

Leptin Receptors and Mechanism of Action

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Introduction

The spontaneous apparition of obesity mutant mice at the Jackson Laboratory (Bar Harbor, ME, USA) was a starting point for a long scientific journey, which climaxed in the discovery of leptin. Using these mutants in pioneer parabiosis experiments, DL Coleman proposed that “the *db/db* mutant mouse overproduced a satiety factor but could not respond to it—perhaps owing to a defective receptor—whereas the *ob/ob* mutant recognized and responded to the factor but could not produce it” (reviewed in [14]). He was right, as proved more than 40 years later when technical progresses in molecular biology allowed cloning the gene of leptin [54, 64] and, 1 year later, the gene of its receptor (LEPR) by virtue of leptin binding

[54, 64]. It was then established that LEPR is mutated in various strains of *db/db* mice [9, 29–31] and in the *fafa* Zucker and *fa^k/fa^k* Koletsky rats [47, 53]. These major breakthroughs were rapidly followed by the identification of rare congenital leptin deficiency and mutations in *LEPR* in subjects with strikingly similar early onset and massive obesity phenotypes as mutant mice [13, 40, 52]. In this chapter, we focus on the genetic abnormalities of *LEPR* gene with dramatic clinical consequences and difficult patient management. In most cases, however, obese patients do not bear mutations in genes related to leptin signaling, but often have abnormally elevated circulating concentrations of leptin. As such, they are considered leptin resistant. Despite a wealth of information on LEPR structure and function, deciphering the molecular mechanisms underlying resistance to leptin remains a challenge. This question is addressed in the first part of this chapter.

LEPR Structure and Mechanism of Action

LEPR Genomic Organization

The genomic organization of the *LEPR* gene is unusual and holds some degree of complexity (Fig. 2.1a). A proximal promoter (P1) produces two distinct transcripts through alternative splicing: *OB-R*, now referred as *LEPR*, and the Leptin Receptor Overlapping Transcript, *LEPROT* [1, 39].

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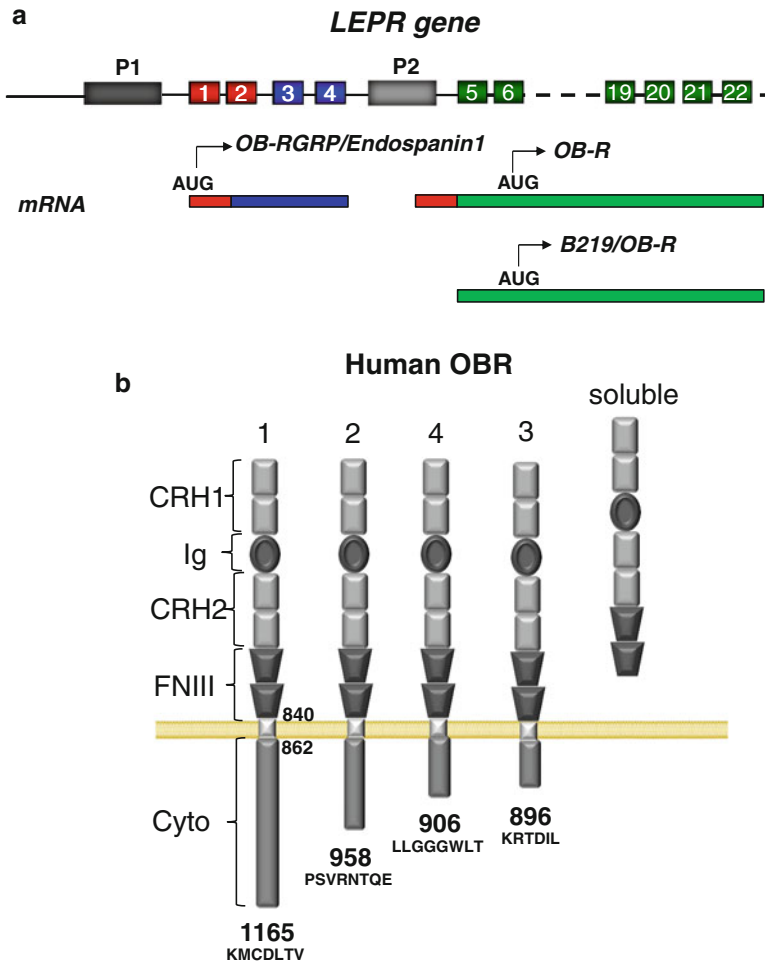


