Isolation of vessel-associated pdgfra-H2BGFP positive cells from murine brain

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Method Article

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Abstract

The _pdgfra_-H2BGFP reporter mouse visualizes expression of Pdgfra by expression of GFP bound to histones. These leads to a strong stabilization of the GFP molecule, leading to a) a strong nuclear signal and b) retention of fluorescence even after the cells no longer express Pdgfra. In the brain of this reporter mouse, the majority of cells expressing GFP are of the oligodendrocyte lineage. However, there is a small subset of cells that are not belonging to this lineage, and present an interesting anatomical location. They are localized in close proximity to the larger vessels in the brain. In order to properly study these cells with single cell sequencing technologies, prior enrichment for the perivascular subtype of Pdgfra expressing cells is recommended. Here, we describe a protocol that allows to focus on this subtype in more detail by first isolating brain vasculature prior to single cell suspension generation. The entire protocol aims to minimize time between brain dissection and FACS sorting, and thus only takes between 60 and 75 minutes.

Introduction

In the adult mouse brain, Pdgfra is mostly expressed by a subset of cells belonging to the oligodendrocyte lineage. However, a more rare type of cell, localized in close proximity to the larger blood vessels of the brain, also express Pdgfra. This protocol describes a method to enrich for the vessel-associated _Pdgfra_-H2BGFP expressing cells specifically. Flowcharts describing the protocol are attached

Reagents

- Animals: Pdgfra$^{tm11\(EGFP)Sor}$, here designated as _pdgfra_-H2B mice. - Collagenase type 2: ThermoFisher Scientific: C6885) - Dulbecco's modified Eagle Serum \(DMEM): ThermoFisher Scientific: 31885-023 - Dulbecco’s modified Eagle Serum \(DMEM) \(without phenol red): ThermoFisher Scientific: 31053-044 - Penicillin/Streptomycin \(P/S): ThermoFisher Scientific: 15140-122 - Dynabeads® Sheep Anti-Rat IgG, Thermofisher Scientific, 11035 - Rat anti-mouse CD31: BD Pharmingen, cat# 553370 - 2 ml Eppendorf tubes, any provider - 100 mm plastic Petri dishes of any provider - PBS with calcium and magnesium added: ThermoFisher Scientific: 14040091 - Fetal Bovine Serum \(FBS): Any provider - EDTA: Any provider - FACS buffer: DMEM without phenol red with 2% FBS and 1% P/S.

Equipment

- DynaMag™-2 Magnet: ThermoFisher Scientific, cat# 12321D - Centrifuge capable of centrifuging 15 ml tubes at +4ºC and 300g. - Tools for brain dissection - Rotator capable of incubation at 37ºC - Optional: Inverted microscope equipped with GFP filters for inspection of isolation efficiency - Sharp scissors

