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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

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kinase), PI3K (phosphatidylinositol 3-kinase) and AMP-activated protein kinase (AMPK) pathways [for an extensive review see [127]].

In a well-accepted model for JAK–STAT signaling, ligand induced receptor activation leads to cross-phosphorylation and activation of JAKs, phosphorylation of cytoplasmic receptor tyrosine residues and the recruitment of signaling molecules like STATs. STATs themselves become a JAK substrate and translocate as phosphorylated dimers to the nucleus to modulate transcription of target genes. The STAT molecule primarily involved in leptin signaling is STAT3 [123], but also activation of STAT1, STAT5 and STAT6 could be shown in cultured cells [5,46,105].

The LR can activate the extracellular signal-regulated kinase 1/2 (ERK1/2) MAPK via the SH2-containing protein tyrosine phosphatase 2 (SHP2) and growth factor receptor-bound protein 2 (Grb2) [4,14,69]. This pathway leads to up-regulation of the immediate early genes *egr-1* and *c-fos* in the hypothalamus [10,26,32].

Several members of the insulin receptor substrate (IRS) family allow leptin induced PI3K activation and phosphatidylinositol 3,4,5-triphosphate (PIP3) production [9,28,63,128]. This in turn leads to the activation of 3-phosphoinositide-dependent protein kinase 1 (PDK1), Akt and cyclic nucleotide phosphodiesterase 3B (PDE3B), a cAMP-degrading enzyme [141].

Finally, leptin can stimulate or inhibit 5′-AMPK activity in a tissue-specific manner [81,82,122].

5. LR activation

5.1. Leptin independent LR oligomerisation at the cell surface

The existence of signaling-inactive, pre-formed receptor complexes on the cellular surface in the absence of ligands has been shown for several cytokine receptors. Examples include the erythropoietin (Epo) receptor [24,72,102], the growth hormone (GH) receptor [12,44], and the IL-6 receptor [111]. Two different experimental set-ups proved that this is likely also true for the leptin receptor: co-immunoprecipitation of differently tagged LRs [1,130,135] and the relatively high basal signal in both fluorescence resonance energy transfer (FRET) and bioluminescence resonance energy transfer (BRET)[1,7,25]. In these experiments, both homo- and heterodimerization of LR isoforms could be shown. The increase in BRET and FRET signals upon leptin treatment is probably a result of reorganization within the pre-formed complexes and/or de novo oligomerization [7,25].

This type of ligand-independent clustering could also be illustrated in isolated plasma membranes [25] and was suggested to occur in solution [27,135]. However, soluble LR ectodomain variants or the isolated CRH2 domain were detected as monomers in more recent electron microscopy (EM) and small-angle X-ray scattering (SAXS) experiments [15,73,83].

5.2. Leptin as a trivalent ligand

The structural and evolutionary relationship between the leptin, G-CSF and IL-6 related cytokine receptor systems allowed the identification of three potential receptor-binding sites (I, II and III). In the hexameric 2:2:2 IL-6:IL-6Rα:gp130 complex, IL-6 interacts via site II with the IL-6Rα; while sites I and III are used to recruit two gp130 receptors via their CRH or IGD domain [11]. G-CSF on the other hand uses only sites II and III to interact with CRH or IGD of two cognate receptors; thereby forming a tetrameric 2:2 assembly [67].

We evaluated the effect of 31 mutations in mouse leptin on binding to and signaling through the LR [96]. Mutations in binding site I (located at the C terminus of helix D) only moderately affect binding and signaling. In the related receptor systems, IL-6 site I residues interact with the gp130 CRH domain, while this site is not used in the G-CSF complex.

Like in G-CSF and IL-6, leptin binding site II residues (at the surface of helices A and C) are crucial for binding to the LR CRH2 domain, but mutations in this region have only limited effect on signaling. Residues R20 and Q75 flank this binding site and their mutation drastically affects binding and LR activation. As these residues likely interact with each other, these mutations could affect the structure and/or stability of the protein. The D23L mutation results in a 60-fold increase in affinity of leptin for the CRH2 domain [116].

Ligand site III has been found to be essential for interaction with the IGD of a shared receptor like in the IL-6 system [11] or that of a cognate receptor as in the G-CSF receptor [119], enabling the ligand to induce necessary conformational changes in the receptor. The precise position and nature of leptin's site III remains a matter of debate. Our mutagenesis study identified residues located at the N terminus of helix D, CD and AB loops, with S120 and T121 as the most important [96]. On the other hand, Niv-Spector et al. used a sensitive, bi-dimensional hydrophobic cluster analysis to identify a hydrophobic stretch in leptin's AB loop (39-LDFI-42) as part of the binding site III [85]. Mutation of both putative sites III results in potent antagonists both in vitro and in vivo [137]. Three alternative models can be proposed to explain how both regions can contribute to binding site III. First, it cannot be excluded that both motifs are part of a larger binding site III. Residue R128 may also locate to this larger site, since its mutation affects signaling but not binding [125]. However, modeling with this larger site requires inter-domain angles that deviate substantially from those found in any of the related cytokines [98]. Second, Niv-Spector and colleagues suggested that the 39-LDFI-42 motif interacts with the LR sequence 325-VFTT-328 [85]. However, modeling shows that this strand is part of the LR's CRH1 and not IGD domain. Once bound with residues 39-LDFI-42 to the CRH1 domain, leptin still could interact with the IGD via the site containing S120-T121. Third and finally, residue F41 was identified as a site I residue in our mutagenesis study [96], suggestion that the 39-LDFI-42 motif may interact as a site I with the CRH2 domain of an additional receptor in an activated LR complex.

5.3. The CRH2 domain as the leptin binding determinant

Binding of leptin to the soluble ectodomain of the LR has been characterized in several studies and using different techniques including isothermal titration calorimetry (ICT) and surface plasmon resonance (SPR). Obtained K_D values ranged from 0.2 to 15 nM in a 1:1 ratio (reviewed in [45,98]). The affinity of leptin for the membrane-bound receptor seems to be somewhat lower: 0.2 to 1.2 nM [45,98].

Fong and colleagues used a series of LR deletion variants to show that the CRH2 domain is the major leptin-binding determinant in the receptor [38]. This is further supported by the observation that the affinity of this domain is comparable to that of the complete ectodomain [38,107].

The crystal structure of the CRH2 domain in complex with an antibody Fab fragment has been determined at 1.95 Å resolution, and is until now the only high-resolution structure for the LR (PDB ID: 3V6O; [15]). This domain has a typical CRH domain structure: two FN III-like subdomains each consisting of an IGD beta-sandwich fold with seven strands in two layers. As in most CRH domains, the C-terminal domain contains a typical WSXWS motif (622-WSXWS-626), while the N-terminal domain is characterized and stabilized by three disulfide bridges.

This crystal structure combined with previous mutagenesis studies identified a region of four hydrophobic residues (503-IFLL-506) located in the EF loop as crucial for leptin binding [15,60,86]. Homology based modeling suggests that this hydrophobic patch interacts with the hydrophobic cleft (L13, L86, L89, and F92) in A–C helix in leptin [60]. CRH2 residues L505 and L506 fit into this hydrophobic cleft and interact with L13 and L86 in leptin. Y472 contributes to binding by interaction with the leptin residues L86, V89 and V6. Residues in the JK loop of the second CRH2 subdomain (F563, E565 and N566) are predicted to interact with leptin helix A [98]. In the crystal structure, the JK loop adopts two different conformations, and this conformational flexibility may suggest that leptin binding to CRH2 involves an induced fit mechanism [15]. Finally, structural insights in the leptin:CRH2 binding could allow

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virtual docking screening for compounds with potential agonistic or antagonistic potential [121].

