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The promise of stem cell research for neuropsychiatric disorders

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Abstract

The study of the developing brain has begun to shed light on the underpinnings of both early and adult onset neuropsychiatric disorders. Neuroimaging of the human brain across developmental time points and the use of model animal systems have combined to reveal brain systems and gene products that may play a role in autism spectrum disorders, attention deficit hyperactivity disorder, obsessive compulsive disorder and many other neurodevelopmental conditions. However, precisely how genes may function in human brain development and how they interact with each other leading to psychiatric disorders is unknown. Because of an increasing understanding of neural stem cells and how the nervous system subsequently develops from these cells, we have now the ability to study disorders of the nervous system in a new way—by rewinding and reviewing the development of human neural cells. Induced pluripotent stem cells (iPSCs), developed from mature somatic cells, have allowed the development of specific cells in patients to be observed in real-time. Moreover, they have allowed some neuronal-specific abnormalities to be corrected with pharmacological intervention in tissue culture. These exciting advances based on the use of iPSCs hold great promise for understanding, diagnosing and, possibly, treating psychiatric disorders. Specifically, examination of iPSCs from typically developing individuals will reveal how basic cellular processes and genetic differences contribute to individually unique nervous systems. Moreover, by comparing iPSCs from typically developing individuals and patients, differences at stem cell stages, through neural differentiation, and into the development of functional neurons may be identified that will reveal opportunities for intervention. The application of such techniques to early onset neuropsychiatric disorders is still on the horizon but has become a reality of current research efforts as a consequence of the revelations of many years of basic developmental neurobiological science.

History and prospective

One of the universal laws of development is the progressive restriction of fate potential of cells as the organism grows in size—restriction that enables the development of specialized cell types. This process, which was thought to be irreversible until recently, is mediated by permanent repression of gene expression by the binding of transcription factors to promoters

and epigenetic marks in the chromatin and on the genomic DNA sequence itself. Hence, the most reliable source of diploid stem cells is the early embryo. Indeed, in the past twenty years, due to technical advances which have allowed us to culture and maintain mouse and human embryonic stem cells, we have learned a great deal about the characteristics and fate potential of these cells. Understanding the biology and potential use of stem cells could represent a major advance for neuropsychiatry and brain sciences.

Neuroimaging studies have revealed structural and functional brain abnormalities in many neuropsychiatric conditions, often preceding the onset of symptoms (Tau and Peterson, 2010). Neuropsychiatric disorders, it can be argued, arise from deviations from the regular differentiation programs of the CNS, leading to altered schemes of connectivity; for example, relatively subtle abnormalities in volume and cell number in prefrontal cortex and basal ganglia are observed in depressive disorder and Tourette syndrome, respectively (Rajkowska et al., 1999, Peterson et al., 2001, Kataoka et al., 2010). In all cases, the abnormalities do not represent a drastic departure from the regular program of development, but are more consistent with quantitative shifts or variations in the programs that build the CNS. Mouse and human stem cells can teach us a great deal concerning how typical differentiation programs are implemented and how they may be modified in disease.

Stem cells are early developing cell types that have not yet lost the ability to develop into all other cell types of the organism. As such, their chromatin is in a “bivalent” conformation, allowing for genes to be ready or poised to be transcribed (Gan et al., 2007, Bernstein et al., 2006). Since stem cells derive from embryos, work with human stem cells has been hampered by ethical concerns. Researchers have been able to use a few human stem cell lines, but there is a lack of a “bank” of human stem cells that encompasses the genetic diversity of human populations. Furthermore, the few available embryonic stem cell lines are different from one another in terms of their potential, and there are concerns over the genetic stability of cells after long-term amplification in vitro. The ethical debate over the destruction of human embryos produced restrictions in federal funding of this research and prohibited the generation of a large number of embryonic stem cell line. Thus, a major effort arose from the scientific community to find alternative sources for the derivation of human stem cells.

One alternative to embryonic stem cells is tissue-specific stem cells from adult humans. Stem cells of the central nervous system, from the subventricular zone (SVZ) of the cerebral cortex and the subgranular zone (SGZ) of the dentate gyrus, are of obvious interest for neuropsychiatric disorders. The technical and ethical challenges of obtaining such human cells have been significant, requiring deep biopsies from the living CNS. In addition, adult neural stem cells from the SVZ and the SGZ are restricted in fate, i.e., normally produce only olfactory bulb and dentate gyrus neurons and display limited potential to become neurons of different types upon transplantation into different CNS regions (Milosevic et al., 2008, Alvarez-Buylla et al., 2000, Lim et al., 2002, Vaccarino et al., 2001). Attempts to use adult mesenchymal stem cells to create neural stem cells, either from bone marrow or other tissues, have also been inconsistent (Fricker-Gates and Gates, 2010). Using either co-culture methods with astrocytes or other cell types (Jiang et al., 2003), specific extracellular factors (Trzaska et al., 2009), or transfection with genes expressed in neural precursors (Dezawa et al., 2004), initially promising results await replication. Thus, bone marrow derived stem cells may also have similar limitations to adult brain-derived neural stem cells with limited potential to divide and differentiate, although they have been used in animal models as sources of growth factors, enzymes and cytokines to stimulate neuronal repair for treatment of autoimmune, neurodegenerative and inflammatory injuries (Momin et al., 2010, Lee et al., 2010, Constantin et al., 2009, Wang et al., 2010, Kranz et al., 2010, Xia et al., 2010).

