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Stem Cells as a Cure For Amyotrophic Lateral Sclerosis

Chaya K. Hirsch

Abstract

Amyotrophic Lateral Sclerosis (ALS) is a fatal motor neuron disease which affects approximately 30,000 Americans at any given time (alsa.org, 2010). The etiology of this terminal disease unfortunately remains an unsolved mystery and has therefore severely limited the ability to find a cure. The use of stem cells to regenerate neurons has been vastly studied and have produced very promising results. However, its practicality as a cure or treatment for neurodegenerative diseases, such as ALS, is greatly compromised. Three different therapies involving stem cells were examined, Embryonic Stem Cells (ESC), induced pluripotent stem cells, (iPSC) and direct reprogramming of adult stem cells into motor neural cells, and their advantages and limitations discussed. While ESC, iPSC and induced motor neural cells (iMNC) may have astonishing potential as a treatment they also have severe limitations ethically, clinically and effectively.

Introduction

Amyotrophic Lateral Sclerosis (ALS), commonly known as “Lou Gehrig’s Disease,” is a fatal neurodegenerative disorder characterized by the slow degeneration of the motor neurons (MNs) of the entire human body, including, but not limited to, the MNs of the cerebral cortex, spinal cord and limb, axial and respiratory muscles. ALS is an adult-onset, rapidly progressive disease, killing 50% of patients within 2 and a half years of symptom onset. There are two types of disease onset, Limb-onset, which is characterized by degeneration of upper and lower motor neurons (UMN and LMN) identified by weakness, fasciculations and spasticity of the limbs, and Bulbar-onset, degeneration of tongue muscles causing weakness and fasciculations in the tongue as well as spastic dysarthria and dysphagia. Eventually, no matter the type of onset, the motor neuron degeneration spreads throughout the body resulting in paralysis of the limbs and eventually the diaphragm, causing respiratory distress and ultimately death (Kiernan, et al. 2011).

ALS has been categorized into two classes, familial ALS, which is genetic and presented through a Mendelian pattern of inheritance, accounts for 5-10% of people with ALS, and Sporadic ALS which accounts for the remaining 90% (Kiernan, et al. 2011).

At present, the etiology remains complex and unresolved and has confounded scientists over the century.

As a result, finding a treatment for ALS has proved to be extremely challenging. The etiology seems to be multifaceted with many genetic and molecular factors contributing directly and indirectly to the degeneration of motor neurons, including gene mutations, neurotoxins, glutamate-induced excitotoxicity, and structural abnormalities of the mitochondrion, sodium potassium pump and axonal structures. Mutations in SOD1 gene generates a toxic gain of function of SOD1 enzyme, formation of free radicals and formation of aggregates due to improper folding of the SOD1 protein, resulting in impaired motor neuron function and neuron death (Kiernan, et al. 2011). The SOD1 gene, which accounts for 20% of familial ALS, is of significant importance because it can transduce mice into effective ALS models for stem cell therapy and other research (Gurney, et al. 1994).

To date, only one drug, Riluzole, remains effective in treating ALS symptoms, by inhibiting glutamate receptors and slowing the disease progression by 3-6 months. This unfortunate lack of a significant cure or treatment may be a result of the inadequacy of diagnostic tests. By the time a patient is diagnosed with ALS, many of their motor neurons have already died, and their muscles atrophied. Since ALS can only be diagnosed once clinical symptoms appear, there is no early detection and therefore, no early prevention (Kiernan, et al. 2011).

