Writing Samples for Amy Van Deusen

Excerpt from Van Deusen A.**,** Kumar S., Calhan O.Y., Goggin S., She J., Williams C., Keeler A., Fread K., Gadani I.C., Deppmann C., and Zunder E. A single-cell mass cytometry-based atlas of the mouse brain. *Nature Neuroscience* [In press].

While numerous studies have cataloged cells present in the brain at maturity[1], many fundamental questions about their development remain unresolved. In particular, the molecular profiles, timing of appearance, and cell-lineage relationships of neural stem cells (NSCs) and intermediate progenitors remain poorly characterized. Mapping the molecular trajectories and cell fate decisions underlying brain development promises to enhance our understanding of developmental and other neurological disorders.

Although previous studies have used immunofluorescence microscopy and single-cell RNA sequencing (scRNA-seq) to characterize cell populations in the developing brain[2–14], single-cell proteomic profiling during brain development has not been reported. To address this knowledge gap, we adapted single-cell mass cytometry[15,16] to profile the developing brains of C57/BL6 mice. Mass cytometry has been used previously to profile glioma[17–20], microglia[21–26], and dorsal root ganglia[27], but has not been applied to study neural cell types in the brain, except for one study of obesity-inhibited adult neurogenesis using only seven neural-specific markers[28]. Protein-level mass cytometry analysis of the developing brain serves as an important complement to previous scRNA-seq studies, because mRNA transcript abundance does not necessarily correlate with functional protein abundance[29]. Furthermore, while single-cell mass spectrometry[30,31] and next-generation sequencing-based methods like CITE-seq[32] and ASAP-seq[33] can profile thousands of proteins per cell, they are limited by lower sensitivity and throughput. In contrast, mass cytometry rapidly and cost-effectively analyzes fewer proteins with high sensitivity in millions of single cells, enabling comprehensive profiling of complex tissues.

By profiling over 24 million cells from these brain regions with mass cytometry, we identified 85 molecularly distinct cell populations and quantified their spatiotemporal dynamics across embryonic and postnatal development. These populations generally overlap with previous RNA-based studies[2–14,34–43], but discrepancies between individual protein-mRNA cognate pairs demonstrate the value of protein-based measurements to capture cell states defined by specific functional biomolecules. Validation with immunofluorescence and RNAScope in situ hybridization (ISH) was used to confirm these relationships. To investigate cell lineage trajectories in the developing brain, we applied URD pseudotime analysis[45], which captured classical neuronal and glial trajectories and predicted two distinct trajectories for producing embryonic oligodendrocyte progenitor cells (OPCs). Although not part of our original study design, our measurements appear to show phagocytic cargo within individual microglia, suggesting a dynamic role for phagocytosis in early brain development. Collectively, this study establishes mass cytometry as a platform to quantify cell types in the developing brain by their protein expression profiles and identify the molecular trajectories underlying their specification.

Bibliography

1. Yuste, R. et al. A community-based transcriptomics classification and nomenclature of neocortical cell types. Nat. Neurosci. 23, 1456–1468 (2020).
2. La Manno, G. et al. Molecular Diversity of Midbrain Development in Mouse, Human, and Stem Cells. Cell 167, 566-580.e19 (2016).
3. Kee, N. et al. Single-Cell Analysis Reveals a Close Relationship between Differentiating Dopamine and Subthalamic Nucleus Neuronal Lineages. Cell Stem Cell 20, 29–40 (2017).
4. Frazer, S. et al. Transcriptomic and anatomic parcellation of 5-HT3AR expressing cortical interneuron subtypes revealed by single-cell RNA sequencing. Nat. Commun. 8, 14219 (2017).
5. Chen, Y.-J. J. et al. Single-cell RNA sequencing identifies distinct mouse medial ganglionic eminence cell types. Sci. Rep. 7, 45656 (2017).

