



Signal Transducer and Activator of Transcription (STATs) Proteins in Cancer and Inflammation: Functions and Therapeutic Implication

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Signal Transducer and Activator of Transcription (STAT) pathway is connected upstream with Janus kinases (JAK) family protein and capable of integrating inputs from different signaling pathways. Each family member plays unique functions in signal transduction and crucial in mediating cellular responses to different kind of cytokines. STAT family members notably STAT3 and STAT5 have been involved in cancer progression whereas STAT1 plays opposite role by suppressing tumor growth. Persistent STAT3/5 activation is known to promote chronic inflammation, which increases susceptibility of healthy cells to carcinogenesis. Here, we review the role of STATs in cancers and inflammation while discussing current therapeutic implications in different cancers and test models, especially the delivery of STAT3/5 targeting siRNA using nanoparticulate delivery system.

Keywords: STAT transcription factors, cancer, inflammation, therapeutic implication, STAT3

INTRODUCTION: THE ROLE OF STAT FAMILY MEMBERS IN CANCER AND INFLAMMATION

The first direct link between STAT family proteins and carcinoma in human derived from research works that demonstrate that constitutively activated STAT3 is crucial for the carcinogenesis of head and neck cancer and multiple myeloma cells (1, 2). There is evidence indicated that antagonizing STAT3 signaling could induce cell death in human U266 myeloma cells (1). Subsequently, several types of solid tumors, leukemia, and lymphomas have been linked with constitutive activation of STAT3. IL-6 autocrine or paracrine loops was recognized as source for composition of STAT3 activity in myeloma and prostate malignant cell lines (3). Transforming growth factor- α (TGF- α)-mediated epidermal growth factor receptor (EGFR) signaling plays a vital role for the activation of STAT3 in some head and neck cancer cell lines (2). Hepatocyte growth factor (HGF) signaling via the receptor, c-MET, is related with the transformation of leiomyosarcoma cells, breast carcinoma cells, melanoma cells, and lung cancer cells in conjunction with SRC kinase which stimulate the expression of STAT3 (4–7).

Evidences have shown that constitutive activation of STAT5 are the leading causes of tumorigenesis (8). STAT proteins are indispensable in coordinating the response of hematopoietic

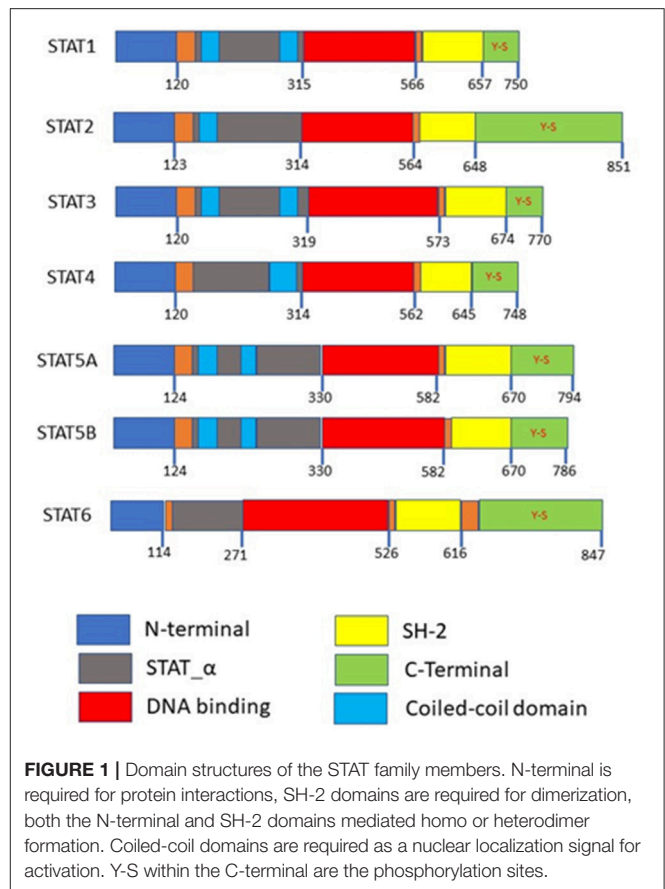
cells to a wide range of cytokines. In fact, STAT5 activation is crucial for cancer progression in chronic myelogenous leukemia (CML) and myeloproliferative disease induced by TEL–JAK2 (9, 10). Aberrant chromosomal translocation BCR–ABL kinase and activation of FMS-like tyrosine kinase 3 (FLT3) receptor tyrosine can induce STAT 5 activation in CML and Acute myeloid leukemia (AML), respectively. However, not all family members in STAT proteins are promoting cancer progression. For instance, activated STAT1 appears to exert pro-apoptotic and anti-proliferative effect as STAT1-null mice lead to higher risk of tumor development than controls (11, 12), these results indicate that STAT1 has the tumor-suppressing properties like TP53 and could be an antagonist for STAT3 and STAT5 activation. All these studies showed that STAT1 unlikely to promote tumor cell growth in human.

Abnormally high activity of the Nuclear Factor Kappa B (NF- κ B) pathway and Cyclooxygenase-2 (COX-2) activity induced by inflammatory mediators or reactive nitrogen oxygen species (RNOS) might aid inflammation-mediated tumorigenesis. STAT3 has close association with inflammation which is subsequently linked with tumor initiation due to mutation in genetic makeup of malignant cells (13, 14), in addition to this different environmental influences such as, stress, carcinogenic agents, smoking and radiations (15, 16). Apart from that, STAT proteins are crucial mediators of immunity against pathogens, and in the advancement of inflammatory disorder (17). Emphatically, initial natural protective event which is connected with STATs was inflammation in which STAT1 shows anti-viral activity while STAT4 and STAT6 involves in polarization of T helper cells. IFN/STAT1 pathway can induce inflammation in several ways, for example increasing the production of chemokines, managing the differentiation and apoptosis of hematopoietic cells and initiating formation of reactive oxygen species and nitric oxide (18). STAT2, STAT4, and STAT6 are stimulated by certain cytokines, such as IL-12, IL-4/IL-13, and IFN- α (19). Development of T helper cells by STAT6 contributes in a positive way in modulating inflammation during allergy while in negative way in autoimmunity (20). Consequently, differentiation of type 1 T helper cells by STAT4 was essential for autoimmune and inflammatory disorders. This model has highlighting the fact that numerous STAT proteins can participate in differentiation of single T helper phenotype, and single STAT protein can be essential for the expansion of numerous T helper subsets (21, 22).

As discussed above, STAT1 protein mainly function as tumor suppressor, suggesting that not all STAT proteins participate in the progression of inflammation and malignancy in human. Since STAT3/5 is directly implicated in oncogenesis and inflammation, the following sections will focus more on their molecular regulation and therapeutic implications of these proteins.

