The Leptin Receptor*
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Of the many genetic obesity syndromes, none have been as intensively studied as ob/ob and db/db mice. These two mutant mice were originally identified over 30 years ago (1, 2) and shown to be a result of two distinct single gene mutations residing on mouse chromosomes 6 (ob) and 4 (db). The phenotypes and pathophysiology of these two mice have been studied for decades and described in well over 1000 publications. However, the nature of the lesions or primary defects was not revealed until very recently. Perhaps the most informative early studies on the nature of the ob and db primary defects were the parabiosis experiments (partial connection of the circulatory systems of animals through grafting) performed throughout the 1970s (3) (reviewed in Ref. 4). Parabiosis of an ob/ob mouse and a lean control resulted in partial normalization of body weight in the ob/ob mutant mouse. This led to the proposal that ob/ob mice were deficient in a circulating factor that could be restored through the blood of the lean animal. However, db/db mice that underwent parabiosis with lean controls did not exhibit body weight normalization. This suggested that db/db mice may be defective in their ability to respond to the putative satiety factor, perhaps because they were defective in the receptor for this molecule.

The Obese (ob) Gene and Its Product, Leptin

Despite intensive interest in the nature of the putative satiety factor missing in ob/ob mice, biochemical strategies failed to identify it. It was not until a genetic/positional cloning strategy was employed that the gene corresponding to the ob locus and its gene product were ultimately identified (5). The wild type ob gene encodes a protein of about 16 kDa that is preceded by a secretory hydrophobic signal peptide. It is expressed in adipose tissue in multiple mammalian species including mice and humans. The development of antibody reagents confirmed that this factor (leptin) is found at high levels in blood, consistent with the previous parabiosis studies (6).

Since the cloning of the ob gene numerous studies have described the regulation of the leptin mRNA and protein. Although the purpose of this review is not to comprehensively examine the growing development of antibody reagents confirmed that this factor (leptin) is found at high levels in blood, consistent with the previous parabiosis studies (6).

The leptin transcript appears to be expressed fairly specifically in adipose tissue (5), although it is also detectable in human placenta on poly(A)⁺ Northern blots. Steady state levels of the leptin mRNA and protein are elevated in a variety of rodent obesity models (6–9). These observations have led to the proposal that leptin serves as an “adipostat,” informing the body of the status of energy storage in the adipose tissue so that appropriate changes in appetite, metabolism, and nutrient partitioning can be signaled via the leptin receptor. Dramatic regulation of the leptin transcript and protein has also been observed in response to short term alterations in food intake (7, 9–11); fasting results in dramatic down-regulation, and excessive caloric intake results in up-regulation. It is therefore plausible that an important role for leptin is mediating the response to starvation (12). Acute effects on leptin mRNA and protein have also been observed in response to a variety of stimuli including glucocorticoids, cytokines, and insulin (10, 13, 14).

There has now also been considerable analysis of leptin regulation in humans. The leptin mRNA is regulated in humans by both changes in the percentage of body fat as well as changes in food intake (6, 15–18). However, the degree of mRNA regulation in humans is less impressive than that seen in rodents. Importantly, at the protein level human leptin is dramatically regulated, with changes approaching those seen in the rodent obesity and fasting (6, 15). The protein is much higher in individuals with an increased percentage of body fat and is down-regulated during body weight loss. This parallel regulation in mice and humans may imply that leptin is functioning in humans as it is in rodents, although further studies are required to directly address this.

An obvious and important question has been whether a significant portion of human obesity can be due to mutations in the ob gene. The ob coding region has been sequenced from hundreds of human individuals, but mutations have not been found (16, 19, 20). Although mutations affecting mRNA levels can reside outside of the coding region, individuals with severely reduced leptin mRNA levels have also not yet been described (15–18), suggesting that the number of such individuals will not be high. On the other hand, genotyping of microsatellite markers that span the ob gene region has suggested linkage of this region with extreme human obesity (21, 22).

Considerable excitement has been generated by the observations that administration of recombinant leptin to rodents results in food intake reduction and weight loss (23–26). Although the potency of leptin is highest in mice that are completely deficient in this protein (ob/ob), significant effects can be seen at higher doses in normal mice and mice with diet-induced obesity. Such studies have brought hope that leptin may be an effective treatment even in some obesity that are not due to leptin deficiencies. Of particular interest are studies that have investigated the effects of centrally administered leptin. These studies showed that leptin injection into the lateral or third brain ventricle produced reduction in food intake and weight loss, strongly implying that leptin could act directly on receptors within the central nervous system (23, 26).