It had been known for a long time that factors present in the cytoplasm of vertebrate eggs are able to reprogram nuclei of fully differentiated cell types, coercing these nuclei to revert to a primordial, stem cell-like, state. In 1997, Ian Wilmut and Keith Campbell at the Roslin Institute in Edinburgh cloned the first animal from an adult somatic cell using these factors, a sheep named “Dolly” (Wilmut et al., 1997). This was followed by the cloning of several vertebrate species by transfer of a somatic cell nucleus into an enucleated egg cell.

In 2007, Yamanaka and colleagues at Kyoto University were able to show that four gene products encoding transcription factors, Oct4, c-Myc, Sox2 and Klf4, were sufficient to reprogram somatic cells into stem cell like-cells (Takahashi et al., 2007). A flurry of subsequent papers from other groups replicated the initial findings (Yu et al., 2007, Wernig et al., 2007, Meissner et al., 2007) and determined that these iPSCs were remarkably similar to embryo-derived stem cells with respect to gene expression profile, epigenetic marks, and fate potential (Mikkelsen et al., 2007). This discovery allowed the derivation of immortal, stem cell-like cells, called induced pluripotent cells or iPSCs, from somatic cell types. Indeed, bona fide iPSCs pass the most stringent tests of pluripotency: germline transmission (the ability to generate germ cells like egg and sperm) and tetraploid complementation (the ability to generate an early stage embryo from two different cell types) (Zhao et al., 2009, Boland et al., 2009). In addition, iPSCs injected in immunocompromised mice were able to form teratomas containing all 3 germ layers, and generated all cell types upon transplantation into mouse embryos. Hence, in a paradoxical way, political and ethical restrictions on human stem cells have spurred a major scientific advance.

In these last few years, iPSC, which are most commonly derived from skin fibroblasts, have been a major focus of investigation. Despite significant efforts, using current techniques, their derivation is still inefficient (at most, a few percent of fibroblasts become iPSCs) and the characterization of “truly” reprogrammed cells versus those that are only partially reprogrammed or unstable has proven difficult. Also, specially for therapeutic purposes, there are unknown but potentially serious risks in those iPSC generation procedures that introduce foreign DNA into cells (Saha and Jaenisch, 2009) and procedures that do not obligatorily involve DNA integration are still at an early stage of evaluation (Seifinejad et al., 2010). Despite the challenges, there are potentially enormous advantages of iPSCs over embryonic and other types of stem cells, as the first are derived from a living person using a non-controversial cell type, can be generated from a specific individual, and maintain his/her genetic constitution and diversity, and can represent a source of differentiated cell types genetically compatible to the person of origin.

Human genomic variation, mental disorders and iPSC

Many neuropsychiatric developmental disorders, including schizophrenia, autism spectrum disorders (ASD) and Tourette’s syndrome, may be the consequence of subtle alterations in the overall scheme of CNS development. This is likely to be caused by a combination of a number of gene variants as well as environmental factors, although in rare cases a single gene variant of strong effect may be sufficient to cause altered development and thus illness. Recent advances in genetics and developmental neurobiology using animal models have unveiled in astonishing depths the fundamental history of CNS development (see the article of JLR Rubenstein in this issue). The development of gene knockout and transgenesis in mouse, transplantation experiments in amphibians and avian embryos, single cell ablation and RNA interference in nematodes and fruit flies, and sophisticated cell culture techniques in a variety of species including human have revealed common laws guiding the morphogenesis and cellular differentiation of the vertebrate CNS, and particularly its most complex portion, the brain. These fundamental studies focus on the commonalities of neural

development amongst different organisms, but are most likely insufficient to understand the developmental origin of psychiatric illnesses.

The characteristics that distinguish the recently evolved primate and human brain from other mammalian brains include the proportionally larger growth of the cerebral cortex, the diversification of cortical area maps and a much more extensive degree of connectivity (Rakic, 1995). It can be argued that these differences in scale and complexity have driven an increase in size of neurons, a larger metabolic demand and an increased proportion of glial cells. Another important aspect of the human brain is its diversity from one individual to another. Not one human is identical to the other with regard to the location of sulci and gyri on the cortical surface, the pattern of cortical area activation in response to stimuli, and other characteristics of the neural network. The much greater variation in both morphology and connectivity amongst human brains is a great challenge for investigators that wish to draw statistical inferences; but it can be considered an important clue for understanding the basis of normal and less typical cognitive functions.

