Divergence between catabolic and anabolic senescence underlies sex dimorphism of osteoarthritis.

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INTRODUCTION: Osteoarthritis (OA) is an aging-associated degenerative joint disease with prominent phenotypes including articular cartilage degradation, chronic inflammation, and bone remodeling. It is the leading cause of disability in elder people. Currently there is no FDA approved disease modifying drugs to treat OA. One of the commonly accepted OA mechanisms is cell senescence, in which senescent cells accumulate in aged tissues and secrete senescence-associated secretory phenotypes (SASPs). The SASPs include chemokines, cytokines, extracellular matrix (ECM), and proteases that degrade ECM. They act on the surrounding tissue microenvironment and contribute to aging-related diseases (ARDs) pathology. We have shown that (1), in OA cartilage, chondrocytes de-differentiate into normal cartilage stromal cells (NCSC), which undergo senescence to become pro-inflammatory osteoarthritis mesenchymal stromal cells (OA-MSC). Sex dimorphism is an important property of ARDs. Women are more prone to erosive osteoarthritis (OA), with a female to male ratio of 12:1. Women have higher risk and experience more pain for knee OA than men (2). However, the fundamental mechanisms causing the sex difference in OA is not clear. In this study, we tested the sex-dependent differences in response to stress through genetically expressing a stress-sensor microRNA in mice. MiR-365 is sensitive to mechano- and inflammation-stress signals and increases its expressions in OA patients (3). We show here that OA pathogenesis presents sex dimorphism in the stress-responsive microRNA level and in catabolic and anabolic gene expression patterns.

METHODS: The use of animals is approved by Lifespan IACUC animal studies committee. All animal studies were performed in accordance with institutional guidelines. To overexpress miR-365 in cartilage tissue, miR-365-flox transgenic mice were crossed with Col2a1-CreER^T mice to generate miR-365; Col2a1-CreER^T mice. Tamoxifen was intraperitoneally injected at 2-week of age to induce miR-365 overexpression. Human OA cartilage was collected from discarded articular cartilage tissues of knee replacement surgery. Patient tissues were used in accordance with the approval from the Institutional Review Board (IRB) of Rhode Island Hospital. RNA was extracted using miRNeasy Mini Kit and reversely transcribed into cDNA using miScript II RT Kit. Quantitative real-time PCR was performed using SYBR Green PCR master mix. All procedures were performed following manufacturer's instruction. For immunohistochemistry, the tissue was fixed, decalcified, dehydrated, paraffin embedded, sectioned, and hydrated. OA pathogenesis was quantified with histology sections of mouse knee joints according to the OARSI grading system. Single-cell RNA sequencing data were retrieved and analyzed from NCBI GEO repository (GSE104782).

RESULTS: To mimic mechanical and pro-inflammatory stress signals, we transiently activated stress microRNA-365 expression in cartilage by tamoxifen induction of the miR-365; Col2a1-CreER^T transgene in both female and male mice (**Fig. 1A**). This stress-microRNA induction resulted in early-onset of joint degeneration in 7-month-old female mice but not in age-matched male mice (**Fig. 1B**). Stress microRNA induced inflammaging and catabolic genes including matrix metallopeptidases MMP3 and MMP13, cytokine IL-1ß in female mice while repressing them in male mice (**Fig. 1C**). In contrast, stress microRNA activated extra- and matrix-cellular genes including proteoglycan ACAN, collagens COL2A1, COL1A1, and COL18A1 in male mice but not in female mice (**Fig. 1D**). Results above suggest that female and male mice show opposite responses to stress signals. To confirm this in human, lesion areas of OA patient cartilage were compared to their non-lesion areas. MiR-365 expression levels were increased in the lesion area in 80% of female but only 50% of male samples (**Fig. 2A**). There are seven cell populations in human OA cartilage (4). We identified three cell populations in the chondrocyte lineage (ProC, HomC, and FC), which are more prevalent in male. Four cell populations were in the cartilage MSC lineage (EC, RegC, preHTC, and HTC), which were more prevalent in female (**Fig. 2B**). Bioinformatic analysis indicated that expression of catabolic genes including cytokines and proteases (MMP3 and IL-1ß) were up-regulated in female OA patients (**Fig. 2C**), while anabolic genes including extracellular matrix (COL1A1 and COL18A1) were up-regulated in male OA patients (**Fig. 2C**). Thus, human OA patients also present a divergence of catabolic and anabolic gene expression between female and male.