6. Yuzwa, S. A. et al. Developmental Emergence of Adult Neural Stem Cells as Revealed by Single-Cell Transcriptional Profiling. Cell Rep. 21, 3970–3986 (2017).
7. Mayer, C. et al. Developmental diversification of cortical inhibitory interneurons. Nature 555, 457–462 (2018).
8. Rosenberg, A. B. et al. Single-cell profiling of the developing mouse brain and spinal cord with split-pool barcoding. Science 360, 176–182 (2018).
9. Tiklová, K. et al. Single-cell RNA sequencing reveals midbrain dopamine neuron diversity emerging during mouse brain development. Nat. Commun. 10, 581 (2019).
10. Wizeman, J. W., Guo, Q., Wilion, E. M. & Li, J. Y. Specification of diverse cell types during early neurogenesis of the mouse cerebellum. eLife 8, e42388 (2019).
11. Romanov, R. A. et al. Molecular design of hypothalamus development. Nature 582, 246–252 (2020).
12. Zhou, X. et al. Cellular and molecular properties of neural progenitors in the developing mammalian hypothalamus. Nat. Commun. 11, 4063 (2020).
13. Kim, D. W. et al. The cellular and molecular landscape of hypothalamic patterning and differentiation from embryonic to late postnatal development. Nat. Commun. 11, 4360 (2020).
14. Lee, D. R. et al. Transcriptional heterogeneity of ventricular zone cells in the ganglionic eminences of the mouse forebrain. eLife 11, e71864 (2022).
15. Bandura, D. R. et al. Mass cytometry: technique for real time single cell multitarget immunoassay based on inductively coupled plasma time-of-flight mass spectrometry. Anal. Chem. 81, 6813–6822 (2009).
16. Bendall, S. C. et al. Single-cell mass cytometry of differential immune and drug responses across a human hematopoietic continuum. Science 332, 687–696 (2011).
17. Leelatian, N. et al. Single cell analysis of human tissues and solid tumors with mass cytometry. Cytometry B Clin. Cytom. 92, 68–78 (2017).
18. Hu, A. X. et al. EPH profiling of BTIC populations in glioblastoma mutiforme using CyTOF. in Brain Tumor Stem Cells: Methods and Protocols (eds. Singh, S. K. & Venugopal, C.) 155–168 (Springer, New York, NY, 2019). doi:10.1007/978-1-4939-8805-1_14.
19. Leelatian, N. et al. Unsupervised machine learning reveals risk stratifying glioblastoma tumor cells. eLife 9, e56879 (2020).
20. Galdieri, L. et al. Defining phenotypic and functional heterogeneity of glioblastoma stem cells by mass cytometry. JCI Insight 6, 128456 (2021).
21. Ajami, B. et al. Single-cell mass cytometry reveals distinct populations of brain myeloid cells in mouse neuroinflammation and neurodegeneration models. Nat. Neurosci. 21, 541–551 (2018).
22. Mrdjen, D. et al. High-dimensional single-cell mapping of central nervous system immune cells reveals distinct myeloid subsets in health, aging, and disease. Immunity 48, 380-395.e6 (2018).
23. Böttcher, C. et al. Human microglia regional heterogeneity and phenotypes determined by multiplexed single-cell mass cytometry. Nat. Neurosci. 22, 78–90 (2019).
24. Böttcher, C. et al. Single-cell mass cytometry of microglia in major depressive disorder reveals a non-inflammatory phenotype with increased homeostatic marker expression. Transl. Psychiatry 10, 310 (2020).
25. Li, S. et al. Microglial deletion and inhibition alleviate behavior of post-traumatic stress disorder in mice. J. Neuroinflammation 18, 7 (2021).
26. Xie, M. et al. TREM2 interacts with TDP-43 and mediates microglial neuroprotection against TDP-43-related neurodegeneration. Nat. Neurosci. 25, 26–38 (2022).
27. Keeler, A. B. et al. A developmental atlas of somatosensory diversification and maturation in the dorsal root ganglia by single-cell mass cytometry. Nat. Neurosci. 25, 1543–1558 (2022).
28. Ogrodnik, M. et al. Obesity-induced cellular senescence drives anxiety and impairs neurogenesis. Cell Metab. 29, 1061-1077.e8 (2019).
29. Liu, Y., Beyer, A. & Aebersold, R. On the dependency of cellular protein levels on mRNA abundance. Cell 165, 535–550 (2016).
30. Lombard-Banek, C., Moody, S. A. & Nemes, P. Single-Cell Mass Spectrometry for Discovery Proteomics: Quantifying Translational Cell Heterogeneity in the 16-Cell Frog (Xenopus) Embryo. Angew. Chem. 128, 2500–2504 (2016).
31. Budnik, B., Levy, E., Harmange, G. & Slavov, N. SCoPE-MS: mass spectrometry of single mammalian cells quantifies proteome heterogeneity during cell differentiation. Genome Biol. 19, 161 (2018).
32. Stoeckius, M. et al. Simultaneous epitope and transcriptome measurement in single cells. Nat. Methods 14, 865–868 (2017).
33. Mimitou, E. P. et al. Scalable, multimodal profiling of chromatin accessibility, gene expression and protein levels in single cells. Nat. Biotechnol. 39, 1246–1258 (2021).
34. Mi, D. et al. Early emergence of cortical interneuron diversity in the mouse embryo. Science 360, 81–85 (2018).
35. Carter, R. A. et al. A single-cell transcriptional atlas of the developing murine cerebellum. Curr. Biol. 28, 2910-2920.e2 (2018).
36. Guo, Q. & Li, J. Y. H. Defining developmental diversification of diencephalon neurons through single cell gene expression profiling. Dev. Camb. Engl. 146, dev174284 (2019).
37. Zhang, Y. et al. Cortical Neural Stem Cell Lineage Progression Is Regulated by Extrinsic Signaling Molecule Sonic Hedgehog. Cell Rep. 30, 4490-4504.e4 (2020).
38. Li, Z. et al. Transcriptional priming as a conserved mechanism of lineage diversification in the developing mouse and human neocortex. Sci. Adv. 6, eabd2068 (2020).
39. Ruan, X. et al. Progenitor cell diversity in the developing mouse neocortex. Proc. Natl. Acad. Sci. U. S. A. 118, e2018866118 (2021).
40. Di Bella, D. J. et al. Molecular logic of cellular diversification in the mouse cerebral cortex. Nature 595, 554–559 (2021).
41. La Manno, G. et al. Molecular architecture of the developing mouse brain. Nature 596, 92–96 (2021).
42. Sarropoulos, I. et al. Developmental and evolutionary dynamics of cis-regulatory elements in mouse cerebellar cells. Science 373, eabg4696 (2021).
43. Turrero García, M. et al. Transcriptional profiling of sequentially generated septal neuron fates. eLife 10, e71545 (2021).