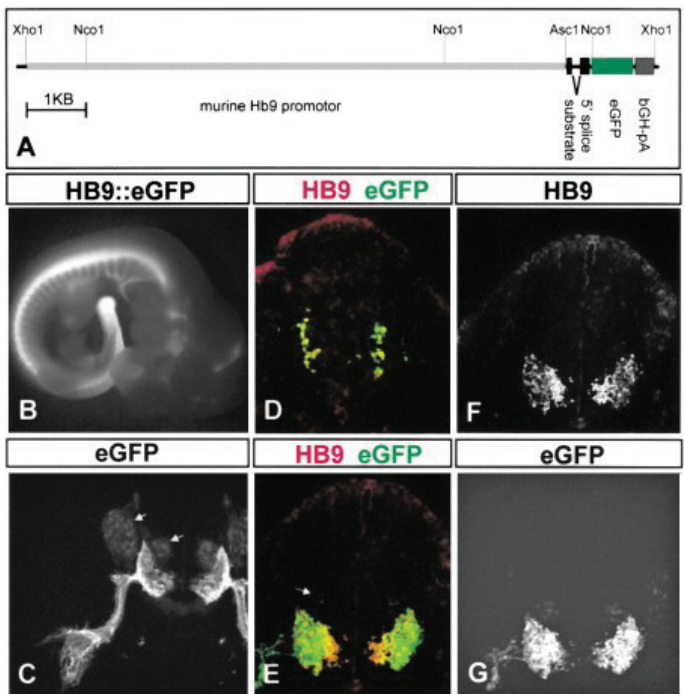
Stem cells, however, provide an astonishing and

effective alternative. They can accomplish what Riluzole cannot, providing hope for a future regenerative cure for neurodegenerative diseases. Stem cells are cells capable of giving rise to new cells of the same type or to different cell types through the process of differentiation. Modern research has discovered that stem cells can be induced into neurons, which can regenerate neurons, reestablish neural connections and protect the remaining neurons from further degeneration when transplanted into mouse or human ALS models and patients (Miles, et al. 2004; Nizzardo, et al. 2013; Peljto, et al. 2010; Son, et al. 2011; Takahashi, Yamanka, 2006; Takahashi, et al. 2007; Umbach, et al. 2012; Vierbuchen, et al. 2010; Wichterle, et al. 2002; Xu, et al. 2009; Yao, et al. 2013) If the safety and efficiency of stem cells can be determined, the progression of ALS can be halted and reversed, rather than just slowed down. Stem cells derived from embryos, called Embryonic Stem cells (ESC) can differentiate in to the three germ layers, ectoderm, mesoderm and endoderm and then be forced through specialized transcription factors or signals to differentiate into functioning neurons in vitro and in vivo (Miles, et al. 2004; Peljto, et al. 2010; Umbach, et al. 2012; Wichterle, et al. 2002). However, ESCs provide an ethical issue because the embryo is sacrificed in exchange for stem cells. Therefor a modern approach was created by inducing differentiated stem cells, including post-natal and adult stem cells, into a pluripotent state, which can then be induced into neurons and other cells through transcription factors with ESC-like efficiency (Nizzardo, et al. 2013; Takahashi, Yamanka, 2006; Takahashi, et al. 2007; Yao, et al. 2013). However, the induction of a stem cell into a pluripotent state requires the use of oncogenes and tumorigenic factors which can be preserved in the induced neuron state. These factors can produce malignant tumors in vivo, a challenging clinical hurdle to overcome (Lee, et al. 2013). The most recent research has been devoted to directly converting stem cells into neurons, skipping the pluripotent state and avoiding the formation of potentially malignant neoplasms (Son, et al. 2011; Vierbuchen, et al. 2010). These three approaches each have advantages and limitations, which will be discussed in further detail, and provide a promising hope for a future cure for ALS.

Embryonic Stem Cells

To begin the process of generating motor neurons from ESC we must understand the developmental stages and the signaling characteristics of motor neurons from the ectoderm. Ectodermal cells develop into a neural progenitor state through the regulating signals, bone morphogenetic protein (BMP), fibroblast growth factor (FGF) and Wnt. These signals play a vital role during development of neural progenitor cells by regulating cell-to-cell interactions and controlling the activation or deactivation of specific genes (Wilson, Edlund, 2001). These neural progenitor cells, which are characterized by the expression of neural biomarkers, Neuronal Nuclei (NeuN), Sex determining region Y- box 1(Sox1), and Neuronal Class III β -Tubulin (TuJ1), can then differentiate into all classifications of neurons through the help of specific transcription factors and signals. Through the action of Sonic hedgehog (Shh) signaling these neural progenitor cells are transformed to a progenitor motor neuron (pMN) domain, which are destined to the fate of spinal motor neurons. These cells in the pMN domain are characterized by the expression of Pax6, Nkx6.1 and Olig2 proteins which each have a role in the differentiation of various spinal motor neurons (Wichterle, et al. 2002). Additionally, these factors direct the expression of Hb9, a transcription factor found in MNs.

