INTRODUCTION: Bone cells can remodel the mammalian skeleton through changes in gene expression and cellular activity by responding to extracellular mechanical signals such as fluid flow. We hypothesized that bone cells may sense fluid flow via the primary cilium, a microtubule-based organelle that has been shown to mediate flow-induced cellular responses in kidney cells. We have shown that primary cilia exist in MC3T3-E1 and mouse periosteal osteoblasts [1] and wanted to determine whether primary cilia exist in osteocytes. To investigate this, we looked for and found primary cilia in MLO-Y4 osteocytes and in vivo mouse osteocytes.

In studies of kidney cells, mechanical stimulation of primary cilia results in calcium mobilization at the base of the cilium and following fluid shear stress in bone cells [4].

To determine whether primary cilia act as mechanosensors, we examined two outcome variables, intracellular calcium release and prostaglandin E₂ release, which have been shown to be independently stimulated by fluid flow [4, 5]. We hypothesized that there would be a correlation between calcium mobilization and the presence of a primary cilium. Since gap junctional communication can transmit calcium signals from cell-to-cell, we also hypothesized that this correlation would increase if gap junctional communication was blocked.

METHODS: Primary Cilia in Bone Osteocytes. We confirmed the existence of primary cilia in bone tissue using immunofluorescent detection of acetylated α-tubulin [6]. Dissected mouse tibiae were fixed, cryosectioned, and incubated with 6-11B-1 anti-acetylated α-tubulin (Sigma) Primary Cilia in MLO-Y4 Osteocytes. MLO-Y4 cells were grown with 10% FBS and 4% calf serum, fixed in 20% methanol and stained for acetylated α-tubulin as described above.

Cilia Removal: RNA interference was used to reduce protein levels of the cilary protein polars. siRNA targeting polars (Invitrogen) was transfected into MC3T3-E1 cells using Xtreme Transfection Agent (Roche). For an investigative trial, we looked for and found primary cilia in MLO-Y4 cells added to cells 72h, cells were washed with PBS, and fresh media was added for 24h before flow experiments. Oscillatory Fluid Flow. Slides were placed in flow chambers 30min prior to the start of each experiment. Slides were exposed to 1Hz, 10 dynes/cm² oscillatory fluid flow for 1h for PGE₂ experiments and for 5min for calcium experiments.

Calcium Correlation Experiments. Real-time intracellular levels of calcium were quantified. Cells were incubated with 10 mM Flura-2/AM (Molecular Probes) for 30min at 31°C. The slides were mounted in a parallel plate flow chamber on an epifluorescence microscope. Flow media consisted of MEM and 2% FBS. A cell response was defined as a transient increase in fluorescent intensity at least 4 times the maximum oscillation recorded during the pre-flow baseline period.

Calcium Correlation Experiments. After calcium experiments, slides were fixed in methanol (−20°C) and stained as described above. The same field of cells was found and scored for primary cilia.

RESULTS: Primary Cilia were found in mouse tibia osteocytes and 65% of MLO-Y4 osteocytes (Figure 1). Primary cilia were found in 69% of MC3T3-E1 osteoblasts, and 62.75% in scrambled siRNA treated MC3T3-E1s. That number dropped to 4.5% in chloral hydrate treated cells and 42% in polars siRNA treated cells. Calcium Responses. Eliminating primary cilia using either chloral hydrate or siRNA against polars did not significantly abrogate cytosolic calcium mobilization in MC3T3-E1 cells (Figure 2). Calcium Correlation: 45% cells with cilia exhibited a calcium response and 37% of cells without cilia exhibited a calcium response (p=0.12, n=364 cells). PGE₂ Release. After exposure to oscillatory fluid flow we saw a 3.4 fold increase in PGE₂ release in untreated cells (p<0.001) and a 1.8 fold increase in PGE₂ in scrambled siRNA treated cells (p=0.01). With primary cilia removed using either chloral hydrate or polars siRNA we saw no significant increase in PGE₂ release after exposure to oscillatory fluid flow (Figure 3).

DISCUSSION: While it has been known for decades that bone is a mechanosensitive organ, the responsible mechanism has been elusive. Primary cilia, once thought to be vestigial organelles, are now being re-examined in development and in kidney tissue as a fluid sensing organelle. We have found primary cilia in both osteoblastic and osteocytic cell lines as well as in osteoblasts and osteocytes in vivo. In kidney cells, primary cilia have been established as a necessary component of the cell’s fluid flow induced calcium response through stretch activated channels at the base of the primary cilium.

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