Efficient isolation of viable primary neural cells from adult murine brain tissue based on a novel automated tissue dissociation protocol

Miltenyi Biotec

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Introduction

Tissue dissociation and preparation of single-cell suspensions with high cell viability and a minimum of cell debris are prerequisites for reliable cellular analysis, cell culture, and cell separation. As dissociation of adult brain requires sophisticated mechanical and enzymatic treatment to successfully disaggregate the tightly connected neural cells, cell analysis is often restricted to embryonic or neonatal rodent tissue. We have set up technologies for dissociation of neonatal brain by combining automated mechanical dissociation using the gentleMACS™ Octo Dissociator with an optimized enzymatic treatment. To extend the analyses to adult neural cells we have further optimized the method by including a novel protocol for removal of debris and erythrocytes, which is crucial for effective cell isolation and culture.

The standardized process allows fast and reproducible dissociation of adult murine brain tissue and was optimized to increase the number of viable cells. Protocols for the magnetic isolation (MACS® Technology) of astrocytes, oligodendrocytes, neurons, microglia, and endothelial cells to high purities were also established and cultivation conditions were optimized to sucessfully cultivate adult neural cell populations. Furthermore, highly purified astrocytes were subjected to single-cell mRNA sequencing analysis in order to characterize neonatal and adult astrocyte diversity. In summary, we present a novel standardized technology to generate highly purified and viable adult neural cells that extends the analysis from neonatal to adult murine brain tissue and facilitates sophisticated cellular and molecular analyses.

Results

1 Automated dissociation of adult mouse brain tissue

We developed a novel process for the automated dissociation of adult rodent brain tissue by combining an optimized enzymatic treatment with gentle mechanical dissociation using the gentleMACS Octo Dissociator with Heaters. Although the dissociation process was optimized to get the highest possible number of viable cells, the resulting cell suspension still contained a significant amount of cell debris (fig. 1A) and red blood cells (fig. 1B), which hampers subsequent cell isolation and cultivation.

To overcome this drawback, we included a novel protocol for removal of debris and erythrocytes, which led to a substantial increase in the percentage of neural cells (fig. 1 C, D). The optimized tissue dissociation process including removal of debris and erythrocytes yielded 3–5×10[6] living cells per adult mouse brain with a viability rate of 70–80%. The required reagents are available as Adult Brain Dissociation Kit, mouse and rat.

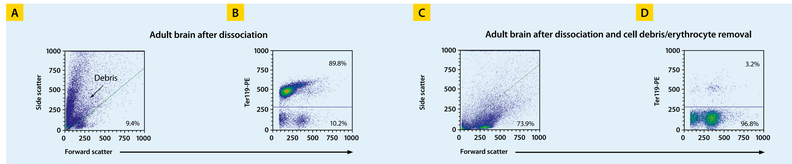

Figure 1

2 Isolation and cultivation of astrocytes from adult mouse brain tissue

After tissue dissociation using the Adult Brain Dissociation Kit, astrocytes were labeled with MACS MicroBeads coupled to antibodies specific for the astrocyte marker ACSA-2 (astrocyte cell surface antigen-2) and isolated using MACS Technology (fig. 2A). Cells were stained with Anti-ACSA-2-PE before and after separation (fig. 2B) for flow cytometry analysis (MACSQuant® Analyzer). Enriched astrocytes showed a purity of 94±5% and a viability of 69±10.3%. A total number of 4×10[5]±0.7×10[5] astrocytes was obtained from one adult mouse brain (n = 15).

Isolated astrocytes were cultivated in MACS Neuro Medium supplemented with MACS NeuroBrew-21 on PLL/Laminin–coated 24-well glass bottom imaging plates. After 7 days, cells were fixed and subjected to immunocytochemical analysis using GLAST- (green) and GFAP (red)–specific antibodies. Cultivated cells formed a dense layer of GLAST/GFAP–positive astrocytes (fig. 2C), whereas only a low number of neurons or oligodendrocytes, and no microglia were detected (fig. 2D).

