,116, 117,122,127,128)
	In vivo: SCCHN xenogragts in mice	Apoptosis ↑, Bcl-X_L↓	
siRNA	*In vitro*: breast cancer, melanoma, glioma and prostate cancer cell lines	VEGF ↓, cell growth ↓, apoptosis ↑, Bcl-2 ↓, cyclin D1 ↓, c-Myc ↓	(15,54,129)
	In vivo: glioma and prostate cancer xenografts in mice	Tumour growth ↓, apoptosis ↑	
MicroRNA			
Anti-let-7a	*In vitro*: malignant cholangiocytes	p-STAT3 ↓	(93)
	In vivo: malignant cholangiocytes xenograft in mice	p-STAT3 ↓, chemotherapy toxicity ↑, tumour growth ↓	
miR-17 family mimic	*In vitro*: human breast cancer cells	STAT3 protein ↓	(91)

IL-6 receptor super-antagonists, such as Sant7, inhibited IL-6-dependent human myeloma cell growth (IC_{50} = 0.16 nM), as well as induced cell death as a pro-apoptotic factor via STAT3 signalling (67,68).

ii) Inhibition of upstream tyrosine kinases JAK or Src with small molecule inhibitors such as AG490, INCB20, PD180970 and Dasatinib: for example AG490, a JAK2-specific inhibitor, blocked constitutive activation of STAT3 causing a significant reduction of Bcl-X_L mRNA expression and induced cell apoptosis in certain human myeloma and prostate cancer cell lines (69). Pan-JAK inhibitor INCB20 was demonstrated to block STAT3 phosphorylation, induce apoptosis and inhibit human multiple myeloma cell growth with an IC_{50} of less than 1 μM, as well as dramatically delay tumour growth in subcutaneous xenograft model in mice (26). Dasatinib (BMS-354825), which inhibits Src tyrosine kinase activity, has shown an anti-tumour effect on head and neck squamous cell carcinoma and non-small cell lung cancer cells with low IC_{50} values *in vitro* (70).

iii) Enhancement of negative regulators: a number of pathways that negatively regulate STAT3 have been identified. These include suppressors of cytokine signalling (SOCS) family proteins which are transcriptionally regulated by activated STAT and form a negative feedback loop to suppress STAT signal by binding to or inhibiting JAKs or by targeting bound proteins to the proteasome degradation pathway. Other negative regulators include: protein inhibitor of activated STAT (PIAS), various tyrosine phosphatises (SHP1, SHP2, CD45), phosphatase and tensin homolog (PTEN), GRIM-19 (gene associated with retinoid IFN induced mortality-19) as well as the ubiquitin-proteasome degradation pathway involved in negative regulation of STAT signalling (71-81). Recently, neurofibromatosis 2 (NF2) tumour suppressor, schwannomin was also demonstrated to inhibit STAT3 phosphorylation (82).

However, although these are all theoretically attractive, any attempt to activate these pathways as a means of downregulating activated STAT3 is problematic owing to redundancy of upstream proteins, meaning that the STAT3 pathway might not be effectively blocked by a single compound. Furthermore, these compounds might inhibit other downstream targets, which the STAT3 signalling are cross-talking with (39,83) and consequently cause undesirable side effects.

RNA interference. Another approach for STAT3 inhibition is to affect translation of STAT3 mRNA by coding RNA interference, such as domain-negative (DN) STAT3 mutants (STAT3β, STAT3D or STAT3F), anti-sense STAT3 oligonucleotides or small interfering RNA (siRNA). Several groups reported that this strategy can inhibit cellular growth and induce apoptosis in multiple human cancer cells *in vitro* and *in vivo*, accompanied with down-regulation of STAT3 target genes. Table III lists

