Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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SHORT COMMUNICATION

Malignancy going viral: ACE2 and TMPRSS2 expression in conjunctival neoplastic diseases

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Angiotensin converting enzyme 2 (ACE2), the human receptor for severe acute respiratory syndrome coronavirus (SARS-CoV-2), is expressed in healthy human conjunctiva (Grajewski et al., 2020). Entry of SARS-CoV-2 into host-tissue occurs after attachment of the viral hemagglutinin protein to ACE2 that is followed by cleavage of the hemagglutinin by its coreceptor transmembrane serine protease, subtype 2 (TMPRSS2) to activate viral entry (Stopsack et al., 2020). Here, we examine the expression of both ACE2 and TMPRSS2 in conjunctival neoplastic lesions. This is an important issue as cancer tissue has been shown to have an impact on expression levels of these entry molecules for SARS-CoV-2 (Kong et al., 2020), while conjunctivitis has been reported as an ocular manifestation of coronavirus disease 2019 (COVID-19)(Xia et al., 2020). Furthermore, ophthalmologists and eye-care personnel have been described at risk for transmission of COVID-19 due to close contact to patients during examination (Rokohl et al., 2020).

After obtaining written informed consent, ACE2 and TMPRSS2 were, for the first time to our knowledge, detected in formalin fixed paraffin-embedded sections of healthy and neoplastic conjunctival tissue using mouse IgG2a anti-human ACE2 (R&D Systems, catalog # MAB933, clone #171606; dilution 1:100) at 5 μg/mL for 60 min at room temperature (RT) and rabbit IgG anti-human TMPRSS2 monoclonal antibody (Abcam, catalog# ab92323, clone EPR3861; dilution 1:000) at 0.477 μg/mL for 30 min at RT. Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using Target Retrieval Solution (pH 9 for ACE2, pH 6.1 for TMPRSS2 Dako, catalog # S2367, S1699). Tissue was stained using DCS DetectionLine, Polylink and Peroxidase Label HRP (DCS, catalog # PD000RP) and as the substrate chromogen AEC+ High Sensitivity (DakoCytomation, catalog # K3461). The counterstain was hematoxylin (blue).

Immunohistochemistry demonstrated ACE2 expression in healthy conjunctiva as well as in conjunctival nevus and melanoma but not in conjunctival carcinoma (Fig. 1 A, D, H, and K). In contrast, we detected TMPRSS2 in all these conjunctival entities, respectively (Fig. 1 B, E, I, and L).

Immunopositivity for ACE2 and TMPRSS2 was specifically confined to the conjunctival epithelium in healthy conjunctiva (Fig. 1A and B) but extended to neoplastic tissue in nevus and melanoma (ACE2 and TMPRSS2, Fig. 1 D, E, H, and I) as well as carcinoma (TMPRSS2, Fig. 1L). Similar results were obtained in all three sections that were performed on the tissue samples of two patients per group.

