Minireview

Modeling the pathophysiology of Parkinson's disease in patient-specific neurons

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Impact statement

Research on the pathophysiology of Parkinson's disease (PD) has generated effective therapies such as deep brain stimulation. A better understanding of PD pathophysiology calls for patient-specific materials amenable for invasive mechanistic studies. In this minireview, I discuss our recent work on oscillatory neuronal activities in midbrain neurons differentiated from induced pluripotent stem cells (iPSCs) of PD patients with parkin mutations. These patient-specific neurons enable a variety of studies previously not feasible in the human system. Further development in stem cell technologies may generate more realistic models for us to decipher PD pathophysiology. These new developments will transform research and development in Parkinson's disease.

Abstract

The 30 trillion cells that self-assemble into a human being originate from the pluripotent stem cells in the inner cell mass of a human blastocyst. The discovery of induced pluripotent stem cells (iPSCs) makes it possible to approximate various aspects of this natural developmental process artificially by generating materials that can be used in invasive mechanistic studies of virtually all human conditions. In Parkinson's disease, instructions computed by the basal ganglia to control voluntary motor functions break down, leading to widespread rhythmic bursting activities in the basal ganglia and beyond. It is thought that these oscillatory neuronal activities, which disrupt aperiodic neurotransmission in a normal brain, may reduce information content in the instructions for motor control. Using midbrain neuronal cultures differentiated from iPSCs of Parkinson's disease patients with parkin mutations, we find that parkin mutations cause oscillatory neuronal activities when dopamine D1-class receptors are activated. This system makes it possible to study the molecular basis of rhythmic bursting activities in Parkinson's disease. Further development of stem cell models of Parkinson's disease will enable better approximation of the situation in the brain of Parkinson's disease patients. In this review, I will discuss what has been found in the past about the pathophysiology of motor dysfunction in Parkinson's disease, especially oscillatory neuronal activities and how stem cell technologies may transform our abilities to understand the pathophysiology of Parkinson's disease.

Keywords: Parkinson's disease, basal ganglia, oscillation, induced pluripotent stem cells, pathophysiology

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Introduction

Life is a DNA-based information processing system that harnesses energy from the environment to produce information (i.e. a reduction in entropy). Information stored in a zygote drives the proliferation and self-assembly of cells that give rise to a blastocyst in day 5 of the human life cycle. The inner cell mass of a blastocyst consists of pluripotent stem cells (PSCs), which eventually generate the 30 trillion cells[1] that constitute the human body. The rest of the cells in a blastocyst produce extraembryonic tissues (e.g. placenta) that support the development of the embryo. The discovery of mouse embryonic stem cells (mESCs)[2] in 1984 makes it possible to study mice by editing the mouse source code (i.e. genome) in these cells and then injecting the genetically modified mESCs to a mouse blastocyst to test how alteration in the source code affects mouse biology.[3] The advent of human embryonic stem cells (hESCs)[4] in 1998 opens up the possibility of doing the same for human biology. Bypassing the ethically thorny need for a human embryo, the derivation of induced pluripotent stem cells (iPSCs)[5] from noncontroversial human somatic cells in 2007 has transformed research on human biology and disease by making it possible to study virtually any human conditions directly. This is particularly useful for

Parkinson's disease,[6] where disease modeling in animals has been challenging,[7] even for monogenic mutations that cause PD in humans.[8]

Basal ganglia motor circuit

As an integral part of the central nervous system, the basal ganglia plays a critical role in computing the instructions that control voluntary motor functions, which are impaired in Parkinson's disease (PD). Voluntary movement (e.g. grasping an object) is an information transformation process, whereby external information (e.g. location of the object) and internal information (e.g. location of the right hand) are transformed in the brain to produce a series of instructions for the coordinated contraction and relaxation of skeletal muscles needed for the intended movement. Decades of research in PD animal models and patients have formulated our current understanding of the basal ganglia circuit, in which the balanced actions of dopamine on the direct pathway and the indirect pathway are disrupted in PD (Figure 1). In a normal brain, the GABAergic medium spiny neurons (MSNs), which account for 95% of neurons in the striatum,[9] receive glutamatergic inputs from many areas of the cortex and dopaminergic inputs from the substantia nigra pars compacta (SNc).[10] The two types of striatal MSNs, expressing either the dopamine D1-class receptors or the D2-class receptors, project directly to the internal segment of the globus palladus (GPi) or indirectly to the GPi through the external segment of the globus palladus (GPe) and the subthalamic nucleus (STN), respectively[11] (Figure 1(a)). Balanced actions of dopamine,[12] delivered by the massive axon arborization of nigral DA neurons,[13] set the appropriate level of activities in the GPi,[14] which inhibits excitatory neurons in the thalamus, a nucleus that integrates and relays information to the motor cortex. Instructions from motor cortex are transmitted through the brain stem and the spinal cord to α motor neurons that control the contraction of skeletal muscles.