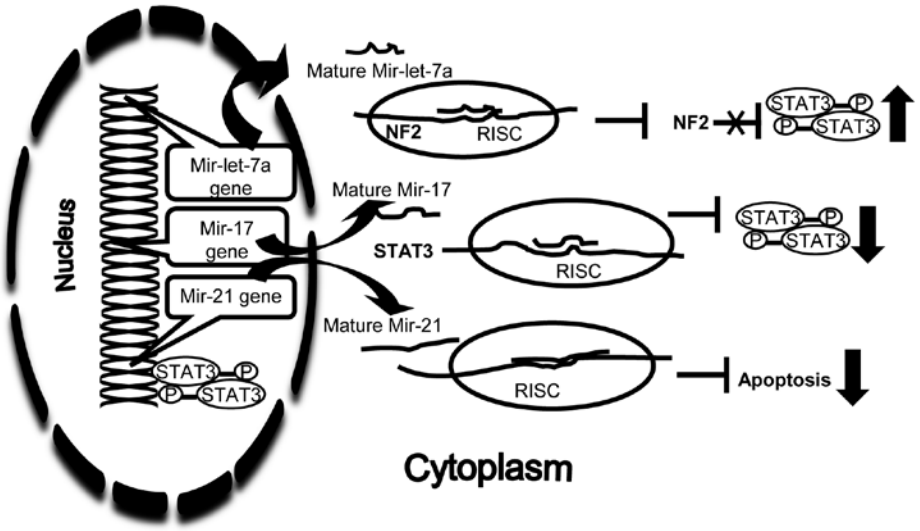

Figure 1. MicroRNAs associated with STAT3 regulation in cancer. Briefly, Mir-let-7a indirectly enhances STAT3 activation through inhibiting NF2 (negative regulator to STAT3), whilst Mir-17 directly downregulates STAT3 protein expression. In addition, mir-21 is a target gene of STAT3. Activated STAT3 binds to mir-21 gene, sequently downregulates the gene expression, and eventually leads to antiapoptosis (RISC, RNA induced silencing complex).

some RNAs used in preclinical studies, which target STAT3 mRNA.

Recently, microRNAs, small non-coding RNA molecules, which act as post-transcriptional regulators have also become the focus of research on regulation of gene activity. MicroRNA binds to the 3' untranslated regions (3' UTRs) of target mRNA in RNA induced silencing complexes (RISC), and negatively regulates gene expression through transcript destabilization and translational attenuation (84). A variety of microRNAs are linked with cancer initiation and development as tumour suppressors or oncogenes (also called oncomirs) (85-90). Several microRNAs can regulate STAT3 protein expression in embryonic development and cellular differentiation (91,92). However, in tumour-related studies, only two microRNAs, mir-17 and mir-let-7a are identified to regulate STAT3 to date (Fig. 1). The Mir-17 family, as a tumour suppressor, directly regulates STAT3 mRNA expression through binding to and silencing STAT3 3' UTRs in a breast cancer cell line (MDA-MB-231) (91). Mir-let-7a, as an oncogene, indirectly modulates STAT3 phosphorylation in malignant human cholangiocytes (93). Neurofibromatosis 2 (NF2), a negative regulator of STAT3 phosphorylation, is a direct target gene of mir-let-7a. As such, let-7a microRNA contributes to elevated STAT3 phosphorylation. Thus, microRNA (anti-let 7a and Mir-17 mimic) which blocks the oncogene or replace the lost suppressor function have the potential for an anti-tumour effect.

Importantly, such gene therapy displayed anti-tumour bystander effects, in which adjacent tumour cells that did not receive the gene therapy also underwent apoptosis (94,95). For example, gene therapy using STAT3β RNA blockade caused the tumour regression seen with massive apoptosis of both transfected and bystander tumour cells.

However, more new techniques will be needed to overcome the small RNA delivery limitations and subsequent immune response in patients (84,96). Some other major problems of RNA delivery include rapid excretion by kidney, degradation in extracellular fluid and permeability to the cell membrane. Nevertheless, approaches using different delivery agents (liposome, nanoparticle and LNA oligonucleotide) have entered preclinical and phase I clinical trials (97,98).

Targeting STAT3 protein directly. The best approach may be to inhibit STAT3 protein directly. The three domains of STAT3: NH2-terminal, DNA-binding and SH2 were identified as selective targets for development of STAT3 inhibitors based on the understanding of the structure and function of STAT3 (99). The most popular target is the SH2 domain, since it is necessary for STAT3 recruiting to any activated receptor. In addition, inhibition of this target can block STAT3 dimerization and consequently inhibit nuclear translocation and STAT3-dependent gene regulation. So far, three categories of STAT3 SH2 inhibitors have been developed including peptides, peptidomimics and small molecule inhibitors. Several phosphotyrosyl peptides (PpYLKTK), tripeptides (PpYL, ApYL) and peptidomimetics (ISS610) were demonstrated to inhibit STAT3 dimerization, cell proliferation and gene regulation *in vitro*. PpYLKTK, PpYL and ApYL, which are STAT3 SH2 domain-binding peptides, blocked STAT3 DNA-binding activity with an IC_{50} of 235, 182 and 217 μM, respectively (100). The most effective yet reported ISS610 disrupted STAT3 dimerization, inhibited cell growth and induced apoptosis, as well as reduced DNA-binding activity with an IC_{50} of 42 μM in cells with pSTAT3 (101). However, poor cell permeability and *in vivo* stability is the limitation to applying peptide-based inhibitors to clinical trials (83).

