IUBMB *Life*, 56(2): 83–88, February 2004

Taylor & Francis
healthsciences

Critical Review

Intracellular Proteins and Mechanisms Involved in the Control of gp130/JAK/STAT Cytokine Signaling

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Summary

Cytokines regulate many cellular responses such as proliferation, differentiation and survival and play regulatory roles in numerous organ systems. The cytokines of the IL-6 family use the membrane glycoprotein gp130 as a signal transducer and signal through the JAK/STAT pathway. As they share a common signal transducer they show some functional redundancy but also exhibit specific biological activities. Considering that gp130 is ubiquitously expressed, the time and place at which gp130 functions *in vivo* appears to be determined by spatially and chronologically regulated expression of specific cytokine-binding receptor chains or cytokines themselves. The study of transgenic and knock-out mice for different members of the gp130 signaling cascade has revealed they are critical in embryo development and play a role in physiological responses as diverse as hematopoiesis, the inflammatory response, nervous system development and survival and myocardial and pituitary proliferation. gp130 cytokines have also been implicated in cellular transformation and the pathophysiology of many tumors. Recently, two new families of proteins that function as negative regulators of cytokine signaling, SOCS and PIAS, have been extensively studied and could be new targets for the treatment of pathologies originated by gp130 signaling disregulation. The ubiquitin-proteosome pathway and the new ubiquitin-like protein SUMO-1 seem to play an important role in SOCS and PIAS mediated inhibition but the mechanisms still remain to be elucidated.

IUBMB *Life*, **56: 83–88, 2004**

Keywords gp130; JAK; STAT; SOCS; PIAS; cytokine.

Received 15 January 2004; accepted 28 January 2004

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ISSN 1521-6543 print/ISSN 1521-6551 online © 2004 IUBMB
DOI: 10.1080/15216540410001668064

INTRODUCTION

Cytokines were originally described as signaling molecules regulating inflammatory and immune responses in multicellular organisms. It is now widely known that they also play important roles in most organs and are key players in the interaction between the immune and neuroendocrine systems (*1*).

The cytokines of the IL-6 family, which include IL-6, leukemia inhibitory factor (LIF), IL-11, oncostatin M (OSM), ciliary neurotrophic factor (CNTF), cardiotropin-1, cardiotrophin-like related cytokine and stimulating neurotrophin-1/B cell-stimulating factor-3, share the membrane glycoprotein gp130 as a common signal transducer. When receptors of this family of cytokines, also known as gp130 cytokines, bind to their respective ligands, they trigger the association of their alpha subunits with gp130 (*2, 3*), which functions as an initial cellular signal transducer (*4*) leading to the activation of the JAK/STAT pathway.

While IL-6 family of cytokines show some functional redundancy for example in the induction of macrophage differentiation, the induction of biosynthesis of acute-phase proteins in hepatocytes or the action in the nervous system, they also exhibit specific biological activities (reviewed in *1, 4, 5*). Since gp130 is ubiquitously expressed, the time and place at which gp130 functions *in vivo* appears to be mainly determined by expression of the respective specific receptor subunits and the cytokines themselves, which synthesis and release are spatially and temporally restricted.

As expected due to the wide array of actions that cytokines have in the organism, there are several tightly regulated control mechanisms to limit their signaling pathways at different levels. Some of these negative controls have already been studied in detail and others have only recently started to be unraveled, being one of the most interesting aspects of cytokine signaling.

GP130 SIGNALING

Human gp130 consists of an extracellular domain of 597 amino acids, a single transmembrane domain of 22 amino acids, and a cytoplasmic domain of 277 amino acids; it encodes a protein with typical characteristics of the cytokine hemopoietic family of receptors, characterized by the presence a cytokine receptor homology region that comprises fibronectin type III domains, with four positionally conserved cystein residues and a WSXWS motif (*2, 4*).