5.4. The IGD and its interaction with leptin binding site III

There is no evidence that leptin directly binds to the IGD, but this domain is nonetheless crucial for LR activation. Deletion of this domain in the membrane anchored receptor completely abolishes signaling [38,134]. The IGD has a similar function in the G-CSF and IL-6 receptor systems, where it interacts with the site III of a ligand bound to another receptor (a second G-CSFR or IL-6Rα). Based on this homology, we identified IGD residues L370, A407, Y409, H417 and H418 in a conserved surface patch in the β-sheet formed by β-strands 3, 6 and 7 as the center of the leptin-binding site [97]. This area shows a positive electrostatic surface potential which may interact with negative electrostatic surface potential of the region that is S120-T121 at the tip of helix D in leptin.

5.5. The membrane-proximal FN III domains

The LR has two FN III domains, while the receptors for G-CSF and IL-6 related cytokines contain three of these domains. LR FN III domains also lack any binding affinity for leptin, but are once again indispensable for receptor activation [38,135]. A deletion variant with an extracellular part consisting of only these domains constitutively (i.e. independent of leptin) activates signaling. This illustrates that these domains can orientate the cytoplasmic tails in such a manner that signaling becomes possible [135]. Furthermore, combined mutation of the two conserved and free FN III cysteine residues (C672 and C751) in the mouse LR completely abolishes signaling [135]. Similarly, mutation of the CRH2 C604 residue drastically affects LR signaling [83]. A role for free cysteine residues in LR activation could also be concluded from the observation that iodoacetamide treatment interferes with the complex formation in solution [83]. Superposition of the LR CRH2 crystal structure and FN III models on the gp130 crystal structure brings residues C604 and C672 sufficiently close to allow the formation of an additional intramolecular disulfide bridge [98].

5.6. The CRH1 and NTD domains

Like the LIF receptor, the LR has an additional CRH domain. In contrast to CRH2, this CRH1 domain has no detectable affinity for leptin and is not strictly required for signaling. Deletion of this domain only reduces the intensity of signaling, which might suggest that this domain allows additional clustering of the receptor [38,134]. However, the role of the CRH1 domain should not be underestimated. First, the *Fa*/*Fa* (Q269P) mutation in this domain causes the obese phenotype of fatty Zucker rats [118]. In a CRH1 homology model, this Q269 residue is in close proximity of the WSXWS motif, and mutation could affect the structure of this domain [98]. Second, the naturally occurring single nucleotide polymorphism Q223R causes obesity in Brazilian multiethnic subjects [29] and increased susceptibility towards protozoan infections in children [31].

No structural or functional information is currently available for the NTD domain.

5.7. LR higher order clustering

We used a JAK-STAT complementation strategy to show that the LR can form higher order clusters (i.e. more than two receptors per activated complex) [134]. In this study, two signaling deficient LR variants (one lacking cytoplasmic tyrosine residues, the other deficient in JAK activation) were only able to signal via the JAK–STAT pathway when they were co-expressed. Given the intrinsic needs for type of signaling, a plausible explanation is to assume higher order clustering. When the extracellular part of the mutants was replaced with that of the strictly dimeric Epo receptor, no complementation of signaling occurred. This higher order clustering could explain why the signaling by LRlo is only marginally susceptible to the presence of excess dominant negative by LRsh [130]. Selective sorting of long and short LR variants could be an alternative or additional explanation [130].

5.8. Model and low-resolution structures of the leptin:LR complex

We compared all available structural, biophysical and mutagenesis data for the LR with structural insights in the mechanisms of G-CSF and IL-6 receptor activation. This led to a model for a 2:4 leptin:LR complex, where two leptin molecules interact with the CRH2 of a two LRs via site II and with the IGD of third and fourth receptors [98]. In this model, the LR CRH2 domains come closer to each other when compared to the G-CSF and IL-6 complexes, likely a consequence of the shorter leptin helices.

The multidomain architecture and the extensive glycosylation of the LR make it technically challenging to determine the structure of the leptin:LR complex by X-ray crystallography. However, two recent studies yielded some structural insights using the low-resolution techniques' single particle EM and SAXS. Negative-stain EM was applied to generate 2D images and 3D reconstructions of the 2:2 leptin:LR quaternary complex, which likely represents the core-signaling configuration [73]. The structures showed significant flexibility in the hinge region between the N- and C-terminal CRH2 subdomains, which is rigidified upon leptin binding thereby orienting the FN III domains into a “leg like” single conformation. In this model, site III of CRH2 bound leptin (via site II) interacts with the IGD of a second receptor in a cooperative manner. No function for the CRH1 (and NTD) could be identified in this study. A similar 2:2 quaternary, core-signaling complex has been proposed based on SAXS experiments [83]. This study also showed that both site III mutants (leptin S120A-T121A and leptin 39-AAAA-42) in complex with the LR ectodomain only form a 1:1 complex, thereby illustrating the importance of these sites in the assembly of the 2:2 complex.

It is of note that higher order LR clustering and the ligand independent LR dimerization were not observed in either of these studies. At this point we cannot exclude the possibility that additional LR:LR interactions (e.g. between FN III domains) occur on the cellular membrane leading to 2:4 or 4:4 leptin:LR complexes.

6. Leptin and LR antagonists

Not only are profound insights in the LR activation mechanisms a prerequisite for the rational design of leptin and LR antagonists, the evaluation of these antagonists can help to further unravel these mechanisms. These agents could have therapeutic value in uncontrolled immune responses in autoimmune diseases, cancer, elevated blood pressure, and certain cardiovascular diseases. At present, four strategies are being used to antagonize leptin signaling: (i) leptin antagonistic mutants; (ii) leptin peptide antagonists; (iii) leptin and LR specific (monoclonal) antibodies or nanobodies; and (iv) soluble LR variants.

6.1. Leptin mutants

Leptin antagonists should be able to compete with endogenous leptin without activating the receptor. Before any structural insights in the LR activation mechanisms, Verploegen and colleagues developed the first leptin antagonist [125]. They could show that human leptin R128Q blocks leptin activity, but not binding, in transfected Ba/F3 cells and that in vivo administration resulted in weight gain and hyperinsulinemia. The antagonistic properties of this substitution appear to be species-specific since similar mutations in ovine and chicken did not result in antagonism [101].

Leptin site III mutants have potential as antagonists. As mentioned earlier, the precise localization and nature of this site remains controversial. Both our leptin S120A-T121A [97] and the leptin 39-AAAA-42 mutant [85] bind with an affinity comparable to wild type leptin, but fail to induce LR clustering in solution [83]. Both muteins behave as potent antagonists in vitro and in vivo [137]. The half-life in circulation and

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thus efficiency when applied in vivo could be increased by pegylation [33] and the antagonistic capacity of the 39-AAAA-42 mutant could further be dramatically increased by an additional D23L substitution [87,116].

6.2. Leptin peptide modulators

Leptin peptide modulators are short peptides derived from original leptin sequences that can act as potent agonists or used as antagonists [reviewed in [68]]. The first 35 Aa leptin-derived peptide, OBGRP 22–56, was designed in 1996 by Samson et al. [106]. The authors showed that it is a potent leptin agonist since administration in rats resulted in inhibition of food intake and weight loss. In vitro, OBGRP 22–56 enhances proliferation of both normal myometrium and myoma cells [76].

Grasso and colleagues designed six peptides corresponding to residues 106–167 of leptin [51]. This sequence was chosen based on the observation that the naturally occurring *ob*-mutation (arginine to a premature STOP codon on position 105) results in an inactive protein. They showed that one of these, LEP-(116–130), was effective in reducing food intake, body weight and glucose levels in leptin deficient animals [52]. Follow-up in vitro experiments and studies in *db*/*db* mice showed that these effects do not require interaction with the LR long form. Nonetheless, LEP-(116–130) was proven to promote luteinizing hormone (LH) and prolactin (PRL) secretion in fasted animal rats [49].

