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JAK-STAT signaling in cancer: From cytokines to non-coding genome

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Abstract

In the past decades, studies of the Janus kinases (JAKs) and signal transducers and activators of transcription (STATs) signaling have uncovered highly conserved programs linking cytokine signaling to the regulation of essential cellular mechanisms such as proliferation, invasion, survival, inflammation and immunity. Inhibitors of the JAK/STAT pathway are used for treatment of autoimmune diseases, such as rheumatoid arthritis or psoriasis. Aberrant JAK/STAT signaling has been identified to contribute to cancer progression and metastatic development. Targeting of JAK/STAT pathway is currently one of the most promising therapeutic strategies in prostate cancer (PCa), hematopoietic malignancies and sarcomas. Notably, newly identified regulators of JAK/STAT signaling, the non-coding RNAs transcripts and their role as important targets and potential clinical biomarkers are highlighted in this review. In addition to the established role of the JAK/STAT signaling pathway in traditional cytokine signaling the non-coding RNAs add yet another layer of hidden regulation and function.

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Potential competing interests

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Understanding the crosstalk of non-coding RNA with JAK/STAT signaling in cancer is of critical importance and may result in better patient stratification not only in terms of prognosis but also in the context of therapy.

Keywords

JAK/STAT signaling; Non-coding RNAs; Prostate cancer; Hematopoietic malignancies; Sarcomas

1 Introduction

The JAK/STAT pathway was discovered through the study that linked interferon-responsive genes to signal transduction [1,2]. Wilks et al. identified a new class of protein-tyrosine kinases, called Janus kinases, or abbreviated, JAK1 and JAK2. JAK1 and JAK2 are members of the JAK family kinases which also comprises of JAK3 and the non-receptor tyrosine-protein kinase 2 (TYK2) [3]. The sizes of JAK proteins ranges from 120 to 140 kDa and they all display seven conserved JAK homology (JH) domains [4]. The C-terminal of JAK kinases consists of two JH tyrosine-catalytic domains (JH1) including the pseudokinase domain (JH2), which was identified in the case of JAK2 to possess serine/threonine kinase activity [5]. An SH₂-like domain containing JH3-JH5 functions as an anchor between the C-terminal and N-terminal domains and is important for cytokine receptor binding and for the regulation of surface expression of the oncostatin M receptor (OSMR) [6,7]. The N-terminus contains the four-point-one, ezrin, radixin, moesin (FERM) domain (JH6 and JH7), which mediates the association of JAKs with cytokine receptors. Mutations in the FERM domain can influence integrity and phosphorylation status of JAKs and interfere with cytokine-dependent stimulation of downstream signaling [8].

Signaling via the JAK-STAT pathway causes, with the exception of mutations, autoactivation of JAK kinases induced by ligand binding, resulting in receptor dimerization and cross-phosphorylation of associated JAK kinases that get activated in a sliding mode, where the JH1 and JH2 domains of juxtaposed JAK kinases move away to allow for kinase activity to commence. This in turn can phosphorylate among many other pathways also the crucial STAT transcription factors that form parallel dimers upon tyrosine phosphorylation and can subsequently move rapidly into the nucleus with the help of GTPase activity.

Activated STAT proteins form either homo- or heterodimers by interacting via their SH₂ domains and subsequently translocate into the nucleus to activate the transcription of target genes [3]. In the minority of cases STATs can also repress gene transcription [9]. In healthy tissues, signaling via the JAK/STAT pathway is a well-controlled process that can be regulated at various levels within the cell. For instance, the tyrosine phosphatases SHP1 and SHP2 can dephosphorylate JAK proteins and thereby limit downstream signaling [10]. The family of protein inhibitors of activated STATs (PIAS) negatively regulates STAT signaling by interfering with the DNA-binding activity of STATs or by a SUMO (small ubiquitin-like modifier) E3 ligase that limits STAT signaling by promoting proteolysis of STATs [11]. Suppressors of cytokine signaling (SOCS) proteins can dampen JAK/STAT signaling by

interfering with the activity of JAK kinases, competing with STATs for binding or by targeting proteins for proteasomal degradation [12,13].

Mutations of *JAK2* have been reported in the majority of patients with myeloproliferative neoplasms such as thrombocythemia, myelofibrosis and polycythemia vera [14,15]. Notably, *CALR* (encoding calreticulin) somatic mutations identified in patients with primary myelofibrosis or essential thrombocythemia [16] lead to hyperactivation of JAK/STAT signaling [17]. *CALR* somatic mutations are mutually exclusive with mutations in both *JAK2* and *MPL* (Thrombopoietin Receptor) and therefore likely an explanation for a milder disease course and greater clinical response to the JAK/STAT inhibitory therapy [18]. Constitutive activation of the JAK/STAT pathway occurs frequently in carcinomas such as PCa, sarcomas and lymphomas e.g. ALK-driven anaplastic large cell lymphomas (ALK+ ALCL) [19–21]. In line with this, a persistent activation of JAK/STAT signaling in tumors is related to advanced tumor growth through tumor-autonomous mechanisms, increased angiogenesis and by modulating tumor-associated stroma as well as the immune system [22,23].