Procedure

Antibody preparation To be done overnight the day before, or minimum one hour before protocol start. 1. Resuspend the Dynabeads® in the vial \(i.e. vortex for >30 seconds, or tilt and rotate for 5 minutes). 2. Transfer 50 µL/brain of Dynabeads® to an Eppendorf tube. 3. Add 1 ml of PBS with 1% BSA and 2 mM EDTA \(referred to as 'washing medium' from now on), and resuspend. 4. Place the tube in the DynaMag™-2 Magnet \(henceforth referred to as 'magnet') for 1 minute and discard the supernatant. 5. Remove the tube from the magnet and resuspend the washed Dynabeads® in the same volume of washing medium as the initial volume of Dynabeads® \(50 µL per brain). 6. Add 10 µL of 0,5 mg/µl CD31 antibodies per brain. 7. Incubate overnight at 4°C or 1 hour at RT with gentle tilting and rotation. For total volumes below 150 µl, use a 200 µl PCR tube instead of a 1,5 ml Eppendorf. 8. Place the tube in a magnet for 1 min and discard the supernatant. 9. Remove the tube from the magnet and add 1 ml washing medium. 10. Place the tube in the magnet for 1 min, discard the supernatant, remove the tube from the magnet and resuspend the Dynabeads® in 50 µL washing medium per brain. Use this suspension in step 13 of the next paragraph **Isolation** 1. Preparation. Weigh two aliquots of 3 mg of collagenase type 2 powder. Keep at -20°C until ready to be used in step 4 and step 23. Note the different volumes of resuspending between the steps. 2. Kill the mouse using an approved method of euthanasia \(terminal anesthesia followed by heart perfusion with HBSS, or cervical dislocation). Remove the brain from the skull. 3. Transfer the brain to a Petri dish and chop it with sharp scissors for about 30 seconds, or until no large pieces are visible. The pieces should be small enough to pass through a cut pipette tip \(see next step). 4. Dissolve 3 mg of collagenase powder in 3 ml of DMEM 5. Transfer the brain pieces to a 5 ml tube and mix them with the collagenase solution. Use a p1000 with cut tip and wash the petri dish. 6. Mix the suspension well using the cut p1000 tip. The solution has to pass the tip freely with no blockage 7. Incubate the suspension for 5 minutes at 37 °C with rotation \(15 rotation per minute) 8. Pass the suspension 10 times through an uncut p1000 tip. The solution has to pass the tip smoothly. 9. Incubate the suspension for 5 minutes at 37 °C with same rotation 10. Wash the tissue suspension with 12 ml of DMEM \(room temperature). 11. Centrifuge the solution at room temperature, 5 minutes at 300g 12. Resuspend the pellet in 4,5 ml of DMEM, and divide the solution in 3 Eppendorf tubes of 2 ml \(i.e. approximately 1,7 ml per tube) 13. Add 15 µL of the antibody+beads solution in each eppendorf \(50 µL/brain). Pipet up and down several times to homogenize before using. 14. Incubate at room temperature for 30 min with gentle rotation 15. Place the three aliquots on the magnet and wait 2,5 minutes 16. Remove the supernatant 17. Add 2 ml of DMEM to the beads, remove the tubes from the magnet and resuspend the beads by gently tilting the tubes end-over-end until the beads are resuspended. 18. Repeat step 15-17 19. Place the tubes on the magnet and wait 2,5 minutes. Remove the supernatant 20. Pool the 3 tubes by resuspending the first pellet in 1,5 ml of DMEM, and use this resuspension to resuspend the pellets of the second and third aliquot. 21. Take 30 µl from the suspension and place a drop on a p100 Petri dish. Verify the presence of microvascular fragments on an inverted microscope with suitable excitation and filters for GFP. If successful, Pdgfra-H2BGFP positive nuclei will be visible at this stage, still attached to larger vascular fragments. See Figure 1 for an example See figure in Figures section. 22. Place the tubes on the magnet and wait 2,5 minutes. Remove the supernatant 23. Resuspend the beads in 1 ml of Collagenase single cell digestion buffer \(3 mg/ml of collagenase type 2 in DMEM) and incubated for 5 minutes at 37 °C with rotation 24. Using a 1ml syringe, pass the suspension firmly,

but without creating bubbles or foam, through a 20 G needle for 10 times. 25. Incubate the single cell suspension for 5 minutes at 37 °C 26. Remove the beads by placing the suspension on the magnet for 2,5 minutes. Remove the supernatant containing the cells. 27. Centrifuge the cells at 300g for 5 minutes at 4 °C 28. Resuspend the cell pellet in 500 µl of FACS buffer. Proceed with FACS

Timing

Antibody preparation: Overnight, or 1 hour prior to protocol start Main protocol from brain dissection to single cells at 4 °C: 60-75 minutes.

Anticipated Results

The protocol should yield an enrichment for the _Pdgfra_-H2BGFP cells that are vessel associated. One brain can yield 500-1000 cells, but for single cell sorting in a 384 well format, use of two brains per plate sort is recommended

