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Invited review

Therapeutic potential of leptin receptor modulators

Antonella Leggio, Stefania Catalano, Rosaria De Marco, Ines Barone, Sebastiano Andò*, Angelo Liguori*

Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, Edificio Polifunzionale, 87036 Arcavacata di Rende, CS, Italy

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ABSTRACT

Leptin, a pleiotropic molecule mainly produced by adipose tissue, was first discovered as a hormone controlling body weight and energy expenditure. In addition, leptin can modulate several processes in peripheral tissues such as immune response, fertility, hematopoiesis and carcinogenesis. The development of molecules that block or stimulate leptin receptor may therefore serve as a potent tool for studying the role of leptin in mammalian physiology and pathology, but it also may open new possibilities for therapy.

This article presents an overview of current knowledge on leptin receptor modulators focusing on leptin mutein, leptin peptide modulators as well as small molecules with heterocyclic structure.

1. Introduction

Leptin, a pluripotent adipokine, is mainly produced by the adipose tissue and results strongly correlated to body fat content. This adipokine reduces food intake and energy storage, and increases energy expenditure. In addition, several studies provided evidences that leptin can be produced by cells other than adipocytes and can regulate various physiological processes. Indeed, leptin affects many peripheral organs, behaving as a mitogen, survival factor, metabolic regulator and angiogenic factor depending on the target tissues. Both leptin deficiency as well as leptin overexpression are associated with different human pathologies. Thus, molecules able to block or stimulate the molecular target of leptin, the leptin receptor (ObR), might represent potential research tools in disease treatment. The major focus of this paper is a systematic review of the leptin receptor modulators developed in the last decades as new therapeutic approaches to modulate the abnormal leptin activity.

2. Leptin and its activity

Leptin, a product of the *ob* gene, is a small nonglycosylated protein (16-kDa) that was discovered in 1994 as a regulator of body weight and energy balance acting in the hypothalamus [1]. Leptin is encoded and synthesized as a 167-amino acid (aa) polypeptide chain with a 21-aa signal sequence at the N-terminus that is cleaved to yield the 146-aa mature protein. The structure of the circulating protein resembles the bundle of four α-helices of the cytokines as the granulocyte colony-stimulating factor (G-CSF) and the interleukin (IL)-6 family [2,3]. The helices A–D of leptin show an up-up-down-down topology [3]. The four antiparallel α-helices are connected by two long crossover links (AB and BC) and one short loop (BC). Leptin seems to have binding sites at similar position as in G-CSF and IL-6. Binding site I is found in helix D and also contains a short residue of the A–B loop. Binding site II is found in the surface of helices A and C, and binding site III is found around the N-terminus of helix D [4] (Fig. 1a).

Abbreviations: ObR, leptin receptor; G-CSF, Granulocyte Colony-Stimulating Factor; BBB, blood–brain barrier; ER-α, estrogen receptor alpha; IGF-I, insulin-like growth factor I; EGFR, epidermal growth factor receptor; CRH, cytokine receptor homology; Ig, immunoglobulin; FNIII, fibronectin type III; JAK2, Janus kinase 2; STAT3, Signal transducer and activator of transcription 3; ERK, extracellular signal-regulated kinase; SOCS3, suppressor of cytokine signaling 3; TNF, tumor necrosis factor; SOLA, superactive ovine leptin antagonist; PEG, polyethylene glycol; LH, luteinizing hormone; PRL, prolactin; rEC, rabbit endometrial cells; LIF, leukemia inhibitory factor; IL-1, interleukin-1; GLP, glucagon-like peptide; PKR, RNA-mediated protein kinase; PDGF, platelet-derived growth factor; 2-AP, 2-aminopurine; JNK, JUN N-terminal kinase; IP3, inositol trisphosphate; cAMP, Cyclic adenosine monophosphate; PKA, protein kinase; mAb, monoclonal antibody; PBMC, Peripheral blood mononuclear cells; NPY, neuropeptide Y.