Figure 1: A: Insertion of green fluorescent protein (eGFP) in the Hb9 promoter. B-G: eGFP expression in an embryonic mouse,



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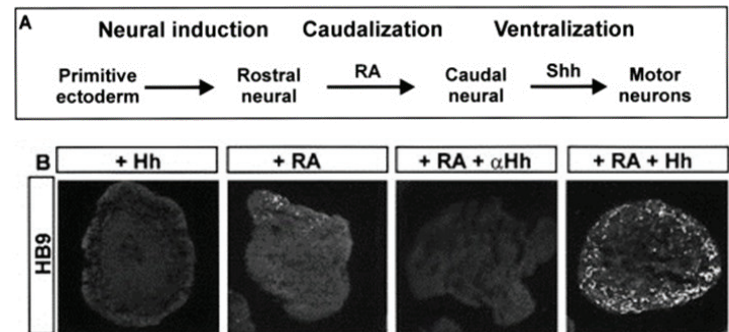
indicative of Hb9 transcription factor and presence of spinal motor neurons (source Wichterle, et al. 2002).

Hb9 is commonly used to identify motor neuron presence in vivo and in vitro research, through the genetic insertion of Green Fluorescent Protein (GFP) in the Hb9 promoter site (Figure 1). The GFP will be expressed and visually viewed if Hb9 is expressed, a factor indicative of motor neuron presence (Wichterle, et al. 2002).

Wichterle et al. began the transformation of ESC into motor neurons by isolating ESCs from a mouse embryo and growing them in culture to produce embryoid bodies (EBs). These EBs produced very few of the neural progenitor state factors, Sox1, NeuN and TuJ1. In contrast, when retinoic acid (RA) was added to the culture, many neurons were detected, evident by Sox1, NeuN and TuJ1 expression. To determine whether RA exposed EBs can be generated into motor neurons specifically, the expression of pMN domain proteins, Pax6, Nkx6.1 and Olig2, was monitored. Few of these proteins were detected and these cells continued to differentiate mostly into interneurons, instead of motor neurons, demonstrating that RA alone cannot sufficiently induce motor neurons. Therefore, Shh was added to RA-exposed EBs, to generate pMN domain cells. After the addition of Shh, expression of Pax6, Nkx6.1 and Olig2 proteins and Hb9 transcription factor was observed, indicating the development of motor neurons. To further prove the involvement and necessity of Shh, a function blocking anti-Hh antibody, which impairs the function of Shh, was added to the RA-exposed EBs. No Hb9 was observed under these conditions. However, when increased levels of Shh were added, the amount of Hb9 increased significantly, in a “concentration dependent manner (Wichterle, et al. 2002),” indicating a RA and Shh dependent motor neuronal pathway (Figure 2).

Figure 2: **A:** generation of motor neurons from the ectoderm of an embryo, requiring the involvement of RA and Shh **B:** expression of Hb9 in ESC exposed to: Shh alone, RA alone, RA with function blocking Hh antibody and RA with Shh. Motor neurons, indicated by white dots, are found in minor amount in RA only culture and in abundance in RA and Shh culture. Absence of motor neurons occur in the absence of RA and in the presence of function blocking Hh antibody. **C:** quantity of GFP/Hb9 cells

grown in RA and different quantities of Shh. -1000 refers to cells grown without RA. (Source: Wichterle, et al. 2002).