Fig. 2.1 *LEPR* gene and protein organization. **(a)** Schematic representation of the human *LEPR* gene on chromosome 1. *LEPR* gene expression is controlled by two promoters. The proximal promoter P1 generates two distinct transcripts (Leptin Receptor Overlapping Transcript (LEPROT) and *LEPR*) through alternative splicing, giving rise to two distinct proteins with no amino acid sequence homology (OB-RGRP/endospinin-1 and *LEPR* short and long isoforms, respectively). The alternative P2 promoter allows the expression of B219 OBR short isoforms. The AUG initiation codons on the mRNA

are indicated by arrows. **(b)** Leptin receptor isoforms. There are five different isoforms of *LEPR* in humans, generated from alternative splicing. All share identical extracellular domains but they differ in the length and sequence of the C-terminal domain. Four of the five isoforms have a transmembrane domain, while the shortest isoform is truncated by proteolysis before the membrane-spanning domain. *CRH* cytokine receptor homology domain, *Ig* immunoglobulin domain, *FNIII* fibronectin III domain. The amino acid sequence at the end of the C-terminal tail is indicated

This overlapping gene structure occurs in humans but is not observed in rodents where the two genes are found distinctly separated. The *LEPR* transcript encodes *LEPR* protein isoforms (see below), while *LEPROT* transcript encodes *LEPR* gene-related protein (OB-RGRP) also called endospinin-1. Endospinin-1 is a 4 transmem-

brane domain-containing protein of 14 kDa with no amino acid sequence in common with *LEPR*. *LEPR/B219* is a third transcript expressed from the leptin receptor gene using a second internal P2 promoter. This transcript generates different short *LEPR* isoforms (B219), which are expressed exclusively in hematopoietic cells.

LEPR Isoforms

LEPR belongs to the class I cytokine receptor family and exists in five different isoforms (Fig. 2.1b). Four of them share the first 862 amino acids and display a unique transmembrane domain contained in exon 16. These four isoforms are generated by alternative splicing and differ in the length and sequence of their intracellular carboxyl terminal domain. The fifth isoform, called “soluble” or “secreted” isoform, binds leptin and circulates in the blood stream, but contains no transmembrane domain. Contrary to rodents, the transcript for the soluble isoform does not exist in humans where the soluble LEPR is generated by proteolytic cleavage of the transmembrane isoforms [36]. The long LEPR isoform (isoform 1 or b) is the one with a full-length intracellular domain capable of activating all the known leptin-induced signaling pathways [10, 11, 54]. The function of the short isoform a (or isoform 3) is less understood and may be involved in leptin transport through the blood brain barrier and in leptin clearance [32, 56]. The specific functions of the other short isoforms are still poorly known.

All five LEPR isoforms contain an N-terminal extracellular domain, which is required for ligand binding. This domain is heavily N-glycosylated, with N-glycosylation level reaching up to 1/3 of the total molecular weight of LEPR [21]. The N-terminal part of LEPR is composed of two conserved cytokine receptor homologous domains, CRH1 and CRH2, separated by a conserved immunoglobulin (Ig) domain. Adjacent to the CRH2 domain two fibronectin type 3 (FNIII) domains are located proximal to the transmembrane region.

LEPR Mechanisms of Action

Cell Surface Localization

Leptin binds and activates LEPR at the cell surface. The majority of LEPR is localized in intracellular compartments, including endoplasmic reticulum, *trans*-Golgi apparatus, and endosomes, with only a minor (10–20 %) fraction of LEPR present at the plasma membrane [4]. This subcellular

distribution has been attributed to partial retention of the receptors in the biosynthetic pathway, coupled to removal from the plasma membrane via ligand-independent constitutive endocytosis [4]. Whether alteration in LEPR trafficking affects cell surface expression and, in turn, leptin signaling has been scarcely investigated. Experimental evidences suggest that Bardet-Biedl syndrome (BBS) proteins physically interact with LEPR. Moreover, the loss of BBS proteins in mice and in reconstituted cell systems perturbs LEPR trafficking and leptin signaling [15, 26, 50]. In other studies, the endospanin-1 protein encoded by the *LEPROT* transcript has been shown to negatively regulate LEPR cell surface expression by facilitating its lysosomal degradation. Silencing of the protein in mouse hypothalamic arcuate nucleus increases LEPR signaling capacity [15, 51, 57]. Regulation of LEPR trafficking by silencing these proteins or by disrupting their interaction with the receptor represents a possible strategy for improving leptin sensitivity.