Weight loss in rodents following leptin administration appears to be due to not only decreases in food intake but also increases in energy expenditure (24, 25). Although the mechanisms of increased energy utilization are likely to be complex, one important component may involve the activation of brown adipose tissue (27).

The observation that leptin deficiencies are not common in human obesity and, in fact, that leptin levels appear to be up-regulated as the percentage of body fat increases has suggested that resistance to normal or elevated levels of leptin may be more important than inadequate leptin production in human obesity (15). This line of thought has been further strengthened by the parallel situation of type II diabetics, many of whom exhibit severe insulin resistance while producing elevated levels of insulin. These observations further stimulated interest in the identification of the receptor for leptin and the analysis of leptin signal reception. They also heightened interest in the db/db mouse, a model of total leptin resistance (23–26) and elevated leptin levels (8).

Cloning of the Leptin Receptor (OB-R)

The identification of the receptor for the leptin protein was realized through an expression cloning strategy (28). Tagged versions of leptin were generated either through a traditional iodination strategy or by generating recombinant fusion proteins between leptin and secreted plasmid alkaline phosphatase. These tagged reagents were then used to identify a tissue source expressing a cell surface leptin binding activity (28, 29). Significant and specific leptin binding was detected in the mouse choroid plexus. To clone this leptin binding activity a murine choroid plexus cDNA library was constructed, and cells transfected with this library were screened with a leptin–alkaline phosphatase fusion protein. From this screen, cDNAs were identified that encoded a cell surface
leptin receptor (OB-R) with an affinity for leptin of about 0.7 nM (28).

Sequencing of the original murine cDNA identified in the expression cloning screen revealed a single membrane-spanning receptor of the class I cytokine receptor family (28). This observation was consistent with a previous structural prediction indicating that the leptin protein would be expected to fold into a cytokine-like structure (30). The closest relatives of OB-R were gp130 (31), the G-CSF receptor (32), and the leukemia inhibitory factor receptor (33). The predicted extracellular domain of OB-R was quite large, about 816 amino acids, while the predicted intracellular domain was fairly short, 34 amino acids, suggesting that this protein might not have signal-transducing capability. However, further screening and analysis of cDNA libraries using the original OB-R cDNA sequence as a guide soon revealed that there were multiple forms of OB-R in both mice and humans, including a long form with an intracellular domain of about 303 amino acids (28, 34–36) (Fig. 1). The intracellular domain of the long form contained sequence motifs suggestive of intracellular signal-transducing capabilities.

The extracellular domains of the short and long forms of OB-R are identical throughout their entire length, since differences in the receptor forms arise from alternative RNA splicing at the most C-terminal coding exon, resulting in OB-R intracellular domains that are interrupted by a partial exon created by the db mutation. This results in a stop codon prematurely terminating the long intracellular domain. The mutation is indicated by an asterisk. Exons and exon segments are not drawn to scale. pa, poly(A) adenylation site.

levels are found in the choroid plexus, lung, and kidney, and somewhat lower levels of expression are detected in nearly all tissues (28). However, subsequent analysis has shown that the vast majority of transcripts detected by these assays are transcripts encoding short intracellular domain forms (OB-Rs) (38). The mRNA species encoding the long intracellular domain is much less abundant. Although this form can be detected by RNase protection or polymerase chain reaction in both mice and humans in nearly all tissues, in most tissues it is expressed at very low levels (38). An exception to this is in the hypothalamus (Fig. 1), where the OB-Rs transcript is expressed at much higher levels (38) and can be detected by in situ hybridization (39). In fact, of the tissues so far tested, only hypothalamus expresses more long form transcript than the most predominant short form (OB-Rs). Within the hypothalamus, the long form transcript has been detected in the arcuate, ventromedial, paraventricular, and dorsomediale nuclei (39), regions previously thought to be important in body weight regulation.

The Mouse Leptin Receptor Is Encoded by the Diabetes (db) Gene

After the cloning of the leptin receptor an important remaining question was whether the gene encoding it corresponded to the db locus, as had been predicted by the parabiosis studies of decades ago. Genetic mapping of the gene encoding OB-R localized its position to a narrow interval on mouse chromosome 4 (28). This position was within the small genetic interval to which the db locus had been mapped by genetic strategies. Sequencing of the gene encoding the leptin receptor from normal and db mice revealed that a mutation was, in fact, present in this gene in db/db mice (original allele) (34, 35). The mutation is a single nucleotide substitution (G → T transversion) within an exon containing the extreme C terminus and 3’-untranslated region of the predominant short intracellular domain form of OB-R (OB-Rs). This mutation results in the generation of a new splice donor site, creating an exon that becomes inappropriately spliced into the transcript encoding the long intracellular domain form of OB-R (Fig. 2). This new exon is composed of the last 6 codons and first 88 base pairs of the 3’-untranslated region of the primary short form (OB-Rs) and is inserted exactly where the long and short intracellular domains diverge. As a result of this insertion, the long form transcript in db/db mice would encode a protein in which the majority of the intracellular domain has been truncated and is identical to the major short form (OB-Rs). The demonstration that the defect in db/db mice is in the OB-R gene provided validation for the importance of this receptor in body weight regulation. In addition, the near identity of the ob/ob (leptin defect) and db/db (OB-R, defect) phenotypes suggests that without OB-R, leptin can exert no control whatsoever over body weight regulation.

