Using the nCounter® Platform for High-Throughput Analysis in Neuroscience Applications

Highlights

- In neuroscience research, it is critical to study the complex structure of the different cell types in the brain, as the chemical, morphological, and functional features of brain cell types are extremely diverse.
- The nCounter® Analysis System, along with the predesigned gene expression panels for Neuropathology and Neuroinflammation, gather a signature profile using different brain cell and immune cell types.
- Two recent studies using the nCounter platform includes: one that classifies pediatric ependymomas using custom antibody probes[1], and another investigates miRNA expression in microglial-derived extracellular vesicles in Alzheimer's Disease[2].
- Canopy Biosciences is designated by NanoString® as a Center of Excellence with services using the nCounter Analysis System, offering experiment and analysis packages for neuroscience research.
- Canopy Biosciences has a CLIA-certified lab for preclinical and clinical trial projects for IHC, nCounter analysis and GeoMx® DSP services analyzing FFPE samples.

Introduction

In neuroscience research, it is important to understand the complex structure of the different cell types in the brain. The problem is figuring out how to identify and track these cell types, as brain cell types vary and are extremely diverse in its anatomical, chemical, and functional characteristics. Gene expression technologies are revealing the cell-type organization of the brain. Our understanding of the cell types that make up the brain is rapidly increasing, driven by newer methods like spatial and single-cell transcriptomics[3].

Although single cell transcriptomics is one approach to study the transcriptomes of individual cells in the brain and CNS, gene expression analysis in the single cell level can be noisy and technically challenging. The transcription profile of the cells in the brain and CNS can be analyzed in a high-throughput manner through a much simpler approach using the nCounter analysis system. NanoString offers highly multiplexed gene expression panels that look at the expression of up to 800 genes associated with neuropathology and neuroinflammation. These panels examine and identify 5 different CNS cell types and 14 different immune cell types to gather a signature profile of biological processes and diseases.

The advantages of using the nCounter System include high reproducibility, ability to work on poor-quality samples with degraded RNA like FFPE, and assays that do not require reverse transcription, target amplification or library preparation – all methods that can introduce bias and variability in the sample prep process.

Using nCounter in Classifying Pediatric Ependymomas

A recent paper authored by scientists from the Children's Memorial Health Institute in Warsaw, Poland included the use of the nCounter Analysis System for proteomic profiling to help classify pediatric ependymomas (Lastowska et al. 2021). Ependymomas (EPN) are tumors of the central nervous system (CNS) that can arise anywhere in the spinal cord, in the supratentorial brain, and in the hindbrain or posterior fossa. Pediatric supratentorial tumors represent about 50% of all intracranial neoplasms[4]. The most frequent tumors of the cerebral hemispheres are gliomas that arise from astrocytes, oligodendrocytes, or ependymal cells[5]. The majority of supratentorial ependymomas

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in children contain oncogenic fusions, such as ZFTA–RELA or YAP1-MAMLD1. In contrast, posterior fossa (PF) ependymomas lack recurrent somatic mutations and are classified based on gene expression or methylation profiling (reviewed in 1). Tumor molecular characterization is clinically relevant, as tumors with ZFTA–RELA translocation and PFA ependymomas are reportedly associated with poor prognosis.

The authors applied a novel and potentially diagnostic approach for the identification of four molecular groups of ependymoma, based on transcription profiling of marker genes, using NanoString nCounter technology. This method enables the analysis of multiplex protein expression using custom antibody probes. The nCounter has previously been successfully tested for the identification of molecular subtypes in medulloblastoma, and the diagnosis of rare pediatric brain tumor[6,7]. The study analyzed 16 supratentorial and 50 PF tumors and identified four molecular types of ependymoma: the RELA+ and YAP1+ markers for supratentorial tumors, and the PFA (subdivided into PFA1 and PFA2) and PFB markers for the posterior fossa ependymomas.