Activation

Resonance Energy Transfer experiments in combination with biochemical and modeling studies started to unravel the molecular mechanism of LEPR activation. LEPR is thought to exist as pre-formed dimers or oligomers at the cell surface [60]. Binding studies in association with site-directed mutagenesis and homology molecular modeling suggest the existence of three different binding sites (I, II, and III) on leptin and support a 2:4 stoichiometry of the leptin–receptor complex [45, 61]. The CRH2 and Ig domains are involved in leptin binding and are required for receptor activation (Fig. 2.2a). Binding site II was shown to be indispensable for high affinity interaction between leptin and CRH2 domain [20, 24]. Engineered leptin mutants with an intact site II but modified site I or site III still bind to the CRH2 domain with strong affinity but are unable to activate the receptor [42, 46, 62]. Binding of two leptin molecules to LEPR triggers a conformational change within the extracellular domain and clustering of receptor dimers to form a hexameric complex (Fig. 2.2a) [45]. Recently, the architecture of the isolated extracellular region of the LEPR in the absence and

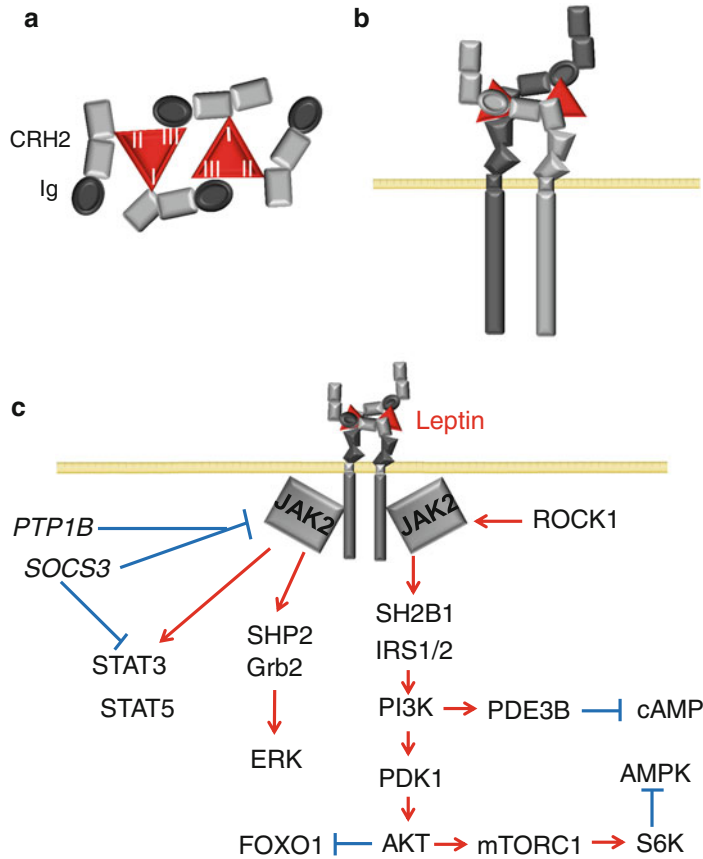


Fig. 2.2 LEPR activation and signaling. (a) Leptin–LEPR hexameric complex based on biochemical results and on molecular modeling. The complex contains two molecules of leptin and four LEPR protomers. Each leptin molecule binds three LEPR molecules through the high affinity binding site II (to CRH2) and via the two lower affinity binding sites I (to CRH2) and III (to Ig domain). (b) Model for the architecture of leptin/LEPR complex unraveled by single-particle electron microscopy [37].

