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Poster Presentations

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SINGLE CELL SOX9 DYNAMICS REVEALS DIFFERENTIAL DNA BINDING IN SUBPOPULATIONS OF HUMAN CHONDROCYTES

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Purpose: SOX9 is the master transcription factor for chondrocyte differentiation, cartilage development and homeostasis. Its impaired function is associated with osteoarthritis (OA) pathophysiology. We studied SOX9 transcription factor dynamics at the single cell level, in the presence of extracellular stimuli, to understand its transcriptional activity in response to these external stimuli. We compared its regulation within subpopulations of healthy, preserved and OA human primary articular chondrocytes (hPCs). We hypothesised that there are subpopulations of cells within a donor, and that these subpopulations respond differently to external stimuli. In effect, the percentage of cells that respond to a stimulus, as well as the quantity of the response, will dictate if and how a patient will respond to a certain therapy.
Methods: We obtained healthy hPCs form Articular Engineering, USA, and isolated preserved and OA hPCs from the knee joints of patients undergoing arthroplasty surgery. We used two healthy, three preserved (i.e. cells were isolated from a macroscopically healthy site of the OA donor) and three OA donors. hPCs were transiently transfected with SOX9-mGFP. Fluorescence Recovery After Photobleaching (FRAP) was used to measure the SOX9 dynamics at the single cell level. hPCs were treated with either anabolic (BMP7) or catabolic factors (WNT3A or IL1β) with or without their inhibitors. Unsupervised hierarchical and K-Means clustering were used to classify the hPC subpopulations, based on distinct SOX9 dynamic rates within the donors, per treatment (Figure 1). FRAP measurements were taken from more than 40 cells per treatment. Altogether, SOX9 dynamics data from more than 3,800 cells were analysed for this study.
Results: We identified at least two clusters of cells in every donor with significantly different SOX9 dynamic rates (Figure 2). In healthy hPCs, over 63% of SOX9 is bound to DNA in one subpopulation (cluster 1), while only 53% of SOX9 was bound to DNA in the other subpopulation (cluster 2). Cells in cluster 1 were highly responsive to differential external stimuli, while the cells in cluster 2 showed minimal response. SOX9-DNA binding was lower in the preserved and OA hPCs, as comparted to healthy hPCs. In one subpopulation of these donors, 38% of SOX9 was bound to DNA (cluster 1) and in the second subpopulation, 53% of SOX9 was bound to DNA (cluster 2). Interestingly, in these donors, cells in both subpopulations responded in a differential manner to external stimuli. Any catabolic signalling decreased SOX9-DNA binding in healthy hPCs. The anabolic factors BMP7 and its inhibitor GREM1, and DKK1, FRZb, and IL1Ra significantly increased SOX9-DNA binding in the preserved and OA hPCs in both the clusters. Changes to the SOX9-DNA binding (Immobile Fraction) in these subpopulations are presented in tables 1 and 2.

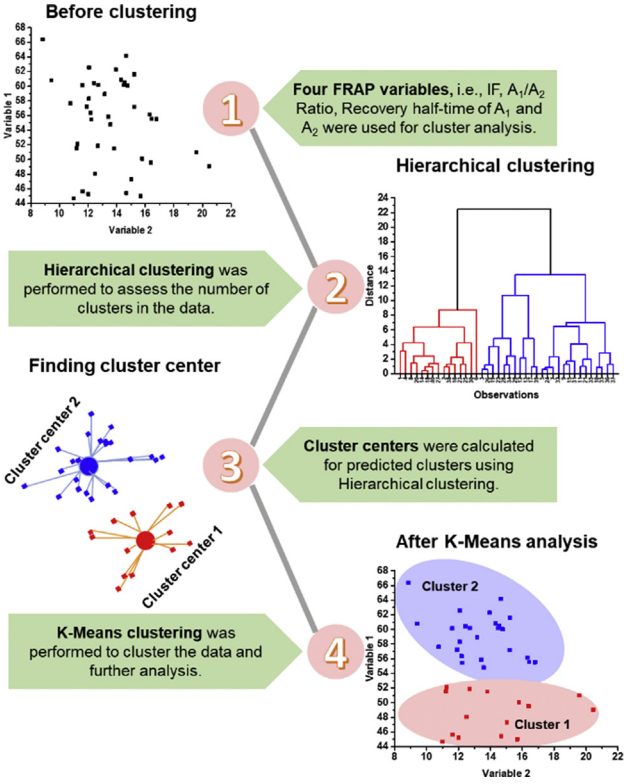

Fig. 1: Workflow of data analysis.