Multiple novel small molecular inhibitors targeting STAT3 SH2 domain were identified and designed through structure-based high-throughout virtual screening and have been demonstrated to inhibit STAT3 dimerization and DNA-binding activity, as well as inhibit cell proliferation and tumour growth in cultured cancer cell lines and in animal models respectively. Table IV summarizes the inhibitors reported to target SH2 domain, as well as the related mechanism studies, including STA-21 and analogue, Stattic, S3I-201 (NSC 74859) and analogue, S3I-M2001, 5,15-DPP, LLL12 and STX-0119. S3I-201 selectively inhibited STAT3 DNA-binding activity, STAT3

Table IV. Preclinical studies of small molecular inhibitors targeting STAT3 SH2 domain.

Name	Studies	Discoveries	(Refs.)
STA 21	Human breast cancer cells, human ovarian carcinoma cells Osteosarcoma and rhabdomyosarcoma	STAT3 DNA-binding activity ↓, STAT3 dimerization ↓, STAT3-dependent luciferase activity ↓ Cells with pSTAT3: survival ↓, viability ↓, growth ↓, apoptosis ↑	(115,130)
STA-21 analogue: LLL-3	Human glioblastoma	STAT3 DNA-binding activity ↓, STAT3-dependent transcriptional activity ↓ Cells viability ↓, apoptosis ↑ *In vivo*: survival ↓, intracranial tumours ↓,contralateral invasion (-)	(131)
Stattic	Human breast cancer cell lines	STAT3 DNA-binding activity ↓, STAT3 dimerization ↓, Cell apoptosis ↑	(132)
S3I-201 (NSC 74859)	Human breast cancer and hepatocellular carcinoma	STAT3 DNA-binding activity ↓, STAT3 dimerization ↓, STAT3-dependent transcriptional activity ↓ Cells growth ↓, apoptosis ↑, cyclin D1 ↓, Bcl-X_L ↓, survivin ↓ *In vivo*: tumour growth ↓	(102,103)
S3I-201 analogue: S3I-201.1066	Human breast, pancreatic and ovarian cancer cells, normal human pancreatic duct epithelial cells	STAT3 DNA-binding activity ↓, STAT3-dependent transcriptional activity ↓ Cell viability ↓, survival ↓, malignant transformation ↓, c-Myc ↓, Bcl-X_L ↓, survivin ↓, MMP-9 ↓, VEGF ↓ *In vivo*: tumour growth ↓	(133,134)
S3I-M2001	Human breast and pancreatic cancer cells, immortalized human breast epithelial cells	STAT3 dimerization ↓, Cells malignant transformation ↓, survival ↓, migration ↓, invasion ↓, Bcl-X_L ↓ *In vivo*: tumour growth ↓	(135)
5,15-DPP	Human breast cell and human embryonic kidney cell	STAT3 DNA-binding activity ↓, STAT3 translocation ↓, STAT3 dimerization ↓, c-myc promoter binding ↓, c-myc protein expression ↓, STAT3 and pSTAT3 unchanged	(136)
LLL12	Human breast and pancreatic cancer cells, glioblastoma cells, hepatocytes, normal lung fibroblasts, medulloblastoma cells and human hepatocytes	STAT3 DNA-binding activity ↓, STAT3-dependent transcriptional activity ↓, STAT3 phosphorylation ↓, Cell viability ↓, colony formation ↓, migration ↓, apoptosis ↑, cyclin D1 ↓, Bcl-2 ↓, survivin ↓ *In vivo*: tumour growth ↓	(137,138)
STX-0119	Human breast cancer cells, and human lymphoma SCC-3 xenografts in mice	STAT3 DNA-binding activity ↓, STAT3 dimerization ↓, apoptosis ↑, c-Myc ↓, survivin ↓, cyclin D1 ↓, Bcl-X_β ↓, *In vivo*: tumour growth ↓	(139,140)

Table V. STAT3 inhibition in clinical trials.