Upon ligand binding, all IL-6 family cytokines recruit gp130 to their receptor complexes. As can be seen in Fig. 1, the nature of these complexes depends on the specific ligand. After association of the IL-6/IL-6R complex with gp130, homodimerization of gp130 occurs. This homodimerization is prerequisite for IL-6 signal transduction. Even the complex of IL-6 and soluble IL-6Rα could generate IL-6-mediated signal transduction, further supporting the notion of the gp130 surface molecule involved in signal transduction (*2*). Soluble gp130 is probably translated from an alternative spliced mRNA and can neutralize IL-6-sIL-6R complexes, thereby acting as an antagonist (*6*). Similar to the IL-6R complex, gp130 is an indispensable component of the IL-11R (*2, 4*). From the close structural similarity between IL-6R and IL-11R, the gp130 homodimer is postulated to be induced also by IL-11 (*2, 4*). The IL-11 receptor complex forms a hexamer, consisting of two molecules each of IL-11, IL-11R and gp130 (*7*). LIF binds to the LIF receptor alpha subunit (LIFRα), which has structural similarity to gp130, and induces the heterodimer LIFR/gp130 (*2, 4*). OSM triggers formation of the LIFR/gp130 heterodimer and was also found to use another heterodimer composed of gp130 and OSM-specific receptor component (OSMRα) (*2, 4*). In the case of CNTF, it binds to the CNTF receptor alpha subunit (CNTFRα), whose extracellular regions are structurally similar to that of IL-6Rα. The resultant CNTF/CNTFRα complex induces the formation of the LIFRα/gp130 heterodimer (*2, 4*). CT-1 has been suggested to induce this type of heterodimer either by direct binding to LIFR or by binding to CT-1 specific receptor (CT-1Rα) (Fig. 1).

gp130 signals through the JAK/STAT pathway (*6*). The cytoplasmic regions of these receptors interact and initiate the signaling cascade, activating associated members of the JAK family of tyrosine kinases (JAK1, JAK2 and Tyk2). The JAKs in turn phosphorylate the specific receptors and gp130 on tyrosine residues, which provides binding sites for molecules that contain an Src-homology-2 (SH2) domain, particularly members of the STAT family, STAT1 and STAT3. Once recruited to the receptors, STAT proteins are also tyrosine-phosphorylated and dimerization occurs immediately through reciprocal phosphotyrosine-SH2 interaction. Dimerized STATS then translocate to the nucleus, where they act as transcription factors (reviewed in *8*). SHP-2 tyrosine phosphatases also have an SH2 domain, are recruited to stimulated gp130 and undergo tyrosine-phosphorylation by JAKs, inducing the Ras/Raf/MAPK pathway via its interaction with GRB2 (*9*).

PHYSIOLOGICAL ACTIONS OF GP130

In vitro studies using recombinant cytokines and different cell lines or primary cell cultures have linked gp130 signaling to a wide array of organ systems and biological functions. gp130 cytokines have been shown to be synthesized in tissues including hematopoietic tissues, reproductive tissues, thymus, heart, liver, pituitary, and nervous system and found to play roles in the regulation of cell differentiation, proliferation, cell survival, hormone secretion and inflammatory response (reviewed in *6*).

Physiological and pathological roles of gp130 have been extensively studied *in vivo* using transgenic and knock-out mice. According to what would be expected by their ubiquitous expression, gene knock-outs gp130 (*10*), STAT3 (*11*) and JAK1 (*12*) lead to mice with lethal phenotypes. gp130 knock-out mice show hypoplastic ventricular myocardium and have greatly reduced numbers of pluripotential and committed

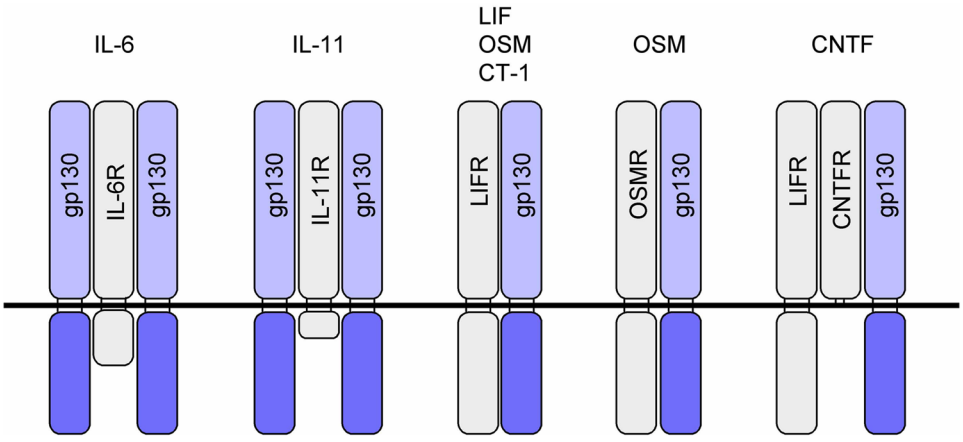

Figure 1. Different gp130 signaling receptor complexes. According to ligand, IL-6 family cytokines induce the formation of different receptor complexes, all of them sharing gp130 as signal transducer.