MOLECULAR REGULATION OF STAT 3/5 PROTEIN ACTIVATION

Accumulating research has shown that how STAT3 constitutively activated in numerous types of cancer, including multiple



myeloma, lymphomas, head and neck squamous cell carcinoma, breast cancer, prostate cancer, and hepatocellular carcinoma (HCC) (23–26). STAT3 generates two isoforms STAT3 α and STAT3 β by alternative splicing (27). STAT5 consists of two isoforms, STAT5a and STAT5b which map to chromosome17q11.2 in human and share 94% structural homology, these two isoforms are transcribed from separate genes and differ primarily at their C-terminus (19, 28). STAT5a is predominantly expressed in mammary gland and mammary tissue while STAT5b is more prevalent in muscle and liver (19, 28, 29). STAT3, STAT5a, and STAT5b belong to a family of STAT proteins along with STAT1, STAT2, STAT4, and STAT6 (30, 31). STAT proteins were discovered initially as latent transcription factors found in the cytoplasm of cells (32, 33). All seven STAT proteins share a common structural motif (Figure 1).

STAT3, STAT5a, and STAT5b can be activated by numerous cytokines and growth factors, including interleukin (IL)-6, EGF, insulin-like growth factor, hepatocyte growth factor, colony-stimulating factor-1, platelet derived growth factor, hormones (growth hormone, insulin) and downstream of some G-protein-coupled receptors (34–37). The binding of these molecules will activate JAK kinases and causing the phosphorylation of tyrosine residues on receptors which allow the SH2 domains of STAT proteins to attach to the phosphorylated receptor (38–40). It is crucial for STAT proteins to undergo tyrosine

phosphorylation for homodimerization or heterodimerization through an SH2 domain-mediated mechanism (40). Particularly, IL-6 is important for the production of HCC (41, 42) and is able to activate STAT3 via IL-6 receptor, gp130 and JAKs (43, 44). Upon attaching of IL-6 to its receptor, dimerization of the gp130 receptor occurs and causes subsequent activation of JAK due to its association with gp130 (25, 45, 46). Three members of the JAK family of proteins, JAK1, JAK2, and TYK2 can be activated by this manner (47, 48). After which, phosphorylation of tyrosine residues on gp130 is induced, providing docking sites for inactive STAT3 monomers (49, 50) causing STAT3 to be phosphorylated at its Tyr⁷⁰⁵ residue (23, 51). Activated STAT3 monomers then dimerize by coupling of the phosphorylated Tyr⁷⁰⁵ remainder on STAT monomer with Src homology 2 (SH2) domain of another STAT monomer (52, 53). Nuclear translocation happens next, which is mediated by importin α /NPI-1 (54, 55). Thereafter, STAT3 dimers attach to STAT3-specific DNA-response elements of desired genes and this brings about transcription of genes (56, 57) Phosphorylation of STAT3 can be induced by c-Src directly or indirectly, in which c-Src may exerts its function downstream following activation of G protein-coupled receptor (GPCRs) or receptor tyrosine kinases (RTKs) (47, 58). Apart from its Tyr⁷⁰⁵ residue, STAT3 can be phosphorylated at its Ser⁷²⁷ as well. BCR-ABL primarily phosphorylates STAT3 at Ser⁷²⁷ while phosphorylation at Tyr⁷⁰⁵ is at a comparatively lesser degree (58). Additionally, the phosphorylation of the Ser⁷²⁷ residue can be moderated by the Ras/mitogen-activated protein kinase (MAPK) pathway when it is induced by IL-6 (36, 59). However, the phosphorylation of Ser⁷²⁷ residue on STAT3 is not affecting the DNA-binding ability of STAT3 (60, 61) (Figure 2).

It was reported that STAT5 can be triggered by cytokines, such as Prolactin (PRL), growth hormone, erythropoietin, thrombopoietin, EGF, IL-2, IL-3, IL-6, IL-7, IL-9, and IL-15 (62). STAT5a and STAT5b are phosphorylated at its Tyr⁶⁹⁴ and Tyr⁶⁹⁹ residue by JAK2 (28). STAT3 and STAT5 isoforms are able to be phosphorylated and consequently initialized by non-receptor tyrosine kinases of the Src kinase families (63, 64), particularly c-Src (65, 66). However, activation of STAT5 by Src kinases will only translocate STAT5b into the nucleus Serine phosphorylation sites for STAT5 isoforms are Ser⁷²⁵ and Ser⁷³⁰ for STAT5a and STAT5b, respectively in murine (67). Another study demonstrated that Ser⁷⁷⁹ in STAT5a was constitutively phosphorylated in multiple tissues (40, 68). Xue et al. (40) demonstrated that both STAT5 isoforms underwent rapid serine phosphorylation in response to IL-2 where the phosphorylation site in STAT5a was Ser⁷⁸⁰ which is not conserved in STAT5b. The mutation of Ser⁷³⁰ in STAT5b stimulated the STAT5 DNA binding activity thus promote the growth hormone-induced β -casein promoter activity (69).

CROSSTALK BETWEEN STATS WITH OTHER TRANSCRIPTION FACTORS IN CANCER AND INFLAMMATION

Constitutively activated STAT3 and STAT5 are causes of concern due to their tumorigenic potential. Various oncogenic pathways