Trial identifier	Country	Drug administration	Study duration	Phase	Patients
NCT00511082	Hong Kong	OPB-31121 oral	12/2007-09/2009	I	Relapsed or refractory non-Hodgkin's lymphoma or multiple myeloma
NCT00657176	Korea	OPB-31121 oral	03/2008-06/2009	I	Advanced solid tumours
NCT00955812	USA	OPB-31121 oral	06/2009-02/2012	I	Advanced solid tumours
NCT01029509	USA	OPB-31121 oral	07/2008-07/2011	I	Advanced leukemias or myelodysplastic syndromes
NCT00696176	USA	STAT3 Decoy intra-tumoural	08/2008-07/2011	0	Surgically resectable head and neck squamous cell carcinoma

dimerization and transcriptional activity, as well as induced growth inhibition and apoptosis in cells with persistent pSTAT3 (102,103). Moreover, in both human breast and hepatocellular cancer xenografts in nude mouse model, S3I-201 significantly inhibited the tumour growth with a dose of 5 mg/kg. OPB-31121, another small molecule inhibitor, inhibits cell proliferation with low IC_{50} in a number of hematopoietic malignancy cell lines through direct blockage of STAT3 phosphorylation in preclinical studies (104). OPB-31121 has been used in clinical trials and is introduced below in the clinical study section.

Collectively, small molecule inhibitors targeting STAT3 directly are the most promising of the possible therapeutic strategies because: i) modulation of upstream regulators by single targeted therapy may not completely block the STAT3 pathway due to multiple regulators and cross-talking among pathways; ii) RNA inhibition requires yet to be identified improved techniques to deal with the delivery issues; iii) the preclinical studies demonstrate that small molecule inhibitors strongly inhibited the growth both of tumour cells *in vitro* and xenografts in animal models with a low IC_{50} encouraging clinical development and reducing the risk of development of resistance.

STAT3 inhibition combined with other targeted therapy or chemotherapy. In addition, synergy may be expected with many other inhibitors, since STAT3 is a complementary pathway for many growth factor pathways. The combination therapy of STAT3 and EGFR inhibitors synergistically and significantly suppressed cancer cell growth and down-regulated activated STAT3 expression in high-grade gliomas and pancreatic cancers (41,105). Furthermore, STAT3 inhibition increased the sensitivity of cancer cells to chemotherapeutic agents such as taxol and cisplatin (105,106). Therefore, STAT3 is an attractive target and some new studies on STAT3 mono-therapy and combination therapy are ongoing.

4. Inhibiting STAT3 in clinical studies

There are several phase 0/I clinical trials of STAT3 inhibition ongoing or not yet reported (Table V). OPB-31121, which inhibits STAT3 phosphorylation, but did not affect JAK kinase, displayed strong anti-proliferation effect in cancer cell lines and in mouse model *in vivo* studies (104). Preliminary data are currently only available from one Korean study reported in the Annual Meeting of the American Society of Clinical Oncology (107) in 21 patients with advanced refractory solid tumours treated by OPB-31121. This showed that toxicities were predominantly grade 1 or 2, with the dose-limiting toxicities (DLT) of grade 3 vomiting and diarrhoea. The MTD has not been determined within the designated dose range. Eight out of 17 patients had the stable disease, with one case more than 12 months. It seems that this oral STAT3 inhibitor is safe and tolerable. More data from different clinical trials of different phases using this kind of drug are required before a clear conclusion can be made.

5. Conclusions

STAT3 play a pivotal role in the initiation, development and progression of cancers, including proliferation, anti-apoptosis, invasion, angiogenesis and immune surveillance evasion. Constitutively activated STAT3 is associated with a wide range of human cancers. As such, STAT3 has been identified as a novel target to treat and prevent cancers with a broad potential application. Several STAT3 inhibitors display anti-tumour effectiveness in preclinical studies. The data for clinical trials using STAT3 inhibitors are emerging. Targeting STAT3 using a specific inhibitor may be a useful cancer treatment approach, with the potential for a broad clinical impact.

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