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Human Cell Models for Schizophrenia

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Abstract

Research of mental disorders that affect mainly unique human traits or higher brain function will benefit greatly from the introduction of live human tissues relevant to account for the phenotypes. Human neuronal cell models allow for precise molecular and functional characterization of patient phenotypes and genetic backgrounds. Sources of human cell types discussed here include cellular reprogramming of patient somatic cell lines (either first to pluripotency or directly to neuronal cells) and biopsy of olfactory tissue. Induced pluripotent stem (iPS) cells are particularly useful to study developmental trajectories and functional activity in many disease-relevant cell types. In fact, several attempts have been made to use iPS cell-derived neurons to study schizophrenia and other psychiatric disease. iPS cell technology consists of very high-cost and laborious experiments that may be ameliorated by a recent, more short-term cell conversion technique to obtain directly induced neuronal (iN) cells from somatic cell lines. Moreover, neuronal cells from olfactory epithelium (OE) biopsy have yielded promising research in that they serve as a reasonable surrogate for the brain without adding any genetic manipulation. These human cell models should be integrated with current clinical psychiatric and functional characterizations as well as animal models to progress the translational and clinical applications of basic research.

Why Do We Need Human Cell Models for Schizophrenia Research?

Human cell models offer a promising strategy to study the biology that underlies schizophrenia and can serve to complement animal and computational models. We define human cell models as central nervous system (CNS)-relevant cells that are enriched or reprogrammed or directly converted from biopsied tissues of patients and normal controls.

The Gap between Animal Models and Human Pathology/Biology

Although rodent models are very useful in addressing some key biological mechanisms that are potentially related to human brain disorders, it is not clear whether mouse or rat neurons can faithfully replicate the pathologies of human brain disorders due to the substantial species differences in neurons, including the following:

1. The unique features of enlarged cerebral cortex in humans are formed through distinct developmental mechanisms to generate cortical neurons, compared with those of rats and mice (Hansen et al. 2010).
2. There is evidence that even the same molecule has a differential spatiotemporal expression pattern in neurons in humans compared to rodents. For example, MeCP2, a molecule responsible for Rett syndrome, is known to have a differential expression pattern in human and mouse brains (Shahbazian et al. 2002).
3. A very recent study reports that development and structures of synapses, basic physical compartments in neuron–neuron communications, are different due to the evolutionary changes regarding the *SRGAP2* gene (Charrier et al. 2012).

Thus, human neurons (if they are available) would be very important to elucidate human-specific characteristics of neurons, which may not be fully covered by rodent models alone. Consequently, such cells may be crucial to clarify molecular mechanisms of brain disorders, especially neuropsychiatric disorders in which human-specific traits may be impaired, and to build assay systems for translational use.

Downfalls of Autopsied/Postmortem Brain Studies

Analysis of autopsied human brains has made important contributions to the field. Transcriptome-profiling experiments show widespread, yet specific, gene expression disturbance across the brain, within multiple cell types and biological processes. Data from these gene expression profiles are utilized in rodent models for the study of disease-relevant molecular cascades (Lin et al. 2012). Information from these vast studies can be gateways to animal model research and extremely informative to tissue culture studies and drug development (Horvath et al. 2011).

Postmortem brain studies are useful, but they have many confounding factors: lifestyle differences, age at time of death, cause of death, varying length of disease/age of onset, and various environmental influences including the effects of medication and substance abuse. All of these factors are especially prevalent among schizophrenia samples. Furthermore, we cannot gain any understanding of functionality from the postmortem brain, and there is no tight link to developmental processes, which likely play a key role in the pathophysiology of schizophrenia (Cascella et al. 2007).

Thus, human cell models may provide complementary approaches to obtain disease-associated molecular and cellular changes.

Utility of Human Cell Models

Recent advances in reprogramming and cell culture technologies have allowed us to obtain human-derived neuronal cells (Yang et al. 2011; Dolmetsch and Geschwind 2011). The advantages of this resource should have great implications for psychiatric disorders, such as schizophrenia, that are uniquely human. Findings from research such as the aforementioned rodent studies and human postmortem brain analyses can provide a foundation for studying human cellular phenotypes *in vitro*. Alterations in neuronal properties, such as arborization, synaptic density, neuronal migration, neuronal connectivity, and signaling, have been found in postmortem and rodent model studies for psychiatric disorders. Thus, functional changes in these biological paradigms may be tested by using human-derived neuronal cells. Furthermore, by employing unbiased assays, especially those for molecular profiling, to human neuronal models, we may be able to build novel hypotheses to unravel the pathophysiology of complex psychiatric diseases (Figure 10.1).