Based on the suggested role of helices A and C in leptin, Gonzales and colleagues designed two leptin peptide antagonists (LPAs) corresponding to amino acids 3–34 (LPA-1) and 70–95 (LPA-2) of human leptin [48]. LPA-2 has the alpha-helical structure found in the native leptin molecule, binds specifically and with high affinity to the LR and potently inhibited leptin-dependent effects in rat endometrial cells [48]. A pegylated variant of the peptide attenuated leptin-induced growth of mouse mammary tumor cells in immunodeficient mice [50]. The precise mechanism of action of these peptides remains elusive.

An alternative design of LPAs was described by Otvos et al. They synthesized four proposed receptor-binding fragments (site I, IIa, IIb, III, and combinations thereof) and tested their effect on cellular proliferation. Agonistic/antagonistic properties greatly depended on the presence of leptin in the assay [90]. A glycosylated site III variant proved to be very effective in vivo: it reduced weight and restored fertility in high-fat diet-induced obese mice [66], clearly suppressed the growth of human breast cancer xenografts [91,92], and reduced the extent of joint swelling and the number of arthritic joints in both mild and more aggressive rheumatoid arthritis models [93].

6.3. Leptin and LR antibodies and nanobodies

Neutralizing antibodies directed against a ligand or its receptor are a classical and effective way to interfere with cytokine signaling. Anti-leptin or anti-LR antibodies were shown to be powerful tools to study the role of leptin in multiple sclerosis. In experimental autoimmune encephalomyelitis (EAE)-susceptible mice, administration of an anti-leptin antibody improved the clinical outcome, slowed disease progression, reduced disease relapses and inhibited the antigen-specific T cell proliferation in proteolipid protein peptide (PLP)-induced EAE [78,104,108]. Similarly, antibody neutralization inhibited leptin-mediated T_H17 responses in lupus-prone mice [132]. Finally, a neutralizing LR antibody clearly improved hemodynamic parameters in a rat coronary artery ligation model for myocardial infarction [99].

Nanobodies, the cloned and isolated variable domain of the heavy-chain antibodies uniquely found in members of the *Camelidae* family [55,71], have become a valuable alternative for classical antibodies. We could show that administration of nanobodies directed against the LR CRH2, IGD or FN III domains increased body weight, body fat content, food intake, liver size and serum insulin levels [136]. A CRH2-specific nanobody exacerbated concanavalin A (Con A) induced hepatitis in wild type mice but not in invariant natural killer T (iNKT) cell deficient animals [124]. Finally, local administration of the same nanobody at low dose adjacent to a tumor decreased tumor mass with no visible effects on body weight or food intake in a mouse model of melanoma [79].

6.4. Soluble LR variants

Soluble LRs compete for leptin binding with the membrane bound receptor. Administration of the LR:Fc fusion protein ameliorated the clinical outcome of PLP-induced EAE [104], and protected against sepsis-mediated morbidity and mortality [114].

7. Future perspectives: uncoupling of leptin physiologic functions

Leptin is a pleoptropic hormone with both beneficial and undesired effects. In some physiological or pathological situations like uncontrolled immune responses in autoimmune diseases, tumorigenesis, elevated blood pressure, and certain cardiovascular diseases, it is desirable to selectively block leptin activity. The use of current leptin and LR antagonists is hampered by the unwanted weight-gain (10–15% per week in rodent models) upon treatment. An ideal leptin (or LR) antagonist would therefore only interfere with peripheral signaling, but not with the central weight regulating effects.

Three studies illustrate the possibility to uncouple leptin physiologic functions. First, loss-of-function mutations in the leptin or LR genes have been linked to infertility in humans and rodents [17,20]. However, Nizard and colleagues reported the pregnancy of a morbidly obese patient with a rare homozygous LR mutation, which was shared by several affected siblings [20]. Despite neonatal hypoglycemia, the child's growth and development have been normal [88]. Second, a L72S mutation in the leptin gene was identified in a 14-year-old child of non-obese Austrian parents [37]. Immunohistochemical analysis showed that this substitution does not affect expression in adipose tissue but the hormone was retained within the cells. The child showed signs of a hypogonadotropic hypogonadism, but in contrast to previous studies only mild obesity (BMI of 31.5) and a normal T cell responsiveness. Third, we could recently demonstrate a similar uncoupling of leptin's metabolic and immune functions in mice [138]. *Fatt*/*fatt* mice carry a spontaneous splice mutation that causes deletion of the complete IGD in all LR isoforms. These animals are hyperphagic and morbidly obese, but, in contrast to other leptin or LR deficient rodents, display only minimal changes in size and cellularity of the thymus. Furthermore, *fatt*/*fatt* mice respond comparable to wild type animals to concavalin A in a model for autoimmune hepatitis. In line with this, treatment of healthy mice with a IGD-specific neutralizing nanobody induced weight gain and hyperinsulinemia, but failed to block development of experimentally induced autoimmune multiple sclerosis, arthritis and hepatitis [138].

Accumulating evidence shows that LR signaling is not strictly dependent on JAK activation. In a first report, Mansour and colleagues showed that the LR is expressed in most cells of the thymus of Wistar rats and that chronic leptin treatment reduced thymic apoptosis [74]. This effect could however not be inhibited by a common JAK2 inhibitor, but appeared sensitive to PI3K inhibition. The same JAK2 inhibitor was also ineffective in blocking focal adhesion kinase (FAK) phosphorylation in several human colon carcinoma cell lines [100]. Finally, leptin was shown to be able to phosphorylate STAT3 and increase viability of human and mice JAK2-null cells [61].

At this point, it is unclear how uncoupling of leptin's physiological functions and/or discrimination between signal cascades and is achieved. Alternative leptin:LR complex formation or trans-activation by other receptor systems could be a valuable explanation. There is currently no experimental evidence for this first hypothesis. Cross-talk of the LR with the insulin-like growth factor-I receptor [94,109], epidermal growth factor receptor [16,89,115] or the estrogen receptor-alpha [8,41] has been described.

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8. Conclusions

The crystal structure of the leptin:LR complex has not been determined. However, detailed mutagenesis studies, homology modeling and low-resolution EM and SAXS leptin:LR structures already provided valuable insights in the mechanism of LR activation. This knowledge allowed the design of several types of leptin and LR antagonists with proven efficacy both in vitro and in vivo. However, several important aspects remain unclear: what is the function of the CRH1 or the NTD domains? What is the exact nature of leptin binding site III or the role of a putative binding site I? How does leptin-induced clustering of the LR engage signaling? Determination of high resolution structures for other domains of the LR or leptin:LR complexes will help to answer these questions. Furthermore, more profound structural insights in the LR activation mechanisms and a better characterization of cell-specific LR cross-talk with other receptors might lead to the design of selective antagonists that allow uncoupling of central and peripheral functions of leptin.

