



# Roles of STAT3 in mediating the cell growth, differentiation and survival signals relayed through the IL-6 family of cytokine receptors

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Members of the IL-6 cytokine family are involved in a variety of biological responses, including the immune response, inflammation, hematopoiesis, and oncogenesis by regulating cell growth, survival, and differentiation. These cytokines use gp130 as a common receptor subunit. The binding of ligand to gp130 activates the JAK/STAT signal transduction pathway, where STAT3 plays a central role in transmitting the signals from the membrane to the nucleus. STAT3 is essential for gp130-mediated cell survival and G1 to S cell-cycle-transition signals. Both *c-myc* and *pim* have been identified as target genes of STAT3 and together can compensate for STAT3 in cell survival and cell-cycle transition. STAT3 is also required for gp130-mediated maintenance of the pluripotential state of proliferating embryonic stem cells and for the gp130-induced macrophage differentiation of M1 cells. Furthermore, STAT3 regulates cell movement, such as leukocyte, epidermal cell, and keratinocyte migration. STAT3 also appears to regulate B cell differentiation into antibody-forming plasma cells. Since the IL-6/gp130/STAT3 signaling pathway is involved in both B cell growth and differentiation into plasma cells it is likely to play a central role in the generation of plasma cell neoplasias. *Oncogene* (2000) 19, 2548–2556.

**Keywords:** IL-6; gp130; STAT3; *pim*; plasmacytoma; Myc

## Introduction

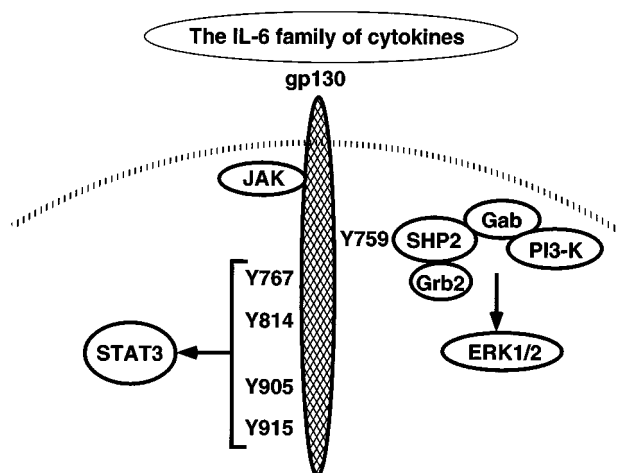
Cytokines and growth factors are pleiotropic in their biological activities and play pivotal roles in a variety of responses, including the immune response, hematopoiesis, neurogenesis, embryogenesis, and oncogenesis (Heldin, 1995; Hirano, 1998; Hunter, 1997; Massague, 1996; Metcalf, 1989; Paul and Seder, 1994; Schlessinger and Bar-Sagi, 1994; Thomson, 1998). For example, interleukin-6 (IL-6), which was identified as a B cell differentiation-inducing factor (Hirano *et al.*, 1986; Teranishi *et al.*, 1982) and as a hybridoma-plasmacytoma growth factor (Van Damme *et al.*, 1987; Van Snick *et al.*, 1986), is a typical cytokine that effects a variety of biological functions, including immunoglobulin production, the acute phase reaction, inflammation, and plasmacytomagenesis, by regulating cell growth, differentiation, and survival (Heinrich *et al.*, 1990; Hirano, 1998; Hirano and Kishimoto, 1990; Sehgal *et al.*, 1989; Van Snick, 1990). Cytokines and growth factors exert their effects through specific receptors. Various signal transduction pathways are activated through distinct

regions of each receptor's cytoplasmic domain. Such pathways include those mediated by the Src and JAK (Janus kinase) tyrosine kinase families, STAT (signal transducer and activator of transcription), Smad, MAPK, and PI3-K (Phosphatidylinositol 3-kinase) (Birge *et al.*, 1996; Darnell, 1997; Heldin, 1995; Heldin *et al.*, 1997; Hirano *et al.*, 1997; Hunter, 1997; Ihle, 1996; Massague, 1996; Pawson and Scott, 1997; Schlessinger and Bar-Sagi, 1994; Taniguchi, 1995). Among these signaling molecules, STAT proteins play a central role in transmitting cytokine signals (Darnell, 1997; Darnell *et al.*, 1994). In this review, we describe how STAT3 is involved in the regulation of cell growth, differentiation, and survival signals through gp130, as a general mechanism of cytokine action. Further, we will discuss the role of the IL-6/gp130/STAT3 signaling pathway in the generation of plasma cell neoplasias.

