The Potential Role of Sirtuin 3 in Mitochondrial Homeostasis In a Murine Model of Rotator Cuff Tendinopathy

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INTRODUCTION: Shoulder musculoskeletal disorders are the most common upper extremity conditions seen by primary care physicians and orthopedic surgeons. Despite the advanced clinical and pathogenetic understanding of rotator cuff tendinopathy, the pathophysiology and treatment optimization of tendinopathy remain under investigation because complete recovery success rates are not yet achieved. Sirtuin 3 (Sirt3), an NAD+-dependent deacetylase protein primarily localized in mitochondria, acts as a principal mitochondrial deacetylase and is expressed in mitochondria-rich tissues. Sirt3 controls various mitochondrial functions, such as energy metabolism and oxidative stress protection. Emerging evidence highlights the key role of mitochondrial dysfunction in the etiology of tendinopathy, with preclinical studies suggesting that modulating mitochondrial homeostasis speeds up rotator cuff repair. However, the specific molecular mechanisms by which mitochondrial regulators, such as the deacetylase Sirt3, coordinate the injury and repair processes of tendinopathy remain undefined. This study aims to investigate the role of Sirt3 in mitochondrial homeostasis in a murine model of rotator cuff tendinopathy and to explore potential therapeutic targets for treating this condition.
METHODS: All procedures received approval from the Institutional Animal Care and Use Committee (IACUC) at Shanghai Sixth People's Hospital. Sirtuin 3 knock-out (Sirt3-KO) mice (Strain NO. T050579) were obtained from GemPharmatech (Nanjing, China). Genotyping was conducted via tail DNA, following the instructions of the Jackson Laboratory. All animals were kept on a standard diet in pathogen-free cages with controlled temperature and humidity. A total of eighty-four male mice (12 weeks old, weighing 25-30g) were used in this study. The primary reason for using only male mice in the experiment is to minimize confounding variables and ensure the stability and reproducibility of the experimental results. Twenty-one Sirt3 KO and twenty-one wild-type mice received bilateral subacromial clip insertion to induce supraspinatus tendinopathy, while another twenty-one Sirt3 KO and twenty-one wild-type mice served as controls without surgery. The mice were euthanized with carbon dioxide gas randomly at 8 weeks post-surgery for histological assessments (hematoxylin-eosin: H&E, alcian blue, and picrosirius red staining), biomechanical testing, mitochondrial- and tendon-related gene expression, SOD activity, and TEM analysis.
RESULTS SECTION: H&E staining showed similar cellularity in group I (Sirt3-KO mice) and group II (wild-type mice), with a gradual increase in cellularity in group III (8 weeks of impingement in Sirt3-KO mice) and group IV (8 weeks of impingement in wild-type mice). Alcian-blue staining revealed abundant stainable mucin with disrupted collagen bundles observed in the impingement groups (III and IV). There were more abnormal discrete fiber bundles and matrix properties in group III than in group IV. Picrosirius-red staining showed organized and aligned collagen bundles with normal crimping in groups I and II. Separation of fiber bundles and loss of fiber demarcation pattern were observed under polarized microscopy in the subsequent impingement groups (III and IV). Severe disorganized collagen structure was observed at 8 weeks after clip insertion in Sirt3-KO mice compared to wild type mice. Biomechanical testing showed no significant difference in the failure force of supraspinatus tendons between the Sirt3-KO control and wild-type control groups. After 8 weeks of impingement, the failure force decreased by 77% in the Sirt3-KO impingement group than in the Sirt3-KO control group, and by 65% in the WT impingement group compared to the WT control group. The failure force in the Sirt3-KO impingement group showed a significant difference compared with the wild-type impingement group. The stiffness data followed a similar trend. The expression of Sirt3 decreased significantly in the Sirt3-KO mice. Following the impingement, Sirt3 expression was significantly downregulated in wild type mice (group IV) compared to the control group (group II; $P<0.05$). There was also a significant difference between the Sirt3 KO group and the WT KO group. The expression of SOD-2 (encoding a mitochondrial antioxidant enzyme) and ATP5F1 (mitochondrial ATP synthase) were decreased in the Sirt3 KO control group (I) compared with the wild-type control group (II). SOD-2 and ATP5F1 expressions decreased significantly after 8 weeks of impingement in both Sirt3 KO (III) and wild-type mice (IV) compared to their respective control groups (I and II). Additionally, there is a significant difference in SOD-2 and ATP5F1 between the KO 8w group and the WT 8w group. Col I expression increased in all impingement groups (III and IV) compared to the control groups (I and II), indicating that fibrosis and new matrix formation contributed to the injury. There was a significantly increased expression of Sox9 in the impingement group (III) compared to the control group (I) of Sirt3-KO mice, with similar significance between the impingement group (IV) and the control group (II) of wild-type mice, indicating that tissue metaplasia occurred with clip insertion. SOD activity was significantly decreased in the control group of Sirt3-KO mice (I) compared to the wild-type mice (II). Compared to the control group of Sirt3-KO mice (I), SOD activity decreased significantly after 8 weeks of impingement (III). A significant difference was observed between the control group (II) and impingement group (IV) of wild-type mice. TEM images revealed changes in nuclear organization and chromatin aggregation of mitochondria in the impingement groups (III and IV) compared to the control groups. Swollen mitochondria with damaged cristae were observed in the impingement groups (III and IV), while the 8-week impingement group of Sirt3-KO mice (III) showed expanded vesicular compartments and a lack of matrix staining with loss of cristae compared to the wild-type group (IV). Arrangement of the cristae decreased in Sirt3-KO mice (III) compared with the wild type mice. (IV). The number of mitochondria per tenocyte decreased in the KO mice compared to the wild-type group. Compared to the two control groups (I and II), the number of mitochondria significantly decreased after the impingement (III and IV). There was also a significant difference in impinged tendons between Sir3-KO mice (III) and wild-type mice (IV).
DISCUSSION: Severe progressive rotator cuff tendinopathy was observed in Sirt3 knockout mice with mitochondrial dyshomeostasis, indicating that Sirt3 might be a potential regulator of mitochondrial homeostasis in the development of rotator cuff tendinopathy.
SIGNIFICANCE/CLINICAL RELEVANCE: Sirt3 might be a potential regulator of mitochondrial homeostasis in the development of rotator cuff tendinopathy; this could be a possible predictor or therapeutic target in the treatment of tendinopathy.
IMAGES AND TABLES: