ISSN:

Gratis

Journal of Nutritional Biology

Mini Review

Significance of CIN85 Analysis in Nutritional Study

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Received: April 20, 2015; Accepted: April 26, 2015; Published: April 28, 2015

Abstract

Nutritional and metabolic processes involve numerous pathways. It is highly significant to clarify the presence and function of signaling molecules involved in each pathway. A Cbl-interacting protein of 85 kDa (CIN85) belongs to a family of ubiquitously expressed adaptor/scaffold proteins. CIN85 is a multiadaptor protein implicated in the regulation of receptor endocytosis, cell division, and the cellular cytoskeleton. Mice deficient in brain-specific CIN85 expression shows aberrant dopamine signaling; insufficient complex formation of endophilins with D2 dopamine receptors (D2DRs) in the striatum, an important center for the coordination of animal behavior and ultimately decreased D2DR endocytosis in striatal neurons in response to dopamine stimulation. As a result, CIN85-deficient mice exhibit the hyperactive phenotype. Here, we show other phenotypes of CIN85 deficient-mice; low levels of bone mineral density and blood glucose. From a perspective of nutritional research, the analysis of CIN85 and its deficiency in mice may lead to new developments in nutritional study.

Keywords: CIN85; CIN85 deficient-mouse; Nutritional research; Bone mineral density; Glucose metabolism

Abbreviations: ADHD: Attention Deficit/Hyperactivity Disorder; CD2AP: CD2-associated protein; CIN85: Cbl-Interacting Protein of 85 kDa; CNS: Central Nervous System; D2DR: D2 Dopamine Receptor; EGFR: Epidermal Growth Factor Receptor; HGFR: Hepatocyte Growth Factor Receptor; IKK-β/NFκB: IκB Kinase-β/nuclear Factor-kappa B; PI 3-kinase: Phosphoinositide 3-kinase; PSD-95: Postsynaptic Density Protein 95; Ruk: Regulator of Ubiquitous Kinase; SETA: SH3 Domain-containing Gene Expressed in Tumorigenic Astrocytes; SH3: Src Homology 3; SH3KBP1: SH3 Domain Kinase Binding Protein 1; VEGFR: Vascular Endothelial Growth Factor Receptor

Introduction

Adaptor proteins are noncatalytic polypeptides that contain one or more domains that can bind to other proteins or nonprotein ligand molecules [1]. These molecules are essential for intracellular signal transduction involved in the regulation of metabolic activity, endocrine action, neuronal function, and cell growth. A Cbl-interacting protein of 85 kDa (CIN85) is a multiadaptor protein containing three Src homology 3 (SH3) domains, a proline-rich region, and a coiled-coil domain [2]. CIN85 controls the spatial and temporal assembly of multiprotein complexes that are involved in the regulation of cell growth, differentiation, migration, and survival [3]. Specifically, CIN85 was shown to link Cbl-epidermal growth factor receptor (EGFR) complexes with endophilin-dependent receptor internalization and to downregulate EGFR signaling [4,5].

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Previously, we reported a novel *in vivo* function of CIN85 in the regulation of postsynaptic dopamine receptor endocytosis in striatal neurons [6]. Mice deficient in CIN85 (CIN85$^{\Delta ex2}$, lacking CIN85 exon 2, CIN85 KO mice) expression show the attention deficit / hyperactivity disorder (ADHD) phenotype. ADHD is one of the most common childhood disorders and can continue through adolescence and adulthood. Symptoms include difficulty staying focused and paying attention, difficulty controlling behavior, and hyperactivity [7]. It remains unclarified what causes ADHD. Various factors have been suggested, among which genes are believed to play certain critical roles in ADHD onset. As a molecular explanation of this phenotype, we concluded that the absence of striatal CIN85 causes insufficient formation of complexes of endophilins with dopamine receptors in the striatum and ultimately suppressed dopamine receptor endocytosis in striatal neurons in response to dopamine stimulation.