The discovery of JAK2 mutations has qualified JAK2 as a promising target for the treatment of myeloproliferative neoplasms [24]. Several small-molecule JAK2 inhibitors have been developed and some are already clinically approved (e.g. Ruxolitinib for the treatment of myelofibrosis [25]) or currently being tested in clinical trials (e.g. Pacritinib or CEP-701) [26–28]. Recently, more sensitive approaches have identified an aberrant IL-6/STAT3 signaling axis to be involved in the regulation of non-coding RNA transcripts, which could represent novel therapeutic targets in malignancies [29]. A recent study highlighted an aspirin-induced blocking of STAT3 phosphorylation through the induction of specific non-coding RNAs in human colorectal cancer [30]. Howe et al. demonstrated that Ribocil, a highly selective chemical modulator of flavin, specifically represses non-coding RNA structures located in messenger RNAs [31]. Thus, novel personalized small molecules against oncogenic non-coding RNAs are being developed [32]. These findings have important implications for our future understanding of non-canonical JAK-STAT signaling in malignant tumors of all three germ layers (carcinomas, sarcomas and lymphomas), which will be discussed in this review.

2 The role of JAK/STAT signaling in prostate cancer

PCa with more than 1.11 million new cases every year worldwide, is the most diagnosed malignancy among adult men and the second most common cause of male cancer deaths [33]. Since PCa development is initially dependent on androgens, therapies for advanced PCa usually involve the reduction of androgens or blocking the ligand-binding site of the androgen receptor (AR) [34]. However, the majority of men undergoing front-line AR deprivation therapy (such as second-generation anti-androgens ARN-509 [35] or enzalutamide) supported by surgery and radiotherapy will develop resistance to these therapies and will progress to therapy-resistant PCa. It is understood that various oncogenic activations of the JAK, STAT, mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K) pathways are frequent events during the multiple stages of carcinogenesis [36–38].

The most common genetic alteration activating PI3K is the genetic ablation of the tumor suppressor PTEN, a negative regulator of PI3K-AKT signaling [39]. The loss of tumor suppressor PTEN provokes the activation of tumor suppressive signals including senescence which could be achieved via a p53-dependent pathway and the induction of the cyclin-dependent kinase inhibitors p21^{CIP} and p16^{INK4A} [40]. Senescence induced by loss of PTEN is critically regulated by the ARF-p53 pathway [41] and leads to the accumulation of p53, p27, p21 and p19^{ARF} [42–44]. Surprisingly, senescence was also shown to occur in human preneoplastic prostate lesions [42], neurofibromas [45] and melanocytic nevi [46], but was completely absent in highly malignant stages of invasive PCa [42] and pancreatic ductal adenocarcinomas [47]. Senescent cells undergo characteristic expression changes, such as increased expression and secretion of IL-6 [48–50]. In response to oncogene-induced senescence, IL-6 is considered to cause cell cycle arrest in a cell-autonomous manner [50,51]. IL-6 is a well-known cytokine activator of the JAK/STAT pathway and a key regulator of cancer progression [52]. Like in many cell types, in prostate epithelial cells IL-6 binds to its receptor promoting formation of the hexameric IL-6 receptor complex with gp130 [53] as the prominent signaling chain and future autophosphorylation of JAK2 [54]. Notably, study of LNCaP cells that lack JAK1 due to epigenetic silencing, showed STAT3 phosphorylation clearly depends on JAK2 [55]. JAK2 also induces phosphorylation and activation of STAT5 which has been identified in 61% of distant metastatic PCa [56]. Activation of STAT5 promotes migration and invasion of PCa cells and increases metastases formation in an *in vivo* experimental metastases assay [57].