It has also been shown that an OB-R defect is responsible for the obesity phenotype of the fa rat (40–42). Genetic mapping studies in the rat had previously shown that the fa mutation mapped to rat
chromosome 5, a region syntenic with the db region on mouse chromosome 4 (43). Sequencing of OB-R in the fa rat revealed a single amino acid substitution in the extracellular domain (41, 42). Although this mutation does not appear to affect binding affinity of the receptor, it strongly affects cell surface expression (42). Whether this mutation also affects signaling potency is currently under study.

The obese phenotype of the Koletsky rat also appears to be due to a defect in OB-R (44). In this case, a codon for an extracellular domain amino acid (common to all OB-R isoforms) has been mutated to a stop codon. This suggests that Koletsky rats are deficient in all cell surface leptin receptors, and therefore these rats are likely to represent the OB-R null phenotype.

**Leptin Receptor Short Forms (OB-Rs)**

The roles of the short intracellular domain forms of OB-R remain to be defined. It is tempting to speculate that the high levels of the short intracellular domain form in the chordee plexus play a role in transporting leptin from the blood into the CSF, where it can then move by diffusion to the brain centers that regulate body weight. It has already been shown that leptin enters the brain by a specific and saturable transport mechanism (45), although a critical role of OB-Rs in this process has not been demonstrated.

This saturable transport process may in fact be a critical feature of leptin resistance. Differences between obese and lean individuals in leptin levels are greater in blood than in CSF (46, 47). This suggests that adipose-derived leptin levels in blood may not be properly translated into rising CSF levels and therefore result in an apparent leptin resistance. This form of leptin resistance would be quite different from the intrinsic cellular resistance that is characteristic of insulin resistance (48). Therefore, it is possible that the central nervous system tissues of even obese individuals may be leptin-responsive if only the elevated leptin levels were capable of reaching these sites. This suggests that a leptin receptor agonist that could more freely cross the blood-brain barrier may be important therapeutically.

Short OB-R forms may play a role not only in transport but also in clearance or as a source of soluble receptor (assuming proteolytic mechanisms exist for releasing the extracellular domain from the cell surface). The reason that several distinct short intracellular domain forms of OB-R are produced is not clear. The different short forms clearly have distinct tissue distributions (35). However, it seems unlikely that there will be significant functional differences demonstrated between the different short forms.

**Leptin Receptor Long Form (OB-RL)**

The mechanism of leptin receptor triggering and signal transduction is of obvious interest both for pharmaceutical considerations as well as insight into the molecular mechanisms of body weight homeostasis. The homology of OB-R to class I cytokine receptors immediately provided important clues as to possible intracellular mediators of leptin receptor activation. The class I cytokine receptors are known to act through JAK and STAT proteins (49, 50). Typically, JAK proteins are associated constitutively with membrane-proximal sequences of the receptor intracellular domain (ICD) and phosphorylate the receptor ICD upon ligand binding. The phosphorylated ICD then provides a binding site for a STAT protein, which is activated upon binding the phosphorylated receptor ICD. The activated STAT proteins then translocate to the nucleus and stimulate transcription.

Transient co-transfection studies have revealed that the OB-Rl protein is capable of activating STAT proteins in response to ligand binding (OB-Rl is inactive in these same assays) (38, 51). Two published reports each show that STAT3 and STAT5 are stimulated in COS cells by OB-R triggering but disagree on the activation of STAT1 and STAT6 (38, 51). Which STAT protein is most important for OB-R regulation of body weight is currently under study.

Reconstitution experiments in hepatoma cells have shown that OB-R is also able to stimulate transcription via response elements known to be stimulated by members of the class I cytokine receptor family (51). As observed for STAT protein activation, the major naturally occurring mouse OB-R short form (also equivalent to the mutant long form in db/db mice) has so far appeared incapable of stimulating transcription (51).

