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translational science

How does diabetes cause susceptibility to COVID-19 in the kidney: new clues provided by organoids

Yun Xia1, P. Toby Coates2 and Masaomi Nangaku3

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Early in the pandemic, diabetes was recognized as a risk factor for poor prognosis in patients with coronavirus disease 2019 (COVID-19). Although diabetes does not appear to increase the risk of infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), individuals with diabetes are more susceptible to severe COVID-19.1 Retrospective cohort studies of hospitalized patients with COVID-19 provided a separate line of evidence that acute kidney injury occurs frequently and is associated with increased in-hospital mortality in patients with COVID-19.2 Despite the recognized importance of diabetes and kidney-related comorbidity in COVID-19, elucidation of potential mechanisms, including the ability of SARS-CoV-2 to infect the kidneys, has been hampered by the limited availability of kidney tissue from patients with COVID-19. Although prior studies demonstrated that human pluripotent stem cell (hPSC)–derived kidney organoids and vascular organoids can be directly infected by SARS-CoV-2,3 only recently did Jansen et al. provide compelling evidence that SARS-CoV-2 is present in the kidneys of patients with COVID-19.4

Human kidney organoids were previously shown to be an amenable model for investigating the direct interaction between SARS-CoV-2 and human kidney tissue; however, established kidney organoid models have not recapitulated critical kidney pathophysiology that is often observed in patients with diabetes. To address this issue and to investigate the role of direct kidney infection by SARS-CoV-2 in the increased disease severity in patients with diabetes, Garreta et al. developed a novel human kidney organoid culture that mimics human kidney tissue exposed to diabetic conditions.5 Using this diabetic kidney organoid model, the authors explored the potential mechanisms underlying the increased disease severity in patients with COVID-19 and diabetes.

What did the study show?
The authors employed high oscillatory glucose treatment to stimulate early diabetic phenotypes in hPSC-derived kidney organoids (Figure 1). Control and diabetic kidney organoids contained comparable nephron-like structures and expressed similar levels of mRNAs of nephron segment-specific, endothelial, and stromal marker genes.5 Although the diabetic condition did not change the composition and integrity of basement membranes, extracellular matrix protein deposition was increased in the tubulointerstitial area in diabetic kidney organoids, reminiscent of kidney fibrosis in diabetic kidney disease (DKD).5 It is noteworthy that the diabetic milieu altered cellular metabolism via upregulating glycolytic genes, including lactate dehydrogenase A (LDHA), while suppressing the mitochondria biogenesis gene PPARg coactivator 1α (PGC1α).5 This “metabolic memory” was preserved in proximal tubular (PT) epithelial cells isolated from diabetic kidney organoids even after long-term culture. Consistent with the
findings in kidney organoids, human proximal tubular cells (HPTCs) derived from patients with diabetes also demonstrated an increase in LDHA mRNA levels and a decrease in PGC1α mRNA levels, alongside increased oxidative phosphorylation, basal respiration, and ATP synthesis, compared with HPTCs derived from control patients.5

In both control and diabetic kidney organoids, angiotensin-converting enzyme 2 (ACE2) expression was primarily detected in PT epithelial cells. Oscillatory glucose treatment significantly upregulated ACE2 expression in diabetic kidney organoids by increasing ACE2 mRNA stability.5 The increase in ACE2 expression in diabetic kidney organoids was associated with enhanced infection by SARS-CoV-2 compared with control organoids. Single-cell RNA-sequencing analysis of control and diabetic kidney organoids with SARS-CoV-2 infection further verified that diabetic kidney organoids contained more cells expressing viral RNA compared with control organoids.5 Likewise, HPTCs derived from patients with diabetes expressed higher levels of ACE2 than control HPTCs, conferring increased susceptibility to SARS-CoV-2 infection.5 Inflammatory processes were upregulated by SARS-CoV-2 infection in both control and diabetic kidney organoids, corroborating recent observations in the kidneys of patients with COVID-19.4

Compared with control organoids, diabetic kidney organoids showed more significant downregulation of glycolytic pathways and more pronounced upregulation of inflammation and diabetes-related pathways on SARS-CoV-2 infection.5

