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Signal Transduction of the Cytokine Receptor

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Cytokines regulate proliferation, differentiation and functions of haemotopoietic cells. Each cytokine possesses a variety of activities on various target cells (pleiotropy) and various cytokines have similar and overlapping activities on the same target cells (redundancy). The nature of these cytokine activities predicts unique feature of cytokine receptors, namely, cytokine has multiple receptors, different cytokines share a common receptor, and different cytokine receptors are linked to common signaling pathways. cDNA cloning of genes for cytokine receptors revealed distinct sets of receptor family with different structural features. The cytokine receptor superfamily consists of a largest family, and contains more than twenty cytokine receptor subunits. This receptor has common structural features in both extracellular and intracellular regions without tyrosine kinase domain. Another striking feature of the receptor is to share common subunit of multiple cytokines, which partly explains the redundancy of activities of some cytokines. Recent studies revealed detailed signaling events of the cytokine receptor, the primary activation of JAK and subsequent phosphorylation of tyrosine residues of receptor, and various cellular proteins. Many SH2 containing adapter proteins play an important role in cytokine signals, and this system has similarities with tyrosine kinase receptor signal transduction. STAT may mainly account for cytokine specific functions as suggested by knockout mice studies. It is of importance to note that cytokine activates multiple signaling pathways and the balance and combination of related signaling events may determine the specificity of functions of cytokines.

Hematopoietic cells are composed of various cells with a homeostasis which is strictly regulated. In contrast to constitutive hematopoiesis, inducible hematopoiesis plays an important role in immune responses and inflammation. Both types of hematopoiesis are regulated by cytokines (Arai et al., 1990). Hematopoietic cells are derived from multipotential stem cells that have the capacity for self renewal and differentiation (Fig. 1) and hematopoiesis involves multiple steps of differentiation and proliferation of hemaopoietic progenitor cells. Stem cells present in a hematopoietic microenvironment are surrounded by stromal cells composed of multiple types of cells, such as macrophages, endothelial cells, fibroblasts, and adipocytes. These cells produce various cytokines. Cytokines produced by activated T cells are components responsible for inducible hematopoiesis. To date, over fifty cytokines, including interleukins (ILs), colony-stimulating factors (CSFs), interferons (IFNs), chemokines, transforming growth factor-β (TGF-β) family, tumor necrosis factor family and growth factors have been identified. Each cytokine exhibits a variety of activities on several target cells (pleiotropy) and synergy and cross-talk are often observed among the activities of cytokines. In addition, many cytokines elicit similar and overlapping activities on the same target cells (redundancy), suggesting that cytokine signaling pathways are non-linear and form a network with considerable cross-talk. Genes for cytokine receptors have been isolated and structures have been elucidated. Studies of signaling mechanisms of cytokine receptors defined the molecular basis for hematopoietic related events.

Cytokine Receptor

Cytokine receptors are classified into distinct sets of families, based on their structural features (Miyajima et al., 1992). Interestingly, structural features and biological activities of cytokines have a significant correlation (Fig. 2). The most prominent group, cytokine receptor super family, is a receptor for so-called α-helical cytokines such as interleukins and colony stimulating factors as well as growth hormone and prolactin. This group is also known as a hematopoietic receptor family or class I cytokine receptor family and most ligands of the receptor family have

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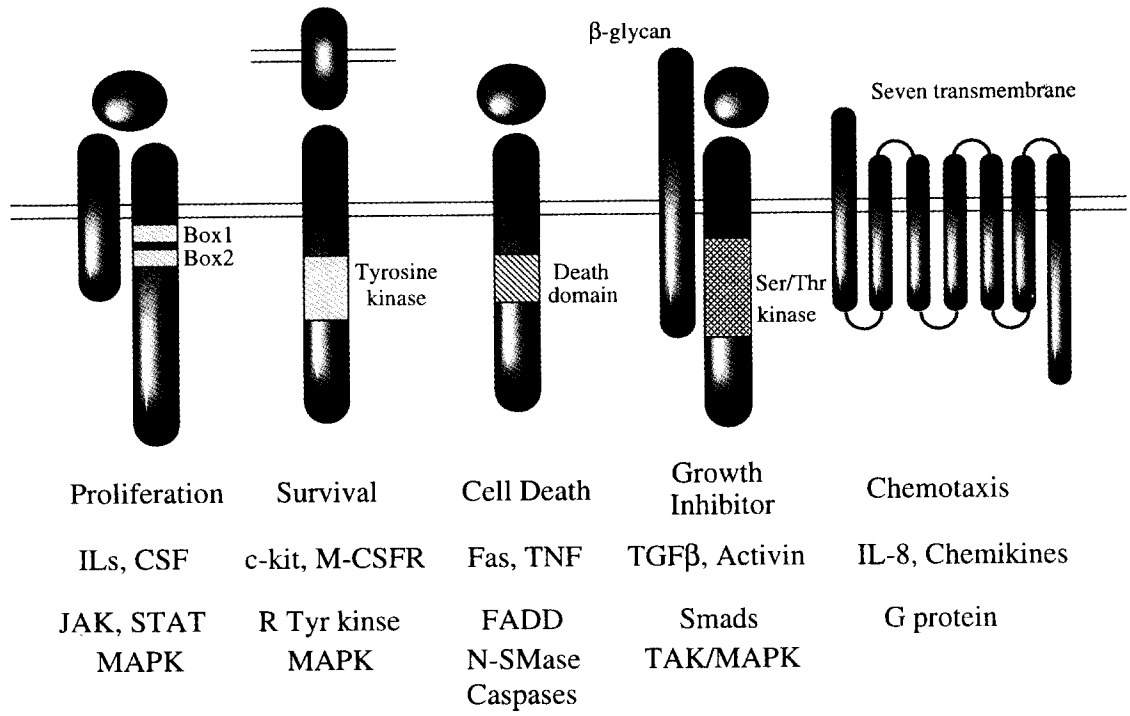

