Michael Burel
2010 Undergraduate Research Award

Derivation of Neural Progenitors from Induced Pluripotent Stem Cells

Ten seconds…that was the time it took me to fall down the rabbit-hole phenomenon that is stem cell research. Although I was editor-in-chief of my high school newspaper at the time, the technical write-up one of our writers produced was enough to invoke a paradigmatic shift in my academic interests. To know that human embryonic stem cells (hESC) possessed the potential to silence diseases by being able to specialize into all human cell types was something I could not ignore. In a field where "irresistibly challenging" only begins to scratch the descriptive surface, I shifted the invested time and great interest in my desires for journalism towards a more biological approach. I digested news article after news article on stem cell research, critically considering its medical and societal implications. I soon developed a particular interest for a late-breaking stem cell technology known as induced pluripotent stem cells (iPSC), a technique that literally turns back the clock on cells to a hESC-like state without embryonic destruction. Upon arriving at UGA, I joined the laboratory of Dr. Stice to investigate the intersection of neurodegenerative diseases and iPSC. The Stice lab has produced exciting research regarding neural progenitors (NPs), a checkpoint cell between the unspecialized hESC state and the terminally differentiated neuronal state. NPs are both easy to culture and able to specialize into the three cell types comprising the central nervous system, making them prime candidates for potential therapies in the neurodegenerative disease realm. I applied iPSC technologies in a neurodegenerative disease context that lead to the development of a project entitled "Derivation of Neural Progenitors from Induced Pluripotent Stem Cells."

Needless to say, the journey from project visualization to execution was demanding. I had arrived at the lab with essentially zero academic knowledge regarding neuronal development

or embryonic biology. I immediately began exhausting and reaping the benefits of the UGA library. Using the GIL catalog, I found textbooks and reference materials—specifically Stem Cell Assays and various methodological protocols—that scaffolded a framework upon which I could build my stem cell knowledge. I began narrowing my search from a more general overview to the NPs and iPSCs that would be the nexus of my research. To do so, I found Web of Science and PubMed to be crucial allies. In Web of Science, I used truncation techniques, negative filtrations, phrase searches, and operators to ensure I encompassed the exact cell type I was studying. Here, I used the reverse and forward citation mapping to backtrack to an original source or to internalize the trajectories that can spawn from a published work, respectively. Ranking by relevancy or citation number also helped me prioritize articles that had evident scientific interest to the rest of the community, and filtering by authors allowed me to familiarize myself with leaders in the field. From here, I used PubMed to review the articles in-depth or to confirm their relevancy by evaluating their abstracts.

It soon became clear to me that one of the keystones of stem cell research is differentiation, a biological process where an unspecialized sell (e.g. hESC) becomes more specialized (e.g. NPs). After piecing together the information I gathered from my extensive literature research, I noticed two things: 1) iPSC-derived NPs could realistically actualize NPs' clinical potential for regenerative transplantation, and 2) no one had differentiated NPs from iPSC before. These two premises were the seeds for my project, which grew quickly. I used articles published in respectable journals such as Cell, Neurobiology of Disease, Science, and Stem Cells to gather differentiation protocols and techniques as well as to understand potential outcomes and benefits of iPSC-to-NP differentiation. My project began producing positive qualitative results; the iPSC were showing morphological, phenotypic changes to the neural fate

while expressing characteristically positive indicators for NPs. The success, however, did not seem to last for long.

After a second repeat of experimentation, the iPSC culture no longer differentiated to the neuronal lineage. Rather, they displayed almost skin cell-like shape and behavior. My agenda transitioned from NP differentiation to determining what exactly was in my culture, and I found solace again in the UGA library's ample resource center. Using E-Journals, GALILEO, and JSTOR databases, I located an article regarding mesenchymal progenitor cell differentiation (the author of which, at one point, also worked in the Stice lab). I noticed the cell types generated by that protocol resembled the phenotype of the cells in my culture. What, then, had caused this sudden transmutation? Returning to Google Scholar and Web of Science, I located a variety of articles—one a review and two other specific case studies—that demonstrated the effects of a growth factor known as TGF-β on cellular transition to the mesenchymal state my cells were displaying. From here, I am in the process of developing another project investigating the role TGF-β plays in the transition my cells exhibited and how that can lead to tumor formation in patients with the neurological disease neurofibromatosis type 1.

Evidently, the tides of stem cell research change quickly, and the vast influx of articles incoming at any one given time is almost too much to absorb. To remain updated, yet focused, I use search queries in LexisNexis and ScienceDaily to keep abreast with recent scientific and related political developments. To keep track of publications, I use RSS feeds through search history manipulation in Web of Science. If any article is published containing my personally contoured keywords, Web of Science will catch them and update me immediately.

Research is a journey. It is continuous, winding, quickly changing, and challenging. Stem cell research in particular encapsulates these qualities perfectly. If anything, the resources

at the UGA library are the signposts along the journey. They point me in the right direction, keep me on track, and inform me of upcoming detours. In a field where so little is known—but so much is at stake—it seems as if the journey towards discovery is just as rewarding as arriving at the destination itself.

Michael Burel
CURO Apprentice

Derivation of Neural Progenitors from Induced Pluripotent Stem Cells

Human embryonic stem cells (hESCs) are pluripotent cells capable of becoming all human cell types. More specifically, hESCs have been shown to differentiate into neural progenitors (NPs), multipotent cells able to differentiate into neurons and neuroglia. Due to their more focused potential and viability *in vitro*, NPs are prime candidates for neurodegenerative disease studies and treatment options. However, hESC-derived NPs not only pose an ethical issue due to initial embryonic destruction but also preclude therapeutic and disease study benefits from being realized. Patients may face immune rejection upon hESC-derived NP transplantation, and creating disease-specific cell lines with the genetic and epigenetic characteristics of a neurological disease proves difficult. This study attempts to circumvent these obstacles by differentiating NPs from induced pluripotent stem cells (iPSCs), genetically reprogrammed somatic cells with hESC-like characteristics. To derive NPs from iPSCs, IMR-90 human lung fibroblasts were subjected to lentiviral vectors encoded for transcription factors promoting pluripotency. The reprogrammed fibroblast cells (now iPSCs) were cultured using a three-phase process utilizing different media and dish coatings to direct iPSC to the neural fate and ultimately the NP identity. Initial immunocytochemical results show successful differentiation of NPs from iPSC with the expression of markers previously observed in hESC-derived NPs (Nestin, Musashi-1, and SOX2). iPSC-derived NPs were further differentiated into terminal neurons, where staining suggests the positive expression of Tuj1, a more mature neuronal marker. The NPs will be differentiated into neuroglia, and positive/negative controls against cell lines will be quantified with real-time PCR and flow cytometry. iPSC-derived NPs could fully actualize the clinical potential NPs possess, opening avenues for the reverse-engineering of neurodegenerative diseases like Parkinson's and Alzheimer's while providing feasible treatment options to curtail the symptoms that dominate affected patients' lives.

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