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The STAT3 pathway as a therapeutic target in head and neck cancer: Barriers and innovations

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SUMMARY

Proteins of the signal transducer and activator of transcription (STAT) family mediate cellular responses to cytokines and growth factors. Aberrant regulation of the STAT3 oncogene contributes to tumor formation and progression in many cancers, including head and neck squamous cell carcinoma (HNSCC), where hyperactivation of STAT3 is implicated in both treatment resistance and immune escape. There are no oncogenic gain-of-function mutations in HNSCC. Rather, aberrant STAT3 signaling is primarily driven by upstream growth factor receptors, such as Janus kinase (JAK) and epidermal growth factor receptor (EGFR). Moreover, genomic silencing of select protein tyrosine phosphatase receptors (PTPRs), tumor suppressors that dephosphorylate STAT3, may lead to prolonged phosphorylation and activation of STAT3. This review will summarize current knowledge of the STAT3 pathway and its contribution to HNSCC growth, survival, and resistance to standard therapies, and discuss STAT3-targeting agents in various phases of clinical development.

Keywords

STAT3; Head and neck cancer

Introduction

Head and neck squamous cell carcinoma (HNSCC) is the sixth leading incident cancer worldwide with 55,000 cases in the United States and 550,000 cases globally in 2014 [1,2]. Despite advances in surgical and radiotherapy techniques, as well as integration of chemotherapy into multimodality treatment paradigms, HNSCC is frequently lethal. Five-year overall survival (OS) is 40–60% and has increased only marginally since 1990 [3]. Incremental improvements in prognosis are largely attributable to changing epidemiology, rather than treatment *per se*. An increasing proportion of oropharyngeal HNSCC is caused

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Conflict of interest

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by oncogenic human papillomavirus (HPV), rather than the classic risk factors of tobacco and alcohol; HPV etiology is associated with improved survival after standard treatments [4,5]. Although two distinct causes of HNSCC exist, environmental carcinogenesis or transformation by HPV oncogenes, both etiologies are associated with aberrant regulation of the signal transducer and activator of transcription (STAT) family [6–8]. However, the transcription factor (TF) signatures of HPV-related HNSCC and HPV-negative HNSCC have been elucidated and differ in respect to the activity of several key TFs, with upregulation of STAT3 and NF-κB gene targets demonstrated in HPV-negative HNSCC [9].

Proteins of the STAT family mediate cellular response to cytokines, such as IL-6, and growth factors. In particular, STAT3 transforms human epithelial cells, thereby meeting the definition of an oncogene [10,11]. Aberrant regulation of STAT3 in HNSCC underlies malignant behaviors, contributing to growth, survival and resistance to standard therapies including chemoradiation and blockade of the epidermal growth factor receptor (EGFR) [12–16]. Aberrant tumoral STAT3 signaling is also immunosuppressive, protecting HNSCC cells from recognition and lysis by cytotoxic T lymphocytes [17,18]. Tumor and lymphocyte STAT3 signaling increases production of immunosuppressive cytokines including TGF-β1, VEGF, IL-6 and IL-10; this cytokine profile negatively regulates innate danger signals, dendritic cell maturation, and cytolysis by effector cells [17–20]. *In vitro* STAT3 inhibition reverses the immunosuppressive phenotype of HNSCC [21]. The association of STAT3 hyperactivation with poor prognosis, resistance to standard therapies, and immune escape makes it a compelling target in HNSCC, particularly in HPV-negative HNSCC where functional studies suggest targeting this pathway may be effective [9]. As for other transcription factors, STAT3 historically has been considered “undruggable.” However, innovative and promising therapeutic strategies are in development. This review will summarize current knowledge of STAT3 pathway activation in HNSCC, and discuss STAT3-targeting agents in various phases of clinical development.

STAT3 activation in HNSCC

The STAT3 transcription factor exhibits its pro-transcription effects in response to signals from upstream receptors including the IL-6 cytokine receptor family, growth factor receptors such as the receptor tyrosine kinases (RTKs) vascular endothelial growth factor receptor (VEGFR) and epidermal growth factor receptor (EGFR), or nonreceptor tyrosine kinases (NRTKs) such as Janus-activated kinases (JAK) and Src family kinases (SFK) [22–24]. Fig. 1 depicts the activation of STAT3 and its target genes in schematic form. First, STAT3 is recruited to the plasma membrane upon binding of cytokines or growth factors to their respective cell surface receptors. STAT3 becomes activated by phosphorylation of a tyrosine residue within its Src homology 2 (SH2) domain (Tyr705), either by the activated RTKs directly, or by intracellular NRTKs. Phosphorylation of STAT3 then induces spontaneous dimerization of the transcription factor via a reciprocal phosphotyrosine–SH2 interaction between two STAT3 molecules. STAT3 can also heterodimerize with STAT1, though the molecular consequence of this interaction remains unknown [25]. Following STAT3:STAT3 dimerization, phospho-STAT3 translocates to the nucleus where dimers bind to consensus sequences on the promoter regions of target genes with the resultant cascade of gene

transcription. Activated STAT3 thus upregulates the transcription of cyclin D1, survivin, and Bcl-$_{xL}$.

