Abstract 2322

Caudatin ameliorated muscle atrophy via Hedgehog signaling in C2C12 cells

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Keywords: Hedgehog (Hh) signaling, Caudatin, Muscle atrophy, Myogenesis, C2C12 cell line

Background: Previous studies have demonstrated that Hedgehog (Hh) signaling is involved in ameliorating muscle atrophy. Different phytochemicals were investigated to show the regulatory effect on muscle atrophy through modulation of the cellular signalings including Hh signaling. However, caudatin, a C-21 steroid identified from Cynanchum plant roots, has not been studied on muscle atrophy. In this study, we hypothesized that caudatin could suppress muscle atrophy by activating the Hh signaling pathway. Methods: To investigate the effect of caudatin on inhibiting muscle atrophy, experiments were conducted using the mouse myoblast-derived C2C12 cell line under various conditions. (i) Muscle atrophy was induced by dexamethasone (DEX) in C2C12 cells and effect of caudatin was investigated. (ii) Muscle atrophy was induced by TNFα in C2C12 cells and effect of caudatin was investigated. (iii) C2C12 cells were differentiated in the presence of caudatin for 5 days to confirm its effect on improving myogenesis. Myotube formation and myosin heavy chain (MHC) expression were assessed in all conditions using Jenner-Giemsa staining and immunofluorescence staining. Additionally, key markers related to Hh signaling, myogenesis, and protein synthesis/degradation were evaluated by western blot. Results: Caudatin demonstrated inhibitory effects on muscle atrophy induced by DEX and TNFα through the activation of Hh signaling. It upregulated AKT signaling, a crucial protein synthesis signal, while concurrently enhancing myotube formation and MHC expression. This improvement was associated with the suppression of muscle atrophy F-box (MAFbx)/atrogin-1 and muscle ring finger 1 (MuRF1) expression, known markers of muscle atrophy. Furthermore, caudatin positively influenced the myogenesis process by increasing the expression of myogenic markers during differentiation. Conclusion: Taken together, caudatin was found to ameliorate muscle atrophy and enhance myogenesis processes through the Hh signaling pathway. These findings suggest that caudatin has the potential to contribute to muscle health homeostasis by counteracting the process of muscle atrophy.

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Abstract 2341

Learning from Nature: strategies for drugging dynamic nuclear proteins

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Conformationally dynamic proteins are at the heart of most cellular machines and frequently dysregulated in disease. Unfortunately, they are often considered 'undruggable' as they are not amenable to targeting by standard drug discovery methodologies. This is particularly true for transcriptional coactivators and activators, proteins that are linked to many disease states yet are have few probes or drugs that engage them. Using transient kinetic studies of activator-coactivator complexes coupled with structural studies, we found that unstructured loop regions adjacent to canonical binding sites within coactivators proteins play a central role in the specific recognition of transcriptional activator binding partners and in allosteric communication between binding sites. Through screening we have found that these sites are amenable to small-molecule targeting and have identified first in-class inhibitors for challenging targets such as Med25. Further, we have developed a screening workflow that enables rapid discovery of submicromolar inhibitors for several transcriptional coactivators and other dynamic nuclear proteins.

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