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Review

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Leptin in normal physiology and leptin resistance

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Abstract Since the discovery of leptin as an adipokine in 1994, much progress has been made in the research about leptin. Circulating leptin binds to leptin receptor, activates STAT3-dependent and STAT3-independent signaling pathways, and plays an effective role in energy homeostasis, neuroendocrine function and metabolism mainly through acting on the central nervous system, especially the hypothalamus. Leptin resistance is considered as a key risk factor for obesity. Various mechanisms have been formulated in order to explain leptin resistance, including impairment in leptin transport, attenuation in leptin signaling, ER stress, inflammation and deficiency in autophagy. Here, we review our current knowledge about leptin action, leptin signaling and leptin resistance, hoping to provide new ideas for the battle against obesity.

Keywords Leptin biology · Leptin function · Leptin signaling · Leptin resistance · Energy homeostasis

1 Introduction

The past decades have witnessed an explosion of the incidence of obesity. Estimates from the World Health Organization (WHO) indicate that worldwide obesity doubled between 1980 and 2008, and as of 2014, at least 600 million adults were obese. Obesity is very closely associated with a myriad of comorbidities, which include hypertension, type 2 diabetes, cardiovascular disease and many types of cancers [1]. Elucidating the mechanisms underlying obesity is urgent and critical in the fight against obesity.

Obesity is characterized by an expanded adipose tissue mass. There are two kinds of adipose tissue: white adipose tissue (WAT) to store energy and brown adipose tissue (BAT) to dissipate energy [2]. BAT has an especially important function in newborns, but gradually disappears or become inactive with age [2]. WAT is not only the largest energy reserve, but also the largest endocrine organ in the body. It has been clearly demonstrated that adipose tissue produces a variety of adipokines and cytokines that regulate important biological processes [3]. Among them, leptin is the first one to be found [4].

The discovery of leptin could be considered the initial milestone for adipokine research. In 1950 and 1966, respectively, two kinds of obese mice derived from homozygous mutations of *ob* and *db* gene were produced in Jackson laboratory [5, 6]. These mice were massively obese, with hyperglycemia, hyperinsulinemia, insulin resistance and peripheral neuropathy [4]. Through parabiosis experiments, scientists postulated the existence of a satiety factor to act on the hypothalamus to regulate food intake and energy consumption [7, 8]. In 1990, successful mapping of the *ob* and *db* gene was reported [9, 10]. Then finally in 1994, Friedman's laboratory cloned *ob* gene through positional cloning [4]. Friedman named this new hormone as "leptin" from the Greek root "lepto", meaning "thin" [11]. A report in 1995 proved that the *db* gene encodes the leptin receptor [12]. Today we know that while ob/ob mice possess a single nonsense mutation in the *ob* gene, leading to a truncated nonfunctional form of leptin

SPECIAL TOPIC: Lipid Metabolism and Human Metabolic Disorder.

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[4], db/db mice have an insertion mutation in the *db* gene that interferes normal splicing of the leptin receptor [13].

In this review, we will focus on leptin function, leptin signaling and recent advances in understanding the possible mechanisms underlying leptin resistance.

2 Leptin biology

Leptin, located on chromosome 7, is a 167-amino acid polypeptide, and molecular weight is 16 kD [14]. It is mainly synthesized and secreted by WAT. However, the placenta, ovary, skeletal muscle, mammary epithelium, bone marrow and lymphoid tissue could also express leptin [15, 16]. The circulating leptin levels are positively correlated with the amount of body fat [17]. Particularly, subcutaneous fat expresses more leptin compared with visceral fat [18]. Women tend to have higher leptin levels than men probably due to larger subcutaneous fat and the influence of sex hormone [19]. Leptin levels also fluctuate according to energy state, with a marked decrease during starvation and an increase in energy surplus states [20]. Other factors including hormones (insulin, estrogen, glucocorticoids, etc.), metabolites (glucose, fatty acid, etc.) and inflammatory cytokines also influence leptin secreting and expression [21–24] (Fig. 1). Besides, leptin levels display a circadian rhythm, with lowest levels in around noon to midafternoon and highest levels between midnight and early morning [25].