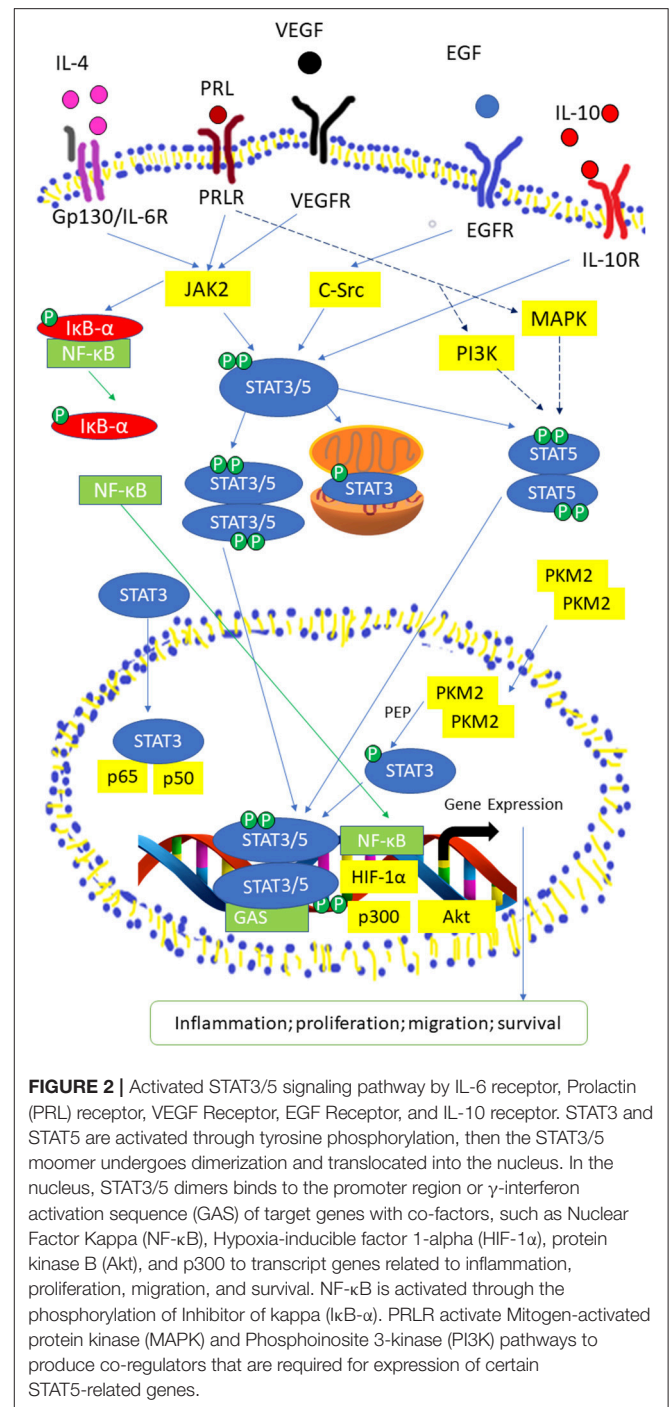


FIGURE 2 | Activated STAT3/5 signaling pathway by IL-6 receptor, Prolactin (PRL) receptor, VEGF Receptor, EGF Receptor, and IL-10 receptor. STAT3 and STAT5 are activated through tyrosine phosphorylation, then the STAT3/5 monomer undergoes dimerization and translocated into the nucleus. In the nucleus, STAT3/5 dimers binds to the promoter region or γ -interferon activation sequence (GAS) of target genes with co-factors, such as Nuclear Factor Kappa (NF- κ B), Hypoxia-inducible factor 1-alpha (HIF-1 α), protein kinase B (Akt), and p300 to transcript genes related to inflammation, proliferation, migration, and survival. NF- κ B is activated through the phosphorylation of Inhibitor of kappa ($\text{I}\kappa\text{B}-\alpha$). PRLR activate Mitogen-activated protein kinase (MAPK) and Phosphoinositide 3-kinase (PI3K) pathways to produce co-regulators that are required for expression of certain STAT5-related genes.

are regulated by STAT3, including cell-cycle progression, apoptosis, angiogenesis, invasion, and metastasis (58, 70). This is because STAT3 is capable of modulating the expression of proteins involved in these pathways, for instance, cell cycle regulator cyclin D1, anti-apoptotic proteins B-cell lymphoma 2 (Bcl-2) and B-cell lymphoma-extra Large (Bcl-xL), Vascular Endothelial Growth Factor (VEGF) (58, 63) and Matrix Metalloproteinase-9 (MMP-9), which performs a main role in

invasion (23, 71). Like STAT3, STAT5 is able to modulate growth and suppression of cell death in a few cancer cell lines, notably association with the Bcl-Ab1 fusion protein in hematopoietic cancers (72, 73). STAT5 is reported to influence the expression of Bcl-2, Survivin, MMP-2, MMP-9, VEGF, and E-cadherin by interacting with MAPK pathway in colorectal cancer cells (72). Interestingly, STAT family also collaborate with other transcription factors, such as RUNX family proteins, p53 and Nuclear factor- κ B, as described below.

RUNX Family Proteins

Runt-related (RUNX) family genes were shown to participate in the progression of human cancers and induce tumors in mouse models (74). The RUNX genes exhibit the properties of oncogenes and tumor suppressors in various cancers and cellular contexts (75). All RUNX family genes attach DNA via the conserved Runt domain, and contain the same heterodimeric binding cofactor, CBF β (75). Mutations in RUNX genes have been correlated with different types of cancers. As one of the developmental regulators in haematopoiesis, functional dysregulation of RUNX1 will lead to leukemia (74, 76). RUNX2 is crucial in breast and prostate cancer progression and is related with osteosarcoma (77, 78). RUNX3 is strongly implicated as tumor suppressor for gastric cancer and the absence of RUNX3 expression is proposed to be linked to gastric cancer because expression of RUNX3 is not observed in more than 45% of the gastric cancer patients (74, 77, 79). JAK-STAT signaling pathway induces interferon- γ (IFN- γ) to express TGF- β signaling pathways inhibitor, Smad7 (80). JAK-STAT signaling pathway indirectly affect the expression of RUNX3 as this gene is closely linked to TGF- β signaling pathways (81–83). By indirect binding of STAT3 and bone morphogenetic proteins (BMP)-specific Smads, BMP pathways interact with Leukemia inhibitory factor (LIF) induce differentiation of astrocytes from neuroepithelial cells (84). Latent form of STAT1 protein binds to RUNX2 and retains it in the cytoplasm thus attenuating its function as a transcription factor in osteoblast and this interaction occurs independent of IFN signaling since phosphorylation of tyrosine-701 of STAT1 is not needed (85). STAT1 is suggested to regulate the role of other RUNX proteins and STAT1 indirectly regulate TGF- β superfamily signaling through the RUNX proteins (86).

p53

The p53 gene is a tumor suppressor gene and a potent inhibitor of cell growth (87). The p53 protein arrests cell cycle progression at several points and promotes cell death of cancerous cells (88). Activated Stat3 was reported to bind to the p53 promoter both *in vitro* and *in vivo*, suppressing the p53 activity in breast cancer cells (88). Both STAT1 and p53 activate the p21 gene promoter (89, 90) and they both bind to p300/CREB binding protein (CBP) at different sites suggest that they may form a complex with CBP to regulate p53-dependent apoptotic signaling pathway (91). Knockout STAT1 gene from a p53-deficient animals leads to rapid tumor development and with a wider spectrum of tumor types (92). Mdm2 protein promotes degradation of the p53 protein by ubiquitination (93), is downregulated by STAT1 (91). Townsend et al. (91) also demonstrated that STAT1 and p53

cooperate in inducing apoptosis in cancer cells. Elevated STAT1 transcription activation is reported to enhance the cytotoxic activity of p53 inducers, such as fludarabine (94). Youlyouz-Marfak et al. (95) showed that agents that are able to induce p53 activation could also promote collateral STAT1 activation. The role of STAT2 in cancer progression and the correlation with p53 remain understudied. Recently Gamero et al. (96) showed that STAT2 is tumorigenic in the absence of p53, STAT2 knockdown in p53 null tumor cells increased protein levels of the marker of epithelial-mesenchymal transition (EMT), E-cadherin while overexpression of STAT2 in p53 null cells reduced E-cadherin protein.

