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Development of urine-derived kidney tubuloid from patients with inherited tubulopathies

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Hereditary kidney tubulopathies are rare diseases that often require lifelong treatment, and there are no therapies available to prevent the progression of kidney injury. Kidney Organoids are 3D structure with complex multi-cellular unit in vitro which resembles kidneys. In contrast to pluripotent stem cell (PSC)-derived organoids, adult stem cell (ASC)-derived epithelial kidney organoids can be derived at any age in a shorter time (typically within 7 days) and can be expanded over many passages while remaining genetically stable.

Urine-derived kidney tubuloids would enable functional studies of organ regeneration, personalized medicine, and drug development *in vitro*. Here, we induced kidney tubuloid in the urine of patients affected by hereditary tubulopathies by establishing an efficient, chemically defined protocol. We collected each urine 300-500cc and repeatedly celled down through centrifugation. We suspended it in basal and matrigel medium (1:1) and added culture media containing R-spondin, Noggin, FGF-10, A8301, epidermal growth factor to increase expansion capacity.

In a previous study, we succeeded in obtaining urine-derived kidney organoids in autosomal dominant polycystic kidney disease (ADPKD). We established tubuloid lines from 6 of 7 patients (efficacy as 85.7%) within 7 days after seeding and were expandable over 3-5 passages. We confirmed *PKD1* gene mutation through whole genome sequencing in some of the tubuloid in ADPKD. Our ADPKD tubuloids represented proximal as well as distal nephron segments, as evidenced by gene expression, immunofluorescence. It was found that the size of ADPKD tubuloid was slightly larger than that of the normal tubuloid without stimulation. When the cysts were induced with forskolin or vasopressin, we investigated that the ADPKD tubuloid was much larger than the experimental group.
In this study, we selected patients (n=7, five were children (< 18 years)) with gene confirmed inherited tubulopathies; nephrogenic diabetes insipidus (NDI) (*AVPR2* mutation, n=2), Dent syndrome (*CLCN5* mutation, n=2), Bartter syndrome type III (*CLCNKB* mutation, n=2), and Gitelman syndrome (*SLC12A3* mutation, n=1).

Kidney tubuloids were established from 2 of 7 patients (efficacy as 28.6%); 1 Dent syndrome, and 1 NDI, within 7 days after seeding consisting of a simple cuboidal epithelium. The quantity and the size gradually increased through subcultures. The tubuloids remained genetically stable over time; are expandable over two weeks (approximately 3-5 passages). We performed LIVE/DEAD stain for one dent syndrome.

The development of urine-derived tubuloid will be remarkably valuable in personalized medicine and drug development for inherited tubulopathies. Further studies to improve efficacy and tubuloid-based high-throughput functional assay are needed.