Leptin receptor (LepR) belongs to long-chain helical cytokines superfamily. Via alternative splicing, *Lepr*, or *db* gene, produces six LepR isoforms (LepRa, b, c, d, e and f) [12, 26]. These isoforms possess the same extracellular domain, but differ by their transmembrane and cytoplasmic domains. LepRb is the only form that contains intracellular motifs of approximately 300 amino acid residues. It is ubiquitously expressed in the body and mediates the main effects of leptin on controlling energy homeostasis and body weight [16]. Besides LepRb, the short isoform LepRa also plays essential roles in mediating leptin internalization and signaling [27, 28]. LepRe is soluble and inhibits the transportation of leptin across the blood–brain barrier (BBB) by reducing the endocytosis of leptin [29].

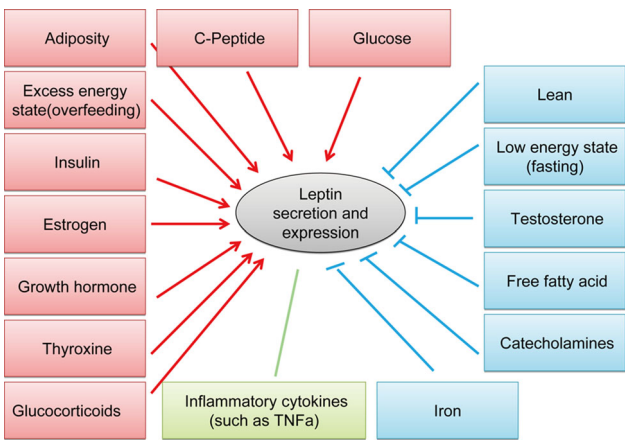

Fig. 1 Factors regulating circulating leptin levels. Notes: The red boxes indicate factors that promote leptin secretion and expression. The blue boxes indicate factors that suppress leptin levels. The green box indicate factor that influences leptin level differently according to the microenvironment

3 Leptin function

Leptin acting on specific populations of neurons in the brain, including hypothalamic, midbrain and brainstem neurons, plays a central role in energy homeostasis and neurofunction [30–32]. Besides, leptin also has multiple roles in metabolism, reproductive system, immune function, etc. [15, 32–35].

3.1 Leptin and the central nervous system

The function of leptin on controlling energy homeostasis and body weight is mainly conducted by the central nervous system. In addition to reducing energy intake via central regulation of appetite and satiety, leptin also promotes energy expenditure and mediates neuroendocrine function and cognition [32].

The leptin receptor LepRb is highly expressed in the brain, particularly in the arcuate (ARC), dorsomedial (DMH), ventromedial (VMH) and ventral premamillary nuclei (PMV) of hypothalamus [36, 37]. Also, these leptin-responsive neurons (designated as the first-order neurons) broadly connect to other neurons in the brain, thus forming a sophisticated neural network [30]. The ARC of the hypothalamus is a critical site of leptin action. Actually, LepRb expression was co-localized with two neuronal populations of ARC: anorexigenic proopiomelanocortin (POMC) neurons and orexigenic agouti-related peptide (AgRP)/neuropeptide Y (NPY) neurons [38]. Leptin signaling activation directly stimulates POMC neurons and thus releases α-melanocyte-stimulating hormone (α-MSH) [30]. α-MSH is an anorexigenic neuropeptide that decreases food intake by binding to and activating melanocortin-4 (MC4R) [39]. On the other side, leptin also inhibits orexigenic neuropeptides AgRP and NPY, which antagonize the α-MSH/MC4R signaling and thus reduce appetite [40]. Recent literature suggests leptin inhibits the rewarding effects of running via LepR-STAT3 modulation of dopamine tone, maybe as an adaptive means to reduce the motivation for feeding [41]. The role of leptin in promoting energy expenditure is mediated by activation of the

adrenergic system [42], the melanocortin system [43], the enhanced activity of BAT [44] and FoxO1 [45]. Leptin also has been associated with neuroendocrine function, demonstrated by hypogonadism and failure of pubertal development in congenital leptin deficiency mice and the various disorders in metabolic and hormone responses with fasting-induced low leptin levels [46]. Furthermore, recent studies indicate leptin could markedly promote hippocampal synaptic plasticity and long-term depression (LTD) of excitatory synaptic transmission and thus improve cognition and memory [47].

