Aging-associated Increase of GATA Binding Protein 4 levels in Chondrocytes Impairs their Regenerative Capacity

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INTRODUCTION: Aging is an inevitable phenomenon resulting in limited functionality, loss of structural integrity, and inability to effectively regenerate, protect, and resist injury and disease. Additionally, aging is the biggest risk factor of osteoarthritis (OA), the world’s most common form of degenerative disease. OA is estimated to affect 32.5 million Americans, with most cases occurring in adults over the age of 45. Yet, the mechanism of age-related OA is not well understood, and there are not any disease-modifying osteoarthritis drugs (DMOADs) that have reached FDA approval. Investigating the mechanisms of healthy aging and molecules involved in age-related pathogenesis might elucidate the mechanisms of age-associated OA onset and progression. In this study, we aimed to compare the transcriptome of young and old human chondrocytes from healthy donors and employ unbiased analysis to define the key molecules that mediate chondrocyte aging.

METHODS: (1) Differences in young and old chondrocytes: Healthy human knee cartilage was isolated from arthritis-free donors with the approval from the University of Pittsburgh Committee for Oversight of Research and Clinical Training Involving Decedents. Cartilage tissues were washed, diced into ~1 mm² pieces, and then digested in 10 mL/g weight cartilage digestion medium with collagenase type II at 1 mg/mL (w/v) on a shaker at 37°C for 16 h. Isolated chondrocytes were seeded in tissue culture flasks at 1x10⁶ cells/cm² and maintained in growth medium. Based on age-related details, young (<45 years) and old (>70 years) chondrocytes were individually plated and collected for RNA sequencing analysis. Tissue samples collected from donors were stained for GATA binding protein 4 (GATA-4) and young and old samples were compared (Fig.1A). (2) Assessment of the role of GATA-4 in Chondrocytes: Young, pooled chondrocytes were cultured in monolayer and transfected with the lentiviral vector containing GATA-4 gene and DTomato or the control lentivirus carrying EGFP for 10 h, which were constructed by VectorBuilder (ID: NM. 0013089093). After transfection, western blot, qPCR, and immunohistochemistry were used to verify the stable expression of GATA-4 in cells. Following the lentiviral transfection of GATA-4 gene in young pooled chondrocytes, the cells were collected and formed into pellets at a cell seeding density of 3 x 10⁶ cells per pellet. Pellets were treated with chondrogenic medium containing transforming growth factor-β3, medium was changed daily for seven days. Upon seven days of chondrogenesis, pellets were collected from qPCR, western blot, and immunohistochemical analysis.

RESULTS SECTION: RNA sequencing results showed that 303 genes are upregulated, and 163 genes are downregulated in aged chondrocytes when compared to young cells (Fig. 1B). IPA Upstream Regulator Analysis was used to predict the key factors that may mediate the difference between young and old cells (Fig. 1C). Interestingly, GATA-4 was predicted to be one key regulator of chondrocyte aging. Results from GATA-4 knock-in in GATA-4 IHC staining depict that aged individuals have higher GATA-4 staining compared to young individuals, confirming that old chondrocytes have higher GATA-4 levels than young cells (Fig 1D). We also explored the function of GATA-4 by overexpressing it in normal young chondrocytes (Fig. 2B). The lentiviral vector-mediated knock-in of GATA-4 significantly impaired the cartilage formation capacity of chondrocytes, indicated by a down regulation in aggrecan (ACAN) and collagen type II (COL2) as well as an upregulation of inflammatory cytokines, interleukin (IL-6) and IL-8, as well as tumor necrosis factor-α (TNF-α). Matrix degrading enzymes, such as Matrix metalloproteinases (MMP) 1, 2, and 12, were also upregulated in cells overexpressing GATA 4 (Fig 2A). Safranin O staining of glycosaminoglycans, and IHC staining of Collagen Type II showed less staining in ECM proteins in the GATA-4 KI groups (Fig 2B).

DISCUSSION: Thus far, we have identified an aging-associated increase of GATA-4 in chondrocytes, which impaired their cartilage formation potential. Interestingly, GATA-4 has been known to be involved in the activation of NF-kB (NF-kB) inflammatory pathway nuclear factor-κB (NF-κB) in tissues such as the synovium and intervertebral disc. NF-kB is known to promote inflammation during disease pathogenesis and is highly upregulated during OA, resulting in the further progression of OA. Further analysis needs to be verified using western blot to ascertain the activation of the NF-kB pathway during GATA-4 induction. Here in, we hypothesize that GATA-4 levels in chondrocytes increase with aging, which contributes to limited cartilage repair, the production of inflammatory cytokines and chemokines through the NF-kB pathway, which finally leads to the onset of OA when other physiological stressors are present.

SIGNIFICANCE/CLINICAL RELEVANCE: The hallmarks of healthy aging that lead to OA have not been identified. Herein, understanding the mechanisms related to aging and the potential onset of OA is essential for treating the vast portion of the population over the age of 45 who suffer from OA. This study analyses the role of GATA-4 to assess the mechanisms of aging to better understand the contribution of aging on OA onset and progression with hopes of identifying therapeutic targets for aged individuals.