The direct utility of neuronal cells from living patients is vast. Functional characterization and cellular properties can be integrated with unique patient

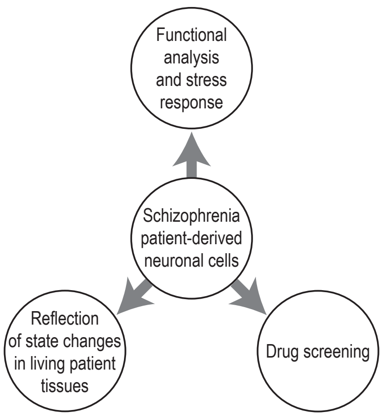

Figure 10.1 Cell models for schizophrenia research. Live sampling of patient tissues provides researchers a unique opportunity to observe functional phenotypes of human neurons. Mechanisms of existing patient abnormalities can be effectively characterized and evaluated at baseline in response to external stimuli and stress. Possible reflection of state changes at the time of biopsy would be useful for longitudinal design, cellular response to treatment as well as identification of biomarkers for diagnosis and prognosis. In addition, live patient tissues would be useful for drug screening and personalized medicine.

attributes such as genes and symptoms. Using electrophysiological recordings, we can address cell autonomous changes as well as functional connectivity among neurons. Easy availability allows for precise molecular and mechanistic characterization of cell lines. Currently, we can link specific genetic abnormalities found naturally in patient samples or through genetic manipulations to live neuronal phenotypes. The advantages of human neuronal models are fully utilized when we wish to address the mechanisms of gene–environment interaction, which is well understood to play a significant role in the etiology of mental illness. Cellular response to stressors or detrimental environmental effects can be implicitly monitored in human cell models. In addition, the translational utility of animal models for bringing new drugs for mental illness to market has fallen short. Testing human cellular response to novel compounds may help to synergize efforts for effective drugs.

Sources of Live Human CNS-Relevant Cells

In this section we describe representative methodologies for human cell models, including their advantages and limitations (see also Table 10.1).

Table 10.1 Comparison among available human neuronal tissues: induced pluripotent stem (iPS) cell, induced neuronal (iN) cell, and olfactory-derived neurons have unique advantages and can be compared to traditional postmortem brain analysis for a better understanding of schizophrenia and other psychiatric diseases. Each cell system also has disadvantages which must be overcome.

	Advantages	Disadvantages
Postmortem brain	• Whole brain • All cell types present	• Many confounding factors • Functional assay not available • Less link to developmental trajectories
iPS cell and embryonic stem cell-derived neurons	• Live neurons • *In vitro* functional study • Examination of developmental trajectories • Directed differentiation of cell type	• Long-term, laborious, and high-cost • Heterogeniety • Epigenetic memory of lineage
iN cells	• Live neuronal cells • *In vitro* functional study • Short-term experiments • Potentially high-throughput	• Limited directed differentiation of cell type • Low conversion rate and maturity of neurons
Olfactory tissues	• Live immature neuronal cells to homogeneity • Homogenous cell population • *In vitro* functional study • Easy for preparation	• May not completely represent brain neurons • May not be able to chase several developmental phases

Induced Pluripotent Stem Cells

Easily accessible patient cells, such as skin fibroblasts, can be reprogrammed to a pluripotent state similar to embryonic stem cells. Theoretically, these iPS cells allow for the production of any cell type from somatic cells. Specifically, iPS cell-derived neurons can give us resources with which to examine developmental trajectories and neuronal functions, in addition to traditional molecular and histochemical tissue analysis.

Advancement of the Technology

First derived from mouse fibroblasts, iPS cells were induced to a pluripotent state by the application of four embryonic stem cell maintenance factors: Oct3/4, Sox2, c-Myc, and Klf4 (Takahashi and Yamanaka 2006). This was quickly followed by iPS cell production from human somatic cells with the four factors: Oct4, Sox2, NANOG, and Lin28 (Yu et al. 2007). Since these first studies, iPS cells can be derived from multiple resources of somatic cells and differentiated into multiple cell types found in brain tissue (Dolmetsch and Geschwind 2011).