3.2 Leptin and the peripheral tissue

LepR, especially LepRa and LepRb, is widely expressed in the body [26, 48]. Undoubtedly, the central nervous system could exert different effects on the peripheral tissue via neuroregulation. Apart from that, evidence is accumulating that leptin also has direct function in the peripheral tissues [48]. For example, in the skeletal muscle, leptin enhances fatty acid oxidation and glucose uptake [49]. In the pancreas, leptin inhibits insulin and glucagon secretion [34, 50]. In the liver, leptin reduces lipid ectopic accumulation [51, 52]. Also, disruption of hepatic leptin signaling improves hepatic insulin sensitivity and protects mice from age- and diet-induced glucose intolerance [53]. Leptin increased glucose utilization of BAT and lipolysis in WAT via sympathetic neurons that directly "envelope" white adipocytes [54, 55]. Also, leptin and insulin act together to promote WAT browning and weight loss [56]. Leptin influences bone metabolism differently through central or peripheral means [32]. Peripherally, in *vitro* and in *vivo* studies indicate leptin interacts with bone marrow stromal cells and osteoblasts to increase overall bone mass [32]. However, disruption of LepR in peripheral tissues has little or none impact on energy metabolism, indicating that leptin functions mainly through the central nervous system to influence energy metabolism [57] (Fig. 2).

4 Leptin signaling

The binding of leptin to LepRb activates a series of signaling pathways, including the janus kinase 2 (JAK2)/signal transducer and activator of transcription 3 (STAT3), src homology-2-containing protein tyrosine phosphatase 2 (SHP2)/growth factor receptor-bound protein 2 (Grb2)/mitogen-activated protein kinase (MAPK), insulin receptor substrates (IRS)/phosphatidylinositol 3 kinase (PI3K)/mammalian target of rapamycin (mTOR), forkhead box O1 (FoxO1), 5′ adenosine monophosphate-activated protein kinase (AMPK) and others. These pathways act synergistically to fully mediate leptin's function.

4.1 Leptin and STAT3-dependent pathways

The JAK2/STAT3 pathway exerts crucial effects on energy homeostasis and neuroendocrine function [58]. Leptin binds to the extracellular domain of LepRb, causing a conformational change that results in autophosphorylation of associated JAK2 tyrosine kinase, which then phosphorylates LepRb in the indicated residues (Tyr^{985}, Tyr^{1077} and Tyr^{1138}). Phosphorylated Tyr^{1138} recruits and phosphorylates the transcription factor STAT3. Phosphorylated STAT3 then homodimerizes and translocates to the nucleus, where it induces transcription of an anorexigenic neuropeptide POMC and suppresses that of orexigenic neuropeptides AgRP and NPY. On the other side, phosphorylated Tyr^{1077} binds to and phosphorylates STAT5; Tyr^{1138} also partially contributes to the activation of STAT5 [59]. Elimination of STAT5 in the brain causes obesity with hyperphagia, although not that severity as STAT3 deletion [60].

JAK2/STAT3 pathways are controlled positively by SH2B adaptor protein 1 (SH2B1) and negatively by suppressor of cytokine signaling 3 (SOCS3), protein tyrosine phosphatase 1B (PTP1B) and T cell protein tyrosine phosphatase (TCPTP). SH2B1 is an adaptor protein that binds to Tyr^{813} and enhances JAK2 activation, thus promoting leptin signaling [61]. SOCS3 is a target gene of JAK2/STAT3 signaling pathway. It attenuates leptin receptor signaling by binding Tyr^{985} on LepRb and inhibiting JAK2, thus providing a pivotal negative feedback mechanism and preventing the overactivation of leptin signaling pathways [62]. Both PTP1B and TCPTP are protein tyrosine phosphatases; PTP1B mediates the dephosphorylation of JAK2 and TCPTP dephosphorylates STAT3, limiting the extent of leptin action [30].

4.2 Leptin and STAT3-independent pathways

In addition to the JAK2/STAT3 signaling pathway, leptin induces a number of STAT3-independent pathways, which are required to fully mediate the effects of leptin on central and peripheral organs. After leptin binding to the LepRb, phosphorylated JAK2 also phosphorylates IRS and activates PI3K pathways both in *vitro* and in *vivo* settings [15, 63]. Both FoxO1 and mTOR/S6K pathways are important downstream effectors of the IRS/PI3K pathway [32]. FoxO1 exerts significant influence on energy balance and glucose homeostasis in POMC, AgRP and steroidogenic factor 1 (SF-1) neurons of hypothalamus [45, 64]. Important roles for mTOR on leptin signaling have been established both in hypothalamus to regulate anorectic effect and in peripheral

