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Elucidation of Regulatory Mechanism and Physiological Role of Magnesium Channel in Renal Epithelial Cells

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Summary

The magnesium balance of whole body is regulated by the kidney which adapts magnesium excretion based on net magnesium absorption from intestine. Chronic magnesium deficiency may be involved in the lifestyle-related diseases such as hypertension, diabetes, and hyperlipidemia and renal failure. Mg^{2+} filtrated by glomeruli is reabsorbed by transcellular and paracellular pathways in renal epithelial cells. Transient receptor potential melastatin 6 (TRPM6) channel is expressed in the apical membrane and involved in the reabsorption of Mg^{2+}. The regulatory mechanism of TRPM6 expression has not yet been cleared.

TRPM6 mRNA and protein were endogenously expressed in rat renal tubular NRK-52E cells. Epidermal growth factor (EGF) increased the TRPM6 expression. The basal and EGF-induced TRPM6 expressions were inhibited by U0126, a MEK inhibitor, and LY294002, a PI3-K inhibitor, but not by SP600125, a JNK inhibitor, and SB202190, a p38 MAP kinase inhibitor. Promoter activity of human TRPM6 was observed in the TRPM6 5'-flanking region from -1,214 to -718. This promoter activity was enhanced by EGF and inhibited by U0126. The mutation of the putative AP-1 binding site (-741/-736) completely inhibited the basal and EGF-induced promoter activity. The introduction of c-Fos or c-Jun siRNA inhibited the basal and EGF-induced promoter activity. A chromatin immunoprecipitation assay revealed that c-Fos and c-Jun bind to the AP-1 binding site within the region of -1,214/-718. These results suggest that EGF up-regulates TRPM6 mRNA expression mediate via the activation of MEK/ERK/AP-1-dependent pathway.

EGF increased the proportion of cells in S phase and decreased that in G1 phase, whereas U0126 and TRPM6 siRNA increased the proportion in G1 phase and decreased that in S phase. Furthermore, cyclin D1 expression was decreased in the U0126-treated or TRPM6 siRNA-treated cells. These results suggest that TRPM6 is involved in the regulation of cell cycles.

EGF is secreted from epithelial cells after tubular injury. We suggest that EGF increases cell proliferation mediated via an increase in TRPM6 expression, resulting in the recovery from tubular injury.