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ZINC40099027 ACTIVATES HUMAN FOCAL ADHESION KINASE BY ACCELERATING THE CATALYTIC ACTIVITY OF THE FAK KINASE DOMAIN

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Focal adhesion kinase (FAK) is a non-receptor tyrosine kinase that regulates gastrointestinal epithelial restitution and mucosal healing. ZINC40099027 (Zn27) promotes intestinal epithelial wound closure and heals mucosal injury in mice by activating FAK. However, whether Zn27 activates FAK directly or indirectly is unknown. We, therefore, sought to understand how Zn27 activates FAK when cells are treated with this compound. We evaluated Zn27 potential modulators of the key phosphatases that inactivate FAK (PTP-PEST, PTP1B, and SHP2), and performed in vitro kinase assays with purified FAK to assess direct Zn27-FAK interaction. In human Caco-2 intestinal epithelial cells, Zn27 stimulated FAK-Tyr-397 phosphorylation despite FAK-Tyr-397 inhibition with PTP-LID and did not affect PTP1B-FAK interaction. Conversely, in vitro kinase assays demonstrated that Zn27 directly activates both 125KDa full-length FAK and its 35KDa kinase domain. Zn27 increased both the apparent Km of ATP for FAK and the maximal activity (Vmax) of the kinase. Zn27 activated FAK-Tyr-397 phosphorylation despite the presence of the FAK inhibitor PF573228 in Caco-2 cells. Increasing PF573228 concentrations completely prevented the activation of the FAK kinase domain in vitro by a normally effective concentration of Zn27. Conversely, increasing Zn27 concentrations dose-dependently activated kinase activity and overcame PF573228 inhibition of FAK, suggesting the direct interactions of Zn27 with FAK may be competitive. These results suggest that Zn27 is a highly potent enhancer of FAK activity that interacts allosterically with the FAK kinase domain to increase FAK stability and activation. [Funding: The National Centre for Research and Development (LIDER/09/0055/18/16NCBR/2017)]
(ER) stress and release of Damage Associated Molecular Patterns (DAMPs), which act on enteric neurons and stimulate the production of neurotransmitters. The influence of ER stress on enteric neuron-derived vasoactive intestinal peptide (VIP) on the expression of electroneutral transporter Na+/H+ exchanger 3 (NHE3) was also examined. Methods: SARS-CoV-2 (2019-nCoV/USA-WA1/2020) was propagated in Vero-E6 cells. Caco-2, a human colon epithelial cell line, expresses the essential SARS-CoV-2 entry receptor ACE2 and was thus used for infection (MOI ~0.01). We used Western blotting to assess the expression of ER stress (phospho-PEK and Xbp1s) and DAMP (HMGB1) markers at 48 hours post-infection. Primary mouse enteric neurons were co-cultured with Caco-2 cells, pretreated for 24 hours with 2 μM tunicamycin to induce ER stress. Supernatants from enteric neurons were then assessed for expression of VIP by ELISA. Primary enteric neurons were treated with HMGB1 or ATF (another form of DAMPs), and the expression of c-FOS, a marker of neuronal activity, was determined by Western blotting and immunofluorescence staining. Results: We found that SARS-CoV-2 infection of Caco-2 cells led to increased expression of phospho-PEK and Xbp1s. Compared to uninfected control, infected Caco-2 cells-secreted HMGB1 into culture media, indicating epithelial production of DAMPs in response to SARS-CoV-2 infection. Tunicamycin was used to induce ER-stress and secretion of HMGB1 by Caco-2, mimicking SARS-CoV-2 infection. Importantly, enteric neurons co-cultured with tunicamycin-treated Caco-2 cells secreted significantly higher levels of VIP. Treated Caco-2 cells with tunicamycin or VIP on the basolateral side led to decreased surface NHE3 expression, suggesting a partial impairment of intestinal electrolyte fluid absorption. Moreover, HMGB1 and ATF both increased the expression of phospho-c-FOS in cultured enteric neurons, indicating DAMP-induced neuronal activation. Conclusions: Our findings demonstrate that enterocytes infected by SARS-CoV-2 release DAMPs with the capacity to induce VIP secretion by the enteric neurons, which in turn acts on enterocytes and inhibits apical localization of NHE3. These findings establish basic mechanisms relevant to diarrheal disease in COVID-19 patients and identify potential targets for the treatment of SARS-CoV-2 infection of the gastrointestinal tract.