Mirroring the diversity and degree of variation in the physical characteristics of the human brain is the variation found in genomic sequence, when comparing individual human genomes (Kim et al., 2008, McCarroll et al., 2008). This genomic variation is to be found in their complement of SNPs (single nucleotide polymorphisms) affecting about 0.1% of the total genomic sequence. In addition to this it has become clear over just the last few years, with the advent of novel genomics technology and also the completion of additional human genome sequences that the degree of variation between two individual genomes is even larger than what can be accounted for by SNPs. The nature of this variation is very complex: in addition to SNPs each genome contains an abundance of copy-number-variation and structural variation (CNV and SV). CNVs consist of all variations leading to changes in amount of genomic material such as deletions and duplications, whereas the term SV indicates other types of structural changes, such as insertions, translocations and inversions. It is now clear that entire blocks of sequence in size from less than 1 kb to several millions bp have been deleted, duplicated, inserted, translocated or inverted in the human genome (Hurles et al., 2008, Korbel et al., 2007, Levy et al., 2007) (Figure 1A). The methodology for efficiently identifying CNV/SV in DNA samples is still evolving and not yet as robust or inexpensive as SNP genotyping. The average number of such CNV/SV per individual is variously estimated between 700 and 1400 depending on the methods chosen for analysis and the ethnicity of the subject (Park et al., 2010, Conrad et al., 2010). In total, CNV/SVs may alter the coding potential of at least 5% of the known genes. A substantial fraction, but by no means all, of their impact on disease can be estimated by analysis of SNPs in linkage disequilibrium with the CNVs.

CNV/SVs are incompletely assessed by commercial SNP arrays and only recently have studies begun to explore them as a potential cause of complex traits, including neuropsychiatric disorders. These CNV/SV are scattered all over the genome, although “hot spots” have been identified, i.e., an 8-megabase (Mb) region in chromosome 22q11.2 and an 18-Mb region at 7q11 (Korbel et al., 2007) (Figure 1B). These two hotspot regions, for example, harbor deletions in two developmental neuropsychiatric disorders of genomic etiology, velocardiofacial syndrome (VCFS) and William-Beuren Syndrome, respectively. Patients with VCFS have a high frequency of learning disorders, ASD and schizophrenia. As mapping studies continue the total extent and degree of complexity of human genomic variation is being unveiled. In parallel to this, an increasing number of studies link CNV/SV to phenotypic effects. Disease-associated CNV/SVs detected so far include both rare variants with large effect and common variants with more modest effect sizes, but carried by a large proportion of the population (Manolio et al., 2009). Rare CNV/SVs include deletions/duplications at 16p11.2, associated with ASD and idiopathic mental retardation,

and deletions at 1q21.1, 15.q13.3 and 22.q11.2, found in schizophrenia (Sebat et al., 2007, Weiss et al., 2008, Stefansson et al., 2008). An increase in the occurrence of *de novo* large deletions has been reported in individuals withASD (Christian et al., 2008, Sebat et al., 2007). Common CNVs, which tend to be smaller in size, are under-ascertained by the current methods. Thus, human genomic variation is widespread and varied and has to be taken into account when trying to understand complex phenotypes, both in terms of causative and modifying variation events, if indeed a complex condition is caused not by a single genetic event of strong effect but a combination of variants each with small effect. The large degree of interindividual variability, mostly rare, raises the crucial question of what may be the best approach for determining which of the multitude of genomic sequence variants carried by an individual is responsible for a given phenotype, especially if the functional consequences of such variants at the protein level are not known. Genome-wide association studies have tended to focus exclusively on statistical evidence, but the assessment of the significance of human genomic variants for disease is going to be difficult on statistical grounds alone, and we need to pay more attention to biology in deciding which genetic variants to pursue for diagnostic and treatment purposes.

Recent studies suggest that gene transcripts expressed in the developing human brain encompass a much larger set of mRNA variants and splice patterns, not found at corresponding stages of animal brain development (Johnson et al., 2009). Unfortunately, how human genetic variation leads to morphological and thus functional variation, the extremes of which may represent mental disorders, is extremely difficult to investigate. It would require the ability to follow neural development in specific individuals at the cellular and systems level, and correlate the particulars of this development to the underlying structure of their genome. The experimental analysis of human neural development in its relation to differences in genetic activity is hampered by seemingly insurmountable challenges. These challenges have so far impeded a rigorous exploration of gene transcripts that are specifically expressed at specific stages of human brain development.

The derivation of iPSCs from skin or other differentiated somatic cells might allow the study of human neural development for individual genomes, albeit in vitro. There is a potentially large impact of this type of studies for psychiatry, neurology, and psychology, if, indeed, they will permit study on how individual, natural genetic variation affects neurodevelopment and how genetic variation is linked to individual differences in brain function and behavior. The success of this approach will depend to a large extent on our ability to recapitulate *in vitro* the biological steps that enable an embryonic stem cell to differentiate into neurons and glia from specific regions of the CNS. If, using patient-specific iPSCs, we can reproducibly recreate key milestones of neural differentiation *in vitro*, leading to a highly coordinated generation of specific repertoires of neuronal cell types, we could, in principle, understand the molecular programs of development that may underpin specific disease phenotypes in human (Figure 2).