Nuclear Factor Kappa B

Nuclear factor- κ B (NF- κ B) is a well-known signaling pathway accountable for inflammation-induced carcinogenesis and anti-tumor immunity. NF- κ B has potential of enhancing expression of different inflammatory mediators by acting as their transcription factor in number of immune reactions, it has been designated as major signaling pathway involved in development of tumor as a result of inflammation (97–99). Alongside, dominant part of STAT proteins especially STAT3 in development of inflammation mediated cancers, it is not surprising to mention that it possesses secret crosstalk with NF- κ B (100–104). These proteins are constantly expressed in carcinoma cells and indispensable for converting cytoplasmic signals from extracellular stimuli and act as nuclear transcription factors needed for modulating genes involved in tumor growth, existence, angiogenesis and invasiveness, as well as genes encoding key cancer-promoting inflammatory mediators (52, 98, 99, 105, 106). Various inflammatory factors encoded by NF- κ B target genes, most notably IL-6, are critical activators of STAT3 (1, 100, 101, 107). While in certain tumors, STAT3 encounters directly with NF- κ B to capture it in nucleus and thus causing its activation in malignancies (102). Eventually, STAT3 and NF- κ B are major regulators of oncogenic and pro-inflammatory genes (105, 106, 108, 109). In comparison to regulated expression of STAT3 and NF- κ B in healthy cells, persistent restricted expression of these genes by uninterrupted activation of STAT3 and NF- κ B will result in chronic inflammation and advancement of cancer cell growth (110).

THERAPEUTIC IMPLICATIONS OF TARGETING STAT3

JAK-STAT Inhibitor

Recently, Debio 0617B is the first-in-class kinase inhibitor that targeting phospho-STAT3 (pSTAT3) and/or pSTAT5 specifically in carcinomas by suppressing the activity of JAK, SRC, ABL, and class III/V receptor tyrosine kinases (RTK). Debio 0617B demonstrated dose-dependent inhibition of pSTAT3 in STAT3-activated cancer cell lines as well as suppressing cell proliferation in several cancer cell lines and in malignant xenografts derived from patients (111). In year 2011, Ruxolitinib (Jakafi[®], Incyte Corp.) was the first United State Food and Drug Administration (USFDA) approved JAK inhibitor which targeting both JAK1 and JAK2 (112). Ruxolitinib was investigated in Clinical Trial Phase 3

as intervention for Myeloproliferative Neoplasms, Polycythemia Vera, Primary Myelofibrosis and Graft-vs-host Disease in year 2018. In comparison with other JAK family members which are ubiquitously expressed in mammals, JAK3 is predominantly expressed in hematopoietic cells and is highly regulated with cell development and activation (113, 114). Signals relayed by the JAK3 protein regulate the growth and maturation of T cells and natural killer cells. Tofacitinib (Xeljanz[®], Pfizer) was approved by the USFDA as a JAK3-selective suppressor for rheumatoid arthritis (RA) therapy in year 2012 and recently approved to treat Ulcerative Colitis (112). However, Tofacitinib still demonstrates restricted selectivity against JAK1 and JAK2 which could lead to outcomes like anemia and neutropenia due to simultaneous suppression of JAK1 and JAK2 (115, 116). Pei et al. developed a 4-aminopiperidine-based compound, RB1 which was extremely selective for JAK3, and reasonable pharmacokinetics properties ($F = 72.52\%$, $T_{1/2} = 14.6$ h) and favorable results of toxicology experiments exhibited by RB1 indicating that it might be a potent candidate for RA treatment (113). Several JAK inhibitors which are currently under developmental phases have been listed in **Table 1**.

Persistent initiation of STAT3 and STAT5 are described in a few human cancer cell lines, and clinical samples (117, 118). However, the development of STAT5 inhibitor has been distinctly slower compared to STAT3, but recent years witness a tremendous effort to fill in this gap (119). For instance, Wingelhofer et al. demonstrated that STAT5 SH2 domain inhibitor, AC-4-130 could directly bind and disturb STAT5 activation, dimerization, nuclear translocation, and STAT5-dependent gene transcription in acute myeloid leukemia (120). On the other hand, a psychotropic drug, pimozide was reported to decrease STAT5 tyrosine phosphorylation, induce cell cycle arrest and cell death in chronic myelogenous leukemia cells (121). In fact, several STAT5 inhibitors in development have advanced to clinical studies and we have summarized these trials in **Table 2**.

Several natural-derived pharmacological agents have been used to target aberrant JAK/ signaling pathway by diverse mechanism(s) including blockage of upstream tyrosine kinases that can phosphorylate STAT3/5; activation of negative regulators of STAT3/5 signaling cascade; abrogation of STAT3/5 dimerization, acetylation, and DNA binding (26, 122). However, these natural-derived STAT3/5 inhibitors have exhibited limited efficacy in clinical trials so far, and additional studies are required to clearly establish their utility as a monotherapy or in combination regimens with existing drugs for cancer patients. Few important natural-derived STAT3/5 inhibitors have been summarized in **Table 3**.

STAT3/5-Targeting RNAi

Inhibition of STAT3 pathway has been studied by several small molecules through either upstream inhibition of cytokine and growth factors, inhibition of STAT3 dimerization, inhibition of STAT3/STAT3 nuclear translocation or inhibition of DNA binding activity (129). Due to cytotoxicity and limitation of specificity, progress of pharmacological inhibitors of STAT3 has been limited. Targeting STAT3 using small molecule inhibitors or other non-specific methods could trigger many undesirable

adverse effects because STAT3 is also expressed in normal tissues and involved in many normal cellular processes (64, 130). Thus, these inhibitors need to be delivered by coupled to delivery agents to increase specificity and avoid causing unwanted side effects.

RNA interference (RNAi) is a valuable research tool for analyzing the function of specific genes in cellular and disease processes and for therapeutic application by specific gene knockdown (131–133). RNAi can be mediated either by inserting small interfering RNAs (siRNAs) directly into the cytoplasm of the host cells or by viral vector expressing short hairpin RNAs (shRNAs) which are processed intracellularly into siRNAs by Dicer (134, 135). After the introducing of siRNAs, they will be integrated into RNA-induced silencing complex (RISC), which one strand will be removed and the RISC will be guided by the remaining antisense RNA strand to stop translation of mRNAs bearing complementary sequences (134). The gene knockdown can last for 1 week in splitting cells and for a few weeks in non-splitting cells (132).

