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Research Report

Using human pluripotent stem cells to study post-transcriptional mechanisms of neurodegenerative diseases

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ABSTRACT

Post-transcriptional regulation plays a major role in the generation of cell type diversity. In particular, alternative splicing increases diversification of transcriptome between tissues, in different cell types within a tissue, and even in different compartments of the same cell. The complexity of alternative splicing has increased during evolution. With increasing sophistication, however, comes greater potential for malfunction of these intricate processes. Indeed, recent years have uncovered a wealth of disease-causing mutations affecting RNA-binding proteins and non-coding regions on RNAs, highlighting the importance of studying disease mechanisms that act at the level of RNA processing. For instance, mutations in TARDBP and FUS, or a repeat expansion in the intronic region of the C9ORF72 gene, can all cause amyotrophic lateral sclerosis. We discuss how interspecies differences highlight the necessity for human model systems to complement existing non-human approaches to study neurodegenerative disorders. We conclude by discussing the improvements that could further increase the promise of human pluripotent stem for cell-based disease modeling.

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1. Introduction

1.1. RNA processing in neurodegenerative diseases

Neurodegenerative conditions represent a spectrum of progressive, incurable and invariably fatal diseases, characterized by age-related progressive loss of neurons, typically with a focal onset and/or subtype-specific selective vulnerability. Recently, mutations affecting proteins involved in RNA processing have been found to underlie many neurodegenerative disorders such

as spinal muscular atrophy (SMA), ALS, and frontotemporal lobar dementia (FTLD), among others (Anthony and Gallo, 2010; Sreedharan et al., 2008; Vance et al., 2009). This accumulating number of disease-causing mutations implicating abnormalities in RNA dependent mechanisms emphasizes the pivotal role of RNA processing in higher vertebrate neural function, and encourages development of new models that replicate these disturbances in order to help identify new therapeutic opportunities.

The functionality and processing of an RNA molecule is largely determined by the arrangement of its associated pro-

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teins, together comprising the ribonuclear particle (RNP). Accordingly, RNA-binding proteins (RBPs) have well-recognized and fundamental roles in coordinating and modulating RNA processing. Such processes are directed by sequence motifs or structural arrangements recognized in the processed RNA molecules themselves; for example, in humans the vast majority of protein-coding genes contain introns, which need to be removed from the primary transcript (pre-mRNA). This process is termed splicing and is orchestrated by the spliceosome, a cellular apparatus that is composed of five small nuclear RNPs (snRNPs), each containing a small nuclear RNA (snRNA) and associated RBPs. In addition to catalyzing the splicing reactions, the spliceosome is also responsible for recognition of key regulatory sequences in the pre-mRNA transcript that define the exon boundaries. Due to the complexity of spliceosome, and its ATP-dependence, pre-mRNA splicing is an energy-demanding process. In spite of this, it is highly conserved during evolution, and the number of introns has increased in accordance with species complexity (Lynch and Conery, 2003). Importantly, regulation of splicing is closely coordinated with other aspects of the mRNA life cycle (Maniatis and Reed, 2002). For instance, splicing factors such as the polypyrimidine tract binding protein (PTBP1) are often the crucial targets for gene silencing directed by microRNAs (miRNA) and the RNA interference (RNAi) pathway. RNAi is an important post-transcriptional pathway that restricts the mRNA available for translation in a sequence-specific manner, and thus ensures that the protein levels of such genes are tightly regulated (Makeyev et al., 2007).

Regulatory RBPs can assist or compete with spliceosomal factors for binding to pre-mRNA, and thereby lead to generation of alternative mRNA isoforms. Alternative splicing permits single genes to be transcribed into multiple different mRNA products, in turn increasing the diversity of translated proteins, a central feature of the evolutionary process. Alternative splicing thus allows diversification of the transcriptome with small changes in the genome itself. Recent studies suggest that the extent of alternative splicing is far greater in humans than previously recognized (Pan et al., 2008). In particular, high-throughput sequencing studies have shown that the largest diversity of alternative RNA isoforms is seen in the mammalian brain (Wang et al., 2008).

The combinatorial complexity generated by the large numbers of RNAs and RBPs commands a high level of precision in choreographing the assembly and fate of the RNPs. Therefore it is no surprise that mutations to any of the factors involved in RNP assembly or functionality can cause disease (Lukong et al., 2008; Nilsen, 2003; Wang et al., 2007; Will and Luhrmann, 2001). In particular, mutations affecting pre-mRNA splicing have been found to underlie many neurodegenerative disorders (Anthony and Gallo, 2010). Mechanisms include mutations that modify the sequence motifs required for correct processing of individual transcripts. Additionally, disease-causing mutations in RBPs or non-coding RNAs, which act as *trans*-acting factors at multiple transcripts, can lead to a more widespread splicing mis-regulation of multiple RNA targets. For example, defects in RNA-dependent mechanisms due to loss or gain of function mutations cause several devastating neurodegenerative diseases, including spinal muscular atrophy (SMA; Cartegni et al., 2006; Talbot and Davies, 2001; Wirth et al., 2006a, 2006b), Duchenne Muscular

Dystrophy (DMD; Disset et al., 2006), frontotemporal dementia with Parkinsonism linked to chromosome 17 (FTDP-17; Liu and Gong, 2008), myotonic dystrophy types 1 (DM1; Timchenko et al., 1996; Wang and Griffith, 1995; Wang et al., 1995) and 2 (DM2; Liquori et al., 2001), fragile X-associated tremor ataxia syndrome (FXTAS; Jin et al., 2007), spinocerebellar ataxia 8 (SCA8; Nemes et al., 2000) and amyotrophic lateral sclerosis (ALS; Sreedharan et al., 2008; Vance et al., 2009). Additionally, recent evidence has implicated hexanucleotide repeat expansions in the 5' untranslated region of C9ORF72 as the most common genetic defect identified to date causing amyotrophic lateral sclerosis and fronto-temporal dementia, with putative mechanisms including altered splicing patterns of the C9ORF72 transcript and nuclear RNA aggregation (DeJesus-Hernandez et al., 2011; Renton et al., 2011).

Transgenic mouse models of many of these disease-causing RBP mutations have now been established. Although a high degree of evolutionary genomic conservation suggests that many RNA to RBP interactions might be similar between higher vertebrates, it is likely that a proportion of such processes may be different or even absent in lower vertebrates and invertebrates due to an evolutionary divergence of RNA transcripts and thus RBP binding sites in the human context. For example, one of the largest increases in TDP-43 binding in cases of FTL is seen in a non-coding RNA molecule, NEAT1, which acts as a scaffold for paraspeckle assembly (Tollervey et al., 2011). Interestingly, our work also shows that a significant number of TDP-43 cross-link sites in NEAT1 are located in large regions of this transcript, which are poorly (if at all) conserved in rodents. Indeed, this weakly conserved non-coding RNA and associated paraspeckles appear to be non-essential in mice (Nakagawa et al., 2011), and is also expressed at low levels in the mouse brain suggesting that these findings may have potentially been missed if studying TDP-43 transgenic mouse models rather than human specimens.

Taken together, the growing list of neurodegenerative diseases characterized by disturbances in RNA processing at the molecular level highlights the need to develop new models of RNA-mediated human diseases using appropriate and clinically relevant neurons and glia that faithfully recapitulate the underlying pathobiology. As well as improving understanding of the underlying mechanisms of specific diseases, this will facilitate development of effective therapeutic strategies.