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References

[1] J. Bacart, A. Leloire, A. Levoye, P. Froguel, R. Jockers, C. Couturier, Evidence for leptin receptor isoforms heteromerization at the cell surface, FEBS Lett. 584 (2010) 2213–2217.
[2] A. Bado, S. Levasseur, S. Attoub, S. Kermorgant, J.P. Laigneau, M.N. Bortoluzzi, L. Moizo, T. Lehy, M. Guerre-Millo, Y. Le Marchand-Brustel, et al., The stomach is a source of leptin, Nature 394 (1998) 790–793.
[3] G. Bahrenberg, I. Behrmann, A. Barthel, P. Hekerman, P.C. Heinrich, H.-G. Joost, W. Becker, Identification of the critical sequence elements in the cytoplasmic domain of leptin receptor isoforms required for Janus kinase/signal transducer and activator of transcription activation by receptor heterodimers, Molecular Endocrinology 16 (2002) 859–872 Baltimore, MD.
[4] A.S. Banks, S.M. Davis, S.H. Bates, M.G. Myers, Activation of downstream signals by the long form of the leptin receptor, J. Biol. Chem. 275 (2000) 14563–14572.
[5] H. Baumann, K.K. Morella, D.W. White, M. Dembski, P.S. Bailon, H. Kim, C.F. Lai, L.A. Tartaglia, The full-length leptin receptor has signaling capabilities of interleukin 6-type cytokine receptors, Proceedings of the National Academy of Sciences of the United States of America 93 (1996) 8374–8378.
[6] B.D. Bennett, G.P. Solar, J.Q. Yuan, J. Mathias, G.R. Thomas, W. Matthews, A role for leptin and its cognate receptor in hematopoiesis, Curr. Biol. 6 (1996) 1170–1180.
[7] E. Biener, M. Charlier, V.K. Ramanujan, N. Daniel, A. Eisenberg, C. Bjørbaek, B. Herman, A. Gertler, J. Djiane, Quantitative FRET imaging of leptin receptor oligomerization kinetics in single cells, Biology of the cell/under the Auspices of the European Cell Biology Organization 97 (2005) 905–919.
[8] N.A. Binai, A. Damert, G. Carra, S. Steckelbroeck, J. Löwer, R. Löwer, S. Wessler, Expression of estrogen receptor alpha increases leptin-induced STAT3 activity in breast cancer cells, International Journal of Cancer. Journal International Du Cancer 127 (2010) 55–66.
[9] C. Bjørbaek, S. Uotani, B. da Silva, J.S. Flier, Divergent signaling capacities of the long and short isoforms of the leptin receptor, J. Biol. Chem. 272 (1997) 32686–32695.
[10] C. Bjørbaek, R.M. Buchholz, S.M. Davis, S.H. Bates, D.D. Pierroz, H. Gu, B.G. Neel, M.G. Myers, J.S. Flier, Divergent roles of SHP-2 in ERK activation by leptin receptors, J. Biol. Chem. 276 (2001) 4747–4755.
[11] M.J. Boulanger, D. Chow, E.E. Brevnova, K.C. Garcia, Hexameric structure and assembly of the interleukin-6/IL-6 alpha-receptor/gp130 complex, *Science* 300 (2003) 2101–2104 New York, N.Y.
[12] R.J. Brown, J.J. Adams, R.A. Pelekanos, Y. Wan, W.J. McKinstry, K. Palethorpe, R.M. Seeber, T.A. Monks, K.A. Eidne, M.W. Parker, et al., Model for growth hormone receptor activation based on subunit rotation within a receptor dimer, Nat. Struct. Mol. Biol. 12 (2005) 814–821.
[13] F. Carbone, C. La Rocca, G. Matarese, Immunological functions of leptin and adiponectin, Biochimie 94 (2012) 2082–2088.
[14] L.R. Carpenter, T.J. Farruggella, A. Symes, M.L. Karow, G.D. Yancopoulos, N. Stahl, Enhancing leptin response by preventing SH2-containing phosphatase 2 interaction with Ob receptor, Proceedings of the National Academy of Sciences of the United States of America 95 (1998) 6061–6066.
[15] B. Carpenter, G.R. Hemsworth, Z. Wu, M. Maamra, C.J. Strasburger, R.J. Ross, P.J. Artymiuk, Structure of the human obesity receptor leptin-binding domain reveals the mechanism of leptin antagonism by a monoclonal antibody, Structure 20 (2012) 487–497 London, England: 1993.
[16] H.-H. Chao, H.-J. Hong, J.-C. Liu, J.-W. Lin, Y.-L. Chen, W.-T. Chiu, C.-H. Wu, K.-G. Shyu, T.-H. Cheng, Leptin stimulates endothelin-1 expression via extracellular signal-regulated kinase by epidermal growth factor receptor transactivation in rat aortic smooth muscle cells, Eur. J. Pharmacol. 573 (2007) 49–54.
[17] F.F. Chehab, M.E. Lim, R. Lu, Correction of the sterility defect in homozygous obese female mice by treatment with the human recombinant leptin, Nat. Genet. 12 (1996) 318–320.
[18] H. Chen, O. Charlat, L.A. Tartaglia, E.A. Woolf, X. Weng, S.J. Ellis, N.D. Lakey, J. Culpepper, K.J. Moore, R.E. Breitbart, et al., Evidence that the diabetes gene encodes the leptin receptor: identification of a mutation in the leptin receptor gene in db/db mice, Cell 84 (1996) 491–495.
[19] D. Cirillo, A.M. Rachiglio, R. la Montagna, A. Giordano, N. Normanno, Leptin signaling in breast cancer: an overview, J. Cell. Biochem. 105 (2008) 956–964.
[20] K. Clément, C. Vaisse, N. Lahlou, S. Cabrol, V. Pelloux, D. Cassuto, M. Gourmelen, C. Dina, J. Chambaz, J.M. Lacorte, et al., A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction, Nature 392 (1998) 398–401.
[21] D.L. Coleman, Effects of parabiosis of obese with diabetes and normal mice, Diabetologia 9 (1973) 294–298.
[22] D.L. Coleman, A historical perspective on leptin, Nat. Med. 16 (2010) 1097–1099.
[23] R.V. Considine, M.K. Sinha, M.L. Heiman, A. Kriauciunas, T.W. Stephens, M.R. Nyce, J.P. Ohannesian, C.C. Marco, L.J. McKee, T.L. Bauer, et al., Serum immunoreactive-leptin concentrations in normal-weight and obese humans, N. Engl. J. Med. 334 (1996) 292–295.
[24] S.N. Constantinescu, T. Keren, M. Socolovsky, H. Nam, Y.I. Henis, H.F. Lodish, Ligand-independent oligomerization of cell-surface erythropoietin receptor is mediated by the transmembrane domain, Proceedings of the National Academy of Sciences of the United States of America 98 (2001) 4379–4384.
[25] C. Couturier, R. Jockers, Activation of the leptin receptor by a ligand-induced conformational change of constitutive receptor dimers, J. Biol. Chem. 278 (2003) 26604–26611.
[26] H. Cui, F. Cai, D.D. Belsham, Leptin signaling in neurotensin neurons involves STAT, MAP kinases ERK1/2, and p38 through c-Fos and ATF1, FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology 20 (2006) 2654–2656.
[27] R. Devos, Y. Guisez, J. Van der Heyden, D.W. White, M. Kalai, M. Fountoulakis, G. Plaetinck, Ligand-independent dimerization of the extracellular domain of the leptin receptor and determination of the stoichiometry of leptin binding, J. Biol. Chem. 272 (1997) 18304–18310.
[28] C. Duan, M. Li, L. Rui, SH2-B promotes insulin receptor substrate 1 (IRS1)- and IRS2-mediated activation of the phosphatidylinositol 3-kinase pathway in response to leptin, J. Biol. Chem. 279 (2004) 43684–43691.
[29] S.F.P. Duarte, E.A. Francischetti, V. Genelhu-Abreu, S.G. Barroso, J.U. Braga, P.H. Cabello, M.M.G. Pimentel, Q223R leptin receptor polymorphism associated with obesity in Brazilian multiethnic subjects, American Journal of Human Biology: The Official Journal of the Human Biology Council 18 (2006) 448–453.
[30] P. Ducy, M. Amling, S. Takeda, M. Priemel, A.F. Schilling, F.T. Beil, J. Shen, C. Vinson, J.M. Rueger, G. Karsenty, Leptin inhibits bone formation through a hypothalamic relay: a central control of bone mass, Cell 100 (2000) 197–207.
[31] P. Duggal, X. Guo, R. Haque, K.M. Peterson, S. Ricklefs, D. Mondal, F. Alam, Z. Noor, H.P. Verkerke, C. Marie, et al., A mutation in the leptin receptor is associated with entamoeba histolytica infection in children, J. Clin. Invest. 121 (2011) 1191–1198.
[32] C.F. Elias, C. Aschkenasi, C. Lee, J. Kelly, R.S. Ahima, C. Bjorbaek, J.S. Flier, C.B. Saper, J.K. Elmquist, Leptin differentially regulates NPY and POMC neurons projecting to the lateral hypothalamic area, Neuron 23 (1999) 775–786.
[33] E. Elinav, L. Niv-Spector, M. Katz, T.O. Price, M. Ali, M. Yacobovitz, G. Solomon, S. Reicher, J.L. Lynch, Z. Halpern, et al., Pegylated leptin antagonist is a potent orexigenic agent: preparation and mechanism of activity, Endocrinology 150 (2009) 3083–3091.
[34] G. Fantuzzi, Three questions about leptin and immunity, Brain Behav. Immun. 23 (2009) 405–410.
[35] I.S. Farooqi, G. Matarese, G.M. Lord, J.M. Keogh, E. Lawrence, C. Agwu, V. Sanna, S.A. Jebb, F. Perna, S. Fontana, et al., Beneficial effects of leptin on obesity, T cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human congenital leptin deficiency, J. Clin. Invest. 110 (2002) 1093–1103.
[36] H. Fei, H.J. Okano, C. Li, G.H. Lee, C. Zhao, R. Darnell, J.M. Friedman, Anatomic localization of alternatively spliced leptin receptors (Ob-R) in mouse brain and other tissues, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 7001–7005.
[37] P. Fischer-Posovszky, J. von Schnurbein, B. Moepps, G. Lahr, G. Strauss, T.F. Barth, J. Kassubek, H. Mühleder, P. Möller, K.-M. Debatin, et al., A new missense mutation in the leptin gene causes mild obesity and hypogonadism without affecting T cell responsiveness, J. Clin. Endocrinol. Metab. 95 (2010) 2836–2840.
[38] T.M. Fong, R.R. Huang, M.R. Tota, C. Mao, T. Smith, J. Varnerin, V.V. Karpitskiy, J.E. Krause, L.H. Van der Ploeg, Localization of leptin binding domain in the leptin receptor, Mol. Pharmacol. 53 (1998) 234–240.
[39] R.C. Frederich, B. Lollmann, A. Hamann, A. Napolitano-Rosen, B.B. Kahn, B.B. Lowell, J.S. Flier, Expression of ob mRNA and its encoded protein in rodents. Impact of nutrition and obesity, J. Clin. Invest. 96 (1995) 1658–1663.
[40] J.M. Friedman, J.L. Halaas, Leptin and the regulation of body weight in mammals, Nature 395 (1998) 763–770.
[41] R. Fusco, M. Galgani, C. Procaccini, R. Franco, G. Pirozzi, L. Fucci, P. Laccetti, G. Matarese, Cellular and molecular crosstalk between leptin receptor and estrogen receptor-alpha in breast cancer: molecular basis for a novel therapeutic setting, Endocrinerelated Cancer 17 (2010) 373–382.
[42] C. Garofalo, E. Surmacz, Leptin and cancer, J. Cell. Physiol. 207 (2006) 12–22.

