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신장 집합관 세포에서 항이뇨호르몬-반응 및 AQP2-조절 micro-RNA 규명

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Vasopressin-responsive miRNAs and AQP2-targeting miRNAs in Kidney Collecting Duct Cells

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Mature microRNA (miRNA) combined with RNA-induced silencing complex acts as an important post-transcriptional regulator. However, miRNAs in the kidney collecting duct cells have not been well understood. We aimed to profile the vasopressin-responsive miRNAs in the kidney inner medullary collecting duct (IMCD) cells, and to identify the aquaporin-2 (AQP2)-targeting miRNAs. Microarray chip assay was carried out in IMCD tubule suspension of rat kidney in the absence or the presence of dDAVP stimulation (10-9 M, 2 h). The results demonstrated 19 miRNAs including both precursor- and mature-miRNAs, as potential candidates that showed significant changes in the expression after dDAVP stimulation ($p<0.05$). Nine mature miRNAs exhibiting more than 1.3-fold changes in the expression on microarray were further examined by real time-quantitative PCR (RT-qPCR). To identify AQP2-targeting miRNAs, in silico analysis was performed. Four miRNAs (miR-32, miR-137, miR-216a, and miR-216b), which were also identified by microarray assay, targeted 3'UTR of rat AQP2 mRNA. In particular, target seed regions of miR-32 and miR-137 were conserved at the 3'UTR of rat and mouse AQP2 mRNA. In situ hybridization revealed the expression of miR-32 and miR-137 in the IMCD cells of rat kidney. Importantly, RT-qPCR and immunoblot analysis demonstrated that dDAVP-induced AQP2 expression was significantly attenuated in mpkCCDc14 cells, when the cells were transfected with miRNA-mimics of miR-32 or miR-137. Moreover, luciferase reporter assay demonstrated a significant decrease of AQP2 translation in mpkCCDc14 cells when they were transfected with miRNA-mimics of miR-32 or miR-137. Taken together, the study provides a novel insight on the regulation of AQP2 expression via RNA interference.

Key Words: 수분통로단백-2, 마이크로알엔에이, 집합관
Aquaporin-2, MicroRNA, Collecting duct