In Parkinson's disease (Figure 1(b)), diminished dopaminergic input to the striatum due to the loss of nigral DA neurons increases the activities of striatal neurons expressing the D2-class receptors and decreases the activities of striatal neurons expressing the D1-class receptors.[15,16] This leads to reduced activities of the GPe and increased activities of the STN.[17] Combined with decreased GABAergic input from D1R-expressing striatal MSNs, the activities of the GPi become abnormally high.[17] The resulting GABAergic suppression of thalamic neurons reduces glutamatergic input to the motor cortex and thus suppresses the vigor of movement.[18] Consistent with this model, lesion of the STN in the MPTP monkey model of PD significantly reduces motor deficits.[19] This leads to the development of deep brain stimulation (DBS),[20] in which an electrode implanted in the STN delivers high frequency

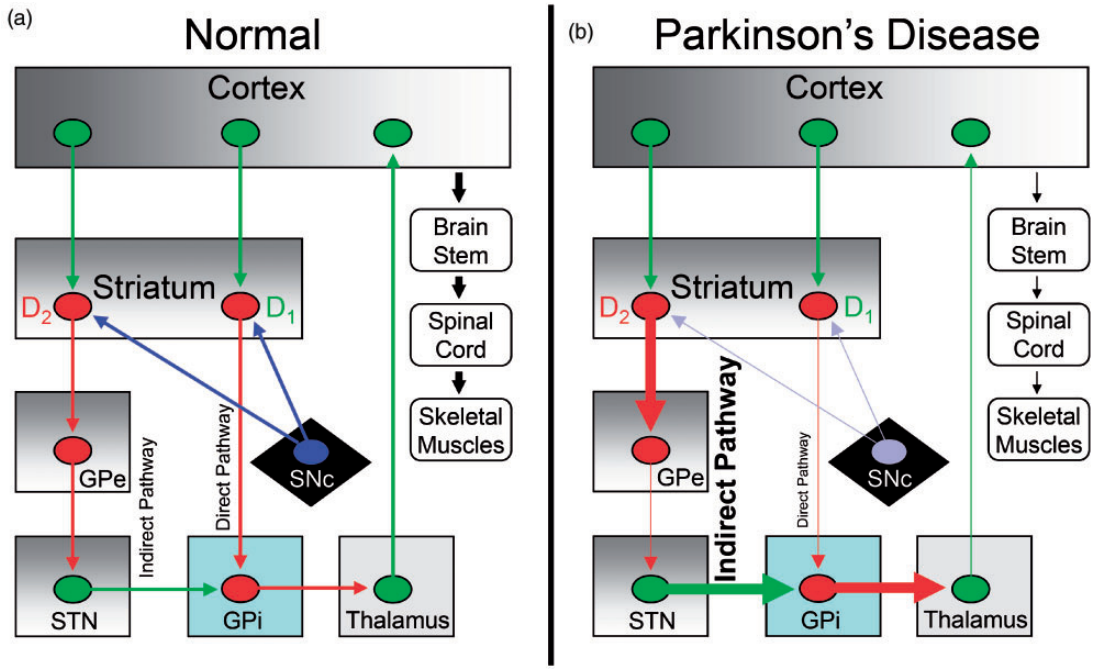

Figure 1. A simplified model for basal ganglia functions in normal subjects and Parkinson's disease patients. (a) In a normal brain, the striatum receives glutamatergic input (green) from many areas of the cortex and dopaminergic input (blue) from the substantia nigra pars compacta (SNc). Balanced actions of dopamine on the GABAergic (red) striatal medium spiny neurons expressing either the D1-class dopamine receptors or the D2-class dopamine receptors converge on the internal segment of the globus palladus (GPi) through the direct pathway and the indirect pathway (via the external segment of globus palladus (GPe) and the subthalamic nucleus (STN)). Information from the GPi is relayed by the thalamus to the motor cortex, which sends instructions through the brain stem and the spinal cord to enable movement (i.e. coordinated contraction and relaxation of skeletal muscles). (b) In Parkinson's disease, loss of nigral DA neurons greatly diminishes dopaminergic input to the striatum. Through unclear mechanisms, this leads to increased activities in striatal neurons expressing the D2-class receptors and decreased activities in striatal neurons expressing the D1-class receptors. Unbalanced activities in the indirect pathway and direct pathway converge to cause over-excitation of the GPi, which inhibits thalamic neurons and thus reducing the vigor of movement. (A color version of this figure is available in the online journal.)

stimulation to override the activities of the STN and thereby restores the normal firing rate in the GPi.[21] This remarkable retreatment demonstrates the validity of the model.

Oscillatory and synchronized neuronal activities in PD

Extracellular recording in the monkey MPTP model of PD[17] and in PD patients[22] has shown that many parts of the basal ganglia, such as the GPe, the STN and the GPi, exhibit synchronized bursts of neuronal activities. Administration of L-DOPA reduces these oscillatory activities.[23] More recent studies in PD patients undergoing DBS surgery show that suppression of β-band (13–35 Hz) oscillation by L-DOPA correlates with an improvement of motor functions.[24] In normal human and monkey brains, activities in these basal ganglia nuclei do not have any obvious pattern.[25] These lines of evidence suggest that normal neurotransmission encoded by aperiodic neuronal activities is disrupted in PD, producing rhythmic and synchronous neuronal activities. The amplitudes of β-band bursts correlate with the durations of these synchronized activities in PD patients undergoing DBS surgery. L-DOPA treatment abrogates long-lasting bursts, leaving only short bursts.[24] The amount of long bursts is positively correlated with the severity of PD symptoms as quantified on Unified Parkinson Disease Rating Scale (UPDRS).