Fig. 1. Structure and function of cytokine receptor. Structural features and biological activities of cytokines have a significant correlation and each receptor activates unique signal transduction pathways. Most haematopoietic cells express multiple different types of receptors and cross-talk between these receptors may determine complexed phenotype of response to cytokines.

strong proliferation promoting activity. The macrophage colony-stimulating factor (M-CSF) and stem cell factor (SCF) receptors, encoded by proto-oncogenes c-*fms* and c-*kit*, respectively, are members of class III of the receptor-type tyrosine kinase (RTK) family which also includes the platelet-derived growth factor receptor. Signals mediated by RTKs are initiated by ligand-induced dimerization of the receptor followed by activation of intrinsic tyrosine kinase. Subsequently, autophosphorylation of RTKs occurs and signals such as MAPK cascade are transduced through phosphorylation of adapter proteins which recognize specific phosphorylated residues of the receptor. The tumor necrosis factor (TNF) receptor family is unique in that some members of this family can transduce signals for cell survival, while others for cell death; and some can even transmit both survival and death signals, depending on the target cells. These receptors contain no known enzymatic activities, instead have so-called death domain which serves as a region for protein - protein interactions for downstream signal transduction. Several signaling cascades such as FADD, caspase pathway lead to cell death by apoptosis, and the TRADD, TRAF, NF-κB pathways which rescue cells from apoptosis (Wallack, 1997). It was also reported that N sphingo-myelinase, a fatty acid mediated signaling pathway, was activated by Fas or TNF. Signals of transforming growth factor-β (TGF-β) family ligands are mediated through heteromeric complexes between type I and type II (β-glycan) receptors, both of which belong to the TGF-β receptor family and type I contains the intrinsic serine/threonine kinase domain within cytoplasmic regions (Dijke et al., 1996). Ligand binding results in formation of functional receptor complexes and induces activation of Smads proteins cascade and TAK/MAPK cascade (Heldin et al., 1997). Unlike previously described cytokine receptors composed of an extracellular and an intracellular region separated by a single transmembrane segment, IL-8 and chemokines transmit signals through receptors with seven transmembrane spanning regions coupled to heterotrimeric G proteins. Detailed analysis of signals of this type of receptor has not yet to be made. Receptors for interferons and IL-1 are classified as the class II cytokine receptor family (Fig. 3). The structure of this receptor family is closely related to class I receptor and is assumed to have evolved from a common ancestor with class I receptor family.

Sharing of Subunit of Cytokine Receptor Superfamily

Many class I cytokine receptors are composed of multiple distinct subunits. In most cases, one or two of these subunits are shared by multiple different receptor complexes (Fig. 4). This system was first identified in receptors for granulocyte-macrophage CSF, IL-3 and IL-5. These receptor complexes are consisted of an α subunit specific for each cytokine and the common β

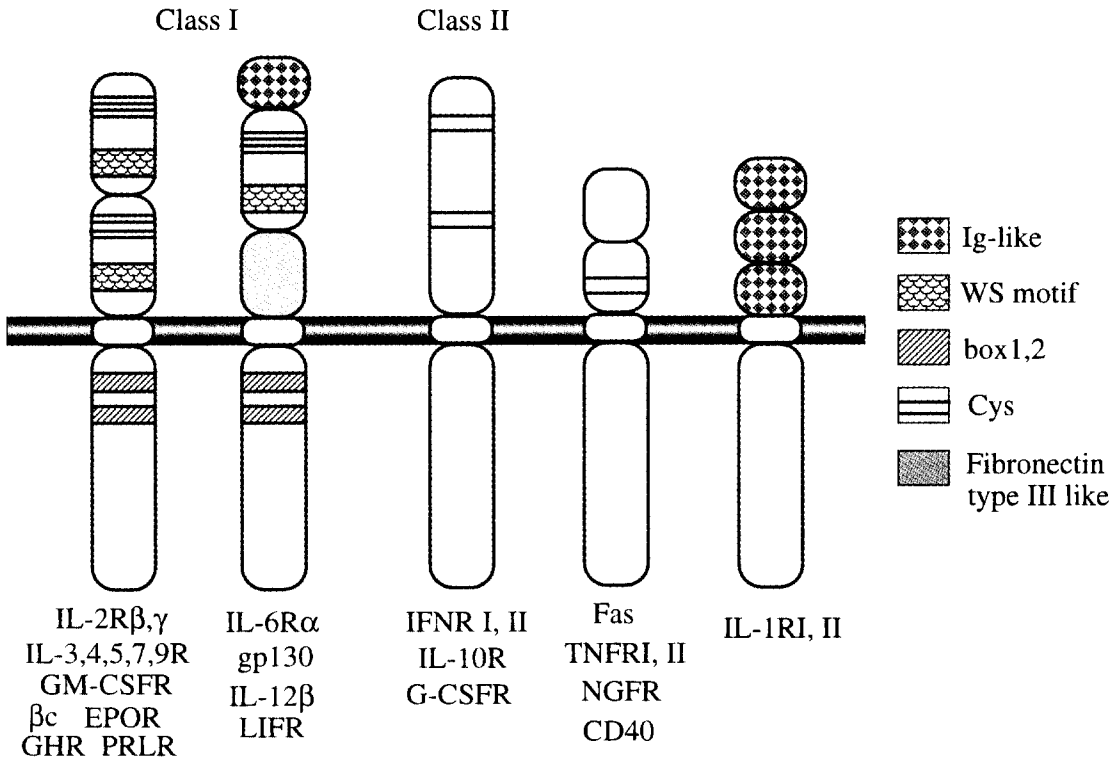

Fig. 2. Type I cytokine receptors. Cytokine receptors do not contain any enzymatic sequence or activity within receptor. Mutation analyses of receptor revealed that most of the conserved motifs are essential for signal transduction of cytokine receptor.

