論　文　内　容　要　旨

Increase in extracellular osmotic pressure causes primary cilia shortening and loss dependently on F-actin and microtubule excessive formation

（細胞外浸透圧の増加はF-アクチンおよび微小管の過剰形成依存的に一次繊毛の短縮および消失を引き起こす）

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Primary cilia are antenna-like structures that protrude from the cell surface in many mammals. They are responsible for mechanical and chemical sensing in development and homeostasis. Primary cilia are built up on the mother centriole, which is docked to the cell surface. Ciliary axonemes are made up of microtubules in a 9+0 arrangement. The mother centrioles are surrounded by a cloud of proteins called pericentriolar materials (PCMs), which are necessary for ciliogenesis and protein trafficking. The purpose of this study is to investigate the morphological response of PCMs as well as primary cilia to increased extracellular osmotic pressure, i.e., hyperosmolarity.

We exposed mIMCD-3 cells, a cell line established from mouse kidney collecting duct epithelium, to various osmotic conditions for 3 hours. Cells were fixed and stained with antibodies against the ciliary marker ARL13B. Primary cilia shortened and disappeared in both an osmotic pressure-dependent and time-dependent manner. To further observe morphological changes of primary cilia under hyperosmotic conditions, time-lapse live imaging was performed using knock-in cells that stably expressed fluorescent protein-labeled ARL13B at physiological levels. Fluorescently labeled primary cilia maintained their length for the first and second 30 minutes, but then shortened and finally almost disappeared by 3 hours. We examined the effects of a stepwise increase in extracellular osmotic pressure, to rule out the possibility that the shortening and disappearance of primary cilia were caused by an instantaneous 2-fold increase in extracellular osmotic pressure. Stepwise increases in extracellular osmotic pressure also caused shortening and loss of primary cilia, as observed with instantaneous hyperosmotic shock.

Next, two proteins of the PCMs, γ-tubulin and ODF2, were examined. These two proteins disappeared from the basal body in an osmotic pressure-dependent and time-dependent manner by increasing extracellular osmotic pressure for 3 hours. In contrast, in hyperosmolarity-exposed cells, Western blot analysis of whole-cell lysates showed that the total amount of the two proteins was maintained at the same level as in control cells. Transmission electron microscopy revealed that the core structure of the centrioles was not degraded by increased extracellular osmotic pressure. These results suggest that the loss of PCM proteins upon elevated extracellular osmotic pressure was caused by delocalization of PCMs. Both shortening and loss of primary cilia and delocalization of PCMs were reversible.

We used a pharmacological approach to investigate the molecular mechanisms underlying the shortening and loss of primary cilia upon elevated extracellular osmotic pressure. Two major cytoskeletons, microtubules and actin fibers, were

investigated. Since both actin fibers and microtubules formation was enhanced by increased extracellular osmotic pressure, experiments were conducted using disrupting agents. Nocodazole, a microtubule-disrupting agent, significantly inhibited hyperosmotic pressure-induced shortening and loss of primary cilia and loss of PCMs from centrioles. Latrunculin A, an inhibitor of actin polymerization, also inhibited hyperosmotic pressure-induced shortening and loss of cilia and loss of PCMs from centrioles. These results indicate that filamentous actin and microtubule hyper-formation underlie hyperosmotic pressure-induced shortening and loss of cilia and delocalization of PCMs.

In summary, elevated extracellular osmotic pressure induces reversible morphological responses of both primary cilia and centrioles dependent on excess formation of F-actin and microtubule. These reversible responses of primary cilia and centrioles may be part of a regulatory mechanism of cellular responses to extracellular osmotic alteration in the kidney collecting duct epithelium, which is exposed to a wide range of urine osmolarities.