Investigating schizophrenia in a "dish": possibilities, potential and limitations

Nicholas J. Bray[1], Shitij Kapur[2], Jack Price[1]

[1]Department of Neuroscience and [2]Department of Psychosis Studies, Institute of Psychiatry, King's College London, London, UK

Psychiatric disorders such as schizophrenia are the most human of conditions, and the idea that they could be studied in a cell culture dish might appear counterintuitive. But advances in genetics and stem cell biology are placing *in vitro* approaches centre stage in furthering our biological understanding of these illnesses.

Genome-wide association studies and screens for rare genetic variants are now implicating a host of novel genes in susceptibility to schizophrenia and bipolar disorder. Concurrently, advances in stem cell biology are providing human nerve cells in a dish, allowing molecular, developmental and pathophysiological events to be studied with considerable veracity.

Here, we examine current human cell culture technologies and ask how far they might go in advancing our understanding and treatment of schizophrenia and other psychiatric disorders.

IMMORTALIZED HUMAN NEURAL CELL LINES

Certain neural populations can be directly obtained from living human subjects and grown as "primary" cultures. However, ethical and practical considerations generally limit the use of primary human brain tissue as a source of neural cells for research. Neural cell lines that have been immortalized through loss of tumour suppressor genes or oncogene induction provide a standardized and potentially limitless alternative.

The principal uses of such cell lines for psychiatric research are as models to explore the intracellular mechanisms of drug action and to investigate the molecular and cellular functions of identified susceptibility genes. This work may lead not only to refinements in current drug treatments, but also to the identification of novel therapeutic targets.

Tumour-derived neural cell lines

Researchers have for many years used human malignancies as a source of cell lines that will readily expand in culture, and several established tumour-derived lines have neural characteristics. Currently, the most commonly used human neural cell line is the SH-SY5Y line, which was originally derived from a metastatic neuroblastoma. This line displays neuronal properties, including neurite outgrowth, neurotransmitter synthesis and receptor expression.

The SH-SY5Y line has been widely used to study intracellular mechanisms of antidepressant and antipsychotic drug action (e.g., 1). Because they endogenously express neural proteins, SH-SY5Y cells are also of utility for investigating the mechanisms of susceptibility genes and the functionality of DNA sequence variants showing association with psychiatric disorders. For example, extracts from these cells have recently been used to demonstrate that the first DNA variant showing "genome-wide significant" association with psychosis alters the binding of a transcription factor which regulates expression of the ZNF804A gene (2).

Immortalized neural stem cell lines

Although they can have neural characteristics, tumour-derived cell lines are limited in the cell types that they can be made to resemble, and usually have major chromosomal abnormalities. Stem cells derived from human fetal brain are multipotent (i.e., they can give rise to a range of neurons and glia) and allow developmental and physiological processes to be studied more faithfully. Clonal neural stem cell lines can be generated by conditional immortalization, whereby a regulated gene that drives cell division is introduced into the cell's genome, allowing controlled expansion and differentiation (3).

Neural stem cell lines with normal chromosomes have been thus established from several human fetal brain regions, including cerebral cortex, hippocampus and striatum. Like tumour-derived cell lines, clonal stem cell lines provide a model system by which to explore the mechanisms of drug treatment and identified susceptibility genes for psychiatric disorders. For example, the hormone cortisol is thought to mediate the negative effect of stress on hippocampal neurogenesis. This has been modelled in a human hippocampal stem cell line, from which an understanding has emerged of how antidepressants counter this effect and restore neurogenic activity (4). Similar cells from human cerebral cortex have been used to model pathogenic changes in the expression of the disrupted-in-schizophrenia-1 (DISC1) gene (5) and to provide the first data on the molecular functions of the schizophrenia/bipolar disorder susceptibility gene ZNF804A (6).

PATIENT-DERIVED NEURAL CELLS

Another approach is to derive and compare neural cells from patient and control subject cohorts. The use of cells from patients permits investigation of pathological processes arising from the combined action of all the genetic susceptibility variants harboured by each individual. These living cell cultures may illuminate processes that are not apparent from case-control comparisons of brain tissue *post-mortem*; in particular, developmental processes which might be particularly relevant to schizophrenia aetiology.