subunit (βc) shared by all. The α subunit binds to the cognate ligand with a low affinity and subsequent heterodimerization of α and β subunits results in formation a high affinity receptor complex. A sharing subunit system was also noted for gp130, second and signal transducing subunit of IL-6 receptor, and common γ subunit (γc), third subunit of IL-2 receptor. Homodimerization of gp130 or heterodimerization between gp130 and LIF receptor is necessary for signal transduction of IL-6, IL-11, OSM, LIF, and CT-1 of receptors (Lindberg et al., 1998). Mutation analysis of gp130 showed that IL-11 receptor and IL-6 receptor recognize overlapping binding motifs on gp130 (Dahmen et al., 1998). Receptors for IL-4, IL-7, IL-9, and IL-15 share γ c. The IL-15 receptor complex shares γc as well as the IL-2 receptor β subunit. These cytokines sharing common subunits have similar biological functions, thus this subunit system may provide, at last in part, the molecular basis for redundancy in cytokine function. How these receptors exhibit specific functions and common functions is a subject of much interest. Analyses of knockout mice of cytokine and its receptor give information for this question. Despite the fact that mice and humans lacking γc have severe immunological defects (Noguchi et al., 1993), in the case of mice (and humans) with a defect in IL-2, the main cause of death is a disease similar to ulcerative colitis (Sadlack et al., 1993). Thymocyte and peripheral T cells are fairly normal in these mice and humans (Schorle et al., 1991). This difference means that phenotypes indicates cytokine(s) rather than IL-2 is mainly responsible for T cell development. The first insight into the notion that γc is used by a factor rather than IL-2 was obtained by comparing findings in patients with either IL-2 defect or γc mutation, X-linked severe combined immune deficiency (X-SCID). Subsequent work on knockout mice revealed that IL-7 is mainly responsible for T cell development (Maeurer et al., 1998). Similarly, in knockout mice of IL-6, LIF has an almost normal phenotype, but mice lacking gp130 is embryonic lethal (Yoshida et al., 1996). Thus, in the sharing receptor system, knockout of shared receptor usually results in a severe phenotype, but defects in other subunits or ligands lead to a relatively subtle phenotype.

Signal Transduction of the Cytokine Receptor

Unique features of the cytokine receptor allow to predict several key factors that determine the nature of the signaling system of the cytokine receptor superfamily. In initial studies of cytokine receptor signals, it was thought that the unique structural features and the rather limited expression in hematopoietic cells of the cytokine receptor superfamily may account for the cytokine or hematopoietic cell specific signaling me-

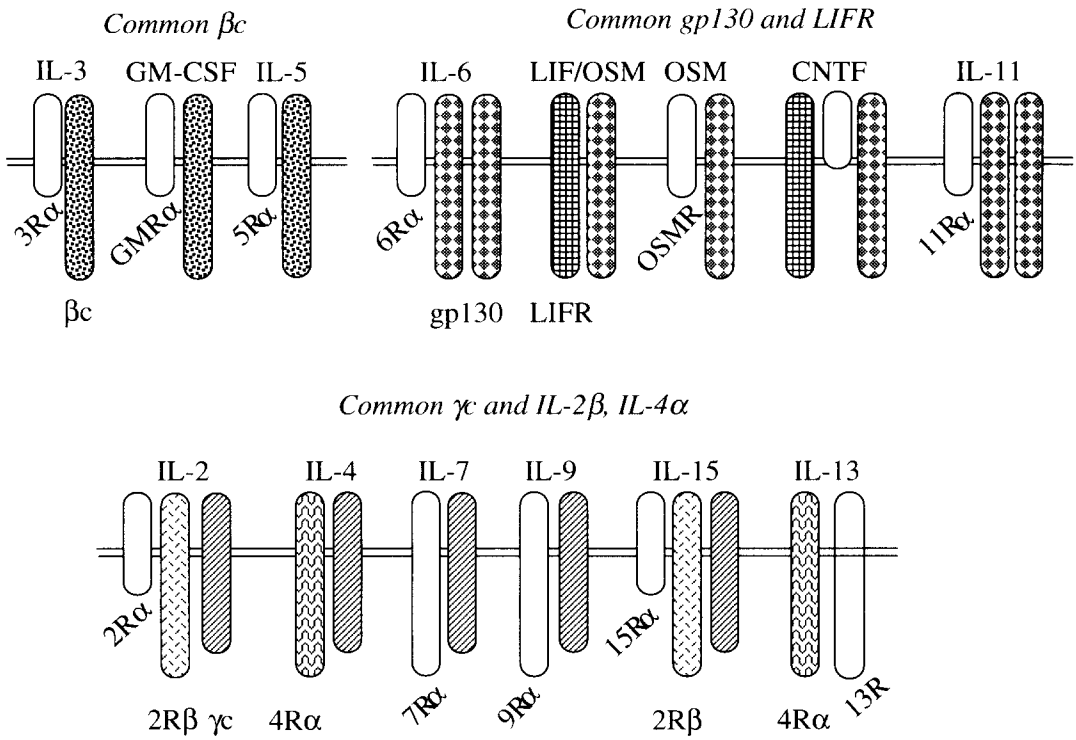

Fig. 3. Sharing subunit of cytokine receptor. Sharing subunit is a unique feature which is founded only for cytokine receptor super family. The βc, γc, gp130 were shared by multiple receptors whose ligands have similar biological activities.

