Primary DCT Cell Culture Using Large Scale DCT Sampling from Mouse Kidneys

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Abstract:

The renal distal convoluted tubule (DCT) is important for the renal control of sodium, potassium, calcium, and magnesium homeostasis. There is increasing need for an *ex vivo* system that allows studying the molecular mechanism of DCT ion transport in native DCT epithelia. Unfortunately, the available DCT cell lines express limited amounts of DCT-specific ion transport proteins. Primary DCT cell culture is difficult to establish as the DCT is a rather short and tedious to isolate nephron portion. Recently, Miller et al. described a novel approach (COPAS) for isolation of specific nephron portions. We adopted this large-particle based flow cytometry technique and used transgenic mice expressing eGFP specifically in the DCT to obtain mouse DCT preparations. Microscopical analysis revealed that almost all DCT cells in sorted tubules are viable and exhibit green fluorescence. Western blot analysis confirmed the significant enrichment of the DCT-specific thiazide sensitive NaCl cotransporter (NCC) in the sorted samples. Marker proteins for other renal tubule segments were not detectable. The rather pure DCT preparations were then seeded either on collagen-coated plastic support or on transwell filters. Sorting under semi-sterile conditions and supplementing the media with antibiotics prevented any detectable bacterial or fungal contamination. After six days in culture, the outgrown cells formed a polarized epithelium with an electrical transepithelial resistance of more than 1000 Ohm.cm2 (cells grown on filters). Quantitative real-time-PCR confirmed significant expression levels of the DCT-specific NCC, which was about 10 times more expressed in cells grown on permeable support than in cells grown on plastic. Thus, COPAS allows rapid, large-scale isolation of DCTs for primary cell culture. The eased DCT primary cell culture will now enable us to study DCT function as well as the regulation of its transport proteins.