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신장 속수질에서 E3 유비퀴틴 연결효소인 NEDD4와 NEDD4L의 특이 기질 단백질 발굴

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Identification of NEDD4 and NEDD4L-specific Substrate Proteins in Kidney Inner Medulla

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Ubiquitylation of proteins by the ubiquitin-proteasome system is a critical mechanism for dynamic control of protein expression levels. Among enzymes mediating the ubiquitylation, E3 ubiquitin-protein ligases confer the specificity to ubiquitylation by recognizing target substrates. Ubiquitylation by HECT E3 ligases, NEDD4 and NEDD4L, has recently gained interests as a central mechanism for understanding the regulation of the epithelial sodium channel (ENaC) in the kidney. Although NEDD4 and NEDD4L are abundantly expressed in the kidney inner medulla, most of the studies on NEDD4-mediated ubiquitylation pathway to date are focused on NEDD4-specific substrate proteins mainly expressed in the cortex, e.g., EnaC expressed in the connecting tubule and cortical collecting duct. Thus, we aimed to identify new substrate proteins of NEDD4 and NEDD4L in the inner medulla where urinary concentrating process occurs. Proteomics analysis (LC-MS/MS analysis) of the WW domains of six His-tagged NEDD4 or NEDD4L which were interacted with the soluble fraction of rat kidney inner medulla homogenates by chemical crosslinking (Sulfo-SMCC) was performed and twenty substrate protein candidates were selected. Among these, S100A11 and pyruvate kinase (PKM2) for NEDD4 and FERM domain-containing protein 6 (FRMD6) for NED4L were specifically chosen to be confirmed as substrates. At present, in vitro ubiquitylation assay and in vivo ubiquitin-dependent protein degradation assay are being applied to examine the specific interaction between the E3 ligase and substrate proteins and subsequent ubiquitylation. Taken together, this study provides new insights into the NEDD4 and NEDD4L-dependent ubiquitin system in kidney inner medulla.

Key Words: E3 유비퀴틴 연결효소, 유비퀴틴화, 집합관
E3 ubiquitin ligase, Ubiquitination, Collecting duct