Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signalling

Janus kinases (JAKs) and signal transducers and activators of transcription (STATs) are essential molecules in cytokine signal transduction pathways, as shown by studies using mutant cell lines, knockout mice and humans with somatic mutations in these genes (reviewed in [1, 2]). The JAKs are commonly found associated with the intracellular domains of cytokine receptors providing the enzymatic activity that they do not possess intrinsically. Following the binding of a given cytokine to their cognate receptor JAKs auto- and/or trans-phosphorylate. These activating events lead to the phosphorylation of the receptor per se and thereby the generation of STAT docking sites. STATs get recruited to the receptor, undergo phosphorylation, dimerise and translocate into the nucleus where they initiate the transcription of target genes (Figure 1) (reviewed in [3]). They can also be phosphorylated, and presumably activated, downstream of several growth factors and oncoproteins [4].

JAKs and STATs in normal homeostasis

Mammalian cells can express four different JAKs (JAK1, JAK2, JAK3 and TYK2) and seven STATs (STAT1-STAT6, including STAT5A and STAT5B). Some STATs have different splicing variants (STAT1, STAT3 and STAT4). These are found in three chromosomal clusters and can also arise post-translationally after, for example, proteolytic processing (STAT5A and STAT5B) (reviewed in [5]). STATs are 750-850 amino acids long and are ubiquitously expressed except for STAT4, which is restricted to myeloid cells, thymus and testis [6]. Their activity is regulated not only through tyrosine but also serine phosphorylation [7-9]. The structure of these molecules is depicted in Figure 2. Of note: the well-conserved SH2 domains are responsible for the association of STAT with tyrosine-phosphorylated motifs in the receptor [10] and for dimerisation with other tyrosine phosphorylated STATs [11, 12]. STAT1 and STAT3 are able to form homo- and heterodimers, as well as tetramers. Near the C-terminus is the transactivation domain (TAD). This domain has a serine residue (amino acids 727 in STAT1 and STAT3) that is necessary for maximal transcriptional activation of some genes [8, 11, 13-15]. Untreated cells have STAT1 and STAT3 randomly distributed in cytoplasm and nucleus [16]. After a few minutes of cytokine treatment, however, they become tyrosine phosphorylated in the...
cytoplasm and translocate to the nucleus [17]. In reality, it is not such a black and white picture and we now know that unphosphorylated STATs can also move into the nucleus and have biological impact (e.g. [18, 19] and reviewed in [3, 19]). When in the nucleus STATs can bind to specific sequences in the DNA and induce the transcription of additional target genes.

Figure 1. Representation of JAK/STAT signal transduction pathways using IL-6 signalling as a paradigm. Ligand (IL-6) binding to the ligand binding unit (IL-6R) induces auto- and trans-phosphorylation of receptor pre-associated JAKs (JAK1, JAK2 and TYK2), phosphorylation of tyrosine motifs in the receptor signalling subunit (gp130), recruitment and re-arrangement of associated STATs (STAT1, STAT3 and, potentially, STAT5) which, upon tyrosine phosphorylation by the JAKs, are released, migrate to the nucleus and activate transcription. MAPKs are also activated via recruitment of SHP2/PTPN11 to tyrosine (Y) at position 759 on gp130, which leads to the transcription of additional target genes.