Leptin employs only epitopes II and III to engage the CHR2 and Ig domain of 2 LEPR protomers, respectively. (c) Signaling pathways triggered upon leptin stimulation. Leptin binds to the long isoform LEPR b and activates ROCK1 and JAK2. JAK2 phosphorylates LEPR b on multiple Tyr, which interact with downstream signaling molecules and activate several signaling pathways. LEPR b signaling is regulated negatively by SOCS3 and PTP1B in a feedback loop

presence of leptin has been characterized by single-particle electron microscopy [37]. The results suggest that leptin and LEPR interact in a tetrameric complex with a 2:2 stoichiometry by engaging only leptin sites II and III (Fig. 2.2b). Thus, the role of binding site I and the overall stoichiometry of the leptin :receptor complex still remains elusive, most likely until we obtain structural information of the complex in its membrane-bound form. Additionally, leptin binding rigidifies the flexible hinge of CHR2 and favors a certain orientation of the FNIII domains that is required for LEPR activation [37, 62]. Based on our knowledge of the activation mechanism of other receptors, it is also conceivable

that changes in microenvironment (interaction with other proteins, lipid environment) or posttranslational (glycosylation) modifications of LEPR could lead to subtle impairment of activation contributing to leptin resistance.

Signaling Pathways

LEPR is devoid of intrinsic enzymatic activity and its signaling capacity relies on the associated Janus family tyrosine kinase JAK2 [3, 48] (Fig. 2.2c). Upon receptor activation, JAK2 phosphorylates three tyrosine residues in the cytoplasmic domain of the long LEPR -b isoform, leading to the recruitment of signaling proteins of the signal transducer

and activator of transcription (STAT) family. The STAT3 pathway is particularly critical and necessary for the effects of leptin on food intake and body weight [2, 41]. STAT3 is recruited to the LEPR-b and is phosphorylated by JAK2 allowing for STAT3 dimerization and translocation to the nucleus to mediate gene transcription. Other intracellular signaling pathways emanating from activated LEPR include the protein tyrosine phosphatase 2 (SHP2)/extracellular regulated kinase (ERK) pathway and the insulin receptor substrate (IRS)/phosphoinositide 3-kinase (PI3K) pathway. The suppressor of cytokine signaling 3 (SOCS3) is induced in response to leptin binding and, in turn, inhibits LEPR signaling via a negative feedback loop ([7, 48], [65]). Other important LEPR inhibitory factors are the protein tyrosine phosphatase-1B (PTP1B) [63] and the T cell protein tyrosine phosphatase TCPTP [35]. Cellular and mouse studies strongly support a role for these factors in leptin resistance at least in rodents [5–8, 35, 63]. Recently, it has been shown that Rho-associated coiled coil-containing protein kinase 1 (ROCK1) activity is required for leptin-induced activation of JAK2 [23]. This finding challenges the common belief that JAK2 is the first kinase activated by leptin. Reciprocal activation of JAK2 and RhoA/ROCK1 brings complexity to the system. Additionally, recent studies highlighted the importance of interacting proteins or lipids for LEPR signaling. Increased association of gangliosides to the LEPR upon leptin stimulation participates in LEPR activation [44]. Low-density lipoprotein receptor-related protein-1 (LRP1) was demonstrated to bind to the leptin–LEPR complex and was shown to be required for LEPR phosphorylation and STAT3 activation [34]. How these intertwined pathways coexist and act to trigger a normal or altered biological response remains elusive.

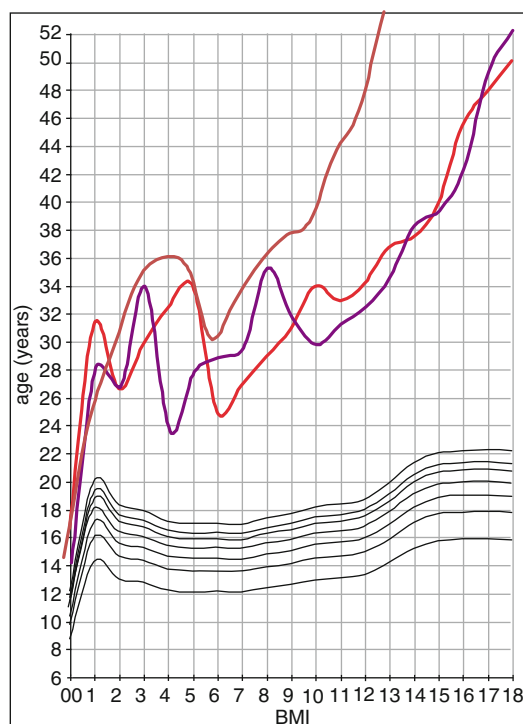
Genetic Abnormalities in Human LEPR

Mutations in LEPR

The first report describing a mutation in the LEPR in three obese subjects from a Kabilian consanguineous family was published in 1998,