Excerpt from Van Deusen A. and McGary M. Overview of chemistry, manufacturing and controls (CMC) for pluripotent stem cell therapies (2015). In: Childers M, ed. *Regenerative Medicine for Degenerative Muscle Diseases*. New York, NY: Springer.

The necessary requirements for completing the Chemistry, Manufacturing, and Control (CMC) section of a Food and Drug Administration (FDA) Investigational New Drug (IND) application as it pertains to the production of pluripotent stem cell (PSC)-based cell therapy products will be described in this chapter. The expectations for IND content are located in 21 CFR 312.23[1] and regulations enacted for Phase 1 investigational products are described in an FDA Guidance for Industry titled "cGMP for Phase 1 Investigational Drugs"[2].

PSC-based products, generated from either embryonic or adult cell sources, are regulated under the general classification of Human Cells, Tissues, or Cellular or Tissue-Based Products (HCT/Ps) under Title 21 of the Code of Federal Regulations Part 1271 (21 CFR 1271)[3]. Additional regulations intended to prevent the introduction, transmission, or spread of communicable disease are contained within section 351 and 361 of the Public Health Service (PHS) Act[4, 5]. Regulatory implementation is primarily determined through risk-based assessments at each clinical phase and varies with the source, manipulation, and intended application of cells used in PSC-based therapies. As the clinical cohort increases in size, the scope and expectations of regulations will expand significantly.

While there are several criteria set out in 21 CFR 1271 Subpart A to determine if a cell therapeutic is exempt from any part of these regulations[6], the necessary *ex vivo* manipulation of cells and their intended use for transplantation into human patients renders the majority of PSC-based cell therapeutics fully regulated under all previously mentioned statutes[7]. Examples of biologically similar HCT/Ps not regulated under 21 CFR 1271 include minimally manipulated bone marrow and blood products. However, the phrase "minimally manipulated" has been controversial, even resulting in a legal battle before the United States District court between the FDA and a cell therapy manufacturer in 2010[8].