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Figures

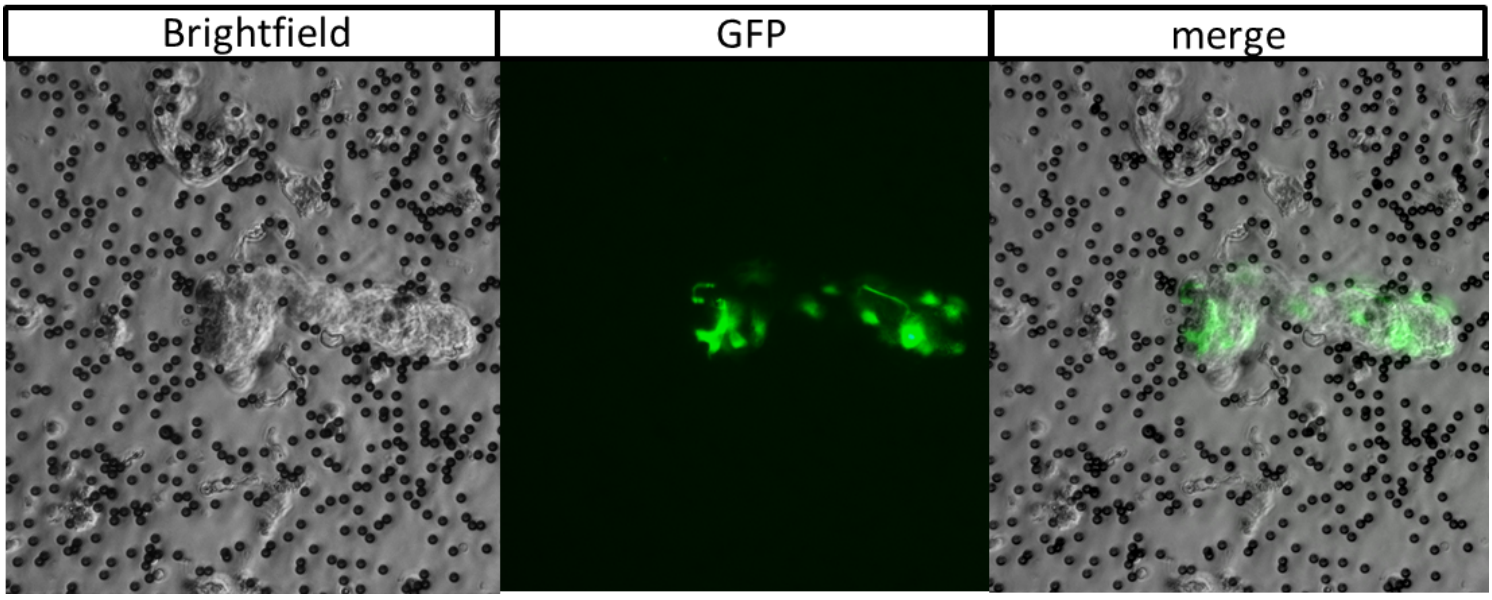

Figure 1

Isolation of GFP positive vessel associated cells This figure illustrates the result after step 21. Note the presence of the beads as round, opaque balls, and appearance of vascular fragments. The central right

large fragment clearly contains GFP positive cells as seen on the middle and right panes. Also, note that the smaller vessels in the image do not contain GFP positive cells, as these cells only associate with the larger vessels in the adult mouse brain. For more information, see the associated publication.