chanism. Subsequent studies revealed that cytokine receptors utilize previously known common signaling systems, such as the Ras/MAPK pathway and phosphatidylinositol-3-OH kinase (PI-3K). Involvement of new members of the MAPK family, such as JNK (c-June kinase) and p38 MAPK has been reported. JNK is activated by various cytokines which promote proliferation (Liu et al., 1997a). Mutation analyses of βc and G-CSF receptors indicated that JNK activation due to is downstream of ras, but the role of JNK activation remains to be clarified (Liu et al., 1997a; Rausch et al., 1997). JNK is apparently involved in apoptosis in both positive and negative manners and balance of activation of erk and JNK may contribute to determine cell fate. Despite the lack of tyrosine kinase domains within the cytoplasmic regions of cytokine receptor superfamily, stimulation by their ligands induces a rapid and reversible tyrosine phosphorylation of various proteins, including the receptor itself. Studies using tyrosine kinase inhibitors also support the notion that tyrosine phosphorylation is a key event in cytokine receptor signaling. The Src family tyrosine kinases were the initial candidate to be associated with the cytokine receptor and to transduce signals. Some members of Src tyrosine kinases were found to bind to the cytokine receptor and roles in STAT activation or proliferation promotion were reported, but these functions are not ubiquitously observed within cytokine receptor signals (Chin et al., 1998; Corey et al., 1998). All of these signaling molecules are not specific for cytokine receptor signaling, but this does not rule out the possibility that each receptor is also linked to a signaling pathway unique to the cytokine receptor family. It is now assumed that JAK and STAT pathways play essential and specific roles in signaling of cytokine receptors.

Role of JAKs in Cytokine Receptor Signal Transduction

The JAK family kinases in mammals consists of JAK1, JAK2, JAK3 and TYK2 (Ihle et al., 1995), members share seven homologous regions termed JAK homology (JH) 1 to 7 domains and have no SH2 and SH3 or PH domains (Fig. 5). The kinase domain is located in the JH1 domain. There is a pseudokinase domain without any obvious kinase activity within the JH2 domain. Thus, it was termed JAK after the Roman god, Janus, who guards gates with two faces (kinase and pseudokinase domains) to keep watch on opposite sides. This family was first identified, independently, either by polymerase chain reaction using a consensus sequence for tyrosine kinases or by low stringency hybridization. No function of the JAK kinase in mammalian cells was known until its role in IFN signaling was recognized. Studies of the IFN receptor signaling

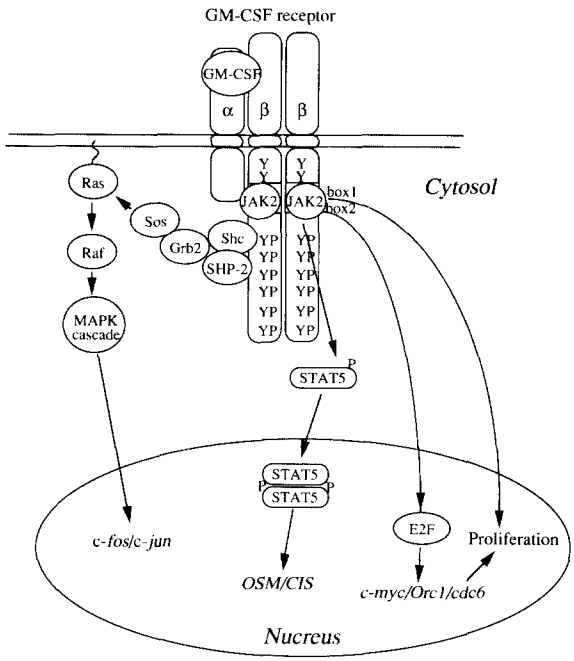

Fig. 4. Signal transduction of GM-CSF receptor. GM-CSF activates at least two distinct signaling pathways. One for activation of c-*myc* and proliferation which requires only box1 region of βc. The other one for activation of MAPK cascade followed by c-*fos*, c-*jun* induction which requires C terminal tyrosine residues in addition of box1 region.

revealed that JAK family kinases are involved in IFN-specific gene expression in cooperation with STAT transcriptional factor (Ihle, 1996). It was elegantly demonstrated with mutant cell lines which were unable to respond to either IFNα/β or IFNγ (Darnell Jr. et al., 1994). By complementation assay, JAK and STAT were identified as essential component of IFNα/β as well as IFNγ signaling. Ihle's group examined the role of JAK kinases in the EPO receptor signaling and found that JAK2 is activated in response to EPO

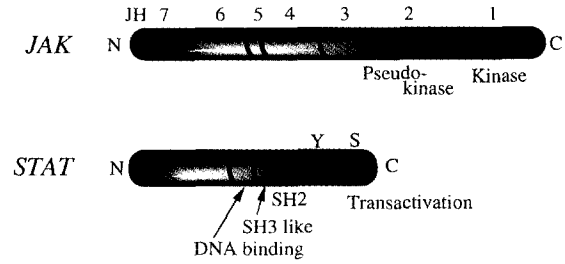

Fig. 5. JAK and STAT. Schematic diagram of structure of JAK and STAT are represented.

