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ABSTRACTS

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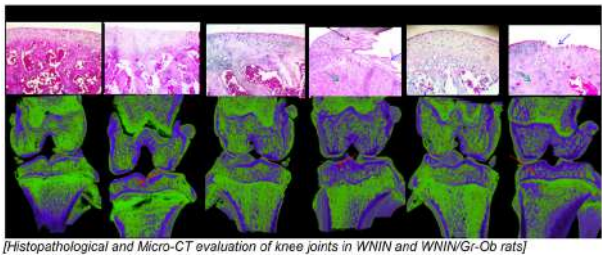
We therefore aim to explore the potential application(s) of these Muts model in OA research to study metabolic and structural alterations (knee joints) with age akin to human OA.
Methods: Knee joints were harvested from female Muts aged 3, 6 and 9 months old and evaluated for OA-like changes using radiography, micro-CT and histopathology and compared against their age-matched Wistar controls (WNIN).
Results: Radiographic assessment showed ossification of soft tissues, osteophyte formation (6 months), subchondral sclerosis and bone cyst (9 months) in Muts. Micro-CT studies revealed significant reduction in subchondral trabecular bone porosity (red arrow) in Muts (6 and 9 months) implying subchondral sclerosis. Histopathological evaluation showed cartilage degeneration (blue arrow), subchondral sclerosis (green arrow), osteophyte (black arrow), bone cyst in Muts (6 and 9 months)—hallmark features of human OA (Ethical clearance obtained).
Conclusions: Our findings advocate for the potential application of these Muts as a befitting rat model in the field of OA research en route the 'natural progression' and spontaneous generation of OA-like changes correlating with human OA.
Obesity, Osteoarthritis, WNIN/Gr-Ob, Animal model, Metabolic Syndrome



[Histopathological and Micro-CT evaluation of knee joints in WNIN and WNIN/Gr-Ob rats]

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The SoxC family transcription factor Sox4 plays a role in osteoarthritis onset by up-regulating ADAMTS4 and ADAMTS5

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Objectives: Osteoarthritisis a common disease affecting joint cartilages. The molecular pathogenesis of osteoarthritis has not been fully understood. Therefore, early diagnostic markers and effective therapeutic agents for osteoarthritis have not been developed. In order to understand the molecular mechanisms of osteoarthritis onset, we attempted to identify transcription factors involved in osteoarthritis onset.
Methods: Superficial zone (SFZ) cells of articular cartilage were treated with retinoic acid (RA) exerting catabolism in cartilage, and subsequently microarray analysis was performed to identify the transcription factors. The identified transcription factors were introduced into C3H10T1/2 cells and SW1353 cells, and the effects on ADAMTS4 and ADAMTS5 expressions were analyzed by RT-qPCR, luciferase and ChIP analyses. Additionally, the effects of the transcription factors were assessed in mouse articular cartilage organ culture system. Finally, expressions of the identified transcription factors were determined in human OA cartilages by performing RT-qPCR..
Results: Microarray analysis revealed that Sox4, a SoxC transcription factor member, is increased about sixfold by RA treatment in SFZ cells. Overexpression of Sox4 or Sox11, another SoxC transcription family member, induced Adamts4 and Adamts5 expression. Luciferase and ChIP analyses also indicated that Sox4 bound to the ADAMTS4 and ADAMTS5 gene promoters and stimulated these gene promoter activities. Sox11, evoked similar effects. Furthermore, introduction of Sox4 adenovirus into the femoral head cartilages showed an increase in Adamts5 expression and articular cartilage destruction. Most importantly, in articular cartilages from human OA patients, mRNA expression of Sox4 and Sox11 was increased along with the degree of joint destruction severity of cartilage degeneration.
Discussion: Sox4 and Sox11 are involved in pathogenesis of osteoarthritis through regulating ADAMTS4 and ADAMTS5 expressions.