*Gp130 is a common subunit of the receptor complexes for the IL-6 family of cytokines*

Gp130 is a common subunit of the receptor complexes for the IL-6 family of cytokines, which includes leukemia inhibitory factor (LIF), ciliary neurotrophic factor (CNTF), oncostatin M (OSM), IL-11, and cardiotrophin-1 (CT-1) (Hibi *et al.*, 1990; Hirano *et al.*, 1997). Studies using transgenic mice expressing the genes encoding IL-6-family cytokines or with targeted disruption of these or the gp130 gene have revealed that gp130-mediated signals are involved in the immune, hematopoietic, nervous, cardiovascular, and endocrine systems, as well as in bone metabolism, inflammation, the acute phase response, plasmacytomagenesis, osteoporosis, liver regeneration, and hepatocyte maturation (Betz *et al.*, 1998; Cressman *et al.*, 1996; Escary *et al.*, 1993; Hilbert *et al.*, 1995a; Hirota *et al.*, 1999; Kamiya *et al.*, 1999; Kopf *et al.*, 1994, 1998; Kumanogoh *et al.*, 1997; Masu *et al.*, 1993; Ohtani *et al.*, 2000; Poli *et al.*, 1994; Ramsay *et al.*, 1994; Romani *et al.*, 1996; Suematsu *et al.*, 1989, 1992; Yoshida *et al.*, 1996) (also see reviews Heinrich *et al.*, 1998; Hirano, 1998). Binding of the IL-6-family cytokines to their receptors leads to the homodimerization of gp130 or heterodimerization of gp130 with other gp130-related receptors (LIF receptor  $\beta$ , OSM receptor  $\alpha$ , and CT-1 receptor  $\alpha$ ), which results in the activation of the gp130-associated Janus kinases (JAK1, JAK2, and TYK2) (Lutticken *et al.*, 1994; Matsuda *et al.*, 1994; Stahl *et al.*, 1994). Subsequently, gp130 is phosphorylated on tyrosine, and the phosphorylated molecule recruits signal-transducing molecules such as SHP2 (protein tyrosine phosphatase 2) and STAT3 (Figure 1) (Akira *et al.*, 1994; Fukada *et al.*, 1996; Stahl *et al.*, 1995; Yamanaka *et al.*, 1996)

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**Figure 1** gp130 generates two major signaling pathways, involving STAT3 and SHP2, through distinct tyrosine residues. Upon the stimulation of gp130, tyrosines (Y) 759, Y767, Y814, Y905, and Y915 of (human) gp130 are phosphorylated by JAK, which is associated with gp130. Y759 is a binding site for SHP2 and required for ERK MAP kinase activation. Y767, Y814, Y905, Y915 have a glutamine at position +3 of tyrosine (YXXQ motif) and are required for STAT3 activation

(also see review Hirano *et al.*, 1997). gp130 contains six tyrosines in its cytoplasmic region (Hibi *et al.*, 1990). Tyrosine 759 (of human gp130) and any one of four other tyrosines in the carboxyl-terminus (Y767, Y814, Y905, and Y915), all of which have a glutamine at position +3 of the tyrosine (YXXQ motif), are required for the tyrosine phosphorylation of SHP2 and STAT3, respectively. Mutating tyrosine 759 reduces the interaction between SHP2 and Grb2 and between SHP2 and Gab1, and lowers the gp130-induced activation of the ERK MAP kinases (Fukada *et al.*, 1996; Nishida *et al.*, 1999; Takahashi-Tezuka *et al.*, 1998), suggesting that SHP2 mediates signals to the ERK MAP kinases through Grb2 and Gab proteins. The essential roles of tyrosine 759 of gp130 and Gab1 for gp130-mediated ERK MAP kinase activation was determined using knock-in mice expressing gp130 mutated at tyrosine 759 and Gab1-deficient mice, respectively (Itoh *et al.*, submitted; Ohtani *et al.*, 2000). In contrast, mutating tyrosine 759 or over-expressing a SHP2 mutant with an inactive catalytic domain enhances the STAT3-mediated biological actions in hepatocytes and neuroblastoma cells (Kim *et al.*, 1998; Symes *et al.*, 1997), suggesting a role for SHP2 in attenuating gp130-mediated signals. This negative regulatory role of the SHP2 signal was shown using knock-in mice expressing gp130 mutated at tyrosine 759, which showed prolonged STAT3 activation and enhanced immunoglobulin (Ig) production and acute phase protein synthesis (Ohtani *et al.*, 2000). After tyrosine phosphorylation, STAT3 forms a homodimer or a heterodimer with STAT1 and enters the nucleus, where it regulates the expression of a specific set of genes (Darnell, 1997; Darnell *et al.*, 1994; Ihle, 1996; Ihle and Kerr, 1995; Hirano *et al.*, 1997).

#### IL-6 and STAT3 in plasmacytomagenesis

A factor that could induce Ig production in Epstein Barr virus (EBV)-transformed B lymphoblastoid cell

lines was identified in 1982, and found to have a molecular weight of 22 kDa and an isoelectric point of 5–6 (Teranishi *et al.*, 1982). This factor was further purified and characterized (Hirano *et al.*, 1984, 1985), and finally cloned (Hirano *et al.*, 1986). The sequence revealed this factor to be identical to others that were known by a variety of names, including plasmacytoma-hybridoma growth factor (Astaldi *et al.*, 1980; Haegeman *et al.*, 1986; Kawano *et al.*, 1988; Nordan *et al.*, 1987; Van Damme *et al.*, 1987; Van Snick *et al.*, 1986; Zilberstein *et al.*, 1986) and it was proposed to rename it interleukin-6 (Hirano, 1998; Hirano and Kishimoto, 1990; Marx, 1988; Sehgal *et al.*, 1989).

