TUMOUR IMMUNOLOGY

Crosstalk between cancer and immune cells: role of STAT3 in the tumour microenvironment

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Abstract | Immune cells in the tumour microenvironment not only fail to mount an effective anti-tumour immune response, but also interact intimately with the transformed cells to promote oncogenesis actively. Signal transducer and activator of transcription 3 (STAT3), which is a point of convergence for numerous oncogenic signalling pathways, is constitutively activated both in tumour cells and in immune cells in the tumour microenvironment. Constitutively activated STAT3 inhibits the expression of mediators necessary for immune activation against tumour cells. Furthermore, STAT3 activity promotes the production of immunosuppressive factors that activate STAT3 in diverse immune-cell subsets, altering gene-expression programmes and, thereby, restraining anti-tumour immune responses. As such, STAT3 propagates several levels of crosstalk between tumour cells and their immunological microenvironment, leading to tumour-induced immunosuppression. Consequently, STAT3 has emerged as a promising target for cancer immunotherapy.

Oncogenic processes are typically viewed independently of the immune response against the tumour. The hallmarks of cancer include tumour-cell proliferation and survival, tumour angiogenesis and metastasis[1]. The activation of proto-oncogenes and oncogenic signalling pathways together with inactivation of tumour-suppressor genes in cancer cells are crucial processes in malignant transformation and progression[1–3]. At the same time, tumour immunologists have uncovered many cellular and molecular mechanisms that mediate tumour escape from natural immune surveillance, which functions as an extrinsic tumour suppressor[4,5]. The paucity of immunological danger signals necessary for immune activation, the increased concentration of immunosuppressive factors and the accumulation of immunosuppressive cells in the tumour microenvironment indicate that immune regulation has an active role in cancer progression.

A major endeavour in the field of cancer immunology has been to understand how cancer cells effect a global shutdown of immune-stimulating molecules, such as co-stimulatory molecules and cytokines, in the tumour microenvironment[5,6]. These effects are typically local, because, with the exception of those with end-stage disease, patients with cancer are not systemically immunodeficient. What is the underlying mechanism that allows a tumour to produce immunosuppressive factors that generate tolerogenic dendritic cells (DCs) and regulatory T cells in the tumour microenvironment[7,8]? Why are the innate immune cells in the tumour microenvironment, including macrophages, natural killer (NK) cells and neutrophils, incapable of killing tumour cells? What allows tumour cells to co-opt immune cells for tumour growth, survival, angiogenesis and invasion[9,10]? The definition of specific molecules and signalling pathways that regulate the tumour microenvironment will provide important targets for cancer immunotherapy.

Recent studies have identified signal transducer and activator of transcription 3 (STAT3) as an important molecule that mediates tumour-induced immunosuppression at many levels. STAT3, which transduces signals from numerous oncogenic proteins and pathways[11–13], is not only a potent negative regulator of T helper 1 (T_H1)-cell-mediated inflammation, but also an important activator of many genes that are crucial for immunosuppression[13–17]. The constitutive activation of STAT3 can be propagated, in part through STAT3-regulated factors such as vascular endothelial growth factor (VEGF) and interleukin-10 (IL-10), from tumour cells to diverse immune cells, mediating a crosstalk between the two, which, in turn, generates immunosuppression involving both innate and adaptive immunity[16–19]. Because STAT3 is also crucial for

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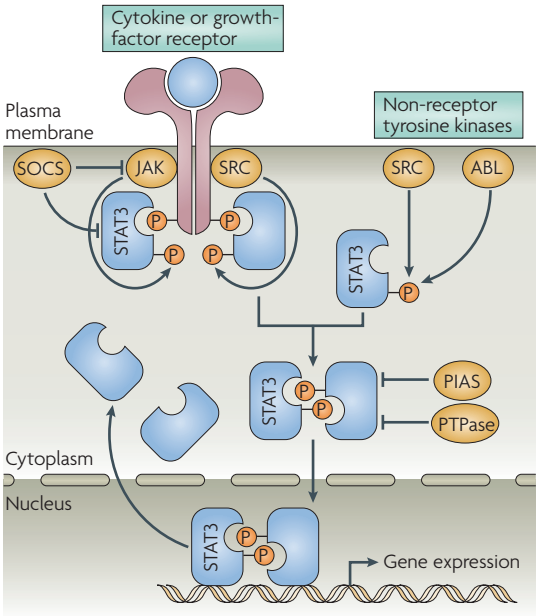

Figure 1 | **Constitutive activation of STAT3 by receptor and non-receptor tyrosine kinases.** Autocrine or paracrine signals activate intrinsic growth-factor-receptor tyrosine kinases, and cytokine-receptor-associated Janus-family kinases (JAKs) and SRC tyrosine kinases, which, in turn, phosphorylate signal transducer and activator of transcription 3 (STAT3). In transformed cells, STAT3 can also be activated by constitutively active non-receptor tyrosine kinases such as SRC and ABL. After tyrosine phosphorylation, STAT3 molecules dimerize and translocate to the nucleus, where they directly regulate gene expression. STAT3 can also be acetylated, which might mediate STAT3 function (not shown). STAT3 signalling is normally tightly regulated by several inhibitory molecules, including suppressor of cytokine signalling (SOCS) proteins, protein inhibitor of activated STAT (PIAS) proteins and protein tyrosine phosphatases (PTPases). In cancer cells, however, overactive receptor and non-receptor tyrosine kinases cause persistent STAT3 phosphorylation and activation.

tumour-cell proliferation and survival, tumour angiogenesis and invasion, a direct link between traditional oncogenesis and immunosuppression is embodied in the STAT3 pathway. We review recent data that provide the first example by which an oncogenic pathway — involving STAT3 — controls the immunological response to tumour cells. Owing to its additional contributions to many other aspects of oncogenesis that negatively affect tumour immunity, we consider how STAT3 has emerged as an important target for effective immunotherapy.

STATs and oncogenesis

STAT signalling pathways were originally discovered in the context of normal cytokine signalling (FIG. 1). STAT1 is obligatory for interferon (IFN) signalling, whereas STAT3 is inducible by interleukin-6 (IL-6) signalling as well as by many other cytokines[20–22]. The activation of STAT signalling pathways requires tyrosine phosphorylation of STAT proteins[23–25]. STAT3 can also be acetylated[26,27], which might mediate some of its biological functions.

