CHEMICALLY AND MECHANICALLY ACTIVATED PIEZO1 LEADS TO CHANGES IN THE EXPRESSION OF ECM-MODULATING FACTORS THAT DIFFER BETWEEN HEALTHY AND HUMAN OA CHONDROCYTES

Keywords: Cell biology, Cartilage, Osteoarthritis

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Background: Osteoarthritis (OA) is the most common degenerative joint disease that causes functional limitations in addition to pain and has become a major global health problem in recent decades. Meta-analysis of studies on patients with knee OA showed that physical exercise can function as a clinically relevant, effective intervention and non-drug treatment for reducing pain and improving physical activity in patients with OA [1]. In chondrocytes, the perception and transduction of mechanical signals leading to changes in Ca2+ is orchestrated by mechanosensitive ion channels [2].

Methods: Piezo1 represents a mechanosensitive cation channel (MSC) that allows Ca2+ influx in chondrocytes and acts as an important mechanotransducer that senses deleterious mechanical stresses (high strains) [3]. Numerous studies have shown that Ca2+ regulation in conjunction with mechanical stimulation is crucial for the behaviour and function of chondrocytes and the balanced assembly of the ECM. The underlying mechanism could influence the expression profile as a consequence of mechanical stimulation. Differences in this regard between primary human chondrocytes isolated from knees of OA patients (pCHOA) and healthy chondrocytes could explain the altered sensitivity to mechanical stimuli in OA.

Results: Mechanical stimulation of chondrocytes was performed by the use of the flexcell system whereas in parallel cells were chemically activated via different concentrations of the Piezo agonist Yoda1. Changes in expression were analysed by qPCR for metalloproteinases (MMP1, 3, 13), inflammatory cytokines (IL6, IL8, IL4) and ECM relevant components (Col-I, Col-II, BMP2). The decreased expression of Yoda1 by siRNA knockdown was used to more clearly map the effect of MSC on ECM under mechanical stimulation.

Conclusion: Performing expression studies in the context of mechanical stimulation and targeting Piezo1 in OA and healthy chondrocytes may reveal OA-specific changes in mechanisms of mechanical signal transduction. Furthermore, this may provide further insight into the extent to which OA cells exhibit differential sensitization to mechanical signals.

Disclosure of Interests: None Declared.

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DEFECTIVE AUTOPHAGY IS ASSOCIATED WITH CHONDROCYTE SENESCENCE AND JOINT DAMAGE

Keywords: Cell biology, Osteoarthritis

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Background: Aging is a main risk factor of many major human diseases, including Osteoarthritis (OA). Compromised autophagy, a hallmark of aging, is involved in joint aging and OA and its activation protects against joint damage and disease. However, targets underlying this defective mechanism are still unknown.

Methods: We performed a quantitative proteomic analysis of defective autophagy by Atg5 knockdown in human OA chondrocytes by using TRIAQ (isobaric tags for relative and absolute quantitation) labeling coupled LC/MS/MS. Protein identification and quantification were performed using Protein Pilot Software v 4.0. Each MS/MS spectrum was searched in the Uniprot/Swissprot database for Homo sapiens. Human cartilage and chondrocytes from healthy, aging and OA subjects were employed to investigate the role of Lamin A/C in aging and disease by western blot, and immunohistochemistry. To test the relevance of Lamin A/C accumulation in joint tissues, a mutant mice model of accelerated aging by genetic deletion of Zinc Metalloproteinase STE24 (Zmpste24−/−) was employed. The functional consequences of Lamin A/C accumulation on macroautophagy, inflammation, and senescence were determined in human chondrocytes. To evaluate the therapeutic effect of regulating Lamin A/C in human chondrocytes, Lonafarnib, an FDA approved drug for Progeria Syndrome, acting as Lamin A/C accumulation inhibitor, was employed.

Results: 24 out of 487 proteins were significantly altered (p<0.05) in response to defective autophagy. Cytoskeleton organization, collagen catabolism, oxidative stress, and aging pathways were affected. Interestingly, Lamin A/C, a nuclear envelope protein implicated in cell senescence and aging, was found upregulated under defective autophagy. Increased Lamin A/C expression was found in human chondrocytes with reduced macroautophagy. Furthermore, increased Lamin A/C expression is found in aging and OA human cartilage. Importantly, Zmpste24 KO mice showed bone damage and intervertebral disc degeneration (IDD), suggesting that Lamin A/C accumulation associated to deficient autophagy is correlated with aging and OA phenotype. Human chondrocyte premature aging by genetic knockdown of Zmpste24, lead to Lamin A/C accumulation, accompanied by a reduction of macroautophagy by MAP1LC3B downregulation, increased inflammation, cartilage degradation and senescence represented by upregulation of NFKB/RELA, MMP13, CDKN1A and CDKN1A respectively. Remarkably, inhibition of Lamin A/C accumulation by Lonafarnib upregulates macroautophagy and protects against inflammation, cartilage degradation and chondrocyte senescence.

Conclusion: The results suggest that increased LMINA due to autophagy defects compromises chondrocyte homeostasis and joint integrity, potentially contributing to systemic maladaptation that occurs in aging. Thus, targeting Lamin A/C might be a promising strategy to develop novel therapeutics for cartilage aging and OA.

Disclosure of Interests: None Declared.

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IMPLICATION OF GLYPICANS AND NOTUM IN BONE MARROW MESENCHYMAL STROMAL CELLS DURING OSTEOSTERIGENIC DIFFERENTIATION IN OSTEOARTHRITIS DISEASE

Keywords: Bone diseases, Cell biology, Osteoarthritis

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