In discussing the roles of IL-6 and STAT3 in plasmacytomagenesis, we would like to emphasize two observations that implicate them strongly. First, IL-6 was identified as a factor that could induce the differentiation of B cells into antibody-forming plasma cells, as described above (Hirano, 1998). Second, plasma cell tumors can be induced by mineral oil or pristane, which induce the formation of chronic granulomatous tissue, leading to plasmacytosis and eventually to plasma cell neoplasias (see review by Potter *et al.*, 1995). Pristane can induce plasmacytoma growth factor (Nordan and Potter, 1986) which is identical with IL-6. Overexpression of IL-6 in transgenic mice induces polyclonal plasmacytosis (Suematsu *et al.*, 1989), and – depending on the genetic background of the mice – plasmacytoma develops eventually (Suematsu *et al.*, 1992). These observations suggested a critical role for IL-6 in plasmacytomagenesis (Hirano, 1991). Consistent with this, it was found that IL-6 is essential for raf/myc plasma cell tumor induction (Hilbert *et al.*, 1995a). Although the development of murine plasma cell tumors induced by raf/myc-containing retroviruses is completely dependent on IL-6, an abl/myc-containing retrovirus induces plasmacytoma independently of it. An interesting finding is that STAT3 is inducible by IL-6 in raf/myc tumors but constitutively activated in abl/myc tumors (Hilbert *et al.*, 1996), suggesting an intimate relationship among IL-6 signals, STAT3, and plasmacytomagenesis, as summarized in the Concluding remarks.

#### STAT3 in cell growth and survival

IL-6 can activate both the ERK1/2 MAP kinase pathway and STAT3 (Figure 1) (Daeipour *et al.*, 1993; Fukuda *et al.*, 1996; Giordano *et al.*, 1997; Ogata *et al.*, 1997; Ohtani *et al.*, 2000) (reviewed in Hirano *et al.*, 1997), but the molecular basis for IL-6-induced cell growth was not known until 1996. The first clue was the observation by Fukuda and his colleagues that STAT3 activation is essential for gp130-induced proliferation of the IL-3-dependent pro-B cell line, BAF/B03 cells (Fukuda *et al.*, 1996). Tyrosine 759 of human gp130, which is required for the tyrosine phosphorylation of SHP2 and activation of ERK1/2 MAP kinase, is essential for the S to G2/M cell cycle progression, but not for preventing apoptosis. On the other hand, the tyrosines in the YXXQ motifs, which are essential for gp130-mediated STAT3 activation, are also required for *bcl-2* induction and cell survival signals. Furthermore, dominant-negative STAT3 inhibits gp130-mediated anti-apoptosis, indicating that at

least two distinct signaling pathways, one for cell cycle progression and one for cell survival, are required for gp130-induced cell growth, and that STAT3 is involved in cell survival (Fukada *et al.*, 1996).

STAT3 is also shown to be required for the Bcl-2-independent survival of T cells in the presence of IL-6 (Takeda *et al.*, 1998). Furthermore, the STAT3 pathway is partially involved in the IL-6-mediated prevention of apoptosis induced by TGF- $\beta$ . In this case, the concomitant activation of the PI3-kinase and Akt pathways is likely to play a major role (Chen *et al.*, 1999). An anti-apoptotic role for STAT3 *in vivo* was first demonstrated using mammary gland epithelial cells from a mouse with a conditional knockout of *stat3*. In this case, the expression of STAT3 had no effect on the expression of *Bcl-x<sub>L</sub>* or *Bax*. Rather, STAT3's anti-apoptotic effect appears to be mediated through the down-regulation of IGFBP-5, which is thought to induce apoptosis by sequestering a growth factor, IGF-1. Therefore, in this case the anti-apoptotic effect of STAT3 is non-cell autonomously mediated through the suppression of a survival factor inhibitor (Chapman *et al.*, 1999). Interestingly, STAT3 is constitutively activated in bone marrow mononuclear cells from patients with multiple myeloma, and in the IL-6-dependent human myeloma cell line, U266. Moreover, U266 cells are inherently resistant to Fas-mediated apoptosis and express high levels of the antiapoptotic protein *Bcl-x<sub>L</sub>* (Catlett-Falcone *et al.*, 1999). Unlike in the case described above, in these cells, inhibition of STAT3 activation inhibits *Bcl-x<sub>L</sub>* expression and induces apoptosis, demonstrating that STAT3 signaling is essential for the survival of myeloma tumor cells and contributes to the pathogenesis of multiple myeloma. All of these results support the idea that STAT3 plays critical roles in gp130-mediated cell proliferation by inhibiting apoptosis in a variety of cells, and of particular interest, those of the B cell lineage and plasmacytoma/myeloma cells.

The involvement of other STAT proteins in cell growth has also been reported. STAT5 is involved in IL-3-mediated cell proliferation in an IL-3-dependent cell line (Matsumura *et al.*, 1999; Mui *et al.*, 1996). Furthermore, *cyclinD1* has been identified as a direct target gene of STAT5 (Matsumura *et al.*, 1999). Targeted disruption of the *stat5a* and *stat5b* genes in mice revealed that STAT5 is needed for IL-2-mediated T cell proliferation and for erythropoietin-mediated anti-apoptosis, in red blood cell progenitors (Moriggl *et al.*, 1999; Socolovsky *et al.*, 1999). Similarly, targeted disruption of the *stat4* and *stat6* genes showed that STAT4 and STAT6 are required for IL-12- and IL-4-dependent lymphocyte proliferation, respectively (Akira, 1999; Kaplan *et al.*, 1996; Takeda *et al.*, 1996; Thierfelder *et al.*, 1996).

