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INCREASED SUSCEPTIBILITY TO IL-1 ACCELERATES OSTEOARTHRITIC CHANGE IN 3D-CULTURED CHONDROCYTES AND IN VIVO ANIMAL MODELS

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Purpose: Mechanical overload applied on the articular cartilage may play an important role in the pathogenesis of osteoarthritis. However, the mechanism of chondrocyte mechanotransduction is not fully understood. Previous study showed that IL-1β released from OA cartilage and the downstream effectors of IL-1β contributed to osteoarthritis progression. Transient Receptor Potential Vanilloid 4 (TRPV4) is a calcium-permeable membrane cation channel that plays a critical regulatory role in the development and maintenance of articular cartilage. We hypothesized that the expression of the IL-1 receptor 1 (IL-1R1) on the surface of chondrocytes would be stimulated by compressive mechanical stress, and subsequent increment of IL-1 susceptibility would be implicated in the OA pathology. We successfully made a three-dimensional (3D) cartilage constructs and maintained it in a cyclic load bioreactor in order to examine chondrocyte responses to the mechanical stress. The purpose of this study was to analyze mechanical stress-induced IL-1R1 expression in 3D-cultured chondrocytes, and the effects of TRPV4 channel regulation on IL-1R1 expression.

Methods: Mouse embryonal carcinoma-derived clonal cell line (ATDC5 cells) was cultured in alginate beads with the growth medium for 6 days. The cells were seeded within collagen gels and type-I collagen scaffold, which enabled ATDC5 cells to maintain chondrogenic phenotype in 3D environment. Histologically, chondrocytes presented with round shape, and mostly synthesized cartilage matrix. The mRNA expression of type-II collagen was enhanced by addition of BMP-2 (Fig.1). Cyclic compressive loading of 40 kPa at 0.5Hz was applied to the 3D cartilage constructs using a cyclic load bioreactor for 3 hours. Thereafter, ADAMTS4 and IL-1R1 mRNA expressions were measured in real-time PCR 6 hours after the cyclic loading. The effects of subtle amount of IL-1β (1pg/ml) was determined with or without compressive stress, and the effects of TRPV4 agonist/antagonist on IL-1-induced ADAMTS4 and MMP3 were determined. Experimental OA was made in IL-1 receptor knockout mice and wild type mice as controls, using techniques of destabilization of the medial meniscus (DMM). OA progression in knee joints was evaluated with Osteoarthritis Research Society International (OARSI) score at 8 weeks postoperatively.

Results: The mRNA levels of ADAMTS4 and IL-1R1 were substantially increased by the excessive compressive stress. Compressive stress plus IL-1β (1pg/ml) upregulated ADAMTS4 and IL-1R1 expressions by 3-fold and 8-fold, respectively, but IL-1β alone failed to do so (Fig.2). TRPV4 agonist suppressed upregulation of ADAMTS4 and IL-1R1 mRNA levels by cyclic compressive stress, conversely, TRPV4 antagonist rather accelerated these mRNA expressions (Fig.3). IL-1 receptor knockout mice exhibited reduced cartilage degradation in DMM-induced experimental OA (Fig.4).

Conclusions: The present study introduced the compressive stress which may more closely mimic physiological condition of articular joint. ATDC5 cells were differentiated toward and maintained mature chondrocyte phenotype by using alginate beads. In normal cartilage tissue, the capacity of chondrocytes to produce active IL-1β remains highly controversial, because compressive stress has not been reported to be capable of triggering IL-1β production by the chondrocytes. In chondrogenic 3D environment with excessive compressive stress, the cells gained IL-1 susceptibility. In this context, the cells could produce ADAMTS4 and MMPs in response to subtle level of IL-1β derived from synovia or cartilage itself, whereas compressive stress activated TRPV4, which in turn downregulated expression of IL-1R1 and control IL-1 susceptibility to maintain cartilage homeostasis. These findings in this study are corroborated with the past insight that TRPV4 contributes to maintain cartilage homeostasis by serving as a mechano- and osmosensor in articular chondrocytes. TRPV4 channel regulation is one of the most important mechanosensor that controls IL-1 susceptibility and prevents development of OA, when excessive mechanical stress is applied on the articular cartilage.

