A novel automated dissociation procedure allows efficient immunomagnetic isolation of viable neural cells from adult mouse brain

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 dissociate the tightly connected neural cells, cell analysis is often restricted to embryonic or neonatal tissues. In the past we have set up technologies for dissociation of neonatal brain by combining automated mechanical dissociation using the gentlEMACS™ Dissociator with an optimized enzymatic treatment. To extend the analysis to adult neural cells we have improved 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, and microglia to high purity were also established and cells were successfully cultured. 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 analysis.

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 dissociation with gentle mechanical dissociation using the gentlEMACS™ Dissociator. Neurons, astrocytes and molecular analyses. We analyzed their transcriptome by single-cell mRNA sequencing from dissociated neonatal (P0–2) and adult mouse brain and identified 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 97.0±0.8% (n = 7), respectively. Cell viability was 87.5±5% for neonatal astrocytes and 95±8% for adult astrocytes. Then, the CST™ Single-Cell Auto Prep System (Ridgway) was used for an automated cell preparation from neonatal and adult astrocytes. Single-cell mRNA sequencing libraries were prepared using the Neuron® mRNA Library Preparation Kit and NextSeq 550 Rapid Kit (Illumina®). Up to now, 75 neuronal and 73 adult ACAS-2 cells were profiled. On average, 1,379 genes were detected in adult astrocytes and 1,399 genes in neuronal astrocytes (t = 1.7). Based on a combination of selection criteria (ANOVA, p ≤ 0.01), average effect size ≥ 2, more than 50% of samples with t ≥ 10 in the group with higher expression 656 genes were expressed at a significantly higher level in adult ACA-2-positive cells relative to adult cells. In contrast, 162 genes were expressed at a significantly higher level in adult ACAS-2-positive cells relative to neuronal cells (fig. 3B). As determined by annotation enrichment analysis, the terms translation, nucleotide metabolism, translational import/ export, and others were enriched with high significance among genes with higher expression level in mouse cells (fig. 3B). In summary, single-cell transcriptome analysis revealed a highly diverse expression profile of neonatal and adult astrocytes.

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 2 h before and after separation (fig. 2B) for flow cytometry analysis (MACSQuant™ Analyzer). Enriched astrocytes showed a purity of 94.6±5% and a viability of 98.3±3%. A total number of 4.0±1.5×105 astrocytes was obtained from one adult mouse brain (n = 10).

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 stained with antibodies against different neural cell types to determine the purity of the enriched astrocyte preparation. The culture showed a well-grown neuronal network as induced by NMDAR (green) and IR Tubulin (red staining) (fig. 4C). Only very few IMP-positive oligodendrocytes, GLAST-positive astrocytes, or CD11b-positive microglia were detected (fig. 4D). In conclusion, single-cell transcriptome sequencing of purified neonatal and adult astrocytes demonstrated distinct expression profiles for neonatal and adult ACSA-2-positive astrocytes.

3 Neuronal 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 97.0±0.8% (n = 7), respectively. Cell viability was 87.5±5% for neonatal astrocytes and 95±8% for adult astrocytes. Then, the CST™ Single-Cell Auto Prep System (Ridgway) was used for an automated cell preparation from neonatal and adult astrocytes. Single-cell mRNA sequencing libraries were prepared using the Neuron® mRNA Library Preparation Kit and NextSeq 550 Rapid Kit (Illumina®). Up to now, 75 neuronal and 73 adult ACAS-2 cells were profiled. On average, 1,379 genes were detected in adult astrocytes and 1,399 genes in neuronal astrocytes (t = 1.7). Based on a combination of selection criteria (ANOVA, p ≤ 0.01), average effect size ≥ 2, more than 50% of samples with t ≥ 10 in the group with higher expression 656 genes were expressed at a significantly higher level in adult ACA-2-positive cells relative to adult cells. In contrast, 162 genes were expressed at a significantly higher level in adult ACAS-2-positive cells relative to neuronal cells (fig. 3B). As determined by annotation enrichment analysis, the terms translation, nucleotide metabolism, translational import/ export, and others were enriched with high significance among genes with higher expression level in mouse cells (fig. 3B). In summary, single-cell transcriptome analysis revealed a highly diverse expression profile of neonatal and adult astrocytes.

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. After 3–6 h, cells were fixed and stained with antibodies against different neural cell types to determine the purity of the enriched neuronal preparation. The culture showed a well-grown neuronal network as induced by NMDAR (green) and IR Tubulin (red staining) (fig. 4C). Only very few IMP-positive oligodendrocytes, GLAST-positive astrocytes, or CD11b-positive microglia were detected (fig. 4D).

In conclusion, single-cell transcriptome sequencing of purified neonatal and adult astrocytes demonstrated distinct expression profiles for neonatal and adult ACSA-2-positive astrocytes.

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 96.0±3% and a viability of 97.6±3.9% (fig. 5A). A total number of 1.2×105±1.0×104 oligodendrocytes was obtained from one adult mouse brain (n = 7). Isolated cells were cultured in MACS Neuro Medium supplemented with MACS NeuroBrew-21 and 50 nM TGF-β and PDGF-AA on PLL-coated substrates. After 5 days, cells were fixed and stained using the CD11b and MOG (green-specific antibodies (fig. 5B). Cultured adult oligodendrocytes showed the typical morphology and almost no contaminating astrocytes, neurons, or microglia (fig. 5C).

6 Microglia were isolated by using CD11b Microbeads, mouse on CD11b Microbeads, rat, respectively. An excellent purity of 96.5±4.8% with a high viability of 95.2±2.0% was obtained (fig. 6A). The separation yielded 4.2×105±1.6×105 viable microglial cells per whole mouse brain (n = 4) and 2–3×106 per adult rat brain (n = 5). Microglia were stained with CD11b and CEDR antibodies and showed no contamination of astrocytes, neurons, or oligodendrocytes (fig. 6B).

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 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 in 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.