

Stem Cell & Regenerative Medicine

Traumatic Spinal Cord Injury at T12 Causing Complete Paraplegia for 12-Years Duration Treated with Autologous Telomerase Positive Stem Cells

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ABSTRACT

A 36-year-old paraplegic female presented to the clinic for bi-monthly pain management below thoracic level, T12. She was absent of cutaneous sensation below level of T12, absent of bladder/rectum function, absent genital function, and could not move around without the use of a wheelchair. She displayed anxiety, depression, and decreased feeling of self-worth. Her intense pain was due to a traumatic spinal cord crush injury from a car accident 12 years previously. To date, no effective pharmacological or regenerative treatment has been developed to treat chronic spinal cord injuries. Advances in stem cell technologies (e.g., embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), neural stem cells (NSCs), NSCs induced from either iPSCs or ESCs, mesenchymal stem cells, medicinal signaling cells, etc.), biomaterials, immune engineering, and nanotechnologies have been applied to regenerative therapies following subacute spinal cord injuries. Although these therapies have proven safe for subacute spinal cord injury in animal models, their efficacy in clinical trials to date has not been demonstrated. The participant was presented with opportunity to join a clinical trial using autologous adult-derived telomerase positive stem cells for amelioration of her neurogenic problems. She agreed to the trial because she wanted to walk. Her first autologous stem cell transplant did not ameliorate any of her symptoms. In retrospect, this was due to the anesthetic having a 100% kill ratio for telomerase positive stem cells. Switching to an anesthetic with a 0% kill ratio allowed the telomerase positive stem cells the potential to restore neurogenic function as previously noted for Parkinson disease, age-related dry macular degeneration, and Alzheimer's disease. Following two telomerase positive stem cell treatments there was restoration of sensation from below her umbilicus to just proximal to her knee joints and restoration of function of her urinary bladder and rectum. Due to the limited time frame following her treatments (e.g., four months), no sustained voluntary control was seen in the musculature of her lower extremities. Her inability to walk following two telomerase positive stem cell treatments prompted her to drop out of the study. Due to restoration of function to damaged structures of the central and peripheral nervous system after following telomerase positive stem cell transplants in this chronic spinal cord injured patient, suggest that TSCs, PSCs, and MesoSCs might have been involved in this restorative process. Since no adverse events were reported during her study, autologous telomerase positive stem cells appeared to be safe for administration. And with restoration of the neurogenic activities during the limited time frame of treatment, administration of telomerase positive stem cells appears to be efficacious in their activities to restore neurogenic function to the tissues absent of those activities for 12 years duration.

Keywords

Adult, Stem Cell, Telomerase, Traumatic Spinal Cord Injury, Regenerative Medicine.

Introduction

High-energy traffic accidents worldwide kill ~1 million people and injure another 50 million people per year. For survivors, traffic accidents not only cause physical impairments, but also post-traumatic stress disorder, anxiety, and depression, which leads to a decrease in quality of life [1,2]. Traumatic spinal cord injury occurs in both high energy impacts (e.g., traffic accidents) in general at all ages or in low energy impacts in populations like the elderly [3]. It often leads to serious sensory and motor dysfunction of the organs and limbs below the injured segment, affecting both psychological and psychosocial well-being of patients and their caregivers [4,5].

Traumatic spinal cord injury (TSCI) is a serious neurological problem due to high rates of morbidity that results in devastating quality of life, expectancy of life, and long-term disability. The annual global incidence of TSCI is ~10 per 80 million people [6-10]. Many people with TSCI manage the consequences of their disability long term without significant levels of pathophysiology, although their individual coping strategies may account for their adjustment [11]. However, those with low self-efficiency and increased pain intensity lead people with TSCI to have a lower quality of life than the general population. Higher levels of depression and anxiety appear as predictive values for lowered quality of life [12,13]. Hence, there has been a heavy economic burden on health systems as well as individuals and their families. This occurs during the acute phase immediately following an injury as well as chronically, long term. The USA has averaged ~\$10 million a year on spinal cord injuries, in both short term and long term costs [4,8,14,15].

Pathophysiology of TSCI

Approximately half of all patients with some form of traumatic spinal cord injury develop neurological dysfunction [8,16]. The pathophysiology of TSCI occurs following both the acute traumatic insult as well as a progressive secondary injury cascade that is characterized by ischemia, apoptotic signaling, and peripheral inflammatory cell proliferation. As the spinal cord lesion matures into the chronic phase, regeneration is severely compromised by the development of astrocyte-fibrous scar tissue surrounding cystic cavities [5]. Neurogenic bladder dysfunction is a common complication of TSCI below T12 and prevents normal daily activities, reduces quality of life, and is generally irreversible [17,18].

Potential Therapies

Neuroprotective treatments, i.e., surgical decompression, corticosteroids, respiratory monitoring, and hemodynamic monitoring, Rho inhibitor, Riluzole, Minocycline, granulocyte-colony stimulating factor, magnesium, therapeutic hypothermia, fibroblast growth factor, neuromodulation, and CSF drainage, have been applied to those with TSCI, but none have been accepted as the standard method of care [5,8,19-22].

Steroid treatment in the early hours (24-48) after injury is aimed at reducing permanent paralysis during the rest of the patient's life [23], however, application of immunosuppressants, i.e., corticosteroids, are controversial [6]. Sacral neuromodulation within 12 weeks of injury was shown to prevent neurogenic detrusor overactivity and preserve bladder capacity and compliance [18]. To date, no neuroprotective treatments have been shown to improve outcomes in individuals with traumatic spinal cord injury, short term or long term [24].

While there is still lack of effective treatments for TSCI [4,6], many new technologies and directions have appeared for improvement following SCI. These new technologies include brain-computer interface, noninvasive brain stimulation, noninvasive sacral nerve stimulation, and stem cell therapy [8]. Indeed, there is emerging hope for stem cell regeneration-based therapy of the damaged central nervous system tissues [25,27].

Regenerative stem cell therapies using different types of stem cells, different transplantation techniques, and with and without scaffolds, have undergone many trials demonstrating both efficacies and their subsequent limitations. Neuroregenerative strategies currently in clinical trials, include Cethