

microRNA-26a deficiency attenuates severity of frozen shoulder in an immobilization model

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INTRODUCTION:

Frozen shoulder is a common disease that affects 2 to 5 % of the total population¹⁾. Severe shoulder pain is often present and subsequent contracture of the shoulder joint is inevitable, therefore leading to a significant influence on the quality of life over a long period of the time. Although the frozen shoulder often improves spontaneously in approximately two years after passing through the inflammatory stage, contracture stage and recovery stage, it has been also reported that symptoms persist for more than seven years in more than half of the cases²⁾.

It is thought that the main pathogenesis of the frozen shoulder is the inflammation of the intra-articular synovium and subsequent fibrosis of the shoulder joint capsule³⁾. Although various pathophysiological studies have been reported on the fibrosis of the frozen shoulder, the full extent of the fibrosis mechanism is still unknown, and revealing the factors related to the fibrosis may help in the treatment of the frozen shoulder.

We focused on microRNA which is a gene expression regulator that negatively regulates target gene expression and has been implicated in a variety of diseases. Although a lot of microRNAs have been reported to be associated with fibrosis, there are no reports on the frozen shoulder. Among miRNAs, it has been reported that decreased expression of miR-26a promotes fibrosis in lungs⁴⁾ and lens⁵⁾, while it inhibits fibrosis in liver⁶⁾. Therefore, we hypothesized that miR-26a might also be related to fibrosis in shoulder joint capsule. The purpose of this study is to investigate the effect of miR-26a on fibrosis in the shoulder capsule using a frozen shoulder model of systemically miR-26a deficient (miR-26a KO) mice.

METHODS:

This study was performed according to protocols approved by Institutional Animal Care and Use Committee at Hiroshima University. Ten-weeks-old male C57BL/6J (wild type: WT) mice and miR-26a KO mice were used. A skin incision was made on the inferior edge of the scapula, and a 3-0 nylon suture passed through the scapula tied with the humeral shaft to immobilize the glenohumeral joint according to the procedure described in a previous report⁷⁾. The right upper limb was immobilized, and the left side was used as an untreated control. The inflammation phase model was defined as 1 week after the immobilization, the contracture phase model was 6 weeks after immobilization, and the recovery phase model was two weeks after removal of the nylon suture after six weeks of immobilization (6+2-week). After being sacrificed, we evaluated the range of motion (ROM) of the shoulder joint, histological analysis (hematoxylin-eosin staining (H.E), Masson's Trichrome (MT) staining, and safranin-O (SafO) staining) and genes expression by real-time PCR. The expression of synovitis and fibrosis related-genes (*Il-6*, *Tnf-α*, *Tgf-β*, *Ctg*, *Colla1*, and *Hgf*) and the expression of miR-26a were evaluated in the shoulder joint capsule of the WT mice during immobilization.

RESULTS SECTION:

In WT mice, the immobilized right shoulder experienced a roughly 50% reduction in ROM after 1 week, as compared to the unaffected left control shoulder. This limitation became more pronounced at the 6-week mark, followed by a slight recovery during the recovery phase (6+2-week), compared to the control shoulder. When comparing WT and miR-26a KO mice, no significant difference in ROM was observed in the control shoulder of the two groups at any time point. However, the immobilized shoulder in miR-26a KO mice consistently showed significantly better ROM in comparison to WT mice (Figure 1). Histological analysis revealed both inflammatory cell infiltration and thickening of the inferior shoulder joint capsule in WT mice after 1 week of immobilization (Figure 2). Over the subsequent 6 weeks, this thickening further progressed (Figure 2). At the recovery phase, some recover of these changes was shown. In miR-26a KO mice, the extent of inflammatory cell infiltration and thickening notably remained less severe than in WT mice at both 1 and 6 weeks (Figure 2). Thus, the miR-26a KO mice exhibited significantly reduced fibrosis compared to WT mice at the 6-week mark. Furthermore, unlike WT mice, miR-26a KO mice did not display cartilage-like formation stained with SafO below the humeral head after 6 weeks. Expression of synovitis and fibrosis-related genes was decreased in the miR-26a KO mice compared with WT mice at 1 and 6 weeks.

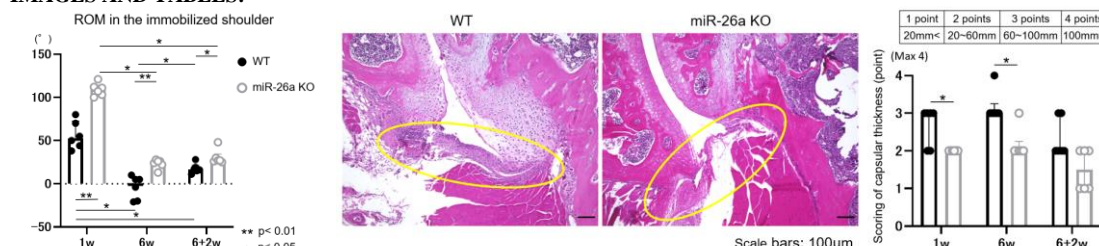
DISCUSSION:

miR-26a KO mice exhibited to attenuate the severity of frozen shoulder in an immobilization model. However, the expression of miR-26a in the shoulder capsule did not upregulate in the immobilized frozen shoulder model. As a results, the reduced severity of frozen shoulder caused by miR-26a deficiency might not be attributed to deletion of miR-26a in synovial cells of the joint capsule. The miR-26a KO mice used in this study represent systemic deficiency model. Our previous study demonstrated that miR-26a modulates bone loss and muscle strength but has no essential role in osteoarthritis⁸⁾. Furthermore, other research groups have reported instances where miR-26a KO mice exhibit excess body fat and dyslipidemia, particularly under stressful conditions such as a high-fat diet⁸⁻¹¹⁾. The attenuation of fibrosis in frozen shoulder could potentially arise from various systems, including the immune system, although the underlying mechanisms remain unclear. Consequently, the relationship between miR-26a and fibrosis remains controversial. To gain a more comprehensive understanding, further investigation is required to ascertain the role of miR-26a in fibrosis, frozen shoulder, as well as its impact on the endocrine and immune systems. The future results from these studies hold the potential to provide novel insights into the mechanisms underlying fibrosis and frozen shoulder, driven by miRNAs like miR-26a, which could subsequently lead to the development of innovative therapeutic strategies.

SIGNIFICANCE/CLINICAL RELEVANCE: microRNA-26a deficiency attenuates severity of frozen shoulder.

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IMAGES AND TABLES:



(Fig.1) In miR-26a KO mice, the immobilized shoulder consistently showed significantly better ROM in comparison to WT mice.

(Fig.2) In miR-26a KO mice, the extent of thickening of the inferior shoulder joint capsule (yellow oval) was notably less severe than in WT mice at 6 weeks, and capsular thickness was significantly thinner than in WT mice at both 1 and 6 weeks.