AI334 and AQ806 antibodies recognize the spike S protein from SARS-CoV-2 by immunofluorescence

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Abstract

The recombinant antibodies AI334 and AQ806 detect by immunofluorescence the spike S protein from SARS-CoV-2.

Introduction

The spike (S) glycoprotein (UniProt #P0DTC2) mediates attachment of coronaviruses to the host ACE2 receptor (through the Receptor-Binding Domain [RBD] in the S1 subunit) and fusion with the host cell membrane (through the S2 subunit) (Yan *et al.*, 2020). Two recombinant antibodies (AI334 and AQ806), originally developed against the SARS-CoV-1 S protein, were previously shown to recognize the SARS-CoV-2 S protein by ELISA (Hammel *et al.*, 2020). Here we show that they successfully detect by immunofluorescence the S protein from SARS-CoV-2 expressed in HEK 293T cells.

Materials & Methods

Antibodies: ABCD_AI334 and ABCD_AQ806 antibodies (ABCD nomenclature, https://web.expasy.org/abcd/) were produced by the Geneva Antibody Facility (http://www.unige.ch/medecine/antibodies/) as mini-antibodies with the antigen-binding scFv portion fused to a mouse IgG2A Fc. The synthesized scFv sequences (GeneArt, Invitrogen) correspond to the sequences of the variable regions of the clones CR3022 (ter Meulen *et al.*, 2006) and VHH-72 (Wrapp *et al.*, 2020), respectively. HEK 293T suspension cells (growing in FreeStyle™ 293 Expression Medium, Gibco #12338) were transiently transfected with the vector coding for the scFv-Fc of each antibody. Supernatants (50-100 mg/L) were collected after 5 days.

Antigen: HEK 293T adherent cells (growing in DMEM, Gibco 11960044, supplied with 10% FBS), cultured on glass coverslips (Menzel-Gläser, 22x22 mm) and transiently transfected 3 days before the experiment with a vector coding for the full-length SARS-CoV-2 S protein (BEI Resources, NR-52310, pCAGGS vector containing the full-length SARS-CoV-2/Wuhan-Hu-1 S glycoprotein coding sequence), were used to detect the viral protein. Non-transfected cells were used as a negative control.

Protocol: The whole procedure was carried out at room temperature. Transfected HEK 293T cells were fixed with PBS + 4% paraformaldehyde (w/v) (Applichem, #A3013) for 30 min, and blocked with PBS + 40 mM ammonium chloride (NH_4Cl) (Applichem, #A3661) for 5 min. Cells were then permeabilized in PBS + 0.2% saponin (w/v) (Sigma, #S7900) for 3 min, incubated in PBS + 0.2% (w/v) BSA (PBS-BSA) for 5 min, and then with the tested anti-S antibodies (final concentration 5 mg/L in PBS-BSA) for 1 h. After 3 washes (10 min) with PBS-BSA, cells were incubated for 30 min in PBS-BSA with secondary goat anti-mouse IgG conjugated to AlexaFluor-488 (1:400, Molecular Probes, #A11029). After 3 washes (10 min) with PBS-BSA, cells were mounted on slides (Menzel-Gläser, 76x26 mm) with Möwiol (Hoechst) + 2.5% (w/v) DABCO (Fluka, #33480). Pictures were taken using a Zeiss LSM700 confocal microscope, with a 63x Neofluar oil immersion objective.

Results

AI334 and AQ806 antibodies specifically detected a signal in HEK cells transfected with the SARS-CoV-2 S protein (Fig. 1). The specificity of the signal was verified by the absence of staining in non-transfected cells (Fig. 1, arrowheads).

References

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Conflict of interest

The authors declare no conflict of interest.

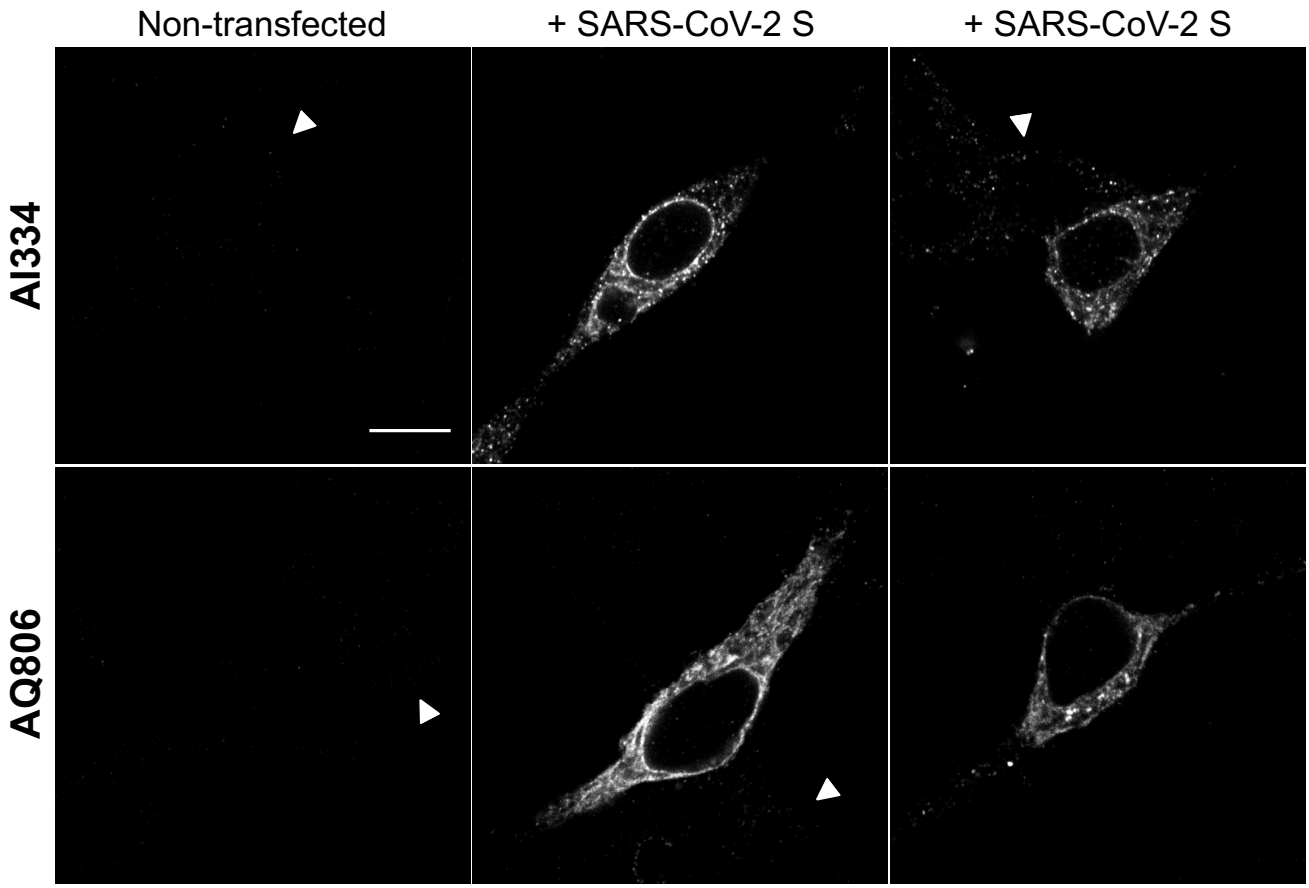

Fig. 1. AI334 and AQ806 labeled HEK cells expressing the SARS-CoV-2 S protein. No (or a very faint) labeling was seen in non-transfected cells (arrowheads). Scale bar: 10 μm.

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