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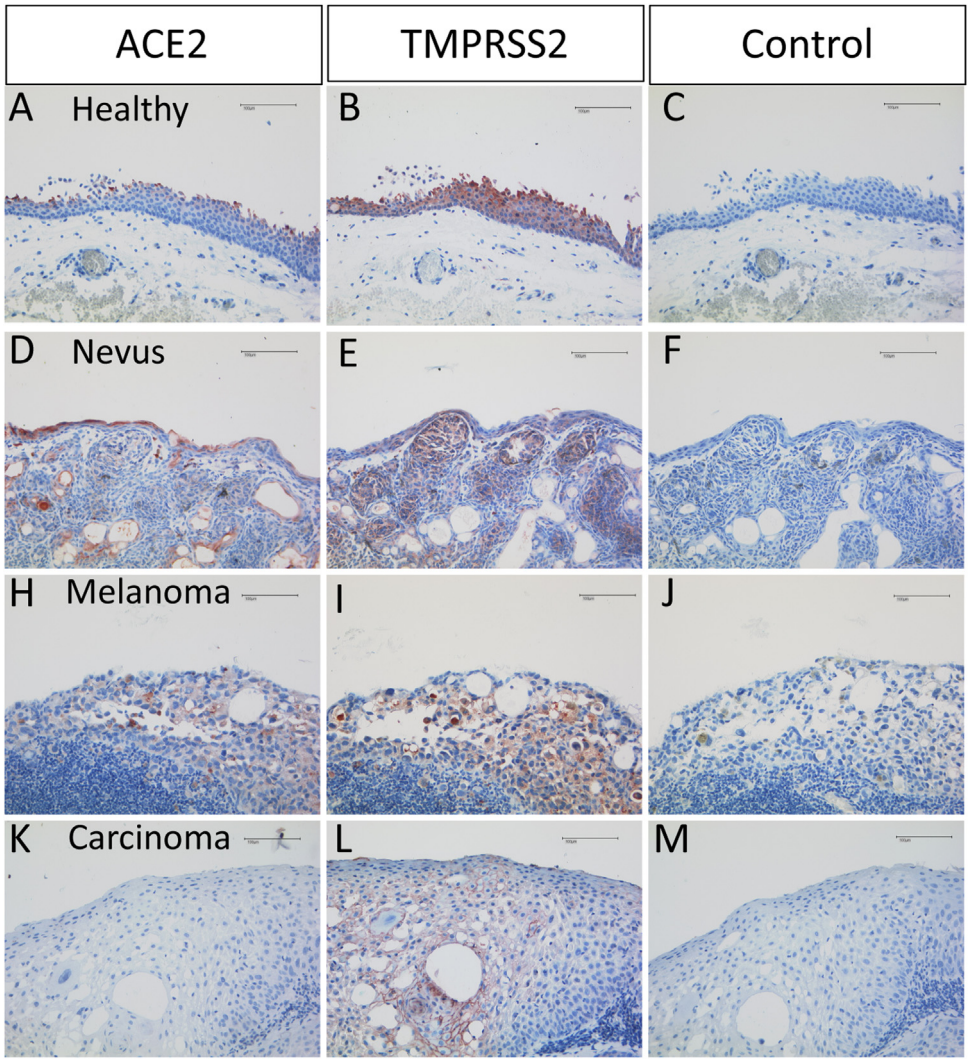

Fig. 1. Angiotensin converting enzyme 2 (ACE2) is expressed in healthy human conjunctiva (A) and in conjunctiva affected by nevus (D) and malignant melanoma (H) but not carcinoma (K), whereas transmembrane protease, serin subtype 2 (TMPRSS2) is expressed in all these tissues, respectively (B, E, I, and L). Scale bar, 100 μm.

Staining without the primary antibody (Fig. 1 C, F, J and M) demonstrated no staining. Human kidney tissue served as a positive control and showed a specific staining of epithelial cells in convoluted tubules, whereas human skin served as a negative control (data not shown).

Our results clearly demonstrate expression of ACE2 and TMPRSS2 in both healthy human conjunctiva as well as conjunctiva affected by the presence of a nevus and conjunctival melanoma. Furthermore, it appears that conjunctival carcinoma might downregulate conjunctival ACE2 expression, although this requires confirmation in a larger study for definitive conclusions. Patients with different types of cancer were shown to have a higher incidence of adverse events and this could also be related to differences in ACE2 and TMPRSS2 expression compared to patients without cancer (Kong et al., 2020). Ocular expression of ACE2 and TMPRSS2 has been demonstrated in human primary conjunctival and pterygium cell lines (Ma et al., 2020). Interestingly, this study showed a decrease of TMPRSS2 expression in association with pterygium, emphasizing the potential of conjunctival pathologies to modify expression of entry factors for SARS-CoV-2.

In summary, our results demonstrate a clear and specific ACE2 and TMPRSS2 expression in healthy and neoplastic conjunctival cells, providing the receptors for entry of SARS-CoV-2. Together, these findings emphasize the urgent need for further research regarding the eye as a possible alternative route for transmission of SARS-CoV-2 and the potential interaction of viral entry and replication with neoplastic and other conjunctival pathologies.

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Ethical statement

All authors agree upon standards of expected ethical behavior for all parties involved in the act of publishing

Conflict of interest

No author has any financial/conflicting/proprietary interests to disclose.

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