Mechanisms of STAT3 hyperactivation in human cancer are incompletely understood. Despite near-universal STAT3 signaling activation in HNSCC, gain-of-function STAT3 mutations have not been observed; neither have activating mutations in upstream growth factor receptors such as EGFR or JAK [26,27]. In general, STATs are positively regulated by upstream cytokine or growth factor receptors or intracellular NRTKs, and negatively regulated by protein tyrosine phosphatase receptors (PTPR). Thus, STAT3 can be constitutively activated either as a consequence of enhanced signaling from positive effectors, or by decreased activity of negative effectors – as observed in HNSCC and glioma cell lines [14,28]. Aberrant protein tyrosine phosphorylation is a hallmark of human cancer. Of all known protein tyrosine phosphatases, the PTPRs comprise the largest family within the human tyrosine phosphatome [29]. Some PTPRs, including *PTPRD* and *PTPRT*, have been reported to function as tumor suppressors because gene mutations or methylation contribute to growth and survival in preclinical models [29]. STAT3 has been reported to be a substrate of *PTPRT* in colorectal cancer models [30], and a substrate of *PTPRD* in glioblastoma cells [31]. This suggests that many members of the PTPR family may be involved in tumor suppression by dephosphorylating STAT3. Of significant interest, mutations in the PTPR gene family have been described in 31% of HNSCC tumors, independent of HPV status, while methylation of *PTPRD* or *PTPRT* has been observed in 60% of the HNSCC cases within the Cancer Genome Atlas (TCGA) [31,32]. The varied distribution and absence of hotspot mutations suggest that these PTPRs function as tumor suppressors. Moreover, many mutations cluster in the catalytic phosphatase domain, supporting that de-phosphorylation of the STAT3 oncoprotein may be important to the hypothesized tumor suppressor function. Such a function was mechanistically corroborated in HNSCC tumor specimens, where selected *PTPRT* mutations correlated with *in situ* up-regulation of phospho-STAT3 expression, as compared to tumors that were *PTPRT* wild type (WT) [32]. Moreover, when WT HNSCC cells were engineered to over-express WT *PTPRT*, decreased STAT3 phosphorylation was observed, whereas transfection of a *PTPRT* phosphatase domain mutation resulted in increased STAT3 phosphorylation. *PTPRT* promoter methylation has been shown to upregulate pSTAT3 expression and is associated with sensitivity to STAT3 inhibition in HNSCC cells [33]. Conversely, mutations in *PTPRD* lead to loss of function and subsequent hyper-phosphorylation of its substrates, including STAT3, and HNSCC cell lines harboring *PTPRD* mutations are more sensitive to STAT3 inhibition [34]. Epigenetic or genetic silencing of PTPRs, negative regulators of the STAT3 pathway, may therefore represent direct drivers for tumor growth in HNSCC by hyperactivation of STAT3. This discovery suggests that tumors harboring PTPR loss-of-function events may be uniquely amenable to STAT3 pathway inhibitors, and *PTPRD* mutation and *PTPRT* promoter methylation may serve as predictive biomarkers for responsiveness to STAT3 blockade. *PTPRD* mutations found in HNSCC are summarized in Table 1 [34].

STAT3 activation and resistance to standard therapeutics

In addition to serving as an oncogene in HNSCC, STAT3 also represents a key resistance mechanism for standard therapeutics including platinum chemotherapy and radiation. Radiation is a modality of therapy paramount to the local control and improved survival of HNSCC, either as a single-modality option in definitive doses or in the adjuvant setting, or in a multimodal approach with chemotherapy. The effects of tumor cell damage, facilitated by damage to DNA, result directly from ionization of DNA or from the action of free radical formation [35,36]. STAT3 has been described as a key mediator of chemoradiotherapy (CRT) resistance in numerous cancers, including gliomas [37–39], breast cancer [40–43], colorectal cancer [44,45], and prostate and cervical cancers [42], in addition to HNSCC.