Figure 2. Structure of JAKs and STATs. JAKs have 7 JAK-homology domains, named JH1-JH7. These form four main regions: the four-point-one, ezrin, radixin and moesin (FERM) domain (blue), the potential SRC homology 2 (SH2) domain (green), and the pseudokinase (purple) and tyrosine kinase domains (magenta). STATs have 6 domains, named N-terminal domain (orange), coil-coiled domain (yellow), DNA binding domain (turquoise), linker domain (navy), SH2 domain (pink) and transcriptional activation domain (red). Key phosphorylation tyrosine (Y) and serine (S) C-terminus residues are also depicted. (Not drawn to scale).
Different cytokines activate distinct sets of JAKs and STATs and, consequently, have the potential to induce different gene expression programmes. Typically, type I IFNs activate JAK1 and TYK2 and induce transcription via a protein complex that is composed of STAT1, STAT2 and IFN-regulatory factor (IRF)-9 (also known as p48). Type II IFN (IFN-γ) leads to the activation of JAK1 and JAK2 and to the formation of STAT1 dimers. These different STAT complexes bind to different DNA consensus sequences and induce distinct sets of genes (with some overlap) [3, 17]. Other cytokines, such as those belonging to the interleukin (IL)-6 family, trigger the phosphorylation of JAK1, JAK2 and TYK2, the accumulation of STAT3 dimers and thus the transcription of STAT3 target genes (Figure 1). STAT1 and STAT5 can also be phosphorylated downstream of IL-6 but the significance of such “activation” is as yet unclear [21, 22]. STAT5 is usually triggered by ligands such as erythropoietin and prolactin and is thus involved in erythropoiesis, breast development and lactation [23]. STAT4 and STAT6 are activated by IL-12 and IL-4, respectively, and are therefore key molecules in regulating T helper cell (Th) 1 and 2 responses [24].

Studies using knockout mice and observations made in patients have largely confirmed initial work in cell lines generated by the Kerr and Stark groups, which were deficient in JAK/STAT signalling components [25]. STAT1-deficient humans and mice are more susceptible to both viral and bacterial infections [26, 27]. STAT2-deficient mice are also more susceptible to viruses and, unsurprisingly, have defects in type I IFN responses [28]. STAT3 knockout mice are embryonic lethal but conditional knockouts have revealed essential roles for the acute-phase response in the liver and the control of IL-6 and IL-10 cytokine responses [22, 29, 30]. Mice that lack STAT4 have most IL-12-mediated responses severely impaired, including the induction of IFN-γ which is important to determine a Th1 response [31, 32]. IL-4, a Th2 cytokine, and IL-13 trigger STAT6 activation and, accordingly, STAT6-deficient animals lack a Th2 response [33, 34] and have defective IL-13 responses [35], respectively. Many cytokines and growth factors can lead to activation of STAT5A and STAT5B. Amongst these are erythropoietin, growth hormone, thrombopoietin, IL-2, IL-3, IL-5, IL-7, IL-9, IL-15 and GM-CSF (reviewed in [36]). STAT5A-deficient animals have pinpointed important roles in mammary gland development and lactation, whereas STAT5B-null animals suggest an important role in growth hormone biology.

### Regulation of JAK/STAT signalling and the consequences of dysregulated activation

Under normal conditions, STATs are active for minutes up to a few hours as these molecules are rapidly down-regulated. STAT activity can be inhibited by phosphatases [37-39], suppressors of cytokine signalling (SOCS, also named JAK-binding proteins and STAT-induced STAT inhibitors) ([40-43] and reviewed in [44]) and protein inhibitors of activated STATs (PIAS) ([45-48] and reviewed in [49]). The SOCS family has 8 members: SOCS1-7 and CIS. These molecules are characterized by an SH2 central domain and by a SOCS box. SOCS can act by distinct mechanisms: they can bind phosphorylated tyrosine residues on receptor chain and JAKs, or bind to STATs and block their recruitment to the ligand. In addition, the SOCS box can target STATs to degradation as it has E3 ubiquitin ligase activity [50]. STATs can also be inactivated directly by protein inhibitors of activated STAT proteins (PIAS) [45, 46]. PIAS can act on different levels: they can bind directly to STATs, thereby blocking protein-DNA interactions; induce degradation of the STATs, or alter STATs localisation [49]. The negative regulatory mechanisms controlling JAK/STAT signalling thus ensure that STAT activation is cyclic and transient (further reviewed in [3]).