Konnikova et al. initially transfected human astrocytoma cell line with STAT3 siRNA, which subsequently led to apoptosis induction via inhibition of anti-apoptotic genes (Bcl-XL and Survivin) expression (136). Later they found that similar cell death was observed in human gastric, primary glioblastoma, and breast cancer cells after transfection with STAT3 siRNA (137). On the other hand, Xiong et al. transfected the human colorectal cancer cell lines SW1116 and HT29 with STAT5 siRNA and the results indicated that STAT5 is involved in promoting cancer cell growth, cell cycle progression, invasion and migration by modifying expression of Bcl-2, p16, p21, E-cadherin, VEGF, MMP, and p27 genes in colorectal cancer (72). Another study showed that human glioblastoma-astrocytoma U87 cells transfected with STAT5 targeting siRNA, caused in particular suppression of STAT5 genes and STAT5 mediated DNA-binding activity as well as significant inhibition of cell invasion (138). Overall, STAT3/5 targeting siRNA is proven to promote apoptosis, cell cycle arrest, and suppress cancer cell invasion in various carcinoma cell-line models, including prostate, esophageal, hepatocellular, ovarian, laryngeal, breast, and colorectal cancer (139–146).

The main challenge to develop siRNA as therapeutics is to overcome the siRNA poor deliverability because naked siRNA are susceptible to degradation by extracellular RNases in the bloodstream and could not pass through cell membranes because of their large molecular weight and net negative charge (132, 147). To overcome the challenges, nanoparticles are commonly used for the delivery of the naked siRNA since they offer protection to the siRNA and assist in endocytosis. This review also presents an update on the current strategies used to deliver STAT3/5 targeting siRNA using viral vectors and nanoparticulate delivery systems.

OTHER STRATEGIES IN TARGETING STAT3/5

Viral Vector

Viruses are natural carriers of genetic information. Previous attempt to transduce B and T lymphocytes using retroviruses

TABLE 1 | JAK inhibitors in clinical trial (phase I not included).

JAK inhibitor	Target of the inhibitor	Disease in clinical trial	Phase of clinical trial	ClinicalTrial.gov (USA) number
Ruxolitinib (INC424)	JAK1,2	Myeloproliferative neoplasms (MPN)	III	NCT00952289
		Polycythemia vera (PV)	III	NCT01243944
		Primary myelofibrosis (MF)	III	NCT02087059
			II	NCT00934544
			II	NCT01732445
		MF, Post-PV, Post-essential thrombocythemia (ET) myelofibrosis	II	NCT01795677
			II	NCT01787552
			II	NCT01693601
			III	NCT01969838
			II	NCT01392443
			II	NCT01445769
			II	NCT00509899
			II	NCT02158858
			III	NCT03112603
			II	NCT02997280
		MF, Acute lymphoblastic leukemia (ALL), MPN, Myelodysplastic syndrome (MDS)	III	NCT03395340
			III	NCT02953678
			II	NCT03099304
			II	NCT02723994
			II	NCT01251965
II	NCT01776723			
II	NCT01712659			
II	NCT00639002			
II	NCT01751425			
II	NCT02164500			
Graft-vs-host disease (GVHD)	II	NCT01877005		
	II	NCT02001181		
	Atopic dermatitis	II	NCT02281552	
		III	NCT00853385	
	RA	II	NCT00147498	
		III	NCT01309737	
	Psoriasis	II	NCT01815424	
		II	NCT01186744	
		II	NCT01976364	
		III	NCT01039688	
Arthritis, RA	III	NCT01458574		
	III	NCT01458574		
Ulcerative colitis	II	NCT02197455		
	II	NCT00658359		
Alopecia areata (AA), Alopecia totalis (AT), Alopecia universalis (AU)	II	NCT00483756		
	II	NCT02265510		
Kidney transplantation	II	NCT02265510		
	II	NCT03450330		
INCB052793	JAK1	Solid tumors, advanced malignancies, metastatic cancer	II	NCT02265510
AZD4205	JAK1	Non-small cell lung cancer	II	NCT03450330
TD-1473	JAK1,2,3	Crohn's disease	II	NCT03635112
Givinostat (ITF2357)	JAK2	MF	II	NCT00606307
		PV	II	NCT00928707
		MPN	II	NCT01761968
Pacritinib	JAK2	MF	II	NCT03645824
Decernotinib (VX-509)	JAK3	RA	II	NCT01590459
		II	NCT01754935	
Baricitinib	JAK1,2	GVHD	II	NCT02759731
		Giant cell arteritis	II	NCT03026504
Lestauritinib (CEP-701)	JAK 2	MF	II	NCT00494585
BMS-911543	JAK2	MF	II	NCT01236352

TABLE 2 | STAT5 inhibitors in clinical trial.

STAT5 inhibitor	Disease in clinical trial	Phase of clinical trial	ClinicalTrial.gov (USA) number
Pioglitazone	CML	II	NCT02888964
Methotrexate and sirolimus	ALL	II	NCT01162551
Tacrolimus	GVHD	II	NCT01927120
Sirolimus			
AT9283	Leukemia	I	NCT01431664

TABLE 3 | Natural-derived STAT3/5 inhibitors.

Inhibitors	Target of the inhibitor	Disease	References
Cryptotanshinone	STAT3	Prostate cancer	(123)
Capsaicin	STAT3	MM	(124)
Curcumin	STAT3	MM	(125)
Cucurbitacin I	STAT3	Osteosarcoma	(126)
Celastrrol	STAT3	MM	(60)
Atriprimod	STAT3	MM	(127)
Sulforaphane	STAT5	CML	(128)

had only 27.2% transduction efficiency after a single transduction (148), however the combination of lentiviral vector system with LacZ reporter fusion system is able to deliver STAT3 shRNAs with high level of transduction efficiency with average 85% in three lymphoma cell lines (131). Yang's team (149) transfected human pancreatic cell line, SW1990 with LV-STAT3siRNA third generation self-inactivating lentivirus vector, significant decrease of VEGF and MMP-2, decrease of cell growth, and decrease of invasion ability of the cancer cells were observed after the transfection. In another study, a single injection of lentiviral vectors encoding *Stat3*-targeting shRNA were able to downregulate *Stat3*, Survivin, and MMP-2 at the same time impair tumor cell survival and invasiveness in melanoma models (150). A lentiviral vector was constructed to co-express Interferon (IFN) genes and siRNA targeting STAT3 to suppress the proliferation of B16 melanoma cells with over 95% transduction efficiency (151). Adenoviral vector was used in transfecting human lung adenocarcinoma A549 cells with cDNA of carboxyl-truncated STAT5a variant that inhibit STAT5 isomers-mediated transcription (152). Due to their immunogenicity and limited transgenic capacity, viral vectors are less commonly utilized in STAT3 RNAi delivery compared to non-viral vectors.