Targeting the STAT3 pathway has been shown to abrogate EGFR inhibitor resistance in HNSCC. EGFR overexpression occurs in the majority of HNSCC, and is associated with advanced stage and reduced overall survival [46–48]. As such, EGFR is a validated therapeutic target. Cetuximab, a monoclonal antibody against EGFR, is U.S. Food and Drug Administration (FDA)-approved for the treatment of locally advanced HNSCC when combined with radiation, as well as for advanced disease when administered during front line treatment with platinum doublet chemotherapy or after platinum failure. STAT3 upregulation and activation via both EGFR-dependent and -independent pathways contributes to intrinsic or acquired resistance to EGFR targeting in HNSCC and other solid tumors. STAT3 activation has been found in the setting of resistance to EGFR tyrosine kinase inhibitors (TKI) in preclinical models of gliomas and HNSCC [14,28]. Resistance to EGFR TKI treatment of non-small cell lung cancer was associated with elevated STAT3 activity in tumors [49]. Combined treatment of HNSCC cell lines with an EGFR TKI and a STAT3 decoy molecule, an oligonucleotide designed to block STAT3 binding to DNA response elements, was associated with enhanced tumor effects relative to EGFR TKI alone [50]. Targeting STAT3 using the decoy oligonucleotide in cetuximab- or TKI-resistant cells sensitizes the cells to EGFR inhibitor treatment *in vitro* and *in vivo* [15]. These findings suggest that targeting the STAT3 pathway may enhance the antitumor effects of EGFR inhibitors and therefore abrogate resistance to anti-EGFR therapies.

Specific targets of the STAT3 pathway

Targeting the STAT3 pathway has been a major focus of drug development, due to its contribution to treatment resistance and immune escape in most epithelial malignancies [13,17,51,52]. Strategies for targeting STAT3 can be conceptualized according to its activation cascade as depicted in Fig. 1. Abrogation of oncogenic STAT3 signaling could be disrupted by (1) inhibition of upstream extracellular or intracellular receptors, thereby decreasing phosphorylation; (2) inhibition of the pSTAT3 SH2 domain, thereby blocking dimerization; (3) inhibition of STAT3-DNA binding, thereby preventing target gene transcription; and 4) inhibition of STAT3 transcription, thereby down-modulating total STAT3 expression. The agents described below are summarized in Table 2 with selected agents shown along the pathway schematic in Fig. 1.

Blocking STAT3 activation: targeting upstream receptors

JAK kinase inhibitors

Inhibiting the phosphorylation and subsequent activation of STAT3 is a logical target for inhibiting the downstream transcription products of STAT3 and can be accomplished by small molecule inhibition of the JAK kinase. Ruxolitinib, an oral small molecule inhibitor of JAK1 and JAK2, is FDA-approved for the treatment of intermediate or high-risk myelofibrosis [53]. Tofacitinib, an inhibitor of JAK3, is FDA-approved for the treatment of rheumatoid arthritis and is being studied in other inflammatory diseases, including inflammatory bowel disease and psoriasis [54,55]. Though nonspecific JAK-STAT3 inhibition has been mentioned to involve cytokine inflammatory activity and downstream transcription products of ruxolitinib [56] and efficacy studies in the tofacitinib trials have suggested that other pathways of inhibition may be affected [57], very little has been published on the effects of JAK inhibition on solid tumors. Another oral JAK1 and JAK2 inhibitor, AZD1480, was shown to abrogate IL-6 induced STAT3 phosphorylation and also suppressed the growth of human solid tumor xenografts with constitutive STAT3 activity [58,59]. In preclinical studies, AZD1480 was shown to inhibit proliferation of eight HNSCC cell lines at low concentrations [60].

These drugs are not without adverse effects, and toxicity is a concern. Ruxolitinib has been associated with cytopenias, gastroin-testinal disturbances, peripheral neuropathy, and metabolic abnormalities [61,62]. However, data from a phase III trial comparing capecitabine plus ruxolitinib to capecitabine plus placebo indicate that ruxolitinib was well tolerated with very few toxicities [63]. Adverse events reported for tofacitinib include hepatic and renal impairment, neutropenia, and an increased incidence of infections, including tuberculosis [64]. Clinical trials involving AZD1480 were terminated due to significant neurotoxicities [65]. Still another JAK2-selective inhibitor, fedratinib, showed promise in a phase III placebo-controlled trial in patients with myelofibrosis where the primary endpoint of spleen response rate was reached [66]. Unfortunately, while early clinical trials of fedratinib demonstrated the drug to be well tolerated [67,68], occurrence of neurotoxicity also forced the discontinuation of clinical development of this drug. WP1066 is a small molecule that blocks STAT3 activation by JAK2 signaling inhibition [69,70]. This molecule was studied in preclinical glioma cell models, but unfortunately exhibited poor efficacy and thus its development was terminated [71].

