CRISPR Interference As An Alternative To The Cre-LoxP System For Loss Of Function Studies In Osteocytes

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The Cre-loxP system has been used to study the function of osteocytes by deleting genes using Cre-driver stains that are active in osteocytes. However, these driver strains, including Dmp1-Cre and SOST-Cre, delete floxed alleles in additional cell types in bone and other tissues. Thus, there is a need to develop loss-of-function (LOF) models with improved specificity. CRISPR interference (CRISPRi) suppresses genes by guiding a nuclease-deficient Cas9-KRAB transcriptional repressor fusion protein (dCas9::KRAB) to transcription start sites via a single guide RNA (sgRNA). Previous studies have shown that global expression of CRISPRi components with a transgene is able to suppress a target gene in mice. Importantly, the level of target suppression was proportional to transgene expression level. Based on this, we hypothesized that expression of dCas9::KRAB under control of a cell-type-specific promoter may lead to suppression of target genes only in cells that express high levels of CRISPRi components and therefore exhibit improved cell type specificity. To test the feasibility of this approach, we produced two knock-in mouse models: one expressing dCas9::KRAB under control of *Dmp1* regulatory elements (Dmp1-dCas9::KRAB) and the other expressing a sgRNA targeting the RANKL gene under the control of U6 promoter (sgRNA^{RANKL}). We first tested sgRNA^{RANKL} mice by crossing them with mice that globally express dCas9::KRAB. Global CRIPSRi of RANKL (gCRi^{RANKL}) led to potent suppression of RANKL in bone and soft tissues, and caused an osteopetrotic phenotype, including failure of tooth eruption and lack of lymph nodes. We then crossed sgRNA^{RANKL} mice with Dmp1-dCas9::KRAB mice, producing Ot_CRi^{RANKL} mice. Consistent with what has been observed in mice in which osteocyte RANKL has been eliminated using the Cre-loxP system, Ot_CRi^{RANKL} mice had high vertebral, femoral, and global BMD compared to littermate controls, beginning at 4 months of age. MicroCT analysis of vertebral cancellous bone at 6 months of age showed that both male and female gCRi^{RANKL} had high cancellous bone mass compared to wild-type mice or mice harboring the individual knock-in alleles. The high cancellous bone mass of gCRi^{RANKL} mice was associated with increased trabecular thickness and trabecular number, and decreased trabecular spacing. These results suggest that CRISPRi can be adapted to perform cell type-specific LOF studies in vivo and may provide a more specific alternative to the Cre-loxP system.