Another question that could be approached using the iPSC technology is that of how, given the substantial similarity in gene number between mammalian species, the substantial differences in brain development amongst mammals are encoded at the genomic level. It seems possible that differences in sequence of non-coding areas of the genome, and therefore in gene regulation, might be a crucial component of such differences. With the term “regulation” we refer here not only to the functional effects of specific transcription factors bound to their enhancers, and thus the regulation of the amount of mRNA expression in space/time, but also the incompletely understood process of alternative splicing of a single mRNA into different types of transcripts. Regulation at the level of control of the rate of translation of specific mRNAs is a comparably important aspect of regulation of protein production that has been much less extensively studied in neural tissues.

Another important component to consider is the regulation of the chromatin due to histone modifications and the methylation of cytosines in the DNA, i.e., epigenetics. The development of iPSCs would enable us to study regulatory processes that establish the dynamic gene networks driving the differentiation of a particular cell type at a particular time, whether determined by DNA sequence variants, mRNA variants, or altered states of chromatin (Figure 2). Hence, iPSCs promise to offer a unique opportunity to begin to understand the direct biological consequences of human gene sequence variation as it applies to the structure and development of the CNS. Importantly, the environmental, hormonal and toxic effects on the differentiation dynamics and related gene expression trajectories can also be explored.

Technology

An unbiased study of how individual, natural genetic variation affects neurodevelopment using iPSCs as a model would have been unthinkable until very recently, given the time and cost required to obtain high quality genomic data. The emergence of *2nd-generation sequencing technology* (also known as next-generation or deep sequencing technology) that allows for cloning-free, *ab initio* (i.e., from the beginning) sequencing with outputs per instrument run of up to several billion bp of genomic sequence is influencing this type of investigations. Modern sequencing technology, used alone or in combination with high-density oligonucleotide arrays, can create genome-wide CNV and SV maps that capture any CNV/SV of at least 1 kb in size, or even smaller.

Although challenging from a bioinformatics standpoint, the decreasing costs and the increased availability of oligonucleotide arrays and deep sequencing technology will allow in the near future a comprehensive examination of structural gene variation, epigenomics, and gene expression. By virtue of combining interrelated datasets of structural DNA variation (including DNA methylation) and variation in transcripts in particular brain areas and cell types, scientists may be able to test the biological and functional implications of natural genetic variants for human neurodevelopment and the risk of neuropsychiatric disorders. For example, the deep sequencing of RNA and DNA isolated from neural stem cells which originated from iPSC of a specific individual may allow us to examine if common and rare individual genetic variation is associated with specific patterns of gene expression during their differentiation. Furthermore, examining the biological properties of these individually-derived neural cells, i.e., proliferation, differentiation, and survival, will enrich our knowledge of the possible biological outcomes of given patterns of gene expression during the cellular processes of neurodevelopment. With the advancement of bioinformatics and the standardization of protocols used to generate and characterize iPSCs, we may be able to eventually understand the combined effects of multiple genetic variants on the cellular processes of neurodevelopment, and assess the impact of epigenetic effects and environmental variables on both gene expression and biological function (Figure 2).

The implications of these emerging technologies for neuropsychiatric disorders are multiple. The complexity of the emerging landscape is staggering, and it is becoming clear that the task ahead is well beyond the capability of a single laboratory or research group. Funding agencies are encouraging the construction and sharing of large datasets. If international consortia will be able to efficiently share information at all levels, future applications of iPSC technology promise to shed light onto disease pathogenesis and influence the classification of neuropsychiatric disorders. Environmental, hormonal and toxic effects on neural differentiation dynamics and related gene expression trajectories can be explored. These technologies may also facilitate drug discovery and the reduction of drug side effects.

The applications of iPSCs in pathological conditions

Of particular interest for psychiatry, neurology, and psychology, iPSC can be derived directly from patients and their family members. As new technologies allow us to dissect in more precise details the genetic differences that distinguish specific patient populations, these cells may provide a biological tool to correlate these genetic differences with cellular differentiation and function in development.

iPSC techniques have already been applied in a few conditions that involve the malfunction of neural cells. Familial dysautonomia, a neuropathy characterized by the loss of autonomic and sensory neurons, has been studied using patient-specific iPSCs (Lee et al., 2009). This approach has revealed that the specific pathology of degeneration in sensory neurons may be due to particularly low levels of the disease-associated I-κ-B kinase complex-associated protein in neuronal precursors. The resulting phenotype in patient-specific iPSCs induced into neuronal fates permitted candidate drugs to be tested for efficacy in ameliorating pathology.

Electrically active human motor neurons have been generated from iPSCs using protocols similar to those used for embryonic stem cells (Karumbayaram et al., 2009). With this technique, iPSCs have been used to improve our understanding of the degenerative processes involved in spinal muscular atrophy (SMA), a disorder of lower motor neurons (Ebert et al., 2009). Ebert and colleagues have reprogrammed iPSCs from a patient with SMA into motor neurons and maintained them in culture where they became progressively smaller and less numerous than similar cells derived from a healthy family member, thus recapitulating the disease in vitro. Spinal motor neuron protein aggregates in patient-derived motor neurons also resembled diseased *in vivo* tissue, but normalized after treatment with valproic acid and tobramycin. iPSCs allowed for the testing of these therapeutic compounds in patient-specific tissue which could open the door to testing other possible therapeutics.

Amyotrophic lateral sclerosis (ALS) has also been studied using patient-derived iPSCs (Dimos et al., 2008). In the case of this work, fibroblasts were taken from an elderly patient with severe familial disease but would still allow themselves to be reprogrammed and cultured with sufficient efficiency for study of the pathology in culture. However, unlike the familial disorders described above in which there is some knowledge of the genetic basis, ALS and many other neurodegenerative disorders including Parkinson disease (below) are predominantly sporadically occurring diseases, with several phenotypes and underlying pathomechanisms proposed. While making the use of patient-specific cells in sporadic diseases may be quite challenging, because of the relatively high disease prevalence there is an even greater promise for determining the specific molecular mechanisms and using this model for drug screening.

Compared to caudal regions of the CNS, the application of iPSC to the study of the brain has been somewhat slower, although dopaminergic neurons in normal and Parkinson's disease patients have been investigated (Soldner et al., 2009). Similarly, a limited number of iPSC lines from a variety of diseases with genetic mutations affecting brain development, including Down syndrome and Huntington's Disease, have been generated, although their differentiation potential has not yet been studied (Park et al., 2008a, Park et al., 2008b).

The iPSC technology could be applied to other neuropsychiatric disorders to validate specific pathogenetic hypotheses and assess their applicability to individual patients and families. For example, for Fragile X syndrome, we know that this disorder is caused by mutations in the *fmr1* gene; however, it is challenging to sort out, amongst all the possible downstream gene products and signaling pathways that could be secondarily affected, those that are implicated in the different aspects of this disorder. In other cases, such a Tourette's

syndrome and ASD, the genetics are likely to be complex, and only rare genetic variants have been identified. In both Fragile X and Tourette's syndrome, patient-derived iPSC could begin linking mutations with specific biological pathways and developmental defects at the neuronal level. For Tourette's syndrome, in particular, a decrease in specific sets of GABAergic interneurons in the basal ganglia has been already implicated from postmortem human studies as a likely mechanism leading to clinical symptoms (Kataoka et al., 2010) and the iPSC model may represent an exciting opportunity to examine the development of these cells. These studies could also help unravel how individual genomic variation impacts on the biological phenotype and ultimately correlates with the clinical spectrum. While there are many other neurological and psychiatric diseases to which iPS techniques could be applied, the technical challenges of this work require a large amount of specialized resources to establish iPSCs from even a single patient. Large research consortiums have committed to creating core facilities for making iPSCs to advance work on a range of diseases.

Interestingly, the forebrain fate appears to be a default fate when embryonic stem cells and even iPSC-derived neuronal progenitors are cultured in the absence of morphogenetic factors; several reports have shown that electrically active forebrain neurons can arise in culture under these conditions (Johnson et al., 2007, Pankratz et al., 2007), but for iPSC the process appears to be somewhat variable (Hu et al., 2010).As is true for human embryonic stem cells, a key quest of the field is to decipher the precise mechanisms whereby iPSC-derived neural cells and their progenies respond to instructive cues, promoting the induction and proper patterning of distinct neuronal subpopulations. Central to the field for neuropsychiatric disorders, recent publications suggest that embryonic stem cells-based *in vitro* differentiation models appear to recapitulate the key milestones, leading to a highly coordinated generation of cortex-specific repertoire of neuronal cell types (Eiraku et al., 2008, Gaspard et al., 2008, Gaspard et al., 2009).

The greatest strides have been made in the use of iPSCs for disorders of the hematopoietic system. To address the malfunctioning erythrocytes found in sickle cell anemia, iPSCs were derived from adult mice with a form of sickle cell anemia and their genetic deficit was corrected by introducing a gene for wild-type globin into the cells (Hanna et al., 2007). When these reprogrammed cells were injected into adult mouse models of the disease, peripheral blood smear analysis and kidney functioning were both normalized. Successful reprogramming in vitro has also been accomplished in fibroblasts taken from individual patients with Fanconi anemia (Raya et al., 2009). By using lentiviral vectors to correct the genetic defects in these differentiated cells, cells could then be de-differentiated into iPSCs and reprogrammed into cells that that gave rise to healthy haematopoetic progenitors without Fanconi pathology *in vitro*.