Remarkably, elevated serum IL-6 levels are associated with poor prognostic outcome for patients with renal cell carcinoma and non-small-cell lung cancer [58–60]. Stromal cells produce high levels of IL-6 in culture [61], whereas basal cells are the main epithelial source of IL-6 in benign prostate tumors [62]. Activated STAT3 was shown by Azare et al., to be strongly activated on the edge of breast tumors, accompanied by CD33 positive cells, a marker of the myeloid lineage [63]. Since deregulation of the JAK/STAT pathway is known to be involved in PCa development, several JAK/STAT inhibitors have been investigated and some are currently being tested in clinical trials, including small molecule STAT3 inhibitors (e.g. STA-21 [64]), JAK kinase inhibitors (e.g. SAR302503 [65] and Ruxolitinib [66]), anti-IL6 antibodies or inhibitors (e.g. Siltuximab [67] and Sant7 [68]). Surprisingly, treatment with Siltuximab (CNTO 328), an anti-IL-6 chimeric monoclonal antibody, did not result in any reduction of prostate specific antigen levels greater than 50 ng/ml (PSA50) or survival advantage in patients with advanced PCa [67,69–71]. Importantly, recent whole genome sequencing studies showed only rare amplifications or activating mutations in tyrosine kinases in PCa [72–75]. Although the therapeutic potential of inhibiting IL-6 or STAT3 reaches beyond PCa, as many tumor types have been described to exhibit deregulated STAT3 activity, the failure of anti-IL-6 treatment in PCa is still unexplained and may be due to an incomplete understanding of the *in vivo* role of IL-6/STAT3 signaling in this tumor type. Based on experimental and clinical observations and the lack of efficacy of IL-6 therapy in PCa patients, loss of *IL-6* or *STAT3* function was found to influence PCa growth, progression and metastasis formation in a *Pten*-deficient PCa mouse model [76]. The tumor phenotypes in all phases of PCa development have shown that the IL-6/STAT3 axis could act as a tumor suppressor in PCa. A loss of IL-6/STAT3 signaling bypassed cellular senescence,

which resulted in aggressive tumor progression and metastasis. ARF (alternative reading frame protein product of the CDKN2A locus) was identified as a STAT3 target gene responsible for this tumorigenic phenotype [76].

Aberrant STAT3 expression frequently results from constitutive activation of upstream members of the signaling pathway or mutations. Acquired *STAT3* mutations have been recently identified in solid malignancies including primary PCa (Fig. 1).

Thus, the STAT3-ARF signaling cascade represents an important and essential driver of loss-of-PTEN induced senescence [77]. STAT3 and ARF are novel prognostic PCa markers and may act as a possible tumor suppressor pathway in a subset of PCa patients [78].

Recent publications identified that STAT3 regulates the expression of various long non-coding RNAs, including transcription factor 7 (*TCF7*) [29] and second chromosome locus associated with prostate-1 (*SChLAP1*) [79]. Importantly, the *SChLAP1* is abundantly expressed in ~25% of PCas and discriminates aggressive tumors from indolent forms of the disease [80]. Another long non-coding RNA, *ANRIL* (antisense noncoding RNA in the INK4 locus) contributes to PCa development [81] and controls cellular senescence [82].

3 JAK/STAT in hematopoietic malignancies

In the hematopoietic system, the JAK/STAT pathway is crucial for cell growth, development, differentiation and survival of immune cells [83]. The importance of this pathway in the hematopoietic system has been demonstrated with various knockout models. For instance, *Jak2*-deficient mice die *in utero* as a result of fetal anemia, while *Jak1*-deficient mice die shortly after birth with immunological abnormalities phenotypically resembling severe combined immunodeficiency [84,85]. The loss of JAK3, which is predominately expressed in natural killer (NK) cells, T-cells and B-cells, resulted in a loss of both T-cells and B-cells due to the absence of IL-7 receptor signaling both in humans and mice [86]. Similarly, *Tyk2*-deficient mice are unable to respond to signals from IL-6, IL-12, IL-10, IL-22 and IL-27 and are more susceptible to viral and bacterial infections due to a lack of type 1 and 2 interferon responses [87]. STAT-deficient mice also exhibit a variation of immunodeficiency phenotypes. *Stat1*-deficiency causes failure to efficiently respond to viral and microbial infections as a result of impaired interferon signaling [88]. As part of IL-6 and IL-21 signaling which is crucial for T_H17 differentiation, mice lacking *Stat3* display defects in T_H17 differentiation and mice with high STAT3 activity showed accumulation of T_H17 cells [89,90]. Indeed, the STAT protein family is part of many transcription factors that are involved in the determination of T-cell fates [91]. STAT4 and STAT6 are considered as T_H1- and T_H2-signature transcription factors, respectively [92,93]. In addition, forkhead box P3 (FOXP3), a known direct target of STAT5, is used for the identification of regulatory T-cells (Tregs) [94]. Cell-type specific functions of STAT5 have been extensively studied, revealing that STAT5 is a key regulator of myelopoiesis, lymphocyte development, differentiation and proliferation [95]. *STAT5*-deficient mice suffer from a severe combined immunodeficiency-like phenotype, which they share largely with *JAK3*- or γ_c -deficient mice [96]. Then again, hyperactivation or overexpression of STAT5 results in an expansion of CD8⁺ T-cells in mice [97].

