

Review

LEPTIN RECEPTORS

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Abstract

Leptin or obesity receptor (Ob-R) is a member of class I cytokine receptor family. Ob-R, expressed in six isoforms, is the product of alternative RNA splicing of *db* gene. According to its structural differences, the receptor's isoforms are divided into three classes: long, short, and secretory isoforms. A long, fully active isoform of Ob-Rb is expressed mainly in the hypothalamus, where it takes part in energy homeostasis and in the regulation of secretory organs' activity. Ob-Rb is also present on all types of immune cells, involved in innate and adaptive immunity. Short leptin isoforms (Ob-Ra, Ob-Rc, Ob-Rd, and Ob-Re) that contain box 1 motif are able to bind JAK kinases (Janus kinases) as well as to activate some other signal transduction cascades. A soluble isoform (Ob-Re) can regulate serum leptin concentration and serve as a carrier protein delivering the hormone to its membrane receptors and is able to transduce the signal into the cell. JAK/STAT pathway plays the major role in leptin signal transduction through membrane receptors. Among all Ob-R isoforms, only full-length isoform (Ob-Rb) is able to fully transduce activation signal into the cell.

Key words: leptin, leptin receptor, leptin receptor isoforms

INTRODUCTION

A search for the biological factor responsible for energy balance and signal transduction to the hypothalamus was initiated by studies on animal models. In 1994, a molecular defect in the obese gene (*Ob*), a gene responsible for the obesity phenotype in *ob/ob* mice was identified using a positive cloning method. *Ob* gene has found on chromosome 6 in mice and on chromosome 7q31.3 in humans and has been shown to encode 4.5 kb-long mRNA. The protein encoded by the *ob* gene has been isolated and named leptin - from the Greek "leptos" meaning thin [1]. It has been demonstrated that leptin biological activity strongly depends on its proper interactions with Ob-R receptors [2].

THE STRUCTURE OF LEPTIN RECEPTORS

Ob-R receptor, encoded by *db* gene has been identified for the first time in a murine vascular plexus using a cloning technique. It is a member of class I cytokine receptors family that, apart from, Ob-R consist of

gp130 subunit of IL-2R, IL-3, IL-4, IL-6, IL-7, LIF (leukemia inhibitory factor), G-CSF (granulocyte-colony stimulating factor), growth hormone, prolactin, and erythropoietin receptors [3, 4]. Ob-R expressed in six isoforms, is the product of alternative RNA splicing of *db* gene (diabetes gene) [5, 6]. According to its structural differences, the receptor's isoforms were divided into three classes: long, short and secretory.

All Ob-R isoforms have a similar, extracellular ligand-binding domain located in N-terminus of the protein. The domain is constituted by 816 amino acids and contains four cysteine residues, WSXWS motif (Trp-Ser-X-Trp-Ser) and a different number of fibronectin III domains. Five isoforms, including the long isoform Ob-Rb and short ones - Ob-Ra, Ob-Rc, Ob-Rd, and Ob-Rf, have a transmembrane domain consisting of 34 amino acids. The intracellular domain of the long isoform consists of 303 amino acids at carboxyl terminus, while intracellular domains of short isoforms are shorter and consists of 32-40 amino acids. Apart from identical extracellular and transmembrane domains, Ob-R isoforms (short and long) have the same sequence of the first intracellular 29 amino acids. This sequence contains a constant box 1 motif and JAK tyrosine kinase. The length of the further intracellular amino acid sequences depends on the alternative mRNA splicing. Ob-Rb isoform contain additional box 2 and SOCS (suppressor of cytokine signaling) motifs. SOCS expression is induced by cytokines [7]. A sixth, secreted Ob-Re isoform, does not contain intracellular and cytoplasmic domains and is secreted to the bloodstream as a soluble receptor (Fig. 1) [6, 8-10].

A long, fully active isoform of Ob-Rb is expressed mainly in the hypothalamus, where it takes part in energy homeostasis and in the regulation of secretory organs' activity. Ob-Rb is also present in all types of immune cells, involved in innate and adaptive immunity (Fig. 2) [7, 9, 11, 12-14].

Lack of a full-length Ob-Rb receptor is responsible for the development of the early obesity phenotype in *db/db* mice and in obese rats. In *db/db* mice, a short Ob-Ra isoform with limited activity is synthesized. The condition leads to diabetes, morbid obesity, and pubertal development disorders. The mice strain is also characterized by cold intolerance and elevated concentration of glucocorticoid hormones. Moreover, *db/db* mice phenotype includes a significantly elevated leptin concentration, with no ability to respond to leptin signal [15].