P188

Association of T-cell and B-cell aberrancies with disease activity in rheumatoid arthritis, psoriatic arthritis and ankylosing spondylitis

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Background: Despite the known autoimmune pathogenesis, the association of the specific T-cell and B-cell aberrancies for a particular rheumatic disease (rheumatoid arthritis (RA), ankylosing spondylitis (AS) and psoriatic arthritis (PsA)) have not yet been fully elucidated.
Methods: Mononuclear cells were isolated from peripheral blood of CTRL (n = 43), RA (n = 42), AS (n = 43) and PsA (n = 42) patients (upon Ethical approval and informed consent). Using flow-cytometry, we identified subpopulations of T-cells: Th1 (CD3 + CD4 + CCR4-CCR6-), Th2 (CD3 + CD4 + CCR4 + CCR6-), Th17 (CD3 + CD4 + CCR4 + CCR6 +), Tc (CD3 + CD8 +); B-cells: naïve (CD19 + IgD + CD27-), unswitched memory (UM, CD19 + IgD + CD27 +), class-switched memory (CSM, CD19 + IgD-CD27 +), double-negative memory (DNM, CD19 + IgD-CD27-) and plasmablasts (CD19 + IgD-CD27 + CD38 +); in addition to activation (CD86) and maturation (CD32) markers, and correlated their frequencies with the disease activity: DAS28 (Disease Activity Index including 28-joint count), ASDAS (AS Disease Activity Score), BASDAI (Bath AS Disease Activity Index). Functional tests included mitogen (PMA/ionomycin)-induced lymphocyte proliferation (Cell Proliferation Dye eFluor670) and activation (CD69). In addition, culture supernatants were used to test the osteoclastogenic response of peripheral monocytes stimulated by RANKL and M-CSF.
Results: Th1 were increased in RA in comparison to CTRL ($p = 0.03$). CD32 was increased on naïve ($p = 0.042$), UM ($p = 0.042$), DNM ($p = 0.07$) from AS, and plasmablasts from RA ($p = 0.026$). In RA, Th17 correlated with aCCP (rho = 0.898, $p = 0.002$) and RF (rho = 0.746, $p = 0.021$), DNM B-cell with RF (rho = 0.854, $p = 0.007$), and CD32 + naïve B-cell with DAS28 (rho = 0.899, $p = 0.015$). In AS, CD32 + DNM B-cells correlated with BASDAI (rho = 0.721, $p = 0.019$) and ASDAS (rho = 0.794,

p = 0.006). In PsA, CD86 + CSM B-cells correlated with DAPSA (rho = 0.786, p = 0.021) and DAS28 (rho = 0.757, p = 0.049). In AS, lymphocytes showed increased activation and proliferation. Finally, T-cell supernatant of RA patients enhanced osteoclastogenesis in vitro (84[52–90] in RA vs. 56[42–62] in CTRL).
Conclusions: The association of the specific lymphocyte subset with a particular rheumatic disease may indicate the role in arthritis pathogenesis and possible usage as a disease marker.

P189

Effects of a ketogenic diet on the progression of osteoarthritis in obese mice

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Osteoarthritis (OA) is prevalent among the obese population, where low-grade metabolic inflammation and a damaging adipokine profile accompany weight-related joint overload.

Histone deacetylase (HDAC) inhibitors have shown the ability to slow-down OA progression in vitro and in vivo, while downregulating protease expression.

beta-hydroxy-butyrate (BHB) is a ketone body whose plasma level increases following a low carb/high fat ketogenic diet (KD). BHB affects histone modifications and gene expression in vitro and in vivo, acting as an HDAC inhibitor.

As KD could simultaneously induce weight-loss, decrease metabolic inflammation and increase BHB circulating levels, it might be beneficial in obesity-linked OA treatment. To directly test this hypothesis, we evaluated the impact of KD in a murine model combining obesity and osteoarthritis (12127-2017110911058255v2).

