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Primary Cilia as Sensors of Mechanical Loading

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1 Introduction

Mechanical loading is a potent regulator of bone remodeling, however, the molecular mechanism through which bone senses mechanical stimuli is unknown. The primary cilium, a microtubule-based organelle that protrudes from the surface of most mammalian cell types [1], is promising candidate for mechanosensing. If primary cilia sense mechanical loading, and regulate bone cell metabolism in response to mechanical cues, then the loss of primary cilia should lead to a reduced sensitivity to mechanical loading.

Primary cilia possess an axonemal core that grows from the mother centriole of the centrosome [2]. Axonemal elongation is achieved by a process called intraflagellar transport (IFT), in which kinesin II drives anterograde movement of IFT particles along the axonemal microtubules [3]. Kinesin II is a heterodimeric protein composed of two subunits, KifSA and KifSB. In some tissues, such as the kidneys, primary cilia have been shown to act as extracellular sensors [4, 5]. Conventional KifSA knockout mice are embryonic lethal, therefore transgenic mice with bone specific knockout of Kif3A are being used to study the role of primary cilia in the response of bone to mechanical loading.

2 Materials and Methods

Transgenic Mice. Transgenic mice with bone specific knockout of KifSA are being created by the breeding strategy shown in **Fig. 1**. Kif3A; Col l-Cremice possess a single functional copy of Kif3A that can be deleted by the expression of Cre recombinase. The genetic, backgrounds of the mice are C57BL/6 and FVB. All experiments are performed after review and approval by Stanford University's Administrative Panel on Laboratory Animal Care and/or the: VAPAHCS Institutional Animal Care and Use Committee.

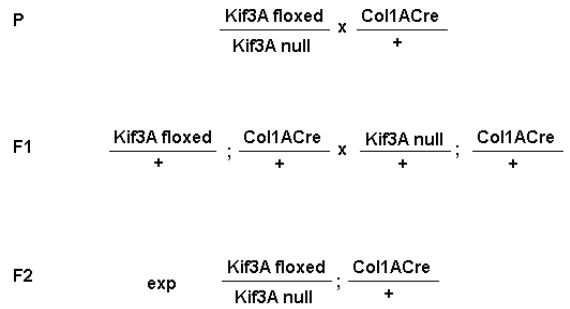

Figure 1 : Schematic illustration of mouse breeding strategy utilized for generation of experimental mice lacking Kif3A in bone tissue.

PCR Genotvpina. Mice are gerabtyped using genomic DNA purified from ear tissue obtained at the time of weaning. Genotyping wilt be performed by polymerase chain reaction (PCR) with the Cre-specific primers, (Cre 5^{1}') CCTGGAAAAT-GCTTCTGTCCGTTTGCC and (Cre 3') GAGT-TGATAGCTGGCTGGTGGCAGATG, and KifSA exon 2-specific primers, (KifSA 5') • AGGGCAGACGGAAGGGTGG, (KifSA 3' wild-type) TCTGTGAGTTTGTGACCAGCC, and (KifSA 3' mutant) GGTGGGAGCTGCAAGAGGG.

Microcomouted Tomography Analysis. Radiographical evaluations of the mice are performed using a vivaCT 40 (Scanco, Hanover, Germany) cone beam in vivo microCT scanner. The proximal tibial metaphyses are scanned and the area constituting 1.0mm in length below growth plate are used in the analysis of trabecular bone. Relative bone volume (BV/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th), and trabecular spacing (Tb.Sp) are evaluated. Cortical bone analysis is conducted in the mid-diaphysis and includes cortical bone area (Ct.Ar), total cross-sectional area (T.Ar), intracortical thickness (Av.Ct.Wi), maximum and

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minimum second moment of inertia (l_{max} and l_{min}), and polar moment of inertia (J).

Ulna Loading Experiments. Beginning with day 1, loading consists of three consecutive daily ulna loading sessions of 1 minute exposure to 2.5N sinusoidal loading for 120 cycles. The unloaded contralateral ulna serves as a control. Calcein labels are administered by subcutaneous injection on days 5 and 9. After euthanization on day 15, the ulnae are embedded in methyl methacrylate and sections are prepared from the mid-diaphysis. Dynamic histomorphometric analysis is conducted on sections to determine the standard measure of bone formation rate at the periosteal surface of loaded and unloaded ulnae of each mouse. The following data is collected from the periosteal surface of each section: total perimeter, single label perimeter, double label perimeter, and double label area. Mineralizing surface, mineral apposition rate, and bone formation rate are calculated from these values [6].

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References

1. Wheatley, D.N., Wang, A.M. and Strugnell, G.E. (1996) *Cell.Biol. Int.* **20**, 73-81.

2. Lin, F., et al. (2003) *Proc. Natl. Acad. Sci. USA* **100**, 5286-5291.

3. Praetorius, H.A. and Spring, K.R. (2001) *J. Membr. Biol.* **184**, 71-79.

4. Dacquin, R., et al. (2002) *Dev. Dyn*. **224**, 245-251.

5. Olsen, B.R., Kolpakova, E., McBratney-Owen, B., Li, X., Zhou, J., and Fukai, N. (2005) *OBM* **2**: 57-65.

6. Robling, AG and Turner, CH. (2002) *Bone* **31**, 562-569.