Therapeutic Inhibition Of Mir-214 By (asp-ser-ser)6-liposome Encapsulating Antagomir-214 In Osteogenic Cells For Promoting Bone Formation In Aged Osteoporotic Rats

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Introduction: MiR-214 in osteogenic cells 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 AI, et al. 2009). However, the therapeutic effects of miR-214 antagomir on the golden osteoporotic model haven’t been well investigated before. 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)6-liposome capsulated antagomir-214 at a dosage of 4 mg/kg every week (Guo B, et al. 2013). Thus, we hypothesized that therapeutic inhibition of miR-214 in osteogenic cells by (Asp-Ser-Ser)6-liposome encapsulating antagomir-214 could promote bone formation 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. Before sacrifice, all the mice were intraperitoneally injected with xylenol orange (30mg/kg) and calcein green (10mg/kg) in a time sequence of 10 and 2 days. After sacrifice, the 5th lumbar vertebrae bodies (LV5) were harvested and subjected to microCT measurement and bone histomorphometric analysis, respectively.

Results: MicroCT analysis showed that the bone mineral density (BMD) and the relative bone volume (BV/TV) in OVX, OVX+Veh and OVX+NC groups significantly decreased when compared to those in OVX-BS Group, respectively. After three months of antagomir-214 administration, the BMD and BV/TV in OVX+AMO Group significantly increased when compared to those in OVX-BS Group and obviously higher than those in OVX, OVX+Veh and OVX+NC groups (Figure A). Meanwhile, better organized micro-
architecture of LVS was also found in OVX+AMO Group when compared that in OVX-BS, OVX, OVX+Veh and OVX+NC groups (Figure B). Consistently, histomorphometric analysis showed that mineral apposition rate (MAR) and bone formation rate (BFR) in OVX, OVX+Veh and OVX+NC groups all significantly decreased compared to those in OVX-BS Group. After three months of antagomir-214 treatment, the MAR and BFR in OVX+AMO Group significantly increased when compared to those in OVX-BS Group and significantly higher than those in OVX, OVX+Veh and OVX+NC groups (Figure C and Figure D).

**Discussion:** Therapeutic inhibition of miR-214 by (Asp-Ser-Ser)6-liposome encapsulating antagomir-214 in osteogenic cells could promote bone formation in aged osteoporotic rat model.

**Significance:** Therapeutic inhibition of miR-214 in osteogenic cells with antagomir-214 encapsulated in (Asp-Ser-Ser)6-liposome would provide a potential bone anabolic strategy to reverse established osteoporosis.

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