During the course of our studies of CIN85 KO mice, we found that these mice show lower bone mineral density (BMD) and blood glucose level than the wild-type mice. In this review, I briefly describe a novel phenotype of CIN85 KO mice regarding the nutritional/metabolic function and the usefulness of CIN85-deficient mice for nutrition/metabolism research.

Structure and Function of CIN85

The CIN85 was independently identified by three research groups in 2000 [2], and is also called regulator of ubiquitous kinase (Ruk) [8], SH3 domain-containing gene expressed in tumorigenic astrocytes (SETA) [9], and SH3 domain kinase binding protein 1 (SH3KBP1) [10]. These genes were isolated from either human (CIN85), rat (Ruk and SETA), or mouse (SH3KBP1) sources and show between 92% and 97% sequence identities, suggesting that they are homologues of one gene. The CIN85 gene is localized on the short arm of the X chromosome (Xp22.1-p21.3) and its length is approximately 353.7 kb in humans. The main 3.2 kb CIN85 mRNA is expressed in all adult and newborn tissues [2,8]. Owing to alternative splicing and the use of different promoters, multiple CIN85 mRNA signals have been detected, which showed a more restricted pattern of expression [9]. CIN85 is composed of three N-terminal SH3 domains, followed by a centrally located proline-rich region and a C-terminal coiled-coil domain [3]. Initially, CIN85 was identified as a negative regulator of the EGFR signaling and phosphoinositide 3-kinase (PI 3-kinase) signaling pathways via its interaction with c-Cbl [2,8]. Then, CIN85 was identified as a central adaptor molecule involved in the recruitment of the endocytic machinery required for the internalization of various cell surface receptors, including receptor tyrosine kinases (RTKs) such as EGFR [4,5], the hepatocyte growth factor receptor (HGFR, Met) [11], and the vascular endothelial growth factor receptor (VEGFR) [12], as well as immunoglobulin IgE receptors in mast cells [13]. Recently, it has been reported that CIN85 is involved in the regulation of the immune system and cytokinesis. Using B cell-specific CIN85 knockout mice, Kometani et al. [14] found that CIN85 links the B cell receptor to IκB kinase-β/nuclear factor-kappa B (IKK-β/NFκB) activation, thereby contributing to T cell-independent immune responses. Haglund et al. [15] reported that Cindr, a *Drosophila* CD2AP/CIN85 ortholog, interacts with anillin and that depletion of either Cindr or anillin gives rise to binucleate cells and fewer intercellular bridges *in vivo*; therefore, Cindr is involved in complete and incomplete cytokinesis in *Drosophila*. In the future, as these reports indicated, a novel function of CIN85 may be identified because CIN85 is expressed ubiquitously.

CIN85 Deficiency Causes Hyperactivity Owing to Aberrant Dopamine Signal

Recently, we have found a novel function of CIN85 in the regulation of the signaling of behavior-related molecules [6]. To investigate the function of CIN85 in the central nervous system (CNS) *in vivo*, we generated mice deficient in the expression of two major CIN85 isoforms (CIN85-xl and CIN85-l) in the brain [6,16]. By homologous recombination, we deleted exon 2 of the CIN85 genomic locus (CIN85$^{\Delta ex2}$). As expected, all CIN85 protein variants encoded by transcripts initiated from promoter #1 (CIN85-xl, CIN85-l, and the shorter CIN85-ΔCP) were abolished in CIN85$^{\Delta ex2}$ mice (CIN85 KO mice).

When subjected to a modified hole-board test [17], which assays spontaneous behavioral factors such as forward and vertical locomotor activity, speed of movement, and exploratory behavior in a novel environment, the CIN85 KO mice showed significantly increased activities, as compared with the wild type. Specifically, the CIN85 KO mice exhibited increased forward locomotor activity, as manifested by increases in total distance travelled, number of line crossings, mean and maximum velocities, as well as turning frequency. In addition, CIN85 KO mice showed enhanced exploratory behavior, namely, entering the board more frequently and exploring a larger number of holes on the board, than the wild-type mice.