In vitro, OB-R seems to have a signaling repertoire similar to the IL-6 type cytokine receptors (51). Additionally, OB-R shares significant homology with both gp130 and the leukemia inhibitory factor receptor (which uses gp130 as a signal-transducing component). However, anti-gp130 antibodies (which are capable of blocking signaling via IL-6 type cytokine receptors) do not block signaling by OB-R in HepG2 cells (51). Therefore, OB-R signaling is unlikely to require interaction with the gp130 signal-transducing component of the IL-6 receptor family.

The question of whether OB-R utilizes a second transmembrane component for signaling is an important one. Several cytokine receptors have been shown to interact with signaling components other than gp130. For example, IL-3, granulocyte-macrophage colony-stimulating factor, and IL-5 all interact with a common chain critical for signal transduction (IL-3Rβ), as do IL-2, IL-4, IL-7, and IL-9 (IL-2Rγ) (49, 50). However, OB-R was found to transduce signals in hepatoma cell lines which do not express IL-3Rβ or IL-2Rγ (51).

OB-R could conceivably interact with a novel signaling chain. Alternatively, it may transduce all relevant signals through homodimerization or homo-oligomerization as has been shown for a number of class I cytokine receptors such as growth hormone receptor, erythropoietin receptor, and G-CSFR (49, 50). To begin to distinguish among these possibilities, chimeric receptors were constructed between the extracellular domain of the G-CSFR and the intracellular domain of OB-R (G-CSFR/OB-R), as well as the reciprocal chimeric receptor encoding the extracellular domain of OB-R and the intracellular domain of G-CSF-R (OB-R/G-CSFR) (58). Both of these receptor fusions were able to signal, and in fact, the signaling repertoire and potency were not significantly different from that of the native receptors. The observation that a G-CSFR/OB-R chimera could transduce signals in response to G-CSF demonstrates that simple homodimerization of OB-R intracellular domains is sufficient for transducing at least some signals. This suggests that OB-R does not require an accessory binding chain and may be activated by simple homodimerization or homo-oligomerization. Furthermore, the ability of the leptin ligand to generate a signal from the OB-R/G-CSFR chimera indicates that the leptin ligand is capable of homo-oligomerizing OB-R extracellular domains, since this is the requirement for G-CSFR intracellular domain triggering.

Intracellular domain mutagenesis of OB-R has shown that there are at least two distinct regions capable of generating intracellular signals (58). A mutant receptor in which the most C-terminal 50 amino acids are removed loses the ability to stimulate transcription via an IL-6 response element. However, this 50-amino acid C-terminal truncation does not affect the ability of OB-R to stimulate transcription via a hematopoietic receptor response element. Similar data were observed when the most C-terminal tyrosine (Tyr-1141) was mutated to phenylalanine (51). In addition, this single amino acid substitution differentially affected the ability of OB-R to activate different STAT proteins (strongly affecting its ability to activate STAT1 and STAT3 and minimally affecting its ability to activate STAT5). The mutational separation of distinct activities emanating from the OB-R intracellular domain suggests that multiple distinct signals may be propagated by OB-R triggering. Which of the identified or as yet unidentified signaling regions is most important for body weight regulation will require the transgenic introduction of mutant receptors into the db/db mouse and testing for complementation of the mutant phenotype.
Future Directions

There are numerous important questions remaining about the role of OB-R in body weight regulation. Identification of the cell types in which direct receptor signaling must occur is important to our understanding of this pathway. It is possible that leptin need only directly bind to a small number of neurons within the hypothalamus and that receptor triggering in this small group of cells transmits the leptin message to the large number of tissues affecting appetite, metabolism, and nutrient partitioning. It is also possible that there are peripheral tissues with sufficient quantities of OB-R, and that direct receptor triggering at these sites is important for leptin to function.

Additionally, the intracellular molecules that mediate signal transduction via OB-R, are of tremendous interest. Although an understanding of some OB-R signal transduction capabilities is well under way, it is critical to define which of these signals, or as yet unidentified signals, is most important in body weight regulation. The observation that OB-R is capable of activating STAT proteins and promoter response elements suggests that leptin binding will result in changes in gene expression within those cells expressing OB-R. The identification of genes induced by leptin in the relevant cell types and the determination of the role they play in body weight regulation will address many still unanswered questions concerning body weight homeostasis.

The nature of leptin resistance is of paramount importance to the understanding of human obesity. It is possible that this resistance occurs as early in this pathway as the transport of leptin from the blood into the brain. Another more remote possibility beginning to be explored (59) is that significant numbers of humans will have a polymorphism in the OB-R gene, which results in the production of receptors with different signaling potency. More likely, perhaps, is the possibility that leptin resistance is a result of flaws within the signal-transducing pathway of the leptin receptor, more analogous to the mechanism of insulin resistance. Discerning between these possibilities and designing pharmaceutical strategies to correct them are the exciting challenges ahead.

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