Owing to its critical roles in hematopoietic development, the JAK/STAT pathway plays an important pro-oncogenic role in lymphoproliferative malignancies. In particular, constitutively activated JAK1/STAT3 and JAK2/STAT5 have been shown to facilitate T-cell transformation [98,99]. While JAK3 has first been shown to be implicated in B-cell malignancies, it has also been shown to be constitutively activated in adult T-cell leukemia [98,100]. Activating somatic mutations of JAK2 and JAK3 are strongly associated with myeloproliferative disorders. The JAK2 V617F mutation is known to occur in 98% of polycythemia vera cases in 50% of essential thrombocytosis cases. The JAK3 A572V mutation was identified as an activating mutation in megakaryoblastic myeloid leukemia [101,102]. However, activating somatic mutations of JAK1, JAK2 and JAK3 have been reported in acute lymphoblastic leukemia, with the majority of the mutations located in the pseudokinase domain. These mutations were shown to be able to transform the Ba/F3, a pro-B cell line, and to activate downstream targets of JAK kinases, such as STAT1 and STAT5 [103,104]. Somatic mutations in JAK1 were identified in 18% of adult acute T-cell lymphoblastic leukemia and in 3% of adult acute B-cell lymphoblastic leukemia patients with poor prognosis [103].

As a consequence of the activation of JAK kinases, STAT hyperactivation has been found in many hematopoietic malignancies. For instance, high STAT3 activity was correlated with unfavorable prognosis in diffuse large B-cell lymphoma (DLBCL) [105–107]. Recently, recurrent somatic mutations have been discovered in the SH₂ domain of STAT3 and STAT5 and in the DNA binding domain of STAT6 in T- and B-cell leukemia patients. STAT3 somatic mutations have been found in 40% of large granular lymphocytic leukemia cases with two hotspots, Y640F and D661Y which were found in 73 and 78 cases, respectively [108–114]. The two mutations were shown to increase STAT3 transcriptional activity, leading to the up-regulation of STAT3 target genes including non-ligand-binding beta chain of the gamma interferon receptor (*IFNGR2*), Bcl-2-like protein 1 (*BCL2L1*), and *JAK2* [108]. Genetic alterations of STAT3 have also been found in other diseases including ALCL, acute T-cell lymphoma, hepatosplenic T-cell lymphoma, $\gamma\delta$ -T-cell-derived lymphomas, NK leukemia, myelodysplastic syndrome, NK/T-cell lymphomas, peripheral T cell lymphoma not otherwise specified (PTCL-NOS), diffuse large B cell lymphoma and angioimmunoblastic T-cell lymphoma (AITL), making up for more than 250 identified cases in total (Fig. 2) [115–120]. In comparison to the large number of patients who were identified with STAT3 mutations, the somatic N642H mutation in STAT5B was reported in less than 100 cases in different hematopoietic malignancy inducing $\gamma\delta$ hepatosplenic T-cell lymphomas, large granular lymphocytic leukemic, T-cell acute lymphoblastic leukemic, enteropathy associated T-cell lymphoma and T-cell prolymphocytic leukemia [121–125]. STAT5B N642H was discovered to be constitutively active together with a number of other constitutively active STAT5 variants, including constitutive active forms STAT5A1*6, STAT5A N642H and STAT5B1*6, by Kitamura and colleagues [126]. The discoveries were made using a system which utilized PCR-driven random mutagenesis together with retrovirus-mediated gene expression [126,127]. The STAT5B1*6 variant was later used to generate a transgenic mouse model, which had a 3- to 4-fold increase in $\gamma\delta$ and NK T-cells [128]. Additionally, the STAT5B1*6 variant was used in a different study to rescue B-cell

development in *IL-7R^{-/-}* mice, indicating that STAT5B activation played a key role in B-cell development [129].

The N642H mutation, located in the SH2 domain of STAT5B, promotes constitutive phosphorylation of STAT5B, which is believed to stabilize the parallel conformation of STAT5 dimers, thus enhancing the transcription of STAT5 target genes. The results of *ex vivo* drug sensitivity tests in leukemic blasts with the STAT5B N642H mutation indicated that this mutant provided leukemic cells with a selective advantage following the current treatment for leukemic progression [123]. Nevertheless, how STAT5B mutations drive the development and the continuation of leukemia is poorly understood. Similar to the discovery of STAT5 activating mutations, activating mutations in the SH₂ domain of STAT6 were also found via mutagenesis scanning experiments [130]. However, the somatic STAT6 D419G/H mutations located in the DNA-binding domain of the protein have only been found in 15 cases of diffuse large B-cell lymphoma [119,131,132]. The mechanism by which STAT6 mutations contribute to cancer development remains to be investigated.

