Temporal And Sex-dependent Effect Of Short-term Simulated Microgravity On Human Meniscus Tissue Model

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Background: Millions of people worldwide suffer from osteoarthritis, particularly knee osteoarthritis (KOA), which is more prevalent and severe in females, although the underlying causes remain unclear. Hormones are a factor, but not the sole cause. The molecular mechanisms of KOA development are complex and not fully understood, but the genes *COL10A1* and MMP13, as well as WNT and NF-kappa B signaling pathways, are activated in chondrocytes in affected joints. Mechanical unloading, such as in microgravity, can lead to OA-like changes and cartilage breakdown, and unloading knee joints in healthy individuals produced MRI changes resembling KOA. Simulated microgravity has been used to study the effects of mechanical unloading on articular chondrocytes, and previous research found that SMG increased expression of COL10A1 and MMP13 in human meniscus models from expanded human meniscus fibrochondrocytes. Methods: Specimens of human meniscus were obtained from 5 male and 5 female donors undergoing partial meniscectomy who had no history of KOA. The primary MFC from each donor were isolated, recovered for 48 hours on human tissue extracellular matrix-coated plates, and then seeded in 3D porous type I collagen cylindrical scaffolds for 48 hours in a defined medium to allow cell attachment and initial matrix production. After the pre-culture period, the engineered models were divided into two groups and cultured under static gravity and SMG conditions for 7 days. The scaffolds were collected on day 0, 1, 3, and 7 and the temporal transcriptome profile were determined using RNA sequencing.

Results: The transcriptomic profile of the engineered tissue models was assessed by RNA sequencing. For all donors combined, principal component analysis (PCA) showed a converging transcriptional trajectory for all donors over 7 days of SMG culture, with 1383 genes significantly regulated on day 7. Among all the significantly regulated genes, several key OA-related markers (COL10A1, MMP13, SPP1) were highly upregulated. Gene set enrichment analysis indicated that inflammation-related pathways (IL-17 signaling pathway and complement cascades) dominated the initial changes along the trajectory, whereas calcification-related pathways (calcium signaling pathway and mineral absorption pathway) and PPAR signaling pathway were heightened at later stages. Sex-related transcriptional differences were observed, with females and males responding differently to SMG at each RNA sequencing time point. Hub protein interaction networks were constructed from the up- and down-regulated gene panels for both sexes to establish mechanistic targets. For females, the hub components of upregulations on both day 3 and day 7 were mainly involved in Wnt signaling (CTSK, IRAK1, JUN, PIK3R1, LEF1, MMP7, NFATC4, ROR2, WNT11), VEGF signaling (KDR, PIK3R1, SPHK1), and NF-kappa B signaling pathways (BIRC3, CXCL12, DDX58, GADD45A, IRAK1, RELB, TRAF1, TRAF5). Whereas for males, the hub components of upregulations were ECM components and matrix remodeling enzymes.

Significance/Clinical Relevance: The results of this study provide new insights into the molecular changes that occur in response to mechanical unloading and the sex differences in the development of KOA. Further understanding of these mechanisms may inform potential therapeutic strategies.