Session: Disease & Treatment — Osteoarthritis

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REGULATION OF GUT COMMENSAL FLORA ATTENUATES OSTEOARTHRITIS THROUGH INTERLEUKIN 17

Dongsheng Yu, Jun Dai, Hongwei Ouyang, Li Dak Sum, Yip Yio Chin
*Center for Stem Cell and Regenerative Medicine, Zhejiang University School of Medicine, China*

**Objective:** To examine the role of interleukin 17 (IL-17) in cartilage destruction during the progression of osteoarthritis (OA), and determine whether regulation of gut commensal flora can attenuate osteoarthritis through IL-17.
**Methods:** OA and normal synovium from mice and human were subjected to immunohistochemistry (IHC) of IL-17 expression. Experimental osteoarthritis was created by destabilization of the medial meniscus (DMM) surgery in MINK1 knock out mice and different conditioned or vancomycin-treated C57BL/6 mice. OA development, gut flora and IL-17 expression were evaluated using SO staining, 16sRNA sequence, SEM, IHC and flow cytometry. Primary cultured mouse chondrocytes were treated with recombinant IL-17.
**Results:** We found that there is an OA subgroup with enhanced IL-17 expression in synovium. IL-17 increased activities of MMP13 and decreased matrix synthesis in chondrocytes. Intra-articular injection of IL-17 accelerated articular cartilage degradation and high IL-17 expression in MINK1 knock-out mice also accelerated OA development. Interestingly, C57BL/6 mice obtained from different microbial conditions displayed difference in IL-17 expression and OA progress. Subsequent 16sRNA sequence analysis demonstrated different composition of gut microbe, including one special bacteria called SFB in the Firmicutes. Regulation of gut microflora by antibiotic attenuated osteoarthritis progress in DMM mice and this alleviation was reversed after colonization of non-SPF mouse fecal microflora.
**Conclusion:** Gut microbe is involved in OA pathology by modulating proinflammatory cytokine IL-17 activity. Regulation of gut microflora maybe a potential therapy in a high IL-17 expression subgroup of OA patients.
http://dx.doi.org/10.1016/j.jot.2016.06.185

Session: Regenerative Medicine — Tissue Engineering

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DIRECTING TENOGENESIS OF STEM CELLS WITH SMALL MOLECULE-BASED NANOFIBERS

Can Zhang, Erchen Zhang, Xiao Chen, Hongwei Ouyang, Li Dak Sum, Yip Yio Chin
*Center for Stem Cells and Regenerative Medicine School of Medicine, Zhejiang University, China*

**Objective:** Epigenetic studies on stem cells have indicated that specific histone alterations and DNA methylation play essential roles in cell differentiation. Different from chemical cue, topographical cue is often used to guide cells to orientate, which is also important in tissue development. Here, we examined the effect of small molecule modified scaffolds to direct tendon progenitor/stem cells (TSPCs) differentiate into tendons *in vitro* and *in vivo*.
**Methods:** The histone deacetylation inhibitor TSA were combined on the aligned nanofibers. TSPCs obtained from Scx-GFP mouse were seeded onto the aligned and TSA modified aligned nanofibers. The gene expression of tendon related genes were examined. Confocal observation was further performed. A rat Achilles tendon defect model was created and implanted with aligned or TSA modified aligned scaffold *in vivo*. The morphology of repaired tissues were analyzed by histological examination and transmission electron microscope. The amount of deposited collagen was quantified using a collagen quantitative assay kit. Mechanical testing was performed for mechanical properties.
**Results:** TSA could release from the scaffold for 24h. Gene expression profile showed that TSA modified align group upregulated the tendon related genes compared to the align group. Scx-GFP as a report system also showed that TSPCs cultured on the TSA-A (i.e. TSA modified aligned scaffold) scaffold maintain strong GFP fluorescence and cells exhibited spindle-shaped morphology, in contrast to weak GFP fluorescence on A (i.e. TSA modified aligned scaffold) scaffold. In rat Achilles tendon repair model, TSA-A treated tendon had superior structural and mechanical properties than A-treated tendon. These findings present a strategy combining well-aligned fiber scaffold with small molecule for tendon regeneration and may assist in clinical regenerative medicine to treat tendon diseases.
**Conclusion:** TSA modified scaffold promotes tendon regeneration by changing the epigenetic and promoting tendon related genes expression both *in vitro* and *in vivo*.
http://dx.doi.org/10.1016/j.jot.2016.06.186