Interestingly, the CIN85 KO mice showed abnormally high levels of dopamine and D2 dopamine receptors (D2DRs) in the striatum [6], an important center for the coordination of animal behavior. Importantly, CIN85 localizes to the postsynaptic compartment of striatal neurons, in which it coclusters with D2DRs and with postsynaptic density protein 95 (PSD-95) [6], which is a scaffold protein involved in the assembly and function of the postsynaptic density complex [18]. Moreover, it interacts with endocytic regulators such as dynamin and endophilins in the striatum. In neurons of the wild-type mice, CIN85 resides postsynaptically and associates with endocytic regulators, such as dynamin and endophilins, and it clearly has a crucial function in stabilizing endophilin binding to D2DRs in the striatum. The internalization of D2DRs is caused by the coordination of functions of these endocytic proteins. As a result, dopamine signals are attenuated, and then the appropriate locomotor activity is maintained. The absence of CIN85 gives rise to insufficient endocytic internalization of D2DRs owing to the defect of endophilin recruitment to the endocytic complex after dopamine stimulation, increasing striatal dopamine receptor expression levels, which can, at least in part, explain the enhanced locomotor and exploratory behavior we observe in the CIN85 KO mice. The resulting increase in the expression levels of surface-associated D2DRs in CIN85 KO mouse striatal neurons and the ensuing hyperactivity phenotype are in line with earlier findings, showing that activation of postsynaptic D2DRs results in increased locomotor activity and that D2DR knockout mice display reduced spontaneous movements [19,20].

Regulation of Appetite and Food Reinforcement by Dopamine

When we compared CIN85 KO and wild-type mice, we found that the KO mice showed deviations in several metabolic parameters, including increased energy uptake, higher lean mass, and lower fat content [6]. We concluded that the deviation from the normal range of these metabolic parameters is due to the hyperactivity of CIN85 KO mice. However, recently, there has been accumulating evidence showing that dopamine signals are involved in appetite and food intake. Epstein *et al*. analyzed the polymorphisms of human D2DR and reported that the D2DR genotype may interact with food reinforcement to influence energy intake [21]. More recently, low expression levels of D2DR and attenuated responsivity of dopamine-target regions (e.g., the striatum) to food and food cues are associated with increased weight [22]. Therefore, dopamine signals mediated by D2DR may interact with food reinforcement to influence energy intake. CIN85 KO mice exhibit aberrant dopamine signaling. CIN85 and CIN85 KO mice seem to be useful in the study of the regulation of appetite and food reinforcement in addition to hyperactivity.

Perspective of Nutritional/Metabolic Study in CIN85 KO Mice

In our current study, we also found that CIN85 KO mice show a significant decrease in BMD as compared with the wild-type mice (Figure 1, left panel). BMD was measured by bone densitometry, also called dual-energy X-ray absorptiometry (DEXA), which is an improved form of X-ray technology. BMD is an index of the amount of minerals (mostly calcium and phosphorus) contained in a certain volume of bone. BMD is reduced in various physiological conditions; aging, insufficient calcium and vitamin D intake, lack of exercise and female menopause. On the other hand, BMD is used in the diagnosis of osteoporosis, determination of the efficiency of drugs against osteoporosis, and prediction of bone fracture. CIN85 KO mice might provide new knowledge in metabolic studies of bone mineral and nutrients.

Furthermore, the blood glucose level in CIN85 KO mice was reduced in both sexes (Figure 1, right pan-

