AI179 single-chain antibody recognizes the Myc tag by Western blotting

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Abstract

The recombinant antibody AI179 against the Myc tag detects a Myc-tagged protein exogenously expressed in human cells by Western blotting.

Introduction

The Myc tag or c-Myc tag is a ten amino acid long peptide derived from the human protein c-Myc (Munro *et al.*, 1986), which is recognized by the monoclonal antibody 9E10 (Evan *et al.*, 1985).

Here we describe the ability of the single-chain variable antibody (scFv) AI179, derived from 9E10, to detect a Myc-tagged marker protein by Western blotting.

Materials & Methods

Antibodies: ABCD_AI179 (ABCD nomenclature, https://web.expasy.org/abcd/) targets the Myc tag epitope EQKLISEEDL. The antibody was produced by the Geneva Antibody Facility (https://www.unige.ch/medecine/antibodies/) as a mini-antibody with the antigen-binding scFv fused to the Fc region of a mouse IgG1. The synthesized scFv sequence (GeneArt, Invitrogen) corresponds to the sequence of the variable regions of the anti-Myc monoclonal antibody 9E10 (Evan *et al.*, 1985). HEK293 suspension cells (growing in FreeStyle™ 293 Expression Medium, Gibco #12338) were transiently transfected with the vector coding for the scFv-Fc. Supernatants (~20-100 mg/l) were collected after 5 days. Cell culture supernatant of the 9E10 hybridoma was used as a control antibody (a gift from Sandra Citi, University of Geneva).

Antigen: HEK293T cells were grown in Dulbecco's Modified Eagle's Medium supplemented with GlutaMAX, 10% fetal bovine serum and penicillin/streptomycin (100 u/ml) and transiently transfected with the expression vector His-Myc-H-Hsp90 (Wang *et al.*, 2009), which allows expression of full-length human heat shock protein 90 (Hsp90) α (UniProt #P07900) with a C-terminal Myc tag. Cells transfected with an unrelated expression vector were used as a negative control.

Protocol: Two days after transfection, cells were pelleted and lysed in 25 mM Tris-HCl pH 7.4, 250 mM sucrose, 5 mM $MgCl_2$, 1% IGEPAL CA-630 (Sigma-Aldrich #I30211), 1 mM DTT, protease inhibitor cocktail. After 10 min of incubation at room temperature, extracts were centrifuged at 16'100 g for 10 min, and the pellet was discarded. Samples were diluted 1:2 and 1:10 using an identically prepared cell lysate of HEK293T transfected with the control vector. 30 µg of proteins of the undiluted samples and the corresponding diluted samples were separated on a 10% SDS-polyacrylamide gel and then transferred to a nitrocellulose membrane. The membranes were blocked for 30 minutes in 5% w/v non-fat dry milk in Tris-buffered saline containing 0.2% Tween 20 (TBST), then incubated for 1 hour at room temperature with the different antibody dilutions in TBST. As a loading control, corresponding sections of the same membranes were probed with an anti-GAPDH antibody (HyTest, #5G4, dilution 1:5000) After incubation, the membranes were washed three times for 15 minutes with TBST. Finally, they were incubated with horseradish peroxidase-coupled goat anti-mouse antibody (Invitrogen #31430, dilution 1:10'000 in TBST) and washed again three times for 15 minutes. Chemiluminescent signals were recorded with a LI-COR Odyssey Fc Imaging System.

Results

Similarly to the original monoclonal antibody 9E10, the single-chain antibody AI179 detects the Myc-tagged Hsp90 protein by Western blotting (Figure 1).

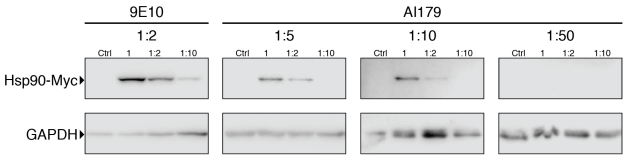

Fig. 1. AI179 detects Hsp90-Myc. Western blots of undiluted sample (1) and of the same volumes of the diluted samples (1:2 and 1:10). A lysate of cells overexpressing an unrelated protein without Myc tag was used as a negative control (Ctrl), and GAPDH as loading control.

References

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Acknowledgments

We thank Sandra Citi for the antibody gift. This work was supported by the Swiss National Science Foundation and the Canton de Genève.

Conflict of interest

The authors declare no conflict of interest.