Data Article

Living cell imaging and Rac1-GTP levels of CXCL12-treated migrating neural progenitor cells in stripe assay

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\textbf{A B S T R A C T}

This data article contains three figures and three videos related to the research article entitled “Applications of Stripe Assay in the Study of CXCL12-mediated Neural Progenitor Cell Migration and Polarization” Zhang et al. (2015) [1], which uses stripe assay to study mouse neural progenitor cell (NPC) migration and polarization. The current article describes the neurosphere method used to culture NPCs. NPCs in neurospheres and monolayer were characterized using immunocytochemistry method with antibodies against two classic NPC markers: nestin and SOX2. The article also describes method to obtain sufficient protein lysates from NPCs in the stripe assay. When protein lysates were subjected to Rac1 affinity precipitation, Rac1-GTP was detected in the pull-down samples. In addition, the articles provides live cell imaging data to better understand CXCL12-mediated cellular migration and polarization.

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1. The NPCs express both nestin and SOX2. The data can be referenced when identifying NPCs in the cultures.

2. Rac1 is an important signaling intermediate for migration and polarization. Sufficient amount of protein lysates can be acquired from stripe assay for Rac1-GTP pull-down. Detection of Rac1-GTP in the pull-down samples will be a valuable benchmark for future studies aiming to identify cell signaling during migration and polarization.

3. Stripe assay can be used to observe NPCs' migration and polarization toward CXCL12 stripes by live cell imaging. This data significantly extends the applications of stripe assay in cell biology.

1. Data

To better understand functional impacts of CXCL12 on NPC biology, NPCs were isolated from E13.5 mouse forebrains and enriched through neurosphere cultures [1]. A majority of mouse NPCs in neurospheres and monolayer (adherent) cultures expressed both nestin and SOX2 (Fig. 1). Because Nestin and SOX2 are markers for NPCs [2,3], the positive staining of nestin and SOX2 in our cultures suggests that these cells are indeed NPCs. Number of living cells are critical to study mechanisms of cellular migration and polarization. Stripe assay could obtain directional migrating cells for protein analysis. Levels of Rac1-GTP increased at 2 min by CXCL12 in the stripe assay (Fig. 2), suggesting that Rac1 is activated in the NPCs by CXCL12 stripes in the assay. Migration and polarization of cells are integrated processes of cell movement. In live cell imaging, NPCs were recorded in stripe assays migrating to CXCL12 stripes in 5 h (Video 1). More importantly, NPCs polarization was also recorded through live cell imaging in stripe assays (Video 2), but not control BSA stripes (Video 3), induced NPC polarization (Fig. 3).

Supplementary material related to this article can be found online at http://dx.doi.org/10.1016/j.dib.2015.09.048.

2. Experimental design, materials and methods

2.1. Characterization of mouse NPCs

Mouse NPCs were fixed using 4% paraformaldehyde (PFA), and permeabilized with 0.4% triton-X in PBS. After blocked by 1% BSA in PBS, mNPCs were incubated with primary antibodies (mouse anti-nestin, 1:200, Millipore; rabbit anti-SOX2, 1:500, Cell Signal Technology) overnight. Cultures were then washed
and incubated with secondary antibodies (Alexa Fluor 568 goat anti-mouse IgG, 1:500, Invitrogen; Alexa Fluor 488 goat anti-rabbit IgG, 1:500, Invitrogen) for one hour at room temperature. Nuclear DNA was labeled with 4',6-diamidino-2-phenylindole (DAPI; Sigma-Aldrich) for 2 min after the secondary antibody at room temperature. Cover slips were mounted on glass slides with mounting medium (Sigma-Aldrich). Triple immunostaining was examined by a Zeiss 710 confocal microscope.

### 2.2. Pull-down assay

Active Rac1 pull-down and detection kit (Thermo scientific) was utilized for the detection of Rac1-GTP level. The assay was performed according to the manufacturer’s instruction. The kit provides a GST-fusion protein containing the p21-binding domain (PBD) of human p21-activated protein kinase 1 (Pak1) along with...
glutathione agarose resin to specifically pull down active Rac1. Briefly, the cell lysates were incubated with the GST-Pak1 beads. Levels of bead-bound GTP-Rac1 and total Rac1 proteins were analyzed by immunoblot using an anti-Rac1 antibody (1:1000; Thermo scientific) and β-actin (Sigma) was as internal reference.

2.3. Living imaging experiment

After transfection of LiveAct-RFP and nuclear DNA stained with Hoechst33342, NPCs were dissociated with accutase (Gibco) into single cells. The stripe-coated dishes were seeded with dissociated NPCs to study living cell migration or polarization. Images were taken by times-series of Zeiss Live cell Imaging System.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.dib.2015.09.048.

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