Supratentorial Ependymomas

The authors did cluster analysis using nine signature genes on samples from supratentorial ependymomas. Two primary clusters were identified: cluster 1, which had significant RELA+ signature gene expression, and cluster 2, which lacked any signature gene expression (Figure 1).

PF Ependymomas

Clustering was done on the 50 patients with posterior fossa ependymomas. Based on the expression of five PFA and five PFB genes, the authors identified two PF clusters (Figure 2). The 7 tumors in Cluster PFB were easily distinguished from the 42 PFA tumors.

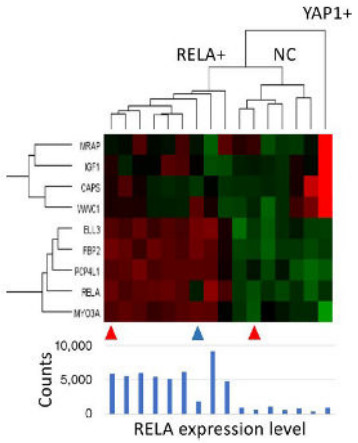

Figure 1. Clustering of supratentorial tumors according to expression levels of pre-selected marker genes (Source: Lastowska et al. 2021)

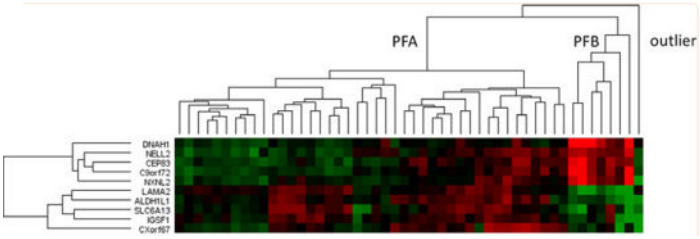

Figure 2. Clustering of PF tumors (Source: Lastowska et al. 2021)

Histopathology

Histopathology results included the RELA fusion-positive ependymomas appearing as solid or solid/focal cystic lesions. The authors commonly found perivascular pseudorosettes, which typically comprised neoplastic cells around the blood vessels, with distinct perivascular anucleate zones (Figure 3A, 3B). Figure 3C shows a high density of neoplastic cells with clear-cell morphology.

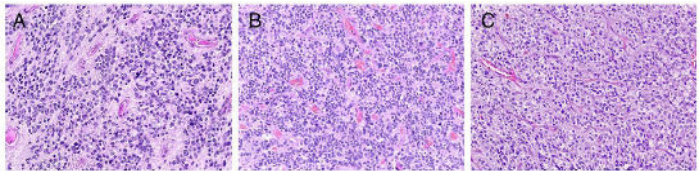

Figure 3. Histopathology of RELA fusion-positive ependymomas (Source: Lastowska et al. 2021)

Using nCounter to Analyze miRNA Expression in Extracellular Vesicles

Another recent paper used a multi-omics analysis approach to study microglia-derived extracellular vesicles or EV's from late-stage Alzheimer's Disease brain samples., wherein EVs may be involved in the progression of Alzheimer's Disease or AD (Cohn W., et al., 2021). The nCounter analysis system was used for the miRNA transcriptomic analysis of the microglial EVs. This proof-of-concept study shows the feasibility of using multiple -omics analyses on small microglial EVs isolated from cryopreserved human brain tissue. Other related studies have revealed that microRNAs (miRNAs) may be considered as potential biomarkers in AD.

Microglia-derived small EV isolation and characterization was done using sucrose-gradient fractions enriched in EVs. Immunoblot analysis (Figure 4) demonstrated the enrichment of exosomal markers, CD63 and CD9, and the microglial marker, CD11B, in small EVs immunoprecipitated with CD11b.