In addition to synthetically-derived compounds, naturally-occurring products inhibit STAT3 function by various mechanisms both *in vitro* and *in vivo*. 2-Methoxystypandrone, a naphthoquinone isolated from roots of the herb *Polygonum cuspidatum*, has activity against STAT3 activation and blocks the STAT3 pathway upstream at JAK2 [72,73]. This compound is not currently under clinical investigation but offers a potential natural alternative for future study.

Currently, many ongoing clinical trials are studying JAK inhibitors in cancer patients. Ruxolitinib is being evaluated in breast cancer (in combination with trastuzumab, NCT02066532; preoperatively in triple negative disease, NCT02041429; in combination with capecitabine, NCT02120417; in combination with exemestane, NCT01594216),

colorectal cancer (NCT02119676), nonsmall cell lung cancer (NSCLC) (NCT02119650), acute myeloid leukemia (AML) (NCT02257138), and lymphoma (NCT01965119). A phase II trial in castrate-resistance prostate cancer was terminated due to lack of clinical response (NCT00638378).

IL-6 receptor inhibitors

Cytokine proteins, including interleukins, regulate cellular growth, proliferation, and signaling in tumor environments. IL-6, an inflammatory cytokine, has been detected in high concentrations in serum of patients with HNSCC and correlates with disease relapse [74]. IL-6 activates the JAK1 and 2 pathway through signal transduction, which leads to the activation of STAT3 by phosphorylation [75], thereby making the IL-6 receptor a target for drug development.

Tocilizumab, a humanized monoclonal antibody (mAb) to the IL-6-receptor-alpha (IL6Rα), is FDA-approved in the treatment of rheumatoid arthritis and juvenile idiopathic arthritis [76–78]. This drug has been studied in other autoimmune disorders, including ankylosing spondylitis and systemic lupus erythematosus [79,80] and is currently being studied in CLL (NCT02336048). A phase I trial in ovarian cancer has been completed, with results forthcoming (NCT01637532).

Another anti-IL-6 mAb, siltuximab, binds highly to IL-6, neutralizing its bioactivity [81]. It has been studied in various malignancies, including myelodysplastic syndrome, prostate cancer, and renal cell carcinoma (RCC) [82–85], and is under study in multiple myeloma (NCT01484275).

Other inhibitors of STAT3 phosphorylation

Flavonoids, such as quercetin, have been found to reduce inflammation [86], and this pathway has been exploited for its effects on the tumor microenvironment. Quercetin is found in fruits, vegetables, leaves, and grains and used as a supplement in many foods and beverages. It has been studied in several disease states, including asthma, fibromyalgia, metabolic syndrome, and cancer. Its antitumor effects were initially described in 2000 and thought to be related to immune stimulation, free radical scavenging, alterations in mitosis, apoptotic induction, and gene regulation [87]. Quercetin was shown to be a potent inhibitor of IL-6 driven STAT3 signaling in glioblastoma cell lines [88], where it also reduced downstream expression of cyclin D1 and MMP-2. Similar results were found in cholangiocarcinoma cell lines, where treatment with quercetin suppressed the JAK2/STAT3 pathway activation with a subsequent decrease in pSTAT3 proteins [89]. Quercetin has also been observed to block tyrosine phosphorylation of JAK2 and STAT3 induced by IL-12 [90] and inhibited the proliferation of melanoma cells [91]. Oral quercetin was given daily to Balb/c mice with colon-25 tumors with an observed reduction in tumor size by day 20 [92]. Mukherjee et al. described the downregulation of IL-6-mediated STAT3 activation by quercetin in a NSCLC cell line [93].

Curcumin is a polyphenol derived from the plant *Curcuma longa* and is the main component of the spice turmeric. A naturally-occurring phenol with a bright yellow color, curcumin is used as a food coloring or additive and has been studied in diseases such as psoriasis,

arthritis, Alzheimer's disease, and various malignancies [94,95]. Anti-inflammatory effects of curcumin were classically attributed to the suppression of NF-κB activation, thereby downregulating the transcription of IL-6 and other inflammatory cytokines [96]. Kim et al. demonstrated that curcumin suppresses the phosphorylation of upstream kinases JAK1 and JAK2, resulting in downstream inhibition of STAT1 and STAT3 phosphorylation and activation [97]. Treatment of activated T-cells with curcumin was shown to inhibit IL-12-induced tyrosine phosphorylation of JAK2 and STAT3 [98] and inhibited STAT3 activation and nuclear translocation in myeloma cells [99]. This was also exhibited when curcumin was administered to murine glioma cell lines [100]. Curcumin decreased STAT3 phosphorylation in both constitutive and IL-6 induced ovarian and endometrial cells, resulting in decreased cell viability, which was shown to be reversible with normalization of pSTAT3 levels within 24 h of curcumin removal [101]. In preclinical studies specifically in HNSCC cell lines, curcumin was shown to inhibit proliferation and invasion by the inhibition of phosphorylation of EGFR and its downstream molecules, including STAT3 [102]. Curcumin also suppressed IL-6 mediated STAT3 phosphorylation [103].