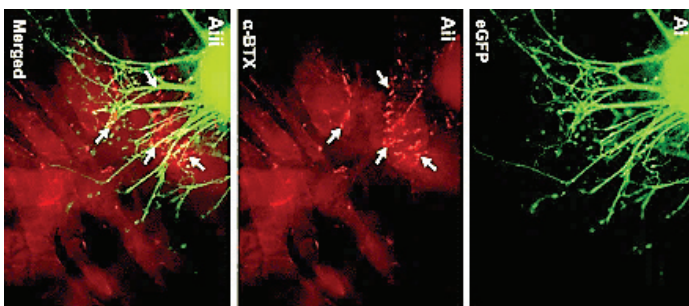
Survival and differentiation of these induced motor neurons was tested in the spinal cord of a mouse in vivo. ESC-derived motor neurons were grafted onto a mouse spinal cord. After 3 days, GFP was detected in the ventral regions of the spinal cord, indicating the ability of these induced motor neurons to survive and differentiate in a living model (Wichterle, et al. 2002). The positioning, trajectory and extension of ESC-derived motor neurons in vivo was then compared with normal motor neuron behavior in embryos. Normal extension along the ventral roots of the spinal cord was detected by use of GFP. Additionally, the motor neurons extended to appropriate muscles, depending on where it was grafted, cervical, thoracic and lumbar segments of the cord (Wichterle, et al. 2002). In another study, two types of ESC-derived MNs, median columnar motor neurons (MMC), which are normally found medially in the spinal cord and innervate axial muscles, and lateral columnar motor neurons (LMC), which are positioned laterally and innervate limb muscles, were mixed together and transplanted in the spinal cord of a mouse. MMC and LMC were found to aggregate in their appropriate medial and lateral regions, next to other motor neurons of their type. In addition to settling in their appropriate regions, the MNs projected along the appropriate pathways, the LMC extending to the limb musculature and the MMC extending to axial musculature (Figure 3) (Peljto, et al. 2010). This implies that ESC-derived MNs can populate all levels of the spinal cord and project along the proper trajectories to specified muscles in vivo.

These studies demonstrate the possibility of generating motor neurons from ESCs and their ability to posi-

tion and project correctly in a host spinal cord. The question still remains if these motor neurons can function as normal motor neurons. In order to use stem cell therapy to treat neurodegenerative disorders, ESC-derived MNs must be able to establish connections, release neurotransmitters, respond to neurotransmitters, and generate action potentials.

MNs containing GFP were prepared through ESC exposure to RA and Shh. Several tests were then conducted to assess MN properties. To determine whether the MNs can connect to muscles, the ESC-derived MNs were placed with myotubes, muscle progenitor cells found in embryo. After 1 day GFP-labeled MNs extended across the myotubes. Additionally, greater than 95% of the GFP labeled MNs that migrated onto the myotubes, expressed ChAT, an enzyme involved in the formation of the neurotransmitter, acetylcholine, indicating the possible ability of the MNs to release cholinergic neurotransmitters (Wichterle, et al. 2002). To establish whether the MNs can send functional neurotransmitters and thereby form a functional connection with muscles, the MNs were cultured together with myotubes and analyzed. After one day, acetylcholine (ACh) receptors, which take up ACh to stimulate muscle contraction, were found on myotubes adjacent to the axons of the ESC-derived MNs (figure 4). These ACh receptors were not observed in the absence of these motor neurons, indicating the ability of ESC-derived MNs to form functional connections with muscles (Miles, et al. 2004).

Figure 4: Ai: axons of GFP-ESC-derived MN. Aii: ACh receptors on myotubes (arrows). Aiii: merged picture of axon and ACh receptor. (Source Miles, et al. 2004).



The effect of neurotransmitters on the ESC-derived MNs was then tested to demonstrate whether GABA, glu-

tamatergic and glycine receptors were present on the MNs and whether their corresponding neurotransmitters can generate a post synaptic action potential on the motor neuron. GABA and glycine, are characteristically inhibitory neurotransmitters in the mature state, however in the embryonic and early postnatal stages they act as excitatory neurotransmitters. Glutamate is an excitatory neurotransmitter which normally generates action potentials in motor neurons. Voltage clamp recording was used to read the membrane potential as the experiment was conducted. In all 3 cases action potentials in the MN membranes were generated when bathed in the neurotransmitters. GABA, glycine and glutamate generated an excitatory post synaptic potential typical of embryonic MNs, demonstrating the ability of these ESC-derived MNs to respond appropriately to neurotransmitters (Miles, et al. 2004).

Next, the possibility of generating multiple trains of action potentials was determined. It seems that the action potential development of ESC-derived MNs develop similarly to MNs in embryo. In early embryonic stages only single trains of action potentials can be fired. In later stages, however, repetitive trains of action potentials can be generated. Similar results were found in ESC-derived MNs, using a current-clamp technique to insert pulses of current. During the first 3 days in culture, only single action potentials could be induced. By the third day, however, repetitive trains of action potentials were detected in 52 out of 61 ESC-derived MNs (See Figure 6) (Miles, et al. 2004).

