学 位 論 文 の 要 旨

論文題目　**Molecular pathogenesis underlying muscle wasting and development of diagnosis tool for kidney disease in mice.**

(骨格筋障害の病態学解析および腎障害の新たな評価モデルの構築)

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Introduction

Skeletal muscle is the most abundant tissue in human body, and it plays a vital role in sustaining life. Several life sustaining activities such as locomotion, whole body metabolic homeostasis and energy expenditure are all dependent on our skeletal muscles. Regrettably, gradual loss of this vital tissue (a process known as muscle wasting or muscle atrophy) is inevitable as we age. Muscle wasting is a debilitating condition that have dire consequences including physical frailty, metabolic disruption, low quality of life and even death. Muscle wasting happens with age, sedentary lifestyle and inactivity resulting from injury and chronic diseases. As life expectancy increases, and sedentary lifestyle and diseases associated with them are on a constant rise, muscle wasting is becoming more and more important public health problem in our aging society. Unfortunately, there is currently no effective therapy that can be used to treat muscle wasting, because the molecular mechanisms driving muscle wasting is still poorly understood. Thus, here, the molecular pathogenesis of skeletal muscle wasting was investigated with a view of identifying novel drug targets. Non-alcoholic fatty liver disease (NAFLD), and diabetes mellitus (DM) disease models have been used for research on muscle wasting, because skeletal muscle disorders are clinically discussed for these two metabolic disease states. Furthermore, given the role of renal injury a common secondary complication of both NAFLD and DM in exacerbating muscle wasting, herein I tried to develop a novel diagnostic tool that can be used to detect this condition at early stage.

1. Satellite cell content and muscle regeneration in a mouse model of NAFLD

Satellite cells (SC) also termed muscle stem cells are the main population of cells responsible for skeletal muscle growth, maintenance, and muscle repair. Given the essential roles of SC in maintaining skeletal muscle health, the first experiment was conducted to investigate the impact of NAFLD on SC population and muscle regeneration. This study demonstrates for

the first time that exposure to NAFLD milieu significantly reduces muscle SC content and function. This depletion of SC pool impaired muscle regeneration in the mouse NAFLD model. The molecular mechanism responsible for this defect appears to be attributed to the elevated expression of proinflammatory cytokines (tumor necrosis factor α [TNFα]) and an oxidative stress marker (Arginase 1, NADPH oxidase-2 [NOX2]). Elevated oxidative stress and inflammation possibly lead to increases in DNA damage and apoptosis of SC in NAFLD mice. Taken together, these results strongly suggest that the impairment of SC content and functions in NAFLD mice is an important molecular event that contributes to muscle wasting associated with NAFLD.

2. **Comparative study on molecular mechanism of diabetic myopathy in two different types of streptozotocin-induced diabetic models**

Several studies have shown that diabetes mellitus (DM) environment induces muscle wasting partially by impairing SC population, activity, and subsequent muscle regeneration. Over the years, a high-dose streptozotocin (STZ) model has been employed to study diabetes induce-muscle wasting also termed diabetic myopathy. However, recent studies have shown that the high STZ treatment could have non-specific cytotoxic effect on other organs including muscle, thereby, making interpretation of results in the literature difficult. To address this concern, the second study aimed to compare diabetic myopathy in a high-STZ model with another well-established STZ model with reduced cytotoxicity (high-fat diet (HFD) and low-dose STZ). The purpose of my study is to identify the molecular mechanism underlying diabetic myopathy in each model that can mirror the pathologies observed in human patients with diabetic myopathy. Although both models exhibit significant reduction in SC content and muscle mass, the molecular cues driving this process in each model differs significantly. This study suggests that both imbalance protein metabolism (i.e., high protein degradation and low protein synthesis) and downregulation of inflammatory pathway partially mediated by elevated blood glucocorticoid levels are involved in muscle wasting in the high-STZ model. On the other hand, chronic inflammation and impaired energy production are considered to be key molecular mechanism underlying muscle wasting in HFD/STZ model. Interestingly, pathophysiological conditions seen in each model mirrors what is observed in diabetic patients.

3. **Serum amyloid A3 promoter-driven luciferase activity enables visualization of diabetic kidney disease**

Since NAFLD and DM almost share common pathological condition, both have related secondary complications (e.g., renal failure and heart failure) that could exacerbate muscle wasting. In particular, failure to detect and treat renal injury at early stage may blunt any gains made in restoring muscle mass. Kidney disease is being managed by standard treatments such as blood glucose and blood pressure control, renin-angiotensin system blockade and reduction of lipid by lipid-lowering agents. However, these therapeutic strategies cannot provide renoprotection if kidney disease has advanced to end stage.

Unfortunately, early diagnosis and noninvasive assessment of renal injury in both human patients and experimental animal models is almost impossible. Thus, to address this problem, the third experiment aims to develop novel noninvasive diagnostic tool to detect kidney injury in two different types of STZ-induced diabetic models. Fibro-inflammatory cues were identified as the major molecular signature underling kidney disease progression in both DM models. Among all the upregulated fibro-inflammatory genes identified, serum amyloid A3 (*Saa3*) was selected as a candidate gene, because of its central role in both fibrosis and inflammation. Thus, Saa3 promoter-driven luciferase reporter (Saa3-promoter luc mice) mice was successfully applied in real-time to visualize renal pathology induced by diabetic fibro-inflammatory cues via non-invasive *in vivo* bioluminescence imaging. Importantly, this new tool was successfully employed to visualize the suppressive effects of insulin treatment on renal fibrosis and inflammation in a diabetic model with moderate dose STZ treatment. Furthermore, Saa3-promoter luc mouse was also able to early detect and quantify non-invasively the nephrotoxicity effect of aristolochic acid. Collectively, these findings indicated that Saa3-promoter luc mice could be widely used in exploring the effectiveness and safety of therapeutic agents on kidney in a non-invasive way.

Conclusion

This study has contributed to our understanding of the molecular mechanisms mediating loss of muscle mass in chronic metabolic disease. The knowledge gained here can be important to solve aging-associated muscle wasting. This study has clearly shown that SC dysfunction is a critical contributor of muscle wasting in mouse model of NAFLD and DM. Given the role of SC in muscle development, maintenance, and regeneration, manipulating their activity may serve as a potential strategy that will be used to treat muscle wasting of human patients. This study strongly suggested that the cascade of events responsible for muscle SC dysfunction in

NAFLD and DM differs in pathology. Importantly, chronic inflammation was implicated as one of the molecular signatures that possibly alters SC activity in both NAFLD and HFD/STZ-induced diabetic model. On the other hand, repression of inflammation drives the impairment of SC number and functions in high-STZ-induced diabetic model. These findings suggest that pharmacological agents directed against components of inflammatory pathways should aim to modulate chronic inflammation and not to totally abrogate it. Furthermore, in addition to defect in SC, this study demonstrated that oxidative stress, low energy production, and imbalance between protein synthesis and degradation play a critical role in the development of diabetic myopathy, giving important hints of multiple targets to treat muscle atrophy associated with chronic disease. This study also presented a reliable diagnostic tool for kidney disease with the ultimate aim of early detection of disease and/or side-effect of drugs in the preclinical phase.