Transduction methods have progressed since the early stages of this technology. The most common method of transduction—integration of transcription factors by viral infection—is adequate for disease modeling purposes, though not for potential transplantation applications (Han et al. 2011). Alternative methodologies, such as those using nonintegrating vectors, excisable lentiviral vectors, proteins that are taken up into the cells to facilitate cell reprogramming, or synthetic mRNA, have since been successfully tested in iPS methodology (Warren et al. 2010; Kim et al. 2010; Kaji et al. 2009; Soldner et al. 2009; Yu et al. 2009). Also, small molecules can be used in addition to or in replacement of factors, but small molecule-only transduction is not yet available (Li et al. 2009). If iPS cells are to be used to study reprogramming or developmental mechanisms, it is best to use the most robust reprogramming method to ensure the most efficient transduction. This is usually accomplished by retrovirus or lentivirus transductions. It is desirable to use robust reprogramming methods when using iPS cells for disease modeling and drug screening. However, nonintegrative methods would reduce the amount of heterogeneity and tumorigenicity among resulting cells. Thus, when considering the use of iPS cells for cell therapy, nonintegration methods are much safer.

iPS cells are most frequently generated from fibroblasts, but have also been established from patient blood. Cells collected from fresh peripheral blood can be used to produce iPS cells efficiently using similar methods to fibroblast reprogramming technology, allowing for an additional patient resource to be easily accessed (Loh et al. 2010; Seki et al. 2010; Staerk et al. 2010). iPS cells have also been produced from immortalized blood cell lines (Choi et al. 2011;

Rajesh et al. 2011). Although this is not yet well established, it is an important advancement because it is the resource most commonly stored in most genetic repositories. Therefore, it is very important for the field of disease research to advance this specific reprogramming technique.

Differentiation into disease-relevant cell types (mainly neurons and glia) and subtypes depends on careful protocols that guide cells throughout developmental stages to specific lineages relevant to disease. Neurons are typically produced from iPS cells by first going through a neural progenitor stage, followed by directed differentiation to neuronal subtypes using neuronal transcription factors and inhibitors of other developmental pathways. Resultant cell types are dependent on factors used and precise timing of culture conditions (Han et al. 2011). iPS cells can be differentiated into glutamatergic, dopaminergic, GABAergic, and motor neurons, as well as astrocytes and oligodendrocytes. Careful cell type specification from iPS cells to neuronal tissues of interest is important for the application of this technology to psychiatric disease. Researchers should aim to produce cell types that are directly relevant to the disease of interest to best elucidate disease-relevant mechanisms (Hansen et al. 2011).

Disease Application

Live neuronal cells derived from humans will be particularly useful for studying developmental trajectories and neuronal characteristics of cells from patients with psychiatric disease. Characterization of iPS cell-derived neurons from patients with psychiatric disorders in the autism spectrum and with schizophrenia has been accomplished (Marchetto et al. 2010; Brennand et al. 2011; Pasca et al. 2011). Although the sample size is extremely small, a pioneering study suggests that neurons from patients with schizophrenia show reduced neurite number, overall connectivity, and levels of glutamatergic receptors and postsynaptic density proteins (Brennand et al. 2011).

The study of patient samples with rare genetic mutations may also be a useful avenue for using patient-derived neuronal cells in schizophrenia research. For example, neuronal cells derived from patients with Rett syndrome, an autism spectrum genetic model, showed morphological, electrophysiological, and early developmental deficits when compared to controls (Marchetto et al. 2010). Similar methods could benefit schizophrenia research through the application of established genetic susceptibilities.

iPS cells also allow for analysis of completely different cell types from the same patient. This is especially useful in the study of systemic disorders. In patients with Timothy syndrome, a rare genetic disorder caused by a mutation in the calcium channel $Ca_v1.2$, iPS cells have been used to show abnormalities in both cardiac and neuronal cells (Pasca et al. 2011; Yazawa et al. 2011). In relationship to this study design, increasing evidence supports the notion that schizophrenia might be a systemic disorder, instead of a mere brain disease (Kirkpatrick 2009). Understanding how genetic abnormalities affect different systems and cell types may help to unify some hypotheses and models of schizophrenia.

