Protocols to examine integrin binding with Talin and protocol to test integrin activation by talin

Feng Ye
University of California at San Diego
Mark Ginsberg
University of California at San Diego

Method Article

Keywords: integrin, talin, integrin activation, integrin binding

Posted Date: January 12th, 2010

DOI: https://doi.org/10.1038/nprot.2009.217

Abstract

Introduction

Integrin activation is an important process in cell biology. Described here are two protocols. One is a general protocol of a cell based assay to test the ability of a protein \(in this case, talin) to activate integrin. The other is a protocol to test the interaction between integrin tail and its tentative binding partner.

Reagents

Cells: CHO cell that stably expressed integrin alphaIIb beta3 \(previously established). Termed A5 cells in the protocol Plasmid: plasmid encoding talin F2F3 domain Antibodies: PAC1 Alexa-647-antimouse IgM Other: Propidium Iodide \(PI) proteins: recombinant talin F2F3 and biotinated model integrin beta3 tail protein and integrin alphaIIb tail protein.

Equipment

FACScalibur flow cytometer.

Procedure

PROTOCOL 1 - Talin F2F3 activates integrin and talin mutations that disrupt integrin beta3 tail binding block activation. 1. On the day before transfection, plate 2~2.5 x 106 A5 cells on a 10cm petridish 2. On the day of transfection the cells should be 50% to 90% confluence. 3. Transfect A5 cells with the appropriate plasmids with fluorescent marker. \(In this case, co-transfect 0.1µg of EGFP plasmid as the transfection marker with either talin F2F3 or pcDNA as control.) 4. wait for 24 hours before FACS 5. Trypsin digest the cells, wash the cells with PBS buffer, and count cells. 6. For each experimental condition add 6 x 105 cells to a FACS tube 7. Pellet cells, 5 min 1000rpm benchtop centrifuge, at RT, decant supernatant 8. Resuspend cells in 50 microliter PBS buffer. Add activation specific antibody, PAC1 to assess the activation state of the integrins and incubate at room temperature for 30mins. 9. Add 500 microliter PBS buffer to wash the cells, then pellet the cells for 5 min at 1000rpm benchtop centrifuge. Decant supernatant. 10. Resuspend the cells with 50 microliter PBS buffer, add Alexa647-antiMouse IgM, incubate on ice for 30mins. 11. Add 1 microliter PI, incubate for another 5 mins. 12. Add 500 microliter PBS buffer to wash the cells, then pellet the cells for 5 min at 1000rpm benchtop centrifuge. Decant supernatant. 13. Resuspend the cells with 500 microliter PBS buffer and analyze the amount of PAC1 binding, which is a reflection of the integrin activation state. 14. Repeat the experiment with F2F3 mutant that does not bind to integrin beta3 tail. **PROTOCOL 2 - Integrin beta3 tail and talin binding assay**. 1. Wash Neutravidin agarose beads 3 times with saline. 2. Dissolve recombinant model integrin cytoplasmic domain \(biotinated) at 10mg/ml in dH2O. 3. Immobilize integrin cytoplasmic domains to the neutravidin beads at 0.8mg per 1ml 50% slurry beads. Incubate at 4 degree overnight. 4. wash the beads twice with

saline 5. wash the beads three more times with the desirable integrin tail-cytoplasmic protein binding buffer. \(in the case of talin, we use PN buffer consists of 20 mM PIPES, 50 mM NaCl, 150 mM Sucrose, 1mM Na3VO4, 50 mM NaF, 40 mM Na4P2O7*10H2O, 0.1% Triton X-100, pH 6.8) 6. Take a sample to run on SDS Page to determined the concentration of the integrin tail proteins on the beads. 7. Set up 700 microliter PN buffer containing recombinant talin F2F3 protein and F2F3 mutant at a protein concentration of around 20nM. 8. Take a sample of 20 microliter as the input control 9. add 40 microliter of neutravidin slurry \(50% slurry) to the 700 microliter F2F3 solution. Make sure the total amount of tail proteins are the same for different integrin tails based on the protein concentration measure in step 6. 10. Incubate the binding mixture at 4 degree overnight with end over end rocking. 11. Wash the beads for 5 times with PN buffer \(by spinning down at 2000rpm for 4 mins). 12. Elute the bound protein with 20 ul of SDS-PAGE loading buffer and analyze the eluent by SDS-PAGE. 13. After the electrophoresis is finished, cut off the gel below 25K to stain for the integrin tail capturing to make sure that equal amount of integrin tails are present for the experiments. 14. Transfer the rest of the gel to a nitrocellulose membrane and blot the membrane for talin binding with the appropriate antibody. 15. Compare the amount of integrin tail binding between F2F3 and F2F3 mutants.

Timing

2 days

References

Wegener KL, Partridge AW, Han J, Pickford AR, Liddington RC, Ginsberg MH, Campbell ID. Structural basis of integrin activation by talin. "Cell. 2007 Jan 12;128\(1):171-82.":http://www.ncbi.nlm.nih.gov/pubmed/17218263?itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum&ordinalpos=1 Calderwood DA, Fujioka Y, de Pereda JM, García-Alvarez B, Nakamoto T, Margolis B, McGlade CJ, Liddington RC, Ginsberg MH. Integrin beta cytoplasmic domain interactions with phosphotyrosine-binding domains: a structural prototype for diversity in integrin signaling. "Proc Natl Acad Sci U S A. 2003 Mar 4;100\(5):2272-7.":http://www.ncbi.nlm.nih.gov/pubmed/12606711?itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum&ordinalpos=4 Partridge, _et al_. Transmembrane domain helix packing stabilizes integrin alphaIIbbeta3 in the low affinity state. "_J Biol Chem._ 2005 Feb 25; **280** \(8):7294-300.":http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15591321 Hughes _et al_., Breaking the integrin hinge. A defined structural constraint regulates integrin signaling "_J Biol Chem_. 1996 Mar 22; **271** \(12):6571-4.":http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=8636068