(Witthuhn et al., 1993). In addition, JAK2 associates with the region of EPO receptor required for mitogenic action and the tyrosine phosphorylation of cellular proteins induced by EPO. Subsequent studies showed that all cytokines activate JAK and STAT (Table 1) (Watanabe et al., 1996a). Although the role in signaling remains to be clarified, tyrosine kinase type receptors such as EGF or SCF may activate JAK and STAT.

The essential role for JAK3 in lymphoid development was noted in patients with severe combined immuno deficiency (SCID) carrying a mutation in JAK3 (Macchi et al., 1995; Russell et al., 1995) and in JAK3 knock-out mice (Nosaka et al., 1995; Thomis et al., 1995). In all cases, the phenotypes were similar to γc-deficient ones. JAK2 knockout mice is embryonic lethal due to the absence of a definitive erythropoiesis (Neubauer et al., 1998; Parganas et al., 1998). Cells derived from JAK2 defective mice failed to respond to EPO, TPO, IL-3, GM-CSF and IFNγ, but the response to G-CSF, IFNα was unaffected. Disruption of JAK1 showed runt at birth, failure to nurse and perinatal death. Jak1$^{-/-}$ cells fail to respond to cytokines utilize class II cytokine receptors, γc and gp130 (Rodig et al., 1998). All these results indicate an essential role of JAKs in signal transduction.

Table 1. Activation of JAK and STAT by cytokines

Group	Cytokine	JAK	STAT
βc	GM-CSF, IL-3, IL-5	JAK1, JAK2	STAT5
gp130(+LIFR)	IL-6, IL-11, LIF, CNTF, OSM	JAK1, JAK2, (Tyk2)	STAT3, (1)
γc	IL-2, IL-7, IL-9, IL-15	JAK1, JAK3	STAT3, 5
IL-4Rα+γc	IL-4	JAK1, JAK3	STAT6
IL-4Rα+IL-13R	IL-13	JAK1	STAT6
	IL-12	JAK2, Tyk2	STAT3, 4
	G-CSF	JAK1, JAK2	STAT1, 3
	EPO	JAK2	STAT5
	TOP	JAK2	STAT1, 3, 5
	GH	JAK2	STAT5
	PRL	JAK1, JAK2	STAT5
	Leptin	JAK2	STAT3, 5, 6
IFNR (class II R)	IFNα/β	JAK1, Tyk2	STAT1, 2, 3
	IFNγ	JAK1, JAK2	STAT1
	IL-10	JAK1, Tyk2	STAT1, 3, (5)
RTK	EGF	JAK1	STAT1, 3
	PDGF	JAK1, JAK2, Tyk2	STAT1, 3
	M-CSF	JAK1, Tyk2	STAT1, 3, 5

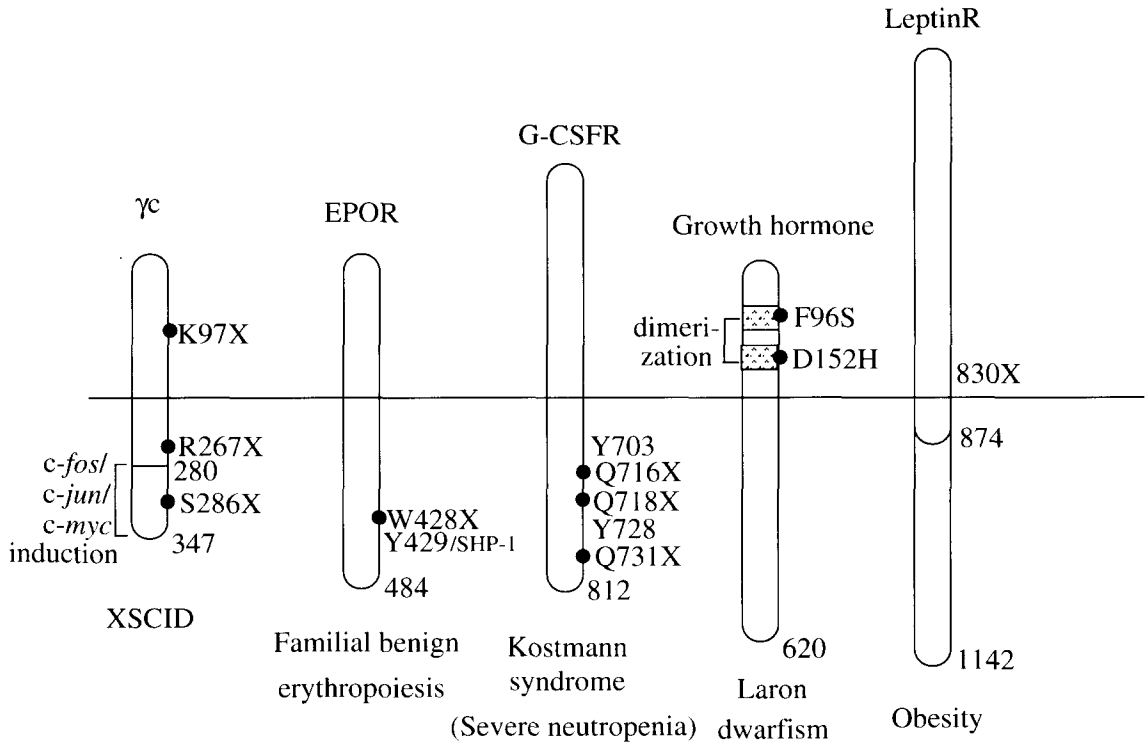

Fig. 6. Cytokine receptor and disease. Several diseases which has mutation within cytokine receptors were reported. Recent detailed studies of receptor and its signal transduction serve molecular basis to understand the mechanism of diseases.

