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Minireview

Leptin: From structural insights to the design of antagonists

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ABSTRACT

After its discovery in 1994, it soon became clear that leptin acts as an adipocyte-derived hormone with a central role in the control of body weight and energy homeostasis. However, a growing body of evidence has revealed that leptin is a pleiotropic cytokine with activities on many peripheral cell types. Inappropriate leptin signaling can promote autoimmunity, certain cardiovascular diseases, elevated blood pressure and cancer, which makes leptin and the leptin receptor interesting targets for antagonism. Profound insights in the leptin receptor (LR) activation mechanisms are a prerequisite for the rational design of these antagonists. In this review, we focus on the molecular mechanisms underlying leptin receptor activation and signaling. We also discuss the current strategies to interfere with leptin signaling and their therapeutic potential.

Contents

Abbreviations: AMPK, 5′-AMP-activated protein kinase; BRET, bioluminescence resonance energy transfer; CNTF, ciliary neurotrophic factor; CRH, cytokine receptor homology; EAE, experimental autoimmune encephalomyelitis; EM, electron microscopy; Epo, erythropoietin; ERK, extracellular signal-regulated kinase; FAK, focal adhesion kinase; FRET, fluorescence resonance energy transfer; FN III, fibronectin type III; G-CSF, granulocyte-colony stimulating factor; gp130, glycoprotein 130; Grb2, growth factor receptor-bound protein 2; ICT, isothermal titration calorimetry; IGD, immunoglobulin-like domain; IL, interleukin; iNKT, invariant natural killer T; IRS, insulin receptor substrate; JAK, Janus kinase; LIF, leukemia inhibitory factor; LPA, leptin peptide antagonist; LR, leptin receptor; LRlo, LR long form; LRsh, LR short form; MAPK, mitogen-activated protein kinase; OSM, oncostatin M; PDE3B, cyclic nucleotide phosphodiesterase 3B; PDK1, 3-phosphoinositide-dependent protein kinase 1; PI3K, phosphatidylinositol 3-kinase; PIP3, phosphatidylinositol 3,4,5-triphosphate; PLP, proteolipid protein peptide; SAXS, small-angle X-ray scattering; SHP2, SH2-containing protein tyrosine phosphatase 2; SPR, surface plasmon resonance; STAT, signal transducer and activator of transcription.

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1. Introduction

Leptin is best known for its dramatic effect as a satiety signal, since mouse strains lacking leptin signaling components are hyperphagic and obese [54]. The hormone is mainly, but not exclusively, produced by adipocytes and its serum levels positively correspond with the energy stored in the fat mass [23,39,54]. Leptin functions as a negative feedback adipostat or an efferent satiety signal by activation of the LR in the feeding centers of the hypothalamus [120]. Loss-of-function mutations in the leptin or LR genes [18,20,84,139], or genetic ablation of leptin's central signaling [127,131] results in obesity and increases the risk of obesity-related conditions like type 2 diabetes [57].

Ten years after its initial discovery, it became clear that leptin is more than a satiety signal, and rather acts as a 'metabolic switch' by connecting the body's energy stores to high energy demanding processes like immunity and reproduction [35,77]. Indeed, leptin or LR deficiency not only causes severe obesity, but also abnormalities in lipid and glucose metabolism [40], hematopoiesis [6], innate and adaptive immunity [13,34,137], reproduction [17], angiogenesis [117], vascular remodeling [65], blood pressure [75], and bone formation [30]. Furthermore, being overweight or obese is a major risk factor for several types of cancer including prostate [42], breast [19], colorectal [95], renal cancer [70] and myeloma [47].

2. Leptin

The first obese mouse arose by chance in a colony at the Jackson Laboratory in 1949 [59]. A series of parabiosis experiments illustrated that these *ob*/*ob* mice are deficient for a blood-borne factor that regulates feeding and metabolism [21,22]. It took over 40 years before Friedman and colleagues positionally cloned the *ob* gene and demonstrated that it encodes for a hormone that they called leptin (after the Greek 'leptos' for thin) [139]. Administration of recombinant leptin to *ob*/*ob* mice dramatically decreased food intake and increased energy expenditure and weight loss [54,110]. Besides fat tissue, low leptin expression could be shown in placenta, stomach, mammary epithelium and skeletal muscle [2,113,126].