3 years after the identification of the mouse gene. Affected individuals were homozygous for a mutation resulting in abnormal splicing of *LEPR* transcripts and generating a receptor lacking both transmembrane and intracellular domains [13]. Since then, several groups have identified LEPR mutations in extremely obese individuals. In 2007, Farooqi et al. sequenced the LEPR gene in 300 subjects with hyperphagia and severe early-onset obesity and estimated the prevalence of pathogenic *LEPR* mutations in this specific population around 3 % [19]. Other groups discovered LEPR mutations in single families or isolated individuals, such as in two cousins from Egypt [38]. Kakar et al. also identified a homozygous c.1603p5G>C mutation at the splice donor site of the exon 11-intron 11 junction [25]. This mutation led to out-of-frame skipping of exon 11 and a premature stop codon (p.Arg468Serfs33). Other family cases included the discovery of an homozygous *LEPR* sequence frameshift mutation predicted to generate a truncated protein from a premature stop codon in exon 14 [28]. At variance with other described cases, this mutation was particular in a sense that it was identified in a Caucasian proband with unrelated parents. The father was heterozygous carrier of the mutation and the mother was intriguingly carrying the normal wild type alleles of *LEPR*. Thus the patient inherited from two paternal copies of chromosome 1 (isodisomy). Another genetic anomaly was found in a 7-year old patient with a homozygous 80 kb deletion in the chromosomal 1p31.3 region. The deleted region also comprises the proximal promoter and exons 1 and 2 of the *LEPR* gene and exons 5–19 of the *DNAJC6* gene. The deletion results in the abolishment of *DNAJC6* transcript which encodes auxilin-1, a protein required for clathrin-dependent recycling of synaptic vesicles in neurons, but results also in the loss of *LEPROT* transcript. Whereas previously described mutations of the *LEPR* gene in humans concerned all transcripts, the deletion on *LEPR* gene of one of the proximal promoter with the preservation of the second internal promoter, only abolishes the canonical *LEPR* transcripts leaving *LEPR/B219* transcript fully functional [57]. Finally, whole-exome sequencing of 39 unrelated severely obese Pakistani children

Fig. 2.3 This picture illustrates the very rapid and severe weight gain observed in three French children with *LEPR* mutation [13]. The BMI curves from the mutation carriers are shown in colors, compared to the reference curves for French children



revealed two homozygous *LEPR* mutations (an essential splice site mutation in exon 15 (c.2396-1G>T) and a nonsense mutation in exon 10 (c.1675G>A) were discovered in two probands, who were phenotypically indistinguishable from age-matched leptin-deficient subjects from the same population [49].

Clinical Consequences of *LEPR* Homozygous Mutations

As in leptin-deficient subjects, patients carrying *LEPR* mutations show an early and dramatic increase in body weight, as illustrated by the weight curve of *LEPR* deficient subjects (Fig. 2.3). Evaluating body composition in some *LEPR* mutation carriers showed a large amount of total body fat mass (>50 %) but resting energy expenditure was generally related to body corpulence. Feeding behavior is characterized by major hyperphagia and ravenous hunger [33]. Associated with severe early-onset obesity, hypogonadotrophic hypogonadism and thyrotropic insufficiency are

frequently observed in *LEP* mutated subjects. It has also been described in some but not all subjects an insufficient somatotrophe secretion, associated with moderate growth delay. It is remarkable to mention that in some individuals with leptin deficiency either due to *LEP* or *LEPR* mutations, there is evidence of spontaneous pubertal development and in general improved endocrine functions with time. The follow-up of the first reported *LEPR* deficient sisters revealed a normalization of mild thyroid dysfunction and spontaneous puberty [43]. One of these patients was even able to give birth to a healthy son at the age of 26 years. Despite extreme obesity, no major clinical or metabolic complication occurred during pregnancy. This observation of a natural pregnancy in a woman with homozygous mutation of *LEPR* raises questions about the role of leptin signaling in human reproductive functions.