Technical Limitations

iPS cell technology may have a profound effect on the field of schizophrenia research, but it also has substantial limitations. Production of iPS cells and subsequent cell types requires long-term cell culture, which means that it is a very laborious and high-cost technology. Furthermore, the lifetime of iPS cell cultures is limited, and thus the maturity of neurons produced is also limited. There is a balance between the capabilities of long-term cell culture and maturity of those cells. Improvements to the robustness of conversion through upcoming transduction methods will reduce the strain on research in the future. Commercially available iPS cell lines may help accelerate research in laboratories as well.

There are also limitations within iPS methodologies. Arguably, the most prominent is the heterogeneity between iPS cell clones and the developmental differences which arise from them. Conversion from somatic cell to iPS cell is low within a cell line, and many iPS cells will be cloned from one converted cell. Therefore, any differences that exist between originally converted cells are amplified. Careful selection of clones can limit the impact of this.

Epigenetic memory of iPS cells and cells derived from them constitutes another limiting factor. Although neurons derived from iPS cells show implicitly neuronal phenotypes, it has been shown that, when compared to embryonic stem cells, epigenetic structure of DNA is still related to the somatic cell type of origin and not fully matched to natural stem cells. These DNA methylation signatures can be altered to resemble more closely the signatures of true stem cells through the use of chromatin-modifying drugs or serial reprogramming and differentiation (Kim et al. 2010).

Future Perspectives for Schizophrenia Research

The association of environmental factors in the etiology and manifestation of psychiatric diseases, including schizophrenia, is well established. However, direct links between diseases and stressors have not yet been determined. Therefore, at least for the immediate future, it is best to use cell lines from patients with a defined genetic background, to be certain that the observed cell phenotypes are directly associated with disease phenotypes. Such research may be expanded to genetic models of mental illness, such as 22q11 and 16p11 mutations.

Direct Cell Conversion

Influenced by the idea that somatic cells can be modified to pluripotency, stem cell biologists attempted to directly induce fibroblasts to other differentiated cells, including neurons. In the area of neuroscience, the first successful example was to convert mouse fibroblasts to functional neuronal cells (Vierbuchen et al. 2010).

Advancement of the Technology

Wernig and colleagues (Vierbuchen et al. 2010) produced iN cells from embryonic and postnatal mouse fibroblasts through lentiviral induction of neuronal transcription factors. Screening of combinations of 19 neuronal and epigenetic reprogramming transcription factors led to the discovery of a three-factor system for reprogramming: the combination of Ascl1, Brn2, and Myt1l. These iN cells express multiple neuronal markers such as βIII-Tub and MAP2, generate action potentials, and form functional synapses.

Conversion of human fibroblasts to iN cells was soon accomplished by using the same three factors used in mouse experiments (Pang et al. 2011). Ascl1, Brn2, and Myt1l will also convert human iPS cells directly to neuronal cells; however, the addition of transcription factor NeuroD1 was necessary to induce conversion of fetal and postnatal human fibroblasts directly to neurons. In addition to immunohistochemical staining for neuronal markers, iN cells generate action potentials, and matured cells make synaptic contacts. The increased complexity of reprogramming from mouse to human cell lines is an example of the vast evolutionary changes to human biology. This further highlights the importance and utility of obtaining human neuronal cells.

The direct conversion of human somatic cells to neuronal cells is a great advancement for psychiatric disease research, but methods need to be made more efficient and robust for further studies. Higher conversion rates, especially of adult cell lines, and improved maturity of iN cells will be important for schizophrenia studies consisting primarily of an adult population with complex molecular and functional phenotypes to be studied. The field has begun to make efforts in the right direction. For example, miRNA-mediated conversion of fibroblasts to iN cells improves efficiency of human iN cell conversion (Yoo et al. 2011). When combined with the transcription factors NeurD1, Ascl1, and Myt1l, miR-9 and miR-124, which have both been shown to be important for neuronal differentiation and development, yield efficient conversion of fibroblasts to iN cells. The addition of small molecules that regulate important pathways in neuronal development has also led to a robust improvement in iN cell conversion (Ladewig et al. 2012).