Starting in 2009, U.S. companies have been granted approval by the FDA to use human embryonic stem cells for the experimental treatment of spinal cord lesions and amyotrophic lateral sclerosis. Many potential problems need to be resolved before iPSCs can be used in human disease treatment, including the potential development of DNA rearrangements in vitro, the risk for tumor formation, graft rejection and appropriate telomerase levels to extend iPSC life (Naegele et al., 2010). Regardless of their use in cell-based regenerative medicine, iPSCs may provide unprecedented tools for the investigation of disease mechanisms and to test candidate drugs as discussed above for other central nervous system disorders.

Short and long-term tasks ahead

The progress in iPSC technology has spurred a large literature exploring the factors that are necessary and sufficient for the reprogramming process. The reprogramming process is still

remarkably inefficient, as only a few percent of the differentiated cells can become pluripotent under existing conditions. It is becoming clear that a host of genetic factors (i.e., factors that regulate cell cycle arrest) and environmental factors (i.e., levels of oxygen) can dramatically affect the efficiency of induction of iPSC.

At the same time, similarities and differences between iPSC and embryonic stem cells are being defined. Overall, there is a large degree of similarity in both gene expression and epigenetic marks among iPSC and embryonic stem cell lines. However, iPSC are not identical to embryonic stem cells. Comparison among several iPSC cell lines generated by different labs has revealed that, even after extensive passaging, there is a restricted set of genes (a “gene signature”) that characterizes iPSC, regardless of their origin or method of derivation, suggesting that iPSCs represent a unique subtype of pluripotent cells (Chin et al., 2009). Many of the differentially expressed genes represent incomplete silencing of fibroblast genes in iPSCs and failure to fully induce embryonic stem cell genes, thus likely reflecting incomplete resetting of somatic gene expression (Chin et al., 2009).

Several goals must be met before we may be able to apply the iPSC technology to the study of CNS disorders. To this advantage, recent data suggest that iPSC, like embryonic stem cells, differentiate into a forebrain phenotype by default and virtually all respond to patterning by retinoic acid treatment (Hu et al., 2010). However, the efficiency of neural differentiation appears to be lower and more variable in iPSC lines compared to embryonic stem cell lines. This is not affected by source of fibroblasts, age, choice of reprogramming vectors or residual transgene expression and it may be attributable to variable response to neural inducers (Hu et al., 2010). Some of this variability is likely to be caused by inter-individual variability in genetic background and variable epigenetic status among the different lines under investigation, as considered for hESC lines (Osafune et al., 2008). Possible incomplete repression of fibroblast gene expression and secondary consequence of the non-robustness of the neural induction protocol used in these experiments might also be considered to explain the findings. Future refinements of the differentiation protocols may address these questions. While current research has thus far shown overall similarity in gene expression and chromatin architecture between different iPSC lines, these analyses have been done using DNA microarrays, which have limited resolution. Thus, the field must await RNA sequencing data and Chip-Seq data to fully assess the diversity in genetic makeup of iPSC and their variable potential, comparing different iPSC clones isolated from the same as well as different individuals.

Linked to this problem is the choice of an appropriate method for directing the iPSC into the neural lineage, and for the subsequent derivation and growth of neuronal and glial cells specific from a region of the CNS. Several basic protocols currently exist, which have been applied to mouse and human embryonic stem cells. Most of the protocols are 2-dimensional cell culture systems (Cohen et al., 2007, Elkabetz and Studer, 2009) that involve an initial aggregation of the cells into embryoid bodies, followed by the generation of “neural rosettes”, aggregates of neural cells with a defined polarity similar to the in vivo CNS. However, the possibility also exists of using 3-dimensional cell aggregates that maintain a neural tube-like structure (Eiraku et al., 2008). In most cases, the cells can be directed into different fates (forebrain, midbrain, spinal cord) by the use of specific morphogens and culture conditions. For example, motoneurons have been generated by treatment with retinoic acid (Li et al., 2005). However, despite the forebrain “default” phenotype exhibited by iPSC as they differentiate into the neural lineage, methods for differentiating specific forebrain neurons, i.e., excitatory projection neurons, GABAergic neurons, and their regional subtypes in the cortex and basal ganglia, require further work and rigorous validation using established markers. A specific subtype of GABAergic neurons in the basal

ganglia expressing the DARPP32 has been generated from human stem cells (Aubry et al., 2008), raising hope that similar neurons can be developed from iPSC lines in the future.

As methods for developing particular subset of neurons from embryonic stem cells emerge, methods for purifying these subsets from the mixed population have also been developed. The most promising are the development of transgenic line of human embryonic stem cells harboring bacterial artificial chromosomes (BAC) with fluorescent protein reporters (Placantonakis et al., 2009, Lee and Studer, 2010). These and similar methods should be applicable to the iPSC field, and will be particularly important for identifying, purifying and analyzing at the molecular level neuronal subsets from patient populations with specific neuropsychiatric disorders. Despite the challenges inherent in human cell transgenesis, we expect that the future application of these methods to clinical populations will be rewarding.

