

Overexpression of PRDM16 Improves Muscle Function after Rotator Cuff Tears

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DISCLOSURE: H. Zhang, A. Diaz, M. Liu, Y. Hu, H. Kim, B. T. Feeley, and X. Liu have no conflicts.

INTRODUCTION: Muscle atrophy, fibrosis and fatty infiltration (FI) are commonly seen in rotator cuff tears (RCT), which are critical factors that directly determine the clinical outcomes for patients with this injury [1]. Therefore, improving muscle quality after RCT is crucial in improving clinical outcome of tendon repair. In recent years, it is discovered that adults have functional brown/beige fat (BAT) [2]. Besides thermogenic function, BAT also serve as an endocrine organ. BAT can secrete batokines (brown adipokines) to promote muscle growth [3]. PRDM16, a PR-domain containing protein, was discovered with the ability to determine the brown fat cell fate and stimulate its development [4]. PRDM16 drives the molecular phenotype of brown fat cells and knockdown of PRDM16 ablates the genetic program of brown fat. Thus, the goal of this study is to discover the role of PRDM16 in regulating muscle function after massive tendon tears through a transgenic mouse model with high levels of PRDM16. We hypothesize that the overexpression of PRDM16 would improve muscle function and ameliorate fibrosis and FI.

METHODS: Transgenic aP2 driven PRDM16 over-expression mice [5] and C57BL/6J mice underwent unilateral supraspinatus (SS) tendon transection and suprascapular nerve transection (TTDN) as described previously (N=8 in each group) [6]. Digi Gait was performed to evaluate forelimb function at 6 weeks post the TTDN injury. Bilateral SS muscles, interscapular brown fat, epididymal white fat and inguinal beige fat were harvested for analysis. The expression of PRDM16 in adipose tissue was detected by Western Blot. Masson's trichrome staining was conducted to evaluate the muscle fibrosis and Oil Red O staining was used to determine the fat infiltration. Muscle fiber type was determined by MHC expression via immunostaining. All data was presented in the form of mean±SD. T-test and Two-way ANOVA analysis was performed to determine a statistically significant difference between groups. Significance was considered when $P < 0.05$.

RESULTS SECTION: Western blot data showed an increased expression of PRDM16 protein in both white and brown fat in PRDM16-overexpression mice compared to wild type (WT) mice (Figure 1A). Even though PRDM16 overexpression had no effect on increasing muscle weight (Figure 1B), it significantly improved the forelimbs function with longer brake, stance and stride time, larger stride length and paw area in mice after RCT (Figure 1C). PRDM16 overexpression showed no effect in improving fibrosis (Figure 2A), however, it significantly reduced the fatty infiltration area (%) after injury (Figure 2B). Additionally, compared to WT mice, PRDM16 overexpression significantly induced the MHC-IIx fiber percentage in supraspinatus muscle after TTDN (Figure 3).

DISCUSSION: PRDM16 expression is highly enriched in BAT, and it activates a robust brown fat phenotype when expressed in white fat cell progenitors. In this study, we found higher expression of PRDM16 in white and brown fat in transgenic mice compared to WT mice. In addition, results from our study demonstrated an improved forelimb function due to the overexpression of PRDM16. BAT has been reported to secrete several growth factors like IGF1 and Follistatin that promote muscle growth [3]. Except for its role in thermogenesis, a previous study showed that genetic loss of PRDM16 could mimic the effect of aging in promoting fibrosis [7]. Even though no change was found in fibrosis, PRDM16 overexpression led to significantly less fatty infiltration in muscle after RCT. Interestingly, we also found the overexpression of PRDM16 significantly increased the MHC-IIx fiber type after RCT. The functional role of MHC-IIx fiber type in rotator cuff muscle metabolism and function remains unknown. Further study is needed to explore their relationship between BAT and muscle fiber type after rotator cuff injury.

SIGNIFICANCE/CLINICAL RELEVANCE: Overexpression of PRDM16 significantly improved muscle function and reduced fatty infiltration after rotator cuff tears. Promoting BAT activity is beneficial in improving rotator cuff muscle quality and shoulder function after RCT.

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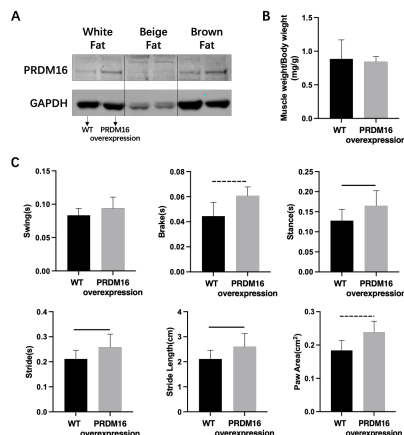


Figure 1. (A) Western-blot of PRDM16 in adipose tissue showed increased PRDM16 protein expression in both white and brown fat in PRDM16 overexpression mice compared to WT mice. (B) No significant difference of relative supraspinatus muscle weight (normalized to body weight) in PRDM16 overexpression and WT mice at 6 weeks after TTDN. (C) PRDM16 overexpression mice have significantly improved upper limb function with multiple parameters in Digigait compared to WT mice at 6 weeks after TTDN. Solid lines indicate $P < 0.05$ and dashed line indicates $P < 0.01$.

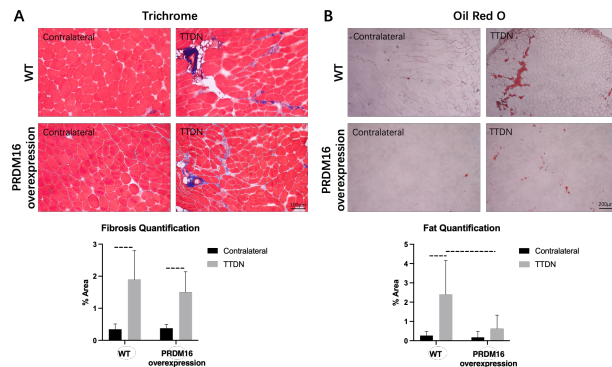


Figure 2. Histology analysis of supraspinatus muscle in PRDM16 overexpression and WT mice at 6 weeks after TTDN. Upper panel: (A) Typical Masson's Trichrome staining images of supraspinatus muscle. Scale bar: 100µm. (B) Typical Oil Red O staining images of supraspinatus muscle. Scale bar: 200µm. Lower panel: Quantification of fibrotic or fatty tissue area/total muscle cross section area (%) in supraspinatus muscles. Dash line indicates $P < 0.01$.

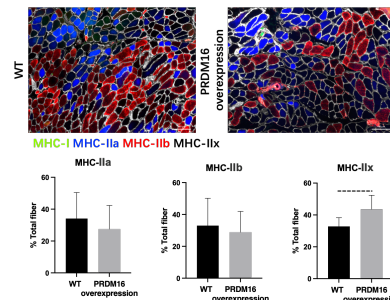


Figure 3. Upper panel: Typical images of muscle fiber type staining of supraspinatus muscle in PRDM16 overexpression and WT mice at 6 weeks after TTDN. Lower panel: Quantification of MHC-IIa, MHC-IIb and MHC-IIx fibers over total myofibers (%). Scale bar: 100µm. Dashed line indicates $P < 0.01$.