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RESEARCH HIGHLIGHT

The role of leptin in the central nervous system remyelination

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Leptin is identified as a mouse obesity gene, which is also preserved in humans. Leptin receptor is highly expressed in the hypothalamus relative to other tissues; therefore, the function of leptin is mainly attributed to hypothalamic control of food intake and body weight. Although the expression of leptin receptors is not limited to the hypothalamus but is also present in other regions of the central nervous system (CNS), such as the spinal cord, the functions of leptin and leptin receptor in the CNS have not been fully clarified. In this research highlight, we focus on the novel function of leptin in CNS remyelination in pathologic conditions, such as the demyelination mouse model. Because remyelination is a crucial process for repair of neuronal networks after injury and wound healing, knowledge of the underlying molecular mechanism of remyelination is useful to establish a therapeutic strategy against demyelinating diseases. We only revealed the role of leptin in remyelination at a histological level; however, a behavioral analysis and evidence of the beneficial effect of leptin for humans may add to knowledge of the effect of leptin on remyelination function.

Keywords: multiple sclerosis; obesity; oligodendrocyte; adipocytes; fibroblast growth factor

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Leptin and leptin receptor (ObRb)

Leptin is identified as the product of the obesity (*ob*) gene in mice [1]. The wild type *ob* gene encodes the leptin protein of 16 kDa, which is preceded by a secretory hydrophobic signal peptide. Leptin is expressed in the placenta, especially in the endometrium [2] and mammary epithelial cells [3], but the main source of leptin is adipocytes [1]. Leptin regulates food intake and energy expenditure in adult mammals via activation of the hypothalamic long form of the leptin receptor [4, 5]. Leptin receptor is encoded by the diabetes (*db*) gene and was identified by cloning of the gene that binds to leptin [6]. Leptin receptor consists of multiple isoforms, which are generated by alternative splicing: four types of splice variants (ObRa-ObRd) in humans [7], five types (ObRa-ObRe) in mice [8], and six types (ObRa-ObRf) in rats [7]. Leptin receptors are divided into secretory (ObRe), long (ObRb), and short forms (ObRa, ObRc, ObRd, ObRf).

Although the expression of leptin receptor is different among the isoforms, ObRb, expressed at a high level in the hypothalamus relative to other tissues, accounts for the majority of leptin action.

Leptin receptor (ObRb) has a single transmembrane domain and belongs to the gp130 family of cytokine receptors [6]. Leptin-binding to ObRb initiates a signaling cascade beginning with the activation of the constitutively receptor-associated Janus Kinase-2 (Jak2), a tyrosine kinase [9]. Activation of JAK2 promotes phosphorylation of multiple residues on the intracellular domains of ObRb-Tyr_{985}, Tyr_{1077}, and Tyr_{1138} [9, 10]. Phosphorylation of Tyr_{985} recruits the SH2-containing tyrosine phosphatase-2 (SHP2; PTPN11) [11, 12] to activate an extracellular signal regulated kinase (ERK) cascade [13], which promotes cell growth and proliferation [14, 15]. Phosphorylation of Tyr_{1077} and Tyr_{1138} also promotes activation of signal cascades (STAT3 and STAT5, respectively) that relate to cell growth [16, 17].

Remyelination

CNS myelin is formed by the oligodendrocyte, which functions to allow saltatory conduction of nerve impulse and to provide trophic support to the axons that they ensheath. Demyelination, occurring in response to CNS injury, is critical for the development of neurological deficits [18]. Therefore, promotion of myelin repair (remyelination) is considered to be prerequisite for the recovery of neurological function in CNS disease with demyelination. Remyelination of an oligodendrocyte is developed by their precursor cells, oligodendrocyte precursor cells (OPCs), which are widely distributed in the mammalian CNS and hold proliferation potential throughout the lifespan of the organism [19]. OPC proliferation is enhanced by treatment with growth factors such as platelet-derived growth factor (PDGF) and basic fibroblast growth factor (FGF2) [20]. So far, the molecular mechanism of remyelination has been attributed to the factors produced by the CNS cells, such as astrocytes [21, 22], microglia [23, 24], and vasculature [25], because the CNS is normally separated from the periphery in the presence of a vascular barrier (blood brain barrier [BBB], blood spinal cord barrier). However, several CNS regions (e.g. circumventricular organs) lack a BBB, and circulating factors leak into the CNS [26]. In addition, the presence of a transport system at the BBB enables some factors in circulation to cross into the CNS [27]. Moreover, we recently reported that CNS remyelination is promoted by peripheral hormones, such as fibroblast growth factor (FGF) 21 [28]. With regard to leptin, leptin level in the cerebrospinal fluid (CSF) is correlated with that in plasma in humans [29]. In addition, in vitro experiments investigating the transport capacity revealed that a short form of leptin receptor, ObRa, contributes to transcellular leptin transport in Madin Darby Canine Kidney cells [30]. These findings suggest that circulating leptin may penetrate into the CNS.