Although the impact of the JAK/STAT pathway on cancer development and progression has become apparent, targeting this pathway is challenging due to its pleiotropic nature. Considerable effort has been made to develop JAK/STAT inhibitors and currently many JAK kinase inhibitors are being tested in clinical trials for leukemia [133]. Two of the most promising JAK inhibitors are Ruxolitinib (INCB028050) and Tofacitinib (CP-690550). Ruxolitinib potently targets JAK1 and JAK2, while Tofacitinib inhibits JAK1, JAK2 and JAK3 with nanomolar potency JAK1 (IC₅₀ = 3.2 nM), JAK2 (IC₅₀ = 4.1 nM) and JAK3 (IC₅₀ = 1.6 nM) [134,135]. In early 2016, Ruxolitinib was used in 27 active clinical trials for leukemia, most of which are currently in phase II and target myeloproliferative disorders, in addition to trials of the drugs for acute lymphocytic leukemia and Hodgkin's Lymphoma, respectively. Tofacitinib entered clinical trials as a treatment for rheumatoid arthritis and many phase II trials have been completed [134,136]. In addition to JAK-targeting drugs, various other kinase inhibitors exist that were developed against other kinases but also inhibit members of the JAK family. For example, Lestaurtinib (CEP-701) was developed as an FLT3 and TrkA inhibitor, but was later reported to have inhibitory effect against JAK2 and has been used as a therapy for acute myeloid leukemia [137]. Another JAK2 inhibitor is SAR302503, which was used for patients who were previously treated with Ruxolitinib. SAR302503 recently completed a phase II clinical trial for Polycythemia Vera and Essential Thrombocythemia [138]. Notably, Ruxolitinib treatment significantly reduced levels of long non-coding RNAs, indicating that their expression is STAT-dependent [139]. As for the inhibition of the JAK kinases, targeting of members of the STAT protein family is very attractive in the search for effective cancer therapies. There are many strategies for targeting STAT proteins including but not limited to using peptides to bind and to inhibit STAT production and activation or using small molecules to bind directly to the SH₂ domain of the transcription factor [140]. Since STAT3 and STAT5 are the most active family members of all seven STAT proteins in many cancers, many attempts are being made in the quest for specific STAT3 or STAT5 inhibitors. From the many inhibitors developed, only a few have entered phase I clinical trials for lymphoma, such as AZD9150 and OPB-31121. AZD9150 is a 16 oligonucleotide antisense molecule, which binds to the 3' untranslated region of the STAT3 mRNA leading to an arrest in STAT3 protein production. OPB-31121 is a small

molecular inhibitor that has been demonstrated to bind to the SH₂ domain of STAT3/5 to inhibit their activation and block downstream functions [137,141]. In addition, a number of inhibitors targeting the SH₂ domain of STAT5 are presently in development with promising potential [142,143].

4 The JAK/STAT3 signaling pathway in soft tissue and extraosseous sarcomas

The role of STAT3 in sarcoma pathogenesis is still under investigation; however there is important evidence, which suggests that STAT3 has an oncogenic role in sarcoma tumor formation. In Ewing sarcoma (ES), the second most common primary bone- and soft tissue malignancy in children, STAT3 is overexpressed in many cases. Perturbation of STAT3 expression has been shown to limit ES cell growth and to inhibit migration and invasion [144–146]. Furthermore, Oncostatin M and LIFR (leukemia inhibitory factor receptor) and cytokines from the IL-6 family can induce cell growth and proliferation by activation of STAT3 and MYC. The ES tumor microenvironment produces IL-6 which leads to apoptosis resistance in ES tumors, thereby enhancing tumor progression. Tumor-associated macrophages (TAMs) are stromal cells that secrete growth factors and cytokines involved in cancer progression and tumor invasion, metastasis, and angiogenesis [147–149]. TAM infiltration is associated with poor prognosis in ES [150]. STAT3 overexpression in ES is positively correlated with TAM infiltration. However, it remains unclear how STAT3 is involved in ES tumor progression. In addition, the microRNA *Let-7a*, a direct target of EWS-FLI1 which is suppressed in ES was shown to negatively regulate macrophage infiltration. *Let-7a* overexpression suppresses ES cell growth and proliferation via targeting the cell cycle, especially cyclin-dependent kinase 6 (CDK6) [151]. It has become clear through recent large sequencing studies that non-coding RNAs play an important role in regulation of ES oncogenesis. Marques Howarth et al., identified Ewing sarcoma-associated transcript 1 (*EWSAT1*) as a key regulator of EWS-FLI that occur as a consequence of oncogenic translocation events [152].

