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Review

Engineering cytokine receptors to control cellular functions

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ABSTRACT

Cytokine receptors function as an interface between a cell and the extracellular milieu, and play a pivotal role in cell fate, because they recognize specific ligands via their extracellular domain and trigger signal transduction via their intracellular domain. Recent advances in unraveling the mechanism of cytokine receptor-mediated signal transduction have allowed us to engineer cytokine receptors with distinct functions, which have a potential for use in biotechnology. This paper reviews the history and current topics of receptor engineering.

Contents

1. Overview of cytokine receptors

Cytokines are a series of protein factors that function as intercellular signaling mediators to control animal cell growth and differentiation. Cytokines include growth factors, neurotrophic factors, hematopoietic factors, colony-stimulating factors, lymphokines, monokines, interleukins, interferons and chemokines. Cytokine receptors are categorized by their structural characteristics: type I/II cytokine receptor family, tumor necrosis factor receptor family, immunoglobulin superfamily, chemokine receptor family and transforming growth factor (TGF)-β receptor family. Of these subfamilies, this review focuses on the type I cytokine receptors, and the receptor tyrosine kinases (RTKs) that belong to the immunoglobulin superfamily [1,2].

Many cytokines were cloned in the 1980s using an expression-cloning method in COS cells [3,4]. By this method, a cDNA library was constructed and divided into pools, followed by transfection into COS cells. By measuring the transiently expressed cytokines in the culture media, the cDNA pool showing cytokine activity was selected. The selected cDNA was repeatedly subjected to transfection and selection cycles, resulting in acquisition of a cDNA clone. Before this method was established, the cytokines were categorized on the basis of their physiological activity, but identification of the cDNA sequence revealed that some series of differently named cytokines encoded the same polypeptide chain, indicating functional pleiotropy of cytokines. Some cytokines also have functional redundancy in terms of their actions. For example, granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-3 (IL-3) have similar multi-colony stimulating activity, which has been clearly explained by the fact that GM-CSF receptor (GM-CSFR) and IL-3R share a common β chain subunit, βc [5].

X-ray crystallographic analysis revealed that cytokines share a structural characteristic of four α-helix bundles in an up–up–down–down pattern [6–11]. Nevertheless, this structural

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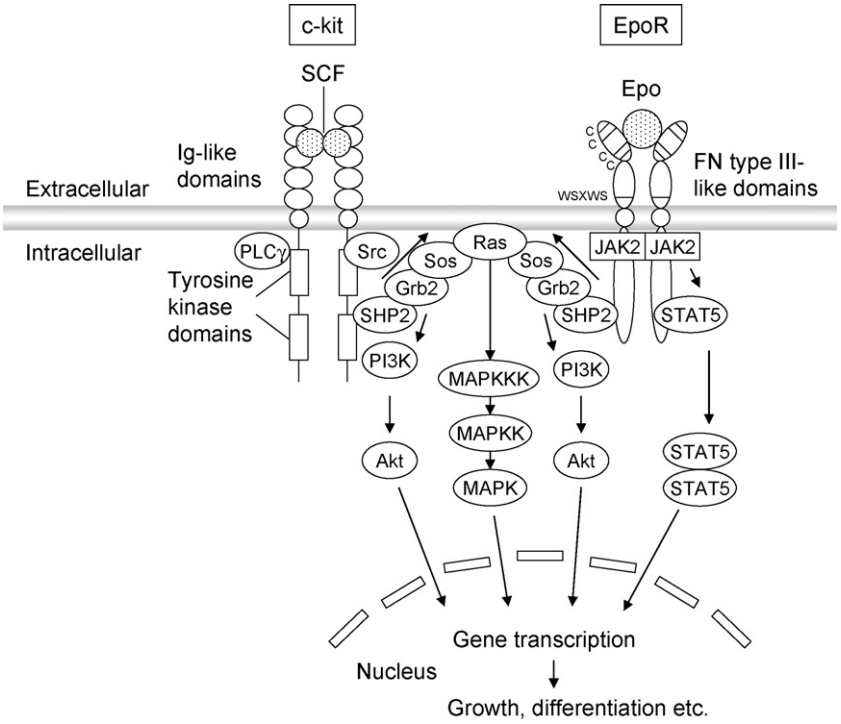

Fig. 1. Structural motifs and signal transduction pathways for c-Kit and EpoR as representatives of RTKs and type I cytokine receptors, respectively.

motif is not essential for binding to cytokine receptors, because many agonists and antagonists with no structural similarity to cytokines have been reported. Monoclonal antibodies [12,13], small peptides [14–16] and even organic compounds [17–19] have been shown to act as agonists.