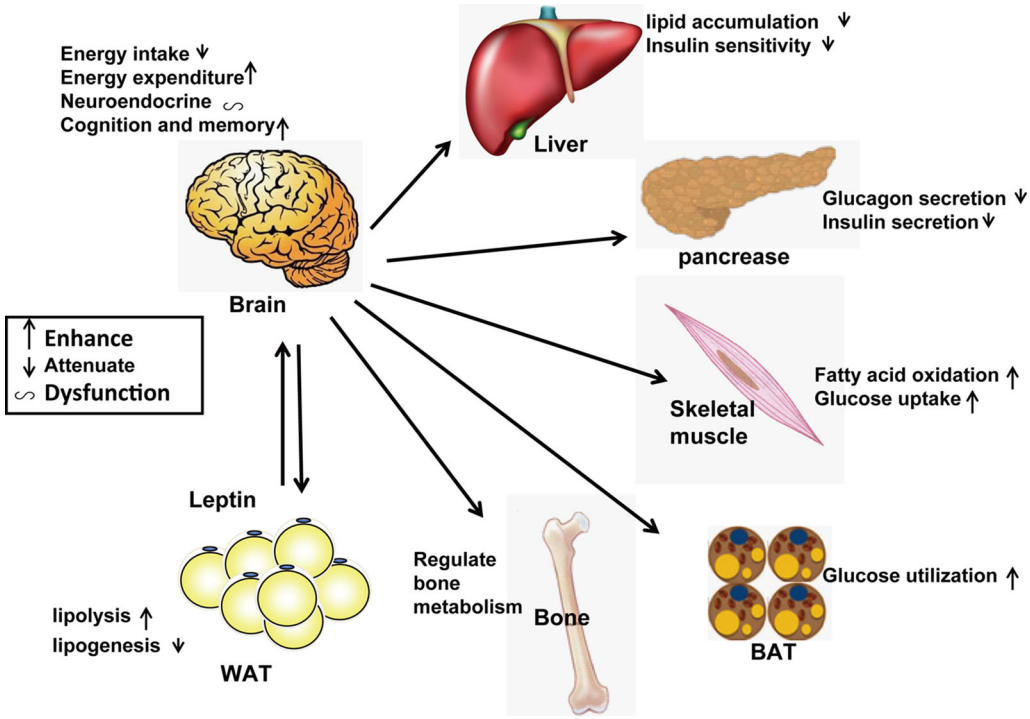

Fig. 2 (Color online) Effects of leptin in the central nervous system and peripheral tissues. Leptin can affect energy homeostasis and neuron function in the CNS through a variety of mechanisms. In the peripheral tissues, leptin exerts different effects in different tissues through both direct and indirect means. Modified from references [34, 51]

tissues to regulate lipid metabolism and inflammation [64–66]. JAK2 phosphorylation also activates SHP2 by binding to phospho-Tyr^{985}, which then recruits the adaptor protein Grb2 to prompt activation of ERK1/2 and improve the effect of leptin on energy homeostasis [15]. The condition of AMPK pathway is complicated. While leptin activates AMPK and thus promotes fatty acid oxidation in skeletal muscle, it exerts inhibitory effect on AMPK in the hypothalamus and help to exert leptin's anorexigenic response [15]. Other MAPK pathways, namely JNK and p38 MAPK, are mainly participate in the peripheral function of leptin [15, 30] (Fig. 3).

5 Leptin resistance

Since the discovery of leptin in 1994, people have high expectation for leptin to combat obesity. Actually, leptin replacement treatment could improve or normalize the neuroendocrine and metabolic abnormalities in leptin deficiency conditions such as lipodystrophy, hypothalamic amenorrhea and congenital leptin deficiency (CLD) [67]. Metreleptin, an analog of the human hormone leptin being developed by Amylin Pharmaceuticals, was approved in the USA in 2014 for use in diseases associated with leptin deficiency such as lipodystrophy [68]. However, in most cases, obese individuals have elevated plasma leptin concentrations. Exogenous leptin administration exerts poor effect on weight reduction in the majority of human obesity [69, 70]. This phenomenon is referred to as leptin resistance [71]. Mechanisms underlying leptin resistance are multifactorial and complicated. Impairment in leptin transportation, attenuation in leptin signaling, endoplasmic reticulum (ER) stress, deficiency in autophagy and inflammation are the most common proposed reasons for obesity-associated leptin resistance (Fig. 4).