The molecular mechanism of activation of JAK has been implicated by analogy with the model proposed for growth factor receptors that have a tyrosine kinase domain. Several lines of evidence showed that box1 is required and is sufficient for activation or interaction of JAK with the cytokine receptor and the box1-like motif of the IFNα receptor 1 is assumed to be responsible for TYK2 interaction. JAK binds constitutively to cytokine receptors such as βc, EPO, and gp130 receptors. After ligand stimulation, dimerization of cytokine receptor subunits follows dimerization of associated JAKs and results in cross-phosphorylation of JAKs.

STATs and Cell Differentiation

Six members of STAT family, STAT1 to 6, with similar structural features have been identified (O'shea, 1997) (Fig. 5). A DNA binding domain is located in the amino-terminal half, and SH3-like and SH2 domains are in the carboxy-terminal end (Ihle, 1996). All STAT binding sites are very similar with the consensus site being TTCCXGGAA. The DNA-binding domains of STAT proteins are highly conserved and the binding specificity is in the carboxy-terminal end, as revealed by chimeric molecules constructed from different STATs (Schindler et al., 1995). There is a conserved tyrosine residue in C terminal region and this residue plays an essential role in the dimerization of STAT. The serine residue locates in the more C terminal region of the tyrosine residues, which phosphorylates Erk-dependent and -independent pathways. It appears to be involved in STAT transcriptional activity at least in the case of STAT1 and STAT3 (Chung et al., 1997). As is the case with other SH2 containing proteins, STAT is assumed to recognize specific sequence surrounding tyrosine residues of the receptor and to bind to phosphorylated specific tyrosine residues (Gerhartz et al., 1996; Stahl et al., 1995). This recognition may account for the specificity of type of STAT activation by each cytokine.

As observed in the IFN system, STAT proteins are involved in the activation of cytokine-specific genes (Darnell, 1997). Molecular cloning of STAT3 and STAT5 (MGF) clearly showed that these STAT proteins are involved in cytokine-specific gene regulation (Akira et al., 1994; Wakao et al., 1994; Zhong et al., 1994). Using dominant negative STAT3, it was shown that G-CSF utilizes STAT3 for neutrophil differentiation, but another pathway appears to be utilized for MPO gene expression (Shimozaki et al., 1997). For neurite outgrowth, STAT4 negatively regulates MAP kinase-dependent neurite outgrowth in PC12 cells (Ihara et al., 1997). The phenotype of knockout mice of STAT indicates the role of STAT in cytokine specific functions *in vivo*. No STAT1, STAT4, STAT5, and STAT6 knockout mice revealed overt developmental abnormality. STAT1 knockout mice are sensitive to infection by microbial patho-

gens and viruses and fail to respond to IFN, but do respond normally to other cytokines (Durbin et al., 1996; Meraz et al., 1996). STAT4 knockout mice have impaired in IL-12 functions such as the induction of INFγ, proliferation and cytolytic function of natural killer cells and Th1 differentiation and the development of Th2 cells is enhanced (Durbin et al., 1996; Kaplan et al., 1996b; Meraz et al., 1996; Thierfelder et al., 1996). In contrast, STAT6 knockout mice lack the IL-4 induced Th2 response and IgE class switching (Kaplan et al., 1996a; Shimoda et al., 1996; Takeda et al., 1996). Although STAT5 is activated by various cytokines such as EPO, IL-3/IL-5/GM-CSF, prolactin, growth hormone, TPO, the phenotype of STAT5A knockout mice develop normally (Liu et al., 1997c). However, it is evident that mammary lobuloalveolar outgrowth during pregnancy was curtailed, and lactation after parturition failed. These results suggest that STAT5a is an obligate mediator of mammaopoietic and lactogenic signaling, but retardation of proliferation by GM-CSF in bone marrow-derived macrophages of STAT5a deficient mice has been reported (Feldman et al., 1997). STAT3 knockout mice died between embryonic days 6.5 and 7.5 indicating that STAT3 is essential for early embryonic development (Takeda et al., 1997).

Interestingly, the mice died earlier than gp130-deficient mice which died about 12.5 days. There is probably an unknown ligand which does not use gp130 for early STAT3-dependent embryonic development of mice.

The role of STAT in cell proliferation is debatable. Mutation analyses of the receptor domain of IL-4, GM-CSF and EPO receptors show a lack of correlation between cell growth and STAT6 (by IL-4) and STAT5 (by GM-CSF, EPO) (Klingmuller et al., 1997). In contrast, dominant negative STAT5 partially suppressed IL-3 induced proliferation (Mui et al., 1996). Activation of hematopoietic cell proliferation through gp130 depends on activation of both STAT3 and SHP-2 (Kim et al., 1998). On the other hand, activation of SHP-2 is not required for acute-phase plasma (APP) proteins in hepatic cells, which indicates that signaling for APP gene induction and proliferation promotion differ qualitatively. Requirement of STAT3 in Src induced cell transformation is also indicated (Trukson et al., 1998). Taken together, it is speculated that STAT3 may be involved in cell proliferation and other STATs may play a role in cell differentiation. It has also been suggested that STAT1 mediates cell growth arrest by IFNγ through activation of the cyclin-dependent kinase inhibitor p21/WAF1/CIP1 gene. Involvement of STAT5a in IL-2 receptor α subunit induction was also reported (Nakajima et al., 1997). Therefore, STAT5a may participate in cell proliferation by IL-2 indirectly through induction of the IL-2 receptor α subunit. The role of STAT5 in cytokines other than MGF signals and the role of STAT3 in adult tissues remain to be clarified in the near future.