To examine the requirement for ACE2 and other putative receptors for SARS-CoV-2 infection of kidney epithelial cells, the authors employed CRISPR-Cas9 gene editing tools to generate ACE2, basigin (BSG), and neuropilin 1 (NRP1) knockout hPSCs. ACE2 deficiency completely abrogated SARS-CoV-2 infection in ACE2 knockout kidney organoids under both control and diabetic conditions, without affecting the development of kidney structures.5 Reexpression of ACE2 restored susceptibility to SARS-CoV-2 infection. Neither BSG nor NRP1 was required for SARS-CoV-2 infection in kidney organoids, although BSG deletion reduced SARS-CoV-2 mRNA levels in BSG knockout kidney organoids.5

**Why is this study important?**

This study developed the first diabetic kidney organoid model, providing another potential platform to investigate pathophysiology of DKD. Indeed, diabetic kidney organoids displayed some key pathologic features reminiscent of kidneys under diabetic conditions, such as tubulointerstitial fibrosis and upregulation of ACE2 expression, which, in turn, conferred increased susceptibility to SARS-CoV-2 infection.3 However, limitations of the current kidney organoids make it challenging to recapitulate many hallmarks of DKD, including vascular defects, mesangial expansion, and glomerular basement membrane thickening.5 Functional vascularization of kidney organoids, especially glomerular vascularization, will substantially enhance the pathophysiological relevance of the diabetic kidney organoid model. This feat may be achieved via organoid multiplexing with vascular organoids,7 with the facilitation of a microfluidic device.

Diabetic kidney organoids demonstrated metabolic changes at the transcriptomic level.
More importantly, PT epithelial cells isolated from diabetic kidney organoids retained the metabolic memory after withdrawal of oscillatory glucose treatment, similar to PT epithelial cells derived from patients with diabetes. This study made an intriguing observation that oscillatory glucose treatment downregulated PGC1α expression, whereas it upregulated mitochondrial respiration and ATP production in kidney organoids.7 Although PGC1α is upregulated in several models of diabetes where gluconeogenesis is elevated, recent studies demonstrated a renoprotective role of PGC1α via improving fatty acid oxidation.8 Kidney biopsy samples from DKD patients showed a strong reduction in the transcriptional regulators of fatty acid oxidation pathway, whereas most mouse models of DKD neither show changes in PGC1α levels nor develop progressive kidney fibrosis.9 Human kidney organoids may help address discrepancies caused by interspecies differences, while also considering the impact of diabetes medications prescribed to human patients. For example, future studies could examine whether sodium/glucose cotransporter 2 (SGLT2) inhibitors can alleviate some of the pathologic phenotypes in diabetic kidney organoids.

It remains to be elucidated how often SARS-CoV-2 directly infects the kidneys of COVID-19 patients, and whether diabetes exacerbates COVID-19 specifically through ACE2-mediated infection. Jansen et al.4 detected SARS-CoV-2 in all kidney samples from 62 patients with COVID-19; however, another study with kidney samples from 284 patients with COVID-19 only detected SARS-CoV-2 nucleocapsid protein by immunohistochemistry in 3.7% of cases.5 Single-nuclear RNA-sequencing analysis of kidney autopsy tissue from one patient showed that almost all of the 14 identified cell clusters expressed SARS-CoV-2.6 Interestingly, not all cell types in kidney organoids expressed SARS-CoV-2 on infection. Although Jansen et al.4 detected SARS-CoV-2 in PT cells, podocytes, loop of Henle cells, and some stromal cells, Garreta et al. demonstrated predominant PT expression of SARS-CoV-2.5 On the one hand, the observed differences between different kidney organoid models could be explained by the varying differentiation protocols and viral infection schemes employed in each of the studies. On the other hand, differences between studies in patient samples and those in organoids suggest that current kidney organoid models may not precisely mimic the reciprocal interaction between SARS-CoV-2 and human kidney in vivo, because of a lack of functional circulatory and immune systems.

Notwithstanding these limitations, organoid models offer a valuable opportunity to evaluate the efficacy of candidate compounds in suppressing SARS-CoV-2 infection. Jansen et al.4 demonstrated that cotreatment with a protease inhibitor effectively suppressed SARS-CoV-2 RNA levels in kidney organoids, and Garreta et al.5 showed that pretreatment of pyruvate dehydrogenase kinase inhibitor DCA reduced SARS-CoV-2 infection in HPTCs derived from patients with diabetes. Future studies could further refine the diabetic kidney organoid model to enhance its pathophysiological relevance, making it a versatile platform for modeling patient-specific disease phenotypes, assessing novel diabetes risk loci, and evaluating drug efficacy.

DISCLOSURE
All the authors declared no competing interests.

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