1.2. Interspecies differences highlight the need for human model systems

Our incomplete understanding of the pathobiological mechanisms underlying neurodegenerative diseases and the consequent absence of therapies reflects in part the limitations of existing model systems. Classic experimental systems relying on *in vivo* transgenic/lesion and cell line studies, although invaluable and highly informative, are unable to wholly capture the complexity and biology of the human system. Notwithstanding considerable evolutionary conservation between vertebrate neuraxial systems there are important differences that need to be addressed in order to improve the translational hit of experimental studies. Focusing in on the neuro-motor axis as an example, interspecies differences include gross anatomical structure,

intrinsic circuitry, functionality, cellular and molecular processes (Courtine et al., 2007; Hardingham et al., 2010; Lemon et al., 2004; Tuszyński et al., 2002). Some aspects of this evolutionary divergence are discussed below in more detail.

Evolutionary anatomical divergence is most abundant in the more recently developed, rostral regions of the neuraxis (i.e. cerebrum, especially the forebrain) compared to more primitive caudal structures (i.e. spinal cord). Indeed, it is noteworthy that there are major anatomical differences between mouse and human forebrain. For example, the motor cortex is positioned at the rostral most point of the forebrain in adult mice while it occupies a more caudal position in an adult human forebrain. Humans, additionally, have a relatively massive neocortex compared to rodents, including the motor area, which gives rise to the corticospinal tract. The anatomical position of the corticospinal tract is also different; it lies more laterally in the human spinal cord (Rouiller et al., 1996). These anatomical differences would argue for divergent evolutionary processes governing even initial patterning of cortical motor neuron precursors (Zhang, 2006). Some anatomical differences are more subtle; for example, direct synaptic contact between upper and lower motor neurons, which appears to be a recent feature in evolution, is confined to the primary motor cortex and exclusive to higher primates (Eisen, 2009). Furthermore, the upper motor neurons also have a more complex axonal projection repertoire in humans and macaque compared to lower primates and other vertebrates (Lemon et al., 2004), with the most complex projection pattern seen in humans (Kuypers, 1964).

Additional evidence for divergent evolutionary processes comes from electrical stimulation of upper motor neurons in the motor cortex and subsequent analysis of evoked motor responses. These differ markedly between rodents and primates, but an inter-species primate variability is also established (Lemon and Griffiths, 2005; Lemon et al., 2004). For example, excitatory oligosynaptic signals (i.e. involving only a few neurons) can be readily detected in spinal motor neurons of the squirrel monkey following repetitive pyramidal tract stimulation, but not in macaque, which have more complex, polysynaptic corticospinal connections (Lemon et al., 2004). The consequences of such differences are thought to be in conferring the capacity for complex and precisely choreographed recruitment of motor units (Bortoff and Strick, 1993; Lawrence and Kuypers, 1968a, 1968b; Lemon and Griffiths, 2005). The aforementioned development of direct cortical projections to spinal MNs appears to be a recent evolutionary event (Eisen, 2009) and coincides with the acquisition of pincer grip (i.e. precise and coordinated grip between the thumb and index finger; Lemon et al., 2004). This evolutionarily determined increase in complexity is unfortunately mirrored by a distinct increase in the magnitude of neurological disability resulting from disruption of these pathways in primates compared with rodents (Courtine et al., 2005; Freund et al., 2006; Lawrence and Kuypers, 1968a, 1968b; Muir and Whishaw, 1999; Nathan, 1994; Schmidlin et al., 2004).

Evolutionary differences are additionally relevant to further intricate cellular processes including neuroprotective pathways, such as those triggered by synaptic NMDA receptor activity, or neurotrophic factors. While many fundamental mechanisms mediating these processes may be conserved in rodent and human systems, there are likely to be some important

differences. For instance, a significant proportion of functional human transcription factor binding sites are not functional in rodents (Dermitzakis and Clark, 2002). Further evidence for evolutionary divergence comes from a recently identified gene, sulfiredoxin, which is regulated by synaptic NMDAR activity in rat neurons via two activator protein 1 (AP-1) sites (Papadia et al., 2008). Expression of this gene contributes to enhanced antioxidant defenses found in electrically active rodent neurons. However, one of the two AP-1 sites has been lost in the primate lineage, despite being well conserved among other mammals. It would thus certainly be of interest and importance to study the activity-dependent induction of this gene in hESC-derived neurons to further understand its contribution to defense mechanisms in human neurological disease (Hardingham et al., 2010).

It is also important to recognize that several human diseases are difficult to faithfully recapitulate in mice. As an example, SMA results from recessive mutations in the Survival of Motor Neuron 1 (SMN1) gene, which is involved in biogenesis of the spliceosome. Human genome also contains the SMN2 gene, which contains a C→T transition in the exon 7 that leads to alternative splicing of exon 7. About 10–20% of the SMN2 mRNA isoforms encode the full-length SMN protein, allowing the SMN2 gene to partially compensate for mutations in SMN1 gene. Humans are unique in carrying both SMN1 and SMN2, and modeling of mutations in mice is embryonic lethal due to the importance of SMN1 in cell survival (Monani et al., 2000). This therefore dictates either restriction of SMN1 knockout to specific cell-types (Frugier et al., 2000), or concomitant delivery of an SMN2 gene together with SMN1 knockout (Monani et al., 2000). Both approaches have yielded important insights into SMA and potential therapeutic strategies, but the artificial setting must be acknowledged and a more humanized model would be desirable.

Finally, and arguably the strongest reason for the development of new humanized models is that therapeutic strategies that were efficacious in animal disease models have often proved unsuccessful when translated to pre-clinical and clinical human trials (Besselink et al., 2008; Dirksen et al., 2007; Shuaib et al., 2007; Yellon and Hausenloy, 2007). With specific reference to the CNS, the free-radical trapping agent, NXY-059, failed to demonstrate any improvement in the treatment of acute ischaemic stroke relative to placebo in clinical trials (Shuaib et al., 2007) despite having previously shown excellent efficacy in reducing cerebral infarct size and improving functional recovery in primate stroke models (Marshall et al., 2003). Likewise, the recent announcements that dimebon, an apparent neuroprotective agent in rodents (Bachurin et al., 2001), failed phase III clinical trials for both Alzheimer's and Huntington's diseases further highlight the uncertainty surrounding development of therapies in lower organisms (Bezprozvanny, 2010). Therefore, human systems are required to complement lower species systems, and thereby confirm, evaluate as well as discover additional mechanisms and targets of direct relevance to human disease.

2. Stem cells

2.1. Stem cell classification

Human systems can be accessed to some extent clinically in the context of adult-onset disease through opportunistic tissue

biopsy and post-mortem samples. However, these have several limitations other than viability including limited tissue availability, the effects of post-mortem delay on cytoarchitecture, and the difficulty of obtaining material representing the early stages of disease process (Han et al., 2011). Fortunately, the ability to generate defined neuronal cell types from human pluripotent stem cells (hPSCs) offers a unique experimental opportunity to study disease mechanisms. In this context, accurate specification of regionally defined neurons is crucial to establish the most informative in vitro disease model.

Stem cells can be classified as embryonic, foetal and adult, depending on the developmental stage at which they were isolated. This is one of the key determinants of the repertoire of cell types into which they can differentiate, referred to as potency. Maximally potent stem cell types are termed pluripotent and are defined by the following features: i) self renewal capacity and ii) pluripotency (i.e. ability to give rise to any of the cell types that comprise the organism from which they are derived) and iii) teratoma formation (spontaneous formation of tumors derived of multiple cell lineages derived from the three germ layers when injected into immunoprivileged mice). Embryonic stem cells (ESCs) are a type of pluripotent stem cell (PSC) that possess the greatest developmental potential in-vitro, responding predictably to developmental cues. This makes them an attractive prospect for modeling region- and cell-type specific diseases.