DISCUSSION: Sex difference in OA has long been recognized. However, the underlying molecular mechanisms are unclear. We show here that there is a striking divergence of OA pathogenesis between male and females. First, aging related OA is more prevalent in female than in male in both human OA patients and in mouse OA models with cartilage-specific expression of stress microRNA. Second, induction of stress microRNA is associated with early onset of OA in female but not in male. Third, OA pathogenesis is associated with catabolic gene expression in female but with anabolic gene expression in male. Thus, we termed the sex-associated divergence of aging-related OA as catabolic senescence and anabolic senescence. Our data suggests that catabolic senescence is mediated by MSC senescence, manifested in pro-inflammatory cytokines expression and matrix degradation, and prevalent in female. On the other hand, anabolic senescence is mediated by chondrocyte senescence, manifested in fibrosis, osteogenesis, and collagen over- and mis-expression, and prevalent in male. Our *in vivo* analysis of both human and mouse OA models strongly suggest that OA dimorphism is a result of sex-dependent differential responses to external stress signals. To achieve the same tissue defense in response to stress, female prefers to activate catabolism while male prefers to initiate anabolism.

SIGNIFICANCE: Our study provided insights into the mechanism of sex dimorphism in OA. We proposed two senescence pathways, with female preferring the catabolic pathway and male preferring the anabolic pathway. Overall, mechanistic understandings of what causes the sex difference in OA could help to develop sex/gender specific precision medicine to achieve better efficacy in female and male OA patients.

(1) Senescent Tissue-Resident Mesenchymal Stromal Cells Are an Internal Source of Inflammation in Human Osteoarthritic Cartilage. Wenguang Liu et al., Front. Cell Dev. Biol., 9: 725071.

(2) Sex Difference in OA of the hip and knee, Mary O'Connor, J Am Acad Ortho Surg. 2007, 15: Suppl 1 S22-S25.

(3) Mechanical and IL-1β Responsive miR-365 Contributes to Osteoarthritis Development by Targeting Histone Deacetylase 4. Xu Yang et al., Int. J. Mol. Sci. 2016, 17(4), 436.

(4) Single-cell RNA-seq analysis reveals the progression of human osteoarthritis. Ji et al., Ann Rheum Dis, 2019, 78: 100-110.
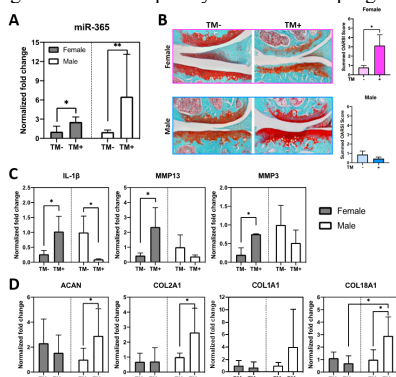
Figure 1. Differences in gene expressions (A, C, D) and OA phenotype (B) between male and female miR-365 transgenic mice.
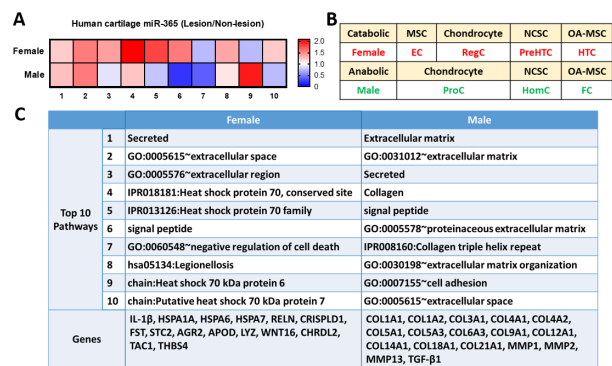

Figure 2. Catabolic and anabolic senescence pathways in human OA.