5.1 Impairment in leptin transportation to the brain

To act centrally, circulating leptin must first enter the brain through the blood–brain barrier. While debate about leptin's exact route of transportation across the central nervous system continues, LepRa, LepRe and megalin have been proposed to be involved in this process [27, 29, 72]. Alternatively, circulating leptin could also directly contact the circumventricular organs (for example, the median eminence), which lack a BBB [73]. During obesity, the cerebrospinal-fluid/serum leptin ratio is decreased [74, 75], indicating impairment in leptin transport [76], which might contribute to leptin resistance. The reasons behind this might be that leptin enters the brain in a saturable manner [77]. As serum leptin levels increase during obesity, the

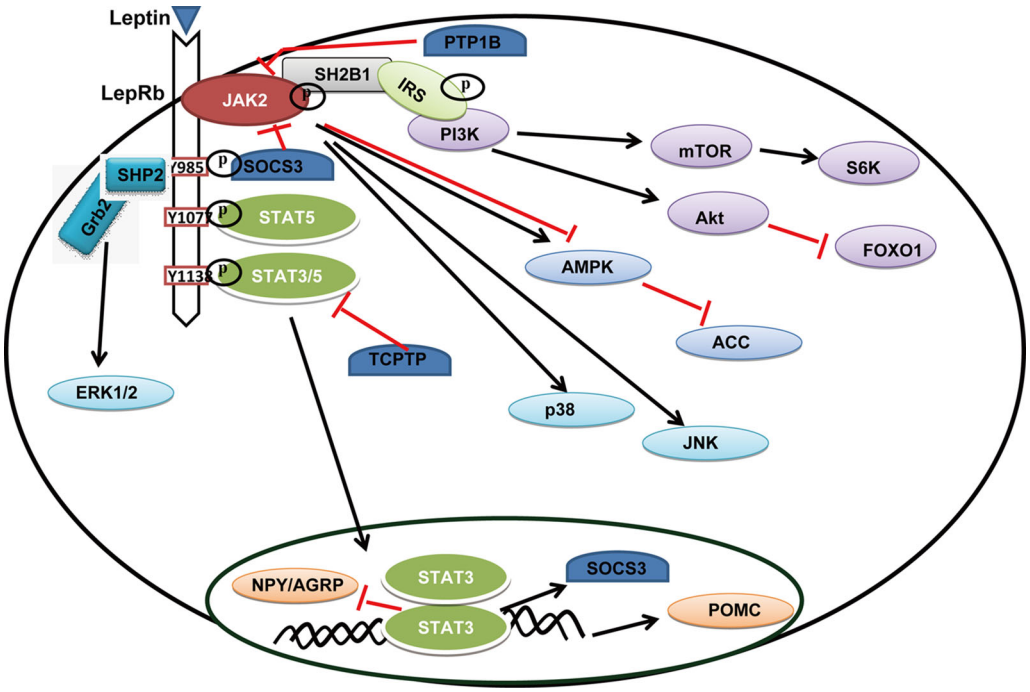

Fig. 3 (Color online) Leptin binds to LepRb and activates JAK2. JAK2 then phosphorylates LepRb on Tyr^{985}, Tyr^{1077} and Tyr^{1138}. Phosphorylated Tyr^{985}, $-Tyr^{1077}$ and $-Tyr^{1138}$ bind to different downstream molecules and activate the JAK2/STAT3, JAK2/STAT5, PI3K/AKT/mTOR, SHP2/Grb2/ERK, JNK and p38 MAPK pathways. Leptin could activate or inhibit AMPK signaling pathway in different cell types. Leptin signaling activation induces the expression of POMC and SOCS3, and attenuates the expression of NPY and AGRP. These pathways act coordinately to regulate energy homeostasis. LepRb signaling is regulated negatively by SOCS3, PTP1B and TCPTP and positively by SH2B1