Session: Disease & Treatment — Osteoporosis

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PREVENTIVE EFFECTS OF POLYGONUM MULTIFLORUM ON GLUCOCORTICOID-INDUCED OSTEOPOROSIS IN MALE RATS

Jingkai Wu [a,b], Jin Li [a,b], Yongjie Yu [a,b], Manru Zhou [a,b], Weimin Yao [a,c], Yajun Yang [a,b], Yuyu Liu [a,b]
[a]*Department of Pharmacology, Guangdong Medical University, China*
[b]*Guangdong Key Laboratory for Research and Development of Natural Drugs, Guangdong Medical University, Zhanjiang, Guangdong 524023, China*
[c]*Affiliated Hospital of Guangdong Medical University, China*

**Objective:** To investigate the effects of PM on glucocorticoid-induced lumbar spine and femur microstructure in male rats.
**Methods:** The crude extract of the PM was extracted by 75% ethanol, 30% ethanol extract of the PM was purified and enriched by the D-101 macro-resin column from the crude extracts, and then determined by HPLC. Male Sprague-Dawley rats (190—230 g, n=180) were randomly divided into eighteen cages, which for nine groups: control, prednisone, prednisone plus calcitriol (CAL), prednisone plus 30% ethanol extract of the PM (H, M, L) dose, prednisone plus crude extract of the PM (H, M, L) dose. Prednisone was administrated orally for 21 weeks in the male rat model of osteoporosis. Meanwhile, PM and extracts mentioned above were administrated in male rats exposed to prednisone. The right femur and fourth LV were collected for the measurement of three-dimensional microarchitecture of micro-CT analysis, detect bone microstructure and bone mineral density, and other related parameters
**Results:** The content of 2, 3, 5, 4′-tetrahydroxystilbene-2-O-β-glucoside (TSG) and combined anthraquinone (CAQ) were 9.20% and 0.15% in the sample of 30% ethanol extract of the PM, and 2.23% and 0.03% in the sample of crude extract of the PM, respectively. Male rats exposed to prednisone exhibited the deteriorated microarchitecture, low BMD, decreased BV/TV. While 30% ethanol extract of the PM (M, L) and crude extract of the PM (H) counteracted the alterations of skeletal characteristics induced by prednisone in male rats, as well as CAL.
**Conclusions:** 30% ethanol extract of the PM (M, L) and crude extract of the PM (H) dose groups can exert a protective influence on bone tissue in GIO male rats.
http://dx.doi.org/10.1016/j.jot.2016.06.187

Session: Disease & Treatment — Cartilage Damage & Repair

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SMALL MOLECULE KARTOGENIN PROMOTES CARTILAGE REGENERATION THROUGH ACTIVATING IL-6-BASED MESENCHYMAL STEM CELL PROLIFERATION

Guangqian Zhou, Xiaolin Li, Tao Liu, Tuanhui Chen, Jun Li, Yuhong Liang
*The Centre for Anti-ageing and Regenerative Medicine of Shenzhen University, China*