The involvement of STAT proteins in cell growth is further supported by the observation that STATs are constitutively activated in cells transformed with Human T cell leukemia virus I (HTLV-I), *v-src*, *abl*, *bcrl-1*, and *v-eyk*, and in some multiple myeloma cells (Besser *et al.*, 1999; Carlesso *et al.*, 1996; Danial *et al.*, 1995; Hilbert *et al.*, 1996; Migone *et al.*, 1995; Turkson *et al.*, 1998; Yu *et al.*, 1995). STAT3 activation is required for transformation with *v-src* (Bromberg *et al.*, 1998; Turkson *et al.*, 1998) and is also implicated in *v-abl*-induced plasmacytomagenesis (Danial *et al.*,

1998; Hilbert *et al.*, 1996). A constitutively active mutant of *Stat3* in immortalized fibroblasts causes cellular transformation, detected by colony formation in soft agar and tumor formation in nude mice, thus acting as an oncogene (Bromberg *et al.*, 1999).

The molecular mechanisms underlying STAT3's involvement in cell cycle progression were demonstrated using an IL-3-dependent pro-B cell line, BAF/B03 (Fukada *et al.*, 1998). In these cells, in the absence of STAT3 activation, gp130 stimulation fails to induce *bcl-2* and cannot prevent growth factor-deprivation-induced apoptosis (Fukada *et al.*, 1996). Fukada and his colleagues made stable transfectants expressing Bcl-2, which could survive upon growth factor deprivation without the activation of STAT3. However, under these conditions, STAT3 activation was required for the gp130-mediated G1 to S phase cell-cycle transition, indicating its importance not only for cell survival but also for cell cycle progression. Furthermore, STAT3 activation is essential for the up-regulation of cyclins D2, D3, and A, and *cdc25A*, and the concomitant down-regulation of p21 and p27 in BAF/B03 cells (Fukada *et al.*, 1998). The requirement for STAT proteins in the down-regulation of p27 has also been demonstrated in *stat6*-deficient lymphocytes in response to IL-4 stimulation, where the down-regulation of p27 seems to depend on the degradation of proteins rather than on altered mRNA expression (Kaplan *et al.*, 1998).

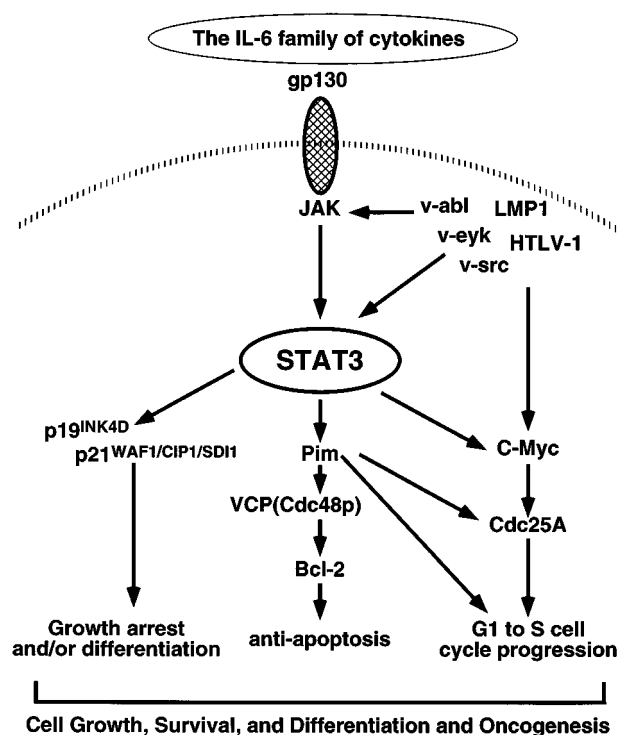
An important issue that remains to be resolved is the identification of the direct target molecules of STAT3 in cell cycle progression. One of the most likely targets is the *c-myc* gene. *c-Myc* is a critical regulator of cell growth, especially for the cell-cycle transition from the G1 to S phase (Henriksson and Luscher, 1996) and for the induction of *cdc25A* (Galaktionov *et al.*, 1996). The *c-myc* gene is induced in response to the proliferative signals elicited by extracellular stimuli, including IL-6. The non-receptor tyrosine kinases *c-src* (Barone and Courtneidge, 1995), *syk* (Minami *et al.*, 1995), and *Jak* (Kawahara *et al.*, 1995; Sakamaki *et al.*, 1992; Watanabe *et al.*, 1996), and the oncogenes, *v-src*, *v-abl*, *bcrl-1*, and *v-akt* all induce *c-myc* mRNA expression (Cleveland *et al.*, 1989; Kuchino *et al.*, 1985; Sovova *et al.*, 1993; Stewart *et al.*, 1995). Kiuchi *et al.* (1999) showed that STAT3 mediates the induction of the *c-myc* gene upon gp130 stimulation. STAT3 binds to a region that overlaps the E2F site in the *c-myc* gene P2 promoter. STAT3 binding to this site is essential for the transcription of *c-myc* in response to IL-6 or gp130 signals. In contrast, STAT5 does not bind to this site, consistent with the fact that induction of *c-myc* by IL-3 is independent of STAT5 (Mui *et al.*, 1996). At the moment, it remains to be resolved whether STAT3 plays any role in *c-myc* gene activation induced by other molecules, such as *v-src*. These findings strongly suggest that *c-myc* is the gene responsible for the G1 to S cell-cycle progression signals that are elicited by STAT3 that has been activated by gp130. However, *c-Myc* alone is not sufficient to compensate for the loss of STAT3 in this process. Stable expression of the *c-myc* gene enhances apoptosis after the deprivation of growth factor, even in the presence of signals activated through a gp130 mutant that is defective in its ability to activate STAT3 (Shirogane *et al.*, 1999), consistent with a role for *c-Myc* in apoptosis (Askew *et al.*, 1991;