[43] H. Ge, L. Huang, T. Pourbahrami, C. Li, Generation of soluble leptin receptor by ectodomain shedding of membrane-spanning receptors in vitro and in vivo, J. Biol. Chem. 277 (2002) 45898–45903.

[44] J. Gent, P. van Kerkhof, M. Roza, G. Bu, G.J. Strous, Ligand-independent growth hormone receptor dimerization occurs in the endoplasmic reticulum and is required for ubiquitin system-dependent endocytosis, Proceedings of the National Academy of Sciences of the United States of America 99 (2002) 9858–9863.

[45] A. Gertler, Development of leptin antagonists and their potential use in experimental biology and medicine, Trends Endocrinol. Metab. 17 (2006) 372–378.

[46] N. Ghilardi, S. Ziegler, A. Wiestner, R. Stoffel, M.H. Heim, R.C. Skoda, Defective STAT signaling by the leptin receptor in diabetic mice, Proceedings of the National Academy of Sciences of the United States of America 93 (1996) 6231–6235.

[47] H. Gogas, M. Trakatelli, N. Dessypris, A. Terzidis, A. Katsambas, G.P. Chrousos, E.T. Petridou, Melanoma risk in association with serum leptin levels and lifestyle parameters: a case–control study, Annals of Oncology: Official Journal of the European Society for Medical Oncology/ESMO 19 (2008) 384–389.

[48] R.R. Gonzalez, P.C. Leavis, A peptide derived from the human leptin molecule is a potent inhibitor of the leptin receptor function in rabbit endometrial cells, Endocrine 21 (2003) 185–195.

[49] L.C. Gonzalez, L. Pinilla, M. Tena-Sempere, E. Aguilar, Leptin(116–130) stimulates prolactin and luteinizing hormone secretion in fasted adult male rats, Neuroendocrinology 70 (1999) 213–220.

[50] R. Gonzalez, A. Watters, Y. Xu, U.P. Singh, D.R. Mann, B.R. Rueda, M.L. Penichet, Leptin-signaling inhibition results in efficient anti-tumor activity in estrogen receptor positive or negative breast cancer, Breast Cancer Research: BCR 11 (2009) R36.

[51] P. Grasso, M.C. Leinung, S.P. Ingher, D.W. Lee, In vivo effects of leptin-related synthetic peptides on body weight and food intake in female ob/ob mice: localization of leptin activity to domains between amino acid residues 106–140, Endocrinology 138 (1997) 1413–1418.

[52] P. Grasso, D.W. White, L.A. Tartaglia, M.C. Leinung, D.W. Lee, Inhibitory effects of leptin-related synthetic peptide 116–130 on food intake and body weight gain in female C57BL/6J ob/ob mice may not be mediated by peptide activation of the long isoform of the leptin receptor, Diabetes 48 (1999) 2204–2209.

[53] E. Haglund, J.I. Sułkowska, Z. He, G.-S. Feng, P.A. Jennings, J.N. Onuchic, The unique cysteine knot regulates the pleotropic hormone leptin, PLoS One 7 (2012) e45654.

[54] J.L. Halaas, K.S. Gajiwala, M. Maffei, S.L. Cohen, B.T. Chait, D. Rabinowitz, R.L. Lallone, S.K. Burley, J.M. Friedman, Weight-reducing effects of the plasma protein encoded by the obese gene, Science 269 (1995) 543–546.

[55] C. Hamers-Casterman, T. Atarhouch, S. Muyldermans, G. Robinson, C. Hamers, E.B. Songa, N. Bendahman, R. Hamers, Naturally occurring antibodies devoid of light chains, Nature 363 (1993) 446–448.

[56] M. Haniu, T. Arakawa, E.J. Bures, Y. Young, J.O. Hui, M.F. Rohde, A.A. Welcher, T. Horan, Human Leptin Receptor. 273 (1998) 28691–28699.

[57] D.W. Haslam, W.P.T. James, Obesity, Lancet 366 (2005) 1197–1209.

[58] S.M. Hileman, J. Tornoe, J.S. Flier, C. Bjorbaek, Transcellular transport of leptin by the short leptin receptor isoform ObRa in Madin–Darby canine kidney cells, Endocrinology 141 (2000) 1955–1961.

[59] A.M. Ingalls, M.M. Dickie, G.D. Snell, Obese, a new mutation in the house mouse, J. Hered. 41 (1950) 317–318.

[60] H. Iserentant, F. Peelman, D. Defeau, J. Vandekerckhove, L. Zabeau, J. Tavernier, Mapping of the interface between leptin and the leptin receptor CRH2 domain, J. Cell Sci. 118 (2005) 2519–2527.

[61] L. Jiang, Z. Li, L. Rui, Leptin stimulates both JAK2-dependent and JAK2-independent signaling pathways, J. Biol. Chem. 283 (2008) 28066–28073.

[62] Y. Kamikubo, C. Dellas, D.J. Loskutoff, J.P. Quigley, Z.M. Ruggeri, Contribution of leptin receptor N-linked glycans to leptin binding, Biochem. J. 410 (2008) 595–604.