Another prominent electrophysiological feature in the PD brain is the widespread synchronization of neuronal activities.[26,27] Normally, the firing of neurons in different basal ganglia nuclei is independent.[28] In PD animal models[28,29] and PD patients,[30,31] synchronization of neuronal activities is found not only in nuclei in the basal ganglia,[27] but also in the cortex.[32] Many different types of neurons in the basal ganglia are autonomous pacemakers. Principal neurons in the GPe, the GPi/SNr, and the STN are fast pacemakers, while striatal cholinergic neurons and nigrastriatal dopaminergic neurons are slow pacemakers.[33] They tonically fire action potentials even in the absence of synaptic inputs. Synaptic inputs disrupt the periodicity of their action potentials and such disruptions are associated with movement.[34] It suggests that information is encoded by these synaptically driven disruptions of pacemaking. In the normal brain, dopamine ensures the active decorrelation in the firing of neurons in the basal ganglia.[35] In PD, substantial reduction of dopamine abrogates this critical function, thus leaving various pacemakers in many parts of the basal ganglia to fire in synchronized bursts, thereby reducing the information content of neurotransmission. DBS in the STN may work by disrupting these pathologically synchronized activities and thus restoring the decorrelated firing state of the network to encode information needed for movement.

Oscillation in a dish

Given the significance of oscillatory neuronal activities in PD and the impracticality in studying patient brains invasively, we have developed a stem cell strategy to study this important pathological hallmark of Parkinson's disease. The complexity of idiopathic PD makes it very difficult to identify the causal relationship between a phenotype and the disease. Many groups, including ours, have studied PD caused by parkin mutations[36] to take advantage of the full power of molecular biology in elucidating the deterministic mechanisms of a monogenic disease. Mutations of parkin, which cause PD with 100% penetrance in diverse genetic backgrounds, represent the most frequent cause of recessively inherited Parkinson's disease.[37] Many parkin mutations are independently arisen in diverse human populations.[38] It suggests that parkin mutations have a deterministic mechanism in causing PD. In contrast, the more frequent LRRK mutations have a penetrance of 24–26% in two large scale studies[39,40] and a very strong genetic founder effect.[41] The most common LRRK2 G2019S mutation can be traced back to a common carrier about 700 years ago in Europe,[42] but the mutation is very rare in the vast population of PD patients in East Asia.[41] PD-causing monogenic mutations in PINK1, DJ1, a-synuclein, VPS35, etc. are considerably less frequent than mutations in parkin.[43,44]

