The loss of mitochondrial inner membrane protein Opa1 causes intervertebral disc degeneration and osteoarthritis in aged mice

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INTRODUCTION: Nucleus pulposus (NP) cells reside in a hypoxic environment and depend on glycolysis for their energy demands and are consequently thought to contain few functional mitochondria. Since mitochondrial function is inextricably linked to its ultrastructure, changes in the quantity, size, and shape result in functional deficiencies and disease states. Dynamic regulation of fission-fusion processes tailors' mitochondrial morphology to the bioenergetic needs of the cell. The failure of these mechanisms raises the possibility of organ dysfunction and degeneration. It has been demonstrated that mitochondrial shape and dynamics are altered in a variety of degenerative and metabolic disorders and aging. We recently showed that deletion of HIF-1 and BNIP3 resulted in altered mitochondrial shape and dysregulated mitochondrial activities in NP cells. In the present investigation, we delineate the importance of Opa1 in disc and cartilage health during aging.

METHODS: To understand the functions of Opa1 *in vivo*, we generated Opa1 conditional knockout mice using Acan1^{CreERT} and characterized the spinal phenotype of Opa1 knockout mice as a function of aging (n=8-10/ genotype). Spines from WT and KO mice were decalcified and mid-coronal disc sections were stained with Safranin O/Fast Green/Hematoxylin and visualized using light microscopy. Modified Thompson scoring was performed on mice of both genotypes. The Picrosirius Red polarized light microscopy was performed at 20 months age mice to determine the presence of fibrotic tissues in the NP compartment using polarized imaging. In addition, we characterized the knee joint phenotypes of mice. To gain mechanistic insights into the contribution of Opa1, we performed metabolic studies on primary NP cells transduced with lentiviral ShOpa1 and ShCtrl. Accordingly, we performed steady state and flux metabolic analysis using [1,2]-^{13}C-glucose and U^{13}C-glutamine labeling. We also evaluated the influence of Opa1 on NP cell real-time bioenergetics using a Seahorse analyzer to measure glycolytic capacity and ATP production rates. Statistical analysis was performed with Prism7. Data distributions were assessed with the Shapiro–Wilk normality test and appropriate parametric or non-parametric tests with post-hoc analysis was used.

RESULTS: We have observed that the deletion of Opa1 in the disc leads to intervertebral disc degeneration, 70% of discs showed NP tissue fibrosis and loss of cells. Interestingly, the remaining 30% of discs showed that NP cells lost their characteristic vacuoles. We also analyzed major disc ECM molecules collagen I, collagen X and COMP. Conditional KO mice evidenced decreased collagen 1 and COMP expression. We noted that loss of Opa1 in knee joints caused severe osteoarthritis. In the medial and lateral joint compartments of the Opa1 cKO mice, there was evidence of significant synovial hyperplasia and/or ossification, and there were substantial osteophytes on medial and lateral tibial plateaus. In cultured primary NP cells, deletion of Opa1 resulted in fragmented mitochondria and aberrant cristae morphology. Interestingly, there was a significant reduction in autophagy and autophagic flux. Interestingly, we noted that loss of OPA1 resulted in structural changes to organelles in addition to mitochondria. In Opa1 knockdown NP cells, while glycolytic capacity was unchanged, glycolytic ATP production was significantly increased, without changes in oxidative ATP generation. 1,2-^{13}C-glucose labeling studies demonstrated decreased PDH flux and altered lipid metabolism on the other hand U^{13}C-glutamine labeling showed dysregulation in the generation of TCA cycle intermediates.

DISCUSSION: In addition to serving as a hub for several cell signaling pathways, mitochondria physically interact with other organelles including endoplasmic reticulum, peroxisomes, endosomes, lysosomes, plasma membrane, and lipid droplets. Our findings showed that Opa1 is critical for the maintenance of organelle morphology in addition to mitochondria and cristae morphology in NP cells. Moreover, evidence suggests that degeneration of the disc and knee joints in old Opa1-cKO mice may have been facilitated by the disruption of autophagy, secretory, and endocytic pathways. Taken together data suggests that Opa1 is vital for maintaining the integrity of mitochondria and other organelles and therefore, tissue homeostasis.

SIGNIFICANCE: Our study illustrated the importance of Opa1 in preserving the health of the knee joint and intervertebral disc during aging. Maintaining a healthy mitochondrial pool is crucial because of its constant interaction with other organelles. The accumulation of faulty mitochondria in age-related pathologies and metabolic disorders has been demonstrated in several studies. Therefore, targeting and modifying the autophagic pathway and restoring mitochondrial function may benefit in designing feasible therapeutic approaches to treat diseases linked to degenerative conditions.

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