ACE2 as a Necessary Portal of Entry for SARS-CoV-2 Salivary Gland Infection: Examining the Controversy

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Objectives. The SARS-CoV-2 virus responsible for the COVID-19 pandemic primarily transmits through inhalation of aerosol droplets produced by coughing, sneezing, and talking. Aerosol droplet transmission produced by the oral cavity along with transcriptional databases reporting SARS-CoV-2 presumptive receptor ACE2 RNA in salivary glands implicated saliva in transmissions. Despite this, salivary glands as a direct site of infection and transmission remains unproven. Our objective was to assess human salivary glands and isolated salivary gland stem/progenitor cells (hS/PCs) for ACE2 and susceptibility to SARS-CoV-2 infection. **Methods.** Tissue and hS/PCs from different patients were used for experiments. RNA and protein were isolated from hS/PCs and analyzed by qPCR and western blots. A genetically modified HEK293T cell line expressing ACE2 at high levels served as positive control. hS/PCs and ACE2 HEK293Ts were processed for flow cytometry for ACE2 detection. A receptor-agnostic fluorescently labeled SARS-CoV-2 spike protein was used to assess direct binding to cells by flow cytometry. Two variants of SARS-CoV-2, the first corresponding to the original alpha virus and the second corresponding to a later circulating omicron variant, were tested. Fresh frozen salivary gland tissue and formalin fixed frozen small intestine tissue, a known infection target, were sectioned and immunohistochemistry (IHC) performed for tissue level ACE2 detection. **Results.** hS/PCs had low transcript levels for ACE2 assessed by qPCR. No ACE2 protein was detected by flow cytometry or western blot. Both fluorescently labeled spike proteins showed high binding to ACE2 HEK293Ts but neither bound to surfaces of salivary hS/PCs. IHC failed to detect ACE2 in salivary glands, but did recognize ACE2 in intestinal sections. **Conclusions.** Based on these data, it is unlikely that healthy salivary glands express ACE2 at levels to support infection via this route. Lack of binding of SARS-CoV-2 spike proteins to hS/PCs indicates these cells are not a primary infection site.