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COMPLEX OSTEOCHONDRAL TISSUE ENGINEERING

H. Cheng [1], Y. Li [1], S. Chow [1], H. Li [1], M. Yuan [1], X. Lu [2], B.P. Chan [1]. [1] *The Univ. of Hong Kong, Hong Kong, China;* [2] *Univ. of Delaware, Newark, DE, USA*

Purpose: Proper zonal organization of cartilage is important for maintaining proper functions of joint tissues. Complex tissue engineering approaches recapitulating the cartilage zonal organization have great potential in treating cartilage injuries and diseases particularly those with compromised osteochondral interface. Here, we aim to develop a stem cell-based biomaterial-assisted tissue engineered osteochondral tissue with proper zonal organization and evaluate its efficacy by comparing with bone marrow stimulation and autograft in a rabbit osteochondral defect model.
Methods: Rabbit bone marrow mesenchymal stem cell were isolated and expanded before microencapsulating them in collagen biomaterials to form cell-matrix microspheres. The mesenchymal stem cells were induced to differentiate towards both the chondrogenic and the osteogenic lineages using chemically defined culture medium. The chondrogenic and the osteogenic micro-tissues so obtained were assembled to form an osteochondral tissue with proper zonal organization. A surgically induced osteochondral defect was created in the load-bearing femoral condyle of adult New Zealand rabbits before the autologous MSC-derived engineered osteochondral tissue was implanted. Osteochondral defect alone (bone marrow stimulation) and autograft were used as the control groups. The articular joint tissues were harvested at 1, 3 and 6 months post-operation for histological, biochemical and biomechanical analyses.
Results: The engineered osteochondral tissue group and the autograft group showed full volume filling immediately after operation. At 1 month post-operation, the engineered osteochondral group showed excellent regeneration outcomes with type II collagen and glycosaminoglycan-rich hyaline cartilage and with partial regeneration of the tide-mark while the defect in the bone marrow stimulation (natural healing) group was still a big hole slightly filled with fibrous tissues without cartilage matrix. The autograft group showed some remodeling but was also positive for cartilage matrix. The histological characteristics of the engineered osteochondral tissues were similar to that of the autograft, with full thickness hyaline cartilage and proper osteochondral zonal organization throughout the 6 months follow up period while the natural healing group showed some regeneration particularly at 3 months post-operation but the nature of the repaired cartilage was still fibrocartilage with mixed type I and II collagen matrix. Moreover, at 6 months post-operation, the natural healing group showed obvious cartilage thinning while the engineered osteochondral tissue group and the autograft group showed nice hyaline cartilage. The O'Driscoll histological score of knees showed that the engineered osteochondral tissue group was the highest among all groups and was statistically significantly different from the bone marrow stimulation group. Biochemical compositional analysis of the cartilage tissues in various groups showed that the glycosaminoglycan to hydroxyproline (marker for collagen) ratio of the engineered osteochondral tissue group was increasing over time and was statistically similar to that of the autograft group throughout the 6 months follow up period but was statistically different than the natural healing group. Biomechanically, the aggregate modulus and the permeability of the engineered osteochondral tissues were statistically similar to that in autograft throughout the 6 months follow up period and the values were even within the 95% confidence interval of the mechanical properties of uninjured (healthy) rabbit cartilage. The natural healing group showed significantly different (lower) biomechanical properties as compared with the other groups.
Conclusions: The engineered osteochondral tissue showed excellent regeneration outcomes histologically, biochemically and biomechanically and these outcomes were statistically comparable with the clinical gold standard the autograft group, throughout the 6 months follow up period. This work demonstrates the promising potential of using complex engineered osteochondral tissues for cartilage regeneration.

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LORECIVIVINT (SM04690), A POTENTIAL DISEASE-MODIFYING TREATMENT FOR KNEE OSTEOARTHRITIS, DEMONSTRATED CARTILAGE-PROTECTIVE EFFECTS ON HUMAN OSTEOARTHRITIC EXPLANTS

V. Deshmukh [1], S. Grogan [2], T. Seo [1], D. Bhat [1], W. Bugbee [2], D. D'Lima [2], Y. Yazici [1]. [1] *Samumed, San Diego, CA, USA;* [2] *Shiley Ctr. for Orthopedic Res., San Diego, CA, USA*

