**LincRNA-Y MODULATES OSTEOGENIC DIFFERENTIATION VIA Wnt/β-CATENIN PATHWAY**

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**Objective:** Bone homeostasis is tightly orchestrated and maintained by the balance between osteoblasts and osteoclasts. Recent studies have broad our knowledge of long non-coding RNAs (lncRNAs) in bone metabolism and more and more emerging evidences have demonstrated that some lncRNAs play important regulatory roles in osteoblast differentiation of MSCs, suggesting a potential therapeutics strategy for bone formation. As a newly discovered large intergenic non-coding RNA, lincRNA-Y was reported to mediate the reprogramming of human pluripotent stem cells. It was also found that this lncRNA induced epithelial-to-mesenchymal transition and contributed to tumorigenesis and metastasis. However, little is known about the role of lncRNA-Y in the osteoblast differentiation of MSCs. Therefore, the aim of our study is to investigate the function of lncRNA-Y in osteogenic differentiation and try to elucidate the underlying mechanism.

**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 lincRNA-Y 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 promotes osteogenic differentiation by serving as a ceRNA for miRNAs, which indicates that it might help to develop a potential therapeutic target for bone formation.

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**INDUCIBLE RESPONSE IN OSTEOCLASTS BY LOW DOSE X-RADIATION THROUGH RELEASE OF ATP AND ACTIVATING P2X7 RECEPTOR**

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

**Methods:** Osteoclasts were randomized into an LDI group (cells exposed to irradiation of 100 mGy), a P2X7−/− LDI group (LDI group with P2X7 receptor detected), 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 osteoclasts. The expression of P2X7 receptor and Cathepsin-K genes’ mRNA in osteoclasts 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 osteoclasts 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 osteoclasts by releasing ATP and activating the P2X7 receptor.

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**INSIGHTS INTO THE BISPHOSPHONATE-HOLIDAY: A PRELIMINARY FTIRI STUDY**

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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 Irys 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%, F-test 0.00368) for the Treatment group.