Estrogen regulates the expression of SARS-CoV-2 receptor ACE2 in differentiated airway epithelial cells

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INTRODUCTION

There are pronounced sex-related differences in the ongoing coronavirus disease 2019 (COVID-19) pandemic, with higher intensive care unit admissions and deaths in males compared with females (3–5). This is consistent with epidemiological reports from prior coronavirus outbreaks, such as the 2002–2003 severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome epidemics (1, 8). Murine studies have confirmed the increased susceptibility of males to pathogenic coronaviruses (CoVs). Interestingly, SARS-CoV-infected female mice treated with an estrogen receptor antagonist have a higher mortality rate compared with their vehicle-treated female counterparts. Overall, this suggests a protective role for estrogen signaling in the setting of a SARS-CoV infection (2).

The novel SARS-CoV-2 depends on angiotensin-converting enzyme 2 (ACE2) for cell entry and engages the serine protease transmembrane protease serine 2 (TMPRSS2) for priming of the viral spike protein (6). Therefore, both ACE2 and TMPRSS2 are crucial for the ability of SARS-CoV-2 to cause infection. Here, we sought to determine whether 17β-estradiol (E2), a primarily female sex steroid, can regulate the gene expression of ACE2 and TMPRSS2 in differentiated normal human bronchial epithelial (NHBE) cells.

MATERIALS AND METHODS

NHBE cells from a female donor were purchased from a commercial source (Lonza) and grown at the air-liquid interface (ALI) on collagen-coated porous cell culture inserts. During the 3-wk differentiation process, the NHBE cells were treated with either 100 nM E2 (Sigma-Aldrich) or vehicle (ethanol). Total cellular RNA was extracted with the RNeasy Mini Kit (Qiagen) according to the manufacturer’s protocol. Complementary DNA was synthesized with the Transcriptor Reverse Transcriptase Kit (Roche). Quantitative RT-PCR was performed with the LightCycler Green 480 SYBR Green I Master on the Roche LC480 Light Cycler (ABI). The following primers were used: 1) human ACE2 forward 5′-GGACCCAG-GAAATGTTGACA-3′ and reverse 5′-GGCTGCGAAAGTGA-CATGA-3′, 2) human TMPRSS2 forward 5′-CTCTGTGCACTAC-CCTGACC-3′ and reverse 5′-ACACCGATTTCTCCTCCTC-3′, and 3) human ribosomal protein S16 forward 5′-GCTTCTCCCTTTC- CGGGTGCG-3′ and reverse 5′-ACACGGATTTGCTACACCGACG-3′. Gene expression was calculated relative to the expression of ribosomal protein S16 (housekeeping gene) and is reported as copies of the gene of interest per 104 copies of ribosomal protein S16, as previously published (10).

RESULTS

Here we demonstrate that E2-treated NHBE cells expressed lower levels of ACE2 mRNA compared with the vehicle-treated controls (Fig. 1A). This E2-driven downregulation of ACE2 expression is particularly relevant, as the efficiency of ACE2 usage by SARS-CoV has been shown to be an important determinant of viral replication and disease severity (6, 9). Furthermore, we also show that the levels of TMPRSS2 mRNA were not affected by E2 treatment (Fig. 1B). Finally, we confirmed prior reports that undifferentiated NHBE cells express very low levels of ACE2 (data not shown) (7). Therefore, it is critical to use fully differentiated NHBE cells grown at the ALI to accurately study ACE2 gene expression.

DISCUSSION

Limitations of our study include the use of a single female donor of NHBE cells. Further studies with NHBE cells from multiple male and female donors are warranted. In addition, it is important to note that the E2 concentration used for our experiments is only seen under physiological conditions during pregnancy and not in nonpregnant women (11). Finally, we...
should emphasize that the observed E2-induced reduction of ACE2 mRNA might not necessarily translate into a reduction of ACE2 protein at the cell surface.

In conclusion, we have shown that E2 can regulate the expression of ACE2 in differentiated NHBE cells. Given the striking sexual dimorphism in the COVID-19 pandemic, it is important to further elucidate the mechanisms by which sex hormones regulate the cellular components required for SARS-CoV-2 infectivity and ability to cause life-threatening disease.

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