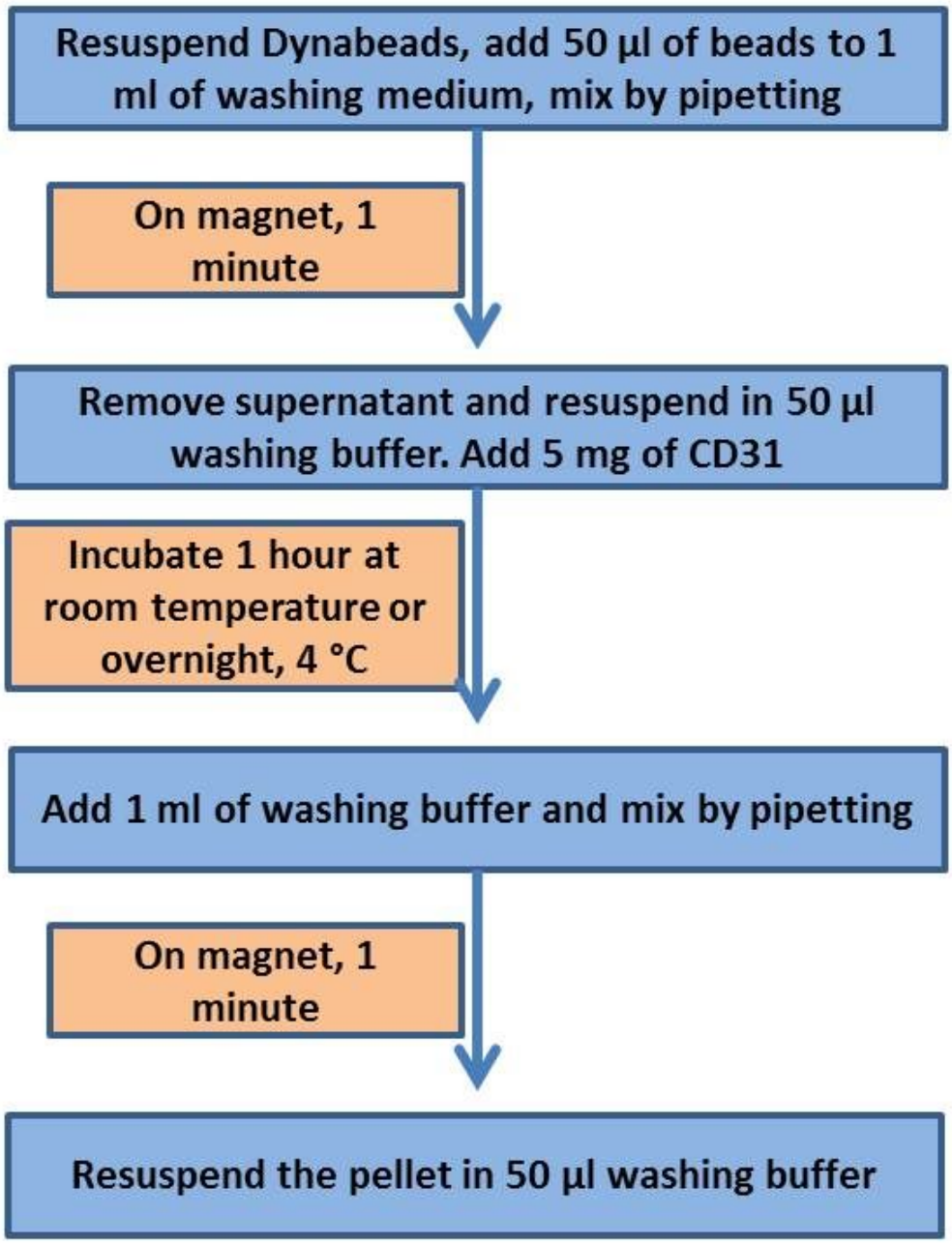

Figure 2

Flowchart Ab Flowchart describing coupling of the CD31 antibodies to the Dynabeads

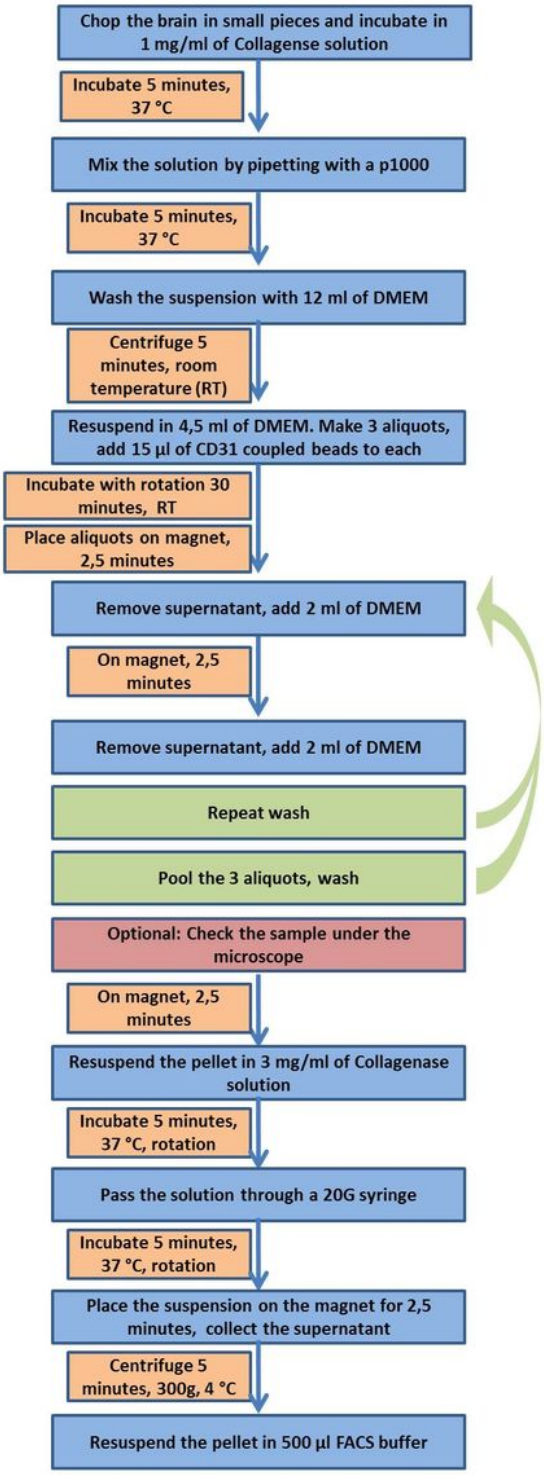

Figure 3

Flowchart peeling Flowchart describing the steps in the isolation protocol