Pancreatic β-cell fate in subjects with COVID-19

Subjects with coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), are still increasing worldwide as of August 2021. The association of COVID-19 with diabetes has been reported in the context of severity, complications such as diabetic ketoacidosis, and the new onset of diabetes. Impairment of insulin-producing pancreatic β-cells is a key contributing factor to the abovementioned diabetic pathogenesis by COVID-19. However, whether β-cells are damaged by SARS-CoV-2 through a direct action remains controversial. Some reports have suggested that SARS-CoV-2 may not directly infect β-cells because of the low expression of angiotensin-converting enzyme 2 (ACE2) and transmembrane serine protease 2 (TMPRSS2), which are host proteins required for SARS-CoV-2 entry into cells, in the endocrine pancreas\(^2\).\(^3\). The serine protease TMPRSS2 primes the viral spike protein, and the spike glycoprotein binds to the ACE2 receptor, allowing SARS-CoV-2 to enter host cells. In addition to ACE2 and TMPRSS2, many other factors, including neuropilin-1 (NRP1), FES Upstream Region (FURIN), heparan sulfate, transferrin receptor (TFRC), Ras-associated binding 7A (RAB7A), cathepsin L (CTSL), transmembrane protein 41B (TMEM41B), TMEM106B, switch/sucrose non-fermenting (SWI/SNF) chromatin remodeling complex members, and other proteins, are reportedly involved in SARS-CoV-2 infection\(^3\).\(^5\). However, whether each factor contributes to the entry of SARS-CoV-2 into β-cells is still unclear. Furthermore, it is uncertain what happens in the β-cells and what changes will occur in the β-cells of subjects with COVID-19. Two recent reports in Cell Metabolism\(^6\).\(^7\) denoted the potential of SARS-CoV-2 to directly infect β-cells and the possible fate of β-cells under COVID-19.

Wu et al.\(^6\) from Stanford University indicated that SARS-CoV-2 infected human β-cells through NRP1, which resulted in increased β-cell apoptosis and reduced glucose-induced insulin secretion (GSIS) (Figure 1). They first confirmed the messenger RNA (mRNA) expression of ACE2, TMPRSS2, NRP1, TFRC, and FURIN in human β-cells by datasets of single-cell RNA sequencing (scRNA-Seq). Notably, immunofluorescence revealed stronger NRP1 and TFRC expression in β-cells but not in α-cells. Both the SARS-CoV-2 nucleocapsid protein (NP) and spike protein (SP) were detected in β-cells at 2 or 6 days post infection (dpi) when healthy human islets were infected with SARS-CoV-2 ex vivo. Pretreatment of human islets with EG00229, an NRP1 antagonist, decreased the NP-positive β-cells after infection with SARS-CoV-2 at 2 dpi, suggesting that SARS-CoV-2 requires NRP1 to enter β-cells. Pathological analysis showed the existence of SARS-CoV-2 NP and spike mRNA in insulin-positive β-cells from 4 out of 7 subjects with COVID-19. NRP1, but not ACE2, expression was elevated in β-cells of COVID-19 donors compared with non-COVID-19 donors. SARS-CoV-2 infection reduced the insulin content in islets and the insulin secretion from β-cells under high glucose conditions, and these functional impairments were recovered, at least in part, by NRP1 antagonism with EG00229. SARS-CoV-2-infected human islets from healthy donors demonstrated an increase in TUNEL-positive apoptotic β-cells. Treatment with the recombinant SARS-CoV-2 SP S1 subunit also induced β-cell apoptosis. They also demonstrated that SARS-CoV-2 SP upregulated the phosphorylation of apoptotic/proapoptotic kinases, such as p21-activated kinase (PAK) and c-Jun N-terminal kinase (JNK), in human islets by phosphoproteomic analysis.

