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Alteration of Uromodulin Expression by Uric Acid Exposure in Primary Culture of Renal Thick Ascending Limb Cells in Rats

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Objectives : Mutations in the uromodulin (UMOD) gene are associated with hyperuricemia and impaired uric acid (UA) excretion. Patients with UMOD deficiency exhibit reduced expression of the renal $Na^+/K^+/Cl^-$ cotransporter 2 (NKCC2), whereas mutations in NKCC2 impair UMOD synthesis. However, the relationship between UMOD expression in the thick ascending limb (TAL) and the pathogenesis of hyperuricemia and hypertension remains unclear, and the underlying molecular mechanisms are yet to be fully elucidated.
Methods : TAL cells were isolated from Sprague-Dawley rat kidneys, primarily cultured, and characterized. The expression levels of UMOD, NKCC2, and CaSR were analyzed using immunoblotting, quantitative PCR, immunohistochemistry, and immunofluorescence. The effects of various stimuli and protein disulfide isomerase (PDI) inhibition on ER-Golgi trafficking were investigated using a transwell co-culture system. Renal tubular markers such as aquaporin 1 (AQP1), Na^+/H^+ exchanger 3 (NHE3), NKCC2, calcium-sensing receptor (CaSR), Na^+/Cl^- cotransporter (NCC), and AQP2 were analyzed for characterization. TAL cells were treated with Na, K, Ca, and UA for 24 hours in a transwell culture system.
Results : Minimal expression of AQP1 and AQP2, and abundant expression of NHE3, CaSR, UMOD, and NKCC2, were observed in TAL cells primarily cultured from rat kidneys. Immunofluorescence staining revealed strong localization of NKCC2 and UMOD around the nucleus and within the cytoplasm. NKCC2 expression increased with Na and UA exposure, but remained unchanged with K and Ca exposure. CaSR expression increased with Na, K, Ca, and UA exposure. UMOD expression decreased following K, Ca, and UA exposure, whereas NaCl exposure increased UMOD expression. PDI inhibition with 16F16 reduced the expression of UMOD, CaSR, and NKCC2, and their distribution shifted closer to the nucleus.
Conclusions : We successfully isolated and characterized TAL cells primarily cultured from rat kidneys. Our findings suggest that UMOD and NKCC2 exhibit characteristic localization in TAL cells and may have a functional interaction.