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Summary

Stem cell research represents the cutting edge of translational medicine, promising patient specific *in vitro* disease models as well as drug and/or cell-replacement therapies. Such research depends on our ability to control cell identity, which for neuroscience includes not only the generation of different neural cell types, but potential for the development of appropriate (and accurate) neural structures. Readers are taken through the process of developing and applying this emerging technology for the purpose of modeling a childhood white matter disorder.

Chapter 2 reviews the state of (neuroscience-focused) stem cell research, as it stood at the outset of experiments reported in the thesis. In particular, it sets the groundwork for readers, by explaining the general concept of cell *potency* (the ability of a cell to become other cell types in the body) within the context of development, the history of understanding/ controlling cell potency, and the molecular mechanisms believed to regulate cell identity.

Chapter 3 examines how different neural progenitor (NP) protocols may generate cells with similar NP-associated markers, but can still exhibit biases/limits in the neural lineages they can produce. In specific, while otherwise similar according to traditional cell-associated markers, NPs derived from induction methods employing 1) single SMAD inhibition, 2) dual SMAD inhibition, and 3) RA administration exhibited different expression profiles for (neural) regional identity markers *HOXB4, LBX1, OTX1*, and *GSX2* (RA administration products having higher *HOXB4* and so putative caudal identity compared to other methods). When differentiated to glial and cortical neuronal lineages: NPs derived from single SMAD induction were found capable of producing astrocytes, but less efficient at producing neurons with mature cortical phenotypes; NPs from dual SMAD induction could equally produce astrocytes and (glutamatergic) cortical neurons; and RA induced NPs produced astrocytes with relative caudal identity (plus high GFAP expression), while neurons had relatively greater GABAergic identity. Taken together, this suggests that method of induction can influence regional identity of NPs and so limitations for efficiency in generating downstream neural cell types. Therefore, care should be taken in choosing the right induction protocol for intended research goals.

In **Chapters 4 and 5**, the development of cerebellar differentiation protocols for use with human pluripotent stem cells (PSCs) is explored. Chapter 4 describes the testing of a 3D protocol, built on a concept that differentiation does not require use of extrinsic patterning factors to carefully reproduce the entire developmental environment cells experience *in vivo*. Rather, free-floating PSC aggregates were allowed to differentiate with minimal patterning factors, specifically those associated with an organizing structure within the desired neural structure, in this case the Isthmic Organizer of the developing cerebellum. Cells with cerebellar associated markers were produced, as well as structures within the 3D aggregates (commonly called "mini-brains" or "organoids") resembling features appropriate to a developing brain, including the mid/hindbrain region. This suggests that when allowed to differentiate freely, PSCs have the capacity to produce intrinsic patterning factors, and so may only need minimal external guidance. Chapter 5 shows how this can be extended to adherent 2D protocols, which may be more convenient for certain research goals, such as calcium-imaging to determine if functional neurons have been produced. In this case, the optional 2D modification was found competent to produce cells with neuron-like firing patterns. In both

the 2D and 3D differentiation protocols, experimental variation was found to be significant and a potential concern for researchers.

Chapter 6 reports the generation of iPSC lines from patients with 4H leukodystrophy (also known as RNA polymerase III-related leukodystrophy), and attempts to model aspects of the disorder using glial and cerebellar differentiation protocols. The goal of the glial protocol was to produce oligodendrocytes, which are responsible for myelination of axons and so involved (in an as yet unknown way) with the hypomyelination seen in 4H patients. The goal of the cerebellar protocol (being the 2D protocol reported in Chapter 5) was to generate cerebellar neurons, most importantly granule cells (followed by Purkinje neurons) which are major components of the cerebellum. Cerebellar atrophy is observed in many 4H patients, particularly those with mutations in the gene encoding the POLR3B subunit (from whom iPSC lines were derived). Significant differences between patient and control lines were not detected in products of either differentiation protocol, based on common cell-associated markers. It was not known if this was due to differences being below experimental variability, or simply nonexistent (perhaps at the stage of development covered by the protocols).

Chapter 7 took the modeling attempted in Chapter 6 one step further. Moving beyond common cell-markers, whole transcriptome analysis was performed on patient and control derived fibroblasts, iPSCs, and cerebellar differentiation products in an attempt to find potential differences in gene expression in a non-hypothesis driven fashion. The results were as interesting as they were unexpected. While no genes with obvious connection to 4H genotype or phenotype were found differentially regulated in patient fibroblasts compared to control, known POLR3 transcription targets were found down-regulated (compared to control) in iPSCs, and a gene with potential relevance to disease pathology (*ARX*) was found down-regulated in cerebellar products. Taken together, this identified what transcription targets were most affected by *POLR3B* mutations (5S rRNA subunits), and suggested a pathway for how altered RNA polymerase III might lead to the various abnormalities observed in 4H patients (across the brain and body). Other genes down-regulated compared to control in cerebellar products hint at an alternative, indirect mechanism for how mutations to POLR3 subunits might disrupt expression of *ARX* and so manifest disease pathology.

Chapter 8 puts the experimental results of the proceeding chapters into proper context, regarding the technology of controlling cell identity, as well as an understanding of the white matter disorder it was used to assess. In addition, it examines the issue of how our attempts to control cell identity is simultaneous relies upon and helps drive an understanding of cell identity itself (and in time perhaps personal identity).