Evan *et al.*, 1992). This result indicates that there must be additional genes that are targets of STAT3 in cell cycle progression. Shirogane *et al.* (1999) identified the proto-oncogenes *Pim-1* and *Pim-2* as targets for the gp130-mediated STAT3 signal (Figure 2). In fact, expression of a *Pim-1* mutant with a defective kinase domain attenuates gp130-mediated cell proliferation. However, the expression of *Pim-1* is not sufficient to compensate for the loss of gp130-mediated STAT3 signal in cell-cycle progression. Only the constitutive expression of *Pim-1* together with *c-myc* fully compensates for the lack of STAT3 in cell-cycle progression, although additional signals generated through gp130 are still required for *Pim-1/c-Myc* induction of cell growth. *Cdc25A*, one of the direct target genes of *c-Myc*, regulates the activity of cyclin-dependent kinases (CDKs) through the dephosphorylation of threonine and tyrosine residues (Galaktionov *et al.*, 1996). Since *Pim-1* phosphorylates *Cdc25A* and activates its phosphatase activity (Mochizuki *et al.*, 1999), *Pim-1* may control the G1 to S transition together with *c-Myc* by activating *Cdc25A*. Furthermore, when a gp130 mutant that is defective in its ability to activate STAT3 is used, *Pim-1* induces *bcl-2* expression and inhibits *c-Myc*-induced apoptosis in a manner that is dependent on gp130-induced signals.

Since *Pim-1* is a serine/threonine kinase, it is likely to act by modifying other signaling molecules or proteins that induce other genes. VCP (valosine containing protein), a mammalian homolog of *Saccharomyces*

*cervisiae* *Cdc48p*, has been identified as one of the target genes for *Pim-1* (Shirogane *et al.*, 1999). The VCP gene encodes an ATPase that belongs to the AAA superfamily (ATPases associated with a variety of cellular activities) (Frohlich *et al.*, 1995), which includes yeast *Cdc48p* and *C. elegans* MAC-1. These ATPases have been suggested to be involved in cell survival, cell-cycle progression, and protein degradation (Dai *et al.*, 1998; Frohlich *et al.*, 1991; Ghislain *et al.*, 1996; Koegl *et al.*, 1999; Madeo *et al.*, 1997; Wu *et al.*, 1999). Forced expression of VCP, or VCP and *c-Myc*, partially rescues the proliferation and anti-apoptosis signals in cells expressing a gp130 mutant that is defective in its ability to mediate STAT3/*Pim-1* signals. Furthermore, expression of a dominant-negative mutant form of VCP inhibits gp130-mediated anti-apoptotic signals, including the induction of *bcl-2*. These data indicate that VCP participates in the STAT3/*Pim-1*-mediated anti-apoptosis function, and is likely to play a role in cell-cycle progression as well.

*Pim-1* is a proto-oncogene that was first identified as a common insertion site in Molony murine leukemia virus (MuLV)-induced T cell lymphomas (Cuypers *et al.*, 1984). *Eμ-Pim-1* transgenic mice are highly susceptible to developing T cell lymphoma after MuLV infection or exposure to a chemical carcinogen, and *c-myc* or *N-myc* genes are activated in the resulting lymphomas (Breuer *et al.*, 1989; van Lohuizen *et al.*, 1989). *Eμ-myc* transgenic mice can also develop B cell lymphomas when *Pim-1* has been activated (van Lohuizen *et al.*, 1991). A variety of cytokines, such as GM-CSF, G-CSF, IL-3, interferon- $\alpha$ , and IL-6 induce the *Pim-1* gene (Jaster *et al.*, 1999; Lilly *et al.*, 1992; Matikainen *et al.*, 1999; Sato *et al.*, 1993). A homolog of *Pim-1*, *Pim-2*, has also been implicated in lymphomagenesis and its expression is regulated by a set of cytokines that is similar to those that induce *Pim-1* (Allen *et al.*, 1997; van der Lugt *et al.*, 1995). Studies by Shirogane *et al.* (1999) indicate that the *Pim* family of proteins plays crucial roles in gp130-mediated cell proliferation. These studies also provide insight into the molecular basis for the synergy between the *Pim* and *c-Myc* activities in cell proliferation and lymphomagenesis, and show a link between STAT3 and cell growth machinery (Figure 2).



