RNAseq-based Analysis of Differential Gene Expression Associated with Tendon Injury in the Mouse Achilles Injury Model

Kairui Zhang[1]
Sohtaroh Izumi[2]
Masatake Matsuoka[2]
Ngozi Akabudike[2]
John Tobias[3]
Louis Soslowsky, PhD[3]
Masahiro Iwamoto[2]
Motomi Enomoto-Iwamoto[2]

[1]Southern Medical University
Guangzhou, China

[2]University of Maryland
Baltimore, MD

[3]McKay Orthopaedic Research Laboratory
University of Pennsylvania

Introduction

Incomplete tendon healing leads to significant mobility restriction, pain and substantial health care costs. Understanding the molecular mechanisms responsible for tendon healing will provide important insights to help develop a focused therapeutic modality to stimulate tendon repair.The goal of this study is to identify the differentially expressed genes associated with tendon injury. We aim to determine the biological pathways that alter in response to tendon injury using the mouse Achilles tendon injury model.

Methods

All animal experiment procedures were approved by the Institutional Animal Care and Use Committee of the Children's Hospital of Philadelphia and University of Maryland.

Tendon surgery

A complete transverse incision was made at the midpoint of the right Achilles tendon in 8-week- old female C57/BL6 mice and the gap was left open[1]. Animals were returned to cage activity and euthanized 1 or 3 weeks after surgery, representing the inflammation/proliferation and repair phases, respectively. Harvested tendons were snap-frozen in liquid nitrogen for RNA preparation.

RNA-seq and analysis

Total RNAs were prepared from uninjured tendons (8 tendons/sample, n=3) and injured tendons (2 tendons/sample, n=3-4) by RNeasy Fibrous Tissue Mini Kit (Qiagen). Stranded RNAseq libraries was generated and indexed by TruSeq RNA library prep kit v2. RNA integrity, cDNA amplification, cDNA fragmentation and barcode ligation were confirmed by Agilent BioAnalyzer.The RNA-seq and data analysis were performed in the Next Generation Sequencing Core at the University of Pennsylvania (Illumina Hiseq 4000, ~40 million reads/lane). After alignment to the mouse genome, mapping to the exons of each gene, and then quantile normalization, differentially expressed genes were identified and analyzed by Ingenuity pathway analysis (IPA) and Gene set enrichment analysis (GSEA).

Results

Comparison of 1-week injury tendon to the uninjured tendon

4537 and 5029 genes were differentially up- and down-regulated, respectively in injured tendons (more than 2-fold, $p<0.01$). GSEA analysis with C2-CP (curated gene sets-canonical pathways) databases showed negative regulation of the gene sets involved in respiratory electron transport and ATP synthesis and positive regulation of the gene sets with O-glycan biosynthesis and mitotic activity (Fig. 1A).

Comparison of 3-weeks injury tendon to the uninjured tendon

3722 and 4240 genes were differentially up- and down-regulated, respectively (more than 2-fold, $p<0.01$). GSEA analysis also showed similar enrichment plot profiles in the gene sets of the respiratory electron transport, TCA cycle and ATP synthesis (Fig. 1B).

Comparison of 1-week injury group to 3-weeks injury group

920 and 739 genes were differentially up- and down-regulated, respectively in 3-week injury group compared to 1-week injury group (more than 2-fold, $p<0.01$). GSEA analysis revealed negative regulation of the gene sets involved in cell cycle regulation and DNA damage, and positive regulation of the gene sets involved in peptide elongation and cytochrome p450 in drug metabolism. The gene sets involved in the respiratory electron transport were up-regulated in the 3-weeks injury group by GSEA analysis (Fig. 1C). The IPA analysis showed consistent results with those by the GSEA analysis: The oxidative phosphorylation, mitochondrial dysfunction and TCA cycle pathways were ranked in top 3 in comparison between 1- or 3-weeks injury and the uninjured group; The cell cycle (chromosomal replication and DNA damage check point) pathways were highly ranked in comparison between 1-week and 3-weeks injury groups.

Discussion

The global gene expression analysis demonstrated strong down regulation of the respiratory electron transfer and ATP synthesis

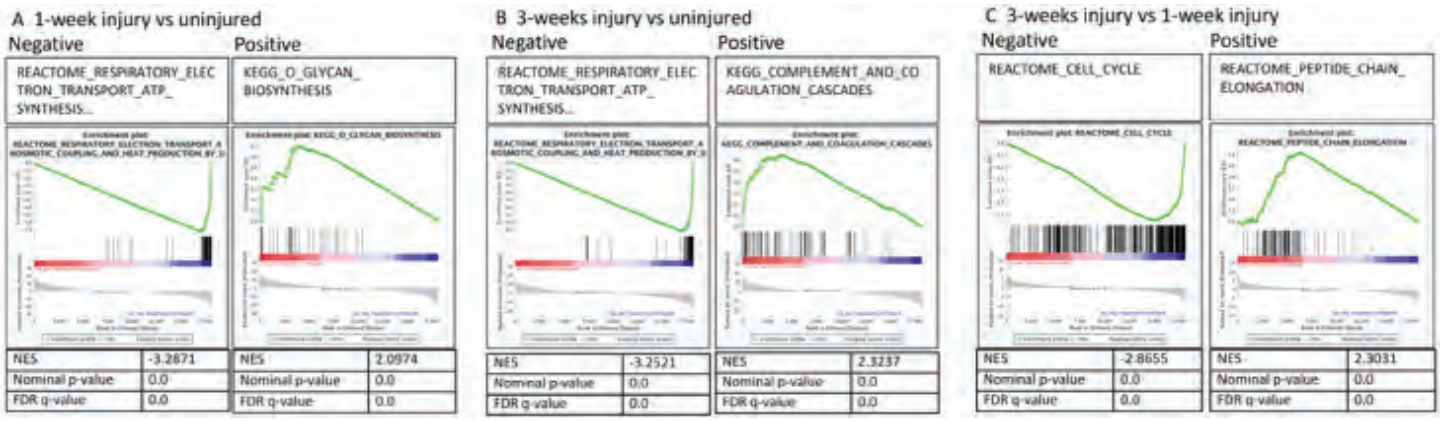

Figure 1

pathways (oxidative phosphorylation pathway) in the early phase of the tendon healing process in mice compared to the normal uninjured tendons. Results may be linked to our findings that mouse injured tendons stimulated lactate synthesis pathway and that the contents of metabolites of the TCA cycle were reduced after injury[2]. Further investigation of energy metabolism in injured tendons is required. Comparison of the gene expression profile between 1-week injury and 3-weeks injury indicate a decrease in cell proliferation, an increase in protein synthesis and a recovery of respiratory electron transfer pathway in the early phase of tendon repair. Reduction in gene expression involved in sensing and protection of DNA damage in the 3-week injury tendon may give negative impact on tendon repair.

Significance & Clinical Relevance

While a large number of clinical and preclinical approaches have been attempted, none result in complete recovery of the mechanical structure and function in injured tendons. This study provides important information to develop a targeted therapeutic modality for tendon repair.

Acknowledgements

We thank the Next Generation Sequencing Core at University of Pennsylvania for RNAseq and data analysis. This study was supported by the Penn Center for Musculoskeletal Disorders Pilot and Feasibility Grant (NIH/NIAMS P30AR050950) and the NIH R01AR070099 Grant.

References

1. Asai *et al.* *Stem Cells* 32, 3266 (2014)
2. Enomoto-Iwamoto *et al.* The ORS 2016 Annual Meeting