Extracellular Matrix and Gelatinases in Disuse-induced Skeletal Muscle Atrophy

INTRODUCTION:
Extracellular matrix (ECM) plays a critical role in maintaining normal structure and function of skeletal muscle. Muscle basement membrane serves as an interface between cells and the rest of matrix. It is also thought to be responsible for force transmission within the muscle [1]. Interruption of the integrity of basement membrane is thought to play a critical role in muscle degradation [2]. Our previous work has demonstrated degradation of type IV collagen and laminin, two major components of basement membrane in skeletal muscle after disuse [3]. Gelatinases are ECM remodeling enzymes, which are thought to be responsible for basement membrane degradation especially. In our previous work, we demonstrated a critical role of gelatinase A (also known as matrix metalloproteinase 2, MMP-2) in disuse-induced muscle atrophy [4]. However, it remains unknown that if the other member of gelatinases—gelatinase B (also known as MMP-9) plays an equally critical role in disuse-induced muscle atrophy. In this study, we investigated the role of gelatinase B (MMP-9) in disuse-induced skeletal muscle atrophy using MMP-9 null mice. We hypothesize that deleting gelatinase B gene will preserve muscle mass after disuse.

METHODS:
Muscle disuse model: 12 weeks old mice MMP-9 knockout mice (kind gift from Dr. Rajabrata Sarkar, UCSF) and age and gender-matched wildtype FVB mice were used to study disuse-induced skeletal muscle atrophy by unilateral Achilles tendon transection as described previously [3]. Mice were sacrificed 2 weeks after surgery. Bilateral gastrocnemius muscles were harvested and weighed immediately after sacrifice. Muscle weight loss was calculated by the percentage of atrophic side to the control side [3]. All procedures and protocols were approved by our institutional animal care committee.

Zymography. Zymography was conducted using 10% gelatin SDS-PAGE gel (Invitrogen) according to manufacturer’s instructions. Histology and immunofluorescent staining. Muscle samples were fixed and paraffinized. Immunofluorescent staining for collagen IV and laminin were performed and the intensity of fluorescence was digitalized using Scion Image™ [3]. Statistical significance was determined by student T-test.

RESULTS SECTION:
2 weeks after disuse, muscle weight loss in MMP-9 null mice was 21.6 ± 4.2% (mean ± SE). Muscle weight loss in wildtype mice was 23.5 ± 2.9% (mean ± SE). There was no significant difference between these two groups (n=6, P=0.7) (Fig. 1). Zymography showed no obvious MMP-9 activity in wildtype mice 2 weeks after surgery (image not shown). However, significant compensative MMP-2 expression with the majority of the active form was observed in MMP-9 null mice compared to wildtype mice (Fig. 2). Immunofluorescent staining showed significant decrease of laminin and type IV collagen staining in atrophic side compared to the control side in MMP-9 null mice (Fig. 3).

DISCUSSION:
Our results showed that deleting MMP-9 gene does not preserve muscle mass from disuse-induced muscle atrophy. This result suggested that MMP-9 may play a less important role in disuse-induced skeletal muscle atrophy compared to MMP-2.

One interesting finding in this study was the compensative MMP-2 expression in MMP-9 knockout mice. Unlike the wildtype mice, the majority of MMP-2 in atrophic gastrocnemius muscle in MMP-9 null mice is the active form. This finding suggested that there was a post-translational regulation of MMP-2 expression in MMP-9 null mice. Degradation of laminin or type IV collagen in basement membrane in disused muscle in MMP-9 null mice may be due to the compensative expression of activated MMP-2. More work is needed to demonstrate the compensation network between gelatinases and its role in maintaining the structure and function of ECM in muscle.

In summary, our current study demonstrated that deletion of a single gene of MMP-9 does not preserve muscle mass from disuse-induced muscle atrophy. MMP-9 may play a less critical role in this form of muscle disorder.

REFERENCES:

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