AZ921 recognizes the endogenous USP7 protein by immunofluorescence in HCT116 cells

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Abstract

The antibody AZ921 derived from a synthetic VHH recognizes the endogenous USP7 protein in HCT116 cells by immunofluorescence.

Introduction

The USP7 protein (UniProt #Q93009) is a member of the Ubiquitin-specific protease family, which comprises more than 50 proteins. This family of proteins functions by removing ubiquitin and ubiquitin chains from various substrates. USP7 is implicated in numerous cellular processes, including virus replication and homeostasis, the p53 pathway, the cell cycle, and the anti-tumor immune response. It represents a promising target in oncology (Al-Eidan *et al*., 2022).

Materials & Methods

Antibodies: VHH G8-1 (ABCD_AZ921, ABCD nomenclature, http://web.expasy.org/abcd/) were selected from the NaliH1 synthetic VHH library according to Moutel *et al*., 2016. Biotinylated full-length USP7 was immobilized on Dynabeads M-280 (Thermo Fisher Scientific #11205D) and used for VHH selection by phage display. The concentration employed was 50 nM in the initial round and 10 nM for both the second and third rounds. The antibody was produced by the Geneva Antibody Facility (http://unige.ch/medecine/antibodies/) as a mini-antibody with the G8-1 VHH antigen binding domain fused to a rabbit IgG Fc.

Antigen: The full length USP7 protein (NM_003470.3, UniProt #Q93009) was produced in SF9 cells using pFastBac (Thermo Fischer Scientific) as described in Reverdy *et al*., 2022. The protein was biotinylated using the EZ-Link™ Biotin Labeling Kit according to manufacturer protocol (Thermo Fischer Scientific, EZ-Link™ Sulfo-NHS-Biotin # 21217).

Cell line: The immunofluorescence study utilized HCT116 cells transfected with a plasmid carrying short hairpin RNA (shRNA) against USP7 and regulated by the doxycycline-inducible T-REx™ system (Invitrogen). Briefly, the HCT116 cell line, which stably expressed the Tet repressor from pcDNA™6/TR, was transfected with the inducible expression plasmid pTER containing the shRNA targeting USP7. Induction of shRNA expression and subsequent inhibition of endogenous USP7 was achieved by treating the cells with doxycycline (1 µg/ml) for 5 days.

Protocol: The whole procedure was carried out at room temperature. Cells were rinsed once with PBS and fixed with PBS + 4% formaldehyde (w/v) for 15 min. Cells were then blocked and permeabilized in PBS + 0.1% triton + 2% BSA for 15 min, washed once (5 min) with PBS + Tween 0.1% (w/v) + BSA 0.2% (w/v) (PBS-T-BSA) and incubated for 1 hour with the AZ921 rabbit mini-antibody (0.25 µg/mL). After 2 washes (10 min) with PBS-T, cells were incubated for 30 min in PBS containing a secondary goat anti-rabbit IgG conjugated to Cy3 (1:400, Merk Millipore #AP132C). After 2 washes (10 min) with PBS, cells were incubated in PBS + Phalloidin Alexa-488 (Invitrogen, #A12379) (1/100e) for 10 min. After one fast wash in PBS, cells were mounted on slides with Möwiol (Hoechst) + 2.5% (w/v) DABCO (Fluka #33480). Pictures were taken using a confocal microscope, with a 40x objective.

Results

The AZ921 antibody labeled the nuclei in doxycycline-untreated cells, as depicted in Fig. 1 (-Dox). This staining pattern is consistent with the known cellular distribution of USP7 (van Loosdregt *et al*., 2013). Upon doxycycline induction, the nuclear staining strongly diminished in cells expressing shRNA against USP7. The effective inhibition of USP7 expression by doxycycline was validated through western blot analysis (data not shown). No signal was observed in the absence of the primary antibody (Fig. 1, No Ab). Based on these findings, we conclude that AZ921 specifically recognizes the endogenous USP7 protein in HCT116 cells.

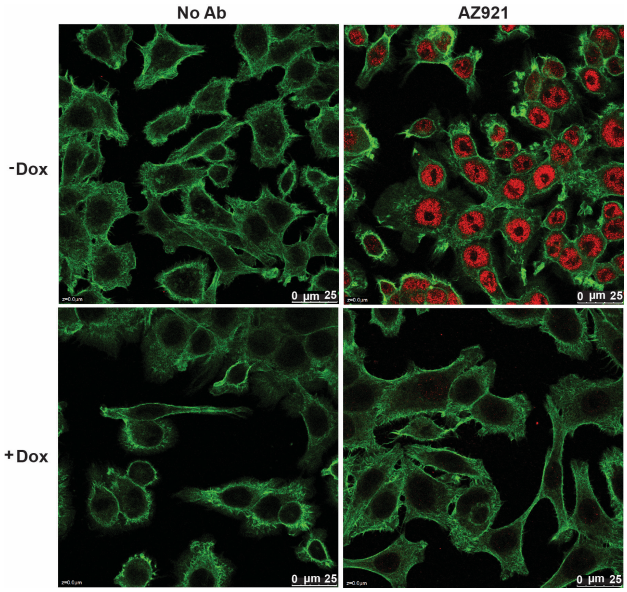

Fig. 1: HCT116 cells were fixed, permeabilized and stained with AZ921 (red) and phalloidin (green). The AZ921 antibody labelled the nuclei of HCT116 cells not treated with doxycycline. Doxycycline treatment inhibited USP7 expression, and abolished staining with AZ291. No labelling was seen when the AZ291 primary antibody was omitted (No Ab). Scale bar: 25 µm.

References

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Conflict of interest

The authors are affiliated and stockholder of Hybrigenics Services SAS