These findings indicate the possibility of generating motor neurons from embryonic stem cells and the ability of these ESC-derived MNs to function as normal motor neurons. Although these findings are remarkable, there is a strong controversy regarding the source of the stem cells, since human embryos have to be sacrificed to apply this therapy to humans. Studies were conducted to determine whether a less controversial method of inducing motor neurons could be employed. The primary alternative method discovered was the induction of pluripotency of a differentiated, non-embryonic stem cell.

Induced Pluripotent Stem Cells:

Pluripotency is an undifferentiated cell state. A cell

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that maintains pluripotency can differentiate into any cell type with the aid of specified factors and signals. By means of specialized transcription factors, differentiated stem cells destined to a certain cell fate can be reprogrammed into a pluripotent stem cell state. These induced pluripotent stem cells (iPSCs) can then differentiate into any cell, including motor neurons, through a similar process to the induction of ESC-derived MNs. Fibroblasts, differentiated stem cells destined to produce connective tissue components, such as collagen and other materials, are most commonly used. Fibroblasts can be isolated from the adult human body as well as embryos, enabling the generation of patient-specific cells from a noncontroversial source. With this research, a patient's own cells can be generated into a pluripotent state and eventually into motor neurons that can be transplanted more safely and efficiently in to a host since they will not be rejected by the patient's immune system (Lee, et al. 2013). The patient will not have to take Immunosuppressant drugs, because the body will recognize the cells as its own, reducing the risk of infections after transplantation.

To reprogram a cell into a pluripotent state, genes found in the regulation and maintenance of pluripotency in embryos were added to a culture of mouse embryonic fibroblast cells. A combination of 24 possible genes were found to induce colonies similar in morphology to ESCs (Figure 6A). To narrow down the candidates, each gene was individually removed to determine whether or not each gene had a positive effect on the induction of pluripotency. The withdrawal of four genes, Oct3/4, klf4, Sox2 and c-Myc, resulted in the absence of any colony formation. The addition of these four genes alone to fibroblast culture generated colonies more similar in morphology to ESCs than the combination of 24. The absence of any one of these genes produced colonies of different morphology than ESCs (Figure 7B), indicating the individual necessity of each of these 4 genes in the renovation of fibroblasts into an ESC-like pluripotent state (Takahashi, Yamanka, 2006).AB

To determine the pluripotency of these iPSCs reprogrammed from fibroblasts by 4 factors, they were injected into mice and monitored. Teratomas consisting all three germ layers, which further differentiated into neural tissues, muscle tissue, cartilage, epithelium and other ma-

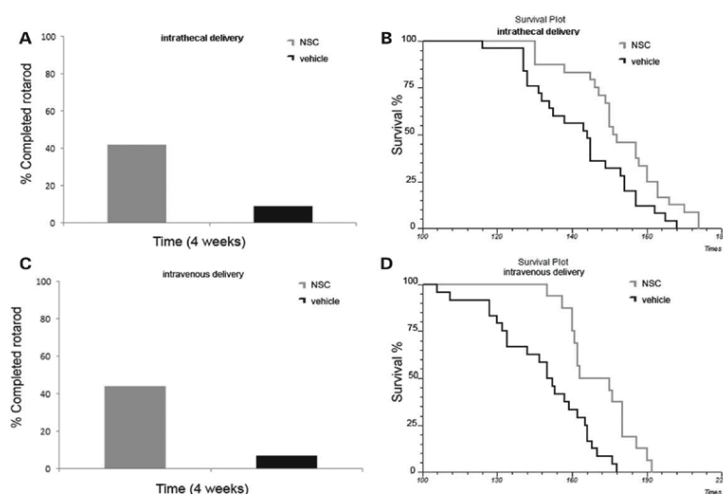
terials, were observed (Takahashi, Yamanka, 2006). The development of these tumors denotes the ability to create pluripotent stem cells, from differentiated cells, that are identical to ESCs in morphology and pluripotency. These studies were similarly applied to adult human fibroblasts which were successfully induced into a pluripotent state with comparable efficiency to mouse embryonic fibroblasts (Takahashi, et al. 2007).

