**Conclusion**

ATP and activating the P2X7 receptor.

**China**

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**Methods**

Human mesenchymal stem cells (hMSCs) were isolated and cultured according to our previous studies. The osteogenic differentiation of hMSCs was evaluated by ALP activity, ARS staining and marker genes expression. The lincRNA-Y overexpression vector was constructed and stably transfected into hMSCS to study its function in osteogenic differentiation. To identify which miRNAs directly target lincRNA-Y, the bioinformatic investigations was performed and the luciferase reporter assay was conducted for further confirmation.

**Results**

In the present study, lincRNA-Y was found to be upregulated during osteogenic differentiation in hMSCS. Further evidence showed that its overexpression promoted while its knockdown suppressed osteoblast differentiation. Interestingly, the lincROR has been identified as a ceRNA or miRNA sponge to regulate the transcriptional factors Oct4, Sox2 and Nanog in human ESCs. According to the prediction, lincRNA-Y functioned as a natural miRNA decoy for miR-138 and miR-145, which all suppressed osteogenic differentiation. The further investigation also showed the lincRNA-Y promoted β-catenin expression, suggesting activating Wnt/β-catenin pathway.

**Conclusion**

Taken together, these findings indicate that lincRNA-Y significantly promoted osteogenic differentiation by serving as a ceRNA for miRNAs, which indicated that it might help to develop a potential therapeutic target for bone repair.

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**Introduction**

Recently, recommendations were made for a bisphosphonate (BP) “holiday” in patients using these drugs to minimise fragility fractures. BPs are known to reduce fracture incidence and increase bone mass in osteoporotic women. Based on the long half-life of BPs in bone (~5.5 yrs.), we hypothesised that bone composition would not be altered by discontinuing BP for less than its “half-life” in bone. BPs bind to mineral in bone with high affinity. Due to variable turnover in different tissues and challenges of measurement, the precise “half-life” or residence-time-in-bone for each BP remains debatable. Alendronate (ALN) is reported to have a half-life >10 years.

**Methods**

To test this hypothesis, we acquired 30 trans-iliac-biopsies from a small subgroup of 1099 patients, from the Fracture Intervention Trial (FIT) Long-term Extension (FLEX) trial. FIT was a multi-centre, double-masked, placebo-controlled trial, in which fracture incidence was documented in 6457 postmenopausal-women, randomised to receive ALN or placebo. FLEX study participants, postmenopausal women who had received ALN therapy (5 or 10 mg) for five years as part of the FIT trial, were randomized to either continue receiving ALN for an additional five years (Treatment group) or were switched to placebo (Discontinued group). Biopsies, obtained ten years were embedded in PMMA; 1-2 um sections of each were prepared in triplicate and the cortical bone (from endosteal-periosteal surface) and intact trabeculae within each biopsy were scanned (6.25 um spatial-resolution) on a Perkin Elmer 300 Infrared Imaging System. These images provide spatially resolved maps of tissue composition. Pixel distribution provides information about the tissue’s compositional heterogeneity. Following data collection, subtraction of embedding media, images were processed using ISIS 5.0 Software, mean and SD in each image from cortical and cancellous bone was calculated for the variables: (i) mineral/matrix ratio, (ii) carbonate/phosphate ratio, (iii) crystallinity, (iv) collagen maturity and (v) acid phosphate substitution along with each of their respective heterogeneities. Comparisons between Discontinued and Treatment groups were made with an unpaired t-test using Welch’s correction. Confidence limits (95%) are shown.

**Results**

Cortical and cancellous parameter means and heterogeneities of their distributions were not significantly different for all variables in the Discontinued and Treatment groups. An exception was cancellous crystallinity heterogeneity which was significantly increased (42%, i.e. 0.038) for the Treatment group.

**Discussion and Conclusions**

Consistent lack of difference between Discontinued and Treatment biopsies provides provisional support for our hypothesis. The observed higher heterogeneity of cancellous crystallinity may reflect the existence of some slightly larger-crystals in the presence of decreased remodelling. These findings agree with previously reported similarity in clinical fracture-risk and BMD in these groups, suggesting that despite a reported difference in the amount of bone lost, bone composition remained unaltered. Conclusions here are limited, due to small sample size; power calculations suggest n=60 would be needed to detect significant differences. Additional studies with more patients and other drugs are required to confirm our hypothesis.

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**Objective**

Our previous research showed that low dose X-irradiation (LDI) can promote callus mineralisation, and stimulate osteoblast proliferation and function. In this study we focused on the relationship of LDI and osteoblasts and the potential mechanism.

**Methods**

Osteoblasts were randomized into an LDI group (cells exposed to irradiation of 100 mGy), a P2X7−/− LDI group (LDI group with P2X7 receptor deleted), and a SHAM group (cells exposed to OmGy). The concentration of ATP in the supernatant was detected by an immunofluorescence staining kit. A TRAP staining method was employed to examine the differentiation of osteoblasts. The expression of P2X7 receptor and Cathepsin-K genes’ mRNA in osteoblasts were evaluated by quantitative real-time polymerase chain reaction (Q-PCR).

**Results**

The releasing of ATP was significantly improved in the LDI group and the P2X7−/− LDI group, with more in the LDI group. TRAP staining showed that LDI enhanced osteoblasts differentiation and maturity. The expression of P2X7 receptor and Cathepsin-K genes’ mRNA increased in the LDI group compared to the P2X7−/− LDI group, and the SHAM group, while the expression of Cathepsin-K declined in the P2X7−/− LDI group.

**Conclusion**

LDI promoted differentiation and function of osteoblasts by releasing ATP and activating the P2X7 receptor.

**Background**

Electromagnetic fields have been reported to be able to improve bone mineral density and are suggested to be a safe and invasive means for treating osteoporosis or healing bone non-unions. However, the action mechanisms and optimal application parameters remain unclear.

**Subjects and Material**

The rat calvarial osteoblasts were expanded in vitro and exposed to 50 Hz 1.8 mT sinusoidal electromagnetic fields (SEMFs) for different daily durations (0.5, 1, 1.5, 2, 2.5 or 3 h per day) for up to 12 days. The alkaline phosphatase (ALP) activity, the colonies stained positive for ALP after 6 days and optimal application parameters remain unclear.

**Subjects and Material**

The rat calvarial osteoblasts were expanded in vitro and exposed to 50 Hz 1.8 mT sinusoidal electromagnetic fields (SEMFs) for different daily durations (0.5, 1, 1.5, 2, 2.5 or 3 h per day) for up to 12 days. The alkaline phosphatase (ALP) activity, the colonies stained positive for ALP after 6 days and the mineralized nodular formed after 12 days were measured, respectively. The gene expression levels of Collagen type I, ALP, and BMP-2 were assayed by real-time PCR. Eighty-four SD rats aged one month were randomly divided into six groups and exposed to same SEMFs for 0 (used as control), 0.5, 1, 1.5, 2, 2.5 and 3 h per day, respectively. After two months, all rats were euthanized, and the femurs and vertebrae were assayed by micro-CT scan, three-point bending tests, and compression tests, as well as bone morphometrical analysis. The serum osteocalcin levels and TRAP-5b levels were measured with an ELISA method.

**Results**

It was found that compared to the non-treated control group, daily exposure for 1.5 h produced the highest number and largest area of ALP+ CFU-F colonies

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