Identification of Scribble interacting proteins from HEK293 cells by Peptide Mass Fingerprinting and Mass Spectrometry

Abstract

The protein Scribble plays a critical role in cell polarity and organisation. In this study, we employed immunoprecipitation to isolate Scribble protein from HEK293 cells and identified it using peptide mass fingerprinting and MS/MS ION search. We compared the protein samples containing GFP only and GFP-Scribble to identify Scribble-specific proteins. The Mascot server analysis of the protein samples revealed the presence of two proteins, ARHG7_HUMAN and GIT1_HUMAN, indicating that these proteins interact with Scribble. ARHG6_HUMAN is a Rho family GTPase that regulates cytoskeleton dynamics, cell migration, and polarity. Meanwhile, GIT1_HUMAN is a scaffold protein that modulates cellular signalling pathways, including cell adhesion and migration. These results suggest that Scribble interacts with ARHG6_HUMAN and GIT1_HUMAN to regulate cellular functions. Overall, our findings provide insights into the role of Scribble in cellular signalling pathways and its potential implications for cellular function and disease pathogenesis

1. good, could name them in the title *[mmetod]*

2. **ARHG6_HUMAN** arh7 *[mmetod]*

Introduction

Scribble is a protein that is essential for cell polarity and adhesion. A scaffolding protein interacts with other proteins to keep epithelial tissues structural and functional. Scribble is also involved in cell signalling pathway control and tissue integrity preservation. Scribble has been reported to interact with several different proteins, including DLG, beta-catenin, and Lgl. These interactions are required for cell polarity control and developing adherens junctions Humbert *et al.* (2006), Zhan L. *et al.* (2005).

Proteomics is a branch of science that focuses on identifying, characterising, and quantifying proteins in a sample. It employs various methods, including mass spectrometry, chromatography, and bioinformatics. Understanding biological systems, particularly protein-protein interactions, requires the use of proteomics. Protein-protein interactions involving Scribble have been identified and characterised using proteomics approaches like co-immunoprecipitation, yeast two-hybrid experiments, and protein microarrays R. H *et al.* (2012), Nola.S *et al*. (2013).

The objective and specific purpose was to identify proteins uniquely interacting with Scribble in the sample immunoprecipitated from HEK293 cells expressing GFP-Scribble. The MS/MS data were processed using the Mascot service.

To summarise, Scribble is an essential protein involved in the control of cell polarity and cell adhesion, and its interactions with other proteins are vital for tissue shape and function preservation. Proteomics approaches have been critical in finding and characterising these interactions, as well as in offering insights into cellular processes and identifying possible therapeutic targets.

3. good *[mmetod]*

Materials and Methods

The following protocol has been adapted from the BS983 practical handbook and Metodieva *et.al.* (2016) for cell transfection, growth, and immunoprecipitation.

Immunoprecipitation

Cells were collected, washed with PBS, and lysed in a solution containing 1% Igepal 40, 50 mM Tris-HCl, pH 6.8, protease and phosphatase inhibitors, and protease and phosphatase inhibitors. The lysates were centrifuged and treated with 4µg anti-GFP antibody or anti-Scribble antibody on a rotator for 45 minutes at RT. 20µl magnetic protein A/G microbeads were added and incubated on the rotator for 1 hour at RT. Beads were washed thrice with 1 ml PBS containing 0.1% Tween 20 and once with 1 ml PBS containing no detergent. Between washing, the magnetic microbeads were pelleted using the Magnarack system. After removing the final wash volume, 20µl of trypsin solution (30 mg/ml trypsin in 25 mM NH4HCO3 containing 1 M urea) was added to the microbeads and digested for 16 hours at 30 °C.

Protein Identification by Peptide Mass Fingerprinting and MS/MS Data [1]

The protein analysis was done with a Mascot database search by the SwissProt database, choosing Mammalia (mammals) from the taxonomy and using the Trypsin enzyme. The peptide charges were 2+, 3+ and 4+ and consolidated the two samples and added 1 to both columns so that there would not be any missing value for further statistical data analysis. To evaluate the statistical significance of MS/MS data with the spectral counts for GFP, Scribble and co-precipitated proteins and computed p-values using the G test and did the adjustment of p-values by the Bonferroni adjustment of multiple comparison tests for the statistical analysis and did true adjusted p-values with the formula =IF(J2>1,1,J2). [2]

Results

We identified the statistically significant protein in sample 2, GFP scribe data at a low spectrum, which showed the mascot annotated the y and b fragments assigned to different parts spectrum.

