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MINIREVIEWS

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The mechanisms of activation of the single chain cytokine receptors: preformed and ligand-induced dimerization

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Despite intensive studies, the mechanisms of cytokine receptor activation are still not completely understood. Historically two models have been proposed: early studies suggested a mechanism involving ligand-induced dimerization of the receptors which results in the activation of downstream signaling pathways, whereas, in the absence of ligands, the receptors are thought to be in a monomeric inactive state; later studies uncovered evidence for the existence of many receptors as inactive preformed dimers, which upon ligand binding undergo conformational changes and/or relative rotation of the receptor molecules. Here we focus on the dimerization status of the single chain cytokine receptors, which are involved in the regulation of hematopoiesis.

Keywords: cytokine receptors, dimerization, hematopoiesis.

Cytokine receptor family. Cytokines are a large group of polypeptide growth factors that bind to their cognate receptors and mediate intracellular signaling events leading to the modulation of gene expression. Most cytokine receptors consist of a multi- or disubunit complex: a unique and specific ligand-binding chain and a signal-transducing subunit, which may be structurally similar to other members of the cytokine receptor superfamily [1, 2]. Type I cytokine receptors can be organized into subgroups by the number of receptor subunits and the use of common signaling chains: single chain family of the receptors; the common β chain (βc) family; gp130 family and the common γ chain (γc) family (Fig. 1). Type II cytokine receptors are always heterodimers and include interferon receptors. The single chain cytokine receptor subfamily includes receptors for growth hormone (GH), prolactin (PRL), erythropoietin (EPO), granulocyte-colony-stimulating factor (GCSFR) and thrombopoietin (TpoR). These receptors share some common structural features: cytokine receptor homology domain (CRH) which can be divided into two sub-domains of approximately 100 amino acids (namely D1 for N-terminal part) and the membrane distal sub-domain (namely D2); WSXWS motif at the C-end of D2 domain, which was shown to be important for the folding and traffic of the receptors; Box1 (proline-rich motif) and Box2 (hydrophobic part) in the intracellular domain of the receptors are conserved and important for JAK2 binding and signal transduction [3–6] (Fig. 1).

Cytokine receptors lack intrinsic kinase activity and rely on the activation of cytoplasmic Janus kinase (JAKs) family proteins that are recruited onto their intracellular domains [7]. Cytokine binding to the receptor extracellular domain elicits a conformational change which rearranges the preformed dimers or promotes the dimer or oligomer formation from monomeric chains

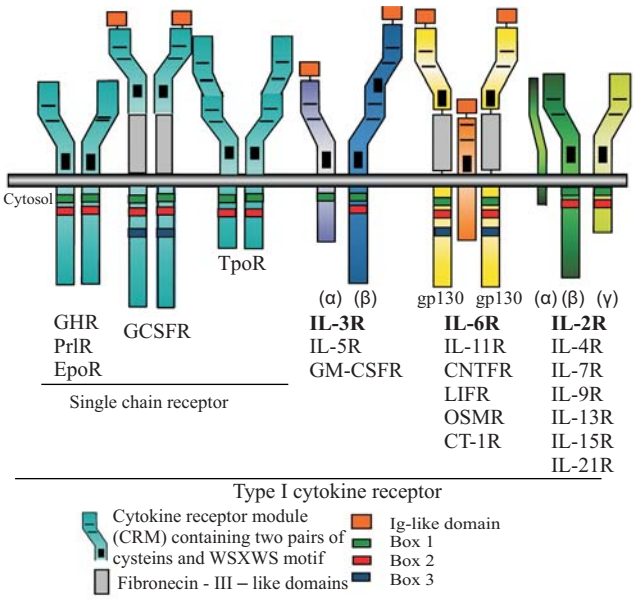

Fig. 1. Schematic representation of type I cytokine receptors subgroups

[8]. This conformation is transmitted to the cytosolic juxta-membrane receptor domain, which brings into close proximity the active and catalytic loops of JAKs enabling them to trans-phosphorylate and activate each other [9]. The activated JAKs phosphorylate the tyrosine residues on receptor cytosolic tails, which recruit several signaling substrates, including, in the first place, the members of the signal transducer and activator of transcription (STAT) family [10].

Since conformational changes in pre-formed dimer or dimerization of two monomer subunits by ligand are two most probable mechanisms of the receptor activation, application of different techniques is important for the investigation of cytokine receptors dimerization. Table briefly summarizes different techniques, which were applied to study dimerization of single chain cytokine receptors.