Curcumin is being actively studied in various malignancies, including colorectal cancer (NCT01490996, NCT01859858), breast cancer (NCT01740323, NCT01975363), chronic lymphocytic leukemia (CLL) (NCT02100423), and prostate cancer (NCT01917890, NCT02064673, and NCT02095717). Studies are also evaluating the cancer prevention abilities of curcumin in familial adenomatous polyposis (NCT00927485, NCT00641147). Quercetin is being studied in prostate and pancreatic cancers (NCT01912820 and NCT01879878, respectively).

Blocking STAT3 dimerization: SH2 domain inhibition

Small molecule inhibitors

Inhibiting the SH2 domain of the STAT3 transcription factor blocks the two major steps required for the formation of STAT3 dimers: first, recruitment of the molecule to the plasma membrane for phosphorylation of Tyr705 by activated RTKs or non-receptor kinases, and second, the subsequent dimerization of two activated STAT3 molecules. Thereby dimer translocation to the nucleus is prevented, and transcription of target genes does not occur. Several small molecules targeting the SH2 domain have been described, and many are in various phases of development.

STA-21, a small molecule antibiotic discovered through computational methods and a virtual library of the SH2 domain, hinders dimerization of STAT3 and downregulates expression of STAT3 target genes in human carcinoma cells with constitutive STAT3 phosphorylation [104]. Despite its pathway inhibitory activity, there is no biochemical evidence to support its binding to the SH2 domain. STA-21 has been studied in patients with psoriasis, where Miyoshi et al. reported clinical improvement in psoriatic skin lesions after topical use of this molecule [105].

Direct dephosphorylation of p-STAT3 by protein tyrosine phosphatases, which includes members of the SH2-domain containing tyrosine phosphatase family (SHP-1 and SHP-2), is another mechanism that leads to downregulation of STAT3 transcription products. SHP-1,

the loss of which has been shown to enhance JAK3/STAT3 signaling in various non-Hodgkin lymphomas [106,107], represents another target in STAT3 modulation. The novel small molecule SC-2001 was shown to inhibit the transcriptional activities of STAT3 by enhancing SH-1 activity in hepatocellular carcinoma (HCC) cells [108]. This molecule has also been studied in combination with sorafenib, a multikinase inhibitor approved for treatment of unresectable HCC, advanced RCC, and thyroid cancer, where it was shown to overcome sorafenib resistance through the SH-1 pathway in HCC cell lines [109].

OPB-51602 is an oral small molecule that has high affinity for the STAT3 SH2 domain with resulting interference in STAT3 activity in numerous *in vitro* and *in vivo* models (Otsuka Pharmaceutical Co., Ltd., unpublished data). A phase I study in advanced solid tumors showed partial responses in two patients with NSCLC who previously were treated with EGFR TKIs [110]. However, a second phase I study in patients with hematologic malignancies demonstrated significant toxicity at doses where clinical responses were observed, and lower doses required a more difficult dosing schedule and no responses were observed, thus further clinical development of OPB-51602 was terminated [111]. A clinical trial to determine safety and tolerability in nasopharyngeal carcinoma patients (NCT02058017) was subsequently terminated.

OPB-31121 has also been shown to have a high affinity for the SH2 domain of STAT3 [112]. Preclinical studies show promise of this drug in hematologic malignancies [113], and phase I studies have been conducted in patients with advanced solid tumors [114] and hematologic malignancies (NCT10129509). Due to an unfavorable pharmacokinetic profile and no objective observed responses, clinical development was halted. Prior to termination of the development of OPB-31121, a phase I/II trial in patient with progressive HCC was completed with results not yet available (NCT01406574).

Pyrimethamine is an anti-parasitic drug used for both the treatment and prevention of malaria. This drug was identified as a STAT3 inhibitor through a chemical library screen and shown to reduce pSTAT3 levels in a human autosomal dominant polycystic kidney disease (ADPKD) cell line [115]. A phase I/II clinical trial is currently studying pyrimethamine for safety and dose-finding in relapsed chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) patients (NCT01066663).

Peptide mimetics

Several peptidomimetic inhibitors targeting the SH2 domain of STAT3 have been derived from sequences in the SH2 domain. The SH2 protein sequences around phosphotyrosine 705 (Pro-pTyr705-Leu-Lys-Thr-Lys) participate directly in STAT3 dimerization, and these sequences served as the starting point [116,117]. ISS-610, a tripeptide mimic, and its interaction with the SH2 domain of STAT3 was combined with structural information from X-ray crystallography of STAT3, leading to the development of an analogue peptidomimetic, S3I-M2001 [117–119]. This compound also exhibited inhibition of STAT3 phosphorylation in NIH 3T3/v-Src and MDA-435-MB cells and tumor growth in human breast tumor xenografts.