Obese mice on a high fat diet (HFD) received knee medial meniscus destabilization at week 16 to induce OA, then were fed one of three diets ad libitum: HFD; KD; Control Diet (CD). 8 weeks later animals were killed and organs collected.

BHB levels increased tenfold in KD group only (0.1 mM for all groups before diet switch, 1.6 mM upon switch to KD; $p < 0{,}001$). Glycemia remained high in HFD (10.2 mM), but decreased in CD (8.2 mM) and even more in KD (6 mM)($p < 0{,}001$). HFD mice continued to gain weight, while CD and KD lost weight. The Kondziela test showed no variation in muscle strength over the 8-week treatment in CD and KD and lower performance in HFD group. MMP13 expression in cartilage of CD was lower than in HFD and even lower in KD (twofold, p = 0.005).

Our results validated the efficacy of KD to induce a raise in BHB levels and weight loss in obese OA mice, without a negative impact on muscle function. We can now further appraise its effects on osteoarthritis in this model.
Keywords: Osteoarthritis, obesity, ketogenic diet, BHB, epigenetics

P191

Development of a novel biologic agent for treating RA

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Rheumatoid Arthritis (RA) is a chronic inflammatory autoimmune disease characterized by serious synovitis accompanying cartilage and bone erosion. Chimeric protein of the extracellular domain of CTLA-4 with IgG1 Fc region (CTLA-4Ig) is a unique biologic clinically used as abatacept. Although CTLA-4Ig shows efficacy in RA patients showing poor responses to a neutralizing antibody for an inflammatory cytokine, the proportion of clinical remission achievement is not yet high. Prevention of joint bone destruction is also not enough. Here, we constructed a chimeric protein construct of CTLA-4Ig fused with anti-RANKL scFv at the C-terminal (CTLA-4Ig ~ scFv) to improve the pharmacological effect of CTLA-4Ig. Anti-RANKL scFv was obtained by screening a phage library displaying randomized scFv. In vitro assay using ELISA confirmed that the binding affinity of CTLA-4Ig ~ scFv to CD86 extracellular domain is about 5 nM, which is comparable with that of CTLA-4Ig. The binding affinity of CTLA-4Ig ~ scFv to RANKL extracellular domain was about 1 nM. Then, we compared the curative effect of CTLA-4Ig ~ scFv with that of CTLA-4Ig using collagen-induced arthritis (CIA) mouse model. Mice with all the paws starting to swell at day 9 after the second immunization were divided randomly to 3 treatment groups; CTLA-4Ig (n = 18), CTLA-4Ig ~ scFv (n = 19) and vehicle (n = 18). Each mouse was administered with CTLA-4Ig 200 μg/day, CTLA-4Ig ~ scFv 200 μg/day or vehicle from day 10 to day 16. At day 17, mice were sacrificed and frozen thin-sections of hindleg knee joints were prepared. Histological assessments of cartilage and bone destruction were performed. CTLA-4Ig ~ scFv significantly reduced ($p < 0.01$) mature osteoclasts formation compared to the vehicle group, while the effect of CTLA-4Ig was not significant. As for the cartilage erosion, tendency of reduction in CTLA-4Ig ~ scFv treated group was observed but the difference was not significant. Collectively, introduction of anti-RANKL scFv to CTLA-4Ig improved the curative effect in CIA mouse model.
Keywords: RA, biologics, RANKL

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Metatarsal open fracture model in rats for in vivo investigation of secondary bone healing

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Objectives: The open and close fracture model frequently utilized for laboratory investigation are associated with varying degree of complications ranging from high degree of fracture comminution to severe associated soft tissue injury which interfere with fracture healing. This study aimed at developing an improved quality and reproducibility of an experimental open fracture model in rat metatarsal with minimal complications.
Methods: Standard open mid shaft transverse metatarsal fracture was produced with bone cutting forceps in 28 rats. The study was approved by the Institutional Animal Care and Used Committee of