JAK deficiency can have severe consequences for an organism, as demonstrated by syndromes such as severe combined immunodeficiency (SCID) and autosomal recessive hyperimmunoglobulin E [51]. On the other hand, JAKs are often found mutated in myeloproliferative disorders (MPDs) and in many leukaeemias. The most common mutation occurs on JAK2 on a valine residue on position 617, which is located on the regulatory pseudokinase domain (Figure 2). This mutation leads to constitutive activation of the kinase and thus constitutive phosphorylation (and, presumably, activation) of STAT3 and STAT5 [52-54]. These observations raise the possibility that JAK kinase inhibitors might prove useful in the management of MPDs and leukaeemias bearing activating JAK mutations and several such inhibitors are currently being evaluated in clinical trials.
Interestingly, we have unravelled an additional role(s) for JAKs in drug resistance induced by signalling in response to fibroblast growth factor (FGF)-2 [55]. Many cancer patients have elevated levels of FGF-2 in their blood, which indicates a poor prognosis on univariate and multivariate analyses [56-58]. In fact, FGF-2 is a potent mitogen and one of the many molecules capable of inducing drug resistance in cancer cells challenged with chemotherapeutic drugs [59-61]. Signalling through the FGF receptor triggers mitogen activated protein kinases (MAPKs) and leads to the assembly of a multi-protein complex, which contains protein kinase C (PKC) ε, v-raf murine sarcoma viral oncogene homologue (B-RAF) and p70 S6 kinase b (S6K2). As a consequence, the translation of a variety of anti-apoptotic genes is upregulated [60, 62, 63]. Such anti-apoptotic molecules thus arm the cell against the noxious effects of chemotherapeutic agents. As several growth factors including FGF-2 can activate JAK/STAT signalling we asked if JAKs and/or STATs might contribute to this novel chemoresistance mechanism. We have discovered that FGF-2 induced phosphorylation of JAKs and their association with the multi-protein complex. Interestingly, downregulation of JAK1, JAK2 or TYK2 expression was sufficient to block FGF-2 survival signals thus leading to cell death of osteosarcoma cells in response to cisplatin [55]. However, to our surprise, silencing of STAT1, STAT3 or STAT5A/B did not impair FGF-2-mediated drug resistance. These observations provide an example of JAK signalling that is independent of STATs. A key question though is whether the effects seen are dependent on JAK kinase activity and/or structure. On-going work with selective JAK kinase inhibitors suggests that the kinase domain is important for transmitting FGF-2-induced drug resistance effects. It is therefore conceivable that JAK inhibitors could also be used to help reverse this form of multi-drug resistance which we have now found to be present in several common cancer types ([55, 61] and unpublished data).

Unlike the JAKs, STATs are not found mutated in cancer cells but are inappropriately activated, presumably as a consequence of dysregulated cytokine/growth factor signalling [4]. In particular, STAT3 and STAT5 are constitutively phosphorylated not just in multiple cancer cell lines but also in many tumour specimens [64-68] As these molecules regulate the transcription of many genes that positively control cell proliferation and cell migration, as well as genes that negatively regulate apoptosis and immune recognition, they may participate directly in tumourigenesis [69]. Indeed, genes such as vascular endothelial growth factor (VEGF), c-MYC, BCL-xL, MCL-1, cyclin D1, JUNB, chemokines, proteases such as uPA and uPAR and, paradoxically, p21\(^{WAF1/CIP1}\), are known transcriptional targets of STAT3 ([70, 71] and reviewed in [72]). This transcriptional profile then explains why STATs have such a wide biological impact, with far-reaching consequences. Importantly, whereas STAT3 is a well-documented oncogene [73], STAT1 has long been seen as a tumour suppressor gene, capable of modulating the immune system and, under certain circumstances, directing it against tumour cells [74]. More recent reports, however, suggest that STAT1 is a double-edged sword and that it can also promote tumourigenesis [75].

