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THE ROLE OF MMP-2, 9, AND 13, IN THE REGULATION OF SKELETAL MUSCLE HYPTERTROPHY FOLLOWING FUNCTIONAL OVERLOAD

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Abstract

Purpose: Skeletal muscle is the most abundant tissue in vertebrates that functions primarily to generate locomotion, and exhibits a high degree of structural and functional plasticity that is largely dependent on the level of activity placed on it. Recent studies have shown that a family of enzymes known as matrix metalloproteinases (MMPs) play an important role in regulating this plasticity. Specifically, MMP-2 and MMP-9 degrade components of the extracellular matrix (ECM) surrounding muscle fibers during periods of growth and repair. However, the mechanisms by which these MMPs mediate this response and how each contributes to muscle repair and remodeling remains largely unknown. The purpose of this study was to examine the relationship between MMP-2, -9, and -13 in functionally overloaded (FO) mouse plantaris muscle, and determine what effect the absence of MMP-2 or MMP-9 has on the expression of MMP-2 and MMP-13 in hypertrophying muscle. METHODS: FO of the plantaris muscle was performed on 10 wild type (WT) and 10 MMP-9 knockout (KO) mice. The plantaris muscle was then harvested at baseline (0-day) and 2- and 14-days after FO. RT-PCR experiments were performed to determine the mRNA expression levels of MMP-2, -9, and -13 in wild type and MMP-9 KO mice. MMP-2 and MMP-9 mRNA expression was significantly higher after 14-days FO compared to baseline (0-day) in both the WT and KO mice. MMP-9 mRNA expression was down-regulated after 14-days FO, consistent with previous findings (R).

Materials & Methods

Functional Overload

- All cells in multicellular organisms are surrounded by an extracellular matrix (ECM). In skeletal muscle, the ability of the ECM to undergo changes in response to increased or decreased activity is an important component of its plastic nature.
- Matrix metalloproteinases (MMPs) are a group of enzymes that regulate the maintenance of the ECM with MMP-2 and MMP-9 being most prevalent in skeletal muscle (1,2).
- Recent studies have identified MMP-13 as another key enzyme required for muscle remodeling via ECM regulation, and has been shown to be up-regulated under atrophying and hypertrophying conditions (3,4).
- Functional overload (FO) is a model used to induce muscle hypertrophy by surgical removal of the major synergists that perform similar functions, i.e., ankle extension.
- Previous studies have shown that MMP-2 and MMP-9 expression occurs in a time-dependent manner with MMP-9 expression up-regulated at 2-days after FO and MMP-2 expression up-regulated at 14-days after FO, suggesting a unique function for each MMP (5).
- In this study MMP-9 knockout (KO) mice were used to further elucidate the role of MMP-9 in regulating skeletal muscle plasticity, and to demonstrate whether other MMPs have overlapping function with MMP-9 after FO.

Results

MMPs are differentially expressed after FO and between WT and KO mice

Step 1: isolation of RNA from plantaris muscle
Step 2: reverse transcription with oligo(dT)20 primers to generate cDNA
Step 3: PCR
Step 4: analysis of RT-PCR products on a 1% agarose gel

Materials & Methods

Agarose gels showing the expression of MMP-2, MMP-9, and MMP-13 in the plantaris muscle in wild type (WT) and MMP-9 knockout (KO) mice at 0-d (baseline), and 2- and 14-d after FO. A 640-bp partial cDNA fragment of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a loading control for each transcript. Lanes M represent the 100 bp DNA ladder. d: day

Conclusions

- MMP-2 and MMP-13 mRNA expression was significantly higher after 14-days FO in both the WT and MMP-9 KO mice compared to baseline (0-day).
- MMP-2 mRNA expression was higher after 2-days FO in WT, but not in the KO mice, though this difference was not statistically significant. The increased expression of MMP-2 could be attributed to the increased relative muscle weight of the plantaris muscle observed in the WT mice after 2-days FO that was not observed in the KO mice at the same time point (data not shown). Our findings are in contrast to what has been observed previously by Calve et al. (6) who showed that MMPs are differentially expressed with MMP-9, not MMP-2, mRNA expression up-regulated at 2-days FO. In fact, no changes in MMP-9 mRNA expression were observed after 2-days FO compared to baseline (0-day).
- MMP-9 mRNA expression was down-regulated after 14-days FO, consistent with previous findings (R).
- MMP-13 mRNA expression was up-regulated after 2- and 14-days FO in both the WT and KO mice, suggesting its importance in skeletal muscle hypertrophy.
- There appeared to be no redundant function of MMP-2 or MMP-13 with MMP-9 in the KO group, suggesting that other factors may be compensating for MMP-9, or that MMP-9 may not be essential under hypertrophying conditions induced by FO.

Future Directions

- Increasing the sample size from all groups at all time points with additional experiments will provide better resolution of the mRNA expression patterns for all MMPs.
- As MMPs are subject to post-translational regulation, determining the active form of these enzymes is essential and should provide a clearer picture of the potential function of each MMP in these studies. Gel zymography will be performed to assess MMP-2 and MMP-9 activity, and Western blot analyses will be performed to assess MMP-13 expression.
- Further elucidation of the relationship between individual MMPs and the mechanisms by which they regulate muscle regeneration, growth, and repair will increase our current understanding of MMP interactions in skeletal muscle and their potential function during muscle adaptation.

References


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