During preclinical and IND phases of development, emphasis is placed on generation of verifiable proof-of-concept studies and prevention of communicable diseases within the laboratory through Good Laboratory Practice (GLP)[9] and Good Tissue Practice (GTP)[10] As a trial advances to Phase 1, current Good Manufacturing Procedures (cGMP) must be more rigorously implemented. As studies progress into even later stages, there is an increased focus beyond safety onto control of the manufacturing process including assessments of product stability and consistency. This largely occurs through evaluation of all generated documentation, including manufacturing and quality control records.

While this chapter will frequently refer to applicable regulations and guidelines, it is not intended to fully recapitulate any section or subsection of the 21 CFR or any FDA Guidance for Industry issued by the Center for Biologics Evaluation and Research (CBER). Rather, this chapter is intended as a general overview of the many regulatory requirements that must be considered in order to generate a complete CMC section for a successful IND application. As there are a multitude of regulations distributed throughout the CFR and US Pharmacopeial Convention (USP), we have provided a list of relevant statutes in Table 7.1.

Bibliography

1. US National Archives and Records Administration. Code of Federal Regulations. IND Content and format. 2013;Title 21,Part 312.23.

2. USFDA Center for Biologics Evaluation and Research. FDA guidance for industry: cGMP for phase 1 investigational drugs. Jul 2008. Available from: http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm070273.pdf.

3. US National Archives and Records Administration. Code of Federal Regulations. Human cells, tissues, and cellular and tissue-based products. 2013;Title 21, Part 1271.

4. US Code. Public Health Service Act. Regulation of biological products. 1999; Title 42, Part 262, Section 351 [cited 4 Aug 2013]. Available from: http://www.fda.gov/RegulatoryInformation/Legislation/ucm149278.htm.

5. US Code. Public Health Service Act. Regulations to control communicable diseases. 1999;Title 42, Part 264, Section 361 [cited 4 Aug 2013]. Available from: http://www.fda.gov/RegulatoryInformation/Legislation/ucm149429.htm.

6. US National Archives and Records Administration. Code of Federal Regulations. General provisions. 2013; Title 21, Part 1271, Subpart A.

7. US National Archives and Records Administration. Code of Federal Regulations. Minimal manipulation means. 2013; Title 21, Part 1271.3.

8. DeFrancesco L. FDA prevails in stem cell trial. Nat Biotechnol. 2012;30(10):906.

9. US National Archives and Records Administration. Code of Federal Regulations. Good laboratory practice for nonclinical laboratory studies. 2013;Title 21,Part 58.

10. US National Archives and Records Administration. Code of Federal Regulations. Current

good tissue practice. 2013;Title 21,Part 1271,Subpart D.

Excerpts from Van Deusen A. and Nasis O. (2013). Commercial opportunities for induced pluripotent stem cells. In: Sell S, ed. *Stem Cells Handbook*, Second Edition. New York, NY. [https://link.springer.com/chapter/10.1007/978-1-4614-7696-2_13#page-1]

14.2.3 *The Ethics of iPS Cell Technologies*

Beyond presenting a novel avenue for patient-specific medicine, the ability to generate iPS cells from adult sources also resolves many ethical barriers surrounding the use of embryonic stem cells. Soon after the first derivation of embryonic stem cells lines from human embryos by Dr. James Thomson at the University of Wisconsin, in Madison[38], controversy began to surround the use of these cells for any purpose. The basis for most objections concerned the origin of these cells, human blastocysts, and the manner in which they are obtained from donor sources.