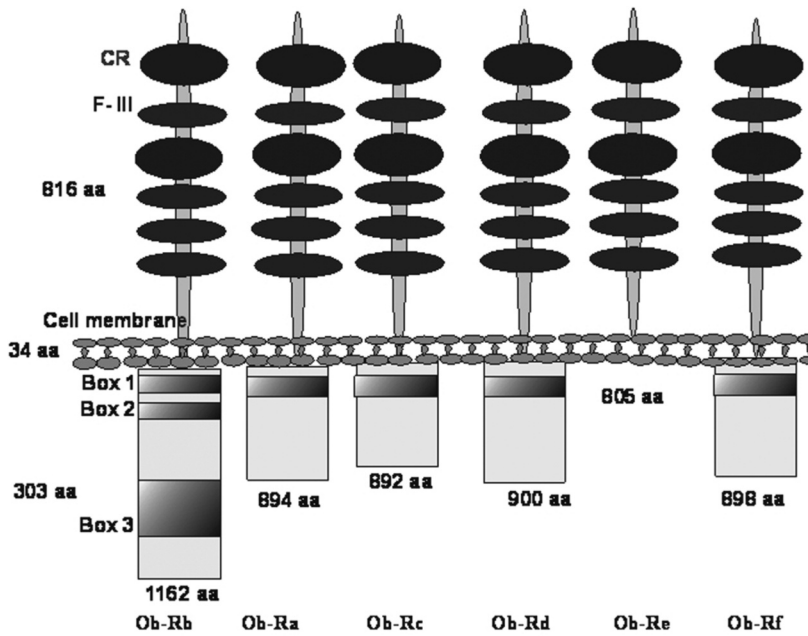


Fig. 1. Leptin receptor (Ob-R) isoforms. CR - cytokine receptor domain, F-III - type III fibronectin domains, Box 1, 2, 3 - constant, intracellular motifs [according to 7, 8].

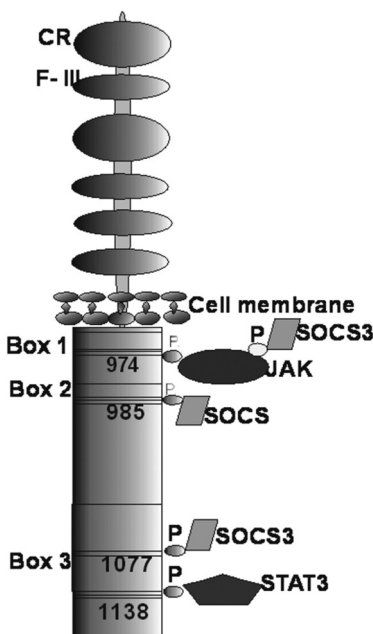


Fig. 2. Ob-Rb, long isoform of leptin receptor: JAK-tyrosine kinases, STAT-signal transducer and activator of transcription, SOCS proteins-cytokine signal transduction inhibitors, P - phosphate residues, CR - cytokine receptor domain, F-III-type III fibronectin domains [according to 7, 17].

Short leptin isoforms that contain box 1 motif are able to bind JAK kinases (Janus kinases) and to activate some signal transduction cascades. However, the effect of short isoform activation differs from that of long isoform activation [16, 17]. Their main function is presumably connected with leptin internalization and degradation [18]. A short isoform, Ob-Ra, is the most common Ob-R isoform that can be found in many various cells and tissues, including kidney, lungs, liver, spleen, and macrophages [6].

A soluble isoform of the receptor, Ob-Re, is probably a result of alternative transcript splicing of db

gene or a consequence of transmembrane Ob-R receptor destruction. Circulating Ob-Re is able to bind serum leptin and to inhibit signal transduction pathways. On the other hand, the receptor can regulate serum leptin concentration and serve as a carrier protein delivering the hormone to its membrane receptors able to transduce the signal into the cell [19].

In normal conditions, only 5-25% of all Ob-R isoforms are present on the cell surface, whereas the majority of receptors are localized within the cell. After ligand binding, the receptors are internalized into early endosomes via clathrin-coated vesicles. Next, the receptor is degraded or effectively recycled to the cell membrane. The process concerns mainly Ob-Ra and Ob-Rb isoforms. A decrease in Ob-Rb expression is much higher than changes in Ob-Ra expression, and short isoform Ob-Ra is much faster recycled to the cell membrane. Relatively weak signal transduction through long Ob-Rb isoform observed in obese, hyperleptinemic patients is related to delayed receptor expression on the cell surface, which may explain leptin resistance in these patients [8, 20, 21].