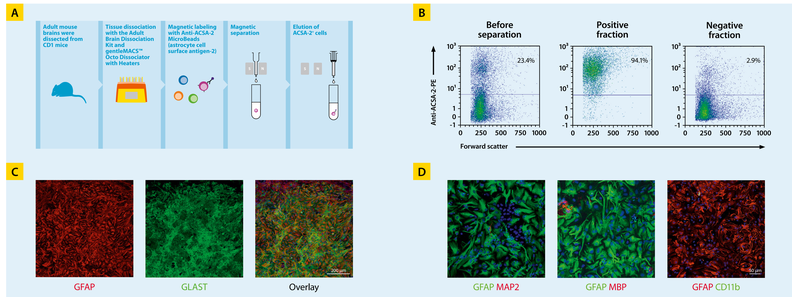

Figure 2

3 Neonatal and adult astrocyte diversity quantified by single-cell mRNA sequencing

In order to characterize astrocyte diversity, we isolated astrocytes from dissociated neonatal (P0–2) and adult mouse brain and analyzed their transcriptome by single-cell mRNA sequencing. Neonatal and adult astrocytes were separated using the astrocyte-specific Anti-ACSA-2 MicroBeads with a purity of 98.4±0.5% (n = 5) and 97.8±0.8% (n = 7), respectively. Cell viability was 87.7±5% for neonatal astrocytes and 74±8.8% for adult astrocytes. Then, the C1™ Single-Cell Auto Prep System (Fluidigm®) was used for single-cell capturing and preparation of cDNA from neonatal and adult astrocytes. cDNA sequencing libraries were prepared using the Nextera® XT DNA Library Preparation Kit and Nextera XT Index Kit (Illumina®) (fig. 3A). Using this approach, 75 neonatal and 73 adult ACSA-2+ cells were profiled. On average, 2,579 genes were detected in adult astrocytes and 3,899 genes in neonatal astrocytes (tpm ≥ 1). Based on a combination of selection criteria (ANOVA; p ≤ 0.01; average effect size ≥ 3; more than 50% of samples with tpm ≥ 10 in the group with higher expression) 856 genes were expressed at a significantly higher level in neonatal ACSA-2-positive cells relative to adult cells. In contrast, 102 genes were expressed at a significantly higher level in adult ACSA-2-positive cells relative to neonatal cells (fig. 3B). As determined by annotation enrichment analysis, the terms translation, nucleotide metabolism, intracellular import/export, and others were enriched with high significance among the genes with higher expression levels in neonatal cells (fig. 3B). In summary, single-cell transcriptome analyses revealed a highly diverse expression profile of neonatal and adult astrocytes.

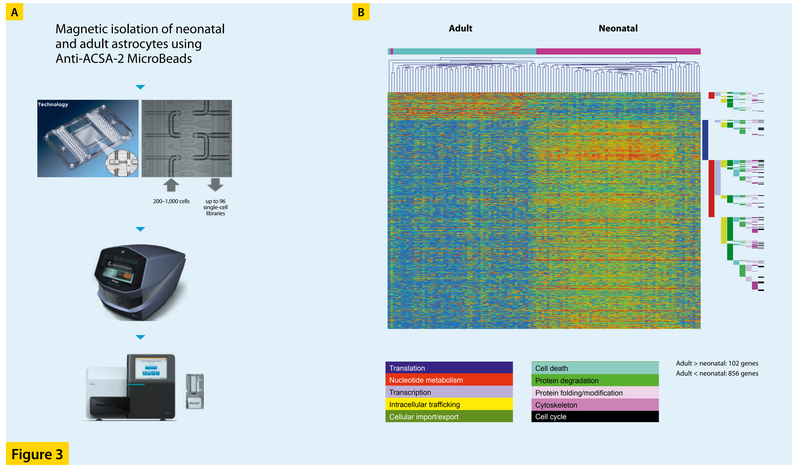

Figure 3

4 Isolation and cultivation of neurons from adult mouse brain tissue

Neurons were enriched by depletion of non-neuronal cells, using the Neuron Isolation Kit, mouse. Magnetically labeled non-neuronal cells were retained within an LS Column placed in a MACS Separator, while the highly enriched unlabeled neuronal cells were collected in the flow-through (fig. 4A). The original cell fraction, the isolated neuronal cells as well as depleted non-neuronal cells were stained with antibodies specific for the non-neuronal cells (fig. 4B). The neuronal cell fraction showed a purity of 86.1±7.4% with only few non-neuronal cells. The separation yielded 1.6×10[5]±5.4×10[4] viable neuronal cells per whole mouse brain with a viability of 63.9±12% (n = 10). Isolated neurons were cultivated in MACS Neuro Medium supplemented with MACS NeuroBrew-21 on PLL-coated substrates and stimulated with 50 ng/mL BDNF on day 1 for 3–6 h. After 7 days cells were fixed and stained with antibodies against different neural cell types to determine the purity of the neuronal cells. The culture showed a well-grown neuronal network as indicated by MAP2 (green) and ßIII Tubulin (red) staining (fig. 4C). Only very few MBP-positive oligodendrocytes, GLAST-positive astrocytes, or CD11b-positive microglia were detected (fig. 4D).

