Modeling Fabry Nephropathy with hPSC-derived kidney organoids

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Dysfunction of endolysosomal pathway due to genetic or acquired conditions impairs the activity of the glomeruli and of the proximal tubules, resulting in common pathological manifestations such as urinary loss of solutes and severe metabolic complications. Fabry disease (FD) is a Lysosomal Storage disorders characterized by kidney dysfunction, caused by mutations in the GLA gene, that encodes for the alpha-galactosidase. The absence of GLA results in the lysosomal accumulation of globotriaosylceramide (Gb3). Glomerular and tubular damage after Gb3 lysosomal accumulation are hallmarks of Fabry nephropathy. Most of the insights on FD mechanisms of disease have been obtained in cell models although the cellular complexity of this disease requires the use of different cell lines (podocytes and proximal tubule epithelial cells) with different culture conditions that may impact with basic cell biology pathways. A multicellular complex system is thus required, and kidney organoids represent a promising platform for the study of complex inherited kidney diseases. We have optimized a protocol for the differentiation of kidney organoids from human pluripotent stem cells (hPSCs) that allows the generation of hundreds of kidney organoids in 3 weeks with a differentiation efficiency of 90%. In order to model FD with kidney organoids we have generated a GLA-KO hPSC line by CRISPR/Cas9 and a GLA-inducibleKO hPSC line by targeting one allele of the AAVS1 safe harbor locus with a cassette expressing the Cas9 and the other AAVS1 allele with a cassette expressing the sgRNA against GLA under the control of a doxycycline-responsive promoter. GLA-KO hPSCs have been used to differentiate kidney organoids that were preliminarily characterized in terms of nephron patterning and Gb3 accumulation, that resulted markedly increased in the proximal tubules. GLA-KO kidney organoids thus represent a new valuable tool to model kidney pathology in FD.