Objectives

Regarding the action of leptin in the CNS cells, especially oligodendrocyte lineage cells, leptin-deficient *ob/ob* mice showed lower brain weight and total brain proteins compared with those in wild-type mice [31]. These decreases are interpreted as the decrease of myelination due to a reduction of oligodendrocytes [32]. Therefore, it is a common view that leptin affects developmental myelination; whereas the precise effect of leptin on remyelination under the diseased condition is unclear. This highlight provides a brief overview of our recent finding about the function of leptin on CNS remyelination.

Leptin promotes OPC proliferation in vitro

At the first step, we analyzed the effect on the proliferation activity of OPCs by leptin treatment. To investigate the direct action of leptin on OPCs, we collected the cells, which were labeled by PDGFRα, an OPC marker from the mouse brain and spinal cord. As mentioned above, myelination/remyelination is started by OPC proliferation. Because we had found the ObRb expression in cultured OPCs obtained from the mouse brain and spinal cord [33], we examined whether leptin treatment promoted OPC proliferation. Cell proliferation activity was correlated with the incorporation of 5-bromo-2-deoxyuridine (BrdU) into the cells. By measurement of the BrdU incorporation activity, treatment with recombinant leptin increased the proliferation of OPCs obtained from the mouse brain and mouse spinal cord, indicating that leptin enables the enhancement of OPC proliferation directly.

ObRb promotes the activation of intracellular signaling, such as ERK phosphorylation. Because gain-of-function studies reported that the constitutive activation of Mitogen-activated protein kinase (MAPK) / ERK kinase 1 (Mek1), an upstream activator of ERK, increases myelin thickness during remyelination [34], we wondered if leptin-mediated OPC proliferation is also dependent on ERK activation. Western blot analysis revealed that leptin treatment promoted ERK phosphorylation in cultured OPCs. We next investigated whether inhibition of ERK activation blocked leptin-enhanced OPC proliferation. We added U0126, a MEK inhibitor, into the OPC culture, and then treated the cells with recombinant leptin. BrdU incorporation analysis revealed that treatment with MEK inhibitor prevented leptin-mediated OPC proliferation. The results

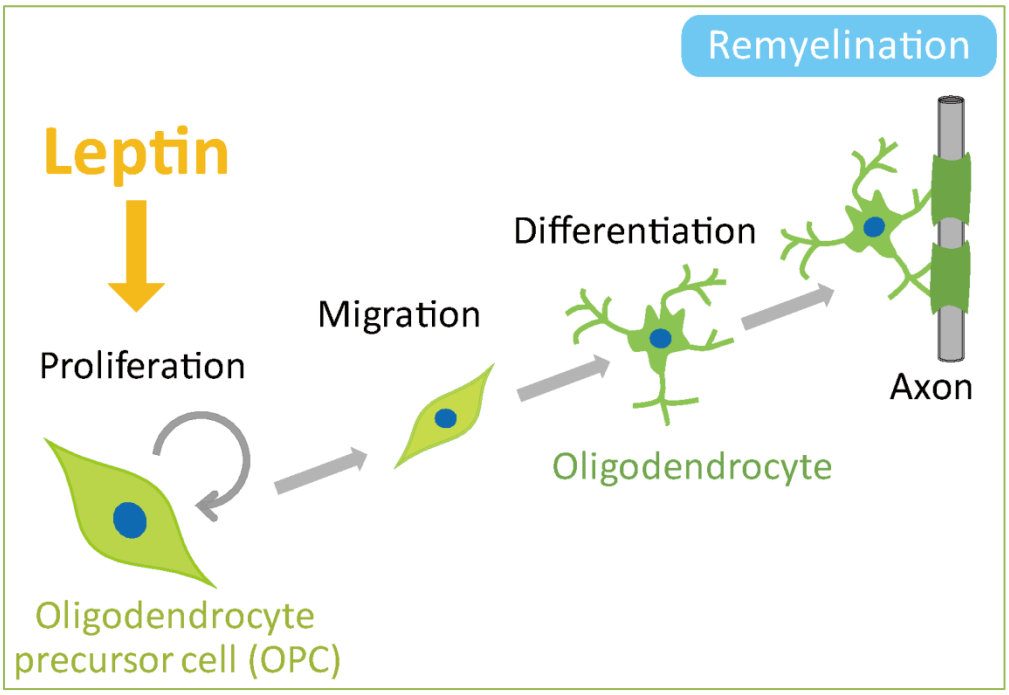

Figure 1. Leptin promotes OPC proliferation. Remyelination is started by OPC proliferation in response to demyelination. The proliferated OPC is considered to migrate into the demyelination site and then differentiate into the mature oligodendrocyte. We found that leptin promotes OPC proliferation without affecting the differentiation of OPC into a mature oligodendrocyte.

showed that leptin promoted OPC proliferation by a process dependent on ERK activation.