Because many cytokine receptors do not have intrinsic tyrosine-kinase activity, ligand engagement leads to the activation of receptor-associated tyrosine kinases, which are usually members of the Janus kinase (JAK) family[23–25]. In addition to cytokines, STATs are also activated by many growth factor receptors with intrinsic tyrosine-kinase activity[28–30]. The growth factor receptors that are known to activate STAT3 include the epidermal growth factor receptors EGFR and HER2 (also known as NEU), FGFR (fibroblast growth factor receptor), IGFR (insulin-like growth factor receptor), HGFR (hepatocyte growth factor receptor; also known as MET), PDGFR (platelet-derived growth factor receptor) and VEGFR (VEGF receptor). As the oncoproteins SRC and ABL are also activators of STAT3, STAT3 is constitutively activated in diverse cancers. Furthermore, many tumour-produced factors, such as IL-10, IL-6 and VEGF, which are crucial for both tumour growth and immunosuppression, activate STAT3 to create an efficient 'feedforward' mechanism to ensure increased STAT3 activity both in tumour cells and in tumour-associated immune cells. In normal cells, and under physiological conditions, the activation of STATs is rapid and transient, because they are negatively regulated by proteins such as suppressor of cytokine signalling (SOCS) and protein inhibitor of activated STAT (PIAS)[31–33].

In the mid-1990s, the first direct links between STAT signalling and oncogenesis were established. Jove and colleagues made the unexpected observation that STAT3 is constitutively activated in cells transformed by an oncoprotein, SRC, which is a non-receptor tyrosine kinase[11]. Definitive evidence that STAT3 contributes to oncogenesis was provided by the finding that interrupting STAT3 signalling blocks the transformation of fibroblasts by SRC oncoprotein[34,35]. A direct role of STAT3 in oncogenesis was shown using a constitutively active STAT3 mutant, which transforms fibroblasts in culture and allows the transformed cells to form tumours in mice[36]. Evidence for an essential role of activated STAT3 in preventing the apoptosis of human tumour cells was first shown in multiple myeloma. STAT3 activity supports tumour-cell survival by upregulating expression of the anti-apoptotic protein BCL-X_L (B-cell lymphoma-2-like 1) (REF 37). Together with these milestone discoveries came the important finding that human cancer cells express constitutively active STAT3 at a high concentration[37,38]. Subsequently, STAT3, and to a lesser extent STAT5, have been shown to be constitutively active in a growing number of diverse human cancer cell lines and tumour tissues[39–41]. In retrospect, because STAT3 and, in many cases, STAT5 are crucial for downstream signalling by specific tyrosine kinases that are oncogenic proteins that commonly bear activating mutations in cancer cells, the high frequency of constitutive activation of STAT3 in human cancers is not surprising[13]. Although BCL-X_L was the first anti-apoptotic factor shown to be regulated by STAT3, many other proteins that are crucial for tumour-cell proliferation and survival have subsequently been found to be regulated by STAT3, including myeloid cell leukaemia sequence 1 (MCL1), survivin, cyclin D1 and MYC[13] (TABLE 1).

Danger signals
A danger signal is normally defined as the pathogen-associated molecular pattern that is recognized by host receptors. Danger signals often trigger the production of cytokines, chemokines and other physiological mediators, such as nitric oxide, leading to immune responses against the pathogen. In the context of this Review, 'danger signals' refer to the similar cytokines, chemokines and other T helper 1-type immunostimulating molecules that are produced by transformed cells on STAT3 inhibition.

The discovery that STAT3 upregulates expression of BCL-X_L, and that interrupting STAT3 signalling induces apoptosis of human cancer cells, prompted an *in vivo* experiment to determine whether STAT3 is a valid target for cancer therapy[42]. A gene-therapy approach using a plasmid expression vector encoding STAT3β, a dominant-negative variant of STAT3 that blocks the oncogenic function of STAT3, was used to induce regression of mouse melanoma B16 tumours. Despite the relatively low *in vivo* transfection efficiency (10–15% of tumour cells were transfected with the vector encoding STAT3β), pre-existing B16 tumour growth was markedly inhibited, and was accompanied by tumour regression and extensive tumour-cell apoptosis that greatly exceeded the number of transfected tumour cells[42]. Inhibiting STAT3 in tumours can, therefore, induce a potent 'bystander' effect, killing tumour cells that do not express the STAT3 dominant-negative protein[43]. A search for the cellular and molecular mechanisms that could account for the anti-tumour bystander effects associated with STAT3 blockade led to the discovery of an important role of STAT3 in angiogenesis through the induction of expression of the pro-angiogenic factor VEGF[44–46]. Since then, many more pro-angiogenic factors have been shown to be STAT3 target genes, including the genes encoding HIF1α (hypoxia-inducible factor 1α), bFGF (basic fibroblast growth factor), HGF (hepatocyte growth factor), MMP2 (matrix metalloproteinase 2) and MMP9 (REFS 47–50; TABLE 1). However, another potent mechanism for the bystander effects associated with blocking STAT3 activity seems to result from the profound role of STAT3 in regulating inflammatory and immune responses in the tumour microenvironment.

Table 1 | **STAT3 promotes oncogenesis and immune evasion**

Function	STAT3-regulated gene products	References
Proliferation and survival	↑MYC	107–109
	↑Cyclin D1/D2	110
	↑BCL-X_L	37,111
	↑MCL1	112,113
	↑Survivin	114–116
	↓p53	117
Angiogenesis	↑VEGF	44,45
	↑HGF	118
	↑bFGF	119
	↑HIF1α	47,120
	↑MMP2	49
	↑MMP9	50
	↓IL-12	16,19,81
	↓IFNβ	16,51
	↓IFNγ	17
	↓CXCL10	16
	↓p53	117
	↓AKT	47
Immunosuppression	↑IL-6	19
	↑IL-10	71,121
	↑TGFβ	71,72
	↑VEGF	44,45
	↓IFNβ	16,51
	↓IFNγ	17
	↓IL-12	16,81
	↓TNF	16,19
	↓CXCL10	16
	↓CCL5	16
	↓MHC class II	16,17,100
	↓CD80	16,17
	↓CD86	16,17

AKT, thymoma viral proto-oncogene 1; BCL-X_L, B-cell lymphoma-2-like 1; bFGF, basic fibroblast growth factor; CCL5, CC-chemokine ligand 5; CXCL10, CXC-chemokine ligand 10; HGF, hepatocyte growth factor; HIF1α, hypoxia-inducible factor 1α; IFN, interferon; IL, interleukin; MCL1, myeloid cell leukaemia sequence 1; MMP, matrix metalloproteinase; MYC, v-myc myelocytomatosis viral-related oncogene; STAT3, signal transducer and activator of transcription 3; TGFβ, transforming growth factor-β; TNF, tumour-necrosis factor; VEGF, vascular endothelial growth factor.

Immune regulation by STAT3 in tumour cells

Expression of immunological danger signals. B16 tumours treated with the dominant-negative STAT3 mutant protein were heavily infiltrated by immune cells. To test whether the infiltration was, at least in part, due to the release of pro-inflammatory mediators, tumour cells cultured in the absence of leukocytes were examined. These experiments showed that inhibiting STAT3 activity in tumour cells led to the upregulation of expression of several pro-inflammatory cytokines and chemokines, indicating that *in vivo* STAT3 inhibition would result in robust leukocyte infiltration into the tumour. Conversely, activation of STAT3 signalling in normal fibroblasts — through either SRC-mediated transformation or overexpression of a constitutively active STAT3 mutant, STAT3C — inhibits the lipopolysaccharide (LPS)-induced release of pro-inflammatory cytokines and chemokines. Taken together, these *in vitro* and *in vivo* findings show that STAT3 activity in tumours can negatively influence the expression of immune-stimulating molecules[16].

Further work revealed a cascade of events in which pro-inflammatory mediators produced by tumour cells owing to STAT3 blockade could activate innate immune cells, including macrophages and neutrophils, leading to their further secretion of pro-inflammatory mediators and increased cytotoxicity against tumour cells[16]. Moreover, levels of STAT3 activity in tumour cells, whether induced or natural, inversely correlate with immune-cell migration *in vitro* and infiltration into tumours *in vivo*[51]. The ability of STAT3 signalling to facilitate immune evasion by the tumour through inhibiting the expression of pro-inflammatory cytokines and chemokines was also shown in rhabdomyosarcoma tumours, many of which are associated with an oncogenic fusion protein, paxillin-3–forkhead (PAX3–FKHR)[18]. PAX3–FKHR interacts physically with STAT3 to alter the transcription of many immune-stimulating cytokines and chemokines,

Tolerogenic dendritic cells
Dendritic cells that can attenuate T-cell-mediated immune responses by anergizing or changing the effector function of antigen-specific T cells.

and MHC class II molecules in tumour cells. *In vivo* studies have shown that the PAX3–FKHR–STAT3 complex affects local cytokine concentrations, thereby suppressing local inflammatory and immunological responses. As a result, the PAX3–FKHR–STAT3 interaction causes more rapid tumour growth in immunodeficient mice compared with immunocompetent mice[18].

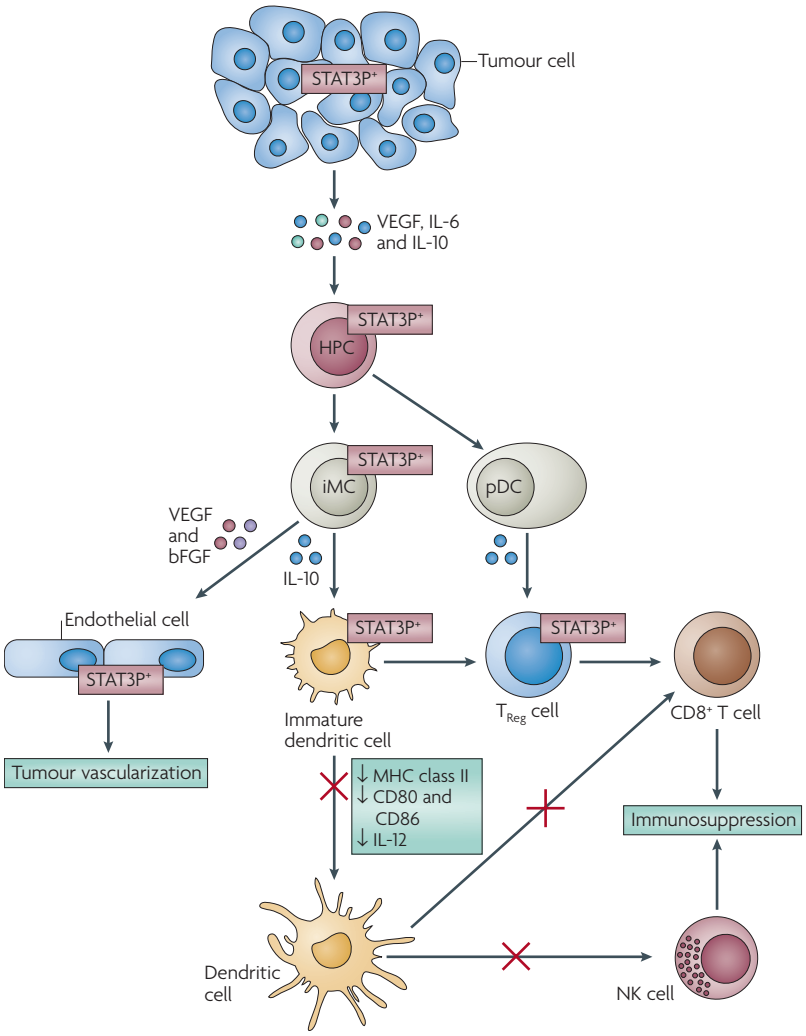

Figure 2 | **STAT3 signalling allows crosstalk between tumour cells and dendritic cells, forming an immunosuppressive network.** Tumour-associated factors such as vascular endothelial growth factor (VEGF), interleukin-10 (IL-10) and IL-6 can not only be upregulated by signal transducer and activator of transcription 3 (STAT3), but are also STAT3 activators. Increased STAT3 activity in haematopoietic progenitor cells (HPCs) promotes the generation of immature myeloid cells (iMCs) and increases the numbers of both immature dendritic cells and plasmacytoid dendritic cells (pDCs), each of which promotes the accumulation of regulatory T (T_{Reg}) cells in the tumour microenvironment. STAT3 activity inhibits the expression of MHC class II molecules, CD80, CD86 and IL-12 by DCs, thereby preventing their maturation and compromising their ability to stimulate the anti-tumour effects of $CD8^+$ T cells and natural killer (NK) cells. The production of tumour-associated factors, such as IL-10, VEGF and basic fibroblast growth factor (bFGF), by myeloid cells in the tumour microenvironment is STAT3 dependent. These factors not only strengthen the immunosuppressive network, but also promote tumour vascularization, which protects tumour cells from apoptosis and counteracts the interferon-γ-dependent effects of $CD8^+$ T cells. STAT3P, tyrosine-phosphorylated (activated) STAT3.

The capacity of STAT3 to inhibit the expression of pro-inflammatory mediators is not unique to tumour cells. STAT3 is also a negative regulator of immune-stimulating molecules in normal immune cells — for example, ablating *Stat3* alleles in macrophages leads to high levels of several pro-inflammatory cytokines similar to those found in tumour cells in which STAT3 signalling is inhibited[14].

Inhibition of DC functional maturation. Although the role of DCs in inducing anti-tumour immunity is well documented, DCs in the tumour microenvironment are usually immature, being characterized by an insufficient level of expression of MHC class II complexes, co-stimulatory signals such as CD80 and CD86, and IL-12 (REFS 7,8,52). Not only are these DCs incapable of activating antigen-specific $CD8^+$ T cells, but they can also induce immune tolerance[7,8]. Tolerogenic DCs are generated in the absence of inflammatory mediators[53], and they can be further blocked from becoming effective antigen-presenting cells by tumour-cell-secreted factors that inhibit their maturation[7,8]. The results of experiments to either induce STAT3 activation in normal fibroblasts or decrease STAT3 activity in transformed cells indicate that tumour-cell STAT3 activity negatively affects DC functional maturation, for example, by suppressing the expression of MHC class II and co-stimulatory molecules, and IL-12 (REF. 16). The STAT3-associated inhibition of DC maturation was mediated, at least in part, by VEGF and IL-10 (REF. 16) (FIG. 2). In tumour cells, the ability of STAT3 to influence DC maturation was confirmed by studies involving PAX3–FKHR-expressing tumour cells[18]. In this case, IL-10, the production of which is STAT3 dependent in tumour cells, was shown to inhibit DC maturation. More recently, it has been confirmed in human melanoma cells with mutated BRAF that STAT3 is required for the production of immunosuppressive factors, such as VEGF, IL-10 and IL-6, which, in turn, inhibit the expression of immune-stimulating molecules, such as IL-12 and tumour-necrosis factor (TNF), by DCs[19]. Inhibiting mitogen-activated protein kinase (MAPK) activity in the same melanoma cells can result in effects similar to blocking STAT3, which indicates that other oncogenic signalling pathways might also have a crucial role in mediating tumour-induced inhibition of DC activity. Indeed, the pro-inflammatory mediators produced by blocking STAT3 in tumour cells led to increased antigen presentation by bone-marrow-derived DCs, thereby activating naive antigen-specific T cells *in vitro*[16]. Furthermore, disrupting STAT3 signalling in tumour cells resulted in the activation of tumour-antigen-specific $CD8^+$ T cells *in vivo*[17].

Effects of STAT3 signalling in immune cells

Tumour-infiltrating DCs and myeloid cells. Because several tumour-associated factors that are known to suppress DC maturation, including IL-6, IL-10 and VEGF[7,8], are activators of STAT3 (REFS 20,22), researchers investigated whether STAT3 signalling in DCs is crucial for the inhibition of their maturation. Blocking STAT3 signalling in DCs — either by a phosphopeptide inhibitor of STAT3

or by ablating the *Stat3* gene — abrogated tumour-induced, as well as IL-10-induced, inhibition of DC functional maturation and decreased the number of immature CD11c$^+$MHC class II^{low}CD86^{low} DCs (REF 16). These findings were confirmed in a study involving tumour cells with activated PAK3–FKHR, whereby blocking STAT3 signalling in DCs neutralized the inhibitory effects of the tumour cells on DC maturation[18]. Another study showed that tumour-derived factors induce STAT3 signalling in immature myeloid cells, thereby preventing them from differentiating into mature DCs[54]. The accumulation of myeloid cells, which form an important immunosuppressive population in the tumour microenvironment, is due therefore, at least in part, to STAT3 activation.

IL-6-mediated suppression of DC maturation has also been shown to be STAT3 dependent[55], and Bruton's tyrosine kinase, which can inhibit DC maturation, does so by activating STAT3 (REF. 56). The *Mx1–Cre–loxP* system was used to ablate *Stat3* alleles in the haematopoietic compartment in adult mice, and this study showed that *Stat3*$^{-/-}$ DCs isolated from tumour-bearing animals expressed more IL-12 than their *Stat3*$^{+/+}$ counterparts after stimulation with LPS[17]. Furthermore, *Stat3*$^{-/-}$ DCs isolated from tumours have higher levels of MHC class II molecules, CD80 and CD86 on their surface, and can present antigens more efficiently to CD4$^+$ T cells compared with *Stat3*$^{+/+}$ equivalents (FIG. 2). These *in vivo* experiments have shown that the constitutive activation of STAT3 restricts immune surveillance of the tumour. Taken together, these studies introduced the concept that abrogation of anti-tumour immunity in tumour-bearing hosts entails a cascade of STAT3 activation, propagating from tumour cells to DCs and myeloid cells, which indicates that tumour-cell STAT3 activity can mediate immune evasion by blocking both the production and sensing of inflammatory signals by various components of the immune system[16] (FIG. 2).

Macrophages, neutrophils and NK cells. The first evidence that STAT3 signalling in macrophages and neutrophils might negatively affect T_H1-type inflammation was provided by Takeda *et al.*, who showed that mice with STAT3-deficient macrophages and neutrophils expressed increased amounts of pro-inflammatory mediators, including cytokines and nitric oxide, after LPS stimulation, which led to chronic enterocolitis[14]. Further evidence that STAT3 signalling in macrophages might inhibit immune stimulation came from the observation that targeted disruption of STAT3 signalling in macrophages led to the activation of antigen-specific CD4$^+$ T cells in response to a normally tolerogenic stimulus *in vivo*[57]. Furthermore, *Stat3*-ablated macrophages effectively broke antigen-specific T-cell anergy *in vitro*, whereas increasing STAT3 activity in macrophages resulted in impaired antigen-specific T-cell responses[57]. It was also shown that blocking STAT3 signalling by using an oligonucleotide decoy for STAT3 in macrophages can activate anti-tumour immune responses in a rat model of breast cancer[58]. Studies using mice with a *Stat3*$^{-/-}$ haematopoietic system showed that lack of STAT3 signalling in both NK cells and neutrophils increases their anti-tumour cytotoxic activity, especially when the cells are exposed to tumour-derived factors[17]. This indicates that tumours can be immunogenic when the responding immune cells lack 'brakes' such as STAT3 (FIG. 2). Further *in vivo* tumour studies showed that the depletion of NK cells, using GM1-specific antibody, partially abrogated STAT3-blockade-induced anti-tumour immune responses[17]. Analysis of NK cells, neutrophils[17] and macrophages (M.K. and H.Y., unpublished observations) in the tumour stroma showed that STAT3 is constitutively activated in all of these cell types. Because the production of several activators of STAT3, including VEGF, IL-10 and IL-6, is upregulated by STAT3 signalling, this pathway seems to have a crucial role in mediating crosstalk between tumour cells and their immunological microenvironment (FIG. 2).

Regulatory T cells. Several recent studies have shown that regulatory T (T_{Reg}) cells have an important role in sustaining the immunosuppressive environment in tumours[8]. T_{Reg} cells selectively accumulate inside tumours, making up most of the tumour-infiltrating lymphocytes at the late stages of tumour progression, as shown in mouse tumour models[17,59]. Tumour-associated T_{Reg} cells efficiently inhibit immune responses mediated by pre-activated CD8$^+$ T cells by suppressing the proliferation and IFNγ production of antigen-specific CD8$^+$ T cells[60]. T_{Reg}-cell-induced suppression of anti-tumour immunity occurs mainly at the tumour site, and the local elimination of T_{Reg} cells can be an effective treatment for well-established cancers[59]. T_{Reg}-cell-mediated suppression of anti-tumour immune responses has been shown to involve IL-10 and transforming growth factor-β (TGFβ)[59,61]. In patients with cancer, an increase in the number of T_{Reg} cells in tumours and lymph nodes has been shown. The T_{Reg} cells isolated from patients with cancer are functional, as shown by the inhibition of nonspecific T-cell activation *in vitro*[62–64].

What might induce T_{Reg}-cell proliferation in the tumour microenvironment? Recent studies in patients with ovarian cancer showed that dysfunctional DCs and tumour-conditioned plasmacytoid dendritic cells (pDCs) contribute to T_{Reg}-cell induction in the tumour microenvironment[64–66]. An alternative mechanism by which T_{Reg} cells accumulate in tumours was revealed by the finding that human ovarian cancer cells and associated macrophages can overexpress CCL22 (CC-chemokine ligand 22), allowing the migration of T_{Reg} cells that express the matching chemokine receptor CCR4 towards the tumour[65]. However, despite the progress made, relatively little is known about the molecular mechanisms that initiate and sustain the dominance of T_{Reg} cells in tumours. Studies involving mice with a *Stat3*$^{-/-}$ haematopoietic system indicate that constitutive activation of STAT3 in DCs and T_{Reg} cells induced by the growing tumour might be crucial for T_{Reg}-cell proliferation and the suppression of tumour-specific CD8$^+$ effector T cells[17]. In addition to a decrease in the number of pDCs in the tumour, ablation of *Stat3* in the haematopoietic system using the *Mx1–Cre–loxP* system is

Mx1–Cre–loxP system
The *Mx1–Cre–loxP* system allows specific gene ablation, mostly in the haematopoietic cell lineages of adult mice. Injection of polyinosinic–polycytidylic-acid oligonucleotides stimulates the production of type-I interferons, which induce Cre recombinase expression through the interferon-sensitive *Mx1* promoter, resulting in the ablation of target gene alleles flanked by *loxP* sites.

T-cell anergy
A state of T-cell unresponsiveness to stimulation with antigen. It can be induced by stimulation with a large amount of specific antigen in the absence of the engagement of co-stimulatory molecules.

Regulatory T (T_{Reg}) cells
A rare population of CD4$^+$ T cells that naturally express high levels of CD25 (the interleukin-2 receptor α-chain) and the transcription factor forkhead box P3 (FOXP3), and that have suppressive regulatory activity towards effector T cells and other immune cells. Absence or dysfunction of T_{Reg} cells is associated with severe autoimmunity. In tumours, T_{Reg} cells are induced and proliferate, thereby suppressing anti-tumour immunity.

Plasmacytoid dendritic cells
A subset of dendritic cells (DCs) that are described as plasmacytoid because of their microscopic appearance that resembles plasmablasts. In humans, these DCs can be derived from lineage-negative stem cells in peripheral blood and are the main producers of type-I interferon (IFN) in response to virus infections. Recent studies have identified a subset of type-I IFN-producing DCs in mice, which are characterized by expression of B220 and Ly6C.

Nucleophosmin/anaplastic lymphoma kinase (NPM/ALK) oncoprotein
An oncogenic fusion tyrosine kinase that is associated with a specific type of non-Hodgkin's lymphoma. The translocation between chromosomes 5 and 2 results in fusion of the amino-terminal part of the ubiquitous nucleolar protein NPM to the cytoplasmic fragment of the receptor tyrosine kinase ALK, creating a hybrid tyrosine kinase with constitutive activity.

accompanied by a reduction in the number of tumour-infiltrating T_{Reg} cells[17]. The decrease in the number of tumour-associated T_{Reg} cells was further associated with a proliferation of $CD8^+$ T cells, leading to a robust $CD8^+$ T-cell-dependent anti-tumour immune response[17]. However, ablation of *Stat3* in T cells, especially those in the thymus, is incomplete in the *Mx1–Cre/Stat3*$^{flox/flox}$ mice treated with polyinosinic–polycytidylic acid (poly I:C), which indicates that, at least in this mouse model, STAT3-associated T_{Reg}-cell proliferation in the tumour is 'extrinsic', being mediated by tumour-associated myeloid cells and DCs. Recent follow-up studies have shown that tumour-infiltrating T_{Reg} cells have increased STAT3 activity compared with their spleen-derived counterparts (M.K. and H.Y., unpublished observations). These findings indicate that STAT3 signalling in T_{Reg} cells is locally induced and might be crucial for their proliferation and function in the tumour stroma.

The idea that constitutively activated STAT3 signalling intrinsic to T_{Reg} cells can promote their proliferation in the tumour is supported by several recent independent studies. Forkhead box P3 (FOXP3)-expressing T_{Reg} cells can develop either in the thymus (natural T_{Reg} cells) or from mature $CD4^+$ T cells in the periphery under appropriate stimuli (induced T_{Reg} cells)[64,67,68]. In addition to secreting IL-10, T_{Reg} cells can also secrete TGFβ, thereby suppressing $CD8^+$ T-cell activation directly or indirectly through DCs[59,64,69,70]. Supporting a crucial role for STAT3 in mediating the generation or proliferation of tumour-associated T_{Reg} cells are findings from a study carried out in mice lacking SOCS3, which is an inhibitor of STAT3, in T cells: STAT3 is required for both TGFβ and IL-10 production by $CD4^+$ T cells[71]. Furthermore, STAT3 can bind directly to the promoter of the gene encoding TGFβ, as well as to the promoter of the gene encoding IL-10 in $CD4^+$ T cells. An important role of cell-autonomous STAT3 in mediating T_{Reg}-cell proliferation and activation is also shown by the finding that nucleophosmin/anaplastic lymphoma kinase (NPM/ALK) oncoprotein induces the T_{Reg}-cell phenotype through STAT3 activation[72]. Specifically, T-cell lymphoma cells carrying NPM/ALK oncoprotein express FOXP3, and secrete IL-10 and TGFβ in a STAT3-dependent manner[72]. A possible involvement of STAT3 in the generation of lung T_{Reg} cells has also been shown[73].

Several studies have also indicated a possible role for STAT5, which is frequently activated in several types of cancer[13], in generating T_{Reg} cells: for example, IL-2-induced FOXP3 expression in human T_{Reg} cells is mediated by STAT signalling, including both STAT3 and STAT5 (REF. 74). Low-dose treatment with IL-2 in individuals with chronic myelogenous leukaemia after allogeneic haematopoietic-stem-cell transplantation resulted in an increased frequency of $CD4^+CD25^+$ T cells and increased expression of FOXP3 in T cells through a STAT3- and STAT5-dependent mechanism[74]. Several other studies have also shown that STAT5, often accompanied by STAT3, is activated in