[63] M. Kellerer, M. Koch, E. Metzinger, J. Mushack, E. Capp, H.U. Häring, Leptin activates PI-3 kinase in C2C12 myotubes via janus kinase-2 (JAK-2) and insulin receptor substrate-2 (IRS-2) dependent pathways, Diabetologia 40 (1997) 1358–1362.

[64] C. Kloek, A.K. Haq, S.L. Dunn, H.J. Lavery, A.S. Banks, M.G. Myers, Regulation of Jak kinases by intracellular leptin receptor sequences, J. Biol. Chem. 277 (2002) 41547–41555.

[65] S. Konstantinides, K. Schäfer, S. Koschnick, D.J. Loskutoff, Leptin-dependent platelet aggregation and arterial thrombosis suggests a mechanism for atherothrombotic disease in obesity, J. Clin. Invest. 108 (2001) 1533–1540.

[66] I. Kovalszky, E. Surmacz, L. Scolaro, M. Cassone, R. Ferla, A. Sztodola, J. Olah, M.P.D. Hatfield, S. Lovas, L. Otvos, Leptin-based glycopeptide induces weight loss and simultaneously restores fertility in animal models, Diabetes Obes. Metab. 12 (2010) 393–402.

[67] J.E. Layton, N.E. Hall, The interaction of G-CSF with its receptor, Frontiers in Bioscience: A Journal and Virtual Library 11 (2006) 3181–3189.

[68] A. Leggio, S. Catalano, R. De Marco, I. Barone, S. Andò, A. Liguori, Therapeutic potential of leptin receptor modulators, Eur. J. Med. Chem. 78C (2014) 97–105.

[69] C. Li, J.M. Friedman, Leptin receptor activation of SH2 domain containing protein tyrosine phosphatase 2 modulates Ob receptor signal transduction, Proceedings of the National Academy of Sciences of the United States of America 96 (1999) 9677–9682.

[70] L.M. Liao, K. Schwartz, M. Pollak, B.I. Graubard, Z. Li, J. Ruterbusch, N. Rothman, F. Davis, S. Wacholder, J. Colt, et al., Serum leptin and adiponectin levels and risk of renal cell carcinoma, Obesity 21 (2013) 1478–1485 Silver Spring, MD.

[71] R. Van der Linden, B. de Geus, W. Stok, W. Bos, D. van Wassenaar, T. Verrips, L. Frenken, Induction of immune responses and molecular cloning of the heavy chain antibody repertoire of lama glama, J. Immunol. Methods 240 (2000) 185–195.

[72] O. Livnah, E.A. Stura, S.A. Middleton, D.L. Johnson, L.K. Jolliffe, I.A. Wilson, Crystallographic evidence for preformed dimers of erythropoietin receptor before ligand activation, *Science* 283 (1999) 987–990 New York, N.Y.

[73] L. Mancour, H. Daghestani, S. Dutta, Ligand-induced architecture of the leptin receptor signaling complex, Mol. Cell 48 (2012) 1–7.

[74] E. Mansour, F.G. Pereira, E.P. Araujo, M.E.C. Amaral, J. Morari, N.R. Ferraroni, D.S. Ferreira, I. Lorand-Metze, L.A. Velloso, E.P. Araújo, Leptin inhibits apoptosis in thymus through a janus kinase-2-independent, insulin receptor substrate-1/phosphatidylinositol-3 kinase-dependent pathway, Endocrinology 147 (2006) 5470–5479.

[75] A.L. Mark, R.A. Shaffer, M.L. Correia, D.A. Morgan, C.D. Sigmund, W.G. Haynes, Contrasting blood pressure effects of obesity in leptin-deficient ob/ob mice and agouti yellow obese mice, J. Hypertens. 17 (1999) 1949–1953.

[76] A. Markowska, A.S. Belloni, M. Rucinski, A.R. Parenti, G.B. Nardelli, K. Drews, G.G. Nussdorfer, L.K. Malendowicz, Leptin and leptin receptor expression in the myometrium and uterine myomas: is leptin involved in tumor development? Int. J. Oncol. 27 (2005) 1505–1509.

[77] G. Matarese, A. La Cava, V. Sanna, G.M. Lord, R.I. Lechler, S. Fontana, S. Zappacosta, Balancing susceptibility to infection and autoimmunity: a role for leptin? Trends Immunol. 23 (2002) 182–187.

[78] Matarese G, Carrieri PB, Cava A La, Perna F, Sanna V, Rosa V De, Aufiero D, Fontana S & Zappacosta S 2005 Leptin increase in multiple sclerosis associates with reduced number of CD4 CD25 regulatory T cells. Proc. Natl. Acad. Sci. U. S. A. 102 5150–5155.

[79] T. McMurphy, R. Xiao, D. Magee, A. Slater, L. Zabeau, J. Tavernier, L. Cao, The antitumor activity of a neutralizing nanobody targeting leptin receptor in a mouse model of melanoma, PLoS One 9 (2014) e89895.

[80] J.G. Mercer, N. Hoggard, L.M. Williams, C.B. Lawrence, L.T. Hannah, P. Trayhurn, Localization of leptin receptor mRNA and the long form splice variant (Ob-Rb) in mouse hypothalamus and adjacent brain regions by in situ hybridization, FEBS Lett. 387 (1996) 113–116.

[81] Y. Minokoshi, T. Alquier, N. Furukawa, Y.-B. Kim, A. Lee, B. Xue, J. Mu, F. Foufelle, P. Ferré, M.J. Birnbaum, et al., AMP-kinase regulates food intake by responding to hormonal and nutrient signals in the hypothalamus, Nature 428 (2004) 569–574.

[82] L. Miyamoto, K. Ebihara, T. Kusakabe, D. Aotani, S. Yamamoto-Kataoka, T. Sakai, M. Aizawa-Abe, Y. Yamamoto, J. Fujikura, T. Hayashi, et al., Leptin activates hepatic 5′-AMP-activated protein kinase through sympathetic nervous system and α1-adrenergic receptor: a potential mechanism for improvement of fatty liver in lipodystrophy by leptin, J. Biol. Chem. 287 (2012) 40441–40447.

[83] K. Moharana, L. Zabeau, F. Peelman, P. Ringler, H. Stahlberg, J. Tavernier, S.N. Savvides, Structural and mechanistic paradigm of leptin receptor activation revealed by complexes with wild-type and antagonist leptins, Structure 22 (2014) 866–877.

[84] C.T. Montague, I.S. Farooqi, J.P. Whitehead, M.A. Soos, H. Rau, N.J. Wareham, C.P. Sewter, J.E. Digby, S.N. Mohammed, J.A. Hurst, et al., Congenital leptin deficiency is associated with severe early-onset obesity in humans, Nature 387 (1997) 903–908.

[85] L. Niv-Spector, D. Gonen-Berger, I. Gourdou, E. Biener, E.E. Gussakovsky, Y. Benomar, K.V. Ramanujan, M. Taouis, B. Herman, I. Callebaut, et al., Identification of the hydrophobic strand in the A–B loop of leptin as major binding site III: implications for large-scale preparation of potent recombinant human and ovine leptin antagonists, Biochem. J. 391 (2005) 221–230.

[86] L. Niv-Spector, N. Raver, M. Friedman-Einat, J. Grosclaude, E.E. Gussakovsky, O. Livnah, A. Gertler, Mapping leptin-interacting sites in recombinant leptin-binding domain (LBD) subcloned from chicken leptin receptor, Biochem. J. 390 (2005) 475–484.

[87] L. Niv-Spector, M. Shpilman, Y. Boisclair, A. Gertler, Large-scale preparation and characterization of non-pegylated and pegylated superactive ovine leptin antagonist, Protein Expr. Purif. 81 (2012) 186–192.

[88] J. Nizard, M. Dommergue, K. Clément, Pregnancy in a woman with a leptin-receptor mutation, N. Engl. J. Med. 366 (2012) 1064–1065.