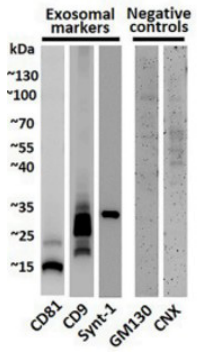

Figure 4. Immunoblot analysis using antibodies against exosome markers (CD9, CD81, and syntenin-1) and negative control markers GM130 and calnexin or CNX. (Source: Cohn W., et al., 2021)

The authors used Tandem Mass Tag (TMT)-based quantitative proteomics, with a total of 1,000 unique proteins identified in the microglia-derived small EV samples from the human parietal cortex (data not shown). Three proteins, fatty acid binding protein 3, heart type (FABP3), mitochondrial copper transporter Solute Carrier Family 25 Member 3 (SLC25A3), and GTPase Atlastin-3(Alt3), were only detected in the NL samples. In contrast, twelve proteins were detected only in the AD samples.

miRNA Profiling of Microglial EVs

The authors identified 105 miRNAs using the nCounter miRNA expression panel. Gene expression analysis identified miRNAs with increased expression, shown in the volcano plot, which revealed that levels of four miRNA - miR-28-5p, miR-381-3p, miR-651-5p and miR-188-5p - increased in microglial EVs from AD cases (Figure 5A).

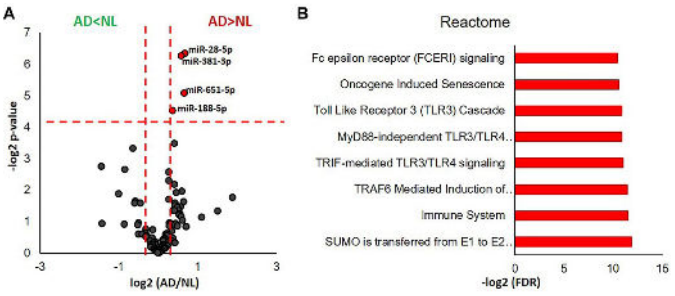

Figure 5. miRNA transcriptional analysis of microglial EVs comparing late-stage AD and normal/low pathology samples. (Source: Cohn W., et al., 2021)

Functional analysis of the miRNA data was done using miRNet, which showed that the SUMOylation, toll-like receptor (TLR), Fc epsilon receptor I (FCERI), and senescence pathways were regulated by the four miRNAs with increased expression (Figure 5B).

Lipidomic Analysis of microglial EVs

The lipidomic analysis demonstrated an increase in cholesterol in AD microglial EVs (data not shown), which is consistent with the phagocytosis of neuronal debris by microglial cells. Cholesterol metabolism has also been associated with immune activation (reviewed in 2). The authors also found a significant increase in the major bis(monoacylglycerol)phosphate (BMP) and monohexosylceramide (MhCer) lipid species, which represents increased lysosomal lipid content in AD microglia[2].

To summarize, the results indicated:

- By using the nCounter system, four miRNAs were upregulated in the AD group and associated with immune and cellular senescence signaling pathways.
- A reduction in levels of homeostatic microglia markers P2RY12 and TMEM119, and increased levels of disease-associated microglia markers FTH1 and TREM2 in CD11b-positive EVs from AD brain compared to NL cases.
- Levels of free cholesterol were elevated in microglial EVs from the AD brain. Lipidomic analysis demonstrated a proinflammatory lipid profile.
- The authors suggest that the analysis of microglia-derived EVs has merit for identifying novel EV-associated biomarkers and for future larger-scale multi-omics studies on patient-derived cell-type-specific EVs.

Summary

Pediatric brain tumors and Alzheimer's Disease significantly impact millions of patients around the globe. It is vital to understand the different cell types in the brain and the immune system response to develop better treatments and new therapies for these diseases. Recent advances in spatial biology offer a deeper understanding of neural processes, diseases and signaling pathways. The nCounter Analysis System offers a simple but powerful tool for proteomic and transcriptomic analysis for neuroscience research. Canopy Biosciences offers comprehensive experiment and analysis packages for researchers looking to analyze changes in gene expression in research, pre-clinical and clinical work. Our extensive expertise with the nCounter assay has been recognized by NanoString as we are a key partner CRO and a Center of Excellence for their services.

References

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