To understand the functions of parkin in human midbrain DA neurons, we generate induced pluripotent stem cells (iPSCs) from PD patients with parkin mutations and normal subjects.[45] In iPSC-derived human midbrain DA neurons, parkin mutations disrupt the precision of dopaminergic transmission by increasing spontaneous, Ca^{2+}-independent DA release, and decreasing dopamine reuptake.[45] Dopamine-induced oxidative stress is significantly increased,[45] because parkin mutations significantly increase the transcription of monoamine oxidases,[45–47] mitochondrial enzymes responsible for the oxidative catabolism of dopamine.[48] We confirm these phenotypes in midbrain DA neurons derived from naïvetropic iPSCs of PD patients with parkin mutations.[49] Parkin mutations significantly reduce the length and complexity of neuronal processes by reducing microtubule stability.[50] The massive axon arborization of nigral DA neurons[13] make them particularly vulnerable to microtubule destabilization,[51] against which parkin protects,[52] through direct binding to microtubules.[53]

When we study the electrophysiology of midbrain neurons differentiated from the same set of iPSCs, we find that activation of D1-class dopamine receptors by the co-application of dopamine and the D2-class receptor blocker sulpiride induces rhythmic bursts of spontaneous excitatory post-synaptic currents (sEPSC) in neurons from PD patients with parkin mutations (Figure 2(b)), but not from normal subjects (Figure 2(a)).[54] Application of D1-class receptor agonist SKF81297 produces the same results.[54] When we reintroduce parkin to neurons derived from PD patients with parkin mutations, oscillation of sESPC is abolished (Figure 2(c)). The PD-causing T240R mutant parkin does not rescue the oscillation phenotype (Figure 2(d)). These data indicate that the phenotype is dependent on parkin.

It is quite remarkable that oscillatory neuronal activities can be generated in iPSC-derived neurons cultured in a dish. The observation that only neurons from PD patients with parkin mutations, but not normal subjects, exhibit the oscillatory bursts suggests that the phenotype, which is

reminiscent of rhythmic bursting of local field potentials in PD patients and animal models,[22] is linked to PD. Another interesting feature is that the oscillatory activities are only induced when D1-class receptors are activated. When dopamine is applied on these neurons, there is only one modest difference between neurons from normal subjects and parkin patients: a delayed increase of sEPSC amplitude in patient neurons.[54] It seems that balanced actions of dopamine on D1 and D2 receptors cannot elicit the intrinsic difference between neurons from normal subjects and parkin patients. When only D1 receptors are activated, there is a significant increase in quantal content in neurons from parkin patients,[54] suggesting that the oscillatory neuronal activities are mediated by a presynaptic mechanism. Based on our previous finding that parkin mutations increase spontaneous release of dopamine in iPSC-derived midbrain DA neurons[45] and the involvement of parkin in vesicle recycling,[55–59] I propose the following model to explain the oscillatory activities (Figure 3). In normal midbrain neurons, parkin may limit the number of vesicles at both dopaminergic terminals and glutamatergic terminals through proteins such as endophilin A,[55] synaptojanin 1, and dynamin.[56] Even when only D1 receptors (D1R) are activated, increased recycling of glutamate vesicles due to activation of PKA by D1R would not cause very big changes in glutamate release. Thus, we do not see a significant increase in quantal content and only a modest increase in the amplitude of sEPSC in normal neurons (Figure 3(a)). In neurons derived from parkin patients, loss of function mutations of parkin may greatly increase the number of vesicles in the basal condition. Activation of D1R further potentiates recycling of glutamate vesicles and thus significantly increases quantal content. The great rise in glutamate release causes marked increase in sEPSC amplitude and frequency, which is periodically interrupted by the exhaustion of vesicles due to the loss of parkin-mediated regulatory mechanism (Figure 3(b)).