* Corresponding authors.
E-mail addresses: sebastiano.ando@unical.it (S. Andò), a.liguori@unical.it (A. Liguori).

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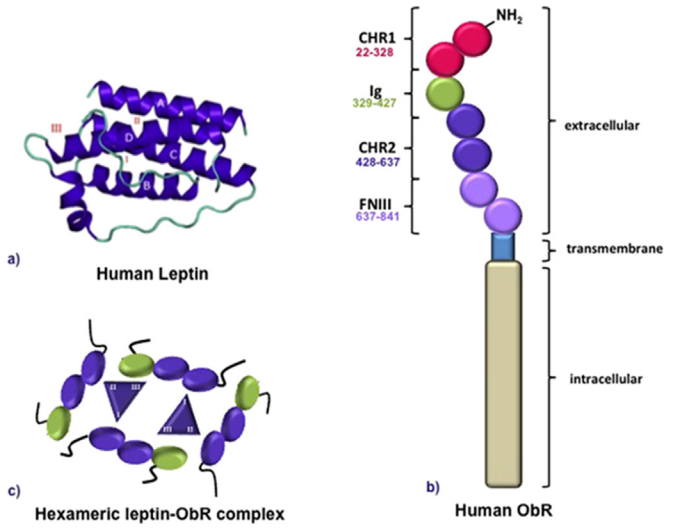

Fig. 1. Leptin and leptin receptor structure. a) Secondary structure of human leptin with indication of binding site (I–III), according to Peelman's model; b) Schematic representation of human ObR; c) Hypotized leptin-ObR exameric complex.

Leptin is mainly secreted in the blood system by adipocytes and, after crossing the blood–brain barrier (BBB), it binds its receptor (ObR) in hypothalamic arcuate nucleus. Here, activation of ObR stimulates anorectic neuropeptides and inhibits the action of orexigenic peptides, resulting in regulation of appetite and energy consumption and, consequently, it controls the size of the fat depots in the body [5,6]. *Ob/ob* mice with mutations in the gene encoding leptin are characterized by hyperphagia and decreased energy expenditure, leading to obesity [7,8]. In obese humans, high leptin plasma levels are correlated with increased fat mass and with the development of leptin resistance [9]. Several potential mechanisms of leptin resistance have been proposed. ObR mutations that decrease leptin binding and impair signaling as well as mutations in target neurons have been described. However, the key mechanism of leptin resistance depends on insufficient leptin transport across the BBB, which may involve saturation and downregulation of short isoforms of ObR [10].

More recent studies showed that leptin can be produced by tissues other than adipocytes and that it can modulate several processes in the peripheral organs, such as immune response, fertility, hematopoiesis, bone remodeling and cognitive functions [11]. Excess leptin levels, for example, contribute to atherosclerosis and increased risk of cardiovascular disease [12,13]. Leptin is also involved in T cell-dependent immunity and autoimmune disease [14,15] as well as being a central mediator of liver fibrogenesis [16]. On a cellular level, leptin has been found to act as a mitogen, metabolic regulator and pro-angiogenic factors [17–19]. Leptin and its receptor are overexpressed in different human cancers, especially in high grade tumors, but are present at minimal levels in nonmalignant tissues [17,18]. The extensive *in vitro* and *in vivo* studies demonstrated that leptin can promote growth, survival and transformation in several cancer cell lines such as breast, colorectal and endometrial cancer cells [17,19–23]. Furthermore, leptin can exert its tumorigenic activities also interacting with different signaling molecules. For instance, leptin signaling in human breast cancer cells enhances aromatase gene expression promoting *in situ* estrogen production [24] and directly transactivates estrogen receptor alpha (ER-α) [25,26]. In addition, it has been reported an interplay between leptin signaling and the transmembrane tyrosine kinase receptor HER2, a member of epidermal growth factor receptor (EGFR) family [27–29]. Also, Saxena et al. [30] have demonstrated the existence of a bidirectional crosstalk between leptin and insulin-like growth factor I (IGF-I) signaling, mediated by synergistic transactivation of EGFR which influences breast cancer cell invasion and migration.

