Characterization of Microglia Progenitor Cells in Mixed Glia Cultures  
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Abstract

Microglia are the innate immune cells of the brain. Our lab has identified in the mouse brain CD11b expressing cells with a microglia-like morphology that express the stem cell marker Nestin and the astrocyte marker GFAP. We hypothesize that this population of cells are microglia progenitors. To further characterize microglia progenitor cells, we used a mixed glia culture system, which has been traditionally used to study microglia cells in vitro. By isolating the cells attached to the culture flask (“attached cells”) using FACS, we successfully isolated Nestin and CD11b positive and negative populations. Using FACS we also sorted the cells free-floating in the culture medium (“floating cells”) and isolated Iba-1, CD11b and Nestin expressing cells. We then confirmed expression of Nestin, Iba-1, and CX3CR1, a receptor expressed by microglia in the brain, by qPCR. These preliminary results begin to shed light on the molecular signature of microglia progenitor cells that are present in mouse neonatal mixed glia culture systems. This study will aid future research efforts aimed at characterizing microglia progenitor cells in different disease paradigms.

Introduction

• Microglia are myeloid cells that regulate innate immune responses in the Central Nervous System (CNS).
• Recent studies have shown that the adult mouse CNS has the capacity to repopulate microglia after genetic or pharmacological depletion.
• Previously the Garden lab has shown that CD11b+ microglia isolated from the adult mouse brain expresses GFAP and the stem cell marker Nestin.

Hypothesis

We hypothesize that microglia progenitor cells can be identified in neonatal mixed glia cultures and that these cells will express CD11b and Nestin.

Methods

Figure 2. Workflow for preparing “floating” and “attached” cells for FACS. Cortices from P3-P4 wild-type mice were isolated and used to create a single suspension that was placed into two T-25 flask and cultured for one week. After one week in vitro, cells floating in the media (“floating cells”) and cells attached to the flask (“attached cells”) were collected separately and fixed using PaxGene, then stabilized in PaxGene Stabilizer over night at 4°C. To prepare for qPCR, we used fluorophore conjugated antibodies against CD11b (eBioscience) and Nestin (Miltenyi Biotec) or Iba-1 (WAKO). Total RNA was extracted from sorted cells and nestin, cx3cr1 and Iba-1 expression was quantified by qPCR.

Results

Figure 3. Sorted attached and floating cells by FACS. “Attached” and “floating” cells were PaxGene fixed for one hour at room temperature and stabilized in PaxGene Stabilizer over night at 4°C. The “attached” cells were stained with fluorophore conjugated antibodies against CD11b (eBioscience) and anti-nestin (Milenyi). “Floating” cells were stained with a rabbit anti-Iba-1 antibody (WAKO) detect Iba-1, then stained with the CD11b and nestin antibodies prior to sorting. All negative and positive population combinations (corresponding quadrants) were collected for the “attached” and “floating” cell samples.

Figure 4. Nestin, cx3cr1, and Iba-1 expression in sorted populations from the “attached” cells. qPCR was performed on all RNA samples using a 96-well plate, house made primers, a 2x PCR master mix and a Universal Probe Library. Nestin and CD11b positive samples showed higher level of Nestin and cx3cr1 expression compared to other sample types.

Conclusion and Future Work

• We isolated Nestin+ and CD11b+ or Nestin− and CD11b− cells using FACS.
• We confirmed Nestin and CD11b expression in the sorted samples by qPCR. Iba-1 was undetected in the sorted “attached cells” sample.
• Nestin, CD11b and Iba-1 expression was undetected by qPCR in the “floating cells.”
• Optimize sorting of “floating cells” with Iba-1 stain. Characterize gene expression signature of “floating cells” by qPCR.

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References