Another important issue is to attain proper maturation of the cells, as later processes pertaining to neuronal differentiation and maturation have been implicated in neuropsychiatric disorders such as ASD and schizophrenia. Hence, it is particularly important to develop protocols that allow morphological and functional aspects of neuronal differentiation to be examined, such as the development of specific neurotransmitter and transporters, and the emergence of functional synaptic connections. Indeed, one of the most remarkable results is that neurons derived from iPSC can display, under appropriate conditions, typical synapses and classic action potentials (Hu et al., 2010, Johnson et al., 2007). It should be also possible to transplant iPSCs in the embryonic rodent CNS, which is permissive to xenogeneic transplantation (Zhang et al., 2001, Muotri et al., 2005, Okuno et al., 2009), in order to examine the development of these cells in the context of the architecture of the CNS. These emerging experiments raise our hope that iPSC could be utilized for understanding how “diseased” iPSCs are altered in functional aspects of the neural network.

Limitations

There are several limitations in working with the iPSC system, some of which are inherent to the model. The differences between iPSC and embryonic stem cells have been discussed above. Ethical issues specific to the use of iPSCs certainly exist although do not pose the limitations of embryonic stem cell use. In order to ensure that ethical concerns do not limit the potential of this technique, researchers in this area have developed strong ethical guidelines to address issues such as informed consent for donors and responsible use of donated cells as they are maintained over time.

There are other concerns that will need to be addressed before iPSC can be used for human cell replacement therapy, particularly the potential of iPSC to accumulate point mutations and CNV/SVs during culture. Hence, their long-term genomic stability will need to be examined.

Another concern stems from the fact that the cell type of origin of iPSC, skin cells or another somatic cell type, remains different from that of true embryonic stem cells, and thus residual gene expression from the cell of origin, which is probably unavoidable, may create differences.

Moreover, there is an important issue of mapping and comparing the genetic and genomic variation in iPSC onto the clinically related variation in human brain function and behavior. This is a separate and major task that is minimally (or not at all) present in the literature at this point.

Perhaps the biggest limitation is the unavoidable fact that neural differentiation *in vitro* is different than what occurs *in vivo*. Therefore, the sophistication of the data that can be derived from such iPSC models in part depends upon the rigor of the culture techniques, and their ability to reflect as much as possible *in vivo* processes. The possibility of using tri-dimensional cell culture systems has been alluded to above; however, *in vivo* embryonic development involves complex interactions among neural, vascular and perhaps even immune cells that are difficult or impossible to mimic in vitro.

Future applications

The most ambitious future application of iPSC technologies is to derive isogenic, relatively differentiated, transplantable cells to assist in cure of neurodegenerative disorders. The hope is to provide a definitive cure for neurological conditions such as Parkinson's, Huntington's and Alzheimer's disorder, as well as many others. If the neurodegenerative conditions are driven by an intrinsic genetic defect, this will be likely reproduced in the transplanted isogenic cells, although it may be fixable by gene therapy. Caution must be exerted in this type of treatment, as the risk of developing teratomas is always present if the transplanted cells are not uniformly differentiated.

A more feasible, but still quite challenging and at the same time well-worthy goal in the near future is the use of iPSC as in vitro models for disorders, allowing the greater understanding of their genetic basis, development of novel diagnostic tools, or novel therapeutic interventions. For disorders beginning early in development, such as ASD, attention deficit hyperactivity disorder, or language and learning disabilities, the potential for detecting deviations of development in patient-derived iPSCs may be even greater than for disorders with later onset; more direct, identifiable links may exist between initial developmental processes that could be monitored in iPSCs and the system dysfunction of these childhood disorders than for disorders that onset after multiple later developmental events. Research with iPSCs will cross-fertilize genomic studies and anatomical/functional studies. For example, identifying that a certain step in cell differentiation occurs in one patient with autism will allow the specific genes and regulatory components involved in that step to be investigated in that patient and others, both in genomic analysis as well as anatomical analysis of post-mortem tissue. As has been shown for SMA and familial dysautonomia, already approved medications may demonstrate the ability to compensate for genetic defects. In addition, new compounds may be identifiable that could promote cellular differentiation processes that are defective or that may correct a cell survival, adhesion, protein turnover, metabolic or synaptic defect. While it is possible that the developmental processes under consideration are to some degree irreversible, this is by no means sure until these models are developed and the potential treatments tested. Thus, an advantage of such individualized developmental models is that preventive measures against later alterations previously unthinkable may be within reach.