Nanoparticles

Non-viral vectors have the edges of low toxicity, easy to be synthesized and mild immune response over viral vectors to introduce siRNA (153). Non-viral siRNA vectors including liposomes and lipid-like materials, polymers, such as polyethyleneimine (PEI), poly D, L-lactic-co-glycolic acid (PLGA), poly (alkyl cyanoacrylate), chitosan and dendrimers, such as PAMAM, positively charged ionic cell penetrating

peptides (CPP), and siRNA bioconjugates, such as cholesterol, antibodies, and aptamers (154). siRNA-PLGA/CSO micelles displayed better cellular uptake and STAT3 gene silencing effectiveness in SKOV3 ovarian cancer cells in comparison with siRNA/CSO complexes at the same N/P ratios with no remarkable differences with lipofectamine 2000 (154). Conventional transfection carriers including polymers and cationic lipids have high cytotoxicity although their transfection efficiency is high. For the cell penetrating peptide based transfection carriers, their efficiency is lower than that of existing lipidic agents due to endosomal trapping although they exhibit better cytotoxicity profiles (155). By incorporating high molecular weight polyethyleneimine cationic lipids into membrane bilayers within the cells can stimulate the introduction of siRNA into the cytoplasm, but it is also generating reactive oxygen species (ROS) and Ca²⁺ discharge (156). Usage of cationic lipids or polymers raised concerns about their suitability for systemic delivery as the result of serum instability, carrier aggregation, and cytotoxicity (157–159). This also lead to investigation of the feasibility of anionic nanoparticles for siRNA delivery (158). We have summarized the STAT3/5-Targeting Nanoparticles in **Figure 3**.

Polyethyleneimine (PEI)

Due to its high toxicity, PEI is not preferable to be utilized as non-viral vector for siRNA delivery (154). To overcome the high toxicity and better STAT3 silencing effect, PEI was modified with stearic acid (StA) to deliver STAT3 siRNA to induce tumor apoptosis in B16 melanoma cells, it showed higher potency in STAT3 silencing with a significant induction of IL-6 secretion, 2.5 times higher expression of cellular Caspase3 and reduction of VEGF production, in comparison to PEI complexes alone (129). The modification of branched PEI with lipids will protect siRNA integrity and improve the siRNA delivery into the cytoplasm (160). The complex of siRNA/PEI/StA showed significant increase of IL-6 secretion, increase of cellular Caspase3 activity and reduction of VEGF production compared to siRNA/PEI (129). PEI also was used in combination with PLGA (161, 162) and Graphene oxide (163). Graphene oxide was functionalized with PEI and polyethylene glycol (PEG) to serve as a plasmid based *Stat3* siRNA carrier in the *in vivo* study of treating mouse melanoma cell line (163).

Poly L-lactic-co-glycolic Acid (PLGA)

PLGA has been widely used as non-viral carrier in STAT3 siRNA delivery in different combinations in recent year. Positively charged Chitosan oligosaccharide (CSO) was added to the STAT3 siRNA-PLGA micelles and the micelles showed high efficiencies of cellular uptake and STAT3 gene silencing in SKOV3 ovarian cancer cells (154). Chitosan was chosen as a condensing agent due to its unique biological activities and when it is fused with siRNA-PLGA conjugates, it can form smaller particles to increase the cellular uptake (154). PLGA nanoparticles can be directly coated with cationic polymers, such as PEI which allow siRNA to attach on their surfaces (164). Combination of non-toxic PLGA and PEI nanoparticles could deliver siRNA cross the blood brain barrier, promoted cell death and arrested cells at G1/G0 stage