In 1996, the United States Congress signed the Dickey-Wicker Amendment banning government funding for creation or destruction of human embryos into law. For 15 years, an ideological battle was waged between researchers, politicians, ethicists, and religious organizations. In 2009, US President Barack Obama issued an executive order that removed restrictions on federal stem cell funding, though this was contested with an injunction for two years[39] before the US District Court for the District of Columbia finally lifted the injunction in 2011. In July 2012, a panel of judges upheld this decision in the US Circuit Court of Appeals for the District of Columbia thereby affirming the legality of embryonic stem cell research in the United States. However, policies governing embryonic stem cell research remain restrictive throughout most of the world including Western Europe, with the exception of the United Kingdom, Sweden and Switzerland where researchers are less limited in their ability to pursue hESC-based work.

The use of iPS cells has the potential to even the stem cell playing field across the globe as it opens up new applications and funding opportunities previously unavailable to thousands of researchers. Bioengineering cells through reprogramming offers an innovative strategy for embryo-independent creation of autologous cell therapies and avoids ethical and political issues surrounding embryonic stem cell work. While much research is still required to validate the use and safety of iPS cells and to fully characterize them in comparison to “gold standard” embryonic stem cells[40], many recent discoveries have brought the goal of regenerative medicine closer than ever to becoming a reality. Indeed, the speed with which iPS technologies could potentially deliver clinical therapies roused officials at the National Institutes of Health to thoroughly examine and codify the informed consent process for iPS cell research in coordination with the US Food and Drug Administration (FDA) in 2012[41].

14.6.4 *Intellectual Property and iPS Cell Technology Licensing*

The immense potential of iPS cell technologies and their subsequent usefulness in a wide variety of research schemes makes them an extremely valuable commodity in today’s marketplace. Therefore, it is of particular importance for researchers and entrepreneurs to protect their inventions, and organizations across the globe are racing to secure rights to use and distribute these technologies. In a nascent environment where smaller iPS cell companies frequently demonstrate a more refined expertise in specific technologies, even the largest organizations appear willing to partner. Thus, it is important to establish scientific ownership of iPS cell technologies as early as possible in the discovery process.

iPS Academia Japan was established to manage the patents and technology rising from Dr. Yamanaka’s discovery of iPS cells at Kyoto University after the institution was granted a patent for iPS cells by the Japan Patent Office in 2008[129]. Since its founding, numerous institutions and companies have applied for licenses to use this technology. In 2011, iPierian (San Francisco, CA) licensed this technology and in 2012, Cellular Dynamics International signed a new agreement to use Yamanaka’s technology and cell lines.

Intellectual property laws vary globally, especially with regard to the applicability of patents to stem cell technologies, making establishing ownership tedious and difficult in some cases. For example, in 2011, the European Parliament courts banned patents on stem cell products citing an “unethical industrial” use of stem cells after a German researcher tried to patent a method of turning human embryonic stem cells into neurons[130]. This placed the UK and Europe in a precarious situation with regard to commercializing any stem cell technology, including iPS cells, and was considered “a blow to years of effort to derive biomedical applications from embryonic stem cells” by leaders in the field[131].

Bibliography

38. Thomson J.A., Itskovitz-Eldor J., Shapiro S.S., Waknitz M.A., Swiergiel J.J., Marshall V.S., et al (1998). Embryonic stem cell lines derived from human blastocysts. *Science*. 282(5391): 1145-7. PubMed PMID: 9804556. Epub 1998/11/06.eng.
39. Katsnelson A. US court suspends research on human embryonic stem cells. *Nature*. 24 August 2010.
40. Izpisua Belmonte J.C., Ellis J., Hochedlinger K., Yamanaka S. (2000). Induced pluripotent stem cells and reprogramming: seeing the science through the hype. *Nature Rev Gen*. 10(12):878-83. PubMed PMID: 19859062. Epub 2009/10/28. eng.
41. Lowenthal J., Lipnick S., Rao M., Hull S.C. (2012). Specimen collection for induced pluripotent stem cell research: harmonizing the approach to informed consent. *Stem Cells Translat. Med*.
129. Cyranoski D. (2008). Japan fast-tracks stem-cell patent. *Nature*. 455(7211):269. PubMed PMID: 18800093.
130. Moran N. (2011). European court bans embryonic stem cell patents. *Nature Biotech*. 29(12):1057-9. PubMed PMID: 22158341.
131. Smith A. (2011). 'No' to ban on stem-cell patents. *Nature*. 472(7344):418. PubMed PMID: 21525916.