**Introduction:** Deregulation of the endogenous stem cells in cartilage tissues is considered to be a part of pathogenesis of osteoarthritis. Kartogenin (KGN) has been reported to exert stimulatory effects on chondrogenesis of mesenchymal stem cells by binding to filamin A that subsequently releases and activates transcription factor CBFβ and by up-regulate the Smad2/3 phosphosphorylation. In this study, we report that KGN is capable of stimulating the proliferation of primary cartilage derived progenitor cells (CPCs).
**Subjects and Methods:** Cartilage derived progenitor cells (CPC) were isolated from rat articular cartilage tissues and KGN stimulated-CPC proliferation was confirmed both *in vitro* and *in vivo*. Slow-cycling cells within cartilage were labeled and counted using RrdU and it specific antibodies. Cell cycle was analyzed using Flow Cytometry and RNA-seq was performed on CPC co-incubated with KGN for 5 days. IL-6 and Stat3 phosporylation were detected with ELISA kit and Western blotting. Cartilage repair/regeneration by oral administration and/or intra-articular injection of KGN was observed in rat knee joint injury models.
**Results:** Our data shows that following 10 mM KGN treatment for a week, the percentage of G2-M phase cells in mitosis reached 9.6%, nearly twice of the control group, which was companied with the doubled total cell number. In the meanwhile, even after 4 weeks stimulation with KGN, Cells were proved to remain the expression of mesenchymal stem cell markers CD90 (93%) and CD105 (98%). As a control, no significant number change was observed in mature human T lymphocyte treated with KGN in the similar manner. Whole RNA-sequencing analysis of KGN-stimulated MSCs showed that significant expression changes of about 20 cell cycle-related genes upon KGN treatment for 72 hours. Among a number of genes found to be significantly changed by the KGN treatment are IL-6 and its receptor Gp130, which reach as much as 6 fold increase

than the control. We further confirmed that the IL-6 level was significantly increased by KGN in both cytoplasm and supernatant media of CPC culture. We further demonstrated that the phosphosphorylation of Stat-3 was up-regulated at the same time. In-articular injection of KGN were also found to increase the number of BrdU-labeled slow-cycling cells. *In vivo* experimental evidences of the increased thickness in articular cartilage with KGN treatment was further confirmed in the rat model that were induced to have knee joint injury. IHC staining of the KGN treatment group showed up-regulate of Stat-3 phosphosphorylation in KGN-treated cartilage.
**Discussion and Conclusions:** Based on the *in vivo* and *in vitro* data, we propose that KGN may improve the number of endogenous cartilage stem cells by promoting their self-renewal in situ while inducing chondrogenesis and its use in cartilage regeneration and repair is definitely worth of further explored.
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http://dx.doi.org/10.1016/j.jot.2016.06.188

## Session: Disease & Treatment — Cartilage Damage & Repair

### 346
### Sox9 SUPPRESSES THE HYPERTROPHY OF CHONDROCYTES BY INHIBITING wnt/β-CATENIN SIGNALING PATHWAY

Kun Chen, Peng Cheng, Min Zhou, Yuting Wang, Anmin Chen
*Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, China*