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DEVELOPING AN ORGANOID MODEL TO UNDERSTAND THE BAT GASTROINTESTINAL EPITHelial RESPONSE TO THE SEVER ACUTE RESPIRATORY CORONA VIRUS-2 (SARS-CoV-2)

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The ongoing COVID-19 pandemic is caused by the severe acute respiratory corona virus-2 (SARS-CoV-2) which has of right now has infected 10% of world’s population and has caused ~1.5 million deaths worldwide. In addition to respiratory symptoms, COVID-19 causes nausea, vomiting and diarrhea in more than half of infected subjects. This indicates that SARS-CoV-2 not only infects the respiratory tract, but also the gastrointestinal. Bats are thought to be the original reservoir for SARS-CoV-2, since SARS-CoV-2 is ~96%-identical to the bat coronavirus RaTG13, which was identified in horseshoe bats. However, coronaviruses fail to cause overt disease in the bats, whereas strong cytopathic effects were observed in human respiratory and gastrointestinal epithelial cells upon SARS-CoV-2 infection. The goal of our research is to compare the response of primary intestinal epithelial cells of bats and human to SARS-CoV-2 infection in order to better understand the cellular mechanisms that allow bats to harbor coronaviruses without developing disease symptoms. To study the SARS-CoV-2 infection in bats, we have, for the first time, established organoids from the stomach, proximal and distal small intestine of three adult Jamaican Fruit Bats (Artibeus jamaicensis). Organoids were successfully generated from both fresh and frozen tissue and could be passaged at least 25 times and frozen and thawed with no apparent changes in growth and morphology. Microscopic analysis showed that bat gastric and intestinal organoids were composed of a simple columnar epithelium and secreted variable amounts of mucus. We also observed spontaneous development of gland and crypt structures, indicating appropriate differentiation (Fig. 1). When seeded on transwell inserts, both gastric and intestinal organoid cells consistently developed a transepithelial resistance, demonstrating intact barrier function. Using confocal microscopy, we showed that both gastric and intestinal organoids from bats expressed the gut hormone ghrelin (GHR), a key regulator of SARS-CoV-2 entry. Our innovative experimental platform will enable us to study multiple aspects of coronavirus infection including viral evolution and determinants of spillover events in a relevant primary cell model system. Importantly, we will utilize the bat organoid model to identify non-pathogenic cellular pathways that enable tolerance to SARS-CoV-2 in the reservoir hosts for this virus, potentially informing novel treatment strategies in human COVID-19 patients.

Methods: Neonatal teams attended all deliveries of COVID+ mothers from March to August 2020. Delayed cord clamping and skin to skin were avoided and all infants were admitted to NICU post-delivery. Infants were tested for COVID-19 at 24 and 48 hours of life. COVID+ mothers did not visit and discharge was arranged with COVID negative family members, if possible. No expressed breastmilk was used in the NICU. Instead, mothers were encouraged to pump and store milk and instructed on how to safely breastfeed at home. Results: A total of 23 singleton deliveries of COVID-19+ mothers were analyzed. The majority of mothers were either African American (n=15) or Hispanic/Latina (n=6). Sixteen COVID+ mothers (76%) were asymptomatic and five (24%) were symptomatic, including one with gastrointestinal symptoms. Mean [range] gestational age and birth weight were 36 [31-40] weeks and 2800 [1300-3500] grams, respectively. All infants tested COVID-19 negative. Average length of stay was 7 [3-30] days, excluding two preterm infants. While all infants received formula or donor breastmilk, 40% of mothers expressed breast milk postpartum and 30% of infants received some breastmilk after discharge. In 50% (n= 15) of cases, infants were discharged home net with their mothers but with COVID-19 negative caregivers. Conclusions: No vertical or horizontal transmission occurred during NICU hospitalization. The absence of skin-to-skin contact after delivery, extended hospital stay, low expressed breastmilk use, and discharge to caregivers outside home environments may affect the transmission of maternal flora to infants. As policies addressing COVID-19 evolve, further studies are warranted on how they affect the neonatal microbiome and may contribute to early life intestinal dysbiosis.