3. Leptin receptor structure and leptin signaling

The activities of leptin are mediated through the transmembrane leptin receptor (ObR), a member of the class I cytokine receptor family. The ObR, encoded by *db* gene, includes six isoform (ObR_{a-f}), resulting from alternative splicing. These isoforms differ in the length of their intracellular tails but share identical extracellular binding domains. The long isoform (ObR_b) contains a long intracytoplasmatic domain, comprising approximately 306 amino acids [31]. Several studies, employing mutein receptor, demonstrated that only the long intracellular domain of ObR_b has full signaling potential. The other isoforms ($ObR_{a,c,d,f}$) exhibiting short cytoplasmatic regions, are involved in leptin transport through the BBB and in other unknown functions. Finally, the ObR_e is a soluble receptor and it does not play a direct role in leptin signaling but it controls leptin circulating levels [32].

The extracellular region of ObR contains several structural domains. At the amino terminal there is a cytokine receptor homology module (termed CRH1), formed by two sub-domains (residues 62–178 and 235–328 in the human leptin receptor). Residues 329–427 adopt an immunoglobulin (Ig)-like fold. The next two sub-domains (amino acids 428–535 and 536–635) form a CRH2 module. In proximity of membrane there are two fibronectin type III (FNIII) fold (Fig. 1b). The membrane-proximal CRH domain, the CRH2, is the main binding site, whereas the other CRH domain, the CRH1, seems to be not essential for leptin binding and receptor activation. Two mutagenesis studies on mouse and chicken receptors identified a four-residue hydrophobic region (^{501}IFFL^{504} of mouse leptin receptor) which is predicted to make contact with Leu^{13} and Leu^{86} of binding site II of mouse leptin [33,34]. Ig-like domain is essential for receptor activation; ObR variants lacking this domain showed unaltered leptin binding but an abolished leptin-dependent signaling [35,36]. Peelman et al. identified residues Leu^{370}, Ala^{407}, Tyr^{409}, His^{417} and His^{418} as the centre of leptin binding site III in IgD [37]. However, the complex structure of leptin and the receptor has not been determined yet. The location of the binding site III on leptin, for example, remains controversial. Peelman et al. studies place binding site III around residues Ser^{120} and Thr^{121} at the N-terminal of helix D of leptin [37], whereas Niv-Spector et al. predict it to be around residues 39–42 [34], which Peelman proposed to be part of binding site I.

The membrane-proximal FNIII domains are also important for the receptor activation, but lack any binding properties [38]. Based on the hexameric IL-6 receptor complex and on the observation that ObR activation requires clustering of more than two ObR chains [35,39], an hexameric complex also for leptin-ObR was proposed [37]. This complex could contain 2 molecules of leptin and 4 molecules of ObR in which each leptin molecule binds three ObR chains through binding sites I–III (Fig. 1c).

Leptin binding to ObR induces activation of multiple intracellular signaling via JAK2 phosphorylation. Three cytoplasmatic tyrosine, Tyr^{985}, Tyr^{1077}, and Tyr^{1138}, are known to be phosporylated and activate the leptin-mediated intracellular cascades [40,41]. Phosphorylation of Tyr^{1138} recruits STAT3 and activates the JAK2/STAT3 pathway, which plays a crucial role in the control of energy balance [42]. Phosporylation of Tyr^{985} leads to recruitment of SH2-containing protein tyrosine phosphatase 2 (SHP2) [43,44] and activation of extracellular signal-regulated kinase (ERK) [45]. Phosporylated Tyr^{985} seems to serve also as a docking site for suppressor of cytokine signaling 3 (SOCS3), which exerts an