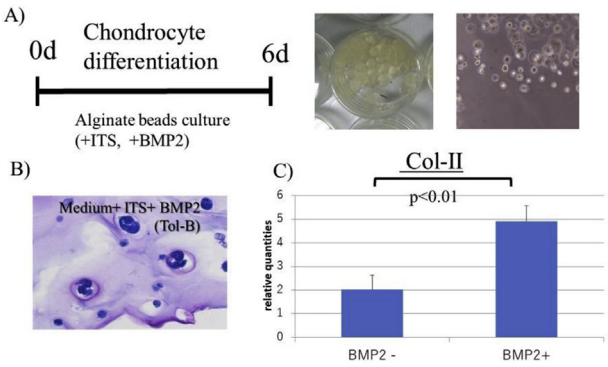

Fig.1 A) Alginate beads culture B) Toluidine blue staining showed increased cartilage matrix synthesis after the alginate beads culture. C) The mRNA expression of collagen typeII increased by addition of BMP-2.

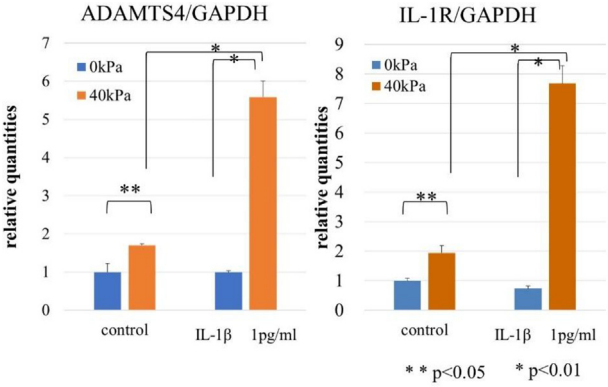

Fig.2 Compressive stress augments IL-1β susceptibility in 3D-cultured chondrocytes: The mRNA levels of ADAMTS4 and IL-1R1 were substantially increased by the excessive compressive stress. Compressive stress plus IL-1beta upregulated ADAMTS4 and IL-1R1 expressions, but IL-1beta alone failed to do so.

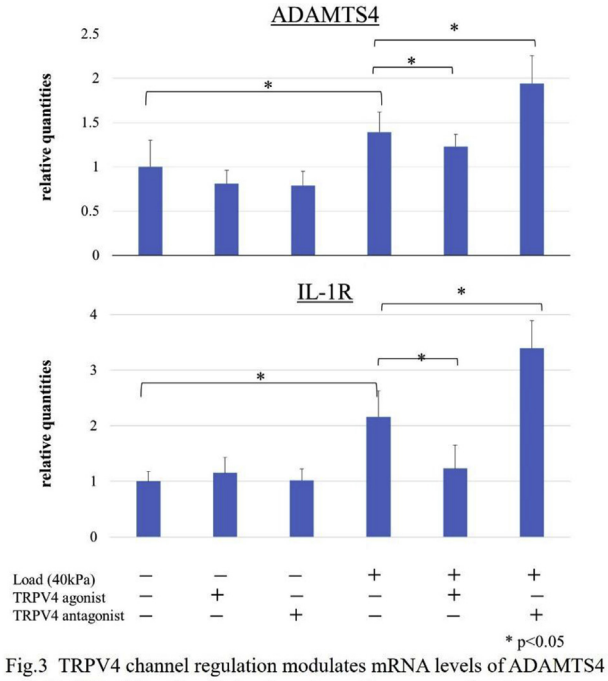

Fig.3 TRPV4 channel regulation modulates mRNA levels of ADAMTS4 and IL-1R in 3D-cultured chondrocytes under compressive stress: TRPV4 agonist suppressed upregulation of ADAMTS4 and IL-1R1 mRNAs by cyclic compressive stress, conversely, TRPV4 antagonist rather accelerated these mRNA expressions.