Mechanism of Activation of the MAPK Cascade

It is assumed that the substrate of JAK is not restricted to STAT. Molecules including several SH2 containing proteins and the receptor itself seem to be involved. In the case of the tyrosine kinase type receptor, the central flow of signaling cascade is initiated by phosphorylation of receptor tyrosine residues by tyrosine kinase followed by binding of SH2 containing signaling molecules to phosphorylate tyrosine residue. SH2 proteins recognize the sequence surrounding tyrosine residue, the result being specific interaction between certain tyrosine residues and SH2 proteins. This model also seems to be applicable for cytokine receptors and JAK (Watanabe and Arai, 1996a). In the case of βc, mutation analysis showed that activation of the MAPK cascade and the subsequent activation of the c-*fos* promoter required cytoplasmic tyrosine residues in addition to the box1 region which is required for JAK2 activation. JAK2 is primarily activated by GM-CSF stimulation and is assumed to phosphorylate receptor tyrosine residues (Watanabe et al., 1996b). Mutation analysis showed that Tyr a.a. 577 is required for Shc phosphorylation and Tyr a.a. 577, 612, and 695 are contribute to SHP-2 phosphorylation (Itoh et al., 1998). Similarly specific utilization of certain tyrosine residues of the cytokine receptor cytoplasmic region is seen within several cytokine receptors such as gp130, and G-CSF (de Koning et al., 1998).

Signaling for Proliferation and Anti-apoptosis

Although most ligands of the cytokine receptor superfamily are strong proliferation promoting factors, signaling events leading to DNA replication and cell proliferation are largely unknown. Mutation analysis of βc showed that activation of the MAPK cascade is not essential for DNA replication, in other words, only the box1 region (JAK2 activation) seems to be essential for DNA replication (Watanabe et al., 1993). This fact indicates that unidentified signaling pathway activated directly from JAK2 may be involved in cell proliferation. Transcriptional activation of c-*myc* mRNA also requires only the box1 region and involvement of E2F transcription factor may play an important role in c-*myc* promoter activation (Watanabe et al., 1995). E2F is involved in transcriptional activation of other cell proliferation related genes such as Orcl, and CDC6, thus, it is assumed that activation of E2F is one critical event linked to the promotion of cell proliferation by cytokines. Upstream signaling events of E2F activation are unknown, however, the involvement of SH3 and the ITAM domain containing protein STAM was noted in c-*myc* activation and cell proliferation (Takeshita et al., 1997).

Although signaling events and the mechanism leading to initiation of cell proliferation are largely unknown,

much attention has been directed to mechanisms of anti-apoptosis activity. Withdrawal of IL-3 from progenitor cell lines or from primary IL-3 dependent cells from bone marrow resulted in apoptosis and there are several IL-3 or GM-CSF dependent cell lines which serve as good models for the study of apoptosis (Packham et al., 1994). In these cells, apoptosis was triggered by factor depletion, and exposing the cells to γ irradiation induced apoptosis at a faster rate than that seen by factor depletion of the same cell line (Canman et al., 1995). Mutation analyses of EPO receptor and βc for γ-irradiation induced or factor depletion induced apoptosis indicate an essential role of JAK2, but not for STATs nor the MAPK cascade in anti-apoptosis (Liu et al., 1997b; Quelle et al., 1998). Involvement of Bcl-2 and Bcl-x_L in anti-apoptosis has been suggested and cytokines regulate related levels of expression (Leverrier et al., 1997). The roles of PI-3K, Akt kinase, and the BAD pathway have been characterized in several systems, including IL-3 signaling (Franke et al., 1997b). BAD heterodimerizes with Bcl-x_L or Bcl-2 and neutralizes their protective effects and promotes cell death (Franke et al., 1997a) and this activity is regulated by phosphorylation of BAD which can be induced by IL-3 (Zha et al., 1996). After IL-3 induces the activation of PI-3K and Akt, a serine-threonine protein kinase, phosphorylation of BAD occurs (Peso et al., 1997; Songyang et al., 1997). In contrast to documented mechanisms of cascade of the PI-3K-Akt-Bad pathway, the role of Ras-MAPK in anti-apoptosis remains to be clarified. Active Ras binds to PI-3K (Rodriguez-Viciana et al., 1994), but it is still unclear whether PI-3K is a downstream of ras in cytokine signals.

Cross Talk between Cytokines

Various mechanisms have been suggested as explanation cross-talk between cytokines in multiple steps for receptor to nucleus. For example, IL-3 or GM-CSF enhances EPO-dependent *in vitro* erythropoiesis by primary hematopoietic progenitors and factor-dependent cells. Physical association between βc and the EPO receptor was noted in these cells (Jubinsky et al., 1997). As a signaling mechanism to explain this coordination, tyrosine phosphorylation of βc by EPO was observed by other group (Chin et al., 1997). Not only the interactions between cytokine receptors but also the engagement of growth factor receptor kinase by cytokine receptor were suggested. Tyrosine phosphorylation of the EPO receptor by c-Kit in response to SCF stimulation has been reported. In a prostate cancer cell line, in which ErbB2 was implicated in the neoplastic transformation, IL-6 induced tyrosine phosphorylation of ErbB2 and abrogation of IL-6 induced MAPK activation by inhibition of ErbB2 activity (Qiu et al., 1998). It is well documented that hematopoietic stem cells and primitive progenitors require both an early acting cytokine such as SCF and a lineage-specific cytokine such as EPO to differentiate to a certain lineage. Regarding mechanisms related to this type of cooperation among cytokines, induction of the EPOR gene by SCF and resulting acquisition of responsiveness to EPO has been reported (Sato et al., 1998).

