Endoplasmic Reticulum (er) Stress Induces Mitochondrial Dysfunction In Chondrocytes

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Introduction:

Mechanisms linking cartilage degradation in osteoarthritis (OA) are not completely understood. During OA, chondrocytes are thought to undergo increased stress as a result of excessive mechanical loading of the joint and from age-related oxidative stress and endoplasmic reticulum (ER) stress. Recent studies have shown that ER stress influences mitochondrial function [1]. Thus, the purpose of the study was to determine if ER stress induces mitochondrial dysfunction in chondrocytes.

Methods:

Cultured primary porcine chondrocytes were stimulated with 2 μM of thapsigargin (TG) and 1μg/ml tunicamycin (TM) for 24 hours to induce endoplasmic reticulum (ER) stress. After treatment, cell lysates were prepared and immunoblotted for ER stress (CHOP and Xbp1) and mitochondrial markers (porin/VDAC). To determine mitochondrial function, chondrocytes were treated with TG and TM for 24 hours and oxygen consumption rate (OCR) was measured by Seahorse XF-24 Extracellular Flux Analyzer (Seahorse Bioscience). The data generated was analyzed by t-test using GraphPad Prism statistical software. P value less than 0.05 was considered statistically significant.

Results:
Treatment of isolated primary chondrocytes with TG and TM induced expression of CHOP (marker from ER stress) and Xbp-1s, an alternative mRNA splicing product that is generated by unfolded protein response (UPR) signaling and plays a major role is ER protein degradation (ERAD) (Fig1). In addition, treatment of chondrocytes with TG and TM significantly decreased the mitochondrial basal respiration rate and ATP turnover (Fig 2A&B). However, immunoblot analysis of porin protein level showed no changes in the expression (Fig 3). Porin [voltage-dependent anion channel (VDAC)] is a mitochondrial outer membrane resident protein, which is often used as a marker for mitochondrial mass index. Taken together our results suggest that ER stress in chondrocytes induces mitochondrial dysfunction without altering mitochondrial mass.

Discussion:

ER stress has been recently shown to play an important role in the development of OA [2]. However, the mechanisms involved are not clearly understood. Here we demonstrate that ER stress induced mitochondrial dysfunction in chondrocytes. Mitochondrial dysfunction has been implicated in several pathways that are involved in OA, including oxidative stress and apoptosis [3]. Further mechanistic studies are underway to delineate the link between ER stress and mitochondrial function and its role in the development of OA. Targeting the ER stress pathway that promotes mitochondrial dysfunction could provide a new target for treatment of OA.

Significance:

This is the first study to establish the direct
link between endoplasmic reticulum (ER) stress and mitochondrial dysfunction in chondrocytes.

Fig 1. Treatment of chondrocytes with thapsigargin and tunicamycin induces CHOP and Xbp1s expression.
Fig 2. Thapsigargin and tunicamycin induces mitochondrial dysfunction. A) A typical oxygen consumption rate graph. B) Individual mitochondrial respiration parameters calculated from A, results are presented per mg of protein.
Fig 3. Treatment of chondrocytes with thapsigargin and tunicamycin did not alter the porin protein levels.

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