[89] O. Ogunwobi, G. Mutungi, I.L.P. Beales, Leptin stimulates proliferation and inhibits apoptosis in Barrett's esophageal adenocarcinoma cells by cyclooxygenase-2-dependent, prostaglandin-E2-mediated transactivation of the epidermal growth factor receptor and c-Jun NH2-terminal kinase activation, Endocrinology 147 (2006) 4505–4516.

[90] L. Otvos, M. Terrasi, S. Cascio, M. Cassone, G. Abbadessa, F. De Pascali, L. Scolaro, D. Knappe, M. Stawikowski, P. Cudic, et al., Development of a pharmacologically improved peptide agonist of the leptin receptor, Biochim. Biophys. Acta 1783 (2008) 1745–1754.

[91] L. Otvos, I. Kovalszky, M. Riolfi, R. Ferla, J. Olah, A. Sztodola, K. Nama, A. Molino, Q. Piubello, J.D. Wade, et al., Efficacy of a leptin receptor antagonist peptide in a mouse model of triple-negative breast cancer, Eur. J. Cancer 47 (2011) 1578–1584.

[92] L. Otvos, I. Kovalszky, L. Scolaro, A. Sztodola, J. Olah, M. Cassone, D. Knappe, R. Hoffmann, S. Lovas, M.P.D. Hatfield, et al., Peptide-based leptin receptor antagonists for cancer treatment and appetite regulation, Biopolymers 96 (2011) 117–125.

[93] L. Otvos, W.-H. Shao, A.S. Vanniasinghe, M.A. Amon, M.C. Holub, I. Kovalszky, J.D. Wade, M. Doll, P.L. Cohen, N. Manolios, et al., Toward understanding the role of leptin and leptin receptor antagonism in preclinical models of rheumatoid arthritis, Peptides 32 (2011) 1567–1574.

[94] T. Ozbay, R. Nahta, A novel unidirectional cross-talk from the insulin-like growth factor-I receptor to leptin receptor in human breast cancer cells, Molecular Cancer Research: MCR 6 (2008) 1052–1058.

[95] R. Pais, H. Silaghi, A.-C. Silaghi, M.-L. Rusu, D.-L. Dumitrascu, Metabolic syndrome and risk of subsequent colorectal cancer, World Journal of Gastroenterology: WJG 15 (2009) 5141–5148.

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[96] F. Peelman, K. Van Beneden, L. Zabeau, H. Iserentant, P. Ulrichts, D. Defeau, A. Verhee, D. Catteeuw, D. Elewaut, J. Tavernier, Mapping of the leptin binding sites and design of a leptin antagonist, J. Biol. Chem. 279 (2004) 41038–41046.

[97] F. Peelman, H. Iserentant, A.S. De Smet, J. Vandekerckhove, L. Zabeau, J. Tavernier, Mapping of binding site III in the leptin receptor and modeling of a hexameric leptin leptin receptor complex, J. Biol. Chem. 281 (2006) 15496–15504.

[98] F. Peelman, L. Zabeau, K. Moharana, S.N. Savvides, J. Tavernier, 20 years of leptin: insights into signaling assemblies of the leptin receptor, J. Endocrinol. 223 (2014) T9–T23.

[99] D.M. Purdham, V. Rajapurohitam, A. Zeidan, C. Huang, G.J. Gross, M. Karmazyn, A neutralizing leptin receptor antibody mitigates hypertrophy and hemodynamic dysfunction in the postinfarcted rat heart, American Journal of Physiology. Heart and Circulatory Physiology 295 (2008) H441–H446.

[100] J. Ratke, F. Entschladen, B. Niggemann, K.S. Zänker, K. Lang, Leptin stimulates the migration of colon carcinoma cells by multiple signaling pathways, Endocr. Relat. Cancer 17 (2010) 179–189.

[101] N. Raver, E. Vardy, O. Livnah, R. Devos, A. Gertler, Comparison of R128Q mutations in human, ovine, and chicken leptins, Gen. Comp. Endocrinol. 126 (2002) 52–58.

[102] I. Remy, I.A. Wilson, S.W. Michnick, Erythropoietin receptor activation by a ligand-induced conformation change, Science 283 (1999) 990–993 New York, N.Y.

[103] F.L. Rock, S.W. Altmann, M. van Heek, R.A. Kastelein, J.F. Bazan, The leptin haemopoietic cytokine fold is stabilized by an intrachain disulfide bond, Hormone and Metabolic Research = Hormon- Und Stoffwechselforschung = Hormones et Métabolisme 28 (1996) 649–652.

[104] V. De Rosa, C. Procaccini, A. La Cava, P. Chieffi, G.F. Nicoletti, S. Fontana, S. Zappacosta, G. Matarese, Leptin neutralization interferes with pathogenic T cell autoreactivity in autoimmune encephalomyelitis, J. Clin. Invest. 116 (2006) 447–455.

[105] C.I. Rosenblum, M. Tota, D. Cully, T. Smith, R. Collum, S. Qureshi, J.F. Hess, M.S. Phillips, P.J. Hey, A. Vongs, et al., Functional STAT 1 and 3 signaling by the leptin receptor (OB-R); reduced expression of the rat fatty leptin receptor in transfected cells, Endocrinology 137 (1996) 5178–5181.

[106] W.K. Samson, T.C. Murphy, D. Robison, T. Vargas, E. Tau, J.K. Chang, A 35 amino acid fragment of leptin inhibits feeding in the rat, Endocrinology 137 (1996) 5182–5185.

[107] Y. Sandowski, N. Raver, E.E. Gussakovsky, S. Shochat, O. Dym, O. Livnah, M. Rubinstein, R. Krishna, A. Gertler, Subcloning, expression, purification, and characterization of recombinant human leptin-binding domain, J. Biol. Chem. 277 (2002) 46304–46309.

[108] V. Sanna, A. Di Giacomo, A. La Cava, R.I. Lechler, S. Fontana, S. Zappacosta, G. Matarese, Leptin surge precedes onset of autoimmune encephalomyelitis and correlates with development of pathogenic T cell responses, J. Clin. Invest. 111 (2003) 241–250.

[109] N.K. Saxena, L. Taliaferro-Smith, B.B. Knight, D. Merlin, F.A. Anania, R.M. O'Regan, D. Sharma, Bidirectional crosstalk between leptin and insulin-like growth factor-I signaling promotes invasion and migration of breast cancer cells via transactivation of epidermal growth factor receptor, Cancer Res. 68 (2008) 9712–9722.

[110] C. Schubring, F. Prohaska, A. Prohaska, P. Englaro, W. Blum, T. Siebler, J. Kratzsch, W. Kiess, Leptin concentrations in maternal serum and amniotic fluid during the second trimenon: differential relation to fetal gender and maternal morphometry, Eur. J. Obstet. Gynecol. Reprod. Biol. 86 (1999) 151–157.

[111] B. Schuster, W. Meinert, S. Rose-John, K.-J. Kallen, The human interleukin-6 (IL-6) receptor exists as a preformed dimer in the plasma membrane, FEBS Lett. 538 (2003) 113–116.

[112] M.W. Schwartz, R.J. Seeley, L.A. Campfield, P. Burn, D.G. Baskin, Identification of targets of leptin action in rat hypothalamus, J. Clin. Invest. 98 (1996) 1101–1106.

[113] R. Señarís, T. Garcia-Caballero, X. Casabiell, R. Gallego, R. Castro, R.V. Considine, C. Dieguez, F.F. Casanueva, Synthesis of leptin in human placenta, Endocrinology 138 (1997) 4501–4504.

[114] N.I. Shapiro, E.V. Khankin, M. Van Meurs, S.-C. Shih, S. Lu, M. Yano, P.R. Castro, E. Maratos-Flier, S.M. Parikh, S.A. Karumanchi, et al., Leptin exacerbates sepsis-mediated morbidity and mortality, Journal of Immunology 185 (2010) 517–524 Baltimore, MD 1950.