Golotimod (SCV-07; γ-D-glutamyl-L-tryptophan) is a novel immunomodulating peptide that inhibits STAT3 signaling and was found to modulate the duration and severity of oral mucositis in animal models that received radiation or a combination of radiation and cisplatin [120]. A subsequent phase 2 trial suggested this drug also favorably attenuated the course of mucositis in patients with HNSCC [121].

Phosphopeptide prodrug

Phosphopeptide prodrugs derived from gp130 Tyr904 represent another class of compounds targeting the SH2 domain of STAT3. These prodrugs were found to be potent inhibitors of STAT3 phosphorylation in several cancer cell lines [122]. Intratumoral injection of PM 73G in an orthotopic xenografts significantly inhibited tumor growth, tumor vascularization, and VEGF expression [123,124]. This suggests that selective inhibition of STAT3 leads to impaired VEGF signaling and inhibition of tumor angiogenesis.

Natural products

Cryptotanshinone, a natural product in the tanshinone class (compounds isolated from *Salvia miltiorrhiza*, an herb used in traditional Chinese medicine [125]), has been identified as a potent STAT3 inhibitor [126]. Because cryptotanshinone rapidly inhibits the phosphorylation of STAT3 Tyr705 in a human prostate cancer cell line while phosphorylation of JAK2 was inhibited in a protracted manner, a JAK2-independent mechanism is hypothesized. Furthermore, cryptotanshinone and STAT3 co-localized in the cytoplasm and the formation of STAT3 dimers was suppressed, providing evidence that the binding site is SH2. The agent also decreased expression of target genes cyclin D1, survivin, and Bcl-$_{xL}$, with similar results found in glioma cell lines [127]. While this compound has not been formally studied in a clinical trial context, its use in traditional Chinese medicine makes it attractive for clinical development.

Inhibition of STAT3-DNA binding

Metal complexes and small molecules

Novel platinum compounds, CPA-1 and CPA-7, are considered analogs of cisplatin and preferentially interfere with STAT3 and disrupt its ability to bind to DNA [61]. This was shown to occur *in vitro*, in mouse fibroblast cells, and in various cell lines including breast, prostate, melanoma, and colon tumor cells. The most potent compound, CPA-7, also induced tumor regression in a murine colon cancer model. This molecule was also active in murine glioma models, inhibiting STAT3 activity and tumor growth and downregulating IL-1β, a pro-inflammatory cytokine [128]. However, Assi et al. showed that CPA-7 elicited effects on peripheral glioma cell lines but not intracranial cells, suggesting that this molecule is unable to penetrate the central nervous system [71] and that further drug development is not warranted.

STX-0119, an N-[2-(1,3,4-oxadiazolyl)]-4-quinolinecarboxa mide derivative, selectively blocked DNA binding activity of STAT3, suggesting the mechanism of binding to either the SH2 or DNA binding domains [129]. This molecule suppressed the expression of various STAT3 target proteins, including c-myc, cyclin D1, and Bcl-$_{xL}$ and the growth of many

cancer cell lines [130]. An oral agent, STX-0119 was found to abrogate the growth of a lymphoma xenograft model, suppressing levels of c-myc, Ki-67, and pSTAT3 within the tumors. These studies were also extended to glioblastoma multiforme stem-like cells (GBM-SC) derived from patient with recurrent GBM tumors where again target gene expression of STAT3 was strongly inhibited [131].

A curcumin analogue, FLLL32, was developed with special biochemical properties for more specificity for STAT3 [132]. This compound decreased STAT3 binding to DNA and cell proliferation in canine and human osteosarcoma cells with decreased levels of both total and pSTAT3. FLLL32 also downregulated pSTAT3 in head and neck squamous cell cancer lines, inducing a potent anti-tumor effect and increased the proportion of apoptotic cells [133].

STAT3 oligonucleotides

Transcription factor decoys consist of nucleotide sequences derived from conserved genomic regulatory elements that are recognized and bound by the transcription factor being targeted. Decoys elicit their biological effects by competitively inhibiting binding of the endogenous transcription factor to corresponding *cis* elements in genomic DNA, thus preventing expression of target genes. Various oligonucleotide molecules have been developed and studied. Antisense oligonucleotides (ASOs) are short sequences of nucleotides developed to alter downstream protein expression [134]. STAT3 oligonucleotide decoy was derived from the conserved hSIE genomic element found in the c-*fos* gene promoter, and was comprised of a 15-bp duplex oligonucleotide with free ends and phosphorothioate modifications of the three 5′ and 3′ nucleotides. It binds specifically to pSTAT3 and blocks its binding to DNA, resulting in inhibition of transcription and potentially tumor cell proliferation [135].

