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PROTEOGLYCAN 4 EXPRESSION PROTECTS AGAINST THE DEVELOPMENT OF OSTEOARTHRITIS

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Purpose: Osteoarthritis (OA) is a common degenerative condition that afflicts more than 70% of the population between 55 and 77 years of age. Although its prevalence is rising globally with aging of the population, current therapy is limited to symptomatic relief and in severe cases, joint replacement surgery. We aim to develop a novel therapeutic modality by intra-articular over-expression of proteoglycan 4. In addition, we aim to investigate the related mechanism in the pathogenesis of OA.
Methods: To study the effects of long-term expression of Prg4 on OA, we generated proteoglycan 4 (Prg4) transgenic mice. To translate localized expression of Prg4 into a therapeutic approach, we delivered Prg4 by intra-articular injection of helper-dependent adenoviral vectors (HDV). To investigate the related mechanism of OA pathogenesis, we performed gene expression profiling of mouse articular cartilage obtained by laser capture micro-dissection (LCM), in vitro cell studies and analysis of human OA gene profiling.
Results: Long-term Prg4 expression under the type II collagen promoter (Col2a1) does not adversely affect skeletal development but protects from developing signs of age-related osteoarthritis. The protective effect is also shown in a model of post-traumatic osteoarthritis created by cruciate ligament transection (CLT). Moreover, intra-articular injection of HDV expressing Prg4 protected against the development of post-traumatic osteoarthritis when administered either before or after injury. Gene expression profiling of mouse articular cartilage obtained by LCM and in vitro cell studies show that Prg4 expression inhibits the transcriptional programs that promote cartilage catabolism and hypertrophy. Analyses of available human OA datasets are consistent with the predictions of this model.
Conclusions: Our data offer long-term intra-articular expression of Prg4 as a potential chondro-protective approach to OA treatment and provide insight into the mechanisms for OA development.

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EARLY CHONDROCYTE HYPERCELLULARITY AND APOPTOSIS MAY BE CORRELATED WITH OSTEOCHONDRAL JUNCTION CHANGE AT OSTEOARTHRITIS ONSET IN DUNKIN-HARTLEY STRAIN GUINEA PIGS

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Purpose: Osteoarthritis (OA) has been one of most prevalent joint disorder which bring heavy burden to social economics. Up to now, the pathogenesis of OA is still not clearly understood. And effective treatment for OA is also lacking. Recently, the early pathological change of OA in cartilage and bone has drawn people's attention. However, the exact early osteochondral change is still unknown. This study aimed to observe the changes of cartilage and osteochondral junction in very early stage (first three months) of OA pathogenesis in Dunkin-Hartley guinea pig spontaneous OA model with Bristol strain 2 guinea pig as OA-free control.
Methods: Female Dunkin-Hartley and Bristol strain 2 guinea pigs ages 1, 2 and 3 months were euthanatize. Bilateral knee joints were excised. One knee joint was scanned with Microfocal Computed Tomography (Micro-CT), and the other knee joint was used for histopathologic and immunohistochemistry evaluation. All evaluations were performed blinded to the strain and time point.
Results: Significant higher chondrocytes density was identified in DH guinea pigs than BS2 guinea pig at 1 (DH 803.3±65.3 vs BS2 523.7±76.6/mm2, $p<0.05$), 2 (DH 1506.7±70.8 vs BS2 910.7±56.0/mm2, $p<0.05$) and 3 (DH 1300.0±144.5 vs BS2 936.2±100.7/mm2, $p<0.05$) months (Figure 1. a & b). Immunohistological analysis revealed that significant higher percentage of PCNA positive chondrocytes was defined in DH strain than BS2 at age of 1 and 2 months (Figure 1 c & d). Morphology of chondrocytes in DH strain appeared hypertrophic at 2 and 3 months comparing to BS2 (Figure 2.a). And hypertrophic chondrocytes clustering was observed at 3 months in DH strain (Figure 2.a). The thickness ratio of calcified cartilage (CC) layer to non-calcified cartilage layer (NCC) was significantly higher in DH strain at three months (DH 0.408±0.064 vs BS2 0.311±0.017, $p<0.05$, Figure 2.b).BMD of osteochondral junction in DH group is higher than BS2 group at and 3 month (DH 0.86±0.07 vs BS2 0.68±0.06, $p<0.05$, Figure 3.e). The thickness of osteochondral junction of DH strain was found significant higher than BS2 at age of 2 and 3 months. Additionally, the porosity of osteochondral junction is significantly lower at DH group at 1 (DH 18.1±3.7 vs BS2 33.1±5.3%, $p<0.05$), 2 (DH 14.7±2.3 vs BS2 24.2±4.2%, $p<0.05$) and 3 month (DH 6.8±1.5 vs BS2 12.0±2.5%, $p<0.05$).
Conclusions: The finding of this study revealed that early change at osteochondral junction, such as thickened osteochondral junction layer with decreased porosity, may lead to early sign of chondrocytes hypertrophy, apoptosis and calcification, as well as chondrocytes hypercellularity in compensation.