**Figure 2** Central roles of STAT3 in gp130-mediated cell growth, survival, and differentiation. STAT3 regulates G1 to S cell-cycle progression as well as the prevention of apoptosis through *c-Myc*, *Pim*-family proteins, VCP, and *Bcl*-family proteins. STAT3 is activated by a variety of oncogenes as well. STAT3 also induces growth arrest and cell differentiation; it is implicated in B cell differentiation into plasma cells. Thus, the IL-6/gp130/STAT3 signal pathway plays crucial roles in both B cell growth and differentiation and in the generation of plasma cell neoplasias (see text for details)

### STAT3 and differentiation

The IL-6 family of cytokines regulates cell differentiation as well as cell growth. For example, they induce the differentiation of B cells into antibody-forming plasma cells and the differentiation into macrophages of the myeloid leukemic cell line, M1. Much evidence supports a central role for STAT3 in these processes. Yamanaka and his colleagues (Yamanaka *et al.*, 1996) first showed that the tyrosine residues of gp130 that are essential for STAT3 activation (the YXXQ motifs) are also required for the gp130-mediated macrophage differentiation and the growth arrest of M1 cells. Furthermore, Nakajima *et al.* (1996) showed that dominant-negative forms of STAT3 inhibit both IL-6-induced growth arrest at G1 and macrophage differentiation in M1 cells. Blocking STAT3 activation inhibits the IL-6-induced repression of *c-myb* and *c-myc*. The same results were reported by Minami *et al.* (1996). A role for STAT3 in G-CSF-induced granulo-

cyte differentiation has also been reported (Shimozaki *et al.*, 1997; Ward *et al.*, 1999). Taken together, these findings demonstrate that STAT3 activation is involved in both cell differentiation and growth arrest.

STAT3 has also been shown to be required for CNTF- and OSM-induced astrocyte differentiation *in vitro* (Bonni *et al.*, 1997; Yanagisawa *et al.*, 1999). The number of GFAP-positive astrocytes is reduced in the brains of gp130-deficient mice (Nakashima *et al.*, 1999a), suggesting a role for STAT3 in astrocyte differentiation *in vivo*. Furthermore, *in vitro* GFAP-positive astrocyte differentiation in response to gp130 stimulation is reduced in fetal brain cells obtained from knock-in mice expressing a mutant gp130 that is defective in its ability to activate STAT3 (Ohtani *et al.*, 2000). Taken together, these data support a role for STAT3 in astrocyte differentiation.

STAT3 is also implicated in B cell differentiation. CD40 is a receptor that is critical for the survival, growth, differentiation, and isotype switching of B lymphocytes. CD40 stimulation induces the activation of JAK3 and STAT3. JAK3 is constitutively associated with CD40, and deletion of the CD40 sequence required for JAK3 binding abolishes the CD40-mediated functions in B cells (Hanissian and Geha, 1997). The missing CD40 signals can be partially compensated for by the EBV latent membrane protein 1 (LMP1), a protein that induces the activation and differentiation of normal B cells (Uchida *et al.*, 1999). LMP1 interacts with JAK3 and activates STAT3 (Gires *et al.*, 1999). These facts indicate that STAT3 is involved in B cell differentiation.

IL-6 is also involved in B cell differentiation (Hirano, 1998). IL-6 transgenic mice exhibit plasmacytosis (Suematsu *et al.*, 1989), while IL-6-deficient mice have defective antibody production (Kopf *et al.*, 1994). The molecular mechanisms through which IL-6 acts on B cells are currently being elucidated. Ohtani *et al.* (2000) have recently generated a series of knock-in mouse lines expressing mutated gp130 in which gp130-dependent STAT3 and/or SHP2 signals are specifically disrupted. IgG2a and IgG2b production increase in mice that express a gp130 mutant that is defective in SHP2 signals, where gp130-mediated STAT3 activation is prolonged; IgG2a and IgG2b production decrease in B cells expressing the mutant gp130 that is defective in STAT3 activation. Furthermore, B cell differentiation into antibody-forming cells in response to the stimulation of both CD40 and gp130 is enhanced in the B cells that are deficient in SHP2 signals, and diminished in the B cells that are deficient in STAT3 signals. All of these results indicate the involvement of STAT3 in gp130-mediated B cell differentiation and a negative role for SHP2 in STAT3 activation through gp130 *in vivo*. STAT3 has also been shown to be constitutively activated in B-1 cells (Karras *et al.*, 1997). Consistent with this observation, the B-1 cell population is decreased in knock-in mice expressing the gp130 mutant that is defective in activating STAT3 (Ohtani *et al.*, 2000). STAT1 and STAT3 activation by BCR stimulation in B cells has also been reported (Karras *et al.*, 1997; Su *et al.*, 1999).

The molecular mechanisms by which STAT3 induces cell differentiation and growth arrest are not well established. However, the cyclin-dependent kinase inhibitors p19<sup>INK4D</sup> and p21<sup>WAF1/CIP1/SDI1</sup> are known to

be involved. IL-6 induces p19<sup>INK4D</sup> mRNA and protein expression without *de novo* protein synthesis in M1 cells in a manner that is dependent on STAT3 activation (Narimatsu *et al.*, 1997). Furthermore, p21 is induced in the osteoblast-like human osteosarcoma cell line MG63, which differentiates upon stimulation with oncostatin M (OSM) or upon gp130 stimulation with IL-6 plus soluble IL-6 receptor (Bellido *et al.*, 1998). This induction is dependent on STAT3 activation. Antisense oligonucleotides that are complementary to p21 mRNA inhibit OSM-induced stimulation of alkaline phosphatase expression and antagonize the protective effect of OSM on anti-Fas-induced apoptosis. These results demonstrate that p21 is a downstream effector of gp130/STAT3 activation and a critical mediator of the pro-differentiation and anti-apoptotic effects of IL-6-type cytokines on human osteoblastic cells (Bellido *et al.*, 1998). The involvement of p21 in IFN-gamma-induced growth inhibition and an essential role for STAT1 have also been reported (Chin *et al.*, 1996).

