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

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Introduction

Tissue disaggregation 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 treatments, cell viability is often lower than 90%. To overcome this drawback, we included a novel protocol for removal of debris and erythrocytes, which is crucial for effective cell isolation and culture.

The standard procedure allows fast and reproducible dissociation of adult murine brain tissue and is optimized to increase the number of viable cells. Protocols for the magnetic isolation (MACS) Technology of astrocytes, oligodendrocytes, neurons, microglia, and endothelial cells were optimized to successfully culture adult neural cell populations. Furthermore, highly purified astrocytes were subjected to single-cell mRNA sequencing. This has allowed for an enhanced understanding of astrocyte diversity. In summary, we present a novel standardized technology to generate highly purified primary neural cells with high viability and a minimum of cell debris. The method for isolating neural cells has also been modified for removal of debris and erythrocytes and is therefore suitable for effective cell isolation and culture.

Results

1 Automated dissociation of adult mouse brain tissue

We developed a novel process for the automated dissociation of adult murine brain tissue by combining automated mechanical dissociation using the cell dissociator with an optimized enzymatic treatment.

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). The optimized tissue dissociation protocol resulted in a cell yield of 3.9±1.9×10⁶ cells per adult mouse brain with a viability of 70–90%. The required reagents are available as Adult Mouse Brain Dissociation Kit, mouse and rat.

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 Microbeads and after magnetic separation (fig. 2B) for flow cytometry analysis.

Isolated astrocytes were cultured 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 CD11b- and CD68-specific antibodies and showed no positive staining for microglia (fig. 3B). Microglia can be cultivated and applied to study the function of microglia.

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

In order to characterize astrocyte diversity we isolated astrocytes from dissociated neonatal (~1–3 days old) and adult (12 months old) mouse brains. Single-cell mRNA sequencing of astrocytes was performed using the astrocyte-specific, Anti-ACSA-2 Microbeads to enrich astrocytes. Cell viability was 87.7±5% for neonatal astrocytes and 94.1% for adult astrocytes. The single-cell mRNA sequencing analysis revealed a significantly higher level of expression of 102 genes in adult ACSA-2-positive cells relative to neonatal cells. In contrast, 19 genes showed a significantly higher level of expression in neonatal cells. Enrichment analysis, the terms translation, nucleotide metabolism, protein degradation, and cellular import/export were enriched among the genes with higher expression levels in neonatal cells as well as depleted non-neuronal cells were stained with antibodies for neuron-specific markers (fig. 4C).

4 Isolation and cultivation of neurons from adult mouse brain tissue

Neurons were enriched by depletion of non-neuronal cells, using the Neuro Isolation Kit, mouse. Magnetically isolated non-neuronal cells were retained within an LS Column placed in a MACS Separator, while the highly enriched neuronal cells were eluted collect in the flowthrough (fig. 4H). The original cell fraction, the isolated neuronal cells (1,000–30,000 cells per 100 μL final cell number) were stained specific for the non-neuronal cells (fig. 4G). The neuronal cell fraction showed a purity of 70–90%, with only 0.08% non-neuronal cells. The separation protocol (fig. 4H–I) enables neuronal cells per whole mouse brain with a viability of 70–90% (n=10). Isolated neurons were cultured 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 neuronal-specific markers to determine neuronal cell type (fig. 4D). Cell viability was 95.2±2.6% and the purity of 96.5±4.6% was obtained (fig. 5B). A total number of 1.2×10⁵±2.5×10⁴ ACSA-2-positive cells were isolated from 100 μL cell fraction.

5 Isolation and cultivation of oligodendrocytes from adult mouse brain tissue

Oligodendrocytes were magnetically isolated using the oligodendrocyte-specific Anti-GLAST Microbeads. The cells were eluted in a purity of 86.1±7.4% with only 5% non-neuronal cells. Isolated cells were cultured in MACS Neuro Medium supplemented with FGF-2 and FGF-3 (fig. 5B). Isolated oligodendrocytes showed the typical branched morphology and almost no contaminating astrocytes, neurons, or microglia (fig. 6C).

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

Microglia cells were isolated by using CD11b Microbeads, mouse or rat (fig. 6B). Neuronal purity was measured using the marker O4 (fig. 6A). Only very few MBP-positive cells or oligodendrocytes were detected (fig. 6B).

Conclusion and outlook

• We present a novel standardized technology to generate highly purified and viable adult neural cells that enables the analysis of microglia and astrocytes in single-cell mRNA sequencing and cell culture.

• Single-cell transcriptome sequencing of purified neural and microglial cells enables the first-time isolation of functional and neural functional cells from adult mouse brain tissue.

• Highly purified adult astrocytes, neurons, oligodendrocytes, and microglia can be cultivated and applied to study the function of individual adult neural cells and their morphological and molecular level.

• The single-cell transcriptome sequencing of purified neuronal and microglial cells allows the study of the function of individual adult neural cells at the morphological and molecular level.