Therapeutic inhibition of miR-214 promotes osteoblastic bone formation of hind limb suspension mice

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Introduction: In our study, we have found increased miR-214 level accompanied by decreased osteoblastic activity (OCN mRNA and ALP mRNA) in whole femur during hind limb suspension in mice. We hypothesized that therapeutic inhibition of miR-214 counteracts decrease in osteoblastic bone formation of hind limb suspension mice. To test the hypothesis in this suspension model, we examined the counteract effect of pre-injection with Antagomir-214 carried by bone targeting delivery system (specifically approaching bone formation surface) on distal femur before hind limb suspension in 6-month-old male mice.

Aim: To examine the effect of Antagomir-214 with the targeted delivery system on bone formation in mice with tail-suspension induced bone loss.

Materials and methods: Mice were either subjected to hindlimb unloading through tail suspension for 28 d just as Luc Malaval reported. Before suspension, 6 mice were sacrificed 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 delivery system (NC, n=6) or the targeted delivery system alone (Veh, n=6) by tail vein injection for three consecutive infections before suspension. 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 for miR-214 and Osteocalcin mRNA levels and Western blot analysis for ATF4 protein level, respectively. Before sacrifice, all the mice were injected intraperitoneally with calcine green (10mg/kg) in a time sequence of 10 and 2 days before euthanasia.

Results: Q-PCR and Western blot analysis of the distal femur showed that miR-214 was significantly increased and ATF4 protein was significantly decreased, respectively, in those suspension mice without Antagomir-214 treatment compared to baseline (before treatment), whereas miR-214 was significantly decreased from baseline and ATF4 protein was remarkably increased from baseline in those mice treated with Antagomir-214, indicating that the administrated Antagomir-214 efficiently worked in vivo (Figure 1). Radioimmunoassay data from serum samples demonstrated that OCN level significantly decreased from baseline in those suspension mice without Antagomir-214 treatment, whereas that was prevented and even significantly increased in Antagomir-214 treatment group. Consistently with Radioimmunoassay data, Q-PCR analysis of distal femur showed that both OCN mRNA and ALP mRNA levels were both significantly decreased in those suspension mice without Antagomir-214 treatment, whereas both of them were significantly increased in those suspension mice treated with Antagomir-214 (Figure 2), suggesting that miR-214 contributes to the decrease in osteoblastic activity in hind limb suspension induced osteoporosis. MicroCT analysis showed that trabecular bone mass was markedly lost (decrease in both BMD and BV/TV) and trabecular architecture were significantly impaired (decrease in Tb.Th, Tb.N and Conn.D) in both Tb.Sp and SMJ in those suspension mice without Antagomir-214 treatment, whereas the suspension-induced deleterious changes in trabecular bone mass and trabecular architecture were efficiently attenuated by Antagomir-214 treatment (Figure 3).

Conclusion: These results suggest that miR-214 plays an important role in inhibiting osteoblastic bone formation in suspension-induced osteoporotic mice. In another word, therapeutic inhibition of miR-214 could efficiently promote osteoblastic bone formation for efficiently attenuating bone loss induced by unloading.

Reference:

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Figure 1 Q-PCR analysis of miR-214 level, Western blot analysis of ATF4 protein level and radioimmunoassay of serum osteocalcin level from baseline mice, 28-day normal control mice (C28) and 28-day hindlimb suspension mice treated with control vehicle (S28+Veh) or Antagomir-214 (S28+AMO) or Antagomir negative control (S28+NC).

Figure 2 Radioimmunoassay of serum osteocalcin level and Q-PCR analysis of OCN and ALP mRNA levels from baseline mice, 28-day normal control mice treated with control vehicle (S28+Veh) or Antagomir-214 (S28+AMO) or Antagomir negative control (S28+NC).

Figure 3 MicroCT analysis of volumetric bone mineral density (BMD) and micro-architecture parameters in distal femur collected from baseline (BS), S28+Veh, S28+NC and S28+AMO mice. Representative 3-D microCT reconstructive images of distal femur collected from baseline, C28, S28+Veh, S28+NC and S28+AMO mice.