In a different study, it was suggested that cyclin D1 and STAT3 are putative direct targets of *Let-7a* and STAT3 can be directly suppressed by *Let-7a* in ES [153,154]. It has been reported that Vorinostat, a histone deacetylase inhibitor (HDACi) in combination with the DNA damaging agent SN-38 and the alkylating agent temozolomide can downregulate Cyclin D1 to induce G0/G1 arrest and promote apoptosis by cleavage of Caspase-3 and PARP in ES cell lines. Vorinostat inhibits HDACs leading to activation of p53, cleavage of Caspase-3, induction of apoptosis and inhibition of Cyclin D1, which all indirectly inhibit STAT3 expression in a negative feedback loop. Disrupting this axis could be considered as a potential therapeutic option to treat ES [144,147,155]. In a similar manner, STAT3 is also considered as an oncogene in osteosarcoma (OS). OS is the most common type of malignant bone tumor in young patients and adolescents, which is very aggressive and often fatal. The poor outcome of OS, particularly in patients with metastatic disease and a five-year survival rate of only 20%, calls for more effective therapeutic strategies that target malignancy-promoting mechanisms. Constitutive STAT3 activation has been demonstrated in OS and was associated with poor prognosis [156]. The down-regulation of STAT3 by the

microRNA-125b suppresses *in vitro* proliferation and migration of OS cells [157]. Higher levels of extracellular signal-regulated kinase 1 and 2 (ERK1/2) and STAT3 are strongly linked to tumorigenesis and chemoresistance. Thus, expression levels of ERK1 and STAT3 might be used as predictive markers for chemotherapy responses in osteosarcoma patients. Treatment of six individual osteosarcoma cell lines with the STAT3 inhibitor S3I-201 led to inhibition of colony formation and induced apoptosis, most probably through disruption of STAT3 target gene expression such as Cyclin D1 [158]. These data could be confirmed by independent experiments showing that cell growth inhibition *in vitro* and delayed tumor growth in a xenograft model were observed after treatment with the specific JAK2/STAT3 inhibitor FLLL32 [156,158]. S-Adenosylmethionine (AdoMet) is a methyl donating chemical compound that circulates in the blood. AdoMet, inhibits proliferation by targeting cell cycle progression and inducing apoptosis. After treatment with AdoMet cell cycle inhibitors such as p53 and p21 increase while protein levels of cyclin A and cyclin E are reduced [159]. Moreover, the AdoMet-induced antiproliferative effects were dynamically accompanied by profound changes in ERK1/2 and STAT3 protein and phosphorylation levels. Thus, targeting and down-regulating ERK1/2 and STAT3 pathways may be a potential therapeutic strategy for osteosarcoma patients [159]. In other soft tissue sarcomas such as fibrosarcoma, angiosarcoma and myxoid liposarcoma (MLS), the levels of STAT3 were also found to be elevated. Fibrosarcoma (FS) is a malignant tumor arising from mesenchymal and fibrous connective tissue characterized by the presence of immature proliferating fibroblasts or undifferentiated spindle cells [160]. FS consists mainly of two distinct tumor subtypes in children and adolescents: infantile FS and FS in adults. While the ETV-NTRK3 fusion transcript is associated with infantile FS, this translocation has not been observed in adult FS [161]. It is evident that PI3-AKT, MAPK and STAT3 pathways are activated in a subset of patients diagnosed with infantile FS. Doxorubicin treatment may activate death receptor pathways, leading to amplified mRNA levels of Cytochrome *c*, Caspase 1, 3, 6, 8, and 9, while decreasing Bcl-2 and STAT3 expression. This indicates that these proteases are important factors in the implementation of doxorubicin-mediated apoptosis in infantile FS.

Angiosarcoma is a vascular tumor, accounting for less than 2% of all soft-tissue sarcomas. Cutaneous angiosarcoma (CAS) is the most common form of angiosarcoma. It is a rare, aggressive malignancy with a poor prognosis that can occur everywhere in the body [162]. While few studies could show that activator protein-1 (AP-1) and activating transcription factor-2 (ATF2) in cooperation with STAT3 regulates cellular proliferation, apoptosis and differentiation in CAS [163], the mechanism behind this is unclear. Furthermore, STAT3 is known to directly transcribe and to interact with several AP-1 family members such as c-FOS or jun proto-oncogene (JUN) family member proteins. Finally, myxoid liposarcoma (MLS) is the second most common subtype of liposarcoma, which metastasizes in up to one third of cases. MLS makes ~30% of all liposarcomas and about 10% of all adult soft tissue sarcomas. The morphology of tumor varies from purely myxoid tumors to highly cellular round cell liposarcomas. It has been reported that tumor-associated macrophage (TAM) infiltration is associated with poor prognosis and that the EGFR/PI3K/AKT/STAT3 pathways control elevated tumor invasion and metastasis in MLS [148,164,165].