**Background:** Due to its low cell density and low proliferative activity, and its avascular character, articular cartilage is virtually unable to regenerate or self-heal upon injury or degeneration by common diseases such as osteoarthritis. Scientists have tried many ways to inhibit the degeneration of chondrocytes or repair the cartilage defect. However, there is still no effective solutions to these problems. Sox9 has been reported as an important transcription factor during the development of cartilage. We have thought to overexpress Sox9 to see its role in chondrocytes.
**Subjects and Methods:** The lentivirus vector was constructed to overexpress the Sox9 in the chondrocytes. And the plasmids contain the full Sox9 and ΔC, ΔN Sxo9 were constructed. These lentivirus and plasmids were transfected to the articular chondrocytes of SD rats. Then western-blot, immunofluorescence, and Alician blue staining were done to test the change of chondrocytes in order to find the role of Sox9 in the chondrocytes.
**Results:** When chondrocytes were transfected with lentivirus to overexpress Sox9, Western-blot showed that the expression of COL2a was upregulated and the expression of COL10 and RUNX2 was downregulated. Meanwhile, the β-catenin and p-GSK-3β were downregulated and the p-β-catenin and GSK-3β were upregulated. And these changes can be relieved when Gsk inhibitor Licl and SB were used. The Alician blue staining showed that when used wnt/β-catenin activator Wnt3A to treat chondrocytes was weaker than the control group and the Sox9 overexpression group was stronger than the control group. These results indicate that the overexpression of Sox9 in chondrocytes can suppress the expression of RUNX2 and COL10 in chondrocytes and suppress the hypertrophy of chondrocytes. And Sox9 acts through inhibiting wnt/β-catenin signaling pathway by activating the GSK-3β. Furthermore, when chondrocytes were transfected with plasmids of Sox9, ΔC and ΔN Sxo9, the Western-blot showed that in Sox9, ΔC Sox9 and ΔN Sxo9 groups, β-catenin COL10 and RUNX2 were downregulated, while p-β-catenin, GSK-3β and COL2a were upregulated. And the ΔC group was changed more significantly than the ΔN Sox9 group. These results suggest that the ΔC part of Sox9 may play the main role in the function of Sox9 in chondrocytes.
**Discussion and Conclusion:** According to the results, Sox9 can suppress the hypertrophy of chondrocytes. And it acts through inhibiting the phosphorylation of GSK-3β, and then inhibit the wnt/β-catenin signaling pathway. This makes Sox9 a promising way to treat the hypertrophy of chondrocytes.
http://dx.doi.org/10.1016/j.jot.2016.06.189

## Session: Regenerative Medicine — Stem Cells & Progenitors

### 395
### STUDY ON THE EFFECT OF PKC-ζ IN SDF-1α/CXCR4 AXIS INDUCED MIGRATION OF RAT BONE MARROW MESENCHYMAL STEM CELLS

Hui Liu, Yonghui Dong, Peng Cheng, Kun Chen, Anmin Chen, Hui Huang
*Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, China*

**Background:** Bone mesenchymal stem cells (BMSCs) are bone marrow-derived cells with multipotent differentiation capability that can differentiate into osteoblasts, adipocytes and chondrocytes. BMSCs have generated a lot of interest because of their potential use in regenerative medicine and tissue engineering. A great deal of studies have shown that the SDF-1α is critical for mesenchymal stem cells migration to injured tissue through interaction with its cognate CXCR4 on the surface of these cells. However, migration signaling pathways required for homing and recruitment of BMSCs are not fully understood. Due to the known effects of PKC-ζ on the migration of cancers and CD34+ progenitor cells, we have sought to investigate the relationship between PKC-ζ and SDF-1α/CXCR4 axis induced migration of BMSCs.
**Subjects and Methods:** The rat BMSCs were cultured by using the whole bone marrow adherence method. The three-line differentiations of BMSCs was conducted and cell surface markers were identified by flow cytometry. Transwell migration assay was used to observe the migrating number of BMSCs that were treated with AMD3100 and PS-ζ in response to SDF-1α. Western blot analysis was used to analyze the phosphorylation of PKC-ζ of BMSCs upon SDF-1α and PS-ζ stimulation. Actin-Tracker Green was applied to illustrate morphology of cell cytoskeleton by staining of the F-actin. Laser scanning confocal microscopy was used to observe the polarization of BMSCs after SDF-1α stimulation.
**Results:** The BMSCs that were cultured in vitro had the potential of multiple differentiation into osteoblasts or adipocytes and highly expressed CD29 and CD90 except CD45.The migrating number of BMSCs increased gradually with the increase of SDF-1α concentration in vitro, and the amount of BMSCs in the filter membrane was approximately maximum when SDF-1α was 100ng/mL, but AMD3100 and PS-ζ were able to cut down the number of BMSCs in the filter membrane. SDF-1α increased the level of phosphorylation of PKC-ζ, while PS-ζ inhibited the phosphorylation of PKC-ζ. Laser confocal microscopy confirmed that the cytoskeleton of BMSCs treated with SDF-1α changes significantly, but AMD3100 and PS-ζ inhibited this effects.
**Discussion and Conclusion:** Our result suggests that SDF-1α induces the migration of BMSCs via affecting the phosphorylation of signaling protein PKC-ζ and changing the cytoskeleton of BMSCs. This may increase the number of transplanted BMSCs migration to injured tissue and organ to promote the rehabilitation.
http://dx.doi.org/10.1016/j.jot.2016.06.190

