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【論文の内容の要旨】

Chronic kidney disease (CKD) has been recognized as a leading public health problem worldwide, and about 13% of the Japanese adult population were predicted to have CKD. CKD can progress to end-stage chronic renal disease, which requires dialysis or kidney transplants in life-threatening conditions. Renal fibrosis is the final common pathway for nearly all forms of CKD, and the progression of tubular interstitial fibrosis coincides with the degree of renal dysfunction. Therefore, the identification of effective therapeutic targets for renal fibrosis has been required.

Because the proximal tubule is a major site for reabsorption of water and solutes, proximal tubular epithelial cells are constantly exposed to various levels of osmotic stress that generates not only osmotic but also mechanical stresses in cells. Higher urine osmolarity has been found to be associated with a higher risk of initiating dialysis, and increased water intake lowers urine osmolality retards the progression of CKD. These results suggest dysfunctions of tubular epithelial cells induced by hyperosmotic conditions play an important role in the pathogenesis and the progression of tubular interstitial fibrosis; however, the effects of hyperosmotic stress on tubular epithelial cells remain unclear.

The purpose of this dissertation is to propose new therapeutic targets for the treatment

of CKD by revealing the cellular responses to hyperosmotic conditions and the underlying mechanisms from the perspective of cell biomechanics. The author examined the effects of hyperosmotic stress on epithelial-mesenchymal transition (EMT) and autophagy of proximal tubular epithelial cells, which have been identified as key events involved in the progression and suppression of renal fibrosis. I also investigated the molecular mechanisms of cellular hyperosmotic responses to identify key molecules that could lead to new drug targets.

Chapter 1 provides background for this dissertation, including the pathophysiology of CKD, the cellular response to hyperosmotic stresses, and the mechanisms of EMT and autophagy. Possible hypotheses for the effects of hyperosmotic stress on tubular epithelial cells and the purpose of this dissertation are also described.

Chapter 2 shows the effects of hyperosmotic stress using mannitol and urea as osmolytes on the morphology and cytoskeletal structure of cultured tubular epithelial cells, which have been known to be intimately involved in sensing and responses of cells to biomechanical stimuli. I showed that 200 mM hyperosmotic mannitol stress (531 mOsmol/L), but not hyperosmotic urea stress, induced a temporary decrease in cell volume at 15 min after the stress application and then disassembly/reorganization of the actin cytoskeleton. These results indicate hyperosmotic mannitol stress could cause cell shrinkage concomitant with changes in the actin cytoskeleton.

Chapter 3 focuses on the effect of hyperosmotic stress on the dynamics of intracellular concentrations of calcium ions (Ca^{2+}) in tubular epithelial cells. Ca^{2+} is an important intracellular signaling factor in the process of converting mechanical forces into biochemical signals within cells, called mechanotransduction, and is also essential for the induction of EMT. Hyperosmotic mannitol stress caused a transient increase in intracellular Ca^{2+} concentration. I also found that changes in actin cytoskeletal structure play an important role in this phenomenon. Furthermore, an antagonist of TRP vanilloid 4 (TRPV4) calcium channels, which act as a sensor of mechanical stimuli, significantly inhibited Ca^{2+} influx in epithelial cells in response to hyperosmotic stress. Thus, the hyperosmotic stress induces a transient Ca^{2+} influx associated with TRPV4 channel activation.

Chapter 4 describes the effect of hyperosmotic stress on the EMT of tubular epithelial cells and its mechanism. Hyperosmotic stress reduced expression of epithelial cell marker, E-cadherin, and an enhanced expression of mesenchymal cell marker, a-smooth muscle actin (a-SMA) proteins, indicating initiation of EMT. I also confirmed that the rearrangement of focal adhesions, which connect the actin cytoskeleton and extracellular matrix and play important roles in the mechanotransduction of cells. Furthermore, the

antagonist of the TRPV4 channel was found to prevent the EMT induction of tubular epithelial cells. The TRPV4 channel is thought to be a therapeutic target for controlling the progression of EMT.

In Chapter 5, the effects of hyperosmotic stress on autophagy, which regulates intracellular homeostasis, are examined. Hyperosmotic stress elevated protein levels of the autophagosome marker LC3-II in tubular epithelial cells 12 h after the stimulation. The inhibition of the actin cytoskeleton reorganization impaired the increase in the LC3-II levels, indicating that hyperosmotic stress induces autophagy mediated by the reorganization of the actin cytoskeleton. I also confirmed that nuclear translocation of transcription factor EB (TFEB), known as a master regulator of autophagy, contributes to hyperosmotic stress-induced autophagy, and this phenomenon was regulated by the activation of transient receptor potential mucolipin 1 (TRPML1) channel, a lysosomal specific Ca^{2+}-permeable ion channel. These findings suggest that activation of TRPML1 channel of tubular epithelial cells is a potential therapeutic target for renal protection by autophagy under hyperosmotic conditions.

Chapter 6 describes the conclusion, which summarizes the results and discusses the utility of TRPV4 channels and TRPML1 channels as new drug targets for the treatment of CKD.