5 JAK-STAT signaling and non-coding RNAs

The ENCODE project has revealed that about 80% of the human genome is actively transcribed whereas only 2% of it codes for the about 20,000 proteins that have been described in humans [166,167]. The functional impact of the huge actively transcribed non-coding RNA sequence is largely unexplored. While non-coding transcripts were initially considered genomic junk or products of transcriptional background noise, this view has dramatically changed over the last decade [168]. In general, non-coding RNAs are discriminated based on their size as either small non-coding RNAs (<200 nucleotides (nt)) or long non-coding RNAs (lncRNAs, >200 nt). [167,168]. The class of small non-coding RNA comprises various subtypes, the best described of which are the microRNAs (miRNAs) that have a length of 18–22 nt and block transcription of their target genes through imperfect base pairing. In the last years miRNAs have been shown to be differentially regulated in all cancer types and they have been implicated in almost all aspects of cancer [169–171]. More than 1000 human miRNAs have been found to date and about two thirds of all proteins are considered to be regulated by miRNAs. For completeness we also mention the small nucleolar RNAs (snoRNAs) that have a length of 100–200 nt and are exclusively localized in the nucleus where they are instrumental for correct mRNA splicing; the siRNAs which are mostly virus-derived and act by base pairing with target mRNA leading to their degradation and finally PIWI protein interacting RNAs (piRNAs) which have a length of 26–31 nt and are exclusively found in testes and germ cells [167,168]. Because RNA-sequencing (5' RNA-Seq) techniques allowed large-scale detection of lncRNAs, these transcripts have recently gained more attention [172–174]. Several recent publications indicate the potential roles of lncRNAs in gene regulation [175,176]. In addition, lncRNAs were also shown to affect physiological processes including stem cell differentiation [177] but also to play a crucial role in various malignant diseases, including breast cancer [178–180].

6 MicroRNAs and their role in JAK-STAT signaling

miRNAs are endogenous, small non-coding RNA molecules that are approximately 22 nt long. They exert their gene-regulatory function on the post-transcriptional level via inhibition of mRNA translation or degradation, depending on the complementarity to their mRNA target [181].

Deregulation of JAK/STAT signaling is found in a variety of disease conditions. Besides mutations in protein-coding genes that can render the pathway constitutively active, various alterations of miRNAs that can modulate this signaling pathway have been described. Many miRNAs were shown to negatively regulate the expression of SOCS proteins, resulting in elevated JAK/STAT signaling activity [182]. miRNA-155 was found overexpressed in breast cancer, leading to aberrant activation of STAT3 signaling by downregulation of the repressor SOCS1 [183]. We have shown a similar mechanism in anaplastic large cell lymphoma without the t(2;5) translocation where miR-155 is highly expressed and downregulates SOCS1 also leading to STAT3 activation [184]. The 3' UTR of the SOCS3 mRNA contains binding sites for a variety of miRNAs, many of which have been discovered to modulate JAK/STAT3 signaling in different tissue and disease contexts. For example, miRNA-30 was shown to be overexpressed in primary glioma tissue, resulting in excessive JAK/STAT3

signaling by targeting the 3' UTR of SOCS3 [185]. Furthermore, miRNA-19a and miRNA-19b were shown to downregulate SOCS3 expression in hepatocytes and in Crohn's disease, respectively [186,187], whereas miRNA-221 seems to limit expression of SOCS3 in PCa cell lines [188]. In a recent interesting study, Zhuang and colleagues showed that cancer cells could deliver miRNA-9 to co-cultured endothelial cells by targeted secretion. This reduces SOCS5 levels in endothelial cells, leading to activation of JAK/STAT signaling and increased motility, providing a potential new mechanism for tumor angiogenesis [189]. In addition, also PIAS proteins were shown to be targeted by miRNAs. For instance, miRNA-18a is overexpressed in gastric cancer and negatively regulates the expression of PIAS3 levels, thereby resulting in increased levels of STAT3 signaling [190].

All the miRNAs described above may act as oncogenes that downregulate critical negative regulators of the JAK/STAT pathway thus leading to aberrant signaling. In contrast other miRNAs were found to exert a tumor suppressor function and are consequently downregulated in malignant diseases. For example, the JAK2-targeting miRNA-216a was identified to be downregulated in pancreatic cancer tissues. Restoration of miRNA-216a levels in a pancreatic cancer cell line was subsequently shown to reduce proliferation and increase apoptosis in cancer cells *in vitro* and *in vivo* [191]. miR-23a and miR-23b were shown to be downregulated in PCa cell lines and clinical patient material. Both, miR-23a and miR-23b target the IL-6R mRNA, coding for an important receptor whose activation triggers a strong JAK/STAT pathway activation [192].

