The role of Wnt10b in sarcopenic muscle and myoblast myogenic function

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INTRODUCTION: Wnts are growth stimulatory factors, binding Frizzled transmembrane receptor family on target cells and promoting cell replication, proliferation and differentiation. Abnormal canonical Wnts pathway causes many diseases. However, the roles of Wnts in sarcopenia and skeletal muscle remains unclear. Based on current evidence and our preliminary data, Wnt10b may be a promising target to regulate myosteatosis in sarcopenia. Our objective is to explore the role of Wnt10b in sarcopenic muscles function and myoblasts myogenic function.

METHODS: 6-month-old SAMP8 sarcopenic mice were allocated to AAV9-*Wnt10b* or AAV9-NC group (n=5 per group) and were sacrificed on month 10 for analysis. Myogenic differentiation induction of C2C12 myoblastic cell line was performed. 2×10^4 cells per well were randomly allocated to SiRNA-*Wnt10b* or SiRNA-NC group (n=3 per group) and samples were collected on Day 5. PCR, Western blot, immunofluorescence staining, *ex-vivo* functional tests for gastrocnemius muscles were used to examine the Wnt-related markers expression, morphological and functional changes. $p = 0.05$ was set as statistical significance and unpaired *t*-test was used to compare the between-group differences.

RESULTS SECTION: Compared with control groups, AAV9-*Wnt10b* significantly reduced the gastrocnemius muscle tetanic strength (609.3 ± 123.5 mN, $p = 0.0011$) and the twitch strength (120.7 ± 46.6 mN, $p = 0.0322$) (**Figure 1**). In *in-vitro* study, SiRNA-*Wnt10b* induced less C2C12 myotube formation and decreased myotube diameter (9.26 ± 1.78 μm, $p < 0.0001$), nuclei number (6.10 ± 0.84, $p < 0.0001$), and fusion index (0.89 ± 2.59, $p = 0.0018$). Wnt10b/β-catenin signaling pathway was inhibited by AAV9-Wnt10b in muscle and SiRNA-Wnt10b in myoblasts in the PCR and Western blot results.

DISCUSSION: After 4-month knocking-down *Wnt10b* in sarcopenic muscles, tetanic muscle strengths decreased significantly, meanwhile the Wnt10b/β-catenin signaling pathway was inhibited. After 5-day myogenic differentiation of myoblasts, knocking-down *Wnt10b* impaired the myoblast myogenic function and the myotube formation. The activation-markers of Wnt10b/β-catenin signaling pathway were down-regulated, while the inhibition-markers up-regulated. All these results indicate that Wnt10b plays an important role in the sarcopenic muscle and myoblast myogenic function.

SIGNIFICANCE/CLINICAL RELEVANCE: In summary, impaired Wnt10b pathway can impede myoblasts myogenic differentiation and decrease the muscle strength in sarcopenic mice, which helps illustrate the underlying mechanism of myosteatosis in sarcopenia and may help to develop therapeutic methods to attenuate the sarcopenia.

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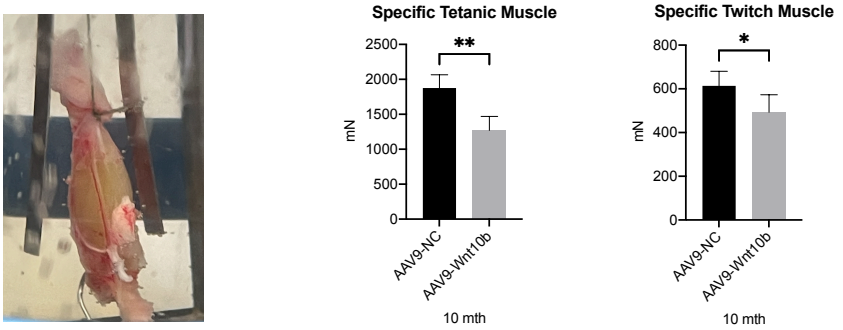

(A) (B)

Figure 1. Muscle and muscle strength of SAMP8 mice in AAV9-NC group and AAV9-*Wnt10b* group (n = 5) in the *ex-vivo* functional test. (A) Gastrocnemius muscles tranfected by AAV9 with green fluorescence; (B) Specific tetanic force and specific twitch force were significantly lower in AAV9-*Wnt10b* group compared with AAV9-NC group on 4 months after transfection. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$.