Cells derived from olfactory neuroepithelium

The olfactory mucosa is a source of accessible adult stem cells that can be harvested through biopsy. These cells can be propagated in culture as neurospheres; that is, as aggregate cultures of neural stem cells and differentiating neural progenitor cells. Cells thus derived have been found to exhibit gene expression differences between schizophrenia patients and controls that implicate neurodevelopmental processes such as axon guidance (7). Cells derived from the olfactory neuroepithelium of schizophrenia patients have also been reported to show alterations in cell cycle dynamics compared with control individuals (8).

Induced pluripotent stem cells

While olfactory neural precursors from patients carry all of the genetic variants that have predisposed them to their illness, they do not provide a perfect model of the regional cell types that are considered to be central to psychiatric disorders, such as those of the cortex or hippocampus. Induced pluripotent stem (iPS) cell technology provides a major advance in this direction.

Imagine you could identify a prospective schizophrenia patient *in utero*, twenty years before the onset of the illness, and take a brain biopsy. You could then culture the patient's own cells, and follow their development as the pathological processes played out. Remarkably, iPS technology, reported in a seminal paper in 2006 (9), permits a close approximation of this. Primary somatic cells – typically from skin – can be taken from a patient and "reprogrammed" into pluripotent stem cells that can give rise to all of the cell types that make up the body, including those of the central nervous system.

Reports are now beginning to emerge in which this technology has been applied to cells taken from psychiatric patients. For example, Brennand et al (10) took skin fibroblasts from schizophrenia patients and healthy controls, reprogrammed them, and then grew neurons from these pluripotent cells. Compared with control cells, neurons derived from patients showed altered expression of genes involved in glutamate, cyclic adenosine monophosphate (cAMP) and wingless-type MMTV integration site family (WNT) signalling, as well as reduced neurite number and synaptic connectivity.

By capturing a patient's entire genome and normal development probably as accurately as possible in a two dimensional culture, iPSCs constitute an unparalleled material for studying neurodevelopmental features of psychiatric disorders *in vitro*. Most recently, it has also proven possible to reprogramme human fibroblasts directly into neuronal ("induced neuronal" or "iN") cells (11), providing a more convenient source of models of individual patient neurons with which to investigate mature cellular pathophysiology and drug treatment response.

However, both of these technologies are very much in their infancy; studies to date have been based on very few samples and future work will be challenged by the variability inherent in the neural cultures themselves, as well as between cell types and subjects (12). Standardized protocols for scaling up these experiments will therefore be necessary for these technologies to reach their full potential.

LIMITATIONS OF CELL MODELS

All cell models have their shortcomings. Although generic human neural cell lines can be used to investigate the molecular and cellular functions of individual susceptibility genes, they do not capture the many, likely interacting, genetic variables that contribute to the development of complex psychiatric disorders. Patient-derived cell lines offer the advantage of capturing each individual's whole genome, but we currently have limited knowledge of which cell types are most relevant to these illnesses and therefore which ones to study.

Analyses of multiple cell types from each patient, when compared with those from control individuals, might indicate cell populations that are generally affected in a given condition, but this will be at considerable expense. In addition, while iPS/iN technology controls environmental variables that can confound investigation of pathogenic mechanisms (e.g., effects of medication), it also loses the effects of environmental factors that contribute to psychiatric illness.

More generally, psychiatric disorders such as schizophrenia are an emergent property of the human brain as a whole, in the context of the individual within society. Although cell models can help elucidate the molecular and cellular basis of these disorders, they therefore have to be considered as only one level of enquiry.

CONCLUSIONS

Cell-based approaches to psychiatric disorders are advancing on two fronts. On one, clonal lines which accurately model cells of the central nervous system are being used in tightly controlled experiments assessing the mechanisms of drug action and identified susceptibility genes for psychiatric disorders, which might in the short term provide the fastest route to improved treatments for these conditions. On the

other, cells derived from patient and control populations are allowing pathological processes arising from the combined action of multiple genetic susceptibility variants to be assessed in "real-time". Although protocols for scaling up induced neural cells are still in development, the combination of accessibility and face validity guarantee their adoption by both academics and drug companies.

While cell models can never capture all of the complexity of psychiatric illness, the derivation and study of defined neural cell types from large patient cohorts may in the not too distant future provide considerable insights into the biology of these disorders, as well as models with which to develop and test novel therapeutics.

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