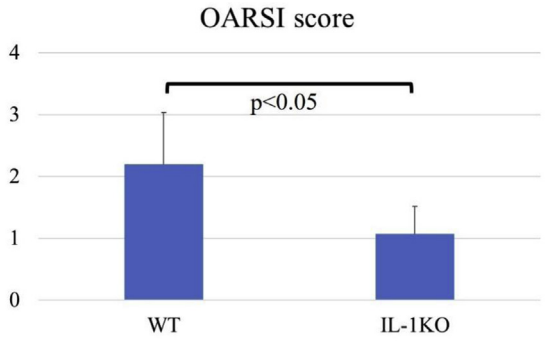

Fig.4 Cartilage degeneration was assessed using the OARSI histological scoring system in WT and IL-1R KO mice 8 weeks after DMM surgery.

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CORRELATION OF SEVERITY OF PRIMARY KNEE OSTEOARTHRITIS WITH THE LIPID PEROXIDATION MARKER IN SYNOVIAL FLUID

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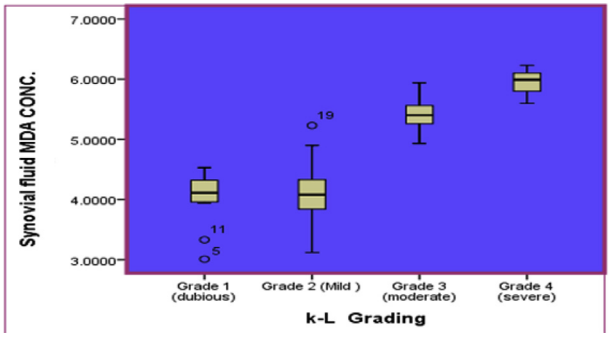
Purpose: The purpose of our study was to find out the correlation between the severity of primary knee osteoarthritis (OA) with the lipid peroxidation marker Malondialdehyde (MDA) in synovial fluid.
Methods: I. STUDY DESIGN A hospital based cross sectional study was conducted on patients attending the Outpatient Department of Orthopaedics of SGRRIM& HS for a period of one year from Jan. 2016 to Dec. 2016. A total no. of 50 patients (26 F +24 M) of primary knee osteoarthritis in the age group of 45-90 years were selected randomly for the study. Inclusion criteria was age equal or greater than 45 years, clinicoradiologically confirmed cases of knee osteoarthritis, patients with acute osteoarthritis symptoms (knee effusion), patients for intra-articular pharmacological injection therapy and patients admitted for knee replacement and arthroscopic lavage. Exclusion criteria was age less than 45 year and more than 90 year, patients with surgery around the same joint in the past, patients with inflammatory joint disease, patients on steroids or long term medications, patients with pain following trauma, serious liver, kidney or cardiac disorder, Other systemic diseases that might cause an increased Oxidative stress. **II. DIAGNOSTIC CRITERIA** ACR (AMERICAN COLLEGE OF RHEUMATOLOGY) for the diagnosis of knee Osteoarthritis. VISUAL ANALOG SCALE was used for scoring the intensity of pain. KELLGREN-LAWRENCE (K-L) RADIOGRAPHIC RATING SYSTEM for grading of KOA. **III. METHODOLOGY** Synovial fluid MDA was measured as an index of synovial fluid lipid peroxidation. All the 50 subjects underwent a biochemical analysis of Synovial fluid MDA that was estimated by Thiobarbituric acid (TBA) reaction described by Dahle's L.K. (1962) and used for tissue fluid (synovial fluid) spectrophotometric assay. Knee joint radiographs were evaluated with the Kellgren-Lawrence grading scale. Furthermore grading of knee OA was correlated with oxidative stress parameters; synovial fluid MDA levels to find out possible association between the oxidative stress induced damage and the disease progression.
Results: Synovial fluid MDA levels of grade 1, 2, 3 and 4 were 3.94±0.4, 4.26±0.47, 5.38±0.20 and 5.96±0.17 respectively. Synovial fluid MDA mean levels were increased with severity of Knee osteoarthritis (K-L grading) that was strongly statistically significant ($p < .001$). The mean of MDA level were unequal according to a one way ANOVA, $F(3, 46) = 76.100$, $p<0.0001$. Pairwise comparison of the mean using Tukey's Honestly Significant Difference procedure indicated that grade 3(M 5.10) & 4(M 5.93) had significant comparisons: subjects in the grade 3 and 4 reported that the MDA levels were significantly ($p <0.0001$) higher than subjects in the grade 1(M 3.94) and grade 2(M 4.26). Grade 1 and 2 comparison was not significant ($p=0.299$). **BOX-WHISKER PLOT** showing distribution of synovial MDA and Kellgren-Lawrence grade. The horizontal bars inside the boxes signify median values, the limit of the boxes denote the 25th and 75th percentiles and the upper and lower whiskers, represent the range=n of samples in each group.