hematopoietic progenitors in the liver and differentiated lineages such as T cells in the thymus (*10*). This indicated a role for gp130 in hematopoiesis during embryogenesis and confirmed the role of gp130 signaling in myocardial development suggested by the transgenic mice having continuously activated gp130 (*13*). A conditional gp130 knockout mice, where gp130 was inactivated by conditional gene targeting after birth, exhibited neurological, cardiac, hematopoietic, immunological, hepatic, and pulmonary defects, confirming the widespread importance of gp130 signaling (*14*). On the other hand, mice deficient in one of the cytokines or their receptors (IL-6, LIF, CNTF, or IL-11R_) displayed milder phenotypes than expected probably due to the redundancy of gp130 cytokines (Table 1). Mice deficient in IL-11Rα showed only female sterility (*15, 16*), whereas IL-6 knock-out mice displayed defects in hematopoiesis (*17*), bone (*18*), liver (*19*) and many inflammatory and immune responses (*20, 21*). Mice lacking LIF exhibited female sterility (*22, 23*) and CNTF deficient mice only presented mild neuronal problems (*24*).

GP130 CYTOKINES AND CANCER

Since IL-6 is involved in normal B-cell development, overproduction of this cytokine is considered to be an important component of the pathogenesis and progression of myeloma (*25*). IL-6 inhibits apoptosis of myeloma cells and causes them to be resistant to chemotherapy due to upregulation of bcl-xl and mcl-1 anti-apoptotic genes. The most common source of IL-6 in myeloma seems to be the bone marrow stromal cells, suggesting that IL-6 is a paracrine rather than an autocrine growth factor in myeloma (reviewed in *26*).

Table 1
Phenotype of gp130 family knock-out mice

Knocked gene	Phenotype
gp130	Embryos die prenatally
	Heart and hematopoietic abnormalities
STAT3	Embryos die prenatally
JAK2	Embryos die prenatally
IL-6	Impaired hematopoiesis
	Defects in acute phase protein synthesis
	Defects in liver regeneration after hepatectomy
	Defects in bone maintenance
	Bactericidal and antiviral response compromised
IL-11Rα	Female sterility
LIF	Female infertility: defective blastocyst implantation
	Postnatal growth retardation
	Reduced thymocite proliferation
CNTF	Gradual loss of motor neurons

Summary of the main phenotypes of gp130 pathway knock-out mice studied so far.

IL-6 has also been identified as an auto-paracrine growth factor in prostate cancer and has been shown to confer resistance of prostate carcinoma cells to etoposide and cisplatin which could be reversed by blocking gp130 expression. IL-6 has also been suggested to promote growth of bladder carcinoma cells, small cell lung cancer cells and cervical carcinoma cells (reviewed in *26*).

IL-6 has an intriguing role in regulating pituitary cell growth. This cytokine stimulates DNA synthesis and cell number of GH3, a pituitary lactosomatotrophic cell line, yet at the same concentrations it inhibits the growth of normal anterior pituitary cells (*27*). Furthermore, IL-6 has inhibitory or stimulatory effects in different tumors (ACTH-, PRL-, GH-secreting and non-functioning adenomas), with no apparent association between the kind of response and tumor type or size (reviewed in *28*). IL-11 and CNTF stimulate the proliferation of pituitary folliculo stellate cells and GH3 cells (*29*). Besides, IL-11 also stimulates the secretion of the angiogenic factor vascular endothelial growth factor (VEGF) by FS cells (*29*). Reduced levels of gp130 protein in GH3 cells (stably transfected with gp130 antisense cDNA) blocked cell growth and hormone secretion stimulated by CNTF, and led to severely impaired *in vivo* tumor development in athymic *nude* mice (*30*) providing evidence supporting a link between gp130 and pituitary abnormal growth.

Constitutive activation of STAT3 has been found in a growing number of murine and human tumors and tumor-derived cell lines (*31*). A constitutively active mutant of STAT3 has been shown to be capable of inducing transformation and thus act as an oncogene (*32*).

