and mineralized nodules, and the highest levels of mRNA expression of osteogenesis-related genes collagen-1, ALP and BMP-2 in the osteoblasts. The *in vivo* studies found that the rats in the 1 h group, 1.5 h group and 2 h group had significantly higher bone mineral densities (BMD) than those of the control group, and the highest BMD was obtained in the 1.5h group. The rats in the 0.5 h group and 3h group were not statistically different in the BMD values with the control. The mechanical tests and bone morphometrical analysis results revealed a similar tendency. The serum osteocalcin level was the highest while the TRAP5b level was the lowest in the 1.5 h group.
Discussion and Conclusion: Our data has demonstrated that SEMF treatment at 1.5 h per day has the strongest osteogenic activity than other duration times in the rat primary osteoblasts. The *in vivo* studies provided further supports that SEMF treatment at 1.5 h per day increased the BMD of growing rats to the greatest extent compared to other durations within 3 h. Our study suggests that the osteogenic effects of SEMFs are duration-dependent and 1.5h per day is the optimal duration for improving peak bone mass and may be used for the prevention and treatment of osteoporosis.
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H19 PROMOTES OSTEOGENIC DIFFERENTIATION BY FUNCTIONING AS A COMPETING ENDOGENOUS RNA

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Objective: Long non-coding RNAs (lncRNAs), extensively transcribed from the mammalian genome, have gained widespread attention in recent years. They serve as important and powerful regulators of various biological activities and play critical roles in a variety of disease progression including differentiation. More and more emerging evidence has demonstrated that some lncRNAs play important regulatory roles in osteoblast differentiation of MSCs, suggesting a potential therapeutic strategy for bone formation. The lncRNA H19, one of the most well-known imprinted genes, is located on human chromosome 11 and it is transcribed only from the maternally inherited allele. Recent researches have highlighted H19 as an active modulator in embryonic placental growth and skeletal muscle differentiation. However, unfortunately, the role of H19 in osteoblast differentiation is largely unknown and its function remains to be characterised.
Methods: Cultures of bone marrow-derived MSCs were established from a healthy donor. The gene encoding human H19 was amplified and cloned into a pBABE retrovirus vector. The H19 overexpression stable hMSCs were generated using retrovirus-mediated gene delivery method as previously described. Osteogenic differentiation was induced according to the published protocols and examined by using ALP activity assay, Alizarin Red Staining, and marker genes expression. Furthermore, the *in vivo* effect of H19 on osteogenesis was evaluated by ectopic bone formation carried out in nude mice. Using bioinformatics tool, the candidate miRNAs targeting were screened out and the direct interaction between H19 and miRNA was identified by using a luciferase activity assay.
Results: In the present study, lncRNA H19 was found to be upregulated during osteogenesis in human mesenchymal stem cells. Stable expression of H19 significantly accelerated *in vivo* and *in vitro* osteoblast differentiation. Meanwhile, by using bioinformatic investigations and RNA immunoprecipitation assays combined with luciferase reporter assays, we successfully demonstrated that H19 functioned as a miRNA sponge for miR-141 and miR-22, both of which were negative regulators of osteogenesis. Further investigations revealed that H19 antagonized the endogenous functions of these two miRNAs and led to the de-repression of their shared target gene β-catenin, which eventually activated the Wnt/β-catenin pathway and hence potentiated osteogenesis. In addition, we also identified a novel regulatory feedback loop between H19 and its encoded miR-675-5p. miR-675-5p was found to directly target H19 and counteracted osteoblast differentiation.
Conclusion: Taken together, these findings indicate that the lncRNA H19 modulates the Wnt/β-catenin pathway by acting as a competing endogenous RNA, which may help to develop a novel therapeutic strategy for promoting fracture healing and bone regeneration.
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Smad7 PARTIALLY KNOCKOUT MOUSE: A NEW ANIMAL MODEL OF OSTEOARTHRITIS

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Objective: Animal models of osteoarthritis (OA) are of considerable importance in elucidating the underlying mechanisms of joint damage and providing proof of concept in the development of pharmacological and biological agents that may modify structural damage in the OA joint. Currently, there is still a lack of an appropriate small animal model of OA which represents the underlying mechanisms. Since transforming growth factor-beta/Smad (TGF-β/Smad) signalling pathway has been identified as a key pathway in osteoarthritis (OA) initiation and progression, we then hypothesise that animal models of OA could be established by the interruption of TGF-β/Smad signalling. This study aims to investigate the role of Smad7, one of the TGF-β/Smad signalling pathway inhibitors, in the initiation and progression of OA, then further to evaluate whether Smad7 partial knockout mouse is a good animal model of OA.
Methods: The genetically engineered Smad7$^{\Delta E1}$ (KO) and wild type (WT) mice (n = 15) at the age of 6, 12, or 24 months were terminated for histological analysis. The anterior cruciate ligament transection (ACLT) or sham operation were performed in both the 6-month old Smad7 KO (n=16) and WT (n=16) mice. Histology, immunohistochemistry (IHC), and micro-computed tomography (CT) analysis were performed to determine the pathological changes in the articular cartilage and subchondral bone after 6 weeks. The knee joints were harvested and subject to histology and IHC examinations.
Results: Histological staining showed that there was no significant difference in the articular cartilage between Smad7 KO and WT mice at 6, 12 or 24 months old. However, cartilage hypertrophic markers (MMP13 and Col X) were significantly upregulated in the intact Smad7 KO mice at 6-months, indicating Smad7 is essential for cartilage homeostasis. In the ACLT surgery model, six weeks after surgery, typical OA phenotype characterised by cartilage destruction, osteophyte formation, and synovium inflammation were all found in the Smad7 KO mice, where only mild degenerative changes were seen in the wild type control mice. Results of Micro-CT showed total bone volume and bone mineral density of subchondral bone were significantly increased in the Smad7 KO mice comparing to the wild type mice after ACLT, indicating a bone hardening in the subchondral bone area. Results of IHC also showed osteogenic marker Osterix was significantly upregulated in the Smad7 KO mice after ACLT, suggesting enhanced bone formation.
Conclusion: Smad7 plays an important role in cartilage homeostasis. Lack of Smad7 may contribute to OA intiation. Smad7 KO mice are susceptible to OA progression under mechanical instability conditions. Smad7 KO mice may be used as an animal model of osteoarthritis to further study the underlying mechanisms.
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TRANSLATIONAL POTENTIAL OF GINSENOSIDE Rb1 IN MANAGING PROGRESSION OF OSTEOARTHRITIS

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Background: Osteoarthritis (OA) is the most common degenerative joint disorder. Inflammatory cytokines play an important role in OA progression. Previous studies have demonstrated that ginsenoside Rb1 would prevent inflammation and apoptosis in chondrocytes. However, we did not find any animal study that reported the effect of Rb1 on attenuation of the severity of osteoarthritis.
Objective: In this study, we used a rat anterior cruciate ligament transection plus medial meniscus resection (ACLT+MMx) model of OA and cell model to investigate whether administration of ginsenoside Rb1 may attenuate the progression of arthritis.
Methods: In the *in vivo* study, the 16-week-old male Sprague-Dawley rats were divided into three groups: Group 1: sham control group; Group 2: Rb1-treated