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Box 1

Definitions

Embryonic stem cells	Cells isolated from the inner cell mass of an early (preimplantation) embryo, which are capable of indefinite self-renewal and have the widest potential, that is, are able to differentiate into all cell types of that organism.
Embryonic neural stem cells	Cells isolated from the embryonic central nervous system (CNS), which self-renew but, depending upon the stage of development, may be already regionally specified, i.e., can differentiate into neural cell types that are typical of the region of origin.
Induced pluripotent stem cells	Cells isolated from fully differentiated tissues such as skin fibroblasts, that upon forced expression of key transcription factors (Oct4,c-Myc, Sox2 and Klf4), alone or in combination with small chemical compounds, revert to a pluripotency state similar to that of embryonic stem cells, characterized by indefinite self-renewal and ability to differentiate into all cell types of that organism.
Fibroblasts	A common cell type with mesenchymal origin that synthesizes and secretes the extracellular matrix and collagen, which maintain the structural framework of many tissues.
Adult neural stem cells	Cells typically isolated from specialized regions of the postnatal CNS, which self-renew and retain the ability to differentiate into the three main CNS cell types: neurons, astrocytes and oligodendocytes. These cells are regionally specified, and typically do not retain the ability to generate the full spectrum of neuronal and glial diversity of the CNS.
Neuronal progenitors	Cells isolated from the embryonic or postnatal CNS, which are the direct progeny of neural stem cells, undergo a pre-programmed number of cell divisions, and differentiate into neural cell types that are typical of the region of origin.
Retroelement	Segments of genetic material that transpose around the genome using an RNA intermediate.
Pluripotent	Characteristics of cells that have the ability to mature into many specific cell types.
Differentiation	The process by which a less specialized cell develops to become more distinct in form and function and takes on a more mature and less pluripotent identity.
Morphogenesis	The process by which form of tissues take shape that involves specification, differentiation, and growth.

Self-renewing	The ability to reproduce the same cell of origin while going through numerous cycles of cell division.
Passaging	The process of maintaining cells in culture by splitting a culture into secondary cultures.
Epigenetics	Stable modifications of DNA and histones in chromatin by methyl and acetyl groups that can alter the availability of DNA for transcription or interaction with DNA binding proteins.
Epigenomics	The study of epigenetic change at a level higher than a single gene and encompassing the entire genome.
Transcription factors	Proteins that bind to DNA alone or by interacting with other proteins to regulate the expression of certain genes and, in this manner, play a role in cell specification and differentiation.
2nd generation sequencing (also next generation or deep sequencing)	Technologies that allow the sequencing of millions of sequence reads in parallel, providing monumental increases in speed and volume of sequence data that can be generated.
Alternative-splicing	The process by which different messenger RNA (mRNA) are made from the transcription of a single gene. These different mRNAs can be translated into different proteins.
Diploid	Cell containing two copies of each chromosome. All somatic cells are diploid, whereas germ cells (eggs and sperm cells) are haploid, which means they contain one single copy of each chromosome and thus half the normal number of chromosomes.

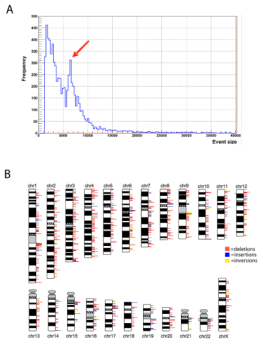

Figure 1. Extensive structural variation in the human genome

A, Predicted length-distribution of CNV/SVs obtained from analysis of next-generation sequencing data (SOLiD 50 bp reads, at ~40–5x coverage) of a single genome (NA18505). The mean length of detected CNV/SVs is around 5 kb. Similar distributions have been previously observed in other studies (Korbel et al., 2007; Levy et al., 2007). Note that by far most detected CNV/SVs are of such a small size that until very recently were out of reach of genomic analysis technologies. The peak detected at approximately 6 kb (red arrow) is at the position presumably corresponding to the size of LINE-1 retroelements deletion and insertion events.

B, CNV/SVs identified in two humans (NA18505, same as for A, and NA15510) using 454/ Roche Paired-End-Mapping (PEM) and mapped onto chromosomal ideograms. Double length horizontal lines indicate CNV/SVs observed in both individuals. The graph is taken, slightly modified, from Korbel et al (2007).

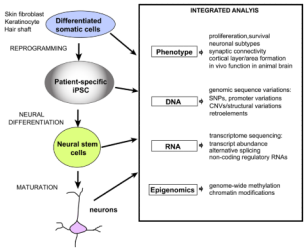

Figure 2. Integrated data analysis using patient-specific iPSCs

The iPSC model provides an ideal opportunity of offering a systems level understanding of the molecular events during the neural differentiation process. This would entail re-programming of somatic cells, derived from patients and healthy controls, into iPSCs and differentiation of these cells into specific neuronal subtypes and structures relevant for a disease. At each step of the process, detailed phenotypic analysis of the cell types is linked to parallel, high-resolution genome-wide studies of genomic sequence variations, gene expression and epigenetic changes. We can build a gene regulatory network based on correlations of levels of gene expression at the different steps during reprogramming and differentiation. We will then gain additional predicting power of this regulatory network by integrating genomic features at multiple levels, i.e. patterns of epigenetic modifications (e.g. histone and DNA methylation), relative locations of genes to CNV/SVs or other structural genomic variations and transposition activity of retroelements. Ultimately, the integration of genetic and epigenetic information of the relevant neuronal cell types derived from patient-specific iPSCs with phenotypic analysis, including cell fate specification in vitro and integration to animal brain in vivo, will provide a critical database toward an in depth understanding of pathways in health and disease.