NEGATIVE REGULATION OF GP130 SIGNALING

Tyrosine phosphatases

Since tyrosine phosphorylation plays a critical role in gp130 signaling, it is not surprising that SHP-1 and SHP-2, two related tyrosine phosphatases with a SH2 domain have been implicated in JAK/STAT negative regulation. Tyrosine dephosphorylation of receptor kinase sites limits further STAT tyrosine phosphorylation cutting the signal off. SHP-1 has been shown to associate and dephosphorylate JAK2 and Tyk2 (*33, 34*), whereas knock in studies in mice where gp130 had been mutated determined that loss in the ability to recruit and activate SHP-2 significantly prolongs STAT3 signaling (*35*). SHP-2 not only has an inhibitory role in the gp130 pathway as it has been demonstrated that it is necessary to link gp130 signaling to the activation of the MAPK pathway via GRB2 (*36*).

SOCS

The Suppressors of Cytokine Signaling (SOCS) are a family of proteins that can down-regulate the JAK/STAT pathway by binding to receptor sites and JAK catalytic sites blocking further STAT protein activation. SOCS1 has been described simulta-

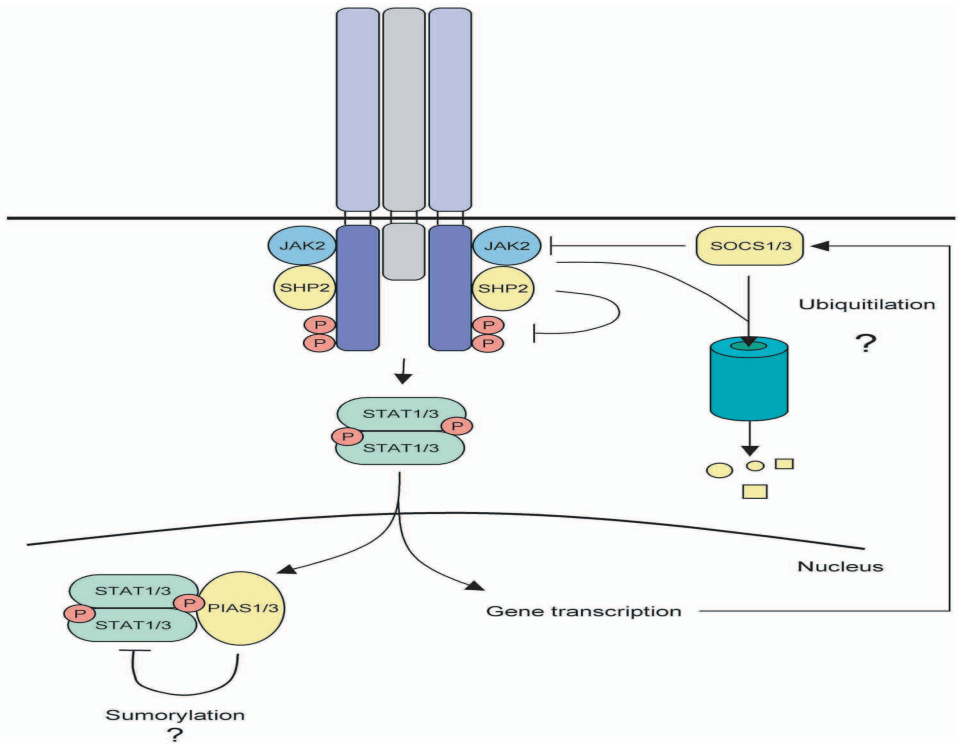

Figure 2. Negative regulatory pathways of gp130 signaling. SHP2 dephosphorylates JAK2 inactivating it, whereas SOCS1 and SOCS3 interact with JAK2 and inhibit its activity. PIAS1 and 3 act at a different level interacting with STATs and blocking their binding to DNA. Question marks indicate that the roles of ubiquitination/proteosome mediated degradation of SOCS and sumorylation of STATs by PIAS proteins are not clear.

neously by three different groups and found to inhibit IL-6 induced receptor phosphorylation and STAT3 activation in macrophages (*37-39*), to interact with JAK2 (*37–39*) and to be induced by IL-6 and LIF, which could be blocked by transfection of a dominant-negative mutant of STAT3 (*37–39*). SOCS proteins have in common a central SH2 domain and a 40 aminoacid C-terminal domain termed the SOCS box (*40*). SOCS1 and SOCS3 have been the most studied of the family and have been found to be involved in the inhibition of gp130 signaling (*41*). Tissue-specific SOCS3 knock-outs were recently generated that underlined the importance of SOCS3 in attenuating IL-6 signaling *in vivo*. SOCS1 has been shown to bind to the catalytic JH1 domain of JAK2 inhibiting JH1 activity (*38*) whereas SOCS3 not only can bind to JAK2 but also binds phosphorylated tyrosine residues in the intracellular domain of gp130 (*42*) and therefore could be acting at two levels.

