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Development of collagen-based matrix that promotes from mesenchymal stromal cells to differentiate into osteocyte-like cells

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Background: Osteocytes differentiate from cuboidal-like osteoblasts and are embedded into soft secreted osteoid in mature bone. The regulation of osteocyte differentiation remains poorly understood, however, collagen as a scaffold will have an influence on it. At the ECTS2019 Congress, we showed that low adhesive scaffold type I collagen (LASCol) has marvelous ability to induce from mesenchymal stromal cells (MSCs) to osteoblasts differentiation in short term. In this study, we report the effects of LASCol to promote differentiation from MSCs into osteocyte-like cells followed after osteoblasts.

Methods: Rat MSCs were cultured on LASCol coated-dish or LASCol gel with osteogenic basal medium. The alkaline phosphatase (ALP) and Alizarin red staining of rMMCs cultured on the LASCol coated dish was observed. The expression of mRNAs related to osteocytes differentiation was analysed with qRT-PCR. In addition, Transmission Electron Microscope (TEM) was used to observe the features of cells in LASCol gel. To investigate local properties of the LASCol gel surface, we measured stiffness of LASCol gel by Scanning Probe Microscope (SPM) and surface images were also acquired.

Results: On both the LASCol coated-dish and the LASCol gel, rat MSCs formed spheroid bodies and highly mineralized extracellular matrix was indicated. After 3 and 5 days culture, the osteocyte-related genes such as *Bglap*, *Dmp1*, *Spp1*, and *Phex* significantly increased ($2.4 < \Delta\Delta Ct < 231$). Interestingly, we confirmed osteocyte-like cells in LASCol gel after 40 days culture. After incubation for 1h at 37°C for gel formation, the stiffness of LASCol gel showed an increase depending on concentration (6.8 nN at 10.5 mg/mL). Probably, LASCol would have the strong ability to induce the differentiation of from rMSCs to osteocyte.

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OCY454 as a novel osteocyte model to study phosphate metabolism

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Background: Phosphate (P) is essential for many processes including cellular signaling and skeletal mineralization. The main regulator is Fibroblast Growth Factor 23 (FGF23), which is secreted by osteocytes, but the exact mechanism underlying FGF23 regulation is poorly understood. Currently used cell models are inappropriate osteoblast lines or slowly differentiating osteocytes. Here, we propose the novel osteocyte cell line OCY454 as it expresses osteocyte differentiation markers within one week of culturing.

Methods: The immortalized murine cell line OCY454 was kept at 33°C for three days to reach confluence, before being moved to 37°C for differentiation. At 37°C, cells were cultured with 0 or 4 mM β-glycerophosphate (β-GP; P donor). qPCR was used to study the expression of genes of interest at day 0, 7 and 11.

Results: *Fgf23* was detectable in the first week of cell culture. Its regulators Dentin matrix acidic phosphoprotein 1 (*Dmp1*) (36.1-fold, p=0.0002), Polypeptide N-acetylgalactosaminyl-transferase 3 (*GalNt3*) (2.65-fold, p=0.0002) and Phosphate Regulating Endopeptidase Homolog X-Linked (*Phex*) (2.75-fold, p=0.029) were all upregulated after 7 days of culture. Furthermore, 4 mM β-GP significantly increased expression of the negative Fgf23 regulator *Dmp1* (2.8-fold, p=0.0011) but decreased expression of positive regulator *GalNt3* (2.7 fold, p=0.0271) and *Fgf23* (4.2 fold, p=0.0230) at day 11 compared to 0 mM.

Conclusion: The osteocyte model OCY454 appears to be suitable to study P metabolism because of its early expression of *Fgf23* and the dynamic effects of β-GP on genes involved in Fgf23 regulation. Future studies will focus on the yet enigmatic mechanisms underlying FGF23 biology and its interplay with phosphate.

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Matrix vesicle biomimetics carrying Annexin A5 and Alkaline Phosphatase bind to native collagen produced by human smooth muscle cell transdifferentiated in osteo/chondrocyte cells

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Vascular smooth muscle cells (VSMCs) transdifferentiated ectopically trigger vascular calcifications, contributing to clinical cardiovascular disease in the aging population. AnxA5 and TNAP play a crucial role in (patho)physiological mineralization. We performed affinity studies between DPPC and 9:1 DPPC:DPPS-proteoliposomes carrying AnxA5 and/or TNAP and different types of collagen matrix: type I, II, I+III and native collagenous extracellular matrix (ECM) produced from VSMCs with or without differentiation, to simulate ectopic calcification conditions. AnxA5-proteoliposomes showed the highest affinity for collagens, specially for type II, reaching 74% binding for 1.8 μg of AnxA5 (protein content into DPPC:DPPS-proteoliposomes). DPPC-proteoliposomes-AnxA5 showed binding of 32%, corresponding to 0.5 mol of AnxA5 bound per mol of coated collagen. TNAP-proteoliposomes bound poorly (not exceeding 20%) in any of the two lipid compositions. The simultaneous presence of TNAP in AnxA5-proteoliposomes disturbed interactions between AnxA5 and collagen. DPPC-proteoliposomes-AnxA5 affinities for ECM from transdifferentiating cells went up 2-fold compared to that from native VSMCs. The affinities of DPPC:DPPS-proteoliposomes were high for ECM from VSMCs with or without differentiation, underscoring a synergistic effect between AnxA5 and DPPS. Co-localization studies uncovered binding of proteoliposomes harboring AnxA5 or TNAP+AnxA5 to various regions of the ECM, not limited to type II collagen. All results were expressed as mean ± SEM. Groups were compared with the one-way ANOVA or Student's t-test ($P < 0.05$). Thus, proteoliposomes as MVs biomimetics are useful in the understanding of mechanisms that regulate the process and essential for the development of novel therapeutic strategies to prevent ectopic mineralization.