## Session: Others

### 402
### Notch1-ANTAGONISTIC APTAMER FOR CHONDROGENIC DIFFERENTIATION OF BONE MARROW STROMAL CELLS

Se-Young Jeong, Mi-Lan Kang, Ji-Eun Kim, Gun-Il Im
*Dongguk University Ilsan Hospital, South Korea*

**Introduction:** Aptamers are oligonucleotide or peptide molecules that bind to a specific target molecule such as small molecules, proteins, nucleic acids, and even cells, tissues and organisms. Aptamers exhibit significant advantages relative to protein therapeutics in terms of size, synthetic accessibility and modification by medicinal chemistry. During murine skeletal development, Notch-1 is strongly localized within the condensing mesenchyme during the early stages of chondrogenesis [1]. Notch signaling is implicated in the repression of chondrogenic differentiation of mesenchymal stem cell (MSC) [2]. The purpose of this study was to investigate the possibility of Notch1-antagonistic aptamer as nucleic acid therapeutics to induce chondrogenic differentiation of human bone marrow stromal cells (hBMSCs).
**Methods:** 1) Cytotoxicity test. hBMSCs ($1\times10^4$ cells/well on 96 well plate) were treated with three Notch1 aptamer clone candidates from 1 to 1000 nM for 7 days. On day 1, 4 and 7, MTT assay was performed. 2) Validation of Notch1-antagonistic aptamer to select Notch1-antagonistic aptamer, expression of Notch1 downstream target genes including Hes and Hey were investigated by RT-qPCR on hBMSCs treated with each of Notch1 aptamer candidate in DMEM/F12. 3) Median effective dose ($ED_{50}$). Pellets of hBMSCs ($2.5\times10^5$ cells, passage 3—5) were cultured in DMEM/F-12 supplemented with dexamethasone ($10^{-7}$ M), BSA (7.5% w/v), L-proline (50 μM), ascorbate-2-phosphate (50 μM), sodium pyruvate (1 mM) and ITS (1% v/v) containing different concentration of Notch1 aptamer (1, 10, 100 and 1000 nM). 4) *In vitro* chondrogenic differentiation. The hBMSCs ($2.5\times10^5$ cells, passage 3—5) were made by pellets and were cultured in chondrogenic medium containing Notch1-antagoistic aptamer (200 nM) from day 0 to 14. 5) Gene expression profiling with oligonucleotide microarrays. Expression profiling was performed by Illumina Expression Beadchip Analysis Service using Illumina HumanHT-12 v4 Expression BeadChip (Illumina, Inc., San Diego, CA, USA). Hierarchical clustering was performed complete linkage algorithm using GeneSpring GX 7.3.1 software (Agilent, Santa Clara, CA, USA).
**Results:** 1) Cytotoxicity test. The MTT data after exposure to the Notch1-antagonistic aptamer candidates from 1 to 1000 nM for 7 days showed no significant cytotoxicity. 2) Validation of Notch1-antagonistic aptamer. The gene expression of hes1 and hey1 significantly decreased in hBMSCs treated with Notch1-antagonistic aptamer candidates for 7 days compared to those of untreated hBMSCs. 3) Median effective dose. The gene expression of *COL2*, *SOX9* and *ACAN* significantly increased in hBMSCs treated with Notch1-antagonistic aptamer (100nM) for 14 days compared to those of untreated hBMSCs. 4) *In vitro* chondrogenic