

Inhibition Of Mir-214 By (asp-ser-ser)6-liposome Capsulated Antagomir-214 In Osteogenic Cells Of Aged Osteoporotic Rats Quantification Analysis For Expression Levels Of Mir-214, Osteocalcin Mrna And Atf4 Protein In Osteogenic Cells

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Introduction: MiR-214 in osteogenic cells plays an important role in regulating bone formation, which directly targets ATF4 to suppress osteogenic differentiation and osteoblastic bone formation (Wang X, et al. 2013). In our previous study, we found that elevated miR-214 level correlated with reduced level of bone formation marker gene expression in bone specimens from postmenopausal fractured patients. We further found that miR-214 expression in osteogenic cells increased with age, whereas bone formation rate decreased with age in ovariectomized mice (Wang X, et al. 2013). An aged ovariectomized rat has been regarded as a golden model for aged postmenopausal osteoporosis (Idris Al, et al. 2009). However, the role of miR-214 within osteogenic cells in regulating bone formation hasn't been well studied in the golden model. Moreover, we have developed a targeted delivery system for ribonucleic acid specifically approaching osteogenic cells, i.e. (Asp-Ser-Ser)6-liposome (Zhang G, et al. 2012). In addition, the stable inhibition of miR-214 in osteogenic cells from the aged osteoporotic rats could be achieved by administration of (Asp-Ser-Ser) -liposome capsulated antagomiR-214 at a dosage of 4 mg/kg every week (Guo B, et al. 2013). Thus, we hypothesized that inhibition of miR-214 in osteogenic cells by (Asp-Ser-Ser)6-liposome capsulated antagomiR-214 could up-regulate ATF protein and osteocalcin mRNA level in the aged osteoporotic rat model.

Methods: Six-month-old female Sprague-Dawley rats were ovariectomized (OVX) then left untreated for 12 months. At the age of 18 months old, ten OVX rats were sacrificed as baseline (OVX-BS). Thereafter, the remaining OVX rats were divided into the following groups: rats treated with Antagomir-214 (OVX+AMO, n=10), rats treated with Antagomir-214 negative control (OVX+NC, n=10), rats treated with (Asp-Ser-Ser)6-liposome (OVX+Veh, n=10) and rats without any treatment (OVX, n=10). The rats in OVX+AMO Group and OVX+NC Group were intravenously administrated with (Asp-Ser-Ser)6-liposome-AMO and (Asp-Ser-Ser)6-liposome-NC at a dosage of 4 mg/kg every week, respectively. The rats in OVX+Veh Group were received (Asp-Ser-Ser)6-liposome alone every week during administration period. Three month later after the first administration, the rats were sacrificed and bilateral tibiae were harvested. The right proximal tibiae was subjected to examine the expression level of miR-214 and osteocalcin (OCN) mRNA using laser captured micro-dissections (LCM) in combination with Q-PCR analysis, while the left proximal tibiae was examined by LCM in combination with western blot for ATF protein expression.

Results: Q-PCR analysis showed that miR-214 level in osteogenic cells was significantly increased and OCN mRNA expression level in osteogenic cells was significantly decreased in OVX, OVX+Veh and OVX+NC groups compared to that in OVX-BS Group, respectively (Figure 1 and Figure 2), while western blot analysis demonstrated that ATF4 protein expression level in osteogenic cells was significantly decreased in OVX, OVX+Veh and OVX+NC groups compared to that in OVX-BS Group, respectively (Figure 3). After three months of antagomiR-214 administration, the miR-214 level within osteogenic cells in OVX+AMO Group was significantly decreased when compared to that in OVX-BS Group and significantly lower than that in OVX, OVX+Veh and OVX+NC groups, whereas the ATF protein and osteocalcin mRNA within osteogenic cells in OVX+AMO Group was significantly increased when compared to that in OVX-BS Group and significantly higher than that in OVX, OVX+Veh and OVX+NC groups.

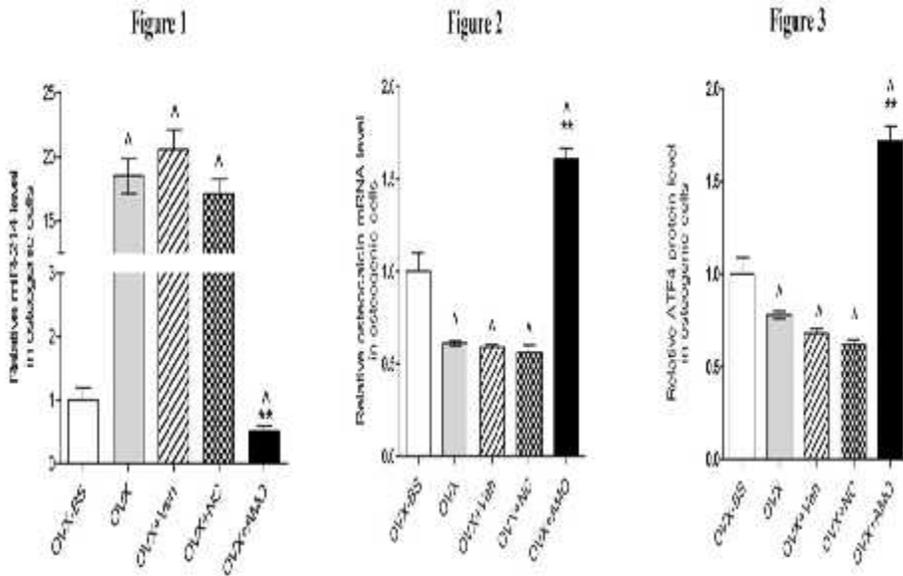
Discussion: Inhibition of miR-214 in osteogenic cells by antagomiR-214 could up-regulate ATF protein and osteocalcin mRNA level in aged osteoporotic rat model implying a potential bone anabolic strategy to reverse established osteoporosis.

Significance: This study indicated a potential bone anabolic strategy to reverse established osteoporosis.

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Note: [^] P<0.05 vs OVX-BS Group; ^{**} P<0.01 vs OVX, OVX+NC, OVX+Vet groups; The miR-214, OCN mRNA and ATF4 protein levels in OVX, OVX+Vet, OVX+NC and OVX+AMO groups were normalized to those in OVX-BS Group, respectively.

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