In regard to neuronal cell type, most investigators used a nondirect approach, including those described above, to produce cultures that coincidently contain a majority of glutamatergic neurons, while obtaining other neuronal types, such as GABAergic and dopaminergic neurons, more rarely. Few groups have directed their conversion to a neuronal fate of interest. However, the addition of the transcription factors FoxA2 and Lmx1a to the original three-factor system directly yields dopaminergic (tyrosine hydroxylase-positive) iN cells (Pfisterer et al. 2011). Furthermore, a minimal set of transcription factors—Ascl1, Nurr1, and Lmx1a—is sufficient to produce induced dopaminergic neuronal cells (Caiazzo et al. 2011). Motor neuronal cells can be produced with the addition of eight motor neuron specification factors (Son et al. 2011).

Disease Application

Widespread application of iN cell technology has not yet occurred in psychiatric disease research. However, Qiang et al. (2011) have observed the conversion of cell lines to iN cells from patients with familial Alzheimer's disease. This is an important proof of concept that adult human iN cells can demonstrate a neuronal cell-specific pathology that can be characterized by histological and electrophysiological methods.

Technical Limitations

iN cell technology is similar to iPS cell technology in its limitations: it still consists of laborious and high-cost experiments, despite having the advantage of being a short-term cell culture. Efficiency of conversion is often low, and maturity of neuronal cells is often limited. In addition, there is a large gap in conversion rate of samples from fetal or newborn patients and adult patients. Thus, the field will need to greatly advance the technology of adult patient cell lines before schizophrenia research can be done well in iN cells. More efficient experiments will eventually relieve this burden from laboratories and make analysis of cultures more informative. Furthermore, epigenetic memory and partial conversion of cells is even more apparent than in iPS cells. In fact, partially converted iN cells can seem to take on an uncharacteristic morphology, yet be positive for neuronal markers like MAP2 (Yang et al. 2011). Researchers may be able to look to improvements in iPS cell technology for help with this. In one iN study, Ladewig et al. (2012) used small molecule inhibitors common to iPS cell neuronal induction for more efficient direct conversion to neuronal cells. iN cell technology is also limited in its ability to reach cell type-specific conversion at high efficiency. For example, in addressing the pathophysiology of schizophrenia, a subclass of GABAergic neurons is needed in parallel to glutamatergic neurons. This may be overcome by referring to novel methodologies that are developed in cell type-specific differentiation of iPS cells.

Future Perspectives for Schizophrenia Research

Cell type-specific conversion will help potential application of iN cells to schizophrenia research. In addition, possible conversion to glial cells, such as astrocytes, microglia, and oligodendrocytes, may also be important for psychiatric disease research and may help to recapitulate schizophrenia as a whole brain disorder. Generation of induced microglia, for example, could benefit a neuroimmune hypothesis of schizophrenia. Furthermore, recent generation of induced neural stem cells from mouse and human fibroblasts could allow for the straightforward study of human neuronal development *in vitro*, omitting the need to begin with a pluripotent state (Ring et al. 2012).

Nasal Biopsy and Olfactory Cells

For over two decades, olfactory neurons via nasal biopsy have been expected as a possible surrogate tissue to study the brain (Trojanowski et al. 1991; Talamo et al. 1989). Due to technical barriers, as described below, this technique has not been widely utilized. Paradoxically, after the limitations of iPS and iN cell technologies became known, the significance of nasal biopsy and olfactory cells has been revisited and underscored.

Advancement of the Technology

The olfactory epithelium (OE) is an easily accessible, direct resource of patient-derived neuronal cells that can be obtained through a simple and relatively noninvasive procedure (Cascella et al. 2007). Early characterizations of OE showed that it is composed of several cell types: structural/supportive cells, neuronal cells which express distinct neuronal markers (e.g., neural cell adhesion molecules and microtubule associated proteins), and basal stem cells that are supposed to give rise to new olfactory neurons (Trojanowski et al. 1991). Thus, immunohistochemical study of OE tissue sections has been used to examine neurodevelopmental processes and disease-relevant molecular changes occurring within the OE tissue of patients and controls (Arnold et al. 2001, 2010). Investigators have also tried to develop methodologies of culturing as well as of differentiating and distinguishing neurons from OE-biopsied tissues.

Following initial studies, improved biopsy methods have increased the amount of neural tissue that can be obtained from one OE biopsy. Introduction of endoscopic sampling from patients have improved the efficiency and quality of samples that had originally been obtained “blindly.” Furthermore, biopsy from the dorsoposterior regions of the nasal septum has been shown to increase the probability of obtaining neuronal cells from the tissue (Feron et al. 1998).