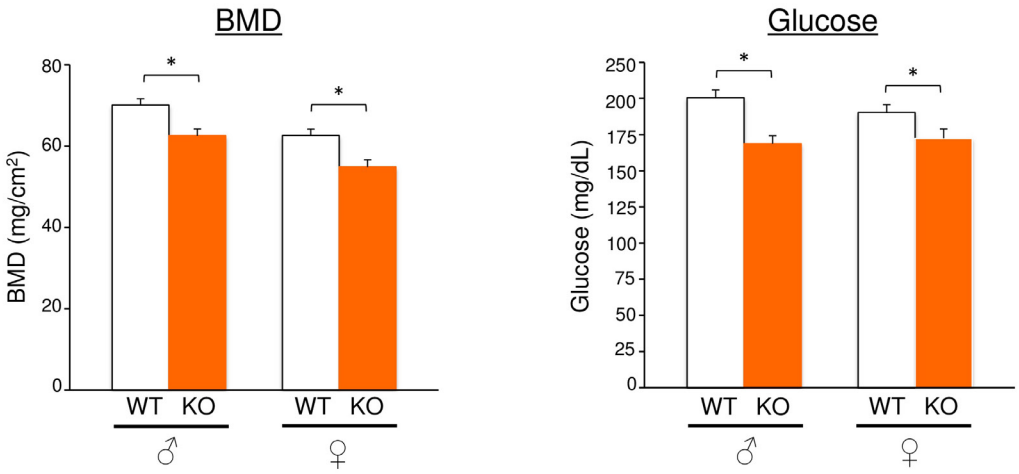

Figure 1: CIN85 KO mice showing abnormal metabolic parameters. Both male (♂) and female (♀) CIN85 KO mice showed decreases in BMD (left panel) and blood glucose level (right panel) as compared with the wild-type mice. n=6-15. *, $p < 0.05$.

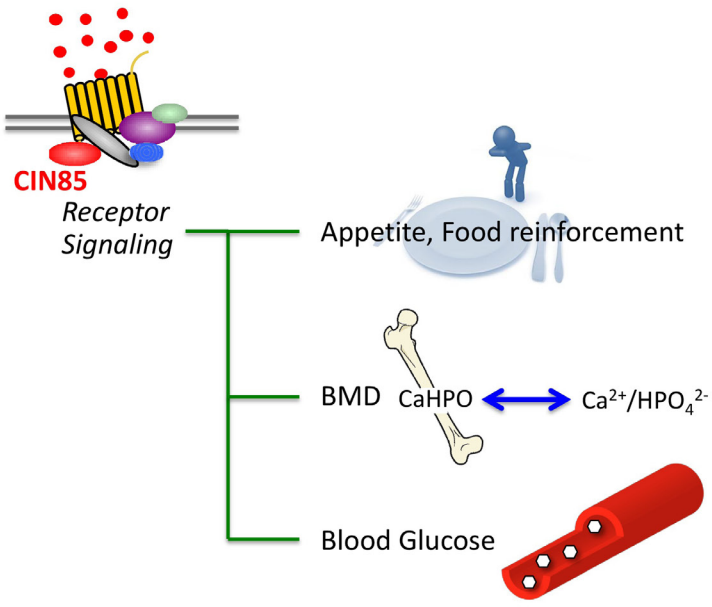

Figure 2: CIN85 KO mice show aberrant dopamine signals and significant decreases in BMD and blood glucose level. CIN85 might have a critical role in appetite regulation and balance between bone mineral and blood glucose levels through receptor signaling. Research on CIN85 and CIN85-deficient mice provides new insights in nutrition and metabolic studies.

el). Initially, CIN85 was noted as a negative regulator of RTKs such as EGFR [4] and HGFR [11]. The insulin receptor also belongs to the family of RTKs [23]. In the presence of CIN85 deficiency, insulin signaling may become excessive. As a result, blood glucose concentration is reduced. It is very interesting to clarify whether CIN85 is involved in glucose homeostasis through the insulin receptor downregulation.

In conclusion, CIN85 is a novel regulator of dopamine receptor signaling, and is involved in controlling behavior. CIN85 KO mice provide new insights in appetite and nutritional studies through the analysis of dopamine signaling, BMD, and glucose metabolism (Figure 2).

Acknowledgements

This work was supported by a Grant-in-Aid for Scientific Research (26460317), and by a Grant-in-Aid for Scientific Research on Innovative Areas (Brain Environment; 24111506) from MEXT of Japan to NS.

Competing interests

The author declares no conflict of interest associated with this review.

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