

Therapeutic Silencing Intra-osseous *Plekho1* For Promoting Bone Formation In A Rat Model Of Aged Postmenopausal Osteoporosis

Baosheng Guo¹, Heng Wu², Lingqiang Zhang³, Aiping Lu², Baoting Zhang⁴, Ge Zhang⁵.

¹Institute for Advancing Translational Medicine in Bone & Joint Diseases, School of Chinese Medicine, Hong Kong Baptist University, Hong Kong, Hong Kong, ²Institute for Advancing Translational Medicine in Bone & Joint Diseases, School of Chinese Medicine, Hong Kong Baptist University, Hong Kong, China, ³State Key Laboratory of Proteomics, Beijing Proteome Research Center, Beijing Institute of Radiation Medicine, Beijing, China, ⁴School of Chinese Medicine, The Chinese University of Hong Kong, Hong Kong, China, ⁵Institute for Advancing Translational Medicine in Bone & Joint Diseases, Hong Kong Baptist University, Hong Kong, China.

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Introduction: Pleckstrin homology domain-containing family O member 1 (*Plekho1*, also called *Ckip-1*) is a newly discovered negative regulator gene of bone formation during bone development and bone maintenance without activating bone resorption (Lu K, et al, 2008). We found the *Plekho1* mRNA expression increased with age, whereas the osteocalcin mRNA expression decreased with age in the bone specimens from the osteoporotic fracture women. Consistently, we further found that *Plekho1* mRNA expression in osteogenic cells increased with age, whereas bone formation rate decreased with age in female rats (Guo B, et al, 2010). It implied that silencing *Plekho1* in osteogenic cells might be a potential therapeutic strategy to promote bone formation in aged osteoporotic women. In our previous study, (Asp-Ser-Ser)₆-liposome has been successfully employed to achieve osteogenic cell-specific delivery for siRNA *in vivo* (Zhang G, et al, 2012). Thus, we hypothesized that *in vivo* administration of *Plekho1* siRNA delivered by (Asp-Ser-Ser)₆-liposome could promote bone formation and improve trabecular architecture in aged osteoporotic women. An aged ovariectomized rat has been regarded as a golden model to test bone anabolic agents for reversing established osteoporosis in aged postmenopausal women, which was utilized in our study to test our hypothesis (Li X, et al. 2009).

Methods: Seventy six-month-old female Sprague-Dawley rats were either ovariectomized (OVX; n=44) or sham-operated (SHAM; n=26) and then left untreated for 9 months. At 9 months post surgery, eight SHAM rats and eight OVX rats were euthanized as baseline before treatment initiation (week 0). Thereafter, the remaining OVX rats were intravenously injected with (Asp-Ser-Ser)₆-liposome-*Plekho1* siRNA (OVX + siRNA group; n=18) or (Asp-Ser-Ser)₆-liposome (OVX + vehicle; n=18) via tail vein every two weeks until euthanasia, respectively (Guo B, et al. 2010). The remaining SHAM rats were also intravenously injected with (Asp-Ser-Ser)₆-liposome every two weeks (SHAM + vehicle; n=18) until sacrifice. Six rats in above each group were sacrificed at week 2, 4, 6 during treat period, respectively. All the rats were intraperitoneally injected with xylenol orange (30mg/kg) and calcein green (10mg/kg) at 10 and 3 days before euthanasia. After euthanasia, the fifth lumbar vertebrae bodies (LV5) were collected and subjected to microCT measurement and histomorphometric analysis, respectively.