SIGNAL TRANSDUCTION PATHWAYS

The major role in leptin signal transduction through membrane receptors is mediated through JAK/STAT (signal transduction and activation of transcription) pathway. Among all Ob-R isoforms, only the full-length isoform Ob-Rb is able to fully transduce an activation signal into the cell. Ob-Rb is considered a fully active receptor, because it contains 3 intracellular motifs necessary to activate the JAK/STAT pathway. The described motifs are the following: box 1 and box 2 that bind JAK tyrosine kinase, and box 3 motif that binds STAT transcription factor [6, 15]. Additionally, in intracellular domain, Ob-Rb contains four tyrosine residues (Tyr974, Tyr985, Tyr1077 and Tyr1138) that activate intracellular signal transduction pathways (Fig. 2) [22]. After ligand binding to the Ob-Rb receptor, a cytoplasmic tyrosine kinase JAK2 binds to box-1 and

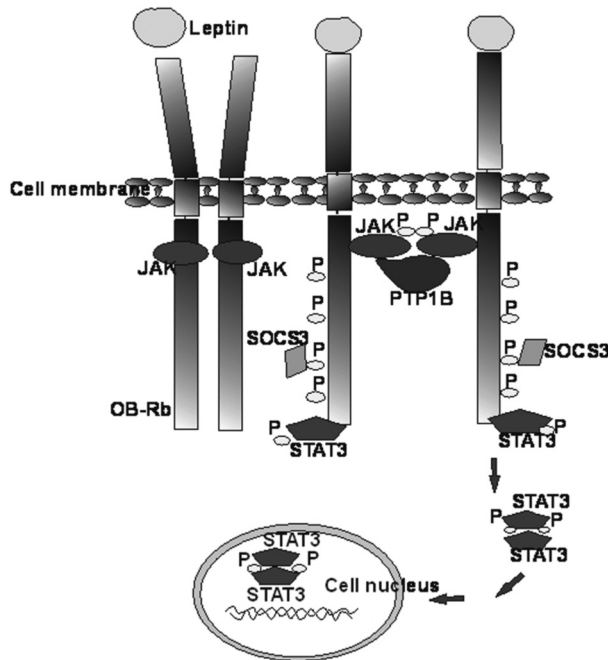


Fig. 3. JAK/STAT pathway activation by Ob-Rb leptin receptor mechanism. After leptin binding, the Ob-Rb receptor changes its conformation that leads to JAK translocation. JAK proteins become activated and start to exert their kinase activity and phosphorylate tyrosine residues of other JAK proteins and of Ob-Rb receptor. Tyr1138 phosphorylation allows STAT3 protein binding and next, STAT proteins become the substrate for JAK proteins related to Ob-R receptor. STAT3 protein phosphorylation leads to dissociation of the protein from the receptor, dimerization and translocation to the nucleus. After the translocation STAT proteins stimulate specific genes expression [according to 17, 24].

box-2 motifs [23]. The activated kinase is autophosphorylated and activates specific tyrosine residues (Tyr 985 and Tyr1138) in the receptor molecule. Next, after Tyr1138 phosphorylation, STAT proteins bind to box-3 motif. STAT proteins (STAT1, STAT3, STAT5, STAT6), including the key signal transduction molecule STAT3, are phosphorylated on tyrosine residues by JAK2 kinases. The process leads to the dissociation of proteins from the receptor, homo- or heterodimerization and translocation of the proteins to the nucleus. In the nucleus, the described proteins serve as transcription factors activating the expression of a suppressor of cytokine signaling 3 (SOCS-3) or protein tyrosine phosphatase 1B (Fig. 3) [17, 24-26].

STAT proteins are activated by leptin and depending on the target tissue; the type of activated protein may differ between various types of target cells [24]. JAK/STAT pathway activation also requires SOCS proteins, mainly SOCS3. SOCS proteins serve as a negative regulator of cytokines' activity. Leptin induces SOCS3 expression by binding to the SOCS3 SH2 domain with Tyr985 or through the inhibition of SOCS3 dependent JAK2 phosphorylation activity [21, 27]. In addition, PTP1B (protein tyrosine phosphatase 1B) is another negative leptin signal transduction modulator that regulates signal transduction through dephosphorylation of JAK2. PTPB1 overexpression de-

creases JAK2 phosphorylation and inhibits leptin-induced SOCS3 and c-fos (proto-oncogene) transcription [28].