Molecular cloning of RTKs was first reported in the early 1980s [20]. The molecular architecture of RTKs is characterized by several repeats of immunoglobulin-like domains in the extracellular domain, single transmembrane domain, kinase-like domain and tyrosine kinase domain (Fig. 1). Upon ligand binding, RTKs are activated by phosphorylation of specific tyrosine residues via the tyrosine kinase domain of two receptor chains. Meanwhile, many type I cytokine receptors have been cloned since the late 1980s using a modified expression-cloning method [21,22]. At first, a cDNA library was transfected to COS cells. The cells expressing a receptor were selected by panning using an anti-cytokine antibody and a cytokine, or cell sorting with a fluorescently labeled cytokine. Repeated transfection/selection cycles resulted in cloning of the receptor that can bind to the cytokine.

Sequencing analysis of cDNA revealed that type I cytokine receptors share the common motif of a conserved four cysteine motif in the extracellular domain, a WSXWS (W: Trp, S: Ser, X: any amino acid) motif in the membrane-proximal portion of the extracellular domain, some immunoglobulin-like or fibronectin type III-like domains and a single transmembrane domain, but without a kinase-like domain in the intracellular domain (Fig. 1) [23]. The last feature is quite different from the RTKs, which have a kinase domain for autophosphorylation.

In the 1990s, X-ray crystallographic analyses of cytokine receptors have been reported. Most of the structures were obtained by crystallizing ligand–receptor complexes, revealing ligand-induced dimerization of the two receptor chains. So far, growth hormone receptor (GHR) [24], prolactin receptor (PRLR) [11], erythropoietin receptor (EpoR) [9,15,25], IL-6R [26,27], IL-4R [10], granulocyte-colony stimulating factor receptor (G-CSFR) [28], epidermal growth factor receptor (EGFR) [29], IL-2R [30,31], IL-15R [32], GM-CSFR [33], c-Fms [34,35] and c-Kit [36,37] have been successfully analyzed by crystallography. Other biochemical analyses, such as the use of cysteine mutants to induce ligand-independent spontaneous dimerization, indicated that receptor dimerization was necessary to trigger signal transduction [38]. Therefore, these crystallographic analyses seemed to have confirmed the result of the biochemical analyses. However, analyses using fluorescence resonance energy transfer (FRET) [39] and more directly, unliganded crystallography of EpoR [40] suggested preformed dimers of two receptor chains. Considering these analyses, ligand-induced dimerization is not sufficient to activate signal transduction, but a conformational change seems to be required. This "ligand-induced conformational change" model was confirmed in EpoR and EGFR with dihydrofolate reductase (DHFR) and β-galactosidase complementation assays, respectively [41,42].

The activated receptors then trigger intracellular signal transduction. The phosphorylated tyrosines on the receptors provide docking sites for SH2 domain-containing signaling molecules, such as SHP1, SHP2, PI-3 kinase, SHIP, Shc, STAT and CIS, leading to their phosphorylation and activation of their respective signaling pathways (Fig. 1). The main pathways are JAK/STAT, PI3K/Akt and Ras/mitogen-activated protein kinase (MAPK) pathways, all of which play a critical role in intracellular signal transduction. The kinases in these pathways transduce a signal via tyrosine/serine/threonine phosphorylation, leading to the signaling cascade. The final targets of the cascade are transcription factors, which regulate the transcription of the target genes. These signal transduction pathways are in a complicated network, which still needs to be elucidated.

With regard to RTKs, the Src family kinases and Ras/MAPK pathway kinases have been shown to play a critical role in intracellular signal transduction (Fig. 1). Src family kinases are intracellular tyrosine kinases that have a unique domain, Src homology 3 (SH3) domain, Src homology 2 (SH2) domain, a kinase domain and a modulation domain. Of these domains, the SH2 domain binds to phosphorylated tyrosine, whereas the SH3 domain recognizes a proline-rich short peptide sequence of around 10 amino acids in the downstream signal transducers. Unlike the Src family kinases,