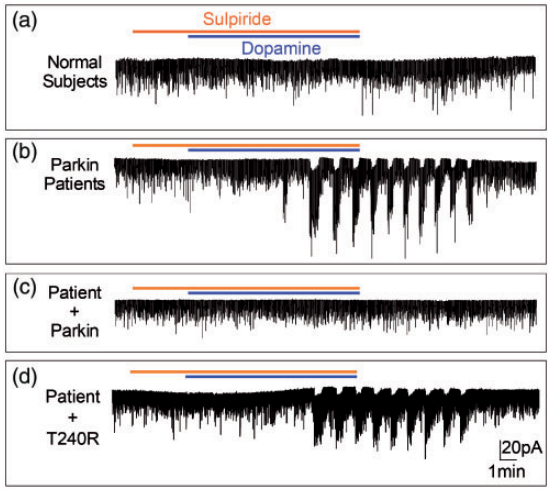

Figure 2. Parkin mutations cause oscillatory activities in iPSC-derived human midbrain neurons. Activation of dopamine D1 class receptors induces only modest changes in spontaneous excitatory postsynaptic currents (sEPSCs) in iPSC-derived midbrain neurons from normal subjects (a), but causes oscillation of sEPSCs in midbrain neurons derived from PD patients with parkin mutations (b). The oscillatory sEPSCs are rescued by overexpression of wild-type parkin (c), but not by its PD-causing T240R mutant (d). Adapted from Zhong *et al*.[54] (A color version of this figure is available in the online journal.)

As the system is highly artificial, there are a number of differences in comparison to the rhythmic bursting of local field potentials in the brain of PD patients. First, oscillation frequency in the dish is only 0.018 Hz (1.1 events/min), much slower than oscillation *in vivo*, particularly the β-band (13–35 Hz) oscillations that are critically involved in PD pathophysiology.[24] The lack of robust delivery of glucose and O_2 in static monolayer culture perhaps makes it hard for iPSC-derived neurons to conduct neurotransmission at physiological frequencies. Second, the dependence on D1R is different from the generally accepted notion that D2R plays a more dominant role in PD, because of the overexcitation of the indirect pathway, which emanates from striatal MSNs expressing D2R.[15,16] The lack of organized neuronal circuits in iPSC-derived neuronal cultures may be related to this difference. Third, dopamine reduces oscillation in PD brains,[24] while activation of D1R induces oscillation in neurons from parkin

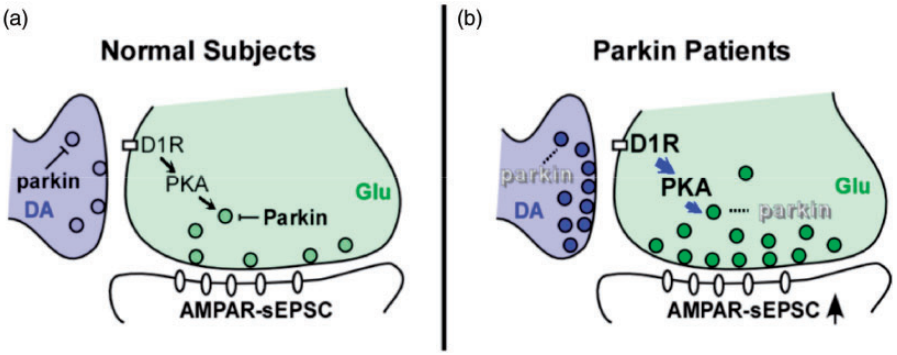

Figure 3. A model on how parkin mutations induce oscillatory neuronal activities. (a) In iPSC-derived neurons from normal subjects, parkin may limit vesicle recycling through interaction with synaptic vesicle proteins. Activation of dopamine D1-class receptors (D1R) does not significantly increase quantal content of glutamatergic transmission. Only moderate enhancement of sEPSC is observed.[54] (b) In neurons derived from parkin patients, mutations of parkin may increase the number of synaptic vesicles in both dopaminergic and glutamatergic terminals. Increased dopamine release, amplified by D1R, results in significantly increased quantal content of glutamatergic transmission.[54] This may cause marked increases in the amplitude and frequency of sEPSC, which is periodically disrupted by the exhaustion of synaptic vesicles. DA: dopamine; Glu: glutamate; PKA: protein kinase A; AMPAR-sEPSC: AMPA receptor-mediated spontaneous excitatory postsynaptic current. Adapted from Zhong *et al*.[54] (A color version of this figure is available in the online journal.)

patients. This difference may also be caused by the lack of complex neuronal circuitry in iPSC-derived neuronal cultures, which are random mixtures of glutamatergic, GABAergic, and dopaminergic neurons, each contributing approximately 1/3 to the population.[54]

Future directions

Imperfect as the current system is, it recapitulates salient features of oscillatory local field potentials in PD patients and animal models. With the rapid development of stem cell technologies, I envision several improvements that will enable the invasive study of PD pathophysiology in patient-derived materials.