Human ESCs (hESCs) were first isolated in 1998 (Thomson et al., 1998), some 17 years after the isolation of their mouse counterparts (Evans and Kaufman, 1981). Human ESCs are isolated from the inner cell mass of the blastocyst (i.e. a pre-implantation embryo), from where cells are micro-manipulated and subsequently grown in culture by a variety of methods (Amit et al., 2003, 2004; Xu et al., 2001). The introduction of chemically defined human ESC culture and differentiation has strengthened the prospect of establishing clinical-grade cells for use in regenerative medicine (Ioannides et al., 2007; Vallier and Pedersen, 2008).

Compared to ESCs, stem cells isolated at later developmental stages (e.g. foetal stem cells) possess restricted phenotypic potentials that are defined by both the tissue and the tissue sub-region from which they are isolated. Given the numbers of cells required for experimental or therapeutic application, regional restriction (e.g. forebrain/midbrain) represents a potential problem because this cannot, at present, be predictably manipulated using extrinsic signals. hESCs thus represent an ideal model system by virtue of their self-renewal capacity and predictable manipulability using extrinsic morphogenetic instruction.

Attempts to derive PSCs by different methods have been partially stimulated by the ethical controversy related to the hESC field. These efforts were largely inspired by initial nuclear transfer experiments from John Gurdon and Ian Wilmut (Campbell et al., 1996; Gurdon et al., 1958), and focus on nuclear reprogramming by various methods, including somatic cell nuclear transfer (SCNT) and cell fusion techniques (Cowan et al., 2005). Both methods have several drawbacks, including the requirement of oocytes in the former and tetraploidy in the latter.

A more recent method of generating patient-specific PSCs is built on the discovery that somatic cell nuclei can be 'reprogrammed' to an embryonic-like state, and these cells are referred to as induced pluripotent stem cells (iPSCs; Takahashi and

Yamanaka, 2006). Importantly, this approach has also been demonstrated using human somatic cells (Takahashi et al., 2007). Induction of the pluripotent state originally necessitated transduction (viral transfection) of four transcription factors, including the oncogenic *c-myc* transcription factor. However, progress has since been made to improve efficiency and/or reduce the need for genetic manipulations (Abujarour and Ding, 2009; Lin et al., 2009; Maherali and Hochedlinger, 2009), including approaches not requiring the use of *c-myc* (Nakagawa et al., 2008). Human iPSCs thus offer a unique opportunity to derive patient specific cell lines to model neurodegenerative diseases.

2.2. Human pluripotent stem cells (hPSCs) as a disease model system

Human PSCs now provide a powerful and unprecedented experimental opportunity to model neurodegenerative disease on account of their competence to developmental signals permitting specification of functional regional sub-type specific neurons (Fig. 1). These include directed differentiation to spinal cord, midbrain and forebrain neurons (Eiraku et al., 2008; Lee et al., 2007; Li et al., 2005, 2009; Perrier et al., 2004; Schulz et al., 2004). These studies provided evidence for the faithful recapitulation of spatio-temporally regulated developmental responsiveness to appropriate extrinsic morphogenetic signals. Although achieving such region-specific differentiation represents a significant advance, the next challenge, which is now beginning to be addressed, is understanding how highly refined sub-region specific human neuronal and glial diversity is generated. For example motor neurons represent a collection of diverse sub-types that in turn exhibit differential disease vulnerability, while nine different subtypes of dopaminergic neuron located throughout the brain (Bjorklund and Dunnett, 2007; Dahlstrom and Fuxe, 1964). Recent studies suggest that in vitro stem cell based systems can begin to capture this diversity (Krencik et al., 2011; Patani et al., 2011). Specifically, these studies have recapitulated rostro-caudal and dorso-ventral patterning of spinal motor neurons (Li et al., 2005; Patani et al., 2011) and demonstrated efficient differentiation into the A9 midbrain dopaminergic neurons that are lost in Parkinson's disease (Perrier et al., 2004; Sanchez-Danes et al., 2011), with confirmation based on expression of region-specific markers, neurotransmitter profiles and electrophysiological activity.

Induced PSCs offer the further advantage over hESCs in enabling study of patient specific lines including disease-causing mutations from routine manipulation of readily available patient material (Han et al., 2011; Wichterle and Przedborski, 2010). To date, proof-of-concept studies using iPSCs from patients with inherited diseases include spinal muscular atrophy (Ebert et al., 2009), familial dysautonomia (Lee et al., 2009) and Rett syndrome (Marchetto et al., 2010) have provided novel insights into disease mechanisms and potential therapeutic targets. For example, in spinal motor-neurons derived from SMA iPSCs, SMN RNA levels were significantly reduced. Both valproic acid and tobramycin (two compounds known to increase SMN levels in pre-clinical models) ameliorated the pathological phenotype observed, demonstrating their potential value as therapies for SMA (Ebert et al., 2009). Disease-specific iPSCs also allow study of the developmental progression of neurogenetic

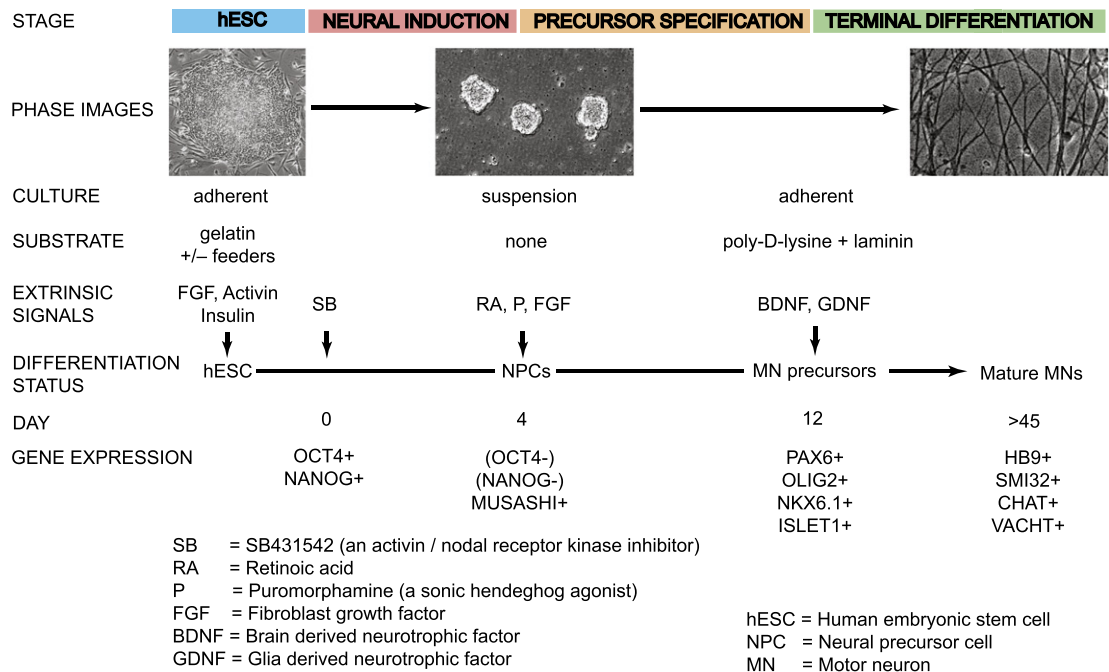


Fig. 1 – Protocol summary for accelerated motor neuron neurogenesis from human embryonic stem cells. Human embryonic stem cells are grown as adherent colonies. These are enzymatically dissociated at the point of sub-confluence and placed in suspension culture on an orbital shaker in chemically defined medium. At day 0, they are treated with 10 μm of the activin and nodal inhibitor SB431542 for 4 days. At day 4, the cells downregulate pluripotency markers OCT4 and Nanog and upregulate neural marker Musashi. The activin and nodal inhibitor SB431542 are withdrawn and 0.1 μm retinoic acid, 1 μm of puromorphamine and 10 ng/ml FGF are added simultaneously for a further 8 days. By day 12, the cells acquire classical motor neuron markers. Specific information including phase micrographs at major phenotypic stages, culture methods, substrates, extrinsic cues, gene/protein expression changes and abbreviations are included in the figure.