2.1. Structure of leptin W100E

Mature leptin is a non-glycosylated 16 kDa protein of 146 Aa. The crystal structure has been solved at 2.4 Å resolution [140]. Since purified human leptin tends to aggregate extensively, the leptin W100E mutant with increased solubility and full biological activity was used in this study. The hormone adopts the four helical bundle cytokine structure with four anti-parallel helices (A, B, C and D) in an up–up–down–down arrangement, two long crossover loops AB and CD (the latter containing a distorted helix of 9 Aa), a short BC link and a 5 Aa kinked helix part of helix D. The crystal structure shows no electron density for the region between residues T27 and G38. Leptin has two conserved cysteine residues (C96 in the CD loop and C146 as the C-terminal residue), which are involved in a solvent exposed disulfide bridge that positions the C-terminal part of helix D to the CD loop. This tethering is essential for structural stability and therefore biological activity [53,103].

Despite low sequence similarity, the inter-helical angles and the characteristics of the crossover loops found in leptin W100E resemble these found in the structures of granulocyte-colony stimulating factor (G-CSF) and the interleukin-6 (IL-6) related cytokines, including IL-6, leukemia inhibitory factor (LIF) and ciliary neurotrophic factor (CNTF). Based on these structural characteristics, leptin is classified as a long-chain cytokine (http://scop.mrc-lmb.cam.ac.uk/scop/data/scop.b.b.dj.b.b.html).

3. Leptin receptor

The LR was first cloned from a mouse choroid plexus cDNA library using an expression cloning strategy by Tartaglia and colleagues [120]. The full-length receptor is 1162 residues long divided in three regions: an extracellular part, a single pass helix trans-membrane domain and an intracellular part. Up to now, six LR isoforms produced by alternative splicing or proteolytic ectodomain shedding [129] have been identified: LRa-f. All these isoforms, except LRe, have an identical extracellular and trans-membrane domain, but differ in the length of their intracellular tail. LRb, also referred to as LR long form (LRlo), has an intracellular domain of 302 Aa and is the only LR isoform capable of efficient signaling. This isoform is highly expressed in specific nuclei of the hypothalamus [36,80,112], a region of the brain that is known to be involved in regulating body weight. However, LRb expression could be shown in a broad range of other cell types, thereby explaining the pleiotropic effects of leptin. LRa (or also referred to as the LR short form, LRsh), LRc, LRd, and LRf have only short (30 to 40 residues) intracellular tails and unique C-termini. It has been suggested that these LR isoforms might have a role in transport of leptin over the blood–brain barrier [58], or renal clearance of the hormone [120]. Finally, the soluble LR variant, LRe, is believed to modulate the leptin bioavailability [43].

3.1. Architecture of the LR extracellular domain

The LR shares highest sequence and structural similarity with the G-CSF receptor and the glycoprotein 130 (gp130) receptor family, including gp130 itself, the LIF and oncostatin M (OSM) receptors [133]. All these receptors belong to the class I cytokine receptor family, which typically contain a so-called cytokine receptor homology (CRH) domain in its extracellular domain. This structure consists of two 100 Aa barrel-like domains, with two conserved disulfide bridges and a WSXWS motif characteristic for respectively the N- and C-terminal parts. The LR contains two such CRH modules (CRH1 and CRH2), separated by an immunoglobulin-like domain (IGD), two membrane proximal fibronectin type III (FN III) domains and a 100 Aa N-terminal domain (NTD) with no sequence similarity to any known proteins.

The LR is heavily N- (and to a lesser extent O-) glycosylated, accounting for 30–70 kDa increases in apparent molecular mass when expressed in eukaryotic cells [56]. Only 2 of the 20 putative N-linked glycosylation sites were found not to be glycosylated. Distribution of these N-glycosylation sites across different domains are as follows: 6 sites in the NTD, 3 in CRH1, 2 in IGD, 2 in CRH2 and 5 in the FN III domains. This N-glycosylation is necessary for optimal leptin binding [62]. Haniu and colleagues showed that of the 28 cysteine residues found in the human LR, 18 were involved in an intramolecular disulfide bridge, organized in in two clusters [56].

4. LR signaling

Like all class I cytokine receptors, the LR has no intrinsic kinase activity, and uses cytoplasmic associated Janus (JAK) kinases for intracellular signaling. These kinases associate with a well-conserved membrane-proximal proline-rich box1 and a less well-defined box2 motif in the receptor [3,64]. Short LR variants lack this box2 motif, which might explain the inefficient JAK activation by these receptors. The LR predominantly activates JAK2 [64], although JAK1 activation has also been demonstrated in certain experimental set-ups [14]. JAK2 activation allows LR signaling via the JAK–STAT (signal transducer and activator of transcription), SHP2-MAPK (SH2 containing protein tyrosine phosphatase 2-mitogen-activated protein