Results: For the microCT data, the bone mineral density (BMD), relative bone volume (BV/TV), trabecular thickness (Tb.Th) and trabecular number (Tb.N) in OVX + vehicle Group were all significantly lower than that in the SHAM Group at week 0, 2, 4 and 6, respectively. After six weeks of treatment, the OVX + siRNA Group showed a significant increase in BMD, BV/TV, Tb.Th and Tb.N at week 6 in relation to week 0 (Figure 1). The trabecular architecture was also improved in OVX + siRNA Group from week 0 to week 6 (Figure 2). Further, the BMD, BV/TV, Tb.Th and Tb.N in the OVX + siRNA Group were significantly higher in OVX + siRNA Group when compared to those in the OVX + vehicle Group at week 6 (Figure 1). Moreover, the Tb.Th in the OVX + siRNA Group almost reached the level in the SHAM Group at week 4 and later. However, the OVX + vehicle Group or SHAM Group showed no significant change in BMD, BV/TV, Tb.Th and Tb.N from week 0 to week 6. For the histomorphometry data, the OVX + siRNA Group showed a continuous increase in both mineral apposition rate (MAR) and bone formation rate (BFR) from week 0 to week 6 and these parameters in the OVX + siRNA Group were significantly higher compared to those in the OVX + vehicle Group at week 6 (Figure1).

Discussion: Therapeutic gene silencing of *Plekho1* in osteogenic cells could promote bone formation and improve trabecular architecture in aged rat model of postmenopausal osteoporosis, which indicated its translational potential for reversing established osteoporosis in aged postmenopausal women.

Significance: Therapeutic gene silencing of *Plekho1* in osteogenic cells could reverse established osteoporosis in aged postmenopausal women.

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Figure 1

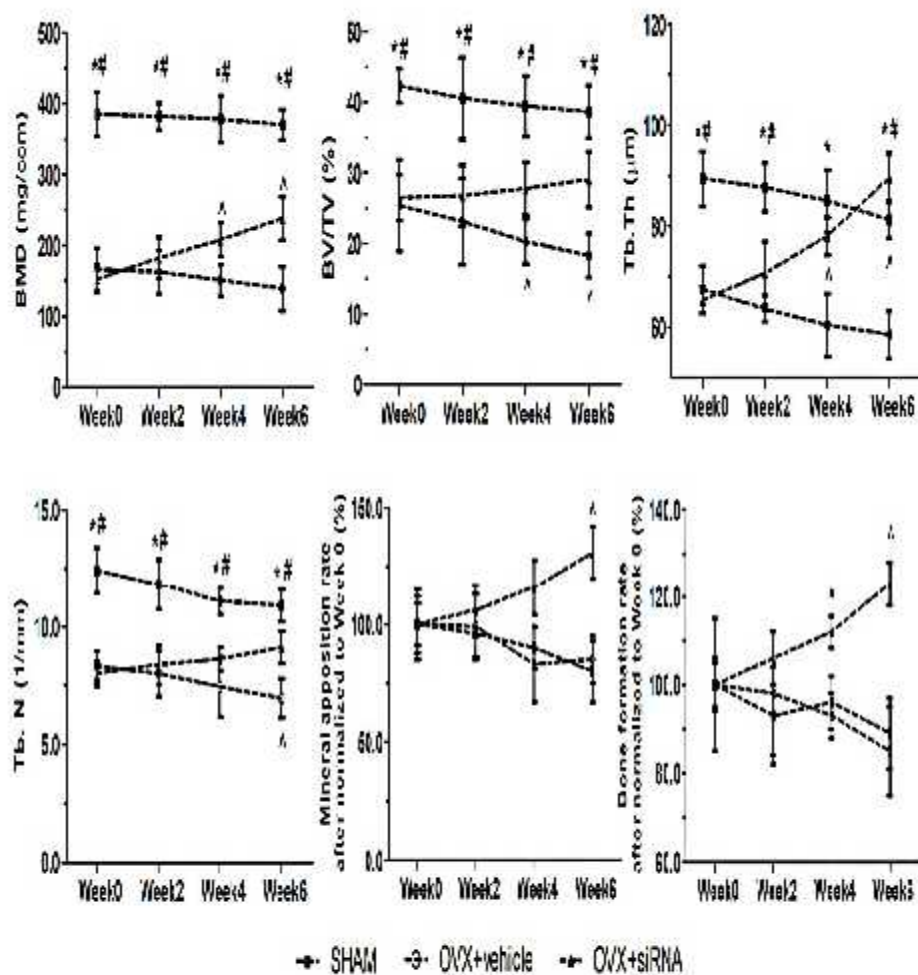


Figure 1 MicroCT and histomorphometric analysis parameters of the LV5 body from the SHAM, OVX, and siRNA groups were measured at 0, 2, 4, and 6 weeks after treatment initiation. n=6-8 for each group at each time point. * $P < 0.05$ OVX + vehicle Group vs. SHAM Group; # $P < 0.05$ OVX + siRNA Group vs. SHAM Group; ^ $P < 0.05$ vs. Week 0.