Constitutive activation of STATs can be seen downstream of many receptor tyrosine kinases (RTKs), such as epidermal growth factor (EGF), hepatocyte growth factor (HGF) and platelet-derived growth factor (PDGF) receptors [72] and downstream of non-RTKs such as ABL [76] and SRC [77]. Interestingly, v-SRC transformation requires STAT3 tyrosine phosphorylation [77]. However, this is not true for all downstream oncogenes. Indeed, RAS requires the presence of unphosphorylated STAT3 in mitochondria [18], which shifts the cell metabolism towards fermentation in a manner that favours tumour cell growth. This raises interesting questions regarding the successful use of STAT3 inhibitors in the clinic [78, 79]. Indeed, it may be insufficient to simply interfere with phosphorylated, mainly nuclear-localised, STAT3 as this will not impair the effects of unphosphorylated STAT3 in mitochondria. Nevertheless, inhibiting molecules such as STAT3 remain worthwhile approaches in cancer treatment and the increasing understanding of how these molecules operate and are regulated will hopefully enable this soon.

**JAK/STAT signalling in lung cancer**

Lung cancer, one of our research interests, causes more deaths per year than any other type of cancer in men [80, 81] and it is the second cause of death in women after breast cancer [82]. The classification of human lung cancer includes two major types: small cell lung
cancer (SCLC) and non-small cell lung cancer (NSCLC). NSCLC includes several sub-types of which adenocarcinoma is the most common [83]. Smoking is responsible for 80-85% of all lung cancer [83]. In 2008 about 1.52 million people around the world were diagnosed with lung cancer and approximately 1.31 million people died from the disease [81]. Lung cancer symptoms are detected in most patients too late [80, 81], which together with lack of effective treatments contribute to a poor prognosis for most patients. Indeed, regional or distant overt metastases are found in more than two-thirds of patients [81] and most of the remaining individuals will have occult metastatic disease. Consequently, most patients are ineligible for curative surgery and, as the remaining radiotherapy, chemotherapy and newer targeted treatment options are non-curative, novel therapeutic approaches and early disease markers are needed [81].

Cytokine responses are highly regulated and often transient. In cancer cells, however, cytokine responses can be dysregulated. For example, IL-6 secretion is often increased in cancer patients and in many cancer cell lines [84]. This can trigger dysregulation of several signalling components and like EGF it can lead to constitutively activated STAT3. STAT3 phosphorylation is, in fact, observed in around 50% primary NSCLC tumours and cancer cells lines derived from them [84-87]. This likely reflects mutations in EGF receptor, increased circulating IL-6, or activation of non-RTKs as discussed earlier.

The main transcription factor induced by IL-6-type cytokines, such as IL-6, oncostatin M (OSM) and leukaemia inhibitory factor (LIF), is STAT3. IL-6 is a pleiotropic cytokine that plays important roles in cell proliferation, differentiation, survival and apoptosis. IL-6 also takes part in immune responses, haematopoiesis and inflammation (reviewed in [21, 88]). In addition, IL-6 can control a variety of responses in many cell types and is a crucial regulator of the nervous system, endocrine system, bone metabolism, amongst others [89]. IL-6 induces transcription of the IL-6 gene via JAK2 and STAT3. This is thought to lead to increased autocrine production of this cytokine observed in different cancer cell lines. Thus, regulating JAK2 and/or STAT3 could reduce IL-6 production, thereby impairing cell growth and enhancing their susceptibility to other treatments [90]. Indeed, blockade of IL-6 signalling in lung cancer-derived cell lines was shown to be enough to inhibit cell growth [84, 91]. It was also shown that some tumours in mice are induced by ras, which can also stimulate secretion of IL-6 in different cell types. Ras-induced transformation in a variety of mouse models appears to require IL-6 and, consistent with that, IL-6 knockout animals were more resistant to ras-induced carcinogenesis ([92] and reviewed in [93]). Accordingly, knockdown, genetic ablation or antibody neutralization of IL-6 can limit tumour growth induced by ras [94]. Interestingly, some human lung adenocarcinomas also have RAS mutated [95]. Paradoxically, IL-6 can under certain circumstances decrease cell growth in some types of lung cancer cells [96]. For example, the growth of Lewis lung cancer carcinoma cells decreased after being transfected with IL-6 [97]. When these cells were treated with an anti-IL-6 antibody they did not proliferate, indicating that growth inhibition was not related to a direct autocrine effect of IL-6 [97]. In contrast, in other NSCLC cell lines, IL-6 caused an increase in growth (A549, Calu3, Calu6, and H23). In the presence of IL-6 antisense phosphorothioated oligonucleotides, cell proliferation was notably reduced. However, neither the presence of monoclonal neutralizing anti-IL-6 antibodies, nor exogenous IL-6, interfered with cell proliferation, or IL-6 synthesis. This probably reflects the now widely recognized importance of cellular background in determining cellular responses and biological outcome [22, 98]. Certainly in lung cancer patients, IL-6 appears to promote and sustain malignancy [71, 99-101]. For example, IL-6 has been found elevated in lung cancer patients and the autocrine production of IL-6 has been shown to lead to constitutive activation of STAT3 and promote lung adenocarcinoma and malignant pleural infusion. Increased levels of circulating IL-6 thus appear to be an adverse prognostic factor for lung cancer patients [100, 101].