Improvements to culture conditions of the biopsy have set the stage for new experiments with human olfactory neurons (Feron et al. 1998). Methodology to culture OE slices has been used to examine *in vitro* cell death, mitosis, neuronal density, and response to a neurotransmitter in both healthy controls and individuals with mental illness (Feron et al. 1999). In addition, OE slice cultures have been used to investigate cell cycle alterations in culture and expression profiles by microarray (McCurdy et al. 2006).

In parallel, efforts to use dissociated OE cell culture for further characterization of the neuronal cells have been made by several groups. Functional activity of olfactory receptor neurons obtained by dissociation of OE tissue has been addressed by measuring intracellular calcium in response to odorants (Rawson et al. 1997; Restrepo et al. 1993). This method was applied to evaluate possible differences in cells from patients with bipolar disorder and controls (Hahn et al. 2005). A more recent study reported that cultures of dissociated OE cells can include neuronal cells that are mature enough to express odorant and neurotransmitter receptors and active signaling mechanisms (Borgmann-Winter et al. 2009).

Cultures from OE tissues can produce neurospheres (i.e., clusters of cells consisting of multipotent progenitors), which in turn generate cells expressing neuronal markers (such as MAP2) and some glial markers (Roisen et al. 2001). Cyclic-AMP, retinoic acid, forskolin, sonic hedgehog, and other media nutrients have improved the neural differentiation and maturation of neuronal cells from neurospheres (Zhang et al. 2004, 2006; Roisen et al. 2001). Interestingly, retinoic acid, forskolin, and sonic hedgehog can elicit motor and dopaminergic characteristics of the neuronal cells, suggesting that olfactory neuronal cells *in vitro* are sensitive to cell fate directions without direct genetic manipulation (Zhang et al. 2006). Isolated cultures of neurospheres produce more numbers of new neurospheres and continue to proliferate over time, and the progenitors from them are restricted to neuronal and glial cell fates (Othman et al. 2005). Progenitor cells obtained from OE neurospheres have characteristics consistent with other stem cells, such as retained telomerase activity and stability of apoptotic activity in culture over time (Marshall et al. 2005a). Neurosphere-derived cells have been used to study gene and protein expression as well as neuronal cell functional activity (Matigian et al. 2010; Fan et al. 2012).

Methods to purify a unique cell population to near homogeniety from biopsied tissues have recently been explored. By using laser capture microdissection, it is possible to purify neuronal layers in which an olfactory neuron receptor marker OMP can be enriched up to thirtyfold more than whole OE tissue (Tajinda et al. 2010). In addition, a protocol that can enrich immature neuronal cells to near homogeneity has also been established (Kano et al. 2012).

Disease Application

Olfactory neuronal cells provide a good surrogate system to study brain disorders, including mental illness. Initially, OE-derived resources, such as cells, were used in research in Parkinson's disease and Alzheimer's disease. Alzheimer's disease is known to accompany odor detection deficits, which provided justification to study OE. In fact, one of the earliest studies with OE found unique pathological changes in the tissue from patients with Alzheimer's disease (Talamo et al. 1989). More recent studies have found that the altered phenotypes found in the OE tissue from patients with Alzheimer's disease correlate with the whole brain pathology that is characteristic of the disease, including amyloid-β accumulation (Arnold et al. 2010). Olfactory dysfunction is also a robust symptom of Parkinson's disease (Doty 2012). Moreover, cells from OE neurospheres from patients with Parkinson's disease show dysregulated gene expression of mitochondrial function, oxidative stress, and xenobiotic metabolism pathways (Matigian et al. 2010), which have previously been linked to Parkinson's disease pathology (Henchcliffe and Beal 2008).

OE tissues could be particularly useful for psychiatric disease research. The psychological processes of motivation, emotion, and fear are closely associated with olfaction as well as the negative symptoms of schizophrenia (Zald and Pardo 1997; Andreasen 1982). Indeed, olfactory deficits have been reproducibly associated with schizophrenia, especially negative symptoms of the disease (Turetsky et al. 2009). A question that arises is whether the olfactory phenotype is due to molecular alteration in OE cells/neurons, or due to a more complicated mechanism, including upstream olfactory circuitry, or both (Sawa and Cascella 2009). A recent study reported that individuals with schizophrenia and their first-degree relatives have different odor detection thresholds for two odorants that differentially activate intracellular cAMP-mediated signaling, indicating that molecular deficits in OE cells/neurons are likely to be, at least in part, associated with the disease pathophysiology (Turetsky and Moberg 2009).

