Transcription Factor Mohawk is essential in Intervertebral Disc Maintenance

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Disclosures:

Introduction: Intervertebral disc (IVD) degeneration is part of the normal aging process, but is also one of the causes of low back pain. Furthermore, the instability of intervertebral discs leads to more serious diseases, such as spinal deformity, spine osteoarthritis (OA), and spinal canal stenosis.

IVD can be divided into two main structures, nucleus pulposus (NP) and annulus fibrosus (AF). NP is a gelatinous homogenous mass centrally located and resists compression. AF located around the NP is organized into layers of TypeIcollagen fibrils and resists tensile stress. Further understanding of the mechanism of AF is essential in attempting to prevent disc degeneration. Mohawk homeobox gene (Mkx) is a transcription factor with relative specificity of tendons and ligaments. Mkx null mice have hypoplastic tendons and TypeIcollagen is decreased in these tendons.1) Also, Mkx is expressed in cells of normal human anterior cruciate ligaments (ACL), and its expression is reduced in ACLs from osteoarthritis joints2). These suggest that Mkx has a potential role in tendon/ligament homeostasis.

Our hypothesis is that Mkx has an important role in homeostasis of AF. We studied this hypothesis in a mouse model 1) by using histological and radiological techniques.

Methods: Venus knock-in Mkx heterozygous mutant mice1) (10 weeks, n=4) were used to evaluate the expression of Mkx by immunohistochemistry. All spines were harvested and made into frozen section (5μm). The expression of Mkx was analyzed using Anti-GFP antibody.

Wild type mice (10 weeks, n=4) were used to evaluate Mkx expression of AF compared to tendons by Quantitative real time-PCR. Total RNA was isolated from Achilles tendons and AF in lumbar spine and tail spine. To evaluate the changes with age, wild type and homozygous type mice were used at each of the time points (10 weeks, 12 months, and 21 months; n=4 in each group at each time point). To evaluate the state of the spine, microcomputed tomography was used after the mice were sacrificed. For histological analysis, all spines were harvested and made into paraffin section (10μm). These sections were stained with hematoxylin and eosin or safranin-O and fast green.

Results: Venus knock-in heterozygous mice (10 weeks) showed Venus expression in outer annulus fibrosus (OAF) strongly, and inner annulus fibrosus (IAF) weakly (Fig1A, B). In addition, Mkx expression of AF was confirmed with mRNA analysis although it was lower than in Achilles tendons (Fig1C). The CT views of lumbar spine did not show a clear difference between wild type and homozygous type at 10 weeks (Fig2a, d) and 12 months (Fig2g, j). However, at 21 months, a difference was observed. No obvious changes were observed in the wild type mice (Fig2a, g, m), but bone spurs were observed at the lumbar spine in homozygous mice (Fig2p).

In histological views of the lumbar spine, both showed normal structures at 10 weeks (Fig2b, c, e, f). At 12 months, wild type was similar to that of 10 weeks (Fig2h, i), but homozygous type showed differences in some discs of the lumbar spine. In hematoxylin and eosin staining, accumulation of huge circular cells were confirmed in the NP. Small round cells resembling chondrocytes proliferate in the IAF. The NP and the IAF were stained more strongly with safranin O (Fig2k, l). In 21 months-old wild type, these changes were confirmed with a few discs and almost all discs were similar to that of 10 weeks and 12 months (Fig2n,o). In contrast, these changes were seen in almost all discs of the lumbar spine in the homozygous type. Additionally, some discs had an anterior bulge (Fig2q, r).

However, in homozygous type, these changes were not seen in all vertebrae. Moderate changes were observed around the cervical-thoracic (CT) junction, but other vertebral discs appeared normal.

Fig1. Confirmation of Mkx expression in AF. Immunohistochemistry of lumbar spine of Mkx heterozygous mouse at 10 weeks (A, B). Venus expression is observed in OAF strongly and IAF weakly (Green: Anti-GFP, Blue: Hoechst). Quantitative real tie-PCR showed that AF of wild type mice at 10 weeks expressed Mkx mRNA. Scale bar represents 100μm.

Fig2. CT and histological changes with age between wild type and homozygous type. Image of Sagittal CT of lumbar spine (L1/2~L6/S) of wild type and homozygous type (a, d, g, j, m, p) showed no clear differences between wild type and homozygous type at 10 weeks (a, d) and 12 months (g, j). At 21 months, homozygous type demonstrated bone spurs of the lumbar spine (p), but not in wild type (m). Hematoxylin and eosin staining (above) and safranin-O and fast green staining (below) of sagittal sections of L3/4 (b, c, e, f, h, i, k, l, n, o, q, r) showed no clear differences at 10 weeks (b, c, e, f). At 12 months, some homozygous type discs showed accumulation of huge circular cells in the NP (white arrow). The NP and the IAF were strongly stained (dark red) with safranin O (k, l). At 21 months, these changes were seen in almost all lumbar spines (q, r). Scale bar represents 300μm.
**Discussion:** Disc degenerative changes in mice were reported in AF puncture models and other degenerative mouse models. The changes of Mkx homozygous mice were almost identical to the degeneration observed in those models, which suggests that disc degeneration is accelerated in Mkx homozygous mice.

A possible mechanism of Mkx in AF is that Mkx maintains the stability of AF. This is supported by the fact that the degenerative changes of Mkx homozygous mice were not seen in all discs but only in discs which are subjected to increased stress (i.e., lumbar spine and CT junction). Mkx is believed to play an important role in regulating the expression of type I collagen in tendon cells. As AF layers also compose of Type I collagen fibrils, it is expected that Mkx has a role in maintaining AF structure and function of resisting tensile stress thus prevents disc degeneration.

**Significance:** Molecular mechanism of disc and especially AF degeneration is still relatively unknown. This study suggests that Mkx has a potential role in AF homeostasis and is a key transcription factor in preventing disc degeneration and spine osteoarthritis.

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**References:**