disease. Importantly, iPSC-derived neuronal models from schizophrenia patients have demonstrated reduced synaptic connectivity compared to control iPSCs. This study went on to show that synaptic connectivity can be enhanced with application of the antipsychotic loxapine (Brennand et al., 2011). This is of particular interest given that the experimental system is necessarily developmentally derived but still appears functional. However, in many cases it is likely that the study of adult-onset disease pathology will require development of new methods (Han et al., 2011). For example, exposure of iPSC-derived midbrain dopaminergic neurons to cell stressors has been used to identify an enhanced stress sensitivity phenotype associated with the LRRK2 G2019S mutation in Parkinson's disease (Nguyen et al., 2011).

With RNA processing being implicated in several neurodegenerative disorders, hPSCs represent an unprecedented resource to gain insight into both development and disease of the central nervous system at the RNA level with high-throughput approaches. In one example, exome sequencing was used to first identify a homozygous insertion in exon 9 of the male germ cell-associated kinase (MAK) in an isolated individual with retinitis pigmentosa (RP) (Tucker et al., 2011). An iPSC model was then derived from this patient to show that the insertion prevents inclusion of exons 9 and 12, which normally occurs upon retinal differentiation.

Recently, RNA-sequencing (RNA-seq) has been used to monitor transcriptional changes of hPSC cells in response to

neuronal differentiation (Lin et al., 2011). A wide-array of changes in gene expression and alternative splicing events were documented, with several disease-associated transcripts also displaying prominent alterations during this transition. This coincided with an increase in expression of the neuron-specific RBP, NOVA1, and decrease in expression of the PTBP1, which play a crucial role in establishing neuron-specific splicing patterns (Ule et al., 2005; Boutz et al., 2007; Makeyev et al., 2007; Licatalosi et al., 2008). Examples include an increase in expression across specific exons in the Neurexin-1 gene associated with schizophrenia and autism spectrum disorders, and isoform-specific changes in the schizophrenia-associated Neuregulin-1 transcript. This suggests a role for these two genes in neurogenesis, and may help explain observed phenotypes in the neuropsychiatric disorders to which these genes are linked. In addition, several disease-associated transcripts and long non-coding RNAs, which display prominent changes during neurogenesis, are the sites of documented single-nucleotide polymorphisms mapped in multiple neuropsychiatric disorders (Lin et al., 2011). It is therefore tempting to speculate that a perturbed role of these transcripts in neurogenesis may be partially responsible for the phenotype associated with these genetic variants, although further work is required to confirm this. An additional study has recently taken a step further and directly looked at RNA processing in an iPSC disease model (Brennand et al., 2011). Following derivation of multiple

neuronal differentiated iPSC lines from control and schizophrenia patients, microarray assessed transcriptional profiling revealed widespread differential regulation of genes, only 25% of which had been identified in previous studies. Many of these could be linked to key schizophrenia associated signaling pathways, and importantly many of these changes could be reversed following treatment with the clinically used antipsychotic agent, loxapine. Collectively, these studies highlight the exciting potential for high-throughput studies of RNA processing in hPSC models to further our understanding of neurological disease.

2.3. The challenges facing human PSCs as a model system for neurodegenerative diseases

The critical prerequisites to establishing accurate and clinically relevant in-vitro disease model systems are summarized in Box 1. First, there are theoretical advantages in moving towards more humanized culture systems, including chemically defined media and feeder free systems (Amit et al., 2004; Stojkovic et al., 2005a, 2005b; Vallier and Pedersen, 2008; Xu et al., 2001; Yao et al., 2006). Additionally, techniques of generating disease-specific iPSCs need to minimize collateral genetic interruption such as *c-myc* activation, while preserving the ability to generate a state of potency that closely resembles that of hESCs. In this regard, a switch towards the use of the closely related *l-myc* has been suggested due to its ability to match, and in some cases increase, the re-programming potential of *c-myc*, while having no apparent ability to promote tumor formation due to the shorter N-terminal region that this *myc* variant possesses (Nakagawa et al., 2010). Studies comparing hESCs with iPSCs derived from established protocols have so far demonstrated that they are genetically and epigenetically similar, but not identical (reviewed in Amabile and Meissner, 2009). Indeed, differences in both gene expression and DNA methylation patterns have been reported (Chin et al., 2009; Lister et al., 2011). The recent derivation of genetically unmodified iPSCs therefore represents a considerable advance (reviewed in Gonzalez et al., 2011). Further, a

move away from viral / integrated transduction may facilitate this issue, and to this end, iPSCs have been generated using delivery of mature miRNA sequences (Miyoshi et al., 2011), non-viral minicircles (Jia et al., 2010), direct delivery re-programming factor RNA (Warren et al., 2010) or recombinant protein (Zhou et al., 2009), and non-viral transposition of doxycyclin-inducible re-programming factors (Tsukiyama et al., 2011), among others.

The ability to study the consequences of disease-causing mutations at physiological dosage against an otherwise normal genetic background from patient iPSC lines is potentially a major advantage of iPSC systems. Generating more accurate iPSC cell controls is therefore of paramount importance, and as such a genetically identical background apart from disease-associated modifications. For example, 'genome editing' of the disease-specific iPSC line by the use of zinc finger nuclease mediated tailored genome engineering can correct the mutation (Cathomen and Schambach, 2010; Sebastiano et al., 2011; Zou et al., 2009, 2011a, 2011b), while the use of iPSCs from unaffected family members of the patient can reduce, but not eliminate, the background genetic differences between patient iPSCs and controls (Ebert et al., 2009). Recent demonstration of variability in the efficiency of neuronal differentiation between iPS and EPS lines suggests that addressing issues of differential lineage restriction capacities of different iPSC lines is also an important issue to consider in the future (Hu et al., 2010). With this in mind, studies establishing predictive markers of differentiation potential will help to avoid current variation between iPSC lines (Bock et al., 2011).

Further refinement of directed differentiation protocols will be required to maximize the efficiency of region- and subtype-specific human neurons for experimental study (Eiraku et al., 2008; Lee et al., 2007; Li et al., 2005, 2009; Perrier et al., 2004; Schulz et al., 2004). This will require improvement of methods for neural conversion from hPSCs (Chambers et al., 2009; Smith et al., 2008), neural patterning (Patani et al., 2009) and subtype diversification. Currently, the field has achieved directed differentiation to major neuraxial regions. However, the remaining challenge is to predictably generate enriched populations of distinct region-specific neuronal subclasses (Patani et al., 2011). Previous work has shown that differentiated hESC cells can be cleared of un-differentiated or partially-differentiated cells, which retain potential for teratoma formation, by incorporating fluorescent markers under the control of cell-type specific promoters (Chung et al., 2006). Sorting of fluorescent cells substantially enriches the differentiated cells, and it is expected that similar results would be achieved with incorporation of antibiotic selection markers. Such approaches may have important implications for facilitating enrichment of distinct region-specific neuronal sub-classes.