To examine the possibility of reprogramming ALS-patient specific cells, an ALS mouse model was prepared by inducing a mouse with the mutant SOD1 gene, initiating motor neuron degeneration in the mouse (Gurney, et al. 1994; Yao, et al. 2013). Fibroblast cells were then isolated from the tail of the SOD1-mutated mouse and introduced to the four transcription factors, Oct3/4, klf4, Sox2 and c-Myc. Expression of ESC markers, such as specialized transcription factors, were observed in the iPSCs, indicating a successful reprogramming of fibroblasts into an undifferentiated state. To confirm whether the iPSCs could be generated into motor neurons with a similar efficiency to ESC-derived motor neurons, RA and Shh were added to the iPSCs. After the addition of RA, ~95% of the iPSC expressed the neural marker Tuj1. Shh was then added to induce a motor neuron fate. ~85% of these MNs expressed ChAT, the enzymatic precursor for ACh neurotransmitter and ~24% expressed Hb9. Whole-cell patch clamp technique was used to determine the electrical potential of these induced MNs. Multiple trains of action potentials were generated suggesting the ability of these induced MN to function normally (Yao, et al. 2013).

To assess the therapeutic potential of using iPSC to treat ALS, neural stem cells (NSCs), generated from iPSCs from human somatic fibroblasts, were delivered to SOD1-mutated mice. The NSCs were treated with RA and Shh to induce motor neurons and injected into the cerebral spinal fluid. They were injected intrathecally or intravenously, rather than direct intraspinal transplantation, to minimize the invasiveness of the therapy and maximize safety. GFP was inserted in the NSCs to trace their trajectory. Analysis of the spine and GFP suggested that the NSCs integrated in appropriate regions of the spinal cord, especially in great quantities in regions of active neuron degeneration. In both cases of delivery, survival of mice were significantly

extended. Intrathecally administered NSCs extended survival by ~10 days in all 24 mice in comparison to vehicle treated control SOD1-mutated mice and all 25 intravenously treated mice survived ~23 days longer than vehicle treated control mice (Figure 7B and D). Although the survival extension seems very limited, it is statistically significant and importantly, the extension remained steady in all the mice. Additionally, in a rotarod test, which evaluates neuromuscular function, NSC treated mice had a much higher rotarod functional outcome, indicating improved neuromuscular function (Figure 7A and C) (Nizzardo, et al.)

Figure 5: **A:** comparison of Rotarod function in control mice and after intrathecal delivery. **B:** survival comparison between control and NSC intrathecally treated mice. **C:** comparison of Rotarod function in control mice and after intravenous delivery. **D:** survival comparison between control and NSC intravenously treated mice. (Source: Nizzardo, et al. 2013) Further studies were conducted to determine whether the induced motor neurons transplanted can prevent further MN degeneration. The number of motor neurons was counted after 140 days in both control and stem cell treated mice. Mice treated with stem cells contained 40% more motor neurons than control mice, a very significant difference. Additionally, axonal density was preserved by 50% in stem cell treated mice while significant reduction was observed in control mice (Nizzardo, et al. 2013). These differences indicate the ability of induced MNs generated from iPSCs to protect against further motor neuron degeneration, thereby indicating the possibility of stem cell therapy to halt the disease progression of ALS.



Clinical Hurdle for iPSCs:

While inducing pluripotency offers a noncontroversial and comparable alternative to ESCs, there is one chief clinical hurdle crucial to overcome. The process of inducing pluripotency in differentiated stem cells requires the use of oncogenes. Genes such as Klf4, c-Myc and others, which are central to inducing pluripotency, are fundamentally interconnected with the networks of benign and malignant tumor formation. The oncogenic network includes proliferation, differentiation and other properties that are major factors in pluripotency (Lee, et al. 2013). In fact, the way researchers detect pluripotency is by injecting these iPSCs into mice where they form teratomas (Lee, et al. 2013; Takashi, Yamanka, 2006; Takashi, et al. 2007; Yao, et al. 2013). The induction of pluripotency can cause malignant tumors by inducing genetic aberrations associated with tumorigenesis. Deletions in tumor-suppressor genes and activation of oncogenes by integration of transcription factors, such as Myc, are some examples of how the induction of pluripotency causes potentially malignant neoplasms (Figure 8).

