Abstract

Currently, there are a number of FDA-approved anti-catabolic drugs for postmenopausal osteoporosis by inhibiting bone resorption, which only prevent further bone loss by altering osteoclast activity and lifespan, but not stimulate osteogenic cells mediating bone formation for reversing postmenopausal bone loss. The only FDA-approved anabolic agent capable of stimulating bone formation is intermittently administered parathyroid hormone (iPTH). However, the dominantly increased bone resorption after 2-year-treatment with iPTH is a great concern. Thus, development of alternative bone anabolic agents is highly desirable.

The applicants of this proposal have reported that casein kinase-2 interacting protein-1 (CKIP-1), abundantly expressed in musculoskeletal system and sparingly expressed in other non-skeletal organs, is a newly discovered intracellular negative regulator of bone formation without activating bone resorption. Thereafter, the applicants have found that CKIP-1 expression in rat bone increased with aging, whereas bone formation decreased with aging. Moreover, abnormal phenotype in non-skeletal organs was not presented in CKIP-1 gene knockout mice even at old age. It suggested that silencing intraosseous CKIP-1 gene could be a potential strategy to promote bone formation in aged osteoporotic patients.

RNA interference (RNAi) is a natural cellular process that regulates gene expression by a highly precise mechanism of sequence-directed gene silencing at the stage of translation, which could be employed to silence CKIP-1 gene in vivo. Recently, the applicants have identified a specific CKIP-1 siRNA sequence with high knock down efficiency across rat, rhesus, and human osteoblast-like cells in vitro, which promoted osteoblast differentiation across the species in vitro and bone formation in adult healthy rats. Further, the applicants have established a targeted siRNA delivery system, i.e. (DSS)$_6$-Liposome, to approach bone formation surface for specifically targeting osteogenic cells to maximally increase the exposure of the CKIP-1 siRNA to bone and remarkably reduce the exposure of the siRNA to non-skeletal organs in adult healthy rats. In addition, the applicants have optimized the RNAi-mediated in vivo periodic CKIP-1 gene silencing protocol at 3.75mg/kg through tail vein injection at an interval of 2 weeks in adult healthy rats without observed adverse events in non-skeletal organs, which was corresponding to our findings in adult healthy mice.

However, the organ-selective distribution, cell-selective gene silencing and bone anabolic effect of the (DSS)$_6$-Liposome-CKIP-1 siRNA have not been examined in an aged osteoporotic animal model even though the corresponding information has been validated in adult healthy rats. Taken together, our hypothesis is that therapeutic RNAi specifically targeting intraosseous CKIP-1 gene could promote bone formation to reverse severe
postmenopausal bone loss. To test this hypothesis, the aged ovariectomy-induced osteoporotic rats will be employed as a severe postmenopausal bone loss model to achieve the following three specific aims: (1) To examine the distribution of the CKIP-1 siRNA delivered by the liposome with and without the target moiety (DSS)₆ at both organ (bone vs non-skeletal organs) and cell (osteogenic and non-osteogenic cells) level; (2) To test the gene knockdown efficiency of the CKIP-1 siRNA delivered by the liposome with and without the target moiety (DSS)₆ at both organ (bone vs non-skeletal organs) and cell (osteogenic vs non-osteogenic cells) level; (3) To evaluate the effect of the CKIP-1 siRNA delivered by the (DSS)₆-Liposome on bone histomorphometric parameters, bone mass, bone structure, bone mechanical properties, bone biochemical markers, and safety for non-skeletal organs.

The current proposal would provide new insight that therapeutic RNAi specifically inhibiting intraosseous CKIP-1 may be a novel bone anabolic treatment strategy to reverse severe postmenopausal osteoporosis.