Another important consideration is optimizing injury paradigms so that they more accurately recapitulate the endogenous pathobiology of the disease in question. To date, the proof-of-concept studies have largely focused on developmental disorders (Ebert et al., 2009; Lee et al., 2009; Marchetto et al., 2010). It remains to be established whether adult onset neurogenetic diseases can accurately be modeled using current methodologies, although the aforementioned recent studies modeling schizophrenia and Parkinson's-disease using iPSCs provide reason for cautious optimism (Brennand et al., 2011; Nguyen et al., 2011). Methods of

Box 1

Summary box.

Challenges for designing accurate in-vitro iPS cell model systems for human neurodegenerative disease:

- 1) Fully humanized culture systems, including chemically defined media and feeder-free systems.
- 2) Techniques of generating iPS cells that minimize genetic disruption.
- 3) More accurate iPS cell controls.
- 4) Addressing differential lineage restriction capacities of different iPS lines.
- 5) Differentiation protocols of region- and subtype-specific neurons.
- 6) Paradigms that accurately recapitulate the endogenous pathobiology.
- 7) Genome-wide understanding of transcriptional, epigenetic and post-transcriptional status of iPS cells.

artificially accelerating the ‘age’ of neurons grown in-vitro might be necessary to allow manifestation of the adult onset disease phenotypes. To begin addressing this issue, an effort should be made to compare gene expression of hPSC-derived regionally defined neurons with their foetal and adult human counterparts with high-throughput methodologies such as RNA-sequencing.

Finally, new methodologies will need to be used to enable a better understanding of the functional defects associated with disease states of iPSCs. Use of refined immunocytochemical and electrophysiological methods can provide insights into the connectivity and properties of synaptic transmission in differentiated iPSCs (Brennand et al., 2011). In addition, genome-wide methods that allow mechanistic studies of transcriptional, epigenetic and post-transcriptional regulation in the iPSCs model system will greatly benefit our understanding of disease progression, and in particular the disturbances of RNA processing that have been reported in several neurodegenerative diseases. First steps in this direction have been to compare the RNA targets of TDP-43 in undifferentiated hESCs with those in human postmortem brain tissue (Tollervey et al., 2011).

3. Concluding remarks

Current therapeutic strategies for neurodegenerative diseases focus on symptomatic treatment and have extremely limited scope for slowing or arresting disease progression, let alone restoring structure and function. However, recent advances in hPSC biology have given rise to unprecedented experimental opportunities to study neurodegenerative disease using clinically relevant model systems, and patient-derived iPSCs now offer an unparalleled human system for in-vitro modeling of disease mechanisms and therapeutic strategies. One major challenge facing this area of iPSC translational research is the ability to generate regionally defined and refined neuronal and glial subtypes from pluripotent stem cells in order to create accurate and clinically relevant disease models, although there have been some recent advances in this area (Krencik et al., 2011; Patani et al., 2011). Subsequently proof-of-concept studies have demonstrated the practical feasibility of using both mouse and human in-vitro iPSC model systems to elucidate both cell-autonomous and non-cell-autonomous mechanisms of neurodegeneration (Di Giorgio et al., 2007, 2008; Nagai et al., 2007).

With respect to RNA processing and neurodegenerative disease, hPSCs now represent the ideal in-vitro model system to study the role of post-transcriptional regulatory networks in neuronal function and dysfunction. The utility of novel methodologies to map the perturbed protein–RNA interactions (Konig et al., 2010; Witten and Ule, 2011; Licatalosi et al., 2008) represents an unparalleled experimental opportunity to gain fundamental insights into the molecular mechanisms of neurodegeneration. Importantly, the first steps of applying such high-throughput technologies to hPSCs have already been made and have resulted in informative data about both disease pathology and potential therapeutic strategy (Brennand et al., 2011; Lin et al., 2011). Future studies can now begin to address the functional ‘logic’ of ribonucleoprotein complex composition, which will allow insights into

how RNA-binding proteins and non-coding RNAs conspire or compete in the regulation of RNA processing and RNA localisation in neurons. Such studies will undoubtedly uncover new functional elements in the non-coding regions of the human genome, and thereby explain how polymorphisms in these regions contribute to neurodegenerative diseases.

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REFERENCES

- Abujarour, R., Ding, S., 2009. Induced pluripotent stem cells free of exogenous reprogramming factors. *Genome Biol.* 10 (5), 220.
- Amabile, G., Meissner, A., 2009. Induced pluripotent stem cells: current progress and potential for regenerative medicine. *Trends Mol. Med.* 15 (2), 59–68.
- Amit, M., Margulets, V., et al., 2003. Human feeder layers for human embryonic stem cells. *Biol. Reprod.* 68 (6), 2150–2156.
- Amit, M., Shariki, C., et al., 2004. Feeder layer- and serum-free culture of human embryonic stem cells. *Biol. Reprod.* 70 (3), 837–845.
- Anthony, K., Gallo, J.M., 2010. Aberrant RNA processing events in neurological disorders. *Brain Res.* 1338, 67–77.
- Bachurin, S., Bukatina, E., Lermontova, N., Tkachenko, S., Afanasiev, A., Grigoriev, V., Grigorieva, I., Ivanov, Y., Sablin, S., Zefirov, N., 2001. Antihistamine agent Dimebon as a novel neuroprotector and a cognition enhancer. *Ann. N. Y. Acad. Sci.* 939, 425–435.
- Besselink, M.G., van Santvoort, H.C., et al., 2008. Probiotic prophylaxis in predicted severe acute pancreatitis: a randomised, double-blind, placebo-controlled trial. *Lancet* 371 (9613), 651–659.
- Bezprozvanny, I., 2010. The rise and fall of Dimebon. *Drug News Perspect.* 23 (8), 518–523.
- Bjorklund, A., Dunnett, S.B., 2007. Dopamine neuron systems in the brain: an update. *Trends Neurosci.* 30 (5), 194–202.
- Bock, C., Kiskinis, E., et al., 2011. Reference Maps of human ES and iPS cell variation enable high-throughput characterization of pluripotent cell lines. *Cell* 144 (3), 439–452.
- Bortoff, G.A., Strick, P.L., 1993. Corticospinal terminations in two new-world primates: further evidence that corticomotoneuronal connections provide part of the neural substrate for manual dexterity. *J. Neurosci.* 13 (12), 5105–5118.
- Boutz, P.L., Stoilov, P., Li, Q., Lin, C.H., Chawla, G., Ostrow, K., Shiue, L., Ares Jr., M., Black, D.L., 2007. A post-transcriptional regulatory switch in polypyrimidine tract-binding proteins reprograms alternative splicing in developing neurons. *Genes Dev.* 21 (13), 1636–1652.
- Brennand, K.J., Simone, A., et al., 2011. Modelling schizophrenia using human induced pluripotent stem cells. *Nature* 473 (7346), 221–225.
- Campbell, K.H., McWhir, J., et al., 1996. Sheep cloned by nuclear transfer from a cultured cell line. *Nature* 380 (6569), 64–66.
- Cartegni, L., Hastings, M.L., et al., 2006. Determinants of exon 7 splicing in the spinal muscular atrophy genes, SMN1 and SMN2. *Am. J. Hum. Genet.* 78 (1), 63–77.
- Cathomen, T., Schambach, A., 2010. Zinc-finger nucleases meet iPSC cells: zinc positive: tailored genome engineering meets reprogramming. *Gene Ther.* 17 (1), 1–3.