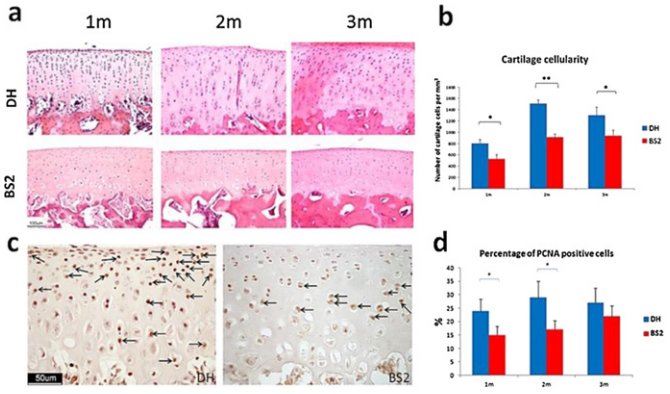

Figure 1. a: H&E staining of knee joints of DH and BS2 strain guinea pigs; b: significant higher chondrocytes cellularity was defined in DH strain than BS2 at 1, 2 and 3 months of age; c: Demographic image of immunohistochemistry of proliferating cell nuclear antigen (PCNA) on sagittal sections of the Tibial end cartilage of DH and BS2 guinea pigs at age of one month; d: significant higher percentage of PCNA positive chondrocyte was defined in DH strain than BS2. * $p<0.05$, ** $p<0.001$.

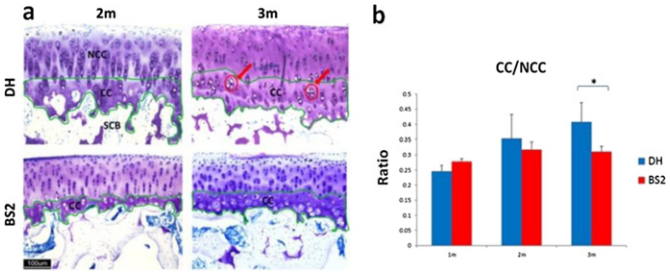

Figure 2. a: Toluidine blue staining of knee joints of DH and BS2 strain guinea pigs. NCC: non-calcified cartilage, CC: calcified cartilage, SCB: subchondral bone. Hypertrophic chondrocytes clustering was observed at 3 months (red circle); b: ratio of CC/NCC was found significant higher in DH strain than BS2 at 3 months. * $p<0.05$.

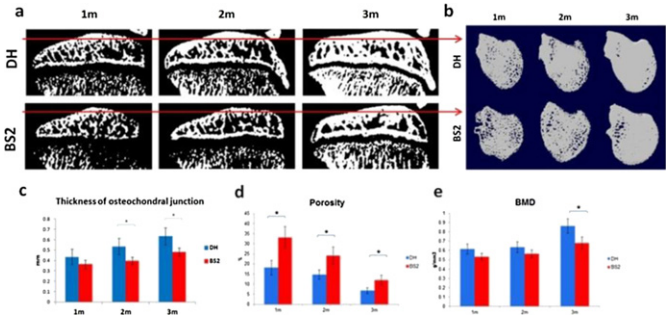

Figure 3. representative Micro-CT image of guinea pig medial knee joint (a) and cross-section view of tibial osteochondral junction of knee joints (b). Quantitative data showing the differences between DH and BS2 strain guinea pigs in thickness of osteochondral junction(c), porosity (d) and BMD (e) , * $p<0.05$.

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ARTICULAR CHONDROCYTES ARE PHYSICALLY CONNECTED THROUGH A CELLULAR NETWORK THAT IS RESPONSIBLE OF THE METABOLIC COUPLING BETWEEN CHONDROCYTES LOCATED IN DIFFERENT LAYERS OF THE TISSUE