Besides for Myc, transcription factors such as Oct4, Klf4 and other core pluripotency factors are associated with tumorigenicity. These factors contain the possibility of maintaining their oncogenic tendency and causing malignant neoplasms when transplanted in humans (Lee, et al. 2013). To ensure patient protection, the clinical expression of these oncogenes will have to be researched further. Other options have to be explored to guarantee the safest and most efficient process of introducing stem cells into a human patient.

Direct Reprogramming of Stem Cells into Motor neurons: In the face of obstacles such as ethical controversy and tumorigenic potential of ESCs and iPSCs respectively, researchers have searched for other methods to apply stem cell therapy to treat ALS. Direct reprogramming of somatic stem cells into motor neurons, skipping the pluripotent state, seems to be an ideal alternative and considerable research has been devoted to this plight.

Vierbuchen et al. describes the process of directly reprogramming fibroblasts into neurons. Both mouse embryonic fibroblasts and mouse post natal fibroblasts were used to

ensure that both somatic and embryonic stem cells express similar efficiency. Five factors, prepared from an original pool of 19 were isolated based on their positive role in inducing neural development, morphology and activity in fibroblasts. Patch-clamp recording was used to identify whether action potentials could be generated in these induced neurons (iNs). A significant quantity of iNs, 85.1% , could elicit action potentials when induced with current. Additionally, iNs were responsive to GABA, glutamine and other excitatory neurotransmitters, indicating properly functioning receptor sites. iNs were placed into an existing neural network in vitro to determine whether the cells could integrate into complex neural networks. After 7 days, normal neuronal behavior was observed (Vierbuchen, et al. 2010). This suggests the capability of iNs to integrate into and cooperate with the complex human neural network, a critical factor in human stem cell therapy.

To directly induce a more specified subtype of neurons, such as a motor neurons, transcription factors that induce motor neuron identity in normal embryonic development were applied first to mouse embryonic fibroblasts, and later to human-derived fibroblasts. Eight candidate factors, responsible for various developmental stages of MNs, in addition to 3 of the 5 general neural transcription factors, described previously, were inserted into mouse embryonic fibroblasts. Hb9-GFP was used to indicate motor neuron presence. Seven factors were deemed necessary to generate induced motor neurons (iMNs). Using a patch-clamp technique, the resting membrane potential was found to be -49.5mV, very similar to ESC-derived MNs, which is -50.5mV. Single and multiple trains of action potentials were elicited using a current-clamp technique in 90% of iMNs. The ability of iMNs to respond to typical inhibitory and excitatory neurotransmitters was then tested by exposure to GABA, glycine and glutamate. The iMNs elicited normal functioning inhibitory and excitatory action potentials upon contact with these neurotransmitters, indicating functional response of iMNs to neurotransmitters (Son, et al. 2011).

To determine the capability of iMNs to form functional synapses with muscles, called neuromuscular junctions, and to stimulate muscle contraction by release of ACh, iMNs and myotubes were co-cultured. Remarkably, the iMNs extended along the myotubes and after several

days began inducing rhythmic contractions in the myotubes. To determine whether the contractions were caused by ACh, curare was added to the culture. Curare is an antagonist of ACh and will inhibit ACh from binding to the ACh receptor site and stimulating muscle contraction. After the curare was added the contractions stopped, indicating the ability of iMNs to develop and dispatch functional ACh neurotransmitters capable of stimulating muscle contraction. iMNs were then transplanted into the spinal cord of chick embryos to determine whether these cells can migrate to appropriate regions, survive and maintain function in vivo. Using Hb9-GFP, proper migration was observed in the spinal cord of the embryo. Additionally, 80% of iMNs extended their axons out of the spine toward the musculature. The same methods were applied to human fibroblasts with similar results (Son, et al. 2011). These findings indicate the similarity of iMNs to ESC-derived MNs and the capability of MNs to function normally both in vitro and in vivo.

These studies determined the ability of differentiated cells to be directly converted into specific subclasses of neurons, including motor neurons, skipping the induced pluripotent state. The use of oncogenes and tumorigenic factors are avoided, because the fibroblasts are not required to pass through an undifferentiated, ESC-like state.