[115] D. Shida, J. Kitayama, K. Mori, T. Watanabe, H. Nagawa, Transactivation of epidermal growth factor receptor is involved in leptin-induced activation of janus-activated kinase 2 and extracellular signal-regulated kinase 1/2 in human gastric cancer cells, Cancer Res. 65 (2005) 9159–9163.

[116] M. Shpilman, L. Niv-Spector, M. Katz, C. Varol, G. Solomon, M. Ayalon-Soffer, E. Boder, Z. Halpern, E. Elinav, A. Gertler, Development and characterization of high affinity leptins and leptin antagonists, J. Biol. Chem. 286 (2011) 4429–4442.

[117] M.R. Sierra-Honigmann, A.K. Nath, C. Murakami, G. García-Cardeña, A. Papapetropoulos, W.C. Sessa, L.A. Madge, J.S. Schechner, M.B. Schwabb, P.J. Polverini, et al., Biological action of leptin as an angiogenic factor, *Science* 281 (1998) 1683–1686 New York, N.Y.

[118] K. Takaya, Y. Ogawa, N. Isse, T. Okazaki, N. Satoh, H. Masuzaki, K. Mori, N. Tamura, K. Hosoda, K. Nakao, Molecular cloning of rat leptin receptor isoform complementary DNAs–identification of a missense mutation in zucker fatty (fa/fa) rats, Biochem. Biophys. Res. Commun. 225 (1996) 75–83.

[119] T. Tamada, E. Honjo, Y. Maeda, T. Okamoto, M. Ishibashi, M. Tokunaga, R. Kuroki, Homodimeric cross-over structure of the human granulocyte colony-stimulating factor (GCSF) receptor signaling complex, Proceedings of the National Academy of Sciences of the United States of America 103 (2006) 3135–3140.

[120] L.A. Tartaglia, M. Dembski, X. Weng, N. Deng, J. Culpepper, R. Devos, G.J. Richards, L.A. Campfield, F.T. Clark, J. Deeds, et al., Identification and expression cloning of a leptin receptor, OB-R, Cell 83 (1995) 1263–1271.

[121] M. Tutone, L. Pantano, A. Lauria, A.M. Almerico, Molecular dynamics, dynamic site mapping, and highthroughput virtual screening on leptin and the Ob receptor as anti-obesity target, J. Mol. Model. 20 (2014), http://dx.doi.org/10.1007/s00894-014-2247-z.

[122] S. Uotani, T. Abe, Y. Yamaguchi, Leptin activates AMP-activated protein kinase in hepatic cells via a JAK2-dependent pathway, Biochem. Biophys. Res. Commun. 351 (2006) 171–175.

[123] C. Vaisse, J.L. Halaas, C.M. Horvath, J.E. Darnell, M. Stoffel, J.M. Friedman, Leptin activation of Stat3 in the hypothalamus of wild-type and ob/ob mice but not db/db mice, Nat. Genet. 14 (1996) 95–97.

[124] K. Venken, S. Seeuws, L. Zabeau, P. Jacques, T. Decruy, J. Coudenys, E. Verheugen, F. Windels, D. Catteeuw, M. Drennan, et al., A bidirectional crosstalk between iNKT cells and adipocytes mediated by leptin modulates susceptibility for T cell mediated hepatitis, J. Hepatol. 60 (2014) 175–182.

[125] S.A.B.W. Verploegen, G. Plaetinck, R. Devos, J. Van der Heyden, Y. Guisez, A human leptin mutant induces weight gain in normal mice, FEBS Lett. 405 (1997) 237–240.

[126] J. Wang, R. Liu, M. Hawkins, N. Barzilai, L. Rossetti, A nutrient-sensing pathway regulates leptin gene expression in muscle and fat, Nature 393 (1998) 684–688.

[127] J. Wauman, J. Tavernier, Leptin receptor signaling: pathways to leptin resistance, Frontiers in Bioscience: A Journal and Virtual Library 16 (2011) 2771–2793.

[128] J. Wauman, A.-S.S. De Smet, D. Catteeuw, D. Belsham, J. Tavernier, Insulin receptor substrate 4 couples the leptin receptor to multiple signaling pathways, Mol. Endocrinol. 22 (2008) 965–977.

[129] J. Wauman, L. De Ceuninck, N. Vanderroost, S. Lievens, J. Tavernier, RNF41 (Nrdp1) controls type 1 cytokine receptor degradation and ectodomain shedding, J. Cell Sci. 124 (2011) 921–932.

[130] D.W. White, L.A. Tartaglia, Evidence for ligand-independent homo-oligomerization of leptin receptor (OB-R) isoforms: a proposed mechanism permitting productive long-form signaling in the presence of excess short-form expression, J. Cell. Biochem. 73 (1999) 278–288.

[131] X. Xu, H. Zeng, D. Xiao, H. Zhou, Z. Liu, Genome wide association study of obesity, Zhong Nan Da Xue Xue Bao Yi Xue Ban = Journal of Central South University. Medical Sciences 38 (2013) 95–100.

[132] Y. Yu, Y. Liu, F.-D. Shi, H. Zou, G. Matarese, A. La Cava, Cutting edge: Leptin-induced RORγt expression in CD4+ T cells promotes Th17 responses in systemic lupus erythematosus, Journal of Immunology 190 (2013) 3054–3058 Baltimore, MD: 1950.

[133] L. Zabeau, D. Lavens, F. Peelman, S. Eyckerman, J. Vandekerckhove, J. Tavernier, The ins and outs of leptin receptor activation, FEBS Lett. 546 (2003) 45–50.

[134] L. Zabeau, D. Defeau, J. Van der Heyden, H. Iserentant, J. Vandekerckhove, J. Tavernier, Functional analysis of leptin receptor activation using a janus kinase/signal transducer and activator of transcription complementation assay, Mol. Endocrinol. 18 (2004) 150–161.

[135] L. Zabeau, D. Defeau, H. Iserentant, J. Vandekerckhove, F. Peelman, J. Tavernier, Leptin receptor activation depends on critical cysteine residues in its fibronectin type III subdomains, J. Biol. Chem. 280 (2005) 22632–22640, http://dx.doi.org/10.1074/jbc.M413308200.

[136] L. Zabeau, A. Verhee, D. Catteeuw, L. Faes, S. Seeuws, T. Decruy, D. Elewaut, F. Peelman, J. Tavernier, Selection of non-competitive leptin antagonists using a random nanobody-based approach, Biochem. J. 441 (2012) 425–434, http://dx.doi.org/10.1042/BJ20110438.

[137] L. Zabeau, F. Peelman, J. Tavernier, Antagonising leptin: current status and future directions, Biological Chemistry, 2014.

[138] L. Zabeau, C.J. Jensen, S. Seeuws, K. Venken, A. Verhee, D. Catteeuw, G. van Loo, H. Chen, K. Walder, J. Hollis, et al., Leptin's metabolic and immune functions can be uncoupled at the ligand/receptor interaction level, Cellular and Molecular Life Sciences, CMLS, 2014.

[139] Y. Zhang, R. Proenca, M. Maffei, M. Barone, L. Leopold, J.M. Friedman, Positional cloning of the mouse obese gene and its human homologue, Nature 372 (1994) 425–432.

[140] F. Zhang, M.B. Basinski, J.M. Beals, S.L. Briggs, L.M. Churgay, D.K. Clawson, R.D. DiMarchi, T.C. Furman, J.E. Hale, H.M. Hsiung, et al., Crystal structure of the obese protein leptin-E100, Nature 387 (1997) 206–209.

[141] A.Z. Zhao, J.-N. Huan, S. Gupta, R. Pal, A. Sahu, A phosphatidylinositol 3-kinase phosphodiesterase 3B-cyclic AMP pathway in hypothalamic action of leptin on feeding, Nat. Neurosci. 5 (2002) 727–728.

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