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論 文 の 要 約

報告番号	㊙甲 乙 第 228 号	氏 名	越智 ありさ
学位論文題目	*N*-Myristoylated ubiquitin ligase Cbl-b inhibitor prevents on glucocorticoid-induced atrophy in mouse skeletal muscle		

Under unloading conditions, such as bed rest or microgravity, skeletal muscle is vulnerable to rapid atrophy. This atrophy is induced by both increased protein degradation and decreased protein synthesis. Proteolysis is enhanced in muscle atrophy primarily due to activation of the ubiquitin-proteasome pathway. Expression of the ubiquitin ligase Casitas B-lineage lymphoma b (Cbl-b) is significantly upregulated under skeletal muscle atrophic conditions. Cbl-b induces muscle atrophy, via its negative regulation of insulin-like growth factor-1 (IGF-1) signaling in skeletal muscle cells through the enhancement of ubiqutination and degradation of insulin receptor substrate 1 (IRS-1). Consequently, the loss of IRS-1 permits the expression of other muscle atrophy-associated ubiquitin ligase (atrogenes), including muscle atrophy F-box protein (MAFbx)/atrogin-1 and muscle RING finger protein-1 (MuRF-1) in a fork head box O (FOXO3)-dependent manner. Thus, Cbl-b is one of several atrogenes, and the inhibition of Cbl-b-mediated ubiquitination of IRS-1 has become an attractive therapeutic target in developing treatments for muscle atrophy. We previously developed a Cbl-b inhibitor for the treatment of unloading-mediated muscle atrophy. This DGphosphrylated(p)YMP penta-peptide, named Cblin inhibited Cbl-b-mediated IRS-1 ubiquitination and was effective against denervation-induced muscle atrophy. We also solved the crystal structure of the Cbl-b tyrosine-kinase binding (TKB) domain:Cblin complex at a resolution of 2.8 Å. However, a high dose of Cblin was necessary to rescue muscle atrophy because Cblin was prone to degradation by aminopeptidases and also showed low efficiency in penetrating cell membranes. In the present study, we sought to increase the inhibitory activity of Cblin by modifying it via N-myristoylation. Interestingly, the N-terminal myristoylation of Cblin rendered the molecule highly resistant to ubiquitination. We also found that N-myristoylated Cblin prevented glucocorticoid-induced skeletal muscle atrophy in vivo. Thus, N-myristoylated Cblin is a novel agent that may be useful for treating muscle atrophy. Using HEK293 cells overexpressing Cbl-b, IRS-1 and ubiquitin, we showed that the 50% inhibitory concentrations of Cbl-b-mediated IRS-1 ubiquitination by N-myristoylated Cblin and Cblin were 20 μM and 90 μM, respectively. Regarding the dexamethasone-induced atrophy of C2C12 myotubes, N-myristoylated Cblin was more effective than Cblin for inhibiting the dexamethasone-induced decreases in C2C12 myotube diameter and IRS-1 degradation. The inhibitory efficacy of N-myristoylated Cblin on IRS-1 ubiquitination in C2C12 myotubes was approximately three larger than that of Cblin. Furthermore, N-myristoylation increased the incorporation of Cblin into C2C12 myotubes approximately 10-fold. Finally, we demonstrated that N-myristoylated Cblin prevented dexamethasone-mediated gastrocnemius muscle wet weight loss, IRS-1 degradation, MAFbx/atrogin-1 expression, and MuRF-1 expression approximately three-fold more effectively than Cblin. Taken together, these results suggest that N-myristoylated Cblin prevents dexamehasone-induced skeletal muscle atrophy in vitro and in vivo, and that N-myristoylated Cblin more effectively prevents muscle atrophy than unmodified Cblin.