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집합관 AQP2 발현 세포막 및 세포내 소포체막에 부착되는 선택적 펩타이드 발굴

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Ligand Peptidomics Identify Peptide Ligands Binding to AQP2-expressing Plasma Membranes and Intracellular Vesicles of Rat Kidney

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Aquaporin- 2 (AQP2), the vasopressin- regulated water channel in the collecting duct principal cells, plays a key role in the regulation of body water balance. We aimed to isolate high- affinity peptide ligands that bind to immunoisolated AQP2- expressing plasma membrane (PM) or intracellular vesicle (ICV) preparations from rat kidney by in vitro phage display technique. Seven phage clones of high frequency were selected, which showed high- affinity to the AQP2- containing PM or ICV fractions compared with non- recombinant T7 insertless phage clone. Control experiments revealed that these phage clones showed lower affinity to H+- ATPase (B1- subunit) containing fractions (PM and ICV). Fluorescein- conjugated peptide labeling was associated with intracellular compartment and PM of primary cultured IMCD cells, relative to absent or very weak labeling using fluorescein-conjugated control peptide. Library analyses identified proteins including annexin A4, synaptotagmin- like protein 4 (slp4), and protein phosphatase 2 regulatory subunit B (PP2B) which have homologous motifs to high- affinity peptide ligands. Semiquantitative immunoblotting demonstrated that whole kidney annexin A4 (PM fractions) and PP2B (ICV fractions) were significantly increased in response to 5 days of dDAVP treatment in rats, whereas no changes were seen in slp4. In conclusion, the proteins selected based on the homologous motifs to the identified high- affinity peptide ligands may play a role in the vasopressin- regulated AQP2 targeting and/or AQP2 expression. This study demonstrated that in vitro phage display technique can be exploited for ligand proteomics to isolate proteins which might be involved in the specific intracellular targeting pathways and protein- protein interactions of the AQP2- expressing vesicles.

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Key Words : 수분통로단백, 집합관, 요농축, 파지디스플레이
Aquaporin, Ligand proteomics, Phage display