Commentary

MOLECULAR METABOLISM

Hunting for the functions of short leptin receptor isoforms*

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Alternate splicing of the leptin receptor gene generates six receptor isoforms (ObRa-f) that share a common extracellular and transmembrane domain, but differ in their intracellular segment. The long ObRb isoform has the longest intracellular domain with 302 amino acids and is the only isoform containing functional JAK2 and STAT binding sites, which are essential for signal transduction and transmission of leptin function. Despite this prominent role of ObRb in mediating the function of leptin, its expression levels are much lower and its tissue distribution much more restricted than those of the other ObR isoforms. Thus, due to the heavy interest in ObRb signaling to elucidate the role of leptin, little research has examined the functional roles of the other, much more abundant, ObR isoforms, including the ObRa isoform.

In this issue of "Molecular Metabolism", Li and colleagues address the functionality of the ObRa isoform by engineering an ObRa knockout (KO) mouse model through deletion of an ObRa-specific exon [1]. The authors first investigated the metabolic phenotype of these mice, and report decreased fasting blood glucose and an improved glucose tolerance during chow feeding without changes in body weight and food intake. However, they observed a slight increase of fat mass and body weight during high fat feeding associated with modest leptin resistance and a small decrease in leptin transport into the cerebrospinal fluid. Overall, the ObRa spliced isoform seems to mediate some of leptin's effects, but compared to ObRb, its role in the control of metabolism appears to be limited. This result seems to be disappointing at the first glance but appears full of sense when taking into account relative expression levels of ObRa and ObRb in the hypothalamus, the major site of leptin action in regulating energy and glucose homeostasis. Indeed, in addition to the phenotypic characterization of ObRa KO mice, the authors also provide for the first time a comprehensive relative expression profile of the different ObR isoforms in central and peripheral tissues. According to their analyses, ObRa mRNA expression levels in the hypothalamus are very low relative to the two major isoforms in this tissue, ObRb and ObRc, which are at least 10 times more abundant than ObRa. Additionally, similar expression levels of ObRb and ObRc are observed in WT and ObRa knockout mice excluding any confounding compensatory effects. Thus, the marginal expression of the ObRa isoform in the hypothalamus is likely to explain the modest changes in energy and glucose homeostasis in ObRa KO mice under chow diet conditions.

Challenging ObRa KO mice with a high fat diet revealed slightly increased fat mass and body weight. In agreement with the very low expression of ObRa in the hypothalamus, leptin responsiveness of the ARC was unchanged in HFD-fed ObRa KO mice. Previous reports have established that the ObRa isoform participates in the transport of leptin into the CNS through the blood brain barrier and that defective transport participates in the establishment and maintenance of the state of leptin resistance [2]. The decrease of leptin transport into the cerebrospinal fluid observed in HFD-fed ObRa KO mice supports this hypothesis. Transport of leptin into the brain may occur through the choroid plexus (CP), blood micro-vessels (V) and circumventricular organs, such as the median eminence, which lack a typical blood brain barrier [3]. Determination of the mRNA levels of the different ObR isoforms in CP and V show that these structures express abundant levels of ObRa, c and e, and to a lesser extent, also ObRb. Based on this observation and that ObRa is thought to be implicated in the transport of leptin, a more obese phenotype would have been expected in high fat-fed mice. The fact that this was not the case might be explained by the substantial up-regulation of ObRb and ObRc isoforms in ObRa KO mice. This compensatory effect renders conclusions concerning the role of ObRa in leptin transport difficult. Furthermore, relative ObR isoform expression levels and possible compensatory effects in ObRa KO mice in the median eminence are currently unknown. This structure is of particular interest due to its close proximity to the hypothalamic arcuate nucleus. Several studies indicate that peripheral tissues also contribute to the effect of leptin on energy and glucose homeostasis [4]. Unfortunately, the ObRa KO model is unable to provide any new insights in this area because the authors show that ablation of the ObRa isoform is largely compensated by the up-regulation of ObRb and ObRc in these tissues leading to similar levels of expression of total ObR. The present work reveals an intriguing compensatory mechanism, which most likely takes place on the level of mRNA splicing generating the different ObR isoforms. Notably, the absence of ObRa in tissues typically expressing significant quantities of this isoform is systematically replaced by ObRb and ObRc isoforms. The biological need to maintain the total ObR expression levels constant is currently not clear. Some tissues, such as the hypothalamus, however, displayed no compensatory increases in other ObR isoforms. This lack of compensation in the hypothalamus could be accounted by the fact that ObRa is expressed at a very low level in the hypothalamus (around 2–3% of all OB-R isoforms) compared to the testis and muscle where OB-Ra mRNA is highly present (around 30%). Increased expression of ObRc in the ObRa KO mice may

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compensate the functional effects of ObRa deletion since the amino-acid sequence of both isoforms differ by only few amino acids at the C-terminal end (…RTDLV for ObRa versus …KVTV for ObRc). This leads to the interesting question whether ObRa and ObRc have partially redundant functions. One way to answer this question would be the generation of double KO mice of these two most abundantly expressed OB-R isoforms.

The study of Li and colleagues clearly places the ObRc isoform in the focus of future work on the leptin receptor. Overall, ObRc is either as abundant (CP, spleen, testis, muscle, small intestine, heart, stomach) or more prevalent than ObRa (cortex, hippocampus, hypothalamus, cerebellum and lung), suggesting that ObRc could be at least as important as ObRa in mediating the functions of leptin. Previous studies had also brought to light a similar or higher expression of ObRc over ObRa [5,6].

Heteromerization between different ObR isoforms has been reported previously [7,8]. Although not shown directly in tissues, engagement of ObRb into heteromers with short ObR isoforms, which are typically expressed in large excess over ObRb, is likely to occur. This would imply that the short isoforms, by forming heteromers with ObRb, could either influence the signaling of the latter or produce a new entity, the heteromer, with its own specific signaling pathways. This option has to be kept in mind for future studies on the role of ObRb versus the short ObR isoforms.

The authors generated a mouse model that would help elucidating the role of ObRa in other biological functions of leptin apart from energy homeostasis. Leptin signaling has been proposed to mediate the regulation of respiration, reproduction, inflammation, immune functions, angiogenesis, bone homeostasis, neuronal plasticity, breast and prostate cancer [9,10]. Ob-Ra and Ob-Rc mRNA are highly abundant in the choroid plexus, the brain microvessels, the lung, spleen, testis, muscle, small intestine, kidney, heart and stomach suggesting their participation in leptin function in those tissues. Recently, a role for ObRa in inflammation has been suggested [11].

The pioneering work of Li et al. opens new perspectives in our understanding of the short ObR isoforms. This work corroborates the involvement of ObRa in leptin transport into the brain and suggests that, compared to ObRb, ObRa has minor effect on energy metabolism. Furthermore, it clearly highlights the potential importance of the ObRc isoform and revealed an unexpected autoregulatory mechanism of the overall amount of expressed ObR in a given tissue. Future studies will have to address the different physiological functions of the short ObR isoforms based on these results.

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