Imbalance of plastin-3 levels disturbs cartilage mechanotransduction and leads to early stage osteoarthritis

Daniela Mählich[1], Ilka Maus[2,3], Maren Dreiner[1], Miriam Weyers[1], Jan-Wilhelm Lackmann[4], Brunhilde Wirth[2,3,5], Anja Niehoff[1,6]
[1]German Sport University Cologne, Institute of Biomechanics and Orthopaedics, Cologne, Germany, [2]University of Cologne, Institute of Human Genetics, Cologne, Germany, [3]University of Cologne, Center for Molecular Medicine, Cologne, Germany, [4]University of Cologne, Cologne Excellence Cluster on Cellular Stress Response in Aging-Associated Diseases (CECAD), Proteomics Facility, Cologne, Germany, [5]University Hospital of Cologne, Center for Rare Diseases Cologne, Cologne, Germany, [6]University of Cologne, Medical Faculty, Cologne Center for Musculoskeletal Biomechanics, Cologne, Germany
d.maehlich@dshs-koeln.de

Disclosures: Daniela Mählich (N), Ilka Maus (N), Maren Dreiner (N), Miriam Weyers (N), Jan-Wilhelm Lackmann (N), Brunhilde Wirth (N), Anja Niehoff (N)

INTRODUCTION: Plastin-3 (PLS3) is an F-actin-binding and -bundling protein and is consequently involved in all processes dependent on F-actin dynamics [1]. Because the actin cytoskeleton is connected via cell surface integrins to the extracellular matrix (ECM), it is of central importance for the transmission of both chemical and mechanical stimuli in both directions inside-out and outside-in the cell [2]. This linkage between the actin cytoskeleton and the ECM plays a crucial role in tissue maintenance and health. In osteoarthritis (OA) an impaired mechanotransduction via the actin cytoskeleton is regarded as an important factor [3]. The aim of our study was to characterize the impact of both *Pls3* knockout (KO) and *PLS3* overexpression (OE) on knee joint articular cartilage mechanotransduction in mice.

METHODS: We studied the influence of ubiquitous *Pls3* KO [4] and *PLS3* OE (homozygous *PLS3V5* transgene) [5] on cartilage homeostasis in comparison to the C57BL/6N (WT) mice. All experimental protocols were performed in agreement with the guidelines of the German animal protection law and were approved by a licensing committee (Institutional review Board: "Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein Westfalen", #§4.19.002, §4.21.026 and #81-02.04.2019.A479). For phenotypic characterization, the knee joint articular cartilage of *PLS3* OE, *Pls3* KO and WT (4-8 animals/genotype) of 6-month-old mice of both sexes were analyzed. Cartilage degeneration was determined using a modified Osteoarthritis Research Society International (OARSI) score [6]. In addition, we isolated chondrocytes from knee joints of newborn WT, *Pls3* KO and *PLS3* OE mice to analyze F-actin structures under unloaded control conditions and mechanical stimulation using the FX4000 Flexcell system. Cyclic tensile strain of 6 % was applied for 30 min on three consecutive days. F-actin structures and effects on the ECM formation were evaluated by immunofluorescence and immunoblotting.
To identify proteins that are regulated depending on PLS3 levels, an unbiased proteome analysis was performed on tibial articular cartilage of 6-month-old mice.

RESULTS SECTION: 6-month-old *PLS3* OE female mice showed a significantly ($p<0.05$) increased OARSI score in comparison to WT mice. Furthermore, in both *PLS3* OE and *Pls3* KO mice we observed a significantly ($p<0.05$) reduced collagen II staining in tibial articular cartilage as well as diminished collagen II levels in protein extracts. Cyclic stretching of primary chondrocytes induced an intense PLS3 staining in chondrocytes of both WT and *PLS3* OE mice. In *Pls3* KO cells, the F-actin level was reduced ($p<0.05$) upon mechanical stimulation while no alterations were observed in PLS3 OE and WT cells. In addition, pro-collagen II levels were reduced ($p<0.05$) in *PLS3* OE and *Pls3* KO chondrocytes following mechanical loading.
In WT and *PLS3* OE chondrocytes, staining intensity of the mechanosensor zyxin was enhanced ($p<0.05$) after mechanical stimulation. In contrast, zyxin did not show any response to mechanical loading in *Pls3* KO cells. Proteome analysis of *Pls3* KO and *PLS3* OE mice revealed enhanced levels of candidates involved in autophagy while *PLS3* OE mice lack proteins of apoptosis.

DISCUSSION: An imbalance of PLS3 led to reduced collagen II levels *in vivo and in vitro*. The reduction might contribute to the early stage OA observed in the corresponding mouse lines. Proteome analysis revealed downregulated apoptosis and upregulated autophagy pathways in *PLS3* OE and *Pls3* KO mice, which might contribute to early stage OA. PLS3 levels might influence many essential OA-relevant cellular processes in addition to mechanotransduction like endocytosis, vesicle trafficking, autophagy and apoptosis.

SIGNIFICANCE/CLINICAL RELEVANCE: PLS3 plays an important role in cartilage mechanotransduction. As altered mechanotransduction is a risk factor for OA development PLS3 could be a novel target, in particular for mechanically induced OA subtypes.

REFERENCES:
[1] Wolff et al. (2021). Cell Mol Life Sci 78(13),5275-5301.
[2] Martino et al. (2018). Front Physiol 9,824.
[3] Blain (2009). Int J Exp Pathol 90(1),1-15.
[4] Neugebauer et al. (2018). Hum Mol Genet 27(24),4249-62.
[5] Ackermann et al. (2013). Hum Mol Genet 22(7),1328–47.
[6] Glasson et al. (2010). Osteoarthritis Cartilage 18,S17-S23.

ACKNOWLEDGEMENTS: This project was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation, FOR2722 to BW & AN (project ID 407176282 and 384170921).