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研究成果報告書

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亜鉛によるメタボとロコモの予防：亜鉛シグナルの理解による安心で安全な社会を目指して

Prevention of Metabolic Syndrome and Locomotive Syndrome with Zinc: Toward a Safe and Secure Society through Understanding Zinc Signaling

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Abstract

In Japan, the gap between average life expectancy and healthy life expectancy has become a major social issue. In particular, locomotive syndrome, which is a risk factor for requiring long-term care, is known to cause deterioration of motor systems such as bones, joints, and muscles, resulting in impairments in daily activities such as walking, standing, and sitting. It has also been suggested that metabolic syndrome in middle age and beyond is behind the need for care due to cerebrovascular disease. In other words, preventing locomotive and metabolic syndrome is considered extremely important for extending healthy life expectancy.

Previous analyses have revealed that the zinc transporter ZIP13 regulates skeletal muscle and adipocyte functions based on the results of studies in humans (EDSSPD3) and mice lacking this transporter activity. In this study, we aimed to answer the questions, "How is the pathophysiology of locomotive syndrome and metabolic syndrome related to the loss of function of ZIP13, which is involved in the regulation of locomotion and energy metabolism?" Previous studies have shown that mice lacking the whole body *Zip13* gene (*Zip13*-KO mice) have an ameliorative effect on metabolic syndrome by increasing browning in white adipose tissue, but it is not clear whether the regulation of browning by ZIP13 is regulated in an adipocyte-autonomous manner. Therefore, we generated mature adipocyte-specific *Zip13*-deficient mice (*Zip13*^{Adipo}-cKO) and analyzed them, and found that *Zip13*^{Adipo}-cKO mice exhibit enhanced lipolysis, preferentially consume lipids, and show resistance to obesity, suggesting a novel ZIP13 function in lipid metabolism that has not been identified before. Furthermore, the expression of Fth1, a ZIP13-binding protein, was increased in *Zip13*^{Adipo}-cKO adipose tissue, and suppression of Fth1 expression reduced lipolysis in *Zip13*^{Adipo}-cKO mice, suggesting that a novel pathway, the ZIP13-Fth1 axis, is involved in the regulation of lipolysis.

Zip13-KO mice showed a marked decrease in muscle strength and activity. To investigate the cause of this, we collected muscle tissue and performed RNAseq and found that myogenesis, muscle contraction, and calcium signaling were decreased in *Zip13*-KO mice. Therefore, we evaluated skeletal muscle differentiation potential using a mouse myoblast cell line C2C12 cells (*Zip13*-KD cells) and an iPS cell line derived from EDSSPD3 patients, in which the *Zip13* gene was knocked down, and found that differentiation into skeletal muscle was markedly suppressed. This suggests that ZIP13-mediated zinc signaling is required for the promotion of skeletal muscle differentiation. Further tissue-specific functional analysis has revealed that the abnormalities in skeletal muscle differentiation described above are due to a role for ZIP13 in *Pdgfra*-positive mesenchymal cells rather than a role for ZIP13 in *MyoD*-positive myoblasts, which is now being analyzed in detail.

Next, we analyzed the downstream signals of ZIP13 involved in the induction of beige adipocyte differentiation. In *Zip13*-KO cells with enhanced beige adipogenesis, an increase in the protein content of PIAS3 was observed, and at the same time, inhibition of beige adipogenesis was observed when PIAS3 was deleted. This indicates that ZIP13 induces beige adipogenesis from progenitor adipocytes by regulating the protein content of PIAS3 through the "ZIP13-PIAS3 axis". Furthermore, we identified regions and amino acid residues important for the binding of PIAS3 and ZIP13 within each molecule. Based on this information, we generated a ZIP13 mutant that does not bind to PIAS3 but retains the ability to transport zinc, and introduced it into Zip13-deficient preadipocytes. The mutant was found to have significantly reduced ability to suppress beige formation by wild-type ZIP13. In other words, direct association between ZIP13 and PIAS3 was required for ZIP13-mediated beige suppression, suggesting that PIAS3

may be a direct downstream molecule of ZIP13. We are currently analyzing the details of the association mode between ZIP13 and PIAS3.