- Chambers, S.M., Fasano, C.A., et al., 2009. Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling. *Nat. Biotechnol.* 27 (3), 275–280.
- Chin, M.H., Mason, M.J., et al., 2009. Induced pluripotent stem cells and embryonic stem cells are distinguished by gene expression signatures. *Cell Stem Cell* 5 (1), 111–123.
- Chung, S., Shin, B.S., Hedlund, E., Pruszkak, J., Ferree, A., Kang, U.J., Isacson, O., Kim, K.S., 2006. Genetic selection of sox1GFP-expressing neural precursors removes residual tumorigenic pluripotent stem cells and attenuates tumor formation after transplantation. *J. Neurochem.* 97 (5), 1467–1480.
- Courtine, G., Roy, R.R., et al., 2005. Performance of locomotion and foot grasping following a unilateral thoracic corticospinal tract lesion in monkeys (*Macaca mulatta*). *Brain* 128 (Pt 10), 2338–2358.
- Courtine, G., Bunge, M.B., et al., 2007. Can experiments in nonhuman primates expedite the translation of treatments for spinal cord injury in humans? *Nat. Med.* 13 (5), 561–566.
- Cowan, C.A., Atienza, J., et al., 2005. Nuclear reprogramming of somatic cells after fusion with human embryonic stem cells. *Science* 309 (5739), 1369–1373.
- Dahlstrom, A., Fuxe, K., 1964. Localization of monoamines in the lower brain stem. *Experientia* 20 (7), 398–399.
- DeJesus-Hernandez, M., Mackenzie, I.R., et al., 2011. Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. *Neuron* 72 (2), 245–256.
- Dermitzakis, E.T., Clark, A.G., 2002. Evolution of transcription factor binding sites in Mammalian gene regulatory regions: conservation and turnover. *Mol. Biol. Evol.* 19 (7), 1114–1121.
- Di Giorgio, F.P., Carrasco, M.A., et al., 2007. Non-cell autonomous effect of glia on motor neurons in an embryonic stem cell-based ALS model. *Nat. Neurosci.* 10 (5), 608–614.
- Di Giorgio, F.P., Boulting, G.L., et al., 2008. Human embryonic stem cell-derived motor neurons are sensitive to the toxic effect of glial cells carrying an ALS-causing mutation. *Cell Stem Cell* 3 (6), 637–648.
- Dirksen, M.T., Laarman, G.J., et al., 2007. Reperfusion injury in humans: a review of clinical trials on reperfusion injury inhibitory strategies. *Cardiovasc. Res.* 74 (3), 343–355.
- Disset, A., Bourgeois, C.F., et al., 2006. An exon skipping-associated nonsense mutation in the dystrophin gene uncovers a complex interplay between multiple antagonistic splicing elements. *Hum. Mol. Genet.* 15 (6), 999–1013.
- Ebert, A.D., Yu, J., et al., 2009. Induced pluripotent stem cells from a spinal muscular atrophy patient. *Nature* 457 (7227), 277–280.
- Eiraku, M., Watanabe, K., et al., 2008. Self-organized formation of polarized cortical tissues from ESCs and its active manipulation by extrinsic signals. *Cell Stem Cell* 3 (5), 519–532.
- Eisen, A., 2009. Amyotrophic lateral sclerosis—Evolutionary and other perspectives. *Muscle Nerve* 40 (2), 297–304.
- Evans, M.J., Kaufman, M.H., 1981. Establishment in culture of pluripotential cells from mouse embryos. *Nature* 292 (5819), 154–156.
- Freund, P., Schmidlin, E., et al., 2006. Nogo-A-specific antibody treatment enhances sprouting and functional recovery after cervical lesion in adult primates. *Nat. Med.* 12 (7), 790–792.
- Frugier, T., Tiziano, F.D., et al., 2000. Nuclear targeting defect of SMN lacking the C-terminus in a mouse model of spinal muscular atrophy. *Hum. Mol. Genet.* 9 (5), 849–858.
- Gonzalez, F., Boue, S., et al., 2011. Methods for making induced pluripotent stem cells: reprogramming a la carte. *Nat. Rev. Genet.* 12 (4), 231–242.
- Gurdon, J.B., Elsdale, T.R., et al., 1958. Sexually mature individuals of *Xenopus laevis* from the transplantation of single somatic nuclei. *Nature* 182 (4627), 64–65.
- Han, S.S., Williams, L.A., et al., 2011. Constructing and deconstructing stem cell models of neurological disease. *Neuron* 70 (4), 626–644.
- Hardingham, G.E., Patani, R., et al., 2010. Human embryonic stem cell-derived neurons as a tool for studying neuroprotection and neurodegeneration. *Mol. Neurobiol.* 42 (1), 97–102.
- Hu, B.Y., Weick, J.P., et al., 2010. Neural differentiation of human induced pluripotent stem cells follows developmental principles but with variable potency. *Proc. Natl. Acad. Sci. U. S. A.* 107 (9), 4335–4340.
- Jia, F., Wilson, K.D., Sun, N., Gupta, D.M., Huang, M., Li, Z., Panetta, N.J., Chen, Z.Y., Robbins, R.C., Kay, M.A., Longaker, M.T., Wu, J.C., 2010. A nonviral minicircle vector for deriving human iPS cells. *Nat. Methods* 7 (3), 197–199.
- Jin, P., Duan, R., et al., 2007. Pur alpha binds to rCGG repeats and modulates repeat-mediated neurodegeneration in a *Drosophila* model of fragile X tremor/ataxia syndrome. *Neuron* 55 (4), 556–564.
- Joannides, A.J., Fiore-Herich, C., et al., 2007. A scaleable and defined system for generating neural stem cells from human embryonic stem cells. *Stem Cells* 25 (3), 731–737.
- Konig, J., Zarnack, K., et al., 2010. iCLIP reveals the function of hnRNP particles in splicing at individual nucleotide resolution. *Nat. Struct. Mol. Biol.* 17 (7), 909–915.
- Krencik, R., Weick, J.P., et al., 2011. Specification of transplantable astroglial subtypes from human pluripotent stem cells. *Nat. Biotechnol.* 29 (6), 528–534.
- Kuypers, H.G., 1964. The descending pathways to the spinal cord, their anatomy and function. *Prog. Brain Res.* 11, 178–202.
- Lawrence, D.G., Kuypers, H.G., 1968a. The functional organization of the motor system in the monkey. I. The effects of bilateral pyramidal lesions. *Brain* 91 (1), 1–14.
- Lawrence, D.G., Kuypers, H.G., 1968b. The functional organization of the motor system in the monkey. II. The effects of lesions of the descending brain-stem pathways. *Brain* 91 (1), 15–36.
- Lee, H., Shamy, G.A., et al., 2007. Directed differentiation and transplantation of human embryonic stem cell-derived motoneurons. *Stem Cells* 25 (8), 1931–1939.
- Lee, G., Papapetrou, E.P., et al., 2009. Modelling pathogenesis and treatment of familial dysautonomia using patient-specific iPSCs. *Nature* 461 (7262), 402–406.
- Lemon, R.N., Griffiths, J., 2005. Comparing the function of the corticospinal system in different species: organizational differences for motor specialization? *Muscle Nerve* 32 (3), 261–279.
- Lemon, R.N., Kirkwood, P.A., et al., 2004. Direct and indirect pathways for corticospinal control of upper limb motoneurons in the primate. *Prog. Brain Res.* 143, 263–279.
- Li, X.J., Du, Z.W., et al., 2005. Specification of motoneurons from human embryonic stem cells. *Nat. Biotechnol.* 23 (2), 215–221.
- Li, X.J., Zhang, X., et al., 2009. Coordination of sonic hedgehog and Wnt signaling determines ventral and dorsal telencephalic neuron types from human embryonic stem cells. *Development* 136 (23), 4055–4063.
- Licatalosi, D.D., Mele, A., Fak, J.J., Ule, J., Kayikci, M., Chi, S.W., Clark, T.A., Schweitzer, A.C., Blume, J.E., Wang, X., Darnell, J.C., Darnell, R.B., 2008. HITS-CLIP yields genome-wide insights into brain alternative RNA processing. *Nature* 456 (7221), 464–469.
- Lin, T., Ambasudhan, R., et al., 2009. A chemical platform for improved induction of human iPSCs. *Nat. Methods* 6 (11), 805–808.
- Lin, M., Pedrosa, E., Shah, A., Hrabovsky, A., Maqbool, S., Zheng, D., Lachman, H.M., 2011. RNA-Seq of human neurons derived from iPS cells reveals candidate long non-coding RNAs involved in neurogenesis and neuropsychiatric disorders. *PLoS One* 6 (9), e23356.
- Liquori, C.L., Ricker, K., et al., 2001. Myotonic dystrophy type 2 caused by a CCTG expansion in intron 1 of ZNF9. *Science* 293 (5531), 864–867.

