ABCD_AC459, ABCD_AC967, ABCD_AK003 and ABCD_AH809 antibodies recognize the human protein EGFR by flow cytometry

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Abstract

The recombinant antibodies ABCD_AC459, ABCD_AC967, ABCD_AK003 and ABCD_AH809 against the human EGFR protein detect their antigen on A-431 cells by flow cytometry.

Introduction

The human EGFR protein (UniProt #P00533) is a transmembrane protein with an extracellular domain that binds the epidermal growth factor protein (EGF) and an intracellular domain with tyrosine kinase activity (Ullrich *et al*., 1984). Human EGFR is known to be highly expressed in the skin carcinoma cell line A-431 making this 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 flow cytometry. These antibodies incorporate variable domains derived from previously characterized anti-EGFR clones, all directed against domain 3 of the extracellular region (see Table 1 for the original clone names and references). Notably, ABCD_AC459 (derived from matuzumab) and ABCD_AC967 (from cetuximab) originate from hybridomas generated through immunization with A-431 cells. Four of the five tested antibodies yielded a positive signal consistent with EGFR binding.

Materials & Methods

Antibodies: ABCD_AC459 (AC459), ABCD_AC967 (AC967), ABCD_AJ171 (AJ171), ABCD_AK003 (AK003), and ABCD_AH809 (AH809) 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 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
AJ171	EG2	Nanobody	Bell *et al*., 2010	180
AK003	7D12	Nanobody	Roovers *et al*., 2011	180
AH809	Zalutumumab	scFv	Bleeker *et al*., 2004	110

Antigen: Antibodies were tested against the endogenous EGFR protein expressed at the surface of A-431 human carcinoma cells (RRID:CVCL_0037).

Protocol: The whole process was carried out at 4°C. $1.5x10^6$ A-431 cells were pelleted by centrifugation 5 min at 300g and washed once with washing buffer (PBS + 0.2% BSA (w/v)). Cells were then incubated for 20 minutes with the tested antibody (5µg/mL) in 1 mL of washing buffer. Negative control samples were incubated under identical conditions without the addition of primary antibodies. After two washes in washing buffer and centrifugation 1 min at 1000g, cells were resuspended and incubated for 20 minutes in the dark with 400 µL of secondary goat anti-rabbit IgG conjugated to Alexa Fluor 488 diluted 1/400 (Molecular Probe #A11034). After three washes in washing buffer and centrifugation 1 min at

1000g, cells were resuspended in 400 µL of PBS and analyzed with a CytoFLEX S4 flow cytometer (Beckman Coulter). Cell debris and dead cells were excluded based on forward and side scatter (FSC/SSC) gating. Fluorescence intensity was recorded using the AF-488 channel.

Results & Discussion

A-431 cells were incubated with primary antibodies or buffer (negative control), followed by staining with Alexa Fluor 488-conjugated secondary antibody. Flow cytometry analysis revealed a marked increase of A-431 cellular fluorescence intensity for the anti-EGFR antibodies AC459, AC967, AK003, and AH809 (Fig. 1). In contrast, only a weak fluorescence signal was detected with the AJ171 antibody. Based on these observations, we conclude that AC459, AC967, AK003, and AH809 successfully recognized endogenous EGFR in A-431 cells. The low signal generated by AJ171 may indicate that this antibody has a reduced affinity for EGFR compared to the other tested antibodies.

While these results are consistent with previous characterizations of the original antibodies as EGFR-specific, we did not directly confirm the identity of the surface antigen. Thus, off-target binding cannot be ruled out. Specificity should be verified in future studies using EGFR-deficient cell lines.

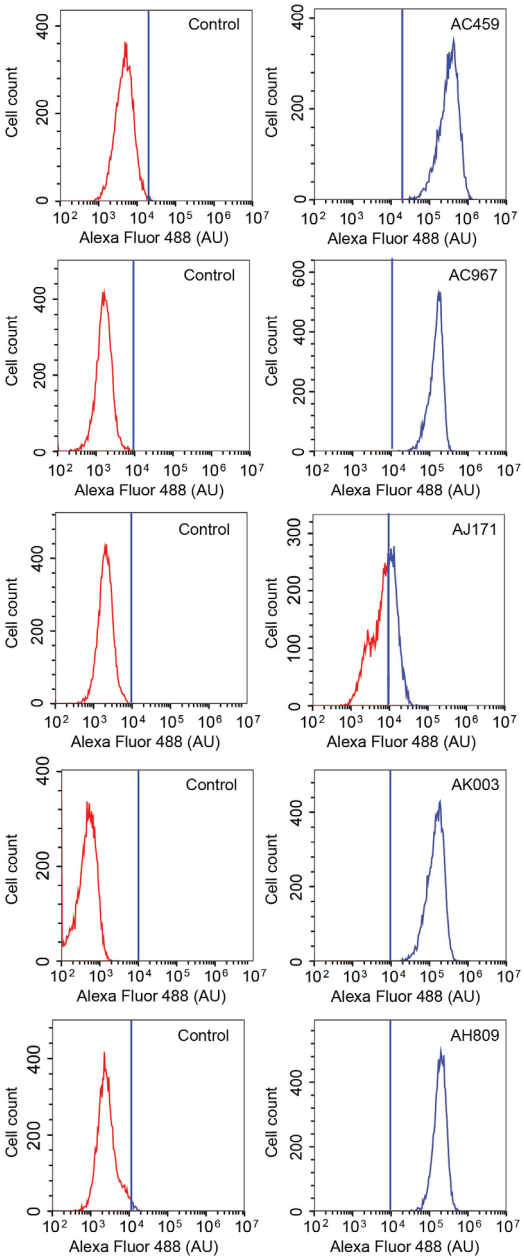

Fig. 1. Mono-parametric flow cytometry analysis showing the Alexa Fluor 488 signal versus the number of events (cell count). The control (left panels) corresponds to A-431 cells that were not incubated with the primary antibody. A vertical blue line indicates the highest level of cellular fluorescence observed in the control sample. Antibodies AC459, AC967, AK003, and AH809 efficiently detect the EGFR protein in A-431 human carcinoma cells.

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Conflict of interest

The authors declare no conflict of interest.

Data Availability Statement

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