Recently, the SOCS box has been found to interact with the Elongin B and C complex, which can recruit Cullin-2, Rb × 1 and the E2 ubiquitin conjugating enzyme (reviewed in *43*). Therefore it has been suggested that SOCS proteins may act as adaptor molecules for an E3 ubiquitin ligase complex that target activated cell signaling proteins to the protein degradation pathway. Accordingly, SOCS1 has been shown to promote ubiquitination and degradation of activated JAK2 and TEL-JAK2, a fusion protein with constitutive JAK activity (*44*). The SOCS box also appears to be important for the regulation of the protein stability of SOCS themselves, since there has been found that JAK mediated phosphorylation of SOCS3 at two tyrosine residues in the SOCS box can inhibit the SOCS3-elongin C interaction and activate proteasome-mediated SOCS3 degradation also suggesting that interaction with elongin C can stabilize SOCS3 protein expression (*45*). The exact role of the SOCS box in the inhibition of gp130 signaling by SOCS proteins remains to be determined.

PIAS

The first member of the PIAS protein family to be cloned was PIAS1, which was cloned in a yeast two hybrid assay by its ability to interact with STAT1. The rest of the members which include PIAS3, PIASx and PIASy were identified later based on sequence similarity to PIAS1. PIAS1 and PIAS3 have been shown to specifically interact with STAT1 and STAT3 respectively and to block their DNA binding activity as well as STAT mediated gene activation. Differing from SOCS proteins, PIAS proteins are constitutively expressed but only associate with their corresponding STATs after they were activated by tyrosine phosphorylation (reviewed in *46*).

Recently, it has been found that *S. cerevisiae* Siz1 and Siz2 proteins are necessary for the covalent attachment of the ubiquitin-related protein SUMO-1 to other proteins and were proposed to function as E3-like factors in the SUMO pathway (*47*). PIAS proteins shared the same RING-related motif with Siz1 and Siz2 and thus were suggested to belong to the same family, suggesting a functional conservation of the E3 like activity. PIAS1, PIAS3 and PIASx have been shown to promote sumorylation of STAT1 at Lys 703. However, mutation of this lysine to arginine, which abolished SUMO modification of STAT1, does not affect the activation of STAT1 or the ability of PIAS1 to function as an inhibitor of STAT1-mediated transcription activation (*48*) suggesting that inhibition of STAT1 by PIAS proteins does not require SUMO modification of STAT1 itself.

SUMO attachment has been described for proteins participating in transcriptional regulation, signal transduction, inflammation, and control of cell growth (*49*) and the mechanisms of action include interference with the ubiquitin-proteosome pathway, protein translocation to the nucleus and regulation of protein–protein interaction, suggesting that sumorylation may be a widespread mechanism for controlling protein activity. Thus, the role of SUMO modification in gp130 signaling has to be further studied.

CONCLUSIONS

gp130 cytokines have been shown to play important roles in very diverse models. As expected due to their actions as regulators of cell proliferation, differentiation and survival, these cytokines have also been shown to be involved in the development and growth of different tumors. Therefore, the study of negative regulators of gp130 signaling presents the interesting possibility to reveal new targets for cancer therapy.

Negative regulatory factors have already been shown to be important in cancer since silencing of the SOCS3 gene locus has been associated with persistent STAT3 activation in hepatocellular carcinoma (*50*) and loss of PIAS3 expression has been detected in a lymphoma with constitutively activated STAT3 (*51*). Additionally, it has been demonstrated that blocking of gp130 expression can dramatically reduce the tumorigenic potential of tumoral pituitary cells (*30*). Despite the extensive research that has been done over the past years on SOCS and PIAS protein families, the role of ubiquitination and sumorylation in gp130 signaling remains an open question.

ACKNOWLEDGEMENTS

This work was supported by grants from the University of Buenos Aires, the Max Planck Institute of Psychiatry, Munich, Germany, the Argentine National Research Council (CONICET) and Agencia Nacional de Promoción Científica y Tecnológica-Argentina.

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