In addition to JAKs and STATs, IL-6 signalling also involves activation of MAPKs (Figure 1). Hirano and colleagues [102] described several experiments carried out with gp130 receptor mutants in order to understand its regulation and the impact of the different pathways activated upon IL-6 signalling. They noted that there appeared to be a compensatory balance between the JAK/STAT and MAPK pathways when either pathway was disturbed. In fact, some-
times gp130 can activate opposite signalling cues. This may explain why/how IL-6 can have multiple functions in different types of cells [102].

PTPN11 (also known as SHP2) has a role not only as an enzyme but also as a protein adapter [103]. Receptor-bound PTPN11 (Y759 on gp130 for IL-6 and Y794 on LIF receptor) can bind GRB2 via two tyrosine residues in the C-terminal (tyrosine 542 and tyrosine 580) that are believed to establish interactions with the GRB2-son-of-sevenless (SOS) complex [10, 104-106], reviewed [21]) This complex, which forms a GDP/ GTP exchanger for RAS, results in MAPK cascade activation in response to IL-6 and LIF. In response to OSM, SH2- and collagen-homology-domain-containing protein (SHC) is recruited instead (Y861 on OSM receptor) and serves as the bridge that links gp130 signalling to MAPK cascades [107]. This also couples to MAPK cascades using GRB2.

Activation of MAPK signalling also involves GRB2-associated binding protein 1 (GAB1), another scaffold protein. This further increases the complexity of the response by allowing the recruitment of additional signalling pathways, such as phospholipase Cy, phosphatidylinositol 3'-kinase (PI3K)/AKT and c-MET (reviewed in [21]). Furthermore, stress-activated kinases (additional MAPK family members) can also be activated by IL-6-type cytokines [108-110]. These molecules have also been implicated in cancer including lung cancer and thus exacerbated production of molecules such as IL-6 not only favour tumourigenesis via STAT activation but potentially also by virtue of inducing these well-known pro-survival and/or mitogenic signals. This, in turn, suggests that successful therapies will likely involve targeting multiple molecules, which may be cross-activated but which elicit different arms of a mitogenic or anti-apoptotic response(s).

Concluding remarks

JAKs and STATs have been identified 20 years ago. Since then, our understanding of their molecular structures and biological functions, both in cellular homeostasis and pathogenesis, has greatly increased. With this knowledge comes the promise of being able to use them as useful and effective targets for the management of several diseases, including a variety of cancers. For this purpose, our efforts to fully understand their cellular roles must continue as this will help to minimise off-targets effects in the clinic and thereby maximise potential benefits to patients.

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Conflict of interest

The authors declare no conflict of interest.

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