Leptin is involved in spontaneous remyelination in vivo

To investigate whether the observation in vitro is consistent with in vivo remyelination, we used a lysophosphatidylcholine (LPC)-induced demyelination model mouse [35, 36], which also reveals spontaneous remyelination [25]. In this model, although we did not detect significant neuronal loss by the measurement of NeuN-positive cell number around the LPC injection site in the spinal cord, damage of the vascular barrier around the lesion site was found [37]. Because vascular barrier disruption causes leakage of circulating factors into the CNS, we hypothesized that LPC injection into the spinal cord increased the exposure of leptin to the spinal cord parenchyma. To investigate this, we measured the leptin level in the spinal cord after LPC injection. As expected, the spinal leptin level in LPC-injection mice was higher than that in control (intact) mice. We detected no significant difference of leptin mRNA level in the spinal cord between LPC injected-mice and intact mice. Therefore, we considered that the increase of leptin level was dependent on the influx of circulating leptin to the CNS without a change of local leptin synthesis in this model. We also confirmed that LPC injection into the spinal cord did not change the leptin protein level in the following peripheral tissues: adipose tissue, liver, kidney, heart, lung, muscle, spleen, and stomach.

To investigate the function of leptin in OPCs, we examined the expression of ObRb in OPCs in adult mice spinal cords. Immunohistochemical analysis showed ObRb expression in PDGFRα-labeled OPCs occurred in the intact mice spinal cords. However, other CNS cells, such as glial fibrillary acidic protein-positive astrocytes and CD11b-positive microglia/macrophages also expressed ObRb. Therefore, we generated OPC-specific leptin receptor knockout mice by crossing tamoxifen-inducible OPC-specific Cre mice and LepR-floxed mice, which reveal the function of leptin in OPCs. We injected LPC into the conditional knockout mice and analyzed the number of proliferating OPCs around the lesion. The number of proliferating OPCs in conditional knockout mice was lower than that in control littermates, indicating that leptin receptor in OPCs is required for OPC proliferation in response to demyelination. Because OPC proliferation contributes to subsequent remyelination, we evaluated the remyelination area by the measurement of the myelin basic protein (MBP)-negative area in the spinal cord. The MBP-negative area in the conditional knockout mice was larger than that in control littermates, indicating that OPC-specific leptin reduction also prevented remyelination. Because remyelination is also required for differentiation from OPC into a mature oligodendrocyte, we investigated the leptin

effect on OPC differentiation. By BrdU tracing analysis, the change of BrdU-labeling OPC number induced by leptin treatment was comparative level in that of glutathione-S-transferase (GST)π-positive mature oligodendrocyte, which incorporated BrdU during the same period. These data suggest that leptin promotes OPC proliferation but does not affect differentiation from OPC to a mature oligodendrocyte. We observed no significant difference of CD11b-positive cell numbers after LPC injection between conditional knockout mice and control littermates, indicating that leptin-mediated OPC proliferation does not depend on microglia.

Leptin promotes OPC proliferation

The above results supported the hypothesis that endogenous leptin contributes to spontaneous OPC proliferation. We finally investigated whether recombinant leptin induced OPC proliferation. To attain the greatest action of recombinant leptin on the OPCs, we performed intrathecal treatment with recombinant leptin continuously. We counted the proliferating OPCs in the spinal cord by immunohistochemical analysis. As the results, recombinant leptin treatment increased the number of proliferating OPCs compared with the control. Therefore, leptin treatment enhanced OPC proliferation after demyelination.

Conclusions

Our findings revealed that leptin has the potential to regulate OPC proliferation in the pathological condition in the CNS (Figure 1). Leptin is known as one of the peripheral hormones, and the function of peripheral hormones on OPC proliferation is consistent with our finding that peripheral FGF21 also promotes OPC proliferation in mice and humans [28]. These studies just focused on OPC proliferation; however, the results provide the possibility that the peripheral environment regulates other types of CNS cells, which supports the previous knowledge that the systemic environment regulates neuronal plasticity [38, 39]. Regarding the utility of peripheral leptin in demyelination, our observation was limited by the histological change in the LPC-injected spinal cord after leptin treatment. Therefore, an investigation of the behavioral benefit by leptin treatment is required to support the therapeutic avenue of leptin for treating demyelination. In addition, evidence of a beneficial effect of leptin in humans is also needed to consider the utility of leptin for treating human demyelinating diseases. In contrast, leptin-based medicine (using an analogue of the human hormone leptin, Metreleptin) has already been developed by pharmaceutical companies and is approved by the United States Food and Drug Administration for use of lipodystrophy. Therefore, the above mentioned investigation may provide an opportunity for the repositioning of existing drugs that target leptin for treating demyelinating diseases.

Conflicting interests

The authors have declared that no conflict of interests exist.

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Author contributions

K.M. drafted the figure, R.M. revised the figure and wrote the manuscript, and T.Y. revised manuscript. All authors reviewed the manuscript.

Abbreviations

CNS: central nervous system; Jak2: Janus Kinase-2; SHP2: SH2-containing tyrosine phosphatase-2; ERK: extracellular signal regulated kinase; MAPK: Mitogen-activated protein kinase; Mek1: MAPK / ERK kinase 1; OPCs: oligodendrocyte precursor cells; PDGF: platelet-derived growth factor; FGF2: basic fibroblast growth factor; BBB, blood brain barrier; CSF: cerebrospinal fluid; fibroblast growth factor; BrdU: 5-bromo-2-deoxyuridine; LPC: Lysophosphatidylcholine; MBP: myelin basic protein; GST: glutathione-S-transferase.

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