In general, analysis of heterozygous relatives showed that obesity was inconstant and not associated with endocrine abnormalities. An intermediate phenotype was described in rodents [12] and it was discussed that dysregulation of body weight

in the *LEPR*^{+/-} heterozygous state might arise from lower expression of the LEPR. If half of functional LEPR is not sufficient to prevent overweight and obesity, it could nevertheless reduce the severity of the phenotype. As described for *MC4R* or *POMC* genes, the penetrance of obesity due to the loss of one copy of *LEPR* gene can be incomplete. Variable clinical expression of the phenotype depends on the localization and the type of mutation, in relation with modulating environmental and genetic factors [18, 22]. Deeper exploration of a sufficient number of patients with one copy of *LEPR* gene is needed to address the relation between heterozygous LEPR mutation and obesity.

Management of Patients

Does the measurement of circulating leptin help in the diagnosis of *LEPR* mutations? Leptin levels were found extremely elevated in some but not all *LEPR* mutation carriers [13, 19, 40]. The specific nature of the first described *LEPR* mutation accounted for very high leptin levels, due to high circulating soluble LEPR shown to be able to trap circulating leptin [27]. Since serum levels of leptin were not disproportionately elevated in patients with other mutation types, it is estimated that serum leptin cannot be used as a marker for leptin-receptor deficiency. Thus, *LEPR* gene screening might be considered in subjects with the association of severe obesity and endocrine dysfunctions but with leptin related to corpulence or moderately elevated [19].

As with most patients with genetic obesity, with the notable exception of leptin-deficient subjects, care of patients with LEPR mutations is difficult and frequently unsuccessful. Factors that could possibly bypass leptin delivery systems are being developed but are not yet available for the treatment of these patients. A first example came with the ciliary neurotrophic factor (CNTF). CNTF activates downstream signaling molecules, such as STAT3, in the hypothalamus area that regulates food intake, even when administered systemically. Treatment with CNTF in humans and animals, including *db/db* mice induced substantial loss of fat

mass [59]. The neurotrophic factor, Axokine, an agonist for CNTF receptor, was under development by the Regeneron Company, for the potential treatment of obesity and its metabolic associated complications. But the phase III clinical trials were stopped due to development of antibodies against Axokine in nearly 70 % of the tested subjects after approximately 3 months of treatment. In addition, Axokine treatment had a small positive effect [16].

Regarding other drug therapies, MC4R located downstream of the LEPR is becoming an attractive candidate drug target. Synthetic ligands and small molecule MC4R agonists have been in vitro tested with variable results [17, 58]. But they have to face specific difficulties of possible side effects due to the widespread expression of MC4R in the brain and the already demonstrated role of MC4R in erectile function [17, 58]. Novel pharmacological MC4R agonists are currently under development. Whether patients with mutation in the *LEPR* gene could benefit from this treatment might be considered.

Finally, bariatric surgery is considered as a long-term efficient treatment for severe obesity [55]. The question of such treatment and its potential efficiency is crucial in patients with monogenic obesity. Currently, data on bariatric surgery in patients with genetic obesity are limited and controversial. In one LEPR-deficient patient, vertical gastropasty was beneficial and sufficient to induce and maintain a 40-kg weight loss (-20 % of the initial weight) over 8 years of regular follow-up [28]. In contrast, a relative failure of surgical therapy was illustrated in a report of rapid weight regain 1 year after bypass in another *LEPR* deficient morbidly obese woman (i.e., the first described patient) [28]. But this patient of low socioeconomic status had extreme difficulties after postsurgical counselling. She was noncompliant with the recommendations provided in this type of purely restrictive surgery and her medical follow-up was very irregular. This report illustrated the important role of environment on the benefice of bariatric surgery especially in case of monogenic obesities or underlined the poor efficiency of bariatric surgery in these patients.

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