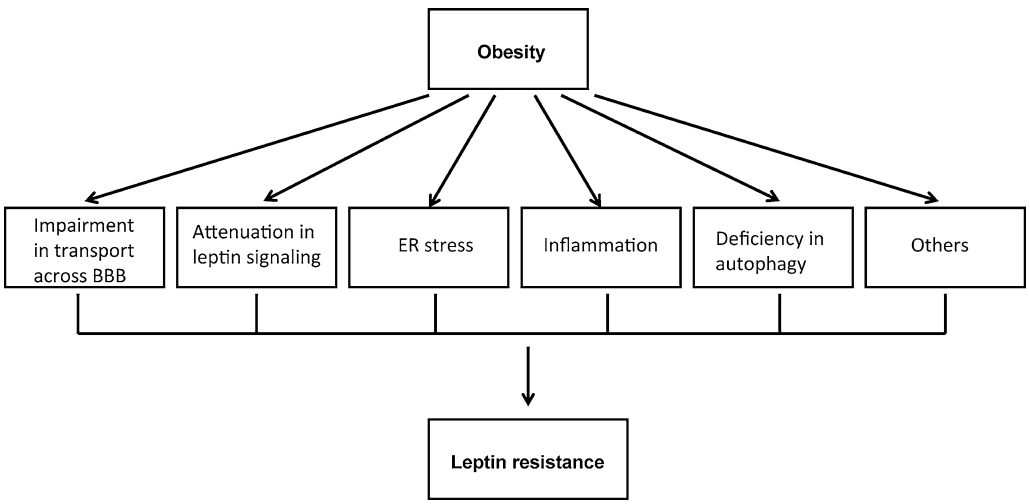

Fig. 4 Proposed mechanisms of leptin resistance. During obesity, impairment in transport across BBB, attenuation in leptin signaling, ER stress, inflammation, deficiency in autophagy and others all influence the function of leptin and lead to leptin resistance

leptin transporter is increasingly saturated and blocks the access of leptin into the CNS [78]. Besides, fatty acids, tumor necrosis factor-a (TNF-a) and triglycerides are reported to influence leptin transporter level [79, 80]. Clearly, more studies are needed to clarify the reason for impaired brain leptin transport and the relationship between this and leptin resistance.

5.2 Attenuation in leptin signaling

Impairment in each component of the leptin signaling pathways could lead to leptin resistance. In diet-induced obese (DIO) mice, leptin signaling (demonstrated by leptin-induced STAT3 phosphorylation) is profoundly decreased in the hypothalamus [81, 82]. As described

above, leptin signaling is negatively regulated by SOCS3, PTP1B, TCPTP and others. The expression levels of hypothalamic SOCS3, PTP1B and TCPTP are elevated in obesity mice and thus might contribute to attenuation of leptin signaling and leptin resistance [83–85]. Also, LepRb mRNA and protein levels were declined in DIO rats with respect to those of normal chow-fed rats [86]. SH2B1 was a positive regulator of leptin signaling, and deletion of the *SH2B1* gene results in leptin resistance, hyperphagia and morbid obesity [87].

5.3 ER stress

Numerous studies have provided evidence that ER stress in hypothalamic neurons can impair leptin signaling [88, 89]. Both increased ER stress and activated unfolded protein response (UPR) in the hypothalamus of obese mice contribute to the compromise of leptin receptor signaling [88]. By contrast, reducing ER stress by chemical chaperones, 4-phenyl butyric acid (PBA) and tauroursodeoxycholic acid (TUDCA), can improve leptin sensitivity [88]. Spliced X-box binding protein 1 (Xbp1) is one of the ER-stress-responsive genes. It has reported that neuron-specific XBP1 knockout mice have ER stress, severe hyperleptinemia, leptin resistance and obesity [88]. On the other hand, overexpression of XBP1 in *vitro* and in *vivo* settings increases leptin sensitivity [88, 90]. There are two possible mechanisms of ER stress resulting in leptin resistance: defective leptin signaling by SOCS3 and PTP1B and impaired POMC processing through diminished expression of hypothalamic mitofusin 2 (Mfn2) [91]. Recently, celastrol, a pentacyclic triterpene extracted from the roots of Tripterygium Wilfordi (thunder god vine) plant, is reported to reduce hypothalamic ER stress and re-establish hypothalamic sensitivity to leptin in obesity [92].

5.4 Deficiency in autophagy

Recent studies indicate autophagy is associated with leptin resistance. Inhibition of autophagy in the hypothalamus by knocking down autophagy-related protein 7 (Atg7) and injecting Atg7-specific small hairpin RNA (shRNA) exhibits obesity and leptin resistance [93]. Particularly, POMC-neuron-specific Atg7-knockout mice display leptin resistance probably due to the inability of leptin to activate and phosphorylate STAT3 [94]. On the other side, AgRP-neuron-specific Atg7-knockout mice lead to reduced body fat and a reduced rebound effect on food intake after fasting, suggesting improvement in leptin sensitivity [95].