7 Long non-coding RNAs (LncRNAs) and their role in JAK-STAT signaling

More than 15,000 lncRNA transcripts have been identified to date. Based on their location with respect to protein-coding genes, lncRNAs can be categorized into different groups ("antisense", "long-intergenic", "sense-overlapping", "sense-intronic" or "processed") [193]. lncRNAs can be spliced, capped, polyadenylated and are actively transcribed by RNA polymerase II [194]. In contrast to protein-coding genes or many miRNAs, the sequences of most lncRNAs are poorly evolutionarily conserved [195]. Although lncRNAs play crucial roles during diverse cellular processes and their aberrant expression was linked to different malignant diseases [178,196–199], the biological role of most lncRNAs remains enigmatic [194].

Besides the known regulatory functions of miRNAs, it has recently become evident that lncRNAs may also contribute to the regulation of JAK/STAT signaling. For example, the lncRNA CASC2 (cancer susceptibility candidate 2) is downregulated in colorectal cancer and was shown to modulate expression levels of the negative JAK/STAT regulator PIAS3 by acting as a sponge for miRNA-18a [200]. As miRNA-18a directly targets the PIAS3 gene, its expression may result in aberrantly increased JAK/STAT3 signaling [190].

In a recent study, Guo and colleagues found that the aspirin-induced lncRNA OLA1P2 directly interacts with tyrosine phosphorylated STAT3 and might inhibit its nuclear import by impairing the formation of phosphorylated STAT3 homodimers [30]. Furthermore, lncRNA-DC that is expressed in human dendritic cells was shown to regulate cellular differentiation by activating STAT3 signaling. Wang and colleagues found that this lncRNA-

DC binds to the STAT3 protein in the cytoplasm and thereby prevents its dephosphorylation by SHP1 [201]. In a recent study hepatocytes were treated with interferon- α (IFN- α) and lncRNAs were measured before and at three time points after IFN- α treatment. About 200 lncRNAs were differentially deregulated, among which lncRNA-CMPK2 was the strongest deregulated. Its induction was dependent on JAK-STAT signaling and its abrogation in hepatocytes led to reduced HCV replication [202]. This year Westermann and colleagues addressed an interesting interaction between the small RNA PinT and coding and non-coding RNA transcripts in host-pathogen interactions using a generic dual-RNA-Sequencing approach. In interspecies correlation analyses the PinT transcript was linked to JAK/STAT signaling [203].

8 Outlook

Inhibition of JAK1 and JAK2 kinases leads to a reduction of STAT3 activity (phosphorylation at Tyr705 and Ser727) and blocks dimerization and translocation of STAT3 to the nucleus. The discovery of specific pharmacological inhibition of JAK1/JAK2 has opened new therapeutic windows for treatment of PCa, sarcomas and myeloproliferative neoplasms. Newly identified functions of miRNAs and lncRNAs in the regulation of cancer progression and metastasis also point to new directions of JAK/STAT targeting in cancer therapy. Collectively, these findings indicate that specific networks of miRNAs and lncRNAs regulate JAK/STAT signaling in distinct tissues and malignancies (Fig. 3). Deregulation of these networks fosters initiation and progression of various malignant diseases. We propose that intervention strategies based on IL-6/STAT3 pathway would require more insights into the regulation non-coding RNAs and other genomic alterations to define their systemic interplay in cancer biology. Non-coding RNAs have been implicated in the regulation of various cellular signaling pathways, hence it is not surprising that they also impact on the JAK-STAT pathway and add yet another layer of complexity to its regulation. Thus, functional *in vivo* validation of non-coding RNAs and JAK/STAT including integrative genomic analysis has emerged to illuminate the disease modeling and therapeutic vulnerability as a useful resource to future clinical trials in solid malignancies.

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Fig. 1. Genetic alterations of STAT3 in solid malignancies. The structure of STAT3 is shown schematically, and the positions of STAT3 somatic mutations identified in primary tumor tissue of patients with solid malignancies are indicated.

Fig. 2.

Genetic alterations of STAT3 in hematopoietic malignancies. The structure of STAT3 is shown schematically, and the positions of STAT3 somatic mutations identified in patients with hematopoietic malignancies are indicated.

Fig. 3.

Integration of non-coding RNAs in regulation of canonical JAK/STAT network. Non-coding RNAs have to be considered in guiding of classical The JAK–STAT pathway. Direct regulation of JAK/STAT signaling by microRNAs, lncRNAs, and RNA-binding proteins promote various oncogenic and tumor suppressor processes and regulate transcription of STAT target genes. Negative regulators of JAK/STAT signaling are displayed in red, non-coding RNAs that promote signaling are displayed in green.