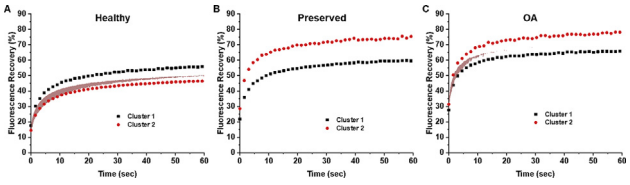

Fig. 2: FRAP curves from one healthy, preserved and OA donors show distinct mobility pattern of SOX9-mGFP of two clusters within a donor.

Table 1. Averaged immobile fraction (% fraction of SOX9 bound to DNA) in cluster 1 of two healthy donors, three preserved donors and three OA donor

	Healthy	Preserved	OA
Control	63.4 ± 3.3	38.7 ± 6.5	38.3 ± 5.6
BMP7	62.0 ± 3.8	44.3 ± 5.2	44.1 ± 5.9
GREM1	44.0 ± 6.0	44.1 ± 6.4	38.9 ± 8.0
WNT3A	53.6 ± 9.5	40.4 ± 5.1	41.7 ± 6.3
DKK1+FRZB	40.5 ± 6.8	41.6 ± 9.0	45.3 ± 10.9
IL1β	59.4 ± 4.3	40.6 ± 9.4	39.7 ± 8.0
IL1Ra	44.1 ± 6.9	43.8 ± 6.1	43.2 ± 7.3

Table 2. Averaged immobile fraction (%, fraction of SOX9 bound to DNA) of cluster 2 of two healthy donors, three preserved donors and three OA donor.

	Healthy	Preserved	OA
Control	52.8 ± 4.4	53.2 ± 4.1	52.2 ± 4.3
BMP7	49.7 ± 3.5	55.6 ± 3.8	54.4 ± 4.3
GREM1	53.4 ± 4.1	53.1 ± 4.4	51.2 ± 5.7
WNT3A	51.9 ± 5.7	51.6 ± 4.3	53.6 ± 4.9
DKK1+FRZB	54.5 ± 5.0	53.1 ± 5.3	51.3 ± 5.3
IL1β	46.9 ± 5.4	52.2 ± 5.7	52.4 ± 5.1
IL1Ra	55.1 ± 4.1	53.3 ± 3.9	52.8 ± 5.0

Conclusions: Our data show that hPCs have at least two subpopulations of cells within a donor, they exhibit a differential expression and mobility pattern of SOX9. These subpopulations within a donor respond differently to the same extracellular stimulation. Our study indicates that the SOX9 overexpression in preserved and OA hPCs will not match DNA binding levels found in the healthy hPCs. This implies that SOX9 overexpression alone will not rescue the healthy chondrocyte phenotype.

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OVEREXPRESSION OF THE MITOCHONDRIAL ANTIOXIDANT PROTEIN PEROXIREDOXIN 3 REDUCES THE SEVERITY OF AGE-RELATED OSTEOARTHRITIS IN MICE

