Sex-specific microRNA profiling reveals distinct mechanisms for onset and progression of aging-associated osteoarthritis in a transgenic mouse model

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INTRODUCTION: Osteoarthritis (OA) is an aging-associated disease characterized by articular cartilage degradation, synovium inflammation, and abnormal ossification. Knee OA is prevalent in older women, who have 40% higher risk compared to age-equivalent man (1). However, the mechanism underlying sexual dimorphism of OA is unclear. It is not known why OA onset occurs earlier in women than men during aging. Furthermore, when OA patients seek help from doctors because of pain and mobility difficulties, the onset of OA may have occurred some time ago. Since OA cases under study are often in the progression stage, it is not clear whether the OA onset shares the same mechanism as OA progression. Past studies indicated that environmental factors such as mechanical and inflammatory stress are involved in triggering OA onset. Many studies however focus on genetic rather than epigenetic mechanisms. MicroRNAs (miRs) are small, single-stranded RNA molecules that regulate multiple genes at the post-transcription levels in response to environmental signals. It has been shown that miRNAs are involved in OA disease pathology (2). However, most studies only compared the miR expression differences between OA and normal samples without distinguishing OA onset from progression or male from females. To fill this gap, we generated a transgenic (TG) mouse model in which a microRNA (miR-365a) responsive to mechanical and inflammatory stress (3) was expressed in articular cartilage under Col2a1-Cre. We found that OA occurred earlier in female than male TG mice thus mimicking aging associated OA in human. Taking advantage of this TG model, we identified miRs associated with the onset and progression of aging associated OA in a sex-specific manner.

METHODS: The use of animals is approved by IACUC animal studies committee. All animal studies were performed in accordance with institutional guidelines. MiR-365-flox mice were crossed with Col2a1-Cre mice to generate miR-365flox+/+; Col2a1-Cre+/+ transgenic mice (TG). These mice overexpress the stress miRNA-365 in articular cartilage, which triggers early onset of OA and promotes progression. The miR-365flox-/-; Col2a1-Cre+/+ littermate mice serve as control (WT). At 6- and 12-months of age, mouse knee articular cartilage samples were collected for RNA extraction (n = 3) using Qiagen miRNeasy Mini Kit. RNA samples were prepared for NanoString nCounter mouse miRNA and mRNA panels per manufacturer's instructions. RNA sequencing was performed at Brown University Genomics Core Facility. Data analysis was performed using nSolver 4.0 and literature search. For immunohistochemistry, the knee joint tissue was fixed, decalcified, paraffin embedded, sectioned, and stained with Safranin-O and Fast Green. OA pathogenesis was quantified with histology sections of mouse knee joints according to the OARSI grading system.

RESULTS: Histology analysis indicated different timing for knee OA onset of female and male mice during aging in a sex-specific manner respectively (Table 1). In females, WT mice were normal while TG mice developed early onset of OA at 6m old (Fig. 1B, 1C). At 12m old, WT mice developed OA onset while TG mice underwent OA progression (Fig. 2A). In male, WT mice were normal at both 6m and 12m old, while TG mice were normal at 6m but developed OA onset at 12m old (Fig. 2B). Profiling of 578 miRs based on miRNA sequencing demonstrated that early OA onset in female was associated with significant up-regulation of miR-342-3p and down-regulation of miR-130a (green arrow) in the 6m old TG mice compared to WT mice (Fig. 1D). Conversely, miR-130a was significantly up-regulated in the 6m-old male TG mice, which had normal cartilage (Fig. 1E). miR-130a down-regulation was also associated with OA onset in female WT mice at 12m old compared to 6m-old with normal cartilage (Fig. 3A). It indicated that miR-130a downregulation was associated with OA onset in females. In contrast, OA onset in male was associated with up-regulation of miR-423-5p and down-regulation of seven miRs (Fig. 2B, 2D). OA progression in females was associated with significant up-regulation of 6 miRs including miR-143 (red arrow) and down-regulation of 5 miRs in 12m-old TG mice compared to WT mice (Fig. 2C). miR-143 was also up-regulated during OA progression in female 12m old TG mice when compared to 6m old with OA onset (Fig. 3B). It indicated that miR-143 up-regulation was associated with OA progression in females.