5.5 Inflammation

Obesity is associated with low-grade chronic inflammation. Activation of the toll-like receptors (TLRs) or hypothalamic IKKβ/NF-κB pathway is reported to induce hypothalamic inflammation and leptin resistance [89, 96]. Deletion of neuronal TLR adaptor molecule MyD88 protects from high-fat diet-induced leptin resistance and obesity [97]. Furthermore, depleting microglia from the mediobasal hypothalamus eliminates inflammation and neuronal stresses and improves leptin sensitivity in mice challenged with excess saturated fatty acids consumption [98]. All these data suggest inflammation may contribute to leptin resistance during obesity.

5.6 Others

Apart from above-mentioned mechanisms, other factors also contribute to the progression of leptin resistance. Firstly, although rare, there are cases of mutations of the leptin receptor and other rare monogenic obesity syndromes [15, 99]. Epigenetic regulation of the leptin signaling circuit could be a potential mechanism of leptin function disturbance [78]. Extracellular circulating factors, such as C-reactive protein, could bind leptin, thus altering its biological actions [100]. Latest study reported circadian dysfunction induced leptin resistance in mice [101]. Finally, hyperleptinemia *per se* might also be a contributing factor of leptin resistance [102]. More studies are warranted to fully clarify the underlying mechanisms of leptin resistance in order to better combat obesity.

5.7 Redefine the concept of “leptin resistance”

It is noteworthy that the term “leptin resistance” is commonly used and appears to connote diverse meanings in distinct circumstances [71]. Recently, Ottaway et al. reported that hyperleptinemic DIO mice retain endogenous leptin physiologic action [103]. That is, endogenous leptin suppress food intake and body weight gain to the same extent in DIO and lean animals [104]. According to this, Myers et al. [104] declared that since there is no pathophysiologic decrease in endogenous leptin action, leptin resistance could be defined as the failure of pharmacologic leptin to increase LepRb signaling and physiologic responses in hyperleptinemic obesity. Besides, there is a discrepancy in the phosphorylation levels of STAT3 (pSTAT3) during obesity from different papers. While Ottaway et al. [103, 105] and some found DIO mice had increased pSTAT3 in the ARC compared with the lean controls; others reported reduced pSTAT3 levels in the obesity [81, 82]. More studies are required to clearly clarify

this issue, and we need to reach a consensus on the exact definition and significance of leptin resistance.

6 Concluding remarks and future perspective

As research continued, many advances have been made about the actions of leptin and the mechanisms of leptin resistance. However, there are still many issues that remain to be addressed.

Firstly, we need to identify the function of short-form leptin receptors and the direct function of leptin in peripheral tissues. To clarify the direct function of leptin in the peripheral tissues, the CNS effect of leptin must first be excluded. Nowadays, numerous studies have been conducted using the cell lines or primary cells derived from peripheral tissues. However, considering the complexity and heterogeneity of the cell lines, the results from different cell lines sometimes are controversial. Also, further studies are needed to elucidate the relative importance of leptin direct function in the periphery tissues.

Secondly, how different pathways downstream of leptin (STAT3, PI3K, ERK, FoxO1, AMPK, etc.) act specifically or coordinately in the regulation of energy homeostasis and other functions. Certain downstream pathway of leptin signaling reacts differentially in discrete subpopulations of LepRb-expressing neurons and neural circuits. It is of great significance to anatomically and biochemically characterize how these neurons and neural circuit mediate energy homeostasis and body weight at the control of various hormonal, neuronal and metabolic signals that cross-talk with leptin.

Thirdly, clarifying the exact definition of "leptin resistance" is important and urgent. Also, since obesity and leptin resistance are closely correlated, an important issue that remains to be clarified is the causal and conditional relationship between obesity and leptin resistance.

And lastly but most significantly, the exploration of new therapeutic drugs is based on more and more comprehensive understanding of leptin action. As leptin resistance is an important risk factor for obesity, but the vast majority of obese individuals are resistant or tolerant to leptin, more studies are required to examine effective measures of improving leptin sensitivity. Also, identifying predictors of leptin responsiveness in order to find the population who might benefit most from leptin-sensitizing agents is profitable.

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Conflict of interest The authors declare that they have no conflict of interest.

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