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Alteration of Uromodulin expression by Uric Acid Stimulation in Primary Culture of Renal Thick Ascending Limb Cells

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Objectives : Uromodulin (UMOD) mutation was known to be associated with hyperuricemia and uric acid (UA) excretion disorder. In patients with uromodulin gene deficiency showed decreased renal NKCC2 and NKCC2 mutation showed decreased UMOD synthesis. The relationship between changes in UMOD of TAL and the occurrence of hyperuricemia or hypertension is unclear, and its molecular mechanism has not been understood yet.
Methods : TAL cells were isolated from Sprague-Dawley rat kidney and were primary cultured and identified. The expression of UMOD as well as NKCC2 and CaSR was measured using immunoblot, immunohistrochemistry, and immunofluorescence methods. Effect of diverse simulation and inhibition of protein disulfide isomerase (PDI) on ER-Golgi was also evaluated using a transwell co-culture system. The expression of both UMOD and renal tubular proteins (AQP1, NHE3, NKCC2, CaSR, NCC and AQP2) was used for identification. TAL cells were incubated in transwell-culture system for 24 hours by treating Na, K, Ca, and UA.
Results : In TAL cell culture, AQP1 and AQP2 were hardly detected. Expression of NHE3, CaSR, UMOD, and NKCC2 was positively expressed. NCC was also strongly expressed in TAL cells than in kidney tissue. The fluorescence staining with NKCC2, uromodulin distribution was strongly stained around nucleus and cytoplasm. NKCC2 protein was increased by Na and UA exposure but was unchanged by both K and Ca. CaSR protein was increased Na, K, Ca, and UA exposure. UMOD protein was decrease by K, Ca, UA exposure, and NaCl (tends to increase). PDI inhibitor, 16F16, was reduced UMOD, CasR, and NKCC2 protein expression and distribution of UMOD and NKCC2 protein was closer to nucleus than to cytoplasm.
Conclusions : We identified successfully TAL culture of isolated from rat kidney through expression of renal tubular markers. We suggest that both proteins were possibility of interaction as characteristically distributed in renal thick ascending limb cells.