Improving data collection

Measuring neuronal activities in the current static monolayer culture system can be improved by using multielectrode array recording, which measures neuronal activities in many neurons at the same time. Alternatively, one can use imaging tools such as GCaMP6 calcium indicators[60] or genetically encoded voltage indicators[61] to measure neuronal activities in either the whole neuronal population or a subset of neurons selectively labeled with a genetically encoded indicator by gene targeting in iPSCs.[49,62] Large scale data analysis will identify patterns of neurotransmission, e.g. whether activities of identified neurons are correlated or not, whether the sequential firing of neurons in synaptic contact encodes any information, whether oscillation in one neuron is spreading to connecting neurons, etc.

Improving the generation of patient-specific neurons

The static monolayer culture system can be used to examine whether oscillatory neuronal activities are found in iPSC-derived midbrain neurons from idiopathic PD patients and PD patients with monogenic mutations in other genes. These studies will tell us how robust the system is for studying the pathophysiology of PD. To do this more effectively, it is necessary to make neurons that are as similar to those *in vivo* as ethically permissible. A currently available method is to generate brain organoids from iPSCs.[63] One can specify organoids with morphogens and tweak the differentiation process to generate midbrain organoids.[64] When the iPSCs are genetically labeled with various activity indicators, it is possible to image neuronal activities of identified neurons (e.g. DA neurons or MSNs). While this approach produces different types of neurons, it is very hard to generate organized circuits that mimic the *in vivo* situation. When organoids grow to a few millimeters in size, the lack of robust delivery of glucose and O_2 starts to kill cells in the middle, which affects neuronal functions on the outer layers. To bypass the problem, one can transplant iPSC-derived neurons, either from monolayer cultures or from organoids, to animal models of PD. This will allow investigators to study the activities of human neurons of identified types in the brain of a PD animal model. But it may be hard to place human neurons in the right location in the animal basal ganglia circuits and expect the human neurons, with a different developmental clock, to integrate into the animal neural network effectively.

An emerging technology may significantly disrupt how we study human diseases including PD, if the ethical issues surrounding the technology can be managed. There have been considerable efforts to generate naïve state human iPSCs that can generate significant amounts of human cells in animal embryos.[65,66] Using rat or mouse embryonic stem cells, which are in the naïve state of pluripotency, a rat pancreas is generated in a mouse[67] and a mouse pancreas is generated in rat.[68] We have developed a new method to convert primed state human pluripotent stem cells (hPSCs) to the naïve state by transient inhibition of mTOR.[69] The naïve hPSCs are maintained in very similar conditions used to culture mouse embryonic stem cells. Because of the similarities in cell states, naïve hPSCs injected in a mouse blastocyst develop with the mouse embryo and generate up to 4% of cells in the chimeric embryos at E17.5. Mature human cells of all three germ layers are generated, including a large amount of human red blood cells, human liver cells, and human ocular cells.[69] Under the current ethics guidelines, it may be problematic to generate a large number of neurons, but not somatic tissues, such as a kidney.[70,71] From a scientific perspective, generation of patient-specific brain tissues in a chimeric animal solves the otherwise intractable problem of incubating each human cell under optimal physiological conditions, such as glucose, O_2, and CO_2 levels. At least 80% of the 30 trillion cells that constitute a human being are red blood cells,[1] which along with many other cells, serve the function of an incubator. The properties of our neurons are shaped by this total immersion incubator from the time that they are generated. Making human neurons in chimeric animals promises to produce the best human neurons, perhaps in the correct circuits, for a variety of applications including studying PD pathophysiology. The ethical debate rests on how we assign moral judgments on a neuron vs. a kidney cell, and how we value patients with Parkinson's disease vs. kidney failure. While our brain is able to render a sense of its superiority, the fact remains that the brain and the kidney are generated from the same genome that encodes an individual for the harmonious operation of all cells in the body. Further development of stem cell technologies for the benefits of patients suffering from disparate diseases is hinged on careful considerations of complex ethical issues by all stakeholders in the society.

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DECLARATION OF CONFLICTING INTERESTS

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