Based on the MASCOT search results, the protein of interest is ARF GTPase-activating protein GIT1 from Homo sapiens. The peptide SLSSPTDNLELSLR was found in the protein and was identified in query 124 with a mass of 1530.790746. The peptide was found in the MS data file SAMPLE 2 gfp_scrib.mgf.txt, and the enzyme used was trypsin, which cuts on the C-terminal side of lysine and arginine residues unless the next residue is proline. The calculated monoisotopic mass of the neutral peptide is 1530.7889. The ions score was 90, and the expected value was 9.9e-08. Out of 128 fragment ions, 17 were matched using the 13 most intense peaks. The protein has a score of 457, a monoisotopic mass of 84289, and a calculated pI of 6.33.

1. **Peptide Mass Fingerprinting...**
only by msms
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2. mascot search parameters not described
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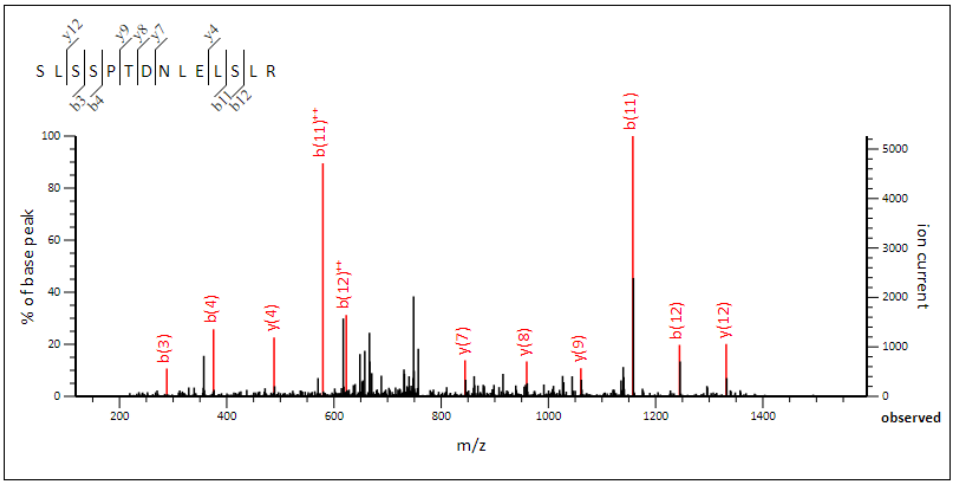

Figure 1. y and b fragments of the most intense peaks of GIT1_HUMAN peptide

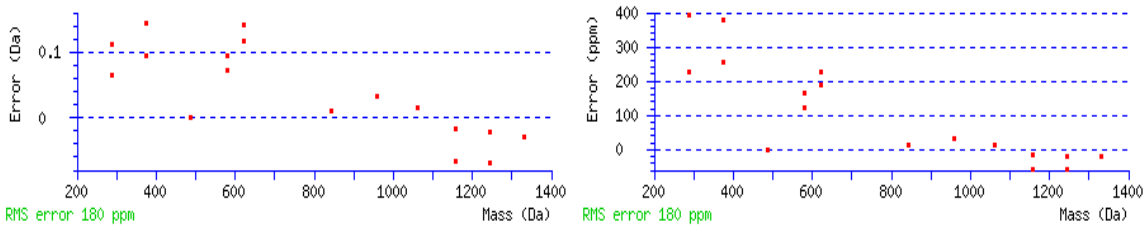

Figure 2. Graph showing an error of the mass management

Score	Mr(calc)	Delta	Sequence
90.0	1530.7889	0.0018	SLSSPTDNLELSLR
14.9	1530.8671	-0.0763	INFTPRVFPTALR
9.4	1530.8505	-0.0597	AEALVETGTVTLSIK
8.6	1530.7348	0.0560	DLDEVSKQELCPR
8.2	1530.8691	-0.0784	IDKMVVSAIVDTLK
8.2	1530.7711	0.0196	EAKPDELMDSKLR
8.0	1530.7063	0.0845	DRGEWDSLTPEAR
7.7	1531.8821	-1.0913	SSLSIALSSGLEKLK
7.7	1530.8116	-0.0208	CALSSPSLAFTPPIK
7.5	1531.7565	-0.9658	RMSLASAGFPPDQR

Figure 3. Table showing ion score with peptide fragments

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1. ok
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Report 2 shows that sample 2 gfp_scrib.mgf.txt contained the peptide VEEGGWWEGTLNGR found in the protein Rho guanine nucleotide exchange factor 7 (ARHG7_HUMAN) from the Homo sapiens species, as recorded in SwissProt. The protein has a score of 502, a mono-isotopic mass of 89955, and a calculated pI of 6.66. The Mascot search was performed with Trypsin as the enzyme, which cuts at the C-terminal side of Lysine and Arginine residues unless the next residue is Proline. The peptide VEEGGWWEGTLNGR had a mono-isotopic mass of 1588.7270, and the MS/MS fragmentation of this peptide was found in Query 140 with a mass of 1588.725560. The peptide had an Ion Score of 96 and an Expect value of 1.4e-08. Of 112 fragment ions, 15 matched using the 14 most intense peaks. These results suggest that the identified peptide is a potential biomarker or target for further investigation in the sample.