- Lister, R., Pelizzola, M., et al., 2011. Hotspots of aberrant epigenomic reprogramming in human induced pluripotent stem cells. *Nature* 471 (7336), 68–73.
- Liu, F., Gong, C.X., 2008. Tau exon 10 alternative splicing and tauopathies. *Mol. Neurodegener.* 3, 8.
- Lukong, K.E., Chang, K.W., et al., 2008. RNA-binding proteins in human genetic disease. *Trends Genet.* 24 (8), 416–425.
- Lynch, M., Conery, J.S., 2003. The origins of genome complexity. *Science* 302 (5649), 1401–1404.
- Maherali, N., Hochedlinger, K., 2009. Tgfbeta signal inhibition cooperates in the induction of iPSCs and replaces Sox2 and cMyc. *Curr. Biol.* 19 (20), 1718–1723.
- Makeyev, E.V., Zhang, J., Carrasco, M.A., Maniatis, T., 2007. The MicroRNA miR-124 promotes neuronal differentiation by triggering brain-specific alternative pre-mRNA splicing. *Mol. Cell* 27 (3), 435–448.
- Maniatis, T., Reed, R., 2002. An extensive network of coupling among gene expression machines. *Nature* 416 (6880), 499–506.
- Marchetto, M.C., Carromeu, C., et al., 2010. A model for neural development and treatment of Rett syndrome using human induced pluripotent stem cells. *Cell* 143 (4), 527–539.
- Marshall, J.W., Cummings, R.M., Bowes, L.J., Ridley, R.M., Green, A.R., 2003. Functional and histological evidence for the protective effect of NXY-059 in a primate model of stroke when given 4 hours after occlusion. *Stroke* 34 (9), 2228–2233.
- Miyoshi, N., Ishii, H., Nagano, H., Haraguchi, N., Dewi, D.L., Kano, Y., Nishikawa, S., Tanemura, M., Mimori, K., Tanaka, F., Saito, T., Nishimura, J., Takemasa, I., Mizushima, T., Ikeda, M., Yamamoto, H., Sekimoto, M., Doki, Y., Mori, M., 2011. Reprogramming of mouse and human cells to pluripotency using mature microRNAs. *Cell Stem Cell* 8 (6), 633–638.
- Monani, U.R., Sendtner, M., et al., 2000. The human centromeric survival motor neuron gene (SMN2) rescues embryonic lethality in *Smn(-/-)* mice and results in a mouse with spinal muscular atrophy. *Hum. Mol. Genet.* 9 (3), 333–339.
- Muir, G.D., Whishaw, I.Q., 1999. Complete locomotor recovery following corticospinal tract lesions: measurement of ground reaction forces during overground locomotion in rats. *Behav. Brain Res.* 103 (1), 45–53.
- Nagai, M., Re, D.B., et al., 2007. Astrocytes expressing ALS-linked mutated SOD1 release factors selectively toxic to motor neurons. *Nat. Neurosci.* 10 (5), 615–622.
- Nakagawa, M., Koyanagi, M., Tanabe, K., Takahashi, K., Ichisaka, T., Aoi, T., Okita, K., Mochiduki, Y., Takizawa, N., Yamanaka, S., 2008. Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. *Nat. Biotechnol.* 26 (1), 101–106.
- Nakagawa, M., Takizawa, N., et al., 2010. Promotion of direct reprogramming by transcription-deficient Myc. *Proc. Natl. Acad. Sci. U. S. A.* 107 (32), 14152–14157.
- Nakagawa, S., Naganuma, T., Shioi, G., Hirose, T., 2011. Paraspeckles are subpopulation-specific nuclear bodies that are not essential in mice. *J. Cell Biol.* 4 (193(1)), 31–39.
- Nathan, P.W., 1994. Effects on movement of surgical incisions into the human spinal cord. *Brain* 117 (Pt 2), 337–346.
- Nemes, J.P., Benzow, K.A., et al., 2000. The SCA8 transcript is an antisense RNA to a brain-specific transcript encoding a novel actin-binding protein (KLHL1). *Hum. Mol. Genet.* 9 (10), 1543–1551.
- Nguyen, H.N., Byers, B., et al., 2011. LRRK2 mutant iPSC-derived DA neurons demonstrate increased susceptibility to oxidative stress. *Cell Stem Cell* 8 (3), 267–280.
- Nilsen, T.W., 2003. The spliceosome: the most complex macromolecular machine in the cell? *Bioessays* 25 (12), 1147–1149.
- Pan, Q., Shai, O., et al., 2008. Deep surveying of alternative splicing complexity in the human transcriptome by high-throughput sequencing. *Nat. Genet.* 40 (12), 1413–1415.
- Papadia, S., Soriano, F.X., et al., 2008. Synaptic NMDA receptor activity boosts intrinsic antioxidant defenses. *Nat. Neurosci.* 11 (4), 476–487.
- Patani, R., Compston, A., et al., 2009. Activin/Nodal inhibition alone accelerates highly efficient neural conversion from human embryonic stem cells and imposes a caudal positional identity. *PLoS One* 4 (10), e7327.
- Patani, R., Hollins, A.J., et al., 2011. Retinoid-independent motor neurogenesis from human embryonic stem cells reveals a medial columnar ground state. *Nat. Commun.* 2, 214.
- Perrier, A.L., Tabar, V., et al., 2004. Derivation of midbrain dopamine neurons from human embryonic stem cells. *Proc. Natl. Acad. Sci. U. S. A.* 101 (34), 12543–12548.
- Renton, A.E., Majounie, E., et al., 2011. A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. *Neuron* 72 (2), 257–268.
- Rouiller, E.M., Moret, V., et al., 1996. Evidence for direct connections between the hand region of the supplementary motor area and cervical motoneurons in the macaque monkey. *Eur. J. Neurosci.* 8 (5), 1055–1059.
- Sanchez-Danes, A., Consiglio, A., et al., 2011. Efficient generation of A9 midbrain dopaminergic neurons by lentiviral delivery of LMX1A in human embryonic stem cells and induced pluripotent stem cells. *Hum. Gene Ther.* Nov 17. [Epub ahead of print].
- Schmidlin, E., Wannier, T., et al., 2004. Progressive plastic changes in the hand representation of the primary motor cortex parallel incomplete recovery from a unilateral section of the corticospinal tract at cervical level in monkeys. *Brain Res.* 1017 (1–2), 172–183.
- Schulz, T.C., Noggle, S.A., et al., 2004. Differentiation of human embryonic stem cells to dopaminergic neurons in serum-free suspension culture. *Stem Cells* 22 (7), 1218–1238.
- Sebastiano, V., Maeder, M.L., et al., 2011. In situ genetic correction of the sickle cell anemia mutation in human induced pluripotent stem cells using engineered zinc finger nucleases. *Stem Cells* 29 (11), 1717–1726.
- Shuaib, A., Lees, K.R., et al., 2007. NXY-059 for the treatment of acute ischemic stroke. *N. Engl. J. Med.* 357 (6), 562–571.
- Smith, J.R., Vallier, L., et al., 2008. Inhibition of Activin/Nodal signaling promotes specification of human embryonic stem cells into neuroectoderm. *Dev. Biol.* 313 (1), 107–117.
- Sreedharan, J., Blair, I.P., et al., 2008. TDP-43 mutations in familial and sporadic amyotrophic lateral sclerosis. *Science* 319 (5870), 1668–1672.
- Stojkovic, P., Lako, M., et al., 2005a. Human-serum matrix supports undifferentiated growth of human embryonic stem cells. *Stem Cells* 23 (7), 895–902.
- Stojkovic, P., Lako, M., et al., 2005b. An autogeneic feeder cell system that efficiently supports growth of undifferentiated human embryonic stem cells. *Stem Cells* 23 (3), 306–314.
- Takahashi, K., Yamanaka, S., 2006. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126 (4), 663–676.
- Takahashi, K., Tanabe, K., et al., 2007. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 131 (5), 861–872.
- Talbot, K., Davies, K.E., 2001. Spinal muscular atrophy. *Semin. Neurol.* 21 (2), 189–197.
- Thomson, J.A., Itskovitz-Eldor, J., et al., 1998. Embryonic stem cell lines derived from human blastocysts. *Science* 282 (5391), 1145–1147.
- Timchenko, L.T., Timchenko, N.A., et al., 1996. Novel proteins with binding specificity for DNA CTG repeats and RNA CUG repeats: implications for myotonic dystrophy. *Hum. Mol. Genet.* 5 (1), 115–121.
- Tollervey, J.R., Curk, T., et al., 2011. Characterizing the RNA targets and position-dependent splicing regulation by TDP-43. *Nat. Neurosci.* 14 (4), 452–458.
- Tsukiyama, T., Asano, R., Kawaguchi, T., Kim, N., Yamada, M., Minami, N., Ohinata, Y., Imai, H., 2011. Simple and efficient method for generation of induced pluripotent stem cells using