Results

In this study, 3 different stem cell therapies were presented and discussed, embryonic stem cells, induced pluripotent stem cells and direct reprogramming of differentiated cells into neurons. All three therapies demonstrated the ability to produce functional motor neurons from stem cells in vitro and some in vivo (Miles, et al. 2004; Nizardo, et al. 2013; Peljto, et al. 2010; Son, et al. 2011; Takahashi, Yamanka, 2006; Takahashi, et al. 2007; Umbach, et al. 2012; Vierbuchen, et al. 2010; Wichterle, et al. 2002; Xu, et al. 2009; Yao, et al. 2013). Embryonic stem cells can successfully generate functional motor neurons using factors and signals found in normal embryonic development (Miles, et al. 2004; Peljto, et al. 2010; Umbach, et al. 2012; Wichtelle, et al. 2002). Because of its ethical controversy alternative methods are being researched. However, ESC-derived motor neurons remains a model for oth-

er research, such as induced motor neurons from pluripotency and induced MNs from differentiated stem cells, because of its success in generating normal embryonic-like motor neurons. The induction of pluripotency from differentiated stem cells demonstrated ESC-like character and could generate into functional neurons (Nizzardo, et al. 2013; Takahashi, Yamanka, 2006; Takahashi, et al. 2007; Yao, et al. 2013). The significance of this method lies in its capability of using adult cells, as well as embryonic cells, to generate motor neurons (Takahashi, et al. 2007). Pluripotency, however, is intricately linked to the oncogenic network (Lee, et al. 2013). Many of the factors used to induce pluripotency are found in the regulation and maintenance of tumor formation (Lee, et al. 2013; Takashi, Yamanka, 2006). These tumorigenic factors can potentially be preserved through the process of generating MNs and cause malignant teratomas when transplanted into humans (Lee, et al. 2013). Direct reprogramming of differentiated stem cells into motor neurons skips the pluripotent state and reduces the risk of tumorigenesis. These directly induced motor neurons can be generated from both embryonic and adult differentiated stem cells, with similar efficiency to ESC-derived MNs (Son, et al. 2011; Vierbuchen, et al. 2010). This method can provide an ideal alternative to the controversial ESC-derived MNs and the tumorigenic iPSCs.

Conclusion

Although the success of inducing motor neurons has been established preclinically, the safety and clinical efficiency of these stem cell therapies in human patients with ALS still have to be determined. Several clinical trials, conducted by “Neuralstem inc.,” “BrainStorm Cell Therapeutics,” and others, are currently underway to evaluate the safety of these methods. If safety can be determined, trials can begin to assess efficiency. While preclinical trials have been successful in generating motor neurons with proper function, clinical success may prove to be more difficult in ALS human patients. Since the etiology remains unknown, the degeneration initiating factors cannot be controlled and the new motor neurons may be subject to the same factors that caused the motor neuron degeneration in the first place. To continue survival, frequent MN transplantations into ALS patients may have to take place

each time degeneration reoccurs. While there are many clinical hurdles to overcome, with further research stem cell therapy can become the cure ALS patients have been waiting for.

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Yao XL, Ye QH, Liu Q, Wan J, Zhen J, Xiang AP, Li WQ, Wang Y, Su H, Lu XL. Motroneuron Differentiation of induced pluripotent stem cells from SOD1G93A mice. PLoS ONE. 2013;8:1-the help of specific transcription factors and signals. Through the action of Sonic hedgehog (Shh) signaling these neural progenitor cells are transformed to a progenitor motor neuron (pMN) domain, which are destined to the fate of spinal motor neurons. These cells in the pMN domain are characterized by the expression of Pax6, Nkx6.1 and Olig2 proteins which each have a role in the differentiation of various spinal motor neurons (Wichterle, et al. 2002). Additionally, these factors direct the expression of Hb9, a transcription factor found in MNs. Hb9 is commonly used to identify motor neuron presence in vivo and in vitro research, through the genetic insertion of Green Fluorescent Protein (GFP) in the Hb9 promoter site (Figure 1). The GFP will be expressed and visually viewed if Hb9 is expressed, a factor indicative of motor neuron presence (Wichterle, et al. 2002).