Data in Brief 5 (2015) 752–755

Contents lists available at ScienceDirect

Data in Brief

journal homepage: www.elsevier.com/locate/dib

Data Article

The dynamic interactome of human Aha1 upon Y223 phosphorylation

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ARTICLE INFO

Article history:
Received 27 August 2015
Received in revised form
15 October 2015
Accepted 19 October 2015
Available online 6 November 2015

Keywords:
Cancer
Hsp90
Interactome
Proteomics
Phosphorylation
Chaperones
Aha1
Co-chaperones

ABSTRACT

Heat Shock Protein 90 (Hsp90) is an essential chaperone that supports the function of a wide range of signaling molecules. Hsp90 binds to a suite of co-chaperone proteins that regulate Hsp90 function through alteration of intrinsic ATPase activity. Several studies have determined Aha1 to be an important co-chaperone whose binding to Hsp90 is modulated by phosphorylation, acetylation and SUMOylation of Hsp90 [1,2]. In this study, we applied quantitative affinity-purification mass spectrometry (AP-MS) proteomics to understand how phosphorylation of hAha1 at Y223 altered global client/co-chaperone interaction [3]. Specifically, we characterized and compared the interactomes of Aha1–Y223F (phospho-mutant form) and Aha1–Y223E (phospho-mimic form). We identified 99 statistically significant interactors of hAha1, a high proportion of which (84%) demonstrated preferential binding to the phospho-mimic form of hAha1.

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium (http://proteomecentral.proteomexchange.org) via the PRIDE partner repository [4] with the dataset identifier PXD001737.

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http://dx.doi.org/10.1016/j.dib.2015.10.028

Specifications table

Subject area	*Biology*
More specific subject area	*Molecular chaperones, Mass spectrometry, phosphorylation*
Type of data	*^{18}O Quant LC–MS/MS Mass spectrometry data*
How data was acquired	*Mass spectrometry.* Thermo Q-Exactive Orbitrap
Data format	**.Raw*
Experimental factors	*HEK293 cells expressing either FLAG–hAha1–Y223E or FLAG–Aha1–Y223F*
Experimental features	*FLAG–hAha1–Y223E or FLAG–Aha1–Y223F complexes were purified by magnetic bead immunoprecipitation and processed by mass spectrometry*
Data source location	*The University of Chicago, Chicago, Illinois, USA*
Data accessibility	The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium (http://proteomecentral.proteomexchange.org) via the PRIDE partner repository with the dataset identifier PXD001737.

Value of the data

- This data provides a comprehensive interactome of human Aha1.
- This study examines the Aha1 interactome quantitatively $\pm$ Y223 phosphorylation.
- Identifies important client and co-chaperone proteins that are specifically altered by Y223 phosphorylation.
- Demonstrates a novel method for regulating Hsp90 function, a key molecule in cancer proliferation.

1. Experimental design, materials and methods

HEK293 cell lines expressing either hAha1–Y223F–FLAG or Y223E–FLAG were grown in Dulbecco's modified Eagle's minimal essential medium (DMEM, Invitrogen) supplemented with 10% fetal bovine serum [1–4]. All cell lines were propagated at 37 °C in an atmosphere containing 5% CO_2. Protein extraction from both HEK293 cells was carried out using methods previously described [5]. For immunoprecipitation, mammalian cell lysates were incubated with anti-FLAG antibody conjugated magnetic beads (Sigma) for 2 h at 4 °C and washed 4 times with fresh lysis buffer (20 mM Tris (pH 7.5), 100 mM NaCl, 1 mM $MgCl_2$, 0.1% NP40, Protease and Phosphatase inhibitor mini tablet, EDTA-free (Pierce). hAha1 complexes were eluted with FLAG peptide (Apex Bio).

2. LC–MS/MS data acquisition

2.1. Trypsin digestion of hAha1–FLAG complexes from SDS-PAGE gels

After hAha1 complexes were obtained, samples were processed as in [6,7]. Purified hAha1–Y223F–FLAG or Y223E–FLAG complexes were loaded onto a 12% MOPS buffered SDS-PAGE gel (Invitrogen) and run for 10 min at 200 v resulting in a $\sim$2 cm "gel plug". The gel was stained with 25 mL Imperial Stain (Pierce) at room temperature, and destained overnight in dH_2O at 4 °C. The gel plugs for each sample to be analyzed were excised by sterile razor blade, divided into 2 sections $\sim$1 cm each, and chopped into $\sim$1 mm^3 pieces. Each section was washed in dH_2O and destained using 100 mM