We present a novel standardized technology to generate highly purified and viable adult neural cells that facilitate sophisticated cellular and molecular analyses. 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.

In order to characterize astrocyte diversity we isolated astrocytes from dissociated neonatal (0-3) and adult mouse brain and analyzed their transcriptome by single-cell RNA sequencing. Neonatal and adult astrocytes were separated using the astrocyte-specific Anti-ACSA-2-PE antibody. The separation yielded 1.6×10^5 cells per adult rat brain (n = 4) and 5.4×10^4 cells per adult mouse brain (n = 7). Isolated cells were cultivated in MACS Neura Medium supplemented with MACS NeuroD21 and 10 ng/mL FGF2 and RA 2× AA on FL-coupled substrates. After 5 days, cells were fixed and stained using O4- and CD68-specific antibodies (fig. 4B). Cultured adult oligodendrocytes showed the typical bipolar morphology and almost no contaminating astrocytes, neurons, or microglia (fig. 4C).

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

Introduction

Tissue dissociation and preparation of single-cell suspensions with high cell viability and a minimum of cell debris are prerequisites for viable cellular analysis, cell culture, and cell preparation. As an alternative to manual mechanical digestion, enzymatic dissociation, or usage of cell-penetrating reagents, a number of mechanical and enzymatic cell dissociation tools have been developed. In a number of cases, cell culture is often restricted to embryonic or neonatal tissue. We have set up technologies for dissociation of neonatal brain by combining automated mechanical dissociation using the GFP®GreenTm 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.

Results

Automated dissociation of adult mouse brain tissue

We developed a novel protocol for the automated dissociation of adult somatic tissue by combining an optimized enzymatic treatment with the mechanical dissociation using the gentler OptoDissociator. It can be used to generate single-cell suspensions of the highest possible number of viable cells, the missing cell suspension contained a significant amount of cell debris (fig. 1B) and red blood cells (fig. 1B), which transport subsequent cell isolation and culture.

Isolation and cultivation of astrocytes from adult mouse brain tissue

After tissue dissociation using the Adult Brain Dissociator kit, astrocytes were labeled with MACS MicroBeads coupled to antibodies specific for the astrocyte marker ACSA-2 (cytokeratin surface antigen) 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. Isolated astrocytes were cultured in MACS Neura Medium supplemented with MACS NeuroD21 on FL-coupled substrates and maintained with 10 ng/mL FGF2 on day 1-3. After 7 days cells were fixed and stained with antibodies specific for the astrocyte marker ACSA-2 (fig. 2C). The neuronal isolation protocol, the isolated neuronal cells as well as the isolated non-neuronal cells were stained with antibodies specific for the non-neuronal cells (fig. 2G). The neuronal isolation protocol showed a purity of 86.1±7.4% with 98.8% astrocytes and 98.9% of the neuronal cells being “untouched” neuronal cells. The isolation strategy with single-cell transcriptome analyses revealed a highly diverse expression profile of neonatal and adult astrocytes.

Neural cells were enriched by depletion of non-neuronal cells using the Neuron Isolation kit and the autoMACS® Pro Separator program. DepNeu (fig. 4G). The original cell factor, the isolated neuronal cells as well as the depleted non-neuronal cells were stained with antibodies specific for the non-neuronal cells (fig. 4G). The neuronal isolation protocol showed a purity of 86.1±7.4% of the neuronal cells. The separation yielded 1.4×10^5 VASH-1-positive neurons per mouse brain with a purity of 91.9% (fig. 4H). Isolated neurons were cultivated in MACS Neura Medium supplemented with MACS NeuroD21 on FL-coupled substrates and maintained with 10 ng/mL FGF2 on day 1-3. After 7 days cells were fixed and stained with antibodies against different neuronal cell types to characterize the isolated neuronal cells. The culture showed a well-grown neuronal network as indicated by specific antibodies. Cultured neurons were also fixed with DAPI (fig. 4D). Only very few cells were labeled with the immature oligodendrocytes, Q170-positive astrocytes, or CD68-positive microglia (detected by fig. 4D).

Conclusion and outlook

- We present a novel automated technology to generate highly purified and viable adult neural cells that enables the analyses from neonatal to adult murine brain tissue and facilitates sophisticated cellular and molecular analyses.
- The adult brain dissociation enables for the first time the isolation of viable and functional neuronal cells from adult murine brain tissue.
- Highly purified adult neurons, microglia, astrocytes, and oligodendrocytes can be cultured and applied to study the function of individual adult neural cells at the morphological and molecular level.
- Single-cell transcriptome sequencing of purified neuronal and adult astrocytes demonstrates the potential to generate expression profiles for neonatal and adult astrocyte-positive astrocytes.