OE biopsies have been used by several groups to study *in vitro* alterations in cells from patients with schizophrenia and other psychiatric disorders. Neuronal cells from the OE tissue show differences in cell adhesion, proliferation, and death in schizophrenia compared to controls (Feron et al. 1999). OE tissues from patients with schizophrenia show reduced density of p75NGFR positive basal cells and increased density in GAP43 positive immature olfactory neurons, as well as increased ratios of immature olfactory neurons or olfactory marker protein positive mature neurons to basal cells, indicating altered development and differentiation within patient tissues (Arnold et al. 2001). In dissociated OE tissue cultures, decreased calcium signaling was observed in cells from patients with bipolar disorder when compared to controls (Hahn et al. 2005). In addition, cell cycle alterations have been found in OE neurosphere-derived cells from patients with schizophrenia and bipolar disorder (McCurdy et al. 2006). Cells showed increased mitosis and expression of cell cycle proteins in patients with schizophrenia, and increased cell death and phosphatidylinositol signaling pathway proteins in those with bipolar disorder. Proliferation rate was also shown to be increased in neurosphere-derived cells from patients with schizophrenia (Fan et al. 2012). Finally, gene and protein expression profiling of OE neurosphere-derived cells from patients with schizophrenia show dysregulated neurodevelopmental pathways (Matigian et al. 2010). In addition to the cellular phenotypes described above, epigenetic profiles of immature olfactory neuronal cells have revealed alterations in oxidative stress response pathways in schizophrenia compared to controls (Kano et al. 2012).

In conjunction with the practicality of OE-derived cells for disease research, OE tissues and cells may also be beneficial for development of therapeutics. The OE itself is a useful area for drug delivery to the brain as it is one of the few areas of the CNS that is readily accessible (Kandel et al. 2000). Therefore, drug development using OE cell culture would provide a direct way to assess the effect of a drug on neural tissue. Consequently, OE-biopsied tissue has previously been used as a tool to evaluate the pharmacological effects of a CNS-acting therapeutics and has revealed biological activity of the astrocyte-targeted drug, thiamphenicol (Sattler et al. 2011).

Technical Limitations

OE tissue can be used to study human neuronal mechanisms and disease characteristics, but it has some drawbacks. First, nobody has fully validated OE-derived cells as CNS neurons that are physiologically relevant at the cell autonomous level or in the context of synaptic connectivity. Second, although OE-derived cells represent a diverse array of molecular signaling pathways relevant to studying brain diseases like schizophrenia, unbiased and extensive studies of whether and how these cells resemble CNS neurons have not yet been conducted.

Future Perspectives for Schizophrenia Research

Given that OE-derived cells are higher throughput, less laborious, less time-consuming, and much less expensive resources when compared to iPS cell-based models, the utility of OE needs be enhanced. To achieve this goal, the resolution of the technical limitations described as above becomes very important. Establishment of further protocols to prepare/enrich homogeneous cell populations, hopefully to fully mature neurons with relevant synaptic formation, is expected. As olfactory deficits are a key phenotype of schizophrenia, especially its negative symptoms, it is very important to study how cellular and molecular changes in OE-derived cells can represent these higher functions.

Link to Animal Models

Gene expression profiling studies of human cells and tissues from patients with schizophrenia and other mental illnesses can provide clues of disease-relevant molecular changes (Lin et al. 2012). However, a major limitation in such human studies is that the information cannot encompass neural circuitry-mediated disease pathology. To compensate for this limitation, human cell study should be linked to research with animal models for the following reasons: First, molecular information obtained from human cell research can be utilized to generate new genetically engineered models, which may be useful in studying the biology that underlies the disease pathology. Second, it will be informative to examine currently available animal models for molecular changes observed in patient cells.