Studies in preclinical models of many human cancers have demonstrated antitumor efficacy of this STAT3 decoy [136–141]. A phase 0 clinical trial of 30 HNSCC patients undergoing surgical resection demonstrated significant downregulation of STAT3 target gene expression in the tumors that received the intratumoral injection of decoy compared with saline controls [135]. The “parent” STAT3 decoy oligonucleotide used in the phase 0 trial is limited by relatively rapid thermal and enzymatic degradation upon systemic administration, thereby limiting broad clinical translation. The parent STAT3 decoy has been modified by adding carbon spacers on both ends creating a cyclic STAT3 decoy, which is resistant to thermal degradation and is stable in serum for up to 12 h.

Further investigation demonstrated antitumor efficacy in HNSCC xenograft models with intravenous administration [135,142]. Toxicology studies demonstrated no evidence of organ hematopoietic toxicity, and tumor growth inhibition was accompanied by downregulation of STAT3 target gene expression in the tumors [142,143]. Currently, the cyclic STAT3 decoy is being developed for a proposed phase I clinical trial and will be a first-in human model.

Decreasing total STAT3 expression

A final target in the STAT3 pathway is the quantitative reduction in STAT3 expression by inhibition of mRNA. ISIS 481464 (AZD9150) is a synthetic ASO that is complementary to mRNA for STAT3 and demonstrated antiproliferative effects in various xenograft models

resulting in reduction of STAT3 mRNA and protein in preclinical monkey and mouse models [144]. In a first-in-human phase I trial in patients with advanced cancers, this ASO was well-tolerated and shown to have anti-tumor activity in patients with lymphoma [145]; a dose-expansion phase II trial in lymphoma is ongoing (NCT01563302). Other active clinical trials studying ISIS 481464 (AZD9150) include phase II studies in patients with malignant ascites (NCT02417753) and metastatic HNSCC (monotherapy or in combination with MEDI4736, NCT02499328). A phase I trial in patients with advanced or metastatic HCC has been completed; results are not yet reported (NCT01839604).

Conclusions

The STAT3 pathway involves complex interactions between cell surface receptors, cytokine signaling, and non-receptor tyrosine kinases, and ultimately directs aberrant protein synthesis, growth, and survival. Although hyperactivation of the STAT3 transcription factor is a hallmark of HNSCC, oncogenic mutations are not described. Rather, aberrant signaling is associated with activation by upstream growth factor receptors and a loss of function of selective PTPRs that de-phosphorylate pSTAT3. Due to the association of STAT3 with oncogenic behavior and resistance to standard therapeutics in HNSCC, it remains a compelling target. Identification of predictive biomarkers for STAT3 dependence is essential, as STAT3-targeting drugs can be associated with hematologic toxicity, in some cases leading to early termination of trials. Balancing toxicity with benefit remains a challenge, especially in the recurrent/metastatic setting, where palliation of symptoms is of utmost importance. Predictive biomarkers for STAT3 dependence could lead to clinical responses to lower doses of STAT3-targeting drugs, thereby also leading to lower treatment-related toxicities. Although no direct STAT3 inhibitor has reached FDA approval, due to inherent challenges in targeting transcription factors, the field is poised for breakthroughs. Promising targets include STAT3 mRNA translation, upstream cell surface receptors, the SH2 domain of STAT3, and binding of the STAT3 dimer to DNA. Ongoing, early phase clinical trials may lead to efficacy studies in HNSCC and other malignancies, ultimately filling a major gap in our therapeutic arsenal against human cancer.

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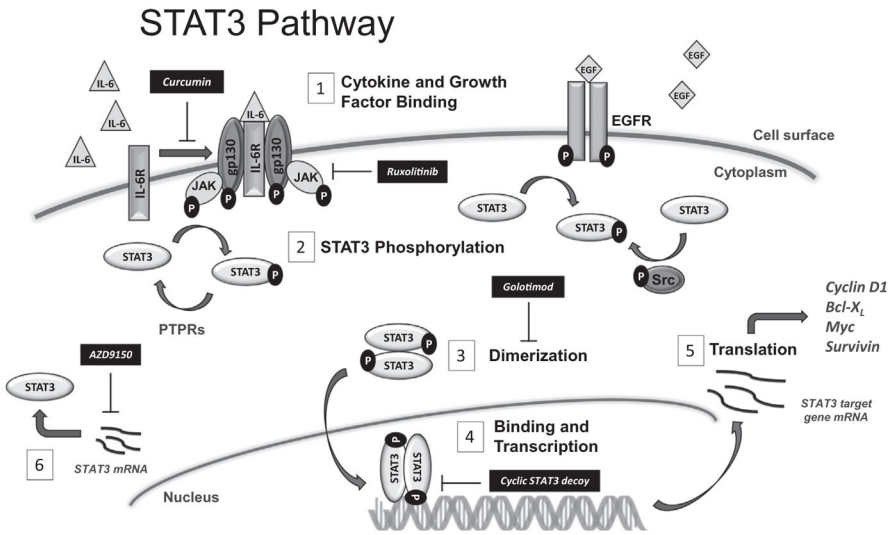

Fig. 1.