In certain cases, STAT3 inhibits cell differentiation. IL-6 induces neurite outgrowth in PC12 cells pretreated with nerve growth factor (NGF). The MAP kinase cascade is essential for the differentiation of PC12 cells (Ihara *et al.*, 1997; Marshall, 1995). Interestingly, stimulation of PC12 cells expressing the gp130 that is defective in its ability to activate STAT3 results in neurite outgrowth without NGF pretreatment. The activity of a dominant-negative STAT3 mimics NGF pretreatment, and NGF suppresses the IL-6-induced activation of STAT3, suggesting that STAT3 might negatively regulate the differentiation of PC12 cells (Ihara *et al.*, 1997). Another case where STAT3 inhibits cell differentiation is in mouse ES cells. The propagation of ES cells in an undifferentiated pluripotent state is dependent on LIF or related cytokines that can stimulate gp130. Boeuf *et al.* (1997) examined the signals induced by LIF in ES cells and found that overexpression of dominant-negative STAT3 abrogates the LIF-regulated transcriptional events. Furthermore, stable expression of dominant-negative STAT3 induces the morphological differentiation of ES cells even in the continuous presence of LIF. These results suggest that STAT3 is a critical component of the LIF signaling pathway, which maintains pluripotent cell proliferation. This process was further studied by Niwa *et al.* (1998), who found that expression of dominant-negative STAT3 in ES cells specifically abrogates LIF-mediated self-renewal and promotes differentiation. They showed that STAT3 plays a central role in the maintenance of the pluripotent stem cell phenotype. Furthermore, studies using an inducible active STAT3 construct showed that STAT3 activation is necessary and sufficient to maintain the undifferentiated state of ES cells (Matsuda *et al.*, 1999). STAT3 activation through the LIF receptor is also required for the LIF-dependent inhibition of ES cell differentiation (Ernst *et al.*, 1999).

#### *STAT3 and cell movement*

IL-6-deficient mice show impaired leukocyte accumulation in their subcutaneous air pouches. The leukocytes of IL-6-<sup>-/-</sup> mice show a normal migratory capability, but the chemokine production by endothelial

cells (ECs) is reduced. Actually, ECs produce chemokines upon stimulation with gp130, indicating that a gp130-mediated signal transduction pathway plays a positive role in local inflammatory reactions by amplifying leukocyte recruitment (Romano *et al.*, 1997). Epidermal cells and keratinocytes that are defective in *stat3* gene expression show impaired growth factor-dependent *in vitro* migration, indicating that STAT3 is required for cell migration in a cell-autonomous manner (Sano *et al.*, 1999). STAT3 also plays a role in the cell movement associated with gastrulation (Yamashita *et al.*, manuscript in preparation), a key step in early embryogenesis involving morphogenetic changes and the specification of cell fates. Consistent with this observation, *Stat3*-deficient mice die around E6-8, when gastrulation takes place (Takeda *et al.*, 1997).

### Concluding remarks

It is now well established that STAT3 is critically involved in cell survival and G1 to S cell-cycle progression induced by the IL-6 family of cytokines and a variety of oncogenes, such as *v-abl* and *v-src* (Figure 2). Pim-1 and c-Myc have been identified as target genes of STAT3 in cell-cycle progression. *Cyclin D2*, *cyclin D3*, and *cyclin A* have also been suggested as downstream targets of STAT3, similar to the role of *cyclinD1* as a target of STAT5. STAT3 plays a major role in gp130-mediated *c-myc* gene induction. On the other hand, STAT3 is involved in growth arrest and differentiation signals, and in certain cases STAT3 mediates apoptotic signals. STAT3 up-regulates p21 to induce cell differentiation (Bellido *et al.*, 1998), yet inhibits p21 induction to exert cell growth signals (Fukada *et al.*, 1998) in different target cells. Furthermore, STAT3 is required to keep ES cells in an undifferentiated pluripotent state in LIF-mediated growth. Thus, numerous observations suggest dual functions for STAT3 in several processes. A variety of other signals, such as other transcription factors, co-activators, and repressors (Chung *et al.*, 1997; Kojima *et al.*, 1996; Murakami-Mori *et al.*, 1997; Nakashima *et al.*, 1999b; Zhang *et al.*, 1997, 1999), which are expressed at different levels in different types of target cells, may modify the activity of STAT3 and determine the final output of STAT3's biological activities.