The use of rodent models for schizophrenia research is discussed by O'Donnell (this volume). In addition to rodent models, nonhuman animal models remain an important tool for neuroscience research. With their extremely well-established nervous systems, small animals (e.g., fly, nematode, zebrafish) provide a well-defined substrate for correlates between molecular and cellular processes and behavior (Burne et al. 2011). For example, *Drosophila* (fruit fly) is very commonly used in genetic manipulation studies and can provide relatively high-throughput gene–behavior relationship data for neuroscience studies. For DISC1, transgenic flies have been linked to effects on behavior and pathways for gene transcription (Sawamura et al. 2008). The nervous system of *Caenorhabditis elegans* (nematode) is completely defined down to the cellular level, including its nervous system (White et al. 1986). Although very simple, its nervous system contains neurons that act very similarly to mammalian cells and interact via common neurotransmitters such as glutamate, GABA, and others (Burne et al. 2011). Mechanistic understanding of molecular pathways that are important for psychiatric disease research, such as for DISC1, can be easily observed in the animal (Brandon and Sawa 2011). Furthermore, investigators have paid attention to zebrafish: due to their transparent bodies, brains in these small animals can be observed in intact, behaving animals. In addition, genetic and molecular manipulations of the zebrafish nervous system can be manipulated in the same way as invertebrates, but their nervous system structure and function is much closer to the mammal. Deletion or duplication of the 16p11.2 chromosomal region in humans has neurocognitive effects, which can produce effects of macro- and microencephaly when the human transcript is inserted into the zebrafish genome (Golzio et al. 2012).

Beyond Human Cell Biology: How Can Human Cell Technology Be Used in a More Translational Sense?

As human cell engineering technologies, such as iPS cells, iN cells, and olfactory cells, continue to advance, the molecular signatures associated with schizophrenia should be able to be clarified. Nonetheless, human brain imaging is crucial to address the important question of how such molecular changes at the cellular level affect the brain function and molecular disposition of the same individual from whom those cells were obtained.

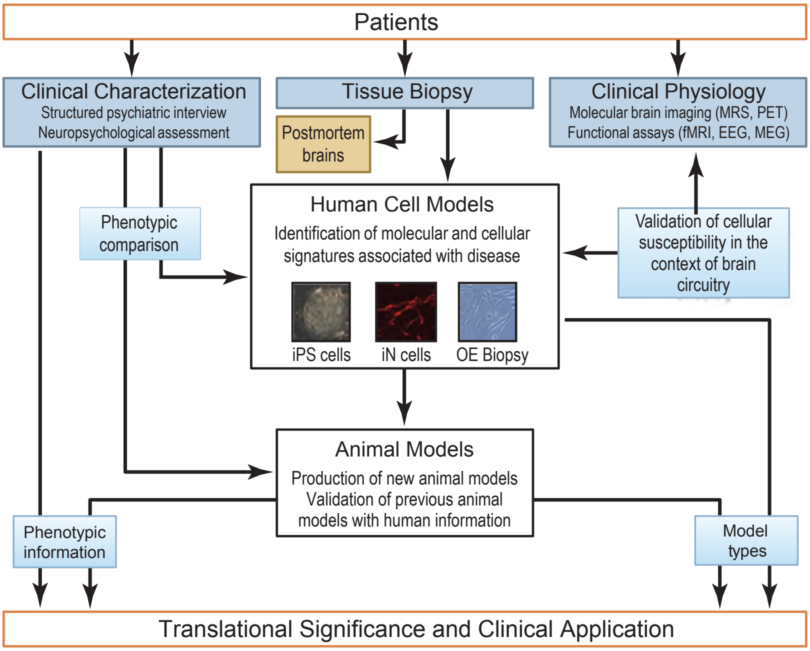

Figure 10.2 Multifaceted study design including cell models. Human cell models of psychiatric disease can be easily integrated with other techniques for better translational research and clinical applications. Aspects of clinical physiology and psychological assessment can be intricately examined at the molecular and cellular levels to clearly identify biological signatures of disease. These cell models can in turn influence and be assimilated with current and future animal models for further understanding of the neurocircuitry and behaviors which those biomarkers represent.

To address brain region-specific molecular changes, positron emission tomography and magnetic resonance spectroscopy are useful modalities. Correlation of molecular and cellular changes in iPS, iN, and olfactory cells with clinical, neuropsychological, and electrophysiological measures will provide us with important information for translational use. A multifaceted study design (see Figure 10.2) involving human cell models represents a promising major approach for schizophrenia research and should be actively pursued. Furthermore, human cell models should be utilized for mechanism-oriented compound screening.

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