Schematic of the STAT3 pathway and therapeutic targets. (1) Cytokines and growth factors, such as IL-6 and EGF, bind to receptors to activate phosphorylation and cell signaling. Curcumin inhibits cell surface signaling, (2) STAT3 molecules are activated by phosphorylation of a tyrosine residue by activated RTKs, such as EGFR, or intracellular NRTKs like JAK or Src. Inactivation by dephosphorylation occurs by PTPRs. Targeted therapies, including the JAK1/2 inhibitor ruxolitinib, inhibit these pathways. (3) Spontaneous dimerization of two phosphorylated STAT3 molecules occurs via the reciprocal phosphotyrosine-SH2 interactions, and the homodimer translocates to the nucleus. Golotimod, an immunomodulating peptide, inhibits homodimerization of STAT3 molecules in the cytoplasm. (4) pSTAT3 homodimer binds to consensus sequences on the promotor regions of target genes. STAT3 decoy molecules are under development to target this step in the STAT3 transcription pathway. (5) The resultant transcripts are translated into pro-proliferative, pro-survival oncogenic proteins. (6) AZD9150 is an antisense oligonucleotide that inhibits the translation of STAT3 mRNA.

Table 1

PTPRD gene mutations identified in HNSCC.

Mutation	Location on gene	
D50E	Immunoglobulin (Ig)	Extracellular domain
T111N	Ig	
Q196H	Second Ig-like domain of the receptor protein tyrosine phosphatase (IG2)	
K204E	IG2	
P249L	IG2	
L308P	IG2	
S384R	Fibronectin type 3 domain (FN3)	
L503I	FN3	
E529Q	FN3	
T820P	FN3	
L1014M	FN3	
L1036P	FN3	
T1100M	Transmembrane region	
L1147F	Transmembrane region	
S1247T	Transmembrane region	
V1270L	Transmembrane region	
P1311T	Transmembrane region	
K1502M	Catalytic domain	

Table 2

Therapeutic agents targeting STAT3.

Drug (company)	Target	Type	Phase of development, human cancer	HNSCC development
Inhibition of upstream receptors				
Ruxolitinib (Incyte Pharmaceuticals, Novartis)	JAK 1/2	Small molecule	I/II/III (FDA-approved myelofibrosis)	Phase I (afatinib combination)
Tofacitinib (Pfizer)	JAK 3	Small molecule	(FDA-approved RA)	–
AZD1480 (AstraZeneca)	JAK 1/2	Small molecule	I (terminated)	–
Fedratinib (Sanofi)	JAK 2	Small molecule	I/II/III	–
Tocilizumab (Genentech)	JAK 3, IL6R	Monoclonal antibody	I/II (FDA-approved RA, juvenile idiopathic arthritis)	–
Curcumin	JAK 1/2, IL6	Natural compound	I/II	Phase 0 (biomarker)
Quercetin	JAK 2, IL-6	Natural compound	I/II	–
Inhibition of STAT3 Domain				
STA-21		Small molecule	(Phase I/II in psoriasis)	–
WP1066		Small molecule	I (brain cancer)	–
OPB-51602 (Otsuka Pharmaceutical)		Small molecule	I	Phase I (nasopharyngeal carcinoma; terminated)
OPB-31121 (Otsuka)		Small molecule	I/II	–
Pyrimethamine		Small molecule	I/II (CLL/SLL)	–
Golotimod (SCV-07; SciClone Pharmaceuticals)		Peptide mimetic	II	Phase II (attenuating oral mucositis)
Inhibition of STAT3-DNA Binding				
STAT3 decoy molecule		Oligonucleotide		Phase 0 (intratumoral injection)
Cyclic STAT3 decoy		Oligonucleotide		–
Inhibition of STAT3 Transcription				
AZD9150 (AstraZeneca); previously known as ISIS 481464 (Isis Pharmaceuticals)		Antisense oligonucleotide	I/II (Advanced cancers, lymphoma, HCC, malignant ascites)	Phase I/II (monotherapy; in combination with MEDI14736)