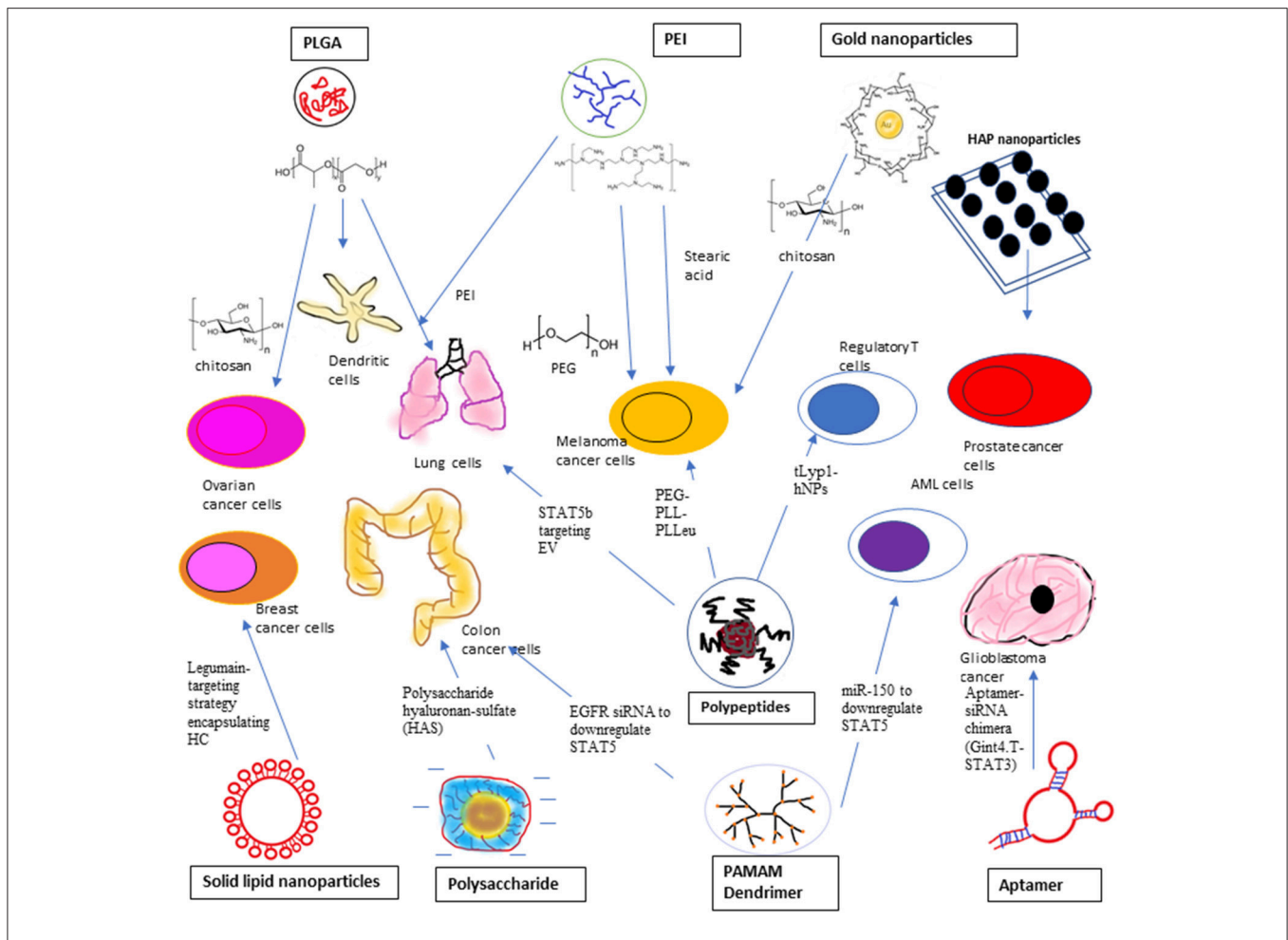


FIGURE 3 | STAT3 siRNA delivery using Polyethyleneimine (PEI), Ploy L-co-glycolic Acid (PLGA), and Inorganic compound, such as gold nanoparticles and Hydroxyapatite (HAP) nanoparticles to target ovarian cancer cells, malfunctioned dendritic cells, lung cancer cells, melanoma cancer cells, and prostate cancer cells. STAT3 siRNA delivery using polypeptides, solid lipid nanoparticles, polysaccharide, and aptamer. Poly (ethylene glycol)-b-poly (L-lysine)-b-poly (L-leucine) (PEG-PPL-PLLLeu) is used to target melanoma cells while Triethylamine hybrid nanoparticles (tLyp1-hNPs) targets Regulatory T-cells. Legumin-targeting strategy is employed to targeting breast cancer cells using encapsulated hydrocarbon carrier. Anionic charged siRNA co-assembled with polysaccharide hyaluronan-sulfate (HAS) to target mouse colon cancer cells. Gint4.T-STAT3 was developed to deliver STAT3 siRNA to PDGFR β ⁺ Glioblastoma cancer cells. PAMAM Dendrimer was used to deliver EGFR siRNA and miR-150 to suppress STAT5 expression in colon cancer cells and AML cells, respectively. Polypeptide PEGylated-anisamide-LPH nanoparticle was used to transfect lung cancer cells with STAT5B targeting non-apeptide, EV.

in vitro and *in vivo*, lead to notable reduce in expression of IL-6 and the angiogenic factor and elevate in caspase3 activity in tumor bearing mice (161). Su's team developed a novel strategy to synthesize PEI-coated paclitaxel-loaded PLGA nanoparticles to target human lung malignant cells and paclitaxel-resistant cell lines (162). *Stat3*-enhanced chemoresistance in lung malignant cells were suppressed by introduction of *Stat3* siRNA thus causing the tumor cells more responsive to paclitaxel (162).

PLGA nanoparticles that containing both STAT3 siRNA, an immune response modifier (imiquimod, R837) and near-infrared (NIR) fluorophores (indocyanine green) enable researchers to monitor the migration of the activated Dendritic cells after the transfection using real time NIR fluorescence imaging (165). Other than siRNA, STAT3 inhibitor JSI-124 was chemically conjugate to PLGA, generated a conjugate which exhibited potent anticancer and STAT3 silencing in immunosuppressed dendritic

cells (166). The PLGA-JSI-124 conjugate worked best when combined with Dendritic cells adjuvant CpG (166).

Inorganic Compounds

To enhance the stability and cell uptake, layer-by-layer chitosan coated gold nanoparticles were used to deliver STAT3 siRNA and co-deliver *Stat3* siRNA and Imatinib (IM) to treat murine melanoma cells using iontophoresis to strengthen the localized skin penetration (167, 168). Gold nanoparticles have demonstrated potential in biomedical application because they have high biocompatibility, chemically inert, nanosized, versatile and have longer plasma circulation (169). In the treatment of melanoma cells, it was the first report to demonstrate *in vivo* efficacy studies of non-invasive iontophoretic administration of anti-cancer agents that was comparable with intratumoral administration (151). Layer-by layer assembly of polyelectrolytes

formed a firm entrapment of siRNA and IM at the same time the electrostatic interactions facilitated adequate slow delivery of therapeutics (151). Plasmid-based *Stat3* siRNA introduced by CaCl₂ modified Hydroxyapatite (HAP) nanoparticles was able to lower the protein expression of *Stat3* and *p-Stat3* in prostate tumor bearing mice at the same time downregulated the expression of *Stat3*-associated downstream genes (170). A hybrid of lipid and polymer vesicles with calcium phosphate as the solid kernel (CaP@HA) was used to introduce STAT3-specific decoy oligonucleotides (STAT3-decoy-ODNs) into TRAZ-resistant HER2-positive breast cancer cells (171). ODNs packaged with CaP@HA showed significantly increased serum stability, cellular transfection, synergistic cytotoxicity and apoptosis *in vitro* compared to normal ODNs (171).

Polypeptides

Poly(ethylene glycol)-b-poly(L-lysine)-b-poly(L-leucine) (PEG-PLL-PLLeu) polypeptide micelles was used for co-encapsulating Toll-like receptor agonist, STAT3 siRNA and OVA antigen to generate nanovaccine in OVA-transfected melanoma cell line (172). PMP/OVA/siRNA simultaneously facilitated the cellular uptake of OVA antigen and siRNA about 3–200-folds, and decreased STAT3 expression in TADCs over 50% both *in vitro* and *in vivo* (172). Melittin derived peptides, P5RHH could conjugated with *Stat3* siRNA to form nanoparticles with small size (190 nm in diameter) with negligible cytotoxicity (155). p5RHH/STAT3 siRNA nanoparticles mediated transfection to impede malignant cell growth, angiogenesis and foam cell formation in mouse melanoma cells while maintaining their size and transfection effectiveness even there are serum proteins around (155).

tLyp1 peptide-conjugated with PEG-DSPE and triethylamine to form hybrid nanoparticles (tLyp1-hNPs) for targeting Treg cells by suppressing of STAT3 and STAT5 phosphorylation at the same time improve the effect of imatinib (173). Imatinib (IMT) has been shown to downregulating Treg cell expression but its application was limited by poor solubility and high cytotoxicity (174). Treg cell targeted tLyp1-hNPs also showed improved survival rate, improved tumor suppression, reduced intratumoral Treg cells, and increased intratumoral CD8⁺ T cells against malignant cells in *in vivo* study when incorporate with anti-cytotoxic T-lymphocyte antigen-4 (CTLA4) antibody (173).

King and Huang (175) developed EEEepYFELV (EV), a non-peptide that inhibit STAT5b phosphorylation and they delivered the inhibitor using a PEGylated-anisamide-LPH nanoparticles to target human lung cancer cells. EV also demonstrated inhibition effect on the phosphorylation of STAT5a without affecting STAT3 phosphorylation (175).