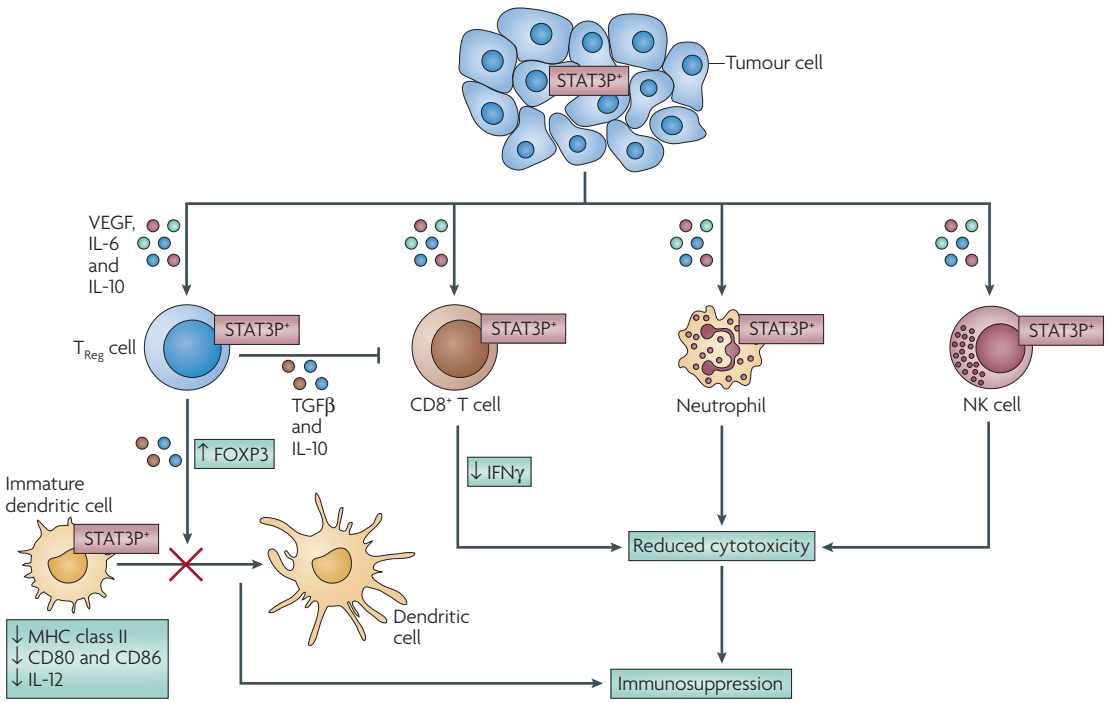

Figure 3 | **STAT3 signalling facilitates communication between tumour cells and diverse immune-cell subsets, including tumour-associated regulatory T cells.** Signal transducer and activator of transcription 3 (STAT3) activity is increased in tumour-associated regulatory T (T_{Reg}) cells. STAT3 signalling in T_{Reg} cells can upregulate the expression of forkhead box P3 (FOXP3), transforming growth factor-β (TGFβ) and interleukin-10 (IL-10), which, in turn, restrain $CD8^+$ effector T cells, as well as dendritic-cell maturation. Natural killer (NK) cells and neutrophils in the tumour stroma also have persistently activated STAT3, which inhibits the tumour-killing activity of both types of effector cell. IFNγ, interferon-γ; STAT3P, tyrosine-phosphorylated (activated) STAT3.

T_{Reg} cells[75–78]. STAT3-promoted production of IL-10 and TGFβ has broad immunological consequences, because these cytokines can inhibit both innate and adaptive immunity. IL-10 and TGFβ secreted by tumour-derived T_{Reg} cells, for example, can inhibit $CD8^+$ T-cell effector function[59,79] and hinder DC functional maturation[61] (FIG. 3).

$CD8^+$ T cells. *Stat3*$^{-/-}$ $CD8^+$ T cells in mice with a *Stat3*-ablated haematopoietic system can produce more antigen-specific IFNγ after vaccination or exposure to a tumour[17]. These data indicate that STAT3 signalling in $CD8^+$ T cells might propagate tumour-associated T_{Reg}-cell function, as recent work showed that IFNγ produced by $CD8^+$ T cells has an important role in abrogating the suppressive effects of antigen-specific T_{Reg} cells[60]. Consistent with a crucial role for STAT3 activation in inducing and sustaining tumour-associated T_{Reg} cells is the observation that IL-10-receptor signalling in $CD8^+$ T cells is crucial for T_{Reg}-cell-mediated suppression[60]. STAT3 activation not only promotes IL-10 production but is required for IL-10-receptor signalling[14,56,71]. These studies also illustrate the intriguing fact that, because growth factors and cytokines that activate STAT3-dependent receptors are also regulated by STAT3, communication between tumour cells and the diverse immune cells in the tumour microenvironment funnels through a single signalling molecule.

STAT3 and immune-mediated tumour production

Recent studies have supported the idea that, in contrast to the surveillance and editing role of the immune system described above, certain inflammatory and immune responses can promote cancer. This concept is highlighted by recent work indicating that the opposing cancer outcomes resulting from IL-12/T_H1 and IL-23/T_H17 (IL-17-producing T helper cell) responses distinguish tumour-inhibiting and tumour-promoting forms of inflammatory response, respectively[80]. Whereas IL-12 expression is associated with T_H1-type inflammation and the infiltration of the tumour by cytotoxic T cells, IL-23 is pro-oncogenic through its upregulation of MMP9 expression, leading to increased tumour angiogenesis but decreased local inflammation and $CD8^+$ T-cell infiltration[80]. STAT3 has been shown to have a role in both inhibiting IL-12 expression and upregulating IL-23 and IL-17 expression[17,81,82]. Preliminary studies support the hypothesis that STAT3 is crucial for IL-23 expression in the tumour milieu (M.K. and H.Y., unpublished observations).

The nuclear factor-κB (NF-κB) signalling pathway is crucial for the initiation and progression of inflammation-induced cancer, as shown by several elegant studies in mouse models[83]. Some inflammatory molecules downstream of NF-κB, such as IL-6 and cyclooxygenase-2 (COX2), are key players in tumour initiation and progression induced by chronic