DISCUSSION: Our study identified miRs associated with OA onset and progression in a sex-specific manner for the first time. The miRs associated with OA onset are different from those with OA progression, and females are different from males. They suggest that molecular mechanism regulating OA onset differs from that of OA progression in a sex-specific manner. Overall, OA onset is more complex in male than female. While OA onset involves up-regulation of miR-342-3p and down-regulation of miR-130a in female (Fig.1D), it involves up-regulation of miR-423-5p and down-regulation of seven miRs in male (Fig. 2D). OA progression also appears to be more complex than OA onset in female, since the progression involves up-regulation of six miRs and down-regulation of five miRs (Fig. 2C). miR-143 appears to be important for OA progression in female because it is upregulated not only during OA progression in TG mice compared to WT mice in 12m-old, but also during the transition from OA onset at 6m to progression at 12m. Thus, we identified four miRs associated with OA onset and progression in a sex-specific manner: miR-130a, miR-143, miR-342-3p, and miR-423-5p. All of them are conserved between mice and humans. It suggests their potentially important role in regulating OA onset and progression in a sex-specific manner during evolution. The miRs associated with early OA onset in female, miR-130a and miR-342-3p, could be particularly important since female is the population most affected by aging associated OA. It has been shown that miR-130a decreases in OA patients thereby increasing TNFa and inflammation in chondrocytes (4), while miR-342-3p regulates IL-4/STAT6 signaling axis (5). Thus, both miRs may be involved in OA onset by regulating joint inflammation in females.

SIGNIFICANCE: Since there is no FDA-approved OA disease modifying drugs currently, identification of miR targets regulating OA onset and progression in male and female respectively will help develop novel therapeutics as personalized medicine with better efficacies.

(1) Sex Difference in OA of the hip and knee. Mary O'Connor, J Am Acad Ortho Surg. 2007, 15: Suppl 1 S22-S25.
(2) MicroRNA expression in osteoarthritis: a meta-analysis. Liu, Yan and Li et. al., Clinical and Experimental Medicine 2023, 23:3737–3749.
(3) MiR-365: a mechanosensitive microRNA stimulates chondrocyte differentiation through targeting histone deacetylase 4. Guan et. Al., FASEB J 2011, 25(12): 4457-4466.
(4) Decreased expression of microRNA-130a correlates with TNF-α in the development of osteoarthritis. Li et. al., Int J Clin Exp Pathol. 2015, 8(3):2555-64.
(5) The IL-4/STAT6 signaling axis establishes a conserved microRNA signature in human and mouse macrophages regulating cell survival via miR-342-3p. Czimmerer et. al., Genome Med., 2016, 8(1):63.

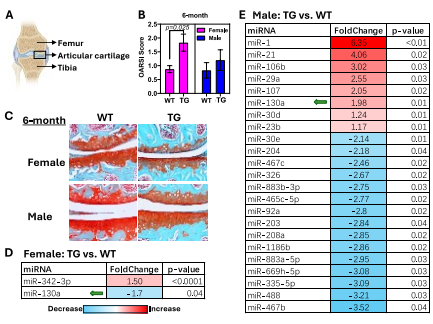

Figure 1. MiRNA profiling for WT and TG mice at 6 months. (A) Structure of mouse knee joint. (B) OARSI scores of mouse knee joint. (C) Safranin-O/Fast green stained mouse knee articular cartilage. (D and E) Differentially expressed miRNAs for female (D) and male (E) TG mice compared to WT mice. Arrows indicate shared miRNA between female and male.

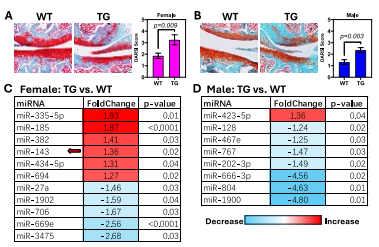

Figure 2. MiRNA profiling for WT and TG mice at 12 months. (A and B) Safranin-O/Fast green stained mouse knee articular cartilage (left) and OARSI scores (right) for female (A) and male (B) mice. (C and D) Differentially expressed miRNAs for female (C) and male (D) TG mice compared to WT mice.

Table 1. Timing for knee OA onset in female and male mice during aging.

Sex	Group	6 months	12 months
Female	WT	Normal	OA onset
	TG	OA onset	OA progression
Male	WT	Normal	Normal
	TG	Normal	OA onset

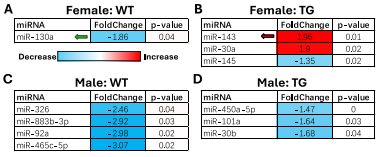

Figure 3. Sex-specific miRNA profiling at 12-month vs. 6-month. Differentially expressed miRNAs comparing 12-month to 6-month were identified for WT mice (A, female; C, male) and TG mice (B, female; D, male).