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The H90-10 single-chain antibody recognizes Hsp90β by immunoprecipitation and Western blotting

Ernest Abboud, Lilia Bernasconi, Didier Picard

Département de Biologie Cellulaire, Université de Genève, Sciences III, CH - 1211 Genève 4, Switzerland

Abstract

The recombinant antibody H90-10 detects the endogenous human heat-shock protein 90 beta (Hsp90β) by immunoprecipitation (IP) and Western blotting.

Introduction

The mouse monoclonal antibody H90-10 specifically recognizes the Hsp90β (UniProt #P08238) isoform of the Hsp90 family (Holt *et al.*, 1999; Barent *et al.*, 1998). Here, we describe the ability of the single-chain variable antibody (scFv) H90-10 to immunoprecipitate the endogenous human Hsp90β and to recognize it by immunoblotting.

Materials & Methods

Antibodies: The ABCD_AO870 antibody (ABCD nomenclature, https://web.expasy.org/abcd) 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 mouse IgG2a. The synthesized scFv sequence (GeneArt, Invitrogen) corresponds to the sequence of the variable regions of the anti-Hsp90β monoclonal H90-10 (Holt *et al.*, 1999; Barent *et al.*, 1998) joined by a peptide linker $(GGGGS)_3$. The H90-10 variable sequences were determined with permission by Brian C. Freeman (University of Illinois, Urbana) from the H90-10 hybridoma originally from David O. Toft (Mayo Clinic, Rochester). HEK293 suspension cells (growing in FreeStyle™ 293 Expression Medium, Gibco #12338) were transiently transfected with the vector coding for the scFv-Fc. Supernatant (~90 mg/L) was collected after 5 days.

Antigen: Both wild-type HEK293T cells, which endogenously express Hsp90β, and their Hsp90β knock-out counterpart (Bhattacharya *et al.*, 2020) were grown in Dulbecco's Modified Eagle's Medium supplemented with GlutaMAX, 10% fetal bovine serum, and penicillin/streptomycin (100 U/ml).

Protocol: Cells were pelleted and lysed in lysis buffer (10 mM Tris-HCl pH 7.5, 1 mM EDTA, 10 mM NaCl, 10 mM Na-molybdate, 10% glycerol, 1 mM DTT, 0.1% Triton X-100) with 1x protease inhibitor complex for 50 min at 4 °C using a Bioruptur™ Twin sonicator. Extracts were centrifuged at 16'100 g for 10 min at 4 °C and the pellets were discarded. 500 µg of proteins were used for the IP. Samples were incubated overnight with murine IgG (Sigma-Aldrich #I5381) as negative control, H90-10, or scFv H90-10, diluted at 1:250, 1:50, and 1:50, respectively. The next day, 50 µl of Dynabeads™ Protein G (Invitrogen #10009D) were added for three hours and then washed 6 times for 10 min with lysis buffer. 50 µg extract (Fig. 1), and 20 µg input extract and IP samples (Fig. 2) were loaded on separate 10% SDS-polyacrylamide gel and then transferred to a nitrocellulose membrane (100 V, 105 min). The membranes were blocked for 60 min with 5% w/v non-fat dry milk in Tris-buffered saline containing 0.2% Tween 20 (TBST), then incubated overnight at 4 °C 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 1:5'000). After washing the membranes three times for 15 min with TBST, they were incubated for 90 min at room temperature with horseradish peroxidase-coupled goat anti-mouse antibody (Invitrogen #31430, dilution 1:10'000 in TBST) and washed again three times for 15 min. The immunoblot of the IP experiment was probed similarly as indicated. Chemiluminescent signals were recorded with a LI-COR Odyssey Fc Imaging System.

Results

The scFv version of H90-10 specifically recognizes Hsp90β (Fig. 1), and it immunoprecipitates Hsp90β as well as or better than the original monoclonal H90-10 (Fig. 2).

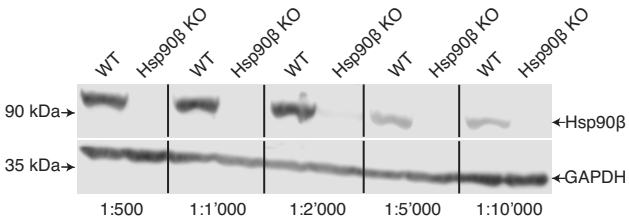

Fig. 1. Immunoblot showing specific recognition of Hsp90β by the scFv H90-10 antibody at different dilutions. Extracts of Hsp90β KO cells were used as the negative control and GAPDH as the loading control.

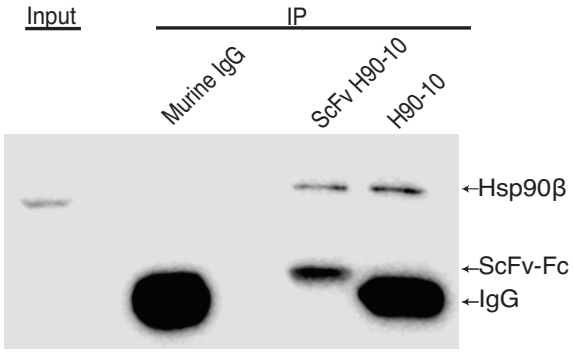

Fig. 2. Immunoblot of an IP experiment comparing the original monoclonal H90-10 and the scFv version, with normal murine IgG as negative control, probed with scFv H90-10 (dilution 1:1'000).

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Conflict of interest

The authors declare no conflict of interest.

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