Purpose: Wnt pathway upregulation contributes to knee osteoarthritis (OA) through osteocyte differentiation, cartilage thinning, and inflammation. Lorecivivint (LOR; SM04690), a novel, small-molecule CLK/DRYK1A inhibitor, demonstrated disease-modifying potential for knee OA via Wnt pathway modulation in preclinical studies. Further studies were performed to evaluate cartilage-protective effects of LOR on human OA explants from total knee replacement (TKR) donors.
Methods: Knee joint tissue from 22 TKR donors was obtained. IRB approval was obtained from Scripps Health. Cartilage was scored using the Outerbridge classification system based on gross appearance (grade 1=least-damaged tissue, grade 4=most-damaged tissue). Cartilage explants (4 mm in diameter) with Outerbridge grades 2-3 were harvested and cultured for 48 hours to reach metabolic stability. These were treated with LOR (10 nM, 30 nM) or DMSO and stimulated with either IL-1β (10 ng/ml) or TNF-α (20 ng/ml) and oncostatin M (OM) (10 ng/ml) or left unstimulated. After 72 hours, supernatants and explants were collected. Gene expression of matrix metalloproteinases (MMP) 1, 3, and 13 were measured by qPCR and levels of MMP1, MMP3, MMP13, ADAMTS4 (a disintegrin and metalloproteinase with thrombospondin motifs), and ADAMTS5 protein were measured in supernatants by ELISA. Glycosaminoglycan (GAG) and nitric oxide (NO) levels were measured in supernatants using the Dimethylmethylene Blue Assay (DMMB) assay and Griess assay, respectively. One-way ANOVA was used for multiple group comparisons.
Results: Treatment with IL-1β or TNF-α and OM led to statistically significant increases in gene expression of MMP1, MMP3, and MMP13 and increased secretion of GAG, MMP1, MMP3, MMP13, ADAMTS4, ADAMTS5, and NO in supernatants compared to unstimulated control. Treatment with LOR decreased both IL-1β-stimulated or TNF-α and OM-stimulated gene expression of MMP1, MMP3, and MMP13 and secretion of GAG, MMP1, MMP3, MMP13,

A

	IL1-β stimulation			TNF-α + OM stimulation		
Protein levels (Supernatants)	Mean ± SEM			Mean ± SEM		
	DMSO	LOR (10 nM)	LOR (30 nM)	DMSO	LOR (10 nM)	LOR (30 nM)
MMP1	4.49 (0.2)	**1.24 (0.03)**	**1.1 (0.02)**	8.23 (0.44)	**1.01 (0.01)**	**1.0 (0.01)**
MMP3	5.97 (0.37)	**1.02 (0.03)**	**1.35 (0.07)**	5.17 (0.32)	**1.1 (0.02)**	**1.08 (0.04)**
MMP13	14.8 (0.49)	**4.57 (0.7)**	**1.93 (0.2)**	70.78 (3.31)	**2.56 (0.17)**	**1.47 (0.08)**
ADAMTS4	1.28 (0.02)	1.04 (0.01)	1.09 (0.02)	1.45 (0.03)	1.03 (0.01)	0.91 (0.02)
ADAMTS5	1.91 (0.1)	**1.03 (0.03)**	**1.15 (0.03)**	2.28 (0.11)	**1.24 (0.02)**	**0.87 (0.03)**

B

	IL1-β stimulation			TNF-α + OM stimulation		
Gene expression (Explants)	Mean ± SEM			Mean ± SEM		
	DMSO	LOR (10 nM)	LOR (30 nM)	DMSO	LOR (10 nM)	LOR (30 nM)
MMP1	1.04 (0.01)	0.81 (0.04)	0.71 (0.05)	1.04 (0.01)	**0.67 (0.03)**	**0.50 (0.03)**
MMP3	1.02 (0.01)	0.82 (0.02)	**0.56 (0.02)**	1.03 (0.01)	**0.73 (0.03)**	**0.71 (0.03)**
MMP13	1.05 (0.01)	**0.60 (0.02)**	**0.60 (0.05)**	1.05 (0.01)	**0.60 (0.03)**	**0.63 (0.03)**

C

	IL1-β stimulation			TNF-α + OM stimulation		
Supernatants	Mean ± SEM			Mean ± SEM		
	DMSO	LOR (10 nM)	LOR (30 nM)	DMSO	LOR (10 nM)	LOR (30 nM)
GAG	1.35 (0.04)	1.05 (0.03)	**0.87 (0.02)**	2.4 (0.07)	2.02 (0.05)	**1.54 (0.04)**
NO	490.46 (18.69)	**0.89 (0.05)**	**0.44 (0.03)**	144.2 (9.6)	**19.79 (2.71)**	**20.09 (2.65)**

ADAMTS4, ADAMTS5, and NO in supernatants compared to treatment with DMSO.
Conclusions: LOR demonstrated potent inhibition of cartilage catabolic enzyme production in human OA explants compared to controls. These cartilage-protective effects support the development of LOR as a potential disease-modifying treatment for knee OA. Human trials are ongoing. **Table:** A) Enzyme protein levels, B) Enzyme gene expression, C) GAG and NO levels. Numbers represent normalized versus unstimulated. ***P*<0.05**, one-way ANOVA.