Meanwhile, Tang et al.\(^7\) from Weill Cornell Medicine showed that SARS-CoV-2 resided in the endocrine pancreas of subjects with COVID-19 and that SARS-CoV-2 provoked β-cell transdifferentiation via the eukaryotic translation initiation factor 2 (eIF2) signaling pathway (Figure 1). They first exhibited high expression of NRP1 in human β-cells, similar to the report from Tang et al.\(^7\). SARS-CoV-2 NP was detected in insulin-positive β-cells as well as in platelet endothelial cell adhesion molecule (PECAM-1)-positive endothelial cells, keratin 19-positive ductal cells, trypsin-positive acinar cells, and vimentin-positive mesenchymal cells in a pathological analysis of pancreas from subjects with COVID-19. The expression of FURIN, CTSL, and SARS-CoV-2 viral RNAs was observed in pancreatic endocrine and exocrine cells, endothelial cells, and immune cells by scRNA-Seq of human islets infected with SARS-CoV-2. SARS-CoV-2 NP was also detected in all cell sets, and the proportion of NP-positive cells was greater in ACE2/NRP1 double-positive cells than in single-positive or double-negative cells. SARS-CoV-2-infected human islets expressed higher chemokines, cytokines, and interferon-stimulated genes. In addition, an Ingenuity pathway analysis further implicated the eIF2 signaling pathway in the effects of SARS-CoV-2 infection on human islets. Then, they focused on the increased expression of α-cell markers and acinar cell markers in β-cells affected by reduced insulin expression in SARS-CoV-2-infected human islets. The proportion of insulin-positive β-cells that also express glucagon, an α-cell marker, or trypsin, an acinar cell marker, was significantly increased under SARS-CoV-2 infection. The ratio of trypsin immunofluorescence-positive cells was...
also elevated in β-cells from subjects with COVID-19. Thus, β-cells in COVID-19 subjects might undergo transdifferentiation. The trajectory analysis of the scRNA-Seq data for SARS-CoV-2-infected human islets determined the process of β-cell transdifferentiation, and eIF2 kinase signals were identified as the most significant pathway under SARS-CoV-2-induced transdifferentiation by ingenuity pathway analysis. The phosphorylation levels of both protein kinase R (PKR), an eIF2 kinase, and eIF2α were elevated in a SARS-CoV-2-infected human β-cell line. High-throughput chemical screening using human pluripotent stem cell-derived endocrine cells identified trans-integrated stress response inhibitor (trans-ISRB) as a potential compound to decrease insulin- and glucagon-positive polyhormonal cells. Remarkably, treatment with trans-ISRB increased insulin expression and reduced glucagon, trypsin, and cell stress-associated genes in SARS-CoV-2-infected human islets or β-cells. These results suggest that trans-ISRB reversed β-cell transdifferentiation under COVID-19 conditions.

Another recent report showed that SARS-CoV-2 directly infected pancreatic β-cells, causing morphological changes in organelles and a decrease in insulin granules, leading to decreased insulin secretion. Each study has suggested the direct infection of β-cells with SARS-CoV-2; however, it is unclear whether NRP1, ACE2, or other factors are important for viral entry into β-cells. It is also unknown whether β-cells are more susceptible to infection than other islet endocrine cells, acinar cells, or endothelial cells. Since there were some cases of COVID-19 in which the SARS-CoV-2 antigen was not detected in β-cells, its associations with severity, ethnicities, and patient background in β-cell infection will also be informative in the future. It is also important to elucidate the infection route of SARS-CoV-2 to islets in the body. Is it transmitted through the bloodstream? Which organ does it migrate from? Experiments with appropriate animal models may be required to unveil the mechanism of transmission to the islets. The functions of islets and β-cells in vivo are closely controlled by interorgan networks with many other organs via humoral factors, nerves, or exosomes. Whether pancreatic β-cell damage in subjects with COVID-19 is due to direct infection of SARS-CoV-2 with β-cells or due to indirect effects, such as other organ damage, cytokine storms, and inflammatory responses, requires further investigation.

Even though diabetes is clearly associated with β-cell failure, the pathogenesis of β-cell dysfunction, especially in type 2 diabetes, remains controversial. β-Cells undergo insulin hyposecretion, adaptive growth defects, apoptosis, autophagy, dedifferentiation, or transdifferentiation, which are caused by metabolic or environmental stresses, including viral infection, during the development of diabetes. While one of these processes could cause β-cell dysfunction, several of these processes probably occur simultaneously under the same stress. The reports by Wu et al. and Tang et al. indicated that β-cell apoptosis and β-cell transdifferentiation were induced by direct infection with SARS-CoV-2. These two aspects of β-cell fate-determining mechanisms are not contradictory and are likely to occur in parallel. A reduced β-cell mass is also a hallmark of both type 1 and type 2 diabetes, and both β-cell apoptosis and β-cell transdifferentiation could be involved in the regulation of the β-cell mass. Further studies are warranted to
evaluate the β-cell mass in subjects with COVID-19.

Interestingly, Tang et al.7 implicated the eIF2-mediated integrated stress response (ISR) as a pathogenic process of SARS-CoV-2-infected β-cells. The ISR is a central regulator of protein homeostasis and is activated by various pathological conditions. A variety of different stresses are sensed by eIF2 kinases, namely, heme-regulated inhibitor (HRI, EIF2AK1), protein kinase RNA-activated (PKR, EIF2AK2), PKR-like endoplasmic reticulum (ER) kinase (PERK, EIF2AK3), and general control nonderepressible 2 (GCN2, EIF2AK4), which phosphorylate eIF2α to activate the ISR. ISR-mediated translational regulation plays a pivotal role in the process of ER stress, which is a major cause of β-cell failure in the development of diabetes. Therefore, increasing attention has been given to the ISR of β-cells in research on the mechanism of β-cell dysfunction. Protective effects of compounds that modulate ISR, such as the delta variant, or variants of interest (VOIs) may also differ in their organ-specific infectivity and pathogenic mechanisms in β-cells. The fight against SARS-CoV-2 will continue to develop more effective therapeutic strategies for diabetes. We sincerely hope for further research in protecting β-cells from COVID-19 to save people.

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DISCLOSURE
The authors declare no conflicts of interest.

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