Therapeutically Targeting miR-214 in osteogenic cells promotes bone formation in mice with established osteoporosis induced by ovariectomy

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Introduction: Emerging evidence indicates that microRNAs play an important role in regulating osteogenic differentiation and osteoblastic bone formation. But, it has been still lack of miRNAs specifically identified in bone specimens from skeletal disorders with reduced bone formation. In the past years, we have screened miRNAs in bone specimens from fractured postmenopausal women across age and ovariectomized mice during bone loss. Then, we found that miR-214 negatively correlated with reduced bone formation in the examined specimens, implying an inhibition effect of miR-214 on osteogenic cell-mediated osteogenesis during age-related reduction in bone formation among postmenopausal women. We used miRBase to predict the targets of miR-214 in mammals. Among the predicted target genes, we focused on ATF4 because it was one of the key transcription factors involved in osteogenesis. Recently, we have developed a targeted delivery system for nucleic acid specifically approaching osteogenic cells at various differentiation stages (Zhang G, et al. Nature Medicine 2011), which facilitated examining the following hypothesis that therapeutically targeting miR-214 in osteogenic cells might promote bone formation in mice with established osteoporosis induced by ovariectomy.

Aim: To examine the effect of Antagomir-214 with the targeted delivery system on bone formation in mice with established osteoporosis induced by ovariectomy.

Materials and methods: Twenty-four six-month-old female mice were ovariectomized and left until 12-month-old. Then, 6 mice were scarified as baseline before treatment (BS Group). The remaining mice were treated with Antagomir-214 with the targeted delivery system (AMO, n=6) or negative control Antagomir with the targeted delivery system (NC, n=6) or the targeted delivery system alone (Veh, n=6) by tail vein injection. Antagomir-214 was injected at a dose of 10 mg/kg body weight on days 1 to 3 for three consecutive infections as one pulsed treatment every two weeks. At 14 month-old later after four pulsed treatment, all the mice were sacrificed. After sacrifice, right distal femur was dissected by microCT examination for trabecular architecture and bone histomorphometry analysis for bone formation, respectively. Left distal femur was examined by Q-PCR and Western blot analysis for miR-214 on osteogenic cell-mediated osteogenesis during age-related reduction in bone formation among postmenopausal women. We used miRBase to predict the targets of miR-214 in mammals. Among the predicted target genes, we focused on ATF4 because it was one of the key transcription factors involved in osteogenesis. Recently, we have developed a targeted delivery system for nucleic acid specifically approaching osteogenic cells at various differentiation stages (Zhang G, et al. Nature Medicine 2011), which facilitated examining the following hypothesis that therapeutically targeting miR-214 in osteogenic cells might promote bone formation in mice with established osteoporosis induced by ovariectomy.

Results: Q-PCR and Western blot analysis showed that miR-214 was significantly increased and ATF4 protein was significantly decreased, respectively, in those OVX mice without AMO treatment compared to baseline, whereas miR-214 was significantly decreased and ATF4 protein was remarkably increased in those OVX mice treated with AMO, indicating that the administrated Antagomir-214 efficiently worked for gene silencing in vivo. Bone histomorphometry analysis revealed that levels of bone formation related parameters were all significantly decreased and that of bone resorption related parameters were moderately increased in those OVX mice without AMO treatment, whereas that of all the bone formation related parameters were significantly increased and that of all the bone resorption related parameters were not remarkably changed in those mice treated with AMO, suggesting that miR-214 gives an important contribution to decrease in both osteogenic lineage differentiation and functionally osteoblastic bone formation without affecting osteoclastogenesis and bone resorption (Figure 1). MicroCT measurement revealed that trabecular bone mass were markedly lost (decrease in both BMD and BV/TV) and trabecular architecture were significantly impaired (decrease in Tb.Th, Tb.N and Conn.D; increase in both Tb.Sp and SMI) in those OVX mice without AMO treatment compared to baseline, whereas trabecular bone mass were significantly increased and trabecular architecture were notably improved by AMO treatment (Figure 2).

Conclusion: Therapeutically targeting miR-214 in osteogenic cells promotes bone formation in mice with established osteoporosis induced by ovariectomy.


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Figure 1 Representative images depicting new bone formation assessed by double calcein labeling. Scale bar=20µm; Objective magnification: 20x.

Figure 2 Representative images depicting three-dimensional trabecular architecture by microCT reconstruction.