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Purpose: Mounting evidence suggests that mitochondrial dysfunction that results in higher levels of mitochondrial reactive oxygen species (ROS) contributes to the development of OA. Excessive levels of ROS not only cause random damage to cellular proteins, DNA, and lipids but also disturb cell signaling promoting catabolic activity. Hydrogen peroxide (H_2O_2) is a key intracellular ROS responsible for the regulation of redox signaling. Levels of intracellular H_2O_2 are controlled by the peroxiredoxins (Prxs) with Prx3 controlling levels of mitochondrial H_2O_2. In prior studies we have reported that generation of excessive mitochondrial H_2O_2 favors activation of p38 over Akt signaling resulting in catabolic activity and cell death. Overexpression of Prx3 using an adenoviral construct counteracted excessive mitochondrial H_2O_2, inhibited p38 activation, promoted Akt activity and chondrocyte survival *in vitro*. The aim of this study was to determine if transgenic overexpression of Prx3 reduces the development of age-related OA in mice in order to test the hypothesis that excessive mitochondrial H_2O_2 contributes to age-related OA *in vivo*.
Methods: Animal studies were approved by the institutional Animal Care and Use Committee. Male mice on a C57BL/6J background were used and included tamoxifen inducible Prx3 generated by crossing aggrecan-Cre mice with flox-stop-flox-Prx3 mice (iPrx3AgCre^{ERT2}), conditional Prx3 in coll-2-expressing cells (Prx3Col2Cre) and germline transgenics to overexpress Prx3 globally (Prx3Tg). Control mice were wild-type littermates for the Prx3Tg mice and flox-stop-floxPrx3 mice used to generate the other two lines. Immunoblotting of cartilage lysates from femoral caps was used to verify the level of Prx3 expression. Stifle joints from 18-month and 24-month-old mice were collected and processed for histology with sectioning and staining of a representative mid-coronal section with hematoxylin and eosin (H&E). Articular cartilage in the medial and lateral tibial plateaus (MTP and LTP) was evaluated using the Articular Cartilage Structure score (ACS), a semiquantitative grading system ranging from 0-12; osteophytes and synovial hyperplasia were scored on a 0-3 scales. Histomorphometric measurements included the thickness and area of articular cartilage (AC), calcified cartilage (CC), and subchondral bone, the area occupied by dead chondrocytes in the AC, and the percent necrosis of the total AC area. Statistical analysis was performed with R Core Team (2017), using a Welch two-sample t-test comparing each experimental group to its control.
Results: Immunoblotting of femoral caps confirmed Prx3 overexpression in the Prx3Tg, iPrx3AgCre and Prx3Col2Cre mice compared to their controls. The summed ACS scores of the MTP+LTP of the 18-month-old mice were significantly lower in the iPrx3AgCre group than the age-matched floxedPrx3 control group (p=0.002), with only a trend toward significance in the 18-month transgenic (Prx3Tg) group (p=0.14) (Figure 1). There were no statistically significant differences between the Prx3Col2Cre and controls (only assessed at 24 months) or the other experimental and control groups at 24-months (Figure 2). There also were no significant group differences in AC area, CC area, subchondral bone area, or percent necrosis. There were trends toward significance in the 18-month-old Prx3Tg group versus the control group in summed osteophyte scores (p=0.09) and summed synovial scores (p=0.06) that were overall lower than the control group.
Conclusions: These results suggest that transgenic overexpression of Prx3 may decrease age-related OA in early-stage disease but may not be enough to combat the effects of ROS in advanced disease, as seen in the 24-month-old mice. The results also suggest that inducible Prx3 in aggrecan-expressing cells may be a more effective option to reduce mitochondrial H_2O_2 than global transgenic overexpression of Prx3. Since Col2Cre expression decreases with age more than AgCre, we are currently testing the possibility that Prx3 overexpression in the 24-month-old mice was reduced in the Col2Cre mice. The lack of a Prx3-knock-out model for comparison is a limitation. However, past studies have shown that Prx3 knock-out mice exhibit metabolic abnormalities that may confound results. Overall, the findings provide support for a role of mitochondrial H_2O_2 in age-related OA but suggest that additional factors also contribute, especially at advanced ages.

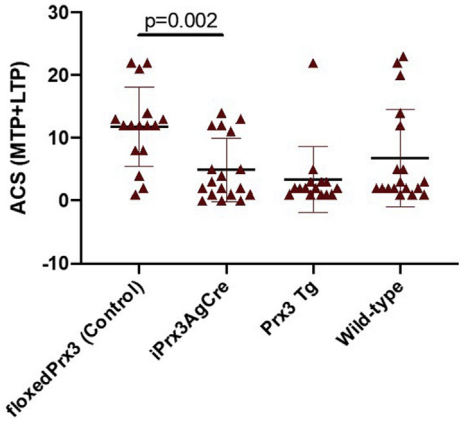

Fig. 1. Comparison of summed (medial and lateral tibial plateau) ACS scores in 18-month old floxedPrx3 control (n=16), Prx3AgeCre (n=18), transgenicPrx3 (n=15), and wild-type mice (n=18).

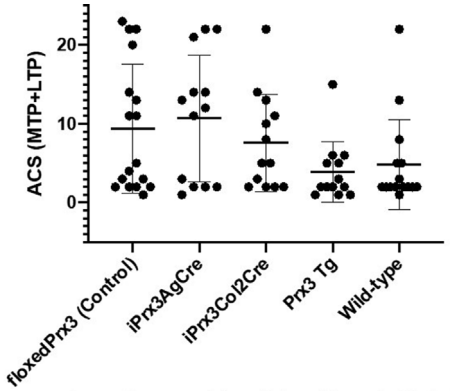

Fig. 2. Comparison of summed (medial and lateral tibial plateau) ACS scores in 24-month old floxedPrx3 control (n=17), Prx3AgeCre (n=13), conditional coll-2 Prx3 (n=13), transgenicPrx3 (n=13), and wild-type mice (n=14).

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CD11B ACTIVATION PREVENTS CHONDROCYTE MINERALIZATION AND OSTEOARTHRITIS PROGRESSION

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