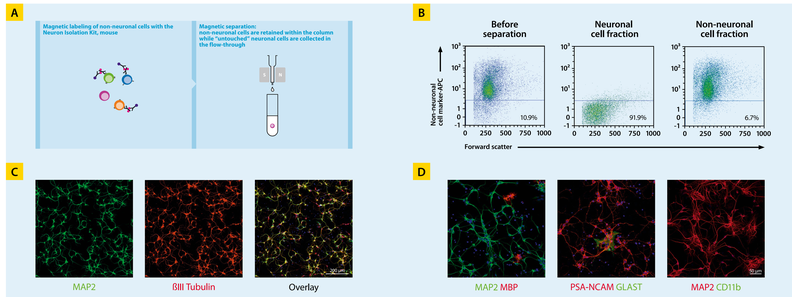

Figure 4

5 Isolation and cultivation of oligodendrocytes from adult mouse brain tissue

Oligodendrocytes were magnetically isolated using the oligodendrocyte-specific Anti-O4 MicroBeads. The cells were enriched to a purity of 90±6.7% and a viability of 76.7±10.3% (fig. 5A). A total number of 1.2×10[5]±2.5×10[4] oligodendrocytes was obtained from one adult mouse brain (n = 7). Isolated cells were cultivated in MACS Neuro Medium supplemented with MACS NeuroBrew-21 and 10 ng/mL FGF-2 and PDGF-AA on PLL-coated substrates. After 5 days, cells were fixed and stained using O4 (red)– and MOG (green)–specific antibodies (fig. 5B). Cultivated adult oligodendrocytes showed the typical branched morphology and almost no contaminating astrocytes, neurons, or microglia (fig. 5C).

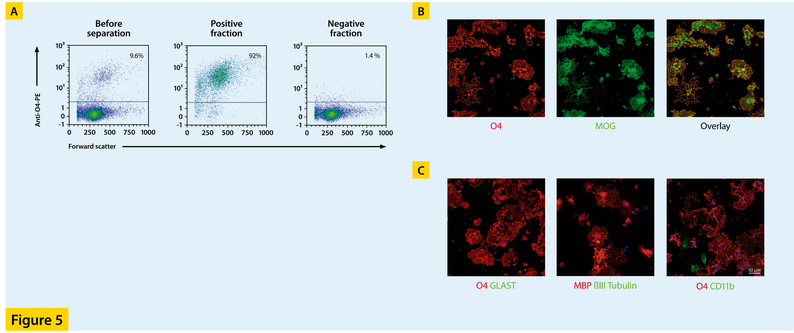

Figure 5

6 Isolation and cultivation of microglia from adult mouse and rat brain tissue

Microglial cells were isolated by using CD11b MicroBeads, mouse or CD11b/c MicroBeads, rat, respectively. An excellent purity of 96.5±4.6% with a high viability of 95.2±2.6% was obtained (fig. 6A). The separation yielded 4.2×10[5]±1.6×10[5] viable microglial cells per whole mouse brain (n = 4) and 2–3×10[6] per adult rat brain (n = 2). Cultivated microglia (7 div) were stained with CD11b- and CD68-specific antibodies and showed no contamination of astrocytes, neurons, or oligodendrocytes (fig. 6B).

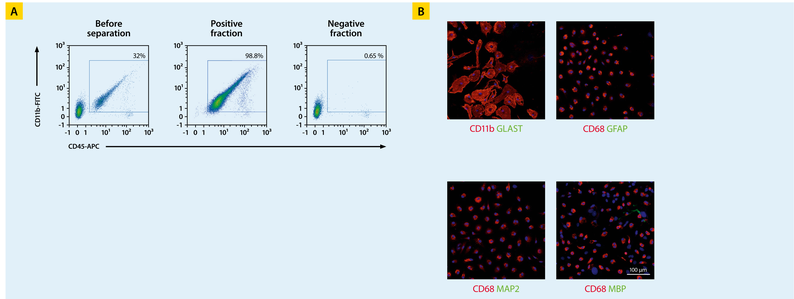

Figure 6

Conclusion and outlook

- We present a novel standardized technology to generate highly purified and viable adult neural cells that expands the analysis from neonatal to adult murine brain tissue and facilitates sophisticated cellular and molecular analyses.
- The Adult Brain Dissociation Kit enables for the first time the isolation of viable and functional neural cells from adult murine brain tissue.
- Highly purified adult astrocytes, neurons, oligodendrocytes, and microglia can be cultivated and applied to study the function of individual adult neural cells at the morphological and molecular level.
- Single-cell transcriptome sequencing of purified neonatal and adult astrocytes demonstrated distinct expression profiles for neonatal and adult ACSA-2-positive astrocytes.

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