Signal transduction pathways activated through a given receptor are thought to act cooperatively with each other to effect the biological activities of a given ligand. However, in certain cases, cytokines can simultaneously generate contradictory signals in the same type of target cell. For example, IL-6, which apparently generates inhibitory signals for cell growth and induces the differentiation of M1 cells, can induce growth signals in M1 cells when STAT3 activation is suppressed (Nakajima *et al.*, 1996). Gp130 simultaneously induces p21, a growth inhibitory signal, and a set of growth-inducing signals from distinct cytoplasmic regions of gp130 in BAF/B03 cells. The induction of p21 through gp130 is suppressed by STAT3, which is also activated by gp130 (Fukada *et al.*, 1998). Neurite outgrowth induced by gp130 stimulation in PC12 cells is totally dependent on ERK MAPK activation through gp130, while STAT3 activated by

gp130 inhibits it (Ihara *et al.*, 1997). These facts indicate that the cytokine receptor can simultaneously activate contradictory signals through its distinct cytoplasmic regions in a given target cell, and the balance between these contradictory signals may determine the final output of the cytokine signal to ultimately express a unified biological activity. Such a mechanism, called 'Signal Orchestration,' may be responsible, at least in part, for the functional pleiotropy that characterizes cytokines and growth factors (Hirano, 1999; Hirano *et al.*, 1997). In this sense, the intensity and/or duration of STAT3 activation may play a crucial role in determining a cell's response to a given cytokine or growth factor.

Finally, the IL-6/gp130/STAT3 pathway appears to play an important role in the generation of plasma cell neoplasias. IL-6 was identified as a B cell differentiation-inducing factor as well as hybridoma/plasmacytoma growth (Hirano, 1998). Gp130 is involved in both cell growth and the differentiation of B cells into plasma cells, and STAT3 is thought to play a central role in exerting these gp130 signals. Furthermore, STAT3 is linked to several oncogenes. It is likely that the IL-6/gp130/STAT3 signaling pathway is essentially involved in the generation of plasmacytoma and myeloma, based on the following observations. (1) Pristane or mineral oil induces plasmacytoma (Potter and Boyce, 1962; Potter *et al.*, 1985). (2) IL-6 is a B cell differentiation-inducing factor (Hirano *et al.*, 1986; Teranishi *et al.*, 1982). (3) IL-6 is a growth factor for plasmacytoma/myeloma (Kawano *et al.*, 1988; Nordan *et al.*, 1987; Van Damme *et al.*, 1987; Van Snick *et al.*, 1986). (4) IL-6-transgenic mice develop plasmacytosis and plasmacytoma (Suematsu *et al.*, 1989, 1992). (5) Ig production is impaired in IL-6-deficient mice (Kopf *et al.*, 1994). (6) IL-6 is essential for *in vivo* plasmacytoma generation (Hilbert *et al.*, 1995a). (7) Raf/myc- and Abl/myc-containing retroviruses induce plasmacytoma (Largaespada *et al.*, 1992; Troppmair *et al.*, 1989). (8) Raf/myc but not Abl/myc requires IL-6 for its plasmacytoma-inducing activity (Hilbert *et al.*, 1995a). (9) v-Abl but not v-Raf activates JAK1/3 and STAT3 (Danial *et al.*, 1995; Hilbert *et al.*, 1996). (10) A v-Abl mutant lacking a binding site for JAK and incapable of activating STAT3 does not support either the survival or proliferation of BAF/B03 cells (Danial *et al.*, 1998). (11) STAT3 is activated in cells transformed by HTLV-1, *v-src*, *abl*, *bc1-abl*, and *v-eyk* and is required for *v-src*-induced transformation (Besser *et al.*, 1999; Bromberg *et al.*, 1998; Carlesso *et al.*, 1996; Danial *et al.*, 1995; Hilbert *et al.*, 1996; Migone *et al.*, 1995; Turkson *et al.*, 1998; Yu *et al.*, 1995). (12) LMP1, required for B cell immortalization by EBV, activates the JAK/STAT pathway (Gires *et al.*, 1999). (13) An active form of STAT3 can transform immortalized fibroblasts (Bromberg *et al.*, 1999). (14) STAT3 is constitutively activated in myeloma cells and required for cell survival through *bcl-x<sub>L</sub>* induction (Catlett-Falcone *et al.*, 1999). (15) STAT3 is required for gp130-mediated cell survival and G1 to S cell-cycle progression signals (Fukuda *et al.*, 1996, 1998). (16) Pim-1 and c-Myc, which together can induce lymphoma, are identified target genes of STAT3 (Shirogane *et al.*, 1999). (17) Pim-1 and c-Myc compensate for a lack of STAT3 in STAT3-mediated cell cycle progression, anti-apoptosis, and *bcl-2* expression in a manner dependent on other

signals through gp130 (Shirogane *et al.*, 1999). (18) CD40, required for the survival, growth, differentiation, and isotype switching of B cells, is associated with JAK3 and activates STAT3 (Hanissian and Geha, 1997). (19) LMP1, which is capable of activating the JAK/STAT pathway, compensates for part of the CD40 function in CD40<sup>-/-</sup> B cells (Uchida *et al.*, 1999). (20) STAT3 is implicated in plasma cell differentiation through gp130 (Ohtani *et al.*, 2000).

Taken together, all of these observations support the hypothesis that the IL-6/gp130/STAT3 signal pathway plays essential roles in the generation of plasma cell neoplasias. The process of plasma cell neoplasia generation can be dissected into at least two parts, 'transformation' and 'differentiation' (Hilbert *et al.*, 1995b). The IL-6/gp130/STAT3 signaling pathway induces B cell growth and plasma cell differentiation. Numerous studies have been performed in the 38 years

since Potter and Boyce (1962) demonstrated that mineral oil induces plasmacytomas in BALB/c mice. These efforts have revealed a critical role for the IL-6/gp130/STAT3 signaling pathway in the generation of plasma cell neoplasias.

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