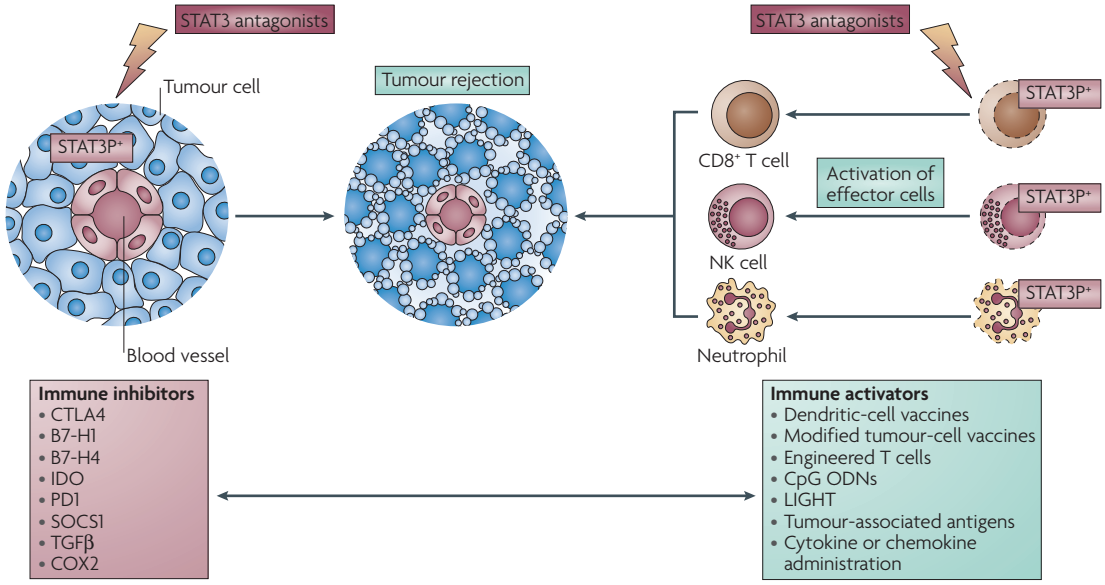

Figure 4 | **Targeting STAT3 for cancer immunotherapy.** Targeting signal transducer and activator of transcription 3 (STAT3) using antagonists can induce tumour-cell apoptosis, inhibit angiogenesis and modify the tumour microenvironment by alleviating the immunosuppressive effects of STAT3. Blocking STAT3 in diverse immune cells can generate potent anti-tumour immunity by decreasing the number of negative immune regulators including immature dendritic cells and regulatory T cells, and by activating effector cells such as $CD8^+$ T cells, natural killer (NK) cells and neutrophils. Optimal anti-tumour immunotherapeutic approaches, however, will probably require combining STAT3 targeting with other promising immunotherapeutic approaches. These include blocking known 'inhibitors' of host immunity, such as those shown in the left-hand box, or using effective immune-activating approaches, including those listed in the right-hand box. COX2, cyclooxygenase-2; CpG ODNs, CpG oligodeoxynucleotides; CTLA4, cytotoxic T-lymphocyte antigen 4; IDO, indoleamine 2,3-dioxygenase; LIGHT, herpesvirus entry mediator ligand; PD1, programmed cell death 1; SOCS1, suppressor of cytokine signalling 1; STAT3P, tyrosine-phosphorylated (activated) STAT3; TGFβ, transforming growth factor-β.

Box 1 | **The multi-faceted role of STAT3 in wound healing and cancer**

Cancers do not create new physiology; rather, they co-opt and dysregulate normal physiological processes. Wound healing is one such process, and cancer draws heavily on its mechanisms. In fact, cancer has been likened to a wound that never completely heals. The fundamental difference between the two is that wound healing is self-limiting, whereas cancer is not[9]. Recent microarray studies showed that signal transducer and activator of transcription 3 (STAT3) regulates numerous genes common to both wound healing and oncogenesis, including those encoding factors that are crucial for growth, angiogenesis, invasion, migration and modulation of coagulation[103]. The capacity of STAT3 to inhibit the expression of pro-inflammatory molecules can also be appreciated in the context of wound healing[104]. A crucial role of STAT3 in both wound healing and transformation was shown by several tissue-specific knockout models: lack of *Stat3* alleles prevents tumour development[105] and impairs wound healing[104]. A recent study involving conditional ablation of *Stat3* or *Socs3* showed that STAT3 has a role in facilitating wound healing mediated by reactive astrocytes after spinal-cord injury, by accelerating the migration of reactive astrocytes to the site of injury and suppressing inflammation[106]. The ability of STAT3 to inhibit inflammation, induce growth, block apoptosis, and stimulate migration, invasion and angiogenesis as shown in cancer, therefore, reflects its role in orchestrating wound healing, an essential physiological process that is extremely complex and highly coordinated.

inflammation[83], and they are also STAT3 activators[22,84]. IL-6, COX2 and MMPs are pro-inflammatory but they are fundamentally different from other immune-stimulating factors downstream of NF-κB that are crucial for tumour inhibition, such as IL-12, CD40, CD80, CD86, CXC-chemokine ligand 10 (CXCL10; also known as IP-10), IFNβ and MHC class II molecules. Although the induced activation of NF-κB and expression of its downstream immune-stimulating genes have a pivotal role in immune-mediated tumour rejection, constitutively active NF-κB in cancer is linked to the upregulation of expression of pro-survival and angiogenic factors, many of which, such as BCL-X_L, survivin, MCL1, VEGF and MMP9, are also regulated by STAT3 (REFS 13,83). Several of the immune-stimulating genes downstream of NF-κB are inhibited by STAT3, including IL-12, TNF, IFNβ, CXCL10, CCL5 (also known as RANTES), CD40, CD80, CD86 and MHC class II molecules[16,17]. These observations indicate a complex relationship between these two pathways such that STAT3 might inhibit the ability of NF-κB to stimulate anti-tumour immunity while acting with NF-κB to promote oncogenic potential.

It is well established that the actions of STAT3 and STAT1 oppose each other, and in cancer cells, whereas STAT3 is constitutively activated, STAT1 is downregulated. In mouse models, Schreiber and colleagues have elegantly shown that both spontaneous and chemically induced cancer is increased in *Stat1*$^{-/-}$ mice[85]. IFNγ–STAT1 signalling has been shown in diverse systems to be crucial for tumour rejection[4]. Systemic treatment of tumour-bearing mice with a small-molecule STAT3 antagonist resulted in the inhibition of phospho-STAT3 in tumour-infiltrating DCs, which, interestingly, coincides with upregulation of phospho-STAT1 in the same DC population[17]. Taken together, these results show that as a key signalling transducer for IL-6, which is not only the activator of IL-23 and T_H17 cells, but also the crucial mediator of NF-κB- and inflammation-associated cancer, STAT3 is probably an important link in immune-system-mediated tumour promotion.

Immune-mediated colitis
An inflammatory disease of the colon most commonly classified as ulcerative colitis or Crohn's disease. Various hereditary and induced mouse models of human colitis have been developed.

Targeting STAT3 for cancer immunotherapy

Recent studies have shown that ablating STAT3 signalling in haematopoietic cells, before or after tumour establishment, can significantly inhibit tumour growth and even cause tumour rejection through immune activation, without affecting STAT3 activity in the tumour cells[17]. Further evidence that inhibiting STAT3 can affect tumour growth without directly inducing tumour-cell death was provided by studies using a small-molecule STAT3 inhibitor and MB49 tumour cells, which are not susceptible to inhibitor-induced direct apoptosis. This work showed that the STAT3 inhibitor induced immune-mediated anti-tumour effects[17]. However, the anti-tumour effects are more marked when the tumour cells are sensitive to direct killing induced by the STAT3 blockade[17], which illustrates the value of approaches targeted at both the tumour and its microenvironment.