Apart from the above described, leptin signaling also is mediated by other pathways. MAPK kinases (mitogen-activated protein kinase), IRS1 (insulin receptor substrate-1), and phosphatidylinositol kinase (PI3K) are important pathways responsible for Ob-Rb receptor activation by leptin in various cells, e.g., T-cells [28, 29]. The MAPK kinase signal transduction pathway is mediated by ERK (estrogen receptor) and p38 kinases. It has been demonstrated in osteoblasts that leptin, after activation of Ob-Rb, induces apoptosis through the activation of MAPK, ERK1/2-dependent activation of cytoplasmic phospholipase A, and subsequent cytochrome c release and activation of caspases 3 and 9 [30]. Osmotic stress, heat shock, and cytokines are able to activate another MAPK family protein, namely p38. In mononuclear cells, after binding to Ob-Rb, leptin increases the level of p38MAPK phosphorylation. In LPS (lipopolysaccharide)-stimulated Kupffer cells, leptin significantly increases TNF α secretion through the activation of p38 and JNK/MAPK (c-Jun N-terminal kinase), whereas in smooth muscle cells it is able to induce hypertrophy through the activation of p38 MAPK [31].

The majority of insulin-mediated biological effects are caused by PI3K activation. PI3K is believed to constitute an important common element for signal transduction pathways activated by insulin and leptin and its receptor. In the central nervous system, adipose tissue, liver, and pancreas, leptin induces a similar pathway to that activated by insulin, including PI3K-dependent activation of PDE3B (phosphodiesterase 3B) and cAMP (cyclic adenosine monophosphate) reduction [32]. It seems that PI3K/PDE3B/cAMP pathway cooperates with the JAK2/STAT cascade and is an important element of leptin signal transduction pathways in the hypothalamus [28].

LEPTIN RECEPTOR GENE POLYMORPHISM

LEPR gene (leptin receptor gene) mutations are extremely rare in human and animals. A single nucleotide substitution (G to A) in exon 6 leads to a deficiency in intracellular and transmembrane domain of the receptor [33]. The mutation caused by premature stop codon insertion at the 3' terminus of LEPRb mRNA was found in *db/db* mice [15], whereas in Zucker rats, the mutation was caused by amino acid substitution (Gln to Pro) at position 269 of the extracellular domain of the receptor. The described mutation resulted in a severe reduction of Ob-R expression on the cell surface and limitation in the leptin-receptor binding. Obese Koletsky rats had a point mutation at 763 position resulting in premature stop codon introduction in the intracellular domain of the receptor that led to a total deficiency in all Ob-R isoforms on the cell surface. Both Zucker and Koletsky rats are characterized by morbid obesity, hyperphagia, hyperlipidemia, and numerous hormonal disorders [34, 35]. In humans, a rare mutation caused by a single substitution of G to A in exon 16 was identified. It leads to an abnormal expression of transmembrane and intracellular do-

mains of receptors. As in case of laboratory animals, humans with LEPR gene defects demonstrate obesity, hyperphagia, pubertal development disorders, and endocrine system abnormalities [33].

LEPR gene polymorphisms seem to be much better described. It has been demonstrated that the polymorphisms may lead to the impairment of signal transduction from the receptor into the cell through premature Ob-Ra, instead of Ob-Rb isoform production, or through a decrease in Ob-R expression on the cell surface and limitation in leptin-receptor interactions. LEPR gene defects influence the development of hyperphagia, obesity, pubertal development disorders, neuroendocrine system regulation impairment, and diabetes caused by β -cell apoptosis in the pancreas [8, 15, 33, 36]. The most commonly seen polymorphism is Gln223Arg, encoding the extracellular domain of the receptor responsible for leptin binding. Glutamine to arginine change may be responsible for an impaired signal transducing capacity of the leptin receptor [36]. A connection between Gln223Arg polymorphism and breast cancer development and progression has been suggested [37]. Genetic interactions between leptin and Gln223Arg polymorphisms of LEPR gene can increase the risk of the development of non-Hodgkin lymphoma in obese patients [38]. It also is suggested that the discussed polymorphisms are related to hemodynamic and metabolic disorders found in obese patients [39]. Overweight and obesity commonly observed after the management of childhood acute lymphoblastic leukemia also may have to do with the Gln223Arg polymorphism of LEPR gene [40].

Conflicts of interest: The authors declare no conflicts of interest in relation to this article.

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