Growth Hormone Receptor (GHR). GH is the major regulator of postnatal growth. GH binds to its receptor (GHR) providing somatogenic, metabolic and differentiative effects. GHR was the first receptor of class 1 cytokine receptors to be cloned. The crystal structures of the bound and unbound forms have been solved suggesting that two receptor subunits bind one molecule of hormone [11]. Two distinct sites within GH molecule engage two GHR extracellular domains at nearly identical contact points on each receptor [11]. Studies on human GHR by co-immunoprecipitation of differently epitope-tagged receptors showed that GHR exists as a ligand-independent dimer [12]. Further studies using fluorescence resonance energy transfer (FRET) and bioluminescence resonance energy transfer (BRET) confirmed that the receptor subunits undergo specific transmembrane interaction independent of hormone binding. An activation mechanism was proposed where the change of conformation in pre-formed dimer activates downstream signaling in response to the hormone binding [13, 14]. Binding of the hormone to the preformed GHR dimer realigns two receptor subunits by rotation and closer apposition, followed by a juxtaposition of the catalytic domains of the associated tyrosine-protein kinase JAK2 below the cell membrane. Atomistic molecular dynamics simulations have been used to explore the conformational changes linked to the binding of human growth hormone (hGH) to the extracellular domains of the human growth hormone receptor (hGHR) suggesting that hormone binding induces the GHR subunit rotation [15]

Prolactin Receptor (PrlR). Prolactin was first shown to stimulate lactation during pregnancy and mammary gland development, but further investigations have proven it to be important in the water and electrolyte balance, growth and development, metabolic functions, immunoregulation and also some neuroendocrine functions [16]. Unlike GHR, where one gene encodes a single transcript, several isoforms of PrlR are generated by alternative splicing [17]. The structure of the extracellular domains of these isoforms appeared to be identical but the length of intracellular domains different. Similar to GHR, two PrlRs have been proven to bind one Prl molecule by engaging two different sites. The Prl molecule was believed to first recruit one PrlR monomer through its binding site 1, thus forming an inactive complex, followed by the addition of another receptor monomer to the binding site 2 of the ligand and activate the complex.

However, the later studies showed that PrlR existed as a preformed dimer on cell membranes, but receptor predimerization is insufficient to trigger signaling in the absence of a ligand [18, 19]. Only upon ligand binding, the receptor undergoes a conformational change to activate the downstream signaling pathways. The structure

Techniques applied to study the dimerezitaion of single chain cytokine receptors

Assays applied	Receptor studied	Reference
Co-immunoprecipitation	GHR, PrlP	[12, 18]
Immunofluorescence Co-Patching	EpoR	[51]
Structural studies (NMR, crystallography)	GHR, PrlR, GSFR, EpoR	[11, 19, 20, 22, 23, 35, 36]
Sedimentation equilibrium analytical ultracentrifugation	EpoR, TpoR	[50, 52]
TOXCAT	TpoR	[49]
FRET (Foster Resonance energy Transfer)	GHR	[13]
BRET (Bioluminescence Resonance energy transfer)	GHR, PrlR	[13, 19]
Cross-linking	TpoR	[53]
Complementation of dihydrofolate reductase	EpoR	[39]
Gaussia princeps luciferase complementation	EpoR, TpoR	[50]

of 2:1 complex between the ECD of two rat PrlR and the human Prl was solved in 2010 [20].

Granulocyte-Colony-Stimulating Factor Receptor. GCSFR, or as it has recently been renamed, CSF3R, the main hematopoietic growth factor that controls neutrophil development. Besides, GCSF plays an important part in the so-called «emergency granulopoiesis» by enhancing the numbers and functions of the neutrophils in response to bacterial infections. Both effects are accomplished by inducing the proliferation and survival of the myeloid precursors, followed by a cell cycle arrest and neutrophilic differentiation [21]. The crystal structure of a 2:2 complex involving human GCSF and the CRM domain of mouse GCSFR has been published in 1999 [22]. Although this structure revealed a significant information concerning the ligand-receptor interactions, it failed to clarify the mechanism of the receptor activation, as the complex did not contain the original Ig-like domain normally present in the receptor. In 2005, the crystal structure of a complex between hGCSF and the Ig-like and CRM (Ig–CRM) domains of human GCSFR (hGCSFR) at 2.8-Å resolution was reported [23]. The 2:2 receptor:ligand signaling complex was obtained by using crossover interactions involving the Ig-like domain of hGCSF-R and the neighboring hGCSF, in a two-fold axis of crystallographic symmetry. This structure is rather distinct from that of the heterogeneous mouse GCSF-R complex [22] resembling more closely the 2:2:2 active complex of human interleukin-6, hIL-6 receptor and human gp130 (the last cytokine receptor is a shared signal transducing unit for several cytokines) [24], and the 2:2 assembly of viral IL-6 and human gp130 [25], rather than the other homodimeric receptors of its subgroup. The Ig-like domain crossover conformation required for GCSFR activation was in agreement with the earlier reports based on the mutational and thermodynamic analyses [26].

Erythropoietin receptor (EpoR). Epo together with its receptor (EpoR) are involved in the production of erythrocytes [27]. Epo or EpoR knock-out mice die at embryo day 12,5 of severe anaemia [28]. However, the recent studies highlighted additional roles of Epo and its receptor in non-hematopoietic processes such as angiogenesis and wound healing [29, 30]. Dimerization of the receptor was highlighted as an important feature of the EpoR activation mechanism. The most studied phenomenon that supports this observation is the constitutively active EpoR mutant, which has a point substitution of arginine to cysteine (EpoR R129C) in the extracellular domain, which was shown to induce intermonomeric disulfide bond formation and activation [31].

