Hypertension is the most common comorbidity associated with unfavorable outcomes in patients with coronavirus disease 2019 (COVID-19). This especially impacts the elderly population with its underlying high rate of hypertension. Emerging evidence also implicates the gastrointestinal tract in COVID-19, with ≈30% to 50% of patients presenting with gastrointestinal symptoms. Nasal, pulmonary, and gastrointestinal epithelia express high levels of ACE2 (angiotensin-converting enzyme 2), the receptor for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) cell entry. While the primary mode of viral transmission is inhalation of respiratory droplets, the gastrointestinal epithelium is the body site with the greatest ACE2 expression. Furthermore, a critical role for gut in COVID-19 pathophysiology is emerging that is potentially relevant for hypertension-COVID-19 comorbidity: (1) ≈30% to 50% COVID-19 patients manifest gastrointestinal symptoms, often before respiratory symptoms; (2) infectious SARS-CoV-2 has been detected in stool, with viral RNA shedding in feces for weeks; (3) all patients with COVID-19 show gastrointestinal symptoms, and some of those bacterial species adversely influence ACE2; (4) gut mucosa exhibits all components of renin-angiotensin system; and (5) the intestinal epithelium supports SARS-CoV-2 replication. These observations led us to hypothesize that increased ACE2 expression in gut epithelium would predispose hypertension patients to COVID-19 infection. We tested this hypothesis in organoid cultures from spontaneously hypertensive rats (SHR) using Wister Kyoto rats (WKY) as controls.

Results and Discussion

We first established culture conditions and compared basic properties of colon organoids between WKY and SHR. Colon was studied based on our previous data demonstrating colonic pathology and altered epithelial gene profile in the SHR. We observed that SHR had 14% shorter colons than WKY (SHR: 0.062 cm/gm versus WKY: 0.072 cm/gm; P<0.001, Figure [A]). This is consistent with observations of decreased colonic length in other chronic inflammatory diseases, such as colitis and could have important implications in absorption of key nutrients and the altered epithelial-microbiota cross-talk we proposed earlier.

Crypts containing colonic stem cells were cultured on the same batch of Matrigel matrix throughout. Organoids reached maximal size in 7 days and there were no differences in organoid size or viability between WKY and SHR (Figure [B]). However, SHR crypts formed 30% fewer organoids than WKY rats (SHR versus WKY, 3 days P<0.001, 5 and 7 days P<0.0001, Figure [C]). This is consistent with decreased colonic length in SHR and suggests that 3-dimensional organoids in culture maintain in vivo properties. Kit67 expression, a nuclear protein marker of cell proliferation, was comparable in both strains (Figure [D]). However, Krt20, a marker for enterocytes and goblet cells, was increased 2.4-fold in the SHR (Figure [D]). This is in line with RNA-seq data from colonic epithelium (Figure [E]) and suggests that decreased growth in SHR may result from increased differentiation or dysbiosis-enhanced epithelial autophagy, a view that needs further exploration.

Next, we compared Ace2 and transmembrane protease serine 2 (Tmprss2) expression. Tmprss2 is a membrane-anchored protease that is critical in the activation of SARS-CoV and SARS-CoV-2 spike protein, a necessary step in ACE2-mediated entry of these coronaviruses into cells. Ace2 mRNA was ≈2-fold enriched in SHR organoids compared to WKY (Figure [E]), with the increase confirmed by both immunostaining and Western blotting (Figure [F]). High magnification images in Figure [F] indicated Ace2 localized to the cell surface. Increased Ace2 mRNA in the SHR epithelium reinforced the preservation of epithelial properties in organoids. Tmprss2 mRNA levels showed a trend towards an increase in SHR organoids (SHR versus WKY, P=0.09, Figure [E]), although this increase was significant in SHR epithelium (Figure [E]). In contrast, B0AT1 mRNA levels were comparable in WKY and SHR. B0AT1 is a neutral amino acid transporter of Solute Carrier Family 6 and after forming the transporter of Solute Carrier Family 6 and after forming the

Ace mRNA was also increased in both SHR organoids and epithelium. ACE, unlike ACE2, is not a receptor for coronavirus entry into cells. However, we can only speculate about the relevance of increases in both ACE and ACE2 in...
Figure. ACE2 is unregulated in colonic organoids of hypertensive rats. 

A. Representative images of colon (left) and the ratio of colon length/body weight of Wistar Kyoto rat (WKY) and spontaneously hypertensive rat (SHR) rats (right), mean systolic blood pressures 211±4 and 137±3 mm Hg, respectively, measured by Tail-Cuff Plethysmography.

B. Phase microscopic images documenting growth of organoids from isolated colonic crypts of WKY and SHR rats stained with Trypan blue, dead cells are blue. Scale bar: 200 µm. Primary colon organoids were grown from isolated crypts (gentle dissociation reagent [Stem Cell Technologies] in Matrigel [BD Biosciences] and organoid growth medium [Stem Cell Technologies] supplemented with recombinant mouse Noggin [PeproTech] and EGF [BioLegend], recombinant human IGF-1, FGF-basic [FGF-2; BioLegend] and R-spondin1 [R&D], Y-27632 [STEMCELL Technologies], and A83-01 [Tocris] described elsewhere [Cell Stem Cell, 2018, 23(6): 787–793. e6]). (n=12 rat colon cultures/group.)

C. Organoid formation efficiency determined 3, 5, and 7 d after culture of isolated colonic crypts from WKY and SHR rats (n=12 per group; left) and 4% PFA-fixed organoids after 5 d in culture (right). (n=12 rat colon cultures/group; left; scale bar: 50 µm).

D. Representative confocal immunofluorescence images of Ki67 and KRT20 in organoids from WKY and SHR (left; scale bar: 50 µm); quantified and normalized fluorescent intensity (middle, n=5 per group). Fixed, permeabilized organoids stained with Ki67 and KRT20 specific antibodies (Abcam) and DAPI. ACE2 (Angiotensin-converting enzyme) and actin antibodies were from Abcam and Western blots were homogenates of organoids (2% SDS-Tris buffer, pH=7.5), run on 12% TGX precast gels, transferred to PVDF membranes (Biorad) and imaged on Odyssey imaging system with infrared light for Li-Cor Biosciences secondary antibodies. Fold change relative to WKY group. Values are means±SEM. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001, and ns P>0.05 vs WKY from SHR group, unpaired t test. PVDF indicates polyvinylidene fluoride.
hypertension and COVID-19. It may be that hypertension is driven by ACE-mediated increases in the vasodeleterious renin-angiotensin system axis. This is counterbalanced by an amplification of the protective ACE2 axis of the renin-angiotensin system during hypertension to maintain homeostasis. But this also increases the receptor for SARS-CoV-2 and, therefore, vulnerability to infection.

In conclusion, we have established organoid cultures from WKY and SHR which demonstrate much of the COVID-19-relevant physiology of the respective in vivo epithelial phenotypes. SHRs show increased Ace2, Ace and expression of Tmprss2. Thus, organoids provide an opportunity to investigate cellular and molecular interactions of components of SARS-CoV-2, the renin-angiotensin system and hypertension. Finally, caution is warranted to prevent over-interpretation of these data before this concept can be validated with our planned studies in organoids from patients with hypertension.

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Disclosures
None.

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