Oscillatory Fluid Flow Influences the Number of Microtubules Attached to the Base of Primary Cilia

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SIGNIFICANCE
We showed that the number of microtubules around primary cilia increase with flow which is possibly a mechanism of altered mechanosensitivity.

INTRODUCTION
A primary cilium is a non-motile cellular antenna that extends from the surface of nearly every cell in the human body. In the past decades a number of studies have revealed the cilium to be a multifunctional antenna, sensing both mechanical and chemical changes in the extracellular environment [1]. In particular, the primary cilium has been described as an organelle involved in fluid flow sensing [2]. Ciliogenesis occurs at a protein based structure called the basal body or mother centriole. The basal body is one of the two centrioles which make up the microtubule organizing center (MTOC). The majority of microtubules assemble from the MTOC contributing significantly to the structural integrity of the basal body and the anchoring of the primary cilium [3, 4]. Furthermore, microtubules act not only to stabilize but also to transmit torque to the cytoskeleton. Changing the microtubule attachment of primary cilia would change primary cilia mechanics and, possibly, mechanosensitivity. Several studies have attempted to count the number of microtubules emanating from the basal body and the results vary from 10 to 100 depending on the cell type [5]. It has also been shown that cellular response to shear stress depends on microtubular integrity [6]. Here for the first time we show an increase of microtubules around primary cilia and a conformation change within the microtubule network in response to fluid flow stimulation.

METHODS
Three cell lines were used in this study: IMCD (Inner medullary collecting duct kidney cells), MLO-Y4 (osteocytes) and hMSC (human bone marrow mesenchymal stem cells). IMCD cells were cultured on type 1 rat tail collagen-coated microscope slides (75 x 38 x 1 mm) in DMEM/F12 supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. MLO-Y4 cells were cultured on type 1 rat tail collagen-coated microscope slides (75 x 38 x 1 mm) in αMEM supplemented with 5% fetal bovine serum and 5% calf serum and 1% penicillin/streptomycin. hMSC cells were cultured on fibronectin-coated microscope slides (75 x 38 x 1 mm) in DMEM low glucose supplemented with 10% fetal bovine serum. All cell types were subjected to oscillatory fluid flow for 1 hour. Oscillatory fluid flow was applied by a Hamilton glass syringe in series with rigid walled tubing and a parallel plate flow chamber in which microscope slides were placed. The syringe was mounted in and driven by a mechanical loading device. The flow rate was selected to yield peak shear stresses of 1 Pa. Control slides were similarly loaded into flow chambers, but were not subjected to fluid shear. To stain microtubules, cells were fixed immediately after oscillatory fluid flow with 10% formalin solution for 10 minutes and permeabilized with 1% triton X-100 in PBS buffer for 4 minutes. Cells were treated in 1% BSA for 1 hour to reduce non-specific binding. Microtubules were first labeled with β-tubulin antibody (Sigma, T5201) for 2 hours and then with Alexa Fluor 560 secondary antibody (Invitrogen, A11031). Cells were imaged with a Leica TCS SP5 laser scanning confocal microscope. Image processing was applied with Matlab in order to remove background noise.

RESULTS
We investigated how microtubule networks change morphology and proliferate with shear stress from oscillatory fluid flow. Fluorescent staining for β-tubulin showed a more buckled and dense microtubule network after being subjected to oscillatory fluid flow (Fig. 1). Significant increases in microtubule numbers were observed with flow in all cell types (Fig. 2). The largest increase was in IMCD cells and the lowest increase was observed in hMSC cells. Furthermore, values for hMSC were significantly higher than those of IMCD and MLOY4 cells, both for flow and no flow conditions (Fig. 3). The increase in microtubules found for MLOY4 cells was only significantly different from the increase for IMCD cells in the no flow situation (Fig. 3).

REFERENCES

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