The studies of EpoR dimerization by bivalent antibodies against the EpoR extracellular domain [32], or Epo-mimetic peptides [33], analysis of chimeric receptor molecules [34] or by employing biochemical studies of the purified EpoR extracellular region [35] supported the idea that receptor homodimerization is an important step in the activation process. The data supporting this idea were also obtained from the 3-dimensional structure analyses of the EpoR extracellular region bound to the Epo or erythropoietic peptide agonists. The EpoR:Epo complex is a dimeric receptor occupied by a single Epo molecule in a 2:1 ratio [36, 37]. Full-length EpoR on the cell surface was shown to be a preformed dimer as well as short forms of EpoR [38, 39]. Mainly the transmembrane domain mediates dimerization of EpoR in the absence of ligand [38]. The study of the Epo:EpoR complex revealed that Epo has 2 discrete binding sites in EpoR with different affinities to Epo [40]. Thus, Epo binds asymmetrically to EpoR dimer.

Thrombopoiein receptor (TpoR). Thrombopoietin and its receptor, Mpl (TpoR), are the primary regulators of megakaryocytopoiesis and play a critical role in the hematopoietic stem biology [41, 42]. Upon ligand binding, Mpl facilitates tyrosine phosphorylation of cytoplasmic signaling proteins and activation of several signaling pathways, including JAK-STAT, MAPK and PI3K [43, 44]. TpoR is a major regulator of megakaryopoiesis and platelet formation, but is also required for maintaining the quiescence of hematopoietic stem cells, regulating proliferation of early myeloid progenitors [45, 46]. The structural modelling and ligand binding affinity experiments showed that first 280 amino acids of the extracellular domain of TpoR are responsible for the Tpo binding and possibly for preventing the rest of the receptor from signaling in the absence of the ligand [47]. The crystal structure of any extracellular part of TpoR is not available so far. Nevertheless, the crystal structure of the receptor-binding domain of human Tpo coupled with a neutralizing Fab fragment was solved in 2004 [48].

The experiments using titration calorimetry showed that human Tpo interacts with soluble Tpo receptor containing the extracellular cytokine receptor homology domains in 1:2 stoichiometry.

Therefore, the model for the activation of TpoR is similar to other single chain receptors. Resent studies confirmed that the transmembrane domain of TpoR and full length TpoR form homodimers in a ligand-independent manner [49, 50], although at lower levels than EpoR.

Most of the studies showed that single chain receptors on the plasma membrane form dimers before ligand binding. This implies that conformational changes are required in preformed dimers to activate downstream signaling upon ligand binding. However, it could not be excluded, that the receptors exist in monomeric and dimeric forms on the cell surface and the ligand could bind to both of them at the same time promoting complex activation. The equilibrium might exist between monomeric and dimeric forms, and high levels of expression might displace the equilibrium towards the dimeric form. What appears certain is that even at high expression levels and near-complete ligand-independent dimerization, the receptors are designed to remain inactive in the absence of cytokine stimulation.

Full understanding of the cytokine receptor activation is a major challenge for the future, as well as application of new techniques to investigate the dimerization issue.

Механізми активації мономерних рецепторів цитокінів: ліганд-незалежна та ліганд-індукавана димеризація

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Резюме

Незважаючи на інтенсивні дослідження, механізми активації цитокінових рецепторів наразі є не зовсім зрозумілими. Історично було запропоновано дві моделі: у ранніх роботах припускали механізм ліганд-індукованої димеризації рецепторів, що призводить до активації більш пізніх сигнальних шляхів, у той час як за відсутності лігандів рецептори перебувають у мономерому неактивованому стані; подальшими дослідженнями виявлено докази існування багатьох рецепторів у формі неактивованих сформованих димерів, які при зв'язуванні ліганда зазнають конформаційних змін та/або мономери яких починають ротаційно переміщуватися відносно один одного. Даний огляд сфокусовано на проблемі димеризації окремих гомодимерних рецепторів цитокінів, які причетні до регуляції кровотворення.

Ключові слова: рецептори цитокінів, димеризація, гематопоез.

Механизмы активации мономерных рецепторов цитокинов: лиганд-независимая и лиганд-индуцированная димеризация

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Резюме

Несмотря на интенсивные исследования, механизмы активации цитокиновых рецепторов до сих пор не полностью изучены. Исторически было предложено две модели: ранние работы предполагали механизм лиганд-индуцированной димеризации рецепторов, что приводит к активации более поздних сигнальных путей, в то время как при отсутствии лигандов рецепторы находятся в мономерном неактивном состоянии; последующие исследования выявили доказательства существования многих рецепторов в форме неактивированных сформированных димеров, которые при связывании лиганда претерпевают конформационные изменения и/или мономеры которых начинают ротационно перемещаться относительно друг друга. Данный обзор сфокусирован на проблеме димеризации отдельных гомодимерних рецепторов цитокинов, вовлеченнх в регуляцию кроветворения.

Ключевые слова: рецепторы цитокинов, димеризация, гематопоез.

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