Figure 2

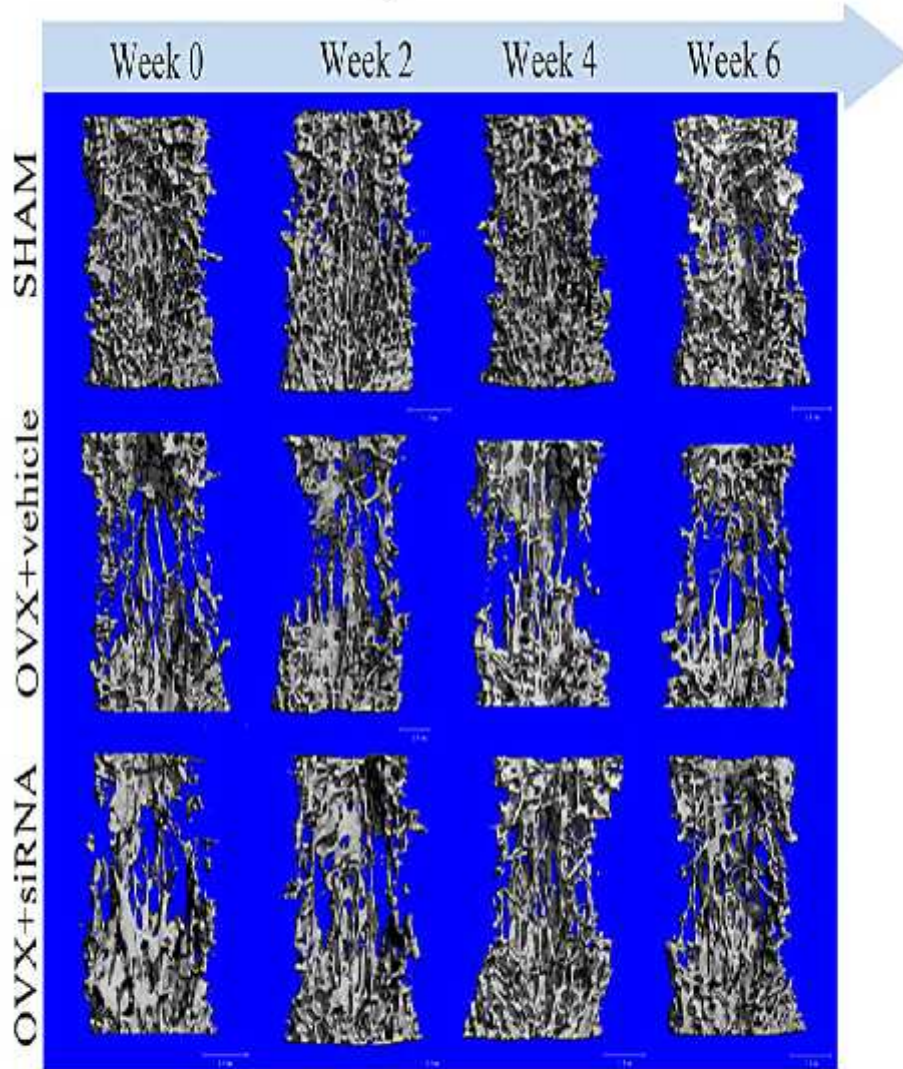


Figure 2 Representative 3D microCT reconstructive images of the LV5 body of the SHAM, OVX + vehicle, and OVX + siRNA groups were obtained at 0, 2, 4, and 6 weeks after treatment initiation.

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