Translation: Screening for Novel Therapeutics With Disease-Relevant Cell Types Derived from Human Stem Cell Models

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The advent of somatic cell reprogramming technologies—which enables the generation of patient-specific, induced pluripotent stem cell and other trans-differentiated human neuronal cell models—provides new means of gaining insight into the molecular mechanisms and neural substrates of psychiatric disorders. By allowing a more precise understanding of genotype-phenotype relationship in disease-relevant human cell types, the use of reprogramming technologies in tandem with emerging genome engineering approaches provides a previously "missing link" between basic research and translational efforts. In this review, we summarize advances in applying human pluripotent stem cell and reprogramming technologies to generate specific neural subtypes with a focus on the use of these in vitro systems for the discovery of small molecule-probes and novel therapeutics. Examples are given where human cell models of psychiatric disorders have begun to reveal new mechanistic insight into pathophysiology and simultaneously have provided the foundation for developing disease-relevant, phenotypic assays suitable for both functional genomic and chemical screens. A number of areas for future research are discussed, including the need to develop robust methodology for the reproducible, large-scale production of disease-relevant neural cell types in formats compatible with high-throughput screening modalities, including high-content imaging, multidimensional, signature-based screening, and in vitro network with multielectrode arrays. Limitations, including the challenges in recapitulating neurocircuits and non-cell autonomous phenotypes are discussed. Although these technologies are still in active development, we conclude that, as our understanding of how to efficiently generate and probe the plasticity of patient-specific stem models improves, their utility is likely to advance rapidly.

The development of effective treatments for neuropsychiatric disorders presents one of the greatest challenges and areas of unmet medical need in the 21st century. Affecting millions of individuals worldwide, neuropsychiatric disorders present a tremendous burden to individuals, their families, and society as whole (1,2). Because the causes of severe mental illnesses are likely to be complex and heterogeneous in nature, the application of new approaches and tools to gain insight into the underling etiology and pathophysiology is critically needed. Recent advances in human genetics have led to an explosion of our understanding of the role that genetic (3) and epigenetic (4) variation plays in determining the susceptibility to a wide range of psychiatric disorders. However, despite these important advances, our increased knowledge has yet to be translated to the discovery and validation of significantly improved, targeted therapeutics (5,6). Moreover, the polypharmacology of many psychopharmacological agents along with poor understanding of their precise mechanism of therapeutic action have limited the development of improved therapeutics that offer either disease-modifying or prophylactic effects, in marked contrast to other disorders, such as rheumatoid arthritis (7).

One of the main challenges to developing next-generation therapeutics for psychiatric disorders is that no common genetic variants of large effect have been identified. Instead, the genetic susceptibility for common disorders such as bipolar disorder, major depression, schizophrenia, and autism is clearly polygenic in nature (3). This polygenicity renders it impossible to create truly genetically accurate models of human psychiatric disorders in animal models that additionally often lack neuroanatomical regions of the brain thought to contribute to such pathologies (8). Moreover, recent comparisons of mouse and human model systems provide a cautionary tale about the challenges in extrapolating from one to the other (9).

Given these challenges and limitations to solely using rodent model systems, the ability to use genetically accurate human models to investigate the molecular and cellular mechanisms of disease and to investigate the step-by-step development of pathophysiology would have a major impact on our understanding of psychiatric disease mechanisms. Moreover, developing human cellular model systems capable of supporting screening for novel targets and lead structures for therapeutic development could help address current bottlenecks in the drug discovery process for psychiatric disorders, leading to the next generation of therapeutics (6). To date, an obstacle in developing such models has been the inaccessibility of the relevant human tissue in patients, making routine sample collection by biopsy infeasible. Even where tissue is available, terminally differentiated cells such as neurons cannot be maintained in culture, so experiments cannot be repeated, and these resources cannot be scaled up to the extent required to use them in chemical screens. Conversely, more accessible tissues such as lymphocytes do not necessarily recapitulate all of the signaling pathways and processes needed to explore pathophysiologic processes in neurons and other brain tissue; this is particularly the case for phenotypes that are not

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cell-autonomous but rather require interaction between multiple cell types.

Fortunately, recent advances in the field of human stem cell biology, namely the ability to create patient-specific induced pluripotent stem cells (iPSCs) that can be differentiated into a growing number of defined cell types with reprogramming technology (10–12), provide new avenues to investigate the pathophysiological mechanisms of psychiatric disorders. This approach allows more accessible cell types, such as dermal fibroblasts from a skin biopsy or lymphocytes in peripheral blood, to be reprogrammed to pluripotent cells. With this technique, which relies on the expression of cocktails of transcription factors, such as OCT4, SOX2, KLF4, and c-MYC (10,12), or OCT4, SOX2, NANOG, and LIN28 (11), there now exist a growing number of human iPSCs models of monogenic psychiatric disorders such as fragile X syndrome (13–15) and Rett syndrome (16–22), along with smaller number of examples of complex, polygenic psychiatric disorders, including schizophrenia (23–26) and bipolar disorder (27).