The emerging picture of STAT3 signalling in both tumours and tumour-infiltrating immune cells indicates that STAT3 is a promising target for therapeutic intervention (FIG. 4). The ability of STAT3 to regulate many aspects of oncogenesis, including tumour-cell proliferation, survival, migration and angiogenesis, reflects its crucial role in wound healing (BOX 1). Although the connection to wound healing raises concern about targeting STAT3, the fact that *Stat3*$^{-/-}$ bone-marrow cells can reconstitute the haematopoietic system of irradiated recipient mice indicates that STAT3 blockade might not lead to severe toxicity. Furthermore, because tumours can be rejected in animals with haematopoietic *Stat3* knockout, increased STAT3 activity in the tumour itself might not be absolutely required as an indicator of therapeutic susceptibility to STAT3 blockade. Although haematopoietic ablation of *Stat3* eventually led to immune-mediated colitis, as reported previously by others[15,86], this effect was not observed until at least 1 month after *Stat3* ablation[17].

Because STAT3 antagonists — either small-molecule drugs or small interfering RNA (siRNA) — are unlikely to completely block STAT3 signalling as gene ablation does, STAT3 targeting might not result in severe autoimmune manifestations. Consistent with this, partial and intermittent blocking of STAT3 with a small-molecule STAT3 inhibitor did not result in any observable autoimmunity or other immune sequelae[17]. Nonetheless, anti-tumour effects were observed almost immediately after *Stat3* knockout or blockade by the STAT3 inhibitor[17]. These findings indicate that there is a significant therapeutic window between anti-tumour immunity and autoimmunity, which could be exploited for cancer therapy. STAT3 blockade would be expected to enhance both innate and adaptive immune responses to tumour antigens. This pleiotropic effect is a potential consequence of the broad role of STAT3 in organizing the immune microenvironment of the tumour in a manner that promotes tumour growth and inhibits immune surveillance. Therefore, blockade of STAT3 might induce a potent anti-tumour effect on its own.

Although transcription factors such as STATs have traditionally been difficult targets for small-molecule inhibition, several STAT3 inhibitors, both rationally

RNA interference
(RNAi). Double-stranded RNAs (dsRNAs) with sequences that precisely match a given gene are able to 'knock down' the expression of that gene by directing RNA-degrading enzymes to destroy the encoded mRNA transcript. The two most common forms of dsRNAs used for gene silencing are short — usually 21-bp long — small interfering RNAs (siRNAs) or the plasmid-delivered short hairpin RNAs (shRNAs).

designed and screened, have been shown to block STAT3 and to induce tumour-cell apoptosis[87–89]. The finding that CPA7, a platinum-IV compound, can selectively inhibit STAT3 (REF. 89) and induce anti-tumour effects *in vivo* provides an incentive for the active screening of more efficacious STAT3 inhibitors[17]. These inhibitors could function at various levels, ranging from the tyrosine-phosphorylation step of STAT3 activation to DNA binding to association with other transcriptional co-activators. The many levels of potential STAT3 inhibition, together with the availability of a STAT3 crystal structure, indicate that rational design and high-throughput screening for STAT3 inhibitors could be fruitful. The advent of RNA interference (RNAi) technology provides opportunities for STAT3 inhibition at the genetic level. Clearly, efficient, targeted delivery of STAT3 antagonists, including both small-molecule drugs and siRNAs, into tumour or tumour-infiltrating cells *in vivo* will be both a major challenge and an exciting opportunity for advancing cancer therapeutics.

Cancer is a complex disease in which the host immune response against the tumour is profoundly suppressed by many mechanisms and cell types. Although STAT3 is an important molecule in the tumour-associated immunosuppressive network, it is highly desirable to combine STAT3 targeting with other inhibitors of the suppressors of the immune response (FIG. 4). Blocking these suppressors, such as CTLA4 (cytotoxic T-lymphocyte antigen 4), B7-H4, SOCS1 and IDO (indoleamine 2,3-dioxygenase), has shown promising efficacy in either animal models or clinical trials[90–94]. Alternatively, STAT3 blockade might synergize with other promising immunotherapeutic agents such as cancer vaccines and systemic agents that promote Toll-like receptor (TLR)-dependent activation of innate immune components and DCs (such as CpG oligodeoxynucleotides)[6,95–97] (FIG. 4). For example, blocking IL-10-receptor signalling, which probably involves inhibition of STAT3 activity, can markedly enhance CpG-oligodeoxynucleotide-induced anti-tumour immunity[98]. That targeting STAT3 signalling can enhance anti-tumour immune responses was also shown by a recent study in which inhibiting the JAK–STAT3 pathway led to better therapeutic outcomes of a DC-based vaccine in mouse models[99].

Concluding remarks

Although the importance of the effects of cancer on the immune system has long been recognized, the molecular basis for the crosstalk between tumour and immune cells remains largely unknown. Recent studies have identified STAT3, an important oncogenic signalling molecule, as mediating this bidirectional communication. In cancer cells, STAT3 is frequently activated, which promotes the expression of factors that are both immunosuppressive and STAT3 activating, including VEGF and IL-10. These tumour-derived factors, in turn, upregulate STAT3 signalling in various immune-cell subsets in the tumour microenvironment, which produce more immunosuppressive factors, including IL-6, IL-10, TGFβ, FOXP3 and VEGF (REFS 19,72,74; M.K. and H.Y., unpublished observations), thereby generating tolerogenic DCs and T_{Reg} cells, and abrogating the function of various immune effector cells. Many of these factors are also growth and angiogenic factors, which directly stimulate tumour-cell proliferation and survival, and indirectly promote tumour progression through angiogenesis and metastasis. The constitutive activation of STAT3 both in tumour cells and in diverse immune cells in the tumour stroma also inhibits the expression of numerous factors and molecules necessary for immune-mediated tumour rejection. These include CCL5, IL-12, TNF, IFNγ, IFNβ, CXCL10, CD40, CD80, CD86 and MHC class II molecules[14,16–18,57,61,81,100]. The list of STAT3-regulated immunosuppressive and immune-stimulating molecules continues to grow.

The ability of STAT3 to broadly and profoundly affect tumour immunity strongly indicates that constitutively activated STAT3 both in tumour cells and in tumour stromal immune cells is an attractive target for cancer immunotherapy. Another unique and appealing aspect of targeting STAT3 for cancer immunotherapy is due to the crucial role of STAT3 in tumour-cell survival and tumour angiogenesis[13,37,47]. Many experiments have shown that tumour rejection mediated by $CD8^+$ T cells is always preceded by the inhibition of tumour-induced angiogenesis[101]. Because targeting STAT3 is expected to decrease the survival and angiogenic potential both of tumour cells and of the tumour stroma, targeting STAT3 could facilitate immune-cell-mediated anti-tumour effects at several levels. Although STAT3 is the first oncogenic target for cancer immunotherapy, other important oncoproteins, such as MAPKs, might have similar roles[19,102]. With the emergence of targeted delivery systems, and small-molecule inhibitors or RNAi technology to block STAT3 and other relevant oncogenic pathways, a new era of molecular targeting for cancer immunotherapy is on the horizon.

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Competing interests statement

The authors declare no competing financial interests.

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