ABCD_AC459, ABCD_ AC967, ABCD_AH809, ABCD_AJ171 and antibodies ABCD_AK003 recognize EGFR by immunofluorescence

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Abstract

The recombinant antibodies ABCD_AC459, ABCD_AC967, ABCD_AH809, ABCD_AJ171 and ABCD_AK003 detect by immunofluorescence the human epidermal growth factor receptor (EGFR) at the surface of A-431 cells.

Introduction

The human epidermal growth factor receptor (EGFR; UniProt #P00533) is a transmembrane protein belonging to the receptor tyrosine kinase family (Ullrich *et al*., 1984). It orchestrates key cellular processes such as growth, survival and differentiation. Aberrant overexpression of EGFR is associated with several types of cancer, establishing it as a relevant target for cancer therapies. Human EGFR is highly expressed at the surface of the skin carcinoma A-431 cell line, a well-established model for studying EGFR-targeting antibodies (Wrann *et al*., 1979). In this study, we evaluated five recombinant antibodies for their ability to detect endogenous EGFR at the surface of A-431 cells by immunofluorescence. The tested antibodies incorporate variable domains derived from previously characterized anti-EGFR clones, all directed against domain 3 of the extracellular region of EGFR (see Table 1 for the original clone names and references). Notably, ABCD_AC459 (derived from matuzumab) originates from an hybridoma generated through mouse immunization with A-431 cells (Murthy *et al*., 1987). The five tested antibodies yielded a positive signal consistent with EGFR binding.

Materials & Methods

Antibodies: ABCD_AC459 (AC459), ABCD_AC967 (AC967), ABCD_AH809 (AH809), ABCD_AJ171 (AJ171) and ABCD_AK003 (AK003) antibodies (ABCD nomenclature, http://web.expasy.org/abcd/) were produced by the Geneva Antibody Facility (http://unige.ch/medecine/antibodies/) and expressed as minibodies, with the antigen-binding portion (VHH or scFv) fused to a rabbit IgG Fc domain (see Table 1 for clone names, formats, and references). VHH and scFv sequences were synthesized by GeneArt (Invitrogen). The scFv heavy and light variable regions were joined by a $(GGGGS)_3$ peptide linker. HEK293 (Human Embryonic Kidney) suspension cells, cultured in HEK TF medium (Xell #861-0001, Sartorius) supplemented with 0.1% Pluronic F68 (Sigma #P1300), were transiently transfected with the vector encoding each antibody. Supernatants (~110–200 mg/L; see Table 1) were collected after 4 days.

Table 1: Clone names, formats, references and production yields (mg/L) of the antibodies used in this study

ABCD	Clone Name	Format	Reference	Yield
AC459	Matuzumab	scFv	Kettleborough *et al*., 1991	200
AC967	Cetuximab	scFv	Goldstein *et al*., 1995	90
AH809	Zalutumumab	scFv	Van de Winkel *et al*., 2004	110
AJ171	EG2	Nanobody	Bell *et al*., 2010	180
AK003	7D12	Nanobody	Schmitz *et al*., 2013	180

Antigen:
Antibodies were tested against the endogenous EGFR protein expressed at the surface of A-431 human carcinoma cells (RRID: CVCL_0037), kindly provided by Prof. Olivier Sorg (University of Geneva). A-431 cells were cultured in DMEM (Gibco, Cat# 11960044) supplemented with 10% fetal bovine serum (FBS).

Protocol: The whole procedure was carried out at room temperature. A-431 cells were fixed with phosphate-buffered saline (PBS) + 4% paraformaldehyde (w/v) (Applichem, #A3013) for 20 min, and blocked with PBS + 40 mM ammonium chloride (NH_4Cl) (Applichem, #A3661) for 5 min. Cells were then permeabilized in PBS + 0.1% triton (w/v) (Sigma, #S7900) for 5 min, washed once (5 min) with PBS + 0.2% (w/v) bovine serum albumin (PBS-BSA), and incubated for 20 min with the recombinant antibodies (5 mg/L in PBS-BSA). After 3 washes (5 min) with PBS-BSA, cells were incubated for 20 min in PBS-BSA with secondary goat anti-rabbit IgG conjugated to AlexaFluor-488 (1:400, Molecular Probes, #A11029). After 3 washes (5 min) with PBS-BSA, cells were mounted on slides (Menzel-Gläser, 76x26 mm) with Möwiol + 2.5% (w/v) DABCO (Fluka, #33480). Pictures

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were taken using a Zeiss LSM700 confocal microscope, with a 63x Neofluar oil immersion objective.

Results & Discussion

Antibodies AC459, AC967, and AH809, AJ171 and AK003 were tested for their ability to detect the EGFR protein on the surface of A-431 cells by immunofluorescence. All tested antibodies produced a signal at the extracellular membrane, consistent with the established localization of EGFR. No signal was observed in the absence of the primary antibody (Fig. 1). Based on these observations, we conclude that all tested antibodies can detect EGFR by immunofluorescence. However, the identity of the recognized surface antigen was not confirmed, and off-target binding cannot be excluded. Therefore, antibody specificity should be validated in future studies using EGFR-deficient cell lines.

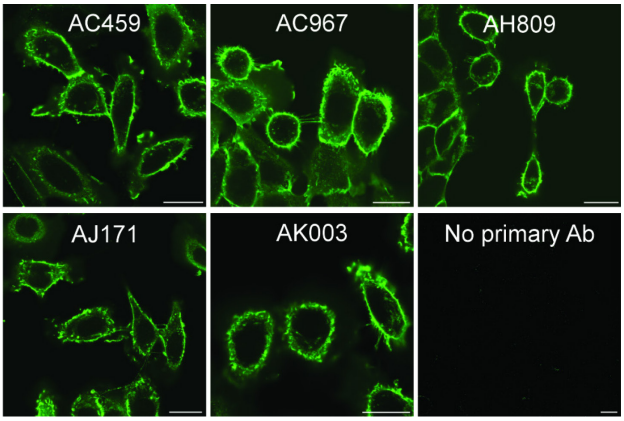

Fig. 1. Immunofluorescence staining of EGFR at the surface of A-431 cells using antibodies AC459, AC967, AJ171, AK003, and AH809. Scale bar: 20 µm

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Conflict of interest

Camille Mary is an editor of Antibody Reports.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.