These new patient-derived cell lines effectively model a human disease genome in a form amenable to in vitro investigation. These models allow access to otherwise difficult or impossible-to-obtain living cells that comprise the human nervous system and, importantly, enable repeated experiments and larger-scale investigations, in contrast to tissue obtained from neurosurgery or through postmortem studies. Overall, disease-specific, human iPSC models provide an emerging, scalable platform from which to build a set of tools and an integrated approach for human chemical neurobiology that will allow: 1) genotype-phenotype correlations to be understood for complex genetic disorders; and 2) development of phenotypic assays capable of supporting high-throughput screening for novel therapeutic agents that target molecular mechanisms not currently modulated by the existing pharmacopeia used to treat psychiatric disorders (Figure 1). As additional encouraging signs of the potential of this approach, outside of the field of psychiatry large-scale therapeutic screening with iPSC-derived disease models has already been successfully applied in a number of examples (28–30), pointing to the generality of the approach for studying human disease biology.

Neurons Generated In Vitro from Multipotent, Self-Renewing, Neural Progenitor Cells

The isolation of multipotent, self-renewing, neural stem and progenitor cells from tissues derived from the rodent central nervous system (CNS) was first described over 2 decades ago by Reynolds and Weiss (31). Building on these studies, Carpenter et al. (32) described the existence of similar multipotent progenitor cells in the human embryonic forebrain that could be expanded in vitro in the presence of basic neurons, astrocytes, and oligodendrocytes. However, these initial studies were limited at the time to working with postmortem human brain tissues, which, for the reasons described in the following text, limited the full potential of human disease modeling. Ultimately, the ability to expand multipotent neural stem and progenitor cells from human pluripotent stem cells as nonadherent neurospheres, adherent monolayer cultures, or 3-dimensional structures that can form synaptically active, excitatory, and inhibitory neuron subtypes by multiple groups over the past few years has brought this approach to modeling human CNS disorders to the forefront (33–44). Examples of these iPSC-derived neural progenitor cells (NPCs) and neurons that can now be generated and used for functional genomic studies and for chemical neurobiology studies are depicted in Figure 2.

As a scalable platform for chemical neurobiology and novel therapeutic discovery, the use of these patient-specific, human iPSC-derived NPCs and differentiated neurons extends previous efforts with rodent stem cell-derived neurons (45–50) as well as efforts to use postmortem, human brain-derived immortalized (51,52), or nonimmortalized (53,54) NPCs. First, although the rodent and human nervous systems share a number of evolutionary conserved properties, there are also radical differences in terms of neurogenesis and neural patterning, most apparent grossly in the lissencephalic nature of rodent brain. Thus, to ultimately develop human disease-relevant neuronal cell models, it will be important to be able to routinely access neural cell types found within regions of the human brain, including the cerebral cortex (8,39,40,55). Second, many aspects of the epigenetic regulation of the genome, including noncoding RNAs and regulator enhancers,
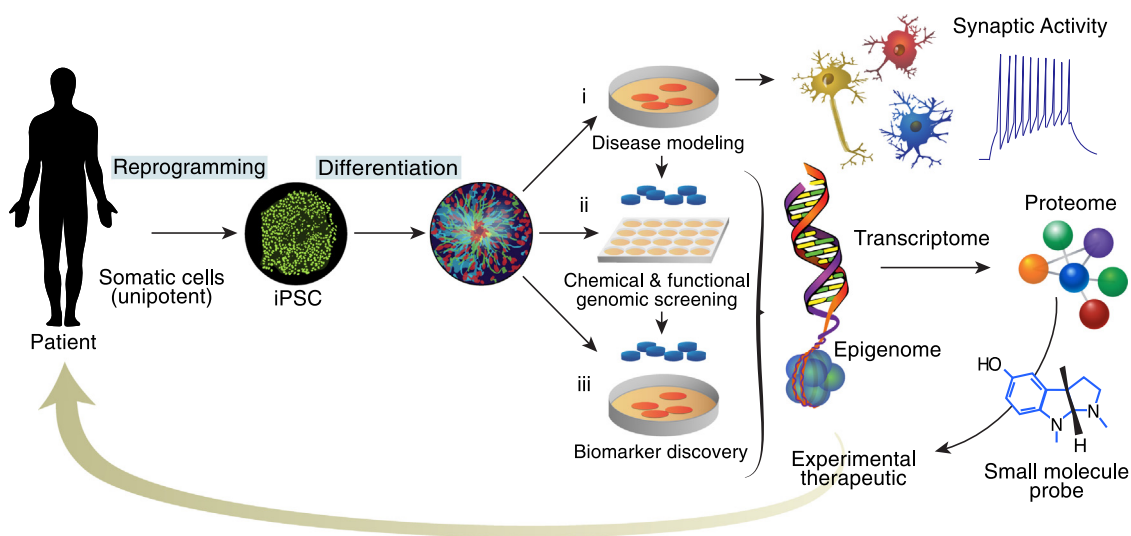
Figure 1. Overview of an integrated platform for biological and therapeutic discovery with patient-specific induced pluripotent stem cell (iPSC) models and chemical neurobiology. Artwork by Applied Art, LLC.