Solid Lipid Nanoparticles

Curcumin can exert anti-inflammatory effects in colitis by inhibiting NK- κ b activation and STAT3 pathway (176). A synthetic analog of curcumin, Hydrazinocurcumin (HC) was synthesized to improve its stability, water solubility, cell permeability and bioavailability to surpass curcumin (177). HC were mixed with 1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE),

Dipalmitoylphosphatidylcholine (DOPC), Cholesterol and DOPE-PEG to generate peptide-lipid conjugated HC nanoparticles to treat breast cancer cell line (178). The study showed that HC encapsulated nanoparticles effectively convert tumor associated M2 macrophage to M1 macrophage with reduced expression of IL-10, TGF- β , p-STAT3, MMP-9, MMP-2, and VEGF, along with elevated expression of IL-12 (pro-inflammatory cytokines) in 4T1 murine breast cancer cell line (178). To enhance targeting capability and solid-tumor penetration, Legumain-targeting strategy encapsulating HC were used by adding Legumain-targeted RR-11a into the liposomal nanoparticles (178, 179). In another study, the suitability of introducing Curcumin in solid lipid nanoparticles (SLN-curc) or d- α -Tocopheryl polyethylene glycol 1,000 succinate (TPGS) nanoparticles (TPGS-curc) was investigated in Hodgkin's lymphoma in mice, which SLN-curc was more superior compared to TPGS-curc in term of pharmacokinetic profile (180).

Non-toxic cationic solid lipid nanoparticles (SLN) was combined with STAT3 decoy oligodeoxynucleotides (ODN) to treat human ovarian cancer cells and cellular uptake of SLN-decoy ODN was comparable to that of Lipo-decoy ODN which is more toxic (181). SLN-STAT3 decoy ODN complexes increased expression of cleaved caspase 3, Bax, Beclin-1 and LC3-II and reduced expression of Bcl-2, pro-caspase 3, Survivin, p-Akt, and pmTOR in human ovarian cancer cell lines (181). Overexpression of STAT3 in Non-Small Cell Lung Cancer (NSCLC) patients is a strong predictor of poor prognosis, cationic solid lipid nanoparticles (cSLN) were used to deliver RNAi-mediating plasmid DNA targeting STAT3 in cisplatin resistant lung cancer cells (182, 183). STAT3 mRNA expression level were lowered by ~5-fold in chemo-resistant Calu1 NSCLC cells after treatment of cSLN:plasmid DNA complexes (K2 and K3) (182).

Reconstituted HDL (rHDL) nanoparticles were utilized to deliver STAT3 siRNA or FAK siRNA to treat human ovarian and colorectal cancer cell lines (184). Combination treatment with STAT3 siRNA/rHDL and docetaxel further impede malignant cell proliferation: Treated the cells with Docetaxel independently caused no impact on cell proliferation, meanwhile STAT3 siRNA/rHDL monotherapy resulted in 19% reduction of proliferation (184).

Polysaccharides

Although anionic charged nanoparticles were less commonly used in siRNA delivery, one research group introduced anionic siRNA nanoparticles co-assembled with polysaccharide hyaluronan-sulfate (HAS) mediated by calcium ion bridges (158). Effective cellular uptake of this anionic siRNA nanoparticles, together with strong gene silencing was observed in several cell types, which include murine primary peritoneal macrophages and human hepatocellular malignant cells (158). Anionic complexes generated via reversible complexation of siRNA and calcium ions in solution were able to downregulate various genes in several cell lines (185). The semi-synthetic HAS was selected because it has multiple functional groups available for ligand attachment and it is more stable compared to non-modified hyaluronan (186). Alginate sulfate (Algs)

with STAT3 siRNA were co-assembled and then followed by bioconjugation of N-acetylgalactosamine (GalNAc) which is specific to the asialoglycoprotein receptors that are overexpressed on hepatocytes to produce a novel tumor specific delivery vector (GalNAc-NPs) (187). GalNAc-NPs enter the cells through ASGPR-mediated endocytosis, these NPs able to induce 67% reduction in STAT3 mRNA levels at 2.5 mM Ca^{2+} (187).

Aptamers

Aptamers can be introduced into the host cells specifically through cell-mediated endocytosis, solving probable problems of other oligonucleotide therapeutics, such as siRNA, shRNA and miRNA (188). A novel aptamer-siRNA chimera (Gint4.T-STAT3) was developed to deliver STAT3 siRNA efficiently and subsequently suppressed the expression of STAT3 in PDGFR β^+ glioblastoma multiforme (GBM) cells, through *in vitro* and *in vivo* studies, demonstrating that this is an effective strategy (189). Gint4.T-STAT3 possesses good serum stability and reduces the expression of STAT3 and its target genes (cMYC, MCL-1, Bcl-2, and Bcl-XL). Moreover, a reduction of pro-caspase 3 and Bcl-XL anti-apoptotic protein levels was also observed (189). PDGFR β is a receptor tyrosine kinase (RTK) which is frequently found in GBM (190). Esposito et al. showed that Gint4.T is capable of passing through a tri-culture *in vitro* model of hematoencephalic barrier, thus making it a suitable carrier targeting GBM cancer stem-like cells (189).

PAMAM Dendrimer

Polyamidoamine (PAMAM) dendrimers are one of the most popular dendrimers. In one study, EGFR antisense oligonucleotides were encapsulated with PAMAM dendrimers to targeting colon cancer cell line (191). The expression level of EGFR was significantly reduced up to 40–50%, consequently the downstream genes of EGFR pathway, MAPK1, and STAT5 expression were down-regulated (191). Jiang et al. (192) developed a nanoparticle delivering system by conjugating FLT3 ligand, amidoamine and PAMAM and used this nanoparticle to deliver miR-150 into AML cells. The inhibition of FLT3 signaling pathway by this nanoparticle suppress the activity of ERK, AKT and STAT5 *in vivo* indirectly (192).

Exosomes

Exosomes are extracellular membrane vesicles with a diameter of 30–120 nm and containing endogenous proteins, genetic material, such as DNA, and lipids (193). Recently, Zhang et al. (194) demonstrated that two exosome subpopulations, Exo-L and Exo-S contained proteins that are involved in regulating secretion pathways including IL-2/STAT5 signaling pathways,

suggesting that these nanoparticles have the potential role for STAT5 targeting treatment.

CONCLUDING REMARKS

In addition to the well-distinguished role of STAT family as transcription factors in causing inflammation and tumorigenesis, we have described various strategies used to regulate STAT3/5 transcription activity either through JAK-STAT inhibitor or RNAi-based targeting system. The findings described in this review indicate that the current understanding of STAT pathway has advanced to the point where different kind of therapeutic interventions are being developed to regulate aberrant activation of STAT protein in order to control chronic inflammation or improve prognosis of several cancers. There is still a need to understand how STAT proteins crosstalk with other signaling pathways and further improve the drug delivery system if we are to develop rational strategies tailored to individual cancers. Based on the progression reported in several studies, STAT3 targeting siRNA using nanoparticulate delivery systems currently offer higher specificity with lesser side effect compared to conventional drug delivery system. These proof-of-concept studies might hold the potential to become mainstream strategies in regulating STAT activation and more works are needed in order to advance these methods into clinical phase.

AUTHOR CONTRIBUTIONS

C-YL and CL conceived the review. C-YL, AA, AN, WW, GS, and CL searched the literature, drafted the manuscript, and critically revised it. C-YL and CL designed and coordinated the manuscript. All the authors read and approved the submitted version of the manuscript.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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