

Age-related gene identification in torn tendon of rotator cuff tear patients

Yibin Liu¹, Hongyi Xin², Jinjin Ma¹

¹South China University of Technology, Guangzhou, China, ²Shanghai Jiaotong University, Shanghai, China
jinjinma@scut.edu.cn

Disclosures: Authors have nothing to disclose.

INTRODUCTION: Higher incidence and poor prognosis of rotator cuff tears (RCTs) were observed in older population. Age-related degeneration in rotator cuff tendon is perceived as the major etiology of RCT. However, the underlying mechanisms remain unclear. We carried out this study to identify differentially expressed genes (DEGs), their functions and molecular pathways of RCT patient tendon samples between the two age groups, which potentially contribute to aging progress in rotator cuff tendons and RCT development.

METHODS: An RNA-seq dataset (GSE180836) of supraspinatus tendon samples (15 degenerative tear and 16 acute tear) was downloaded from Gene Expression Omnibus (GEO) database. Differential expression analyses were first conducted by DESeqs software (version: 1.38.3) on subjects grouped by age[Age \geq 65 (n = 6) vs. Age<65 (n=9)] using samples collected from patients with degenerative tear. Significance threshold was set as $|\log_2\text{FC}| > 1$ and p-value < 0.05. DEGs were used to construct a protein-protein interaction (PPI) network by STRING online platform and clustered base on k-mean clustering method¹. Functional enrichment analyses were then carried out on the candidate DEGs to show the potential biological processes and the number of clusters were determined based on the strength and false discovery rate of GO (gene ontology) terms. Furthermore, candidate genes were validated in the acute tear subsets [Age \geq 65 (n = 2) vs. Age<65 (n=14)] following the same procedure.

RESULTS: Age<65 group was set as the reference group in the degenerative subset and a total of 729 transcripts passed the significance threshold, which mapped to 496 genes. Among these genes, 294 were down-regulated and 202 were up-regulated in the Age \geq 65 group (Fig. 1). DEGs were mapped to proteins and assigned to three clusters in PPI network enriched in specific GO terms potentially related to tendon degeneration (Fig. 2). Three representative GO terms revealed were the following. (1) Collagen-containing extracellular matrix (ECM): collagen synthesis genes COL1A1 and COL1A1 were down-regulated, COL9A2 were up-regulated, and their regulators CTHRC1 and LOX were also down-regulated in Age \geq 65 group). (2) Muscle cell composition and development: genes coding muscle specific protein components (MYH1, MYL2, MYL11, MYLK2, TNNC1 and TNNC3) were down-regulated. (3) Immune response regulation: CD8A, IL1RL1, and HLA-DOB were down-regulated and immune-related cytokines CXCL2 were up-regulated and CXCL9 were down-regulated in advancing age (Fig 2). In the validation analysis performed in the acute tear subset, we found 21 of the same DEGs that were also significantly expressed with the same regulation directions as those in the degenerative tear subset (labeled in Fig. 1).

DISCUSSION: In this study, we identified a set of DEGs associated with advancing age in supraspinatus tendon from degenerative RCT patients, which were potential molecular markers for tendon degeneration and RCTs pathomechanism. The PPI network and functional enrichment analyses revealed three representative DEG clusters that might be associated with tendon aging. (1) Tendon ECM mainly comprises different types of collagens which are crucial to maintain the strength and elasticity of tendon. COL1A1, coding type I collagen, was downregulated in advanced aged group in present study, which was same as previous research reported². COL9A2, coding type IX collagen component, was mostly reported association with cartilage development and intervertebral discs degeneration. The finding of its up-regulation associated with age increase in RCT was novel to date. This new finding may indicate the role of type IX collagen in imbalance of collagen composition when tendon aging. (2) Among the cluster enriched genes coding protein components of muscle, we found MYH1 was significantly down-regulated with advancing age in both degenerative torn tendon and acute torn tendon while MYH1 expression previously has been shown to increase with advancing age³. Other studies discovered that MYH1 was down-regulated in muscles within one week after injury, which suggested that myosin expression was different depending on etiology. (3) Inflammatory infiltrations were common in lesion tissues of RCTs, such as elevated expression of IL-1, IL-6, and CD45, which was considered to be part of the pathology in previous studies. Specific inflammation markers and their impact mechanisms to different stages of the disease demand future comprehensive study. In conclusion, we have identified a series of genes associated with age in torn tendon of RCTs patients, which were mapped to biological processes related to tendon aging and the results provide us with premises for future research with patient samples. The current study has several limitations such as small sample size and lack of other baseline patient and disease factors. Therefore, the analyses did not adjust for known factors that influence the progression and healing of the tears. Future study with prospective longitudinal study design is needed to study the cellular and molecular shifts in the injury site may be responsible for the progression and healing of the RCT patients as they age.

SIGNIFICANCE/CLINICAL RELEVANCE: (1-2 sentences): Current study is clinically relevant to the rotator cuff tear progression and healing after rotator cuff repair as it provides valuable insights into the genetic, molecular, and pathway-level factors contributing to the aging condition.

REFERENCES: 1. Szklarczyk *et al*, *Nucleic Acids Res* 2023. 2. Hawthorne *et al*, *Arthrosc* 2023. 3. Lagou *et al*, *Biology* 2023.

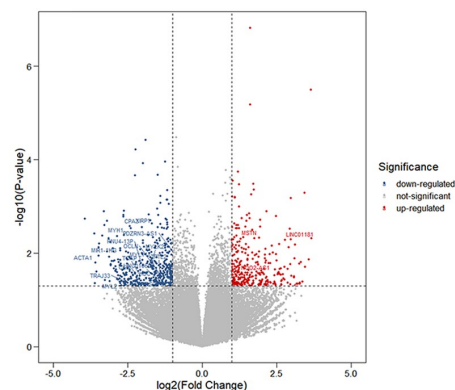


Fig 1. Volcano Plot displayed each gene as a dot positioned based on its Fold Change and P-value. Those passed the significance threshold were highlighted in blue and red, representing down- and up-regulated respectively.

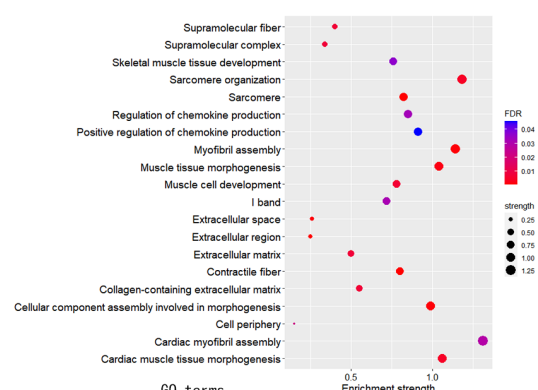


Fig 2. Bubble chart revealed top 20 enriched GO terms ranked by enrichment strength. Bubble sizes indicated enrichment strength and colors indicated FDR.