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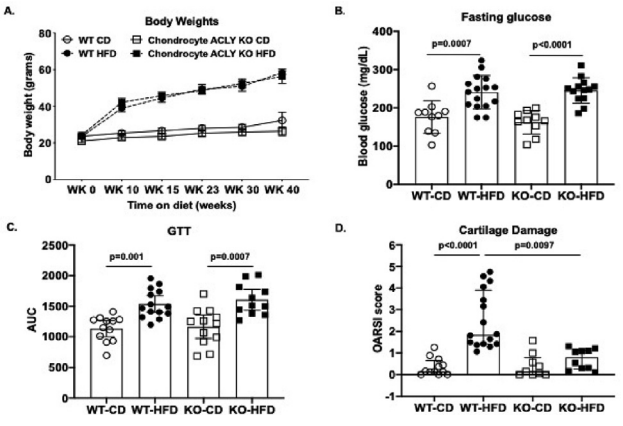
DEFICIENCY OF ATP-CITRATE LYASE (ACLY) IN CHONDROCYTES LIMITS CARTILAGE DAMAGE INDUCED BY OBESITY VIA HIGH-FAT DIET IN MICE

P. Gil Alabarse [1], N. Nguyen [1], R. Wallace [2], R. Liu-Bryan [3]. [1] *VASDHS, San Diego, CA, USA;* [2] *UCSD, San Diego, CA, USA;* [3] *UCSD/VASDHS, San Diego, CA, USA*

Purpose: ATP-citrate lyase (ACLY) is an enzyme located in the cytosol and nucleus that generates acetyl-CoA from mitochondrial-derived citrate. Altered ACLY activity is observed in metabolic disorders, and several core features of metabolic syndrome such as dyslipidemia, obesity and insulin resistance have been linked to OA. Our recent study revealed that ACLY is upregulated in human knee OA chondrocytes. This could cause accumulation of nucleocytosolic acetyl-CoA, leading to increased matrix catabolism via dysregulated histone and transcription factor acetylation. Pharmacologic ACLY inhibition in OA chondrocytes globally reverses these changes and stimulates expression of matrix genes and activation of AMP-activated protein kinase (AMPK) that plays a critical role in cartilage homeostasis. Given obesity is a major risk factor for OA development, in this study, we investigate the role of ACLY in obesity (via high-fat diet)-induced OA in mice *in vivo*.
Methods: ACLYflox/flox and ACAN-Cre/ERT2 mice were used to generate chondrocyte-specific ACLY knockout (KO) mice (ACAN-ACLY$^{fl/fl}$Cre$^{+/-}$) via tamoxifen induction. ACAN-ACLY$^{fl/fl}$Cre$^{-/-}$ mice were used as wild type (WT) control mice. Only male mice were used for the study. At 4 months of age, mice were placed on either a high-fat diet (HFD, 60% kcal fat) or a control diet (10% kcal fat) for 40 weeks. Body weights were monitored at regular interval during the study period and fasting blood glucose levels and glucose tolerance were assessed. Articular cartilage degradation was analyzed on coronal knee joint sections that have been stained with Safranin-O and Fast Green using OARSI scoring system. Statistical analyses were performed by one-way *ANOVA* with Tukey's multiple comparison *post-hoc* test or Kruskal-Wallis with Dunn's multiple comparison post-hoc testusing GraphPad PRISM 8. *P* values less than 0.05 were considered significant
Results: HFD induced obesity in both WT and chondrocyte-specific ACLY KO mice, evidenced by similar amounts of body weight gain in both types of mice (Figure A). No significant differences in body weights were seen between these 2 types of mice on control diet (Figure A). In addition, fasting blood glucose levels were similar between WT and chondrocyte-specific ACLY KO mice on either control diet or HFD (Figure B). However, compared with the control diet, HFD significantly increased fasting blood glucose levels (Figure B) and reduced glucose tolerance (Figure C) in both types of mice. Histology analysis of mouse knee sections reveled that WT mice exhibited moderate cartilage damage after 40 weeks of HFD. The OARSI scores demonstrated a significant difference between WT and chondrocyte-specific ACLY KO mice on HFD (Figure D), with a median (25th-75th percentile) of 1.84 (1.42-3.91) in WT vs. 0.8 (0.27-1.13) in chondrocyte-specific ACLY KO mice ($P < 0.0001$). In comparison, there was little cartilage damage in either WT or chondrocyte-specific ACLY KO mice on control diet with a median (25th-75th percentile) of 0.155 (0.03-0.65) or 0.15 (0-0.79), respectively. Interestingly, osteophyte formation induced by HFD was seen in both types of mice, but the size of osteophyte was smaller in chondrocyte-specific ACLY KO mice, compared to WT mice.
Conclusions: After 40 weeks of HFD, both WT and chondrocyte-specific ACLY KO mice became obese, associated with metabolic dysfunction. However, chondrocyte-specific ACLY KO mice appeared to have significantly less cartilage damage, compared to WT mice, suggesting that inhibition of ACLY may be chondroprotective. Given that ACLY is emerging as a drug target for long-term use in metabolic disorders, its potential as a novel metabolic target for OA is promising.