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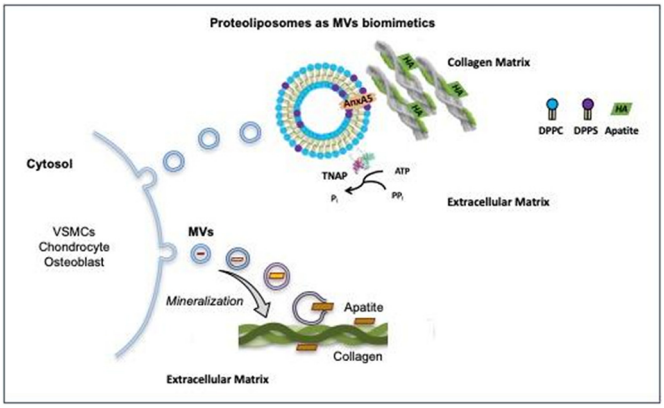

Graphical abstract

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Linc-ROR promotes MSCs chondrogenesis differentiation and releases osteoarthritis through activation of SOX9 via sponging miR-138 and miR-145

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Long noncoding RNAs (lncRNAs) have gained widespread attention in recent years and was known to be involved in multiple musculoskeletal diseases, including osteoarthritis (OA). Emerging evidences have shown that some lncRNAs play important regulatory roles in chondrogenesis differentiation of mesenchymal stem cell (MSCs), suggesting a potential therapeutic strategy for cartilage repair and osteoarthritis treatment. The present study is to characterize the regulation mechanism of long intergenic non-coding RNA, regulator of reprogramming (linc-ROR) in MSCs chodrogenesis as well as OA. Linc-ROR was found to be down-regulated in articular cartilage tissue sample from OA patients compared with healthy control. Meanwhile, linc-ROR expression level was positively correlated with the expression level of chondrogenic gene SOX9 (R^2 =0.64). Linc-ROR expression level of MSCs was also found to be 6 folds higher at day 21 of chondrogenesis induction compared with the initiating time point. Ectopic expression of linc-ROR significantly accelerated MSCs chondrogenesis. By using bioinformatics prediction and luciferase reporter assays, it was demonstrated that linc-ROR functioned as a miRNA sponge for miR-138 and miR-145, both of which were negative regulators of chondrogenesis key factor SOX9. Further investigations revealed that both miR-138 and miR-145 suppressed MSCs chondrogenesis activity as well as SOX9 expression by around 50%, while co-expression of linc-ROR revealed a rescuing effect of chondrogenesis activity and SOX9 expression to around 80-100%. Furthermore, the linc-ROR overexpressed MSCs were encapsulated into hyaluronic acid (HA) hydrogels and transplanted subcutaneously in nude mice. Linc-ROR overexpressing MSCs also showed a higher *in vivo* chondrogenesis activity and SOX9 expression level compared with negative control. Taken together, linc-ROR modulated MSCs chondrogenesis differentiation by acting as a competing endogenous RNA for miR-138 and miR-145, and activating SOX9 expression. Linc-ROR could be considered as a new therapeutic target for the treatment of OA.

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Hypertrophy of chondrocytes compromises their mechanical properties

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Chondrocyte hypertrophy is a key aspect of bone elongation. During hypertrophy chondrocytes enormously increase in cellular volume within a very short time, i.e., 20 folds within 12 hours (Cooper KL et al, Nature 2013). We hypothesized that such an abrupt increase in cellular volume might impair mechanical stiffness of these cells. To address this hypothesis, we compared stiffness of proliferative and hypertrophic chondrocytes within the growth plate.

Knowing that various fixations affect mechanical properties of matrix and cells differently, we have employed live thick tissue sections of bones and analysed cell and matrix stiffness within the growth plate using atomic force macroscopy (AFM, cantilever with 5μm glass microsphere). The Young's modulus was calculated according to the Hertzian contact mechanics model. After the measurements cell viability was confirmed by LIVE/DEAD staining.

The AFM measurements revealed that hypertrophy leads to dramatic softening of the cells with Young's modulus decreasing from 13.6±4.7 kPa (proliferative chondrocytes) to 3.1±1.4 kPa (hypertrophic chondrocytes).

These results show that chondrocyte hypertrophy compromises stiffness of the cells and suggest that these cells may be particularly vulnerable to mechanical loading.

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Activation of caspases and autophagy during cartilage development

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Caspases are proteases acting during inflammation process and apoptosis. The most important caspases activated in apoptotic cascades are caspase-8 and 9 as initiators, the trio of executive caspases, caspase-3, -6 and -7 and caspase-2, which has yet an unclear position. Recently, novel roles of pro-apoptotic caspases in non-apoptotic events such as regulation of autophagy have been reported in different tissues.

Meckel's cartilage is a temporary structure connected with proper mandible development. Recently, autophagy was suggested as an alternative of cell death involved in eliminating the middle segment of Meckel's cartilage. In long bones, formation of the growth plate allows for elongation of the bone and growth plate maturating chondrocytes display an autophagic phenotype. Autophagy in chondrocytes was thus demonstrated as a developmentally regulated process essential for proper bone growth.

This research aimed to: 1) investigate activation of pro-apoptotic caspases within the Meckel's cartilage and long bone development 2) to correlate presence of individual caspases with autophagic markers *in*