Conclusions: Synovial MDA showed positive correlation with Kellgren-Lawrence grading. There was a significant positive correlation between lipid peroxidation MDA level and the severity of the disease process as indicated by the radiological grading. So, synovial MDA could be used as a marker to assess the disease severity of osteoarthritis. Future work in this area will provide a clearer picture to use synovial fluid MDA level as an early marker for measuring oxidative stress in knee joint instead of serum markers. We concluded that oxidative stress as indicated by synovial fluid MDA levels, plays a important role in the aetiopathogenesis of Osteoarthritis. Multicenter placebo-controlled trials focusing on the effect of antioxidant supplementation on synovial fluid MDA levels can provide further insight on this subjects.

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PIEZO1 EXPRESSION IS INCREASED IN RESPONSE TO NON-INVASIVE IMPACT OF MOUSE KNEE JOINT

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Purpose: Cartilage damage brought on by sudden impact and traumatic injury to the joint is a leading cause of post-traumatic osteoarthritis (PtOA). Damage to the cartilage leaves chondrocytes in their catabolic, hypertrophic state. This damage cannot be due to the avascular and aneural environment. As there are currently no disease-modifying drugs available for PtOA, damage is accumulated within the joint, often resulting in the need for early joint replacement which exerts a huge burden on healthcare services. The role of mechanosensitive ion channels in chondrocyte regulation has recently been highlighted in the field, specifically their over-activation in response to impact. We have recently demonstrated the pro-survival effect of silencing Piezo1 in chondrocytes responding to a pro-apoptotic environment. However, the expression profile of Piezo1 in the cartilage in response to impact remains unknown. In this study, we use an established method of non-invasive in vivo impact to investigate the expression of Piezo1 in response to varying levels of impact.
Methods: C57/Bl6 mice were subjected to an established cyclic loading method over a two-week period. The right hind limb was held with 2N force into a non-invasive loading clamp. Peak loads of 2N (control), 9N (high impact), or 11N (destructive impact) were applied for 0.05s in 9.9s cycles, with 40 cycles of this performed on the joint three times a week for two weeks. Mice were permitted to walk as normal for a further six weeks without further loading, and sacrificed on the eighth week. Right

MEAN LEVEL OF MDA ACCORDING TO K-L SEVERITY GRADING OF KNEE OSTEOARTHRITIS

PARAMETER	K-L grade 1 (dubious)	K-l grade 2 (mild)	K-L grade 3 (moderate)	K-L grade 4 (severe)	total average Mean	F	'p'
MDA (**μM/L**)	3.94±0.4	4.26±0.47	5.38±0.20	5.96±0.17	4.86±0.87	76.6	0.0001