- piggyBac transposition of doxycycline-inducible factors and an EOS reporter system. *Genes Cells* 16 (7), 815–825.
- Tucker, B.A., Scheetz, T.E., Mullins, R.F., DeLuca, A.P., Hoffmann, J.M., Johnston, R.M., Jacobson, S.G., Sheffield, V.C., Stone, E.M., 2011. Exome sequencing and analysis of induced pluripotent stem cells identify the cilia-related gene *male germ cell-associated kinase (MAK)* as a cause of retinitis pigmentosa. *Proc. Natl. Acad. Sci. U. S. A.* 23 (108(34)), E569–E576.
- Tuszynski, M.H., Grill, R., et al., 2002. Spontaneous and augmented growth of axons in the primate spinal cord: effects of local injury and nerve growth factor-secreting cell grafts. *J. Comp. Neurol.* 449 (1), 88–101.
- Ule, J., Ule, A., Spencer, J., Williams, A., Hu, J.S., Cline, M., Wang, H., Clark, T., Fraser, C., Ruggiu, M., Zeeberg, B.R., Kane, D., Weinstein, J.N., Blume, J., Darnell, R.B., 2005. Nova regulates brain-specific splicing to shape the synapse. *Nat. Genet.* 37 (8), 844–852.
- Vallier, L., Pedersen, R., 2008. Differentiation of human embryonic stem cells in adherent and in chemically defined culture conditions. *Curr. Protoc. Stem Cell Biol.* (Chapter 1: Unit 1D 4 1-1D 4 7).
- Vance, C., Rogelj, B., et al., 2009. Mutations in *FUS*, an RNA processing protein, cause familial amyotrophic lateral sclerosis type 6. *Science* 323 (5918), 1208–1211.
- Wang, Y.H., Griffith, J., 1995. Expanded CTG triplet blocks from the myotonic dystrophy gene create the strongest known natural nucleosome positioning elements. *Genomics* 25 (2), 570–573.
- Wang, J., Pegoraro, E., et al., 1995. Myotonic dystrophy: evidence for a possible dominant-negative RNA mutation. *Hum. Mol. Genet.* 4 (4), 599–606.
- Wang, G.S., Kearney, D.L., et al., 2007. Elevation of RNA-binding protein *CUGBP1* is an early event in an inducible heart-specific mouse model of myotonic dystrophy. *J. Clin. Invest.* 117 (10), 2802–2811.
- Wang, E.T., Sandberg, R., et al., 2008. Alternative isoform regulation in human tissue transcriptomes. *Nature* 456 (7221), 470–476.
- Warren, L., Manos, P.D., et al., 2010. Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. *Cell Stem Cell* 7 (5), 618–630.
- Wichterle, H., Przedborski, S., 2010. What can pluripotent stem cells teach us about neurodegenerative diseases? *Nat. Neurosci.* 13 (7), 800–804.
- Will, C.L., Luhrmann, R., 2001. Spliceosomal UsnRNP biogenesis, structure and function. *Curr. Opin. Cell Biol.* 13 (3), 290–301.
- Wirth, B., Brichta, L., et al., 2006a. Spinal muscular atrophy and therapeutic prospects. *Prog. Mol. Subcell. Biol.* 44, 109–132.
- Wirth, B., Brichta, L., et al., 2006b. Spinal muscular atrophy: from gene to therapy. *Semin. Pediatr. Neurol.* 13 (2), 121–131.
- Witten, J.T., Ule, J., 2011. Understanding splicing regulation through RNA splicing maps. *Trends Genet.* 27 (3), 89–97.
- Xu, C., Inokuma, M.S., et al., 2001. Feeder-free growth of undifferentiated human embryonic stem cells. *Nat. Biotechnol.* 19 (10), 971–974.
- Yao, S., Chen, S., et al., 2006. Long-term self-renewal and directed differentiation of human embryonic stem cells in chemically defined conditions. *Proc. Natl. Acad. Sci. U. S. A.* 103 (18), 6907–6912.
- Yellon, D.M., Hausenloy, D.J., 2007. Myocardial reperfusion injury. *N. Engl. J. Med.* 357 (11), 1121–1135.
- Zhou, H., Wu, S., et al., 2009. Generation of induced pluripotent stem cells using recombinant proteins. *Cell Stem Cell* 4 (5), 381–384.
- Zhang, S.C., 2006. Neural subtype specification from embryonic stem cells. *Brain Pathol.* 16 (2), 132–142.
- Zou, J., Maeder, M.L., et al., 2009. Gene targeting of a disease-related gene in human induced pluripotent stem and embryonic stem cells. *Cell Stem Cell* 5 (1), 97–110.
- Zou, J., Mali, P., et al., 2011a. Site-specific gene correction of a point mutation in human iPS cells derived from an adult patient with sickle cell disease. *Blood* 118 (17), 4599–608.
- Zou, J., Sweeney, C.L., et al., 2011b. Oxidase-deficient neutrophils from X-linked chronic granulomatous disease iPS cells: functional correction by zinc finger nuclease-mediated safe harbor targeting. *Blood* 117 (21), 5561–5572.