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THE TYROSINE KINASE RECEPTOR EPHA2 IS A DRUG TARGET FOR OSTEOARTHRITIS

M. N. Ferrao Blanco [1], Y.M. Bastiaansen-Jenniskens [1], R. Narcisi [1], M.G. Chambers [2], A.A. Pitsillides [3], G.J. van Osch [1]. [1] *Erasmus Med. Ctr., Rotterdam, Netherlands;* [2] *Eli Lilly and Company, Indianapolis, IN, USA;* [3] *The Royal Vet. Coll., London, United Kingdom*

Purpose: The exact pathogenesis of osteoarthritis (OA) remains unclear and therapeutic options are limited. Accumulating data suggest that the activation of inflammatory signaling pathways promote chondrocyte hypertrophy leading to the modification and degradation of cartilage extracellular matrix typical of OA. Therefore, the aim of this study is to identify a novel therapeutic target for OA, associated with chondrocyte hypertrophy and inflammation.
Methods: To identify targets, we combined previously published microarray data sets: A microarray from different zones of the growth plate of 14-days old C57BL/6 mouse tibiae, a microarray of articular cartilage of an OA mouse model where the destabilization of the medial meniscus was performed in C57BL/6, and a list of genes associated with inflammatory signaling pathways in mice obtained from UniProt. Finally, a human microarray dataset obtained from OA and healthy articular cartilage was used. Combination of datasets revealed candidate targets. To validate the target Ephrin receptor A2 (EphA2), human chondrocytes were isolated from donors who underwent total knee arthroplasty. P3-expanded chondrocytes were encapsulated in alginate beads (4 million cells/ml) and re-differentiated for one week in medium containing 10 ng/mL Transforming Growth Factor (TGF)β1. Receptor tyrosine kinase inhibitor, ALW-41-27, with a high selectivity for EphA2 was added for 24 hours at the concentration of 1 and 10 μM after confirming that this did not affect cell viability based on the lactate dehydrogenase assay and Live/Dead staining. To determine the role of EphA2 in the inflammatory response, TNF-α (10 ng/mL) was used as an inflammatory stimulus and cells were cultured during 24 hours with or without the inhibitor. Interleukin-6 (IL-6) was quantified in the medium using an enzyme-linked immunosorbent assay (ELISA). Nitric oxide (NO) production was quantified in the medium through the Griess reaction. mRNA expression of collagen type 10α1 (*COL10A1*), matrix metalloproteinase 1 (*MMP1*), matrix metalloproteinase 13 (*MMP13*), and interleukin 6 (*IL6*) were determined by qPCR. Each experiment was performed with cells derived from 3 OA donors. Statistical evaluation was performed using IBM SPSS 22.0 and a linear mixed model was used to take into account donor variability. The tests were adjusted for multiple comparisons by a Bonferroni's post hoc comparisons test. Differences were considered statistically significant for P<0.05.
Results: Ephrin receptor A2 (EphA2), a tyrosine kinase receptor, was upregulated in the hypertrophic compared to the proliferative zone of the murine growth plate (fold change= 19, adj. p-value= 1E-07) and its expression was higher in OA versus sham mouse articular cartilage (fold change= 3, adj. p-value= 2E-02) and human OA versus healthy cartilage (fold change= 3, adj. p-value= 1E-08). Addition of the EphA2 activity inhibitor ALW-41-27 dose-dependently reduced the