

論文の内容の要旨

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論文題目

Studies of the skeletal muscle atrophy-responsive factor
and regulatory mechanism
(筋萎縮シグナル応答性分子の発現制御及び機能解析)

Chapter 1: Introduction

The acceleration of aging is a significant issue facing the world today, posing substantial challenges to societal, economic, and healthcare systems. Skeletal muscle, as the largest metabolic organ, plays a pivotal role in the aging process. Sarcopenia, a phenomenon that occurs in aged populations with loss of muscle mass (also known as muscle atrophy), is primarily contributed by a decline in protein synthesis, an increase in protein degradation, and disruptions in structural units of skeletal muscle function, such as endoplasmic reticulum (ER) stress and disorders in neuromuscular junctions (NMJ). Novel regulators of muscle performance are urgently required to be defined and applied to the development of therapeutic interventions against patients suffering severe muscle atrophy symptoms.

Fibroblast growth factors (FGFs) 21, as one of the endocrine FGFs, functions variably in different contexts, with less is known about its direct contribution to skeletal muscle, especially in conditions of neurogenic muscle atrophy (one of the causative mechanisms of sarcopenia). Therefore, in this study, we aim to address the functionality of FGF21 in a model of denervation-induced muscle atrophy and characterize the physiological significance of muscular involvement of FGF21.

Chapter 2: Construction denervation model and identification of FGF21

An experimental denervation model of skeletal muscle atrophy was established, through which we screened the responsiveness of whole FGF members in the context of denervation. Based on expression profiles, FGF21 was found to be exclusively and persistently elevated in the skeletal muscle, rather than other primary FGF21-producing tissues. The upregulated level of FGF21 was negatively correlated with skeletal muscle mass and performance, including declined grip strength of mice, decreased muscle mass, and diminished muscle fiber.

Chapter 3: The regulatory mechanisms of FGF21

By means of transcriptomic analysis, transforming growth factor beta (TGF- β) signaling was screened as an activated signaling associated with muscle atrophy in response to denervation.

Notably, in muscle C2C12 cells, both TGF- β 1 and FGF21 alone could function as muscle atrophy inducers, while TGF- β 1 was also capable of promoting muscle atrophy through FGF21 via a non-canonical JNK/c-Jun pathway, rather than canonical smad2/3 pathway, to exert the synergistic atrophy-provoking effect. Moreover, the pro-atrophic TGF- β 1 was elicited specifically in skeletal muscle, similar to muscle-specific expressed FGF21, again demonstrating the closed association between the two factors. Lastly, the source of denervated muscle-derived TGF- β 1 was determined to be contributed from muscle-resident fibro/adipogenic progenitors (FAPs) in vivo, which released both TGF- β 1 and FGF21 to promote atrophy of myofibers likely through mechanisms demonstrated in muscle C2C12 cells.

Chapter 4: Pharmacologic and physiologic functions of FGF21

By taking advantage of the recombinant adeno-associated virus (rAAV)-mediated gene overexpression system, the pharmacological hyperactivation of FGF21 contributed to impaired muscle performance and can be superimposed with denervation to exacerbate muscle atrophy. By employing genetically Fgf21 knock-out mice, the atrophy-resisting effect of Fgf21 deficiency mediated by attenuated expression of E3 ubiquitin ligases was determined, which was likely achieved by the restoration of functional structures in the skeletal muscle, that is endoplasmic reticulum (ER) and neuromuscular junction (NMJ). The protective effect of Fgf21 deficiency was partly due to its resistance against TGF- β 1-induced ER stress, which disrupted the integrity of NMJ, leading to a subsequent loss of neuronal support and ultimately muscle atrophy. In addition, the expression of glucose transporters enhanced in Fgf21-null muscles might physiologically be favorable to combating muscle atrophy.

Chapter 5: The molecular mechanism of FGF21 action

Upon denervation, the subcellular distribution of Class IIa histone deacetylase (HDAC) members (HDAC4, HDAC5, and HDAC7) was found to be accumulated in the cytoplasm in the presence of Fgf21. The cytoplasmic retention of these HDACs was fulfilled through liver kinase B1 (LKB1) and AMP-activated protein kinase (AMPK) kinases cascade, which mediated the phosphorylation of HDAC4 (as well as HDAC5 and HDAC7) by, resulting in its cytoplasmic location and possibly subsequent functional activity. Through the use of the recombinant adeno-associated virus (rAAV)-mediated gene knockdown system, the functionality of changed subcellular localization of HDAC4 was investigated. The beneficial effects on muscle size due to the lack of Fgf21 were completely abolished by further knockdown of HDAC4 in Fgf21-null muscles, suggesting that cytoplasmic HDAC4 mediates the protective effect of Fgf21 deficiency.

Conclusion

In this study, we constructed a neurogenic muscle atrophy model (referred to as denervation) for screening novel regulators of skeletal muscle homeostasis. Among candidate FGF family members, Fgf21 was identified to be standing out in response to denervation. Our results demonstrated the response of Fgf21 was confined to skeletal muscle and regulated by muscle-specific TGF- β 1 signaling via a non-canonical JNK/c-Jun pathway. By taking advantage of rAAV-mediated gain-of-

function and loss-of-function analysis, we proved that pharmacologically overactivation and physiological deletion of Fgf21 would lead to opposite effects in the same context, which emphasizes the side effect of FGF21-based interventions that have long been underappreciated.

Furthermore, we proceeded to elucidate the underlying mechanisms accounting for the function of Fgf21 under the denervation state, tightly correlating members of FGF, TGF, and HDAC families, which provided the innovative insights of an integral association that cannot be ignored when developing FGF21-based therapeutics in clinical practice.