It is generally accepted that gene regulation through enhancer is achieved by coordinated binding of multiple transcription factors to regulatory elements. Cooperative interactions among STAT through N-terminal domains are required for optimal STAT binding within the IFNγ gene (Xu et al., 1996). Similar cooperative binding with other transcriptional factors such as glucocorticoid receptor and p300/CBP were recognized (Bhattacharya et al., 1996; Stocklin et al., 1996).

Negative Signals of Cytokine Receptor

Recently, mechanism of diminishing the signaling of cytokine receptor is paid much attention. At least three different systems were assumed to inactivate signaling; tyrosine phosphatase SHP-1, which dephosphorylates and deactivate JAKs, degradation pathway of receptor and STAT, negative feedback system, CIS family proteins (Aman et al., 1997; Scharenberg et al., 1996). Two tyrosine phosphatases, SHP-1 and SHP-2 are involved in the cytokine receptor signal, both have tyrosine phosphatase activity in the C terminal end, and two SH2 regions. SHP-1 is expressed in only hematopoietic cells, and SHP-2 expresses ubiquitously. The role of SHP-1 was evidenced in mice lacking SHP-1, termed Motheaten (Chen et al., 1996). The model that binding of SHP-1 to the C terminal tyrosine residue of EPO receptor and dephophorylate JAK2 was proposed (Jiao et al., 1996; Klingmuller et al., 1995). In contrast, SHP-2 acts positively to MAPK cascade activation. SHP-2 may play a role as a docking protein and also dephosphorylate protein which regulates the MAPK cascade negatively; the substrate is yet to be defined. Inactivation of STAT by dephosphorylation in nucleus and degradation by ubiquitin-proteasome pathway were shown (Haspel et al., 1996; Kim et al., 1996). Recently a set of proteins termed CIS (cytokine-inducible SH2 proteins), SSI (STAT-induced STAT inhibitor-1) or JAB (JAK-binding protein) have been cloned (Endo et al., 1997; Naka et al., 1997; Starr et al., 1997). All these proteins can be classified into the same family, "CIS family", based on the structural homology. CIS contains SH2 protein in the center of the molecule and a conserved domain (CIS homology domain) in C terminus. Expression of these proteins is induced by STAT and cytokine signals are inhibited through binding to STAT, receptor or JAK (Matsumoto et al., 1997). Negative effects of one of these proteins, SOCS-3, to the leptin signaling was found in mice *in vivo* model system (Bjorbaek et al., 1998).

Disease and Cytokine Receptor

Cytokines such as G-CSF, EPO and M-CSF are originally identified as activities which produce cells of specific lineage. This is consistent with the idea that diseases which affect cells of restricted lineage may be caused by mutation of lineage-restricted cytokines or its receptor. XSCID (X-linked severe combined immunodeficiency) patients have no or severely decreased number of peripheral T cells. Analysis of peripheral B cells revealed that the patients have mutations within γc. As discussed above, the phenotype of the patient may not be caused by the defect of the IL-2 function. Familial benign erythropoiesis is characterized by increased number of erythrocyte and decrease of EPO concentration in serum. This patient has deletion mutation within EPO receptor lacking C terminal 70 amino acids. This region is termed as hypersensitive domain which acts negatively on EPO functions through phosphatase SHP-1 *in vitro*, as described before. Kostmann syndrome is the most severe congenital neutropenia of peripheral neutrophils characterized by recurrent bacterial infection. The mutation of G-CSF receptor was found with some patients. The region carrying mutation was defined as differentiation inducing region based on the fact that mutated receptor transduces proliferation but not differentiation signals. Patients of Laron dwarfism have mutated growth hormone receptor which binds ligand but defective in signal transduction. The βc knockout mice showed pulmonary alveolar proteinosis suggesting that βc mutation may be related to congenital pulmonary proteinosis in human (Nishinakamura et al., 1995). Leptin which is recently identified as adipocyte-specific hormone regulates adipose-tissue mass through hypothalamic effects. Patient with homozygous mutation in the leptin receptor gene shows early-onset morbid obesity with no pubertal development accompanied by no or reduced secretion of growth hormone and thyrotropin (Clement et al., 1998).

During the past ten years, the structure and the mechanism cytokine receptor signaling events were revealed. Important findings are that cytokine activates multiple signaling pathways within the cells and the same set of signaling components are stimulated by different cytokines. Cytokines activate cellular responses such as proliferation, survival, differentiation, death and variety of cellular functions. It remains to be fully understanded how cytokines regulate specific function in target cells. The discovery of JAK and STAT pathway helped to explain many crucial issues at molecular level. JAKs play important roles in all aspects of cytokine functions, whereas studies with various knockout mice revealed that STATs function in cytokine specific manner. As STATs cooperate with other transcription factors, more complicated mechanism may operates to achieve cytokine specific gene activation. Cytokines activate multiple signaling pathways such as Ras/MAPK, Ras/JNK, PI3K/AKT pathways and other pathway yet to be characterized which is activated directly by JAK. Our next goal is to understand the basic mechanism how cytokines, via multiple cross-talks among these pathways, determine cell fate.

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