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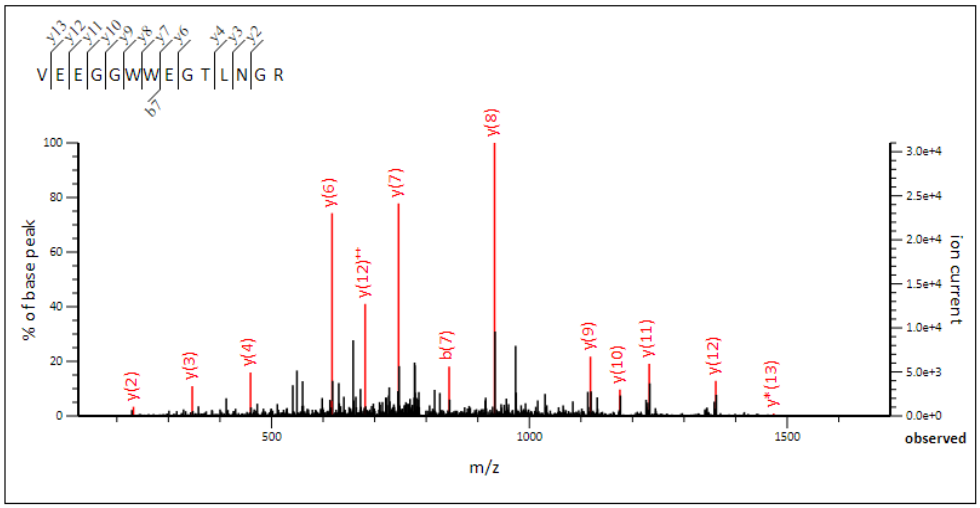

Figure 4: MS spectrum of y and b fragments of the most intense peak of ARHG7_HUMAN peptide

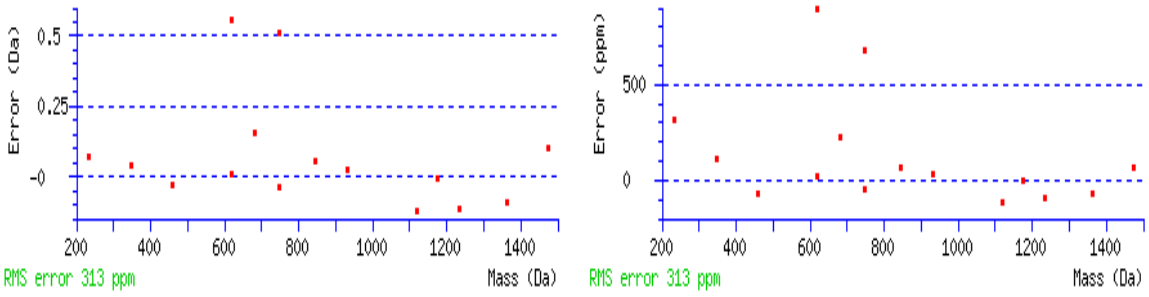

Figure 5. Graph showing an error of the mass management

Score	Mr(calc)	Delta	Sequence
96.1	1588.7270	-0.0015	VEEGGWWEGTLNGR
11.7	1589.8526	-1.1270	TKVFAAVQATNLDGR
11.4	1587.8369	0.8887	NLESIDPQFTIRR
11.1	1587.8077	0.9178	TALAGTRTAQGSGGGAGR
8.7	1588.7079	0.0177	DLENSDEFKSFMK
8.4	1588.8072	-0.0816	KVMLDWAEGNLWK
8.3	1588.9123	-0.1868	SRLGFLGLGGCGLIVK
3.5	1588.8317	-0.1061	VMDNNTTVKMVPIK
2.6	1587.8845	0.8411	GGRIAYQLSVQGLAR
2.3	1588.8130	-0.0875	VEIIMKETVDAEGR

Figure 6. Table showing ion scribe peptide fragments.

Discussion

ARHG7_HUMAN and GIT1_HUMAN are Scribble proteins that play crucial roles in cell polarity, migration, and adhesion. We employed human embryonic kidney cells (HEK293 cells) as a model system to explore these proteins. Mass spectrometry (MS) advances have allowed researchers to investigate proteins in greater depth in recent years. Isolating peptides from proteins and fragmenting them into smaller ions is the first step in an MS/MS ion search. Several researchers in HEK293 cells have employed MS/MS ion search to investigate ARHG7_HUMAN and GIT1_HUMAN. Future studies may use MS/MS ion search to examine these proteins in diverse cell types and circumstances. These proteins may be studied in cancer cells better to understand their involvement in tumor growth and metastasis. We can also utilize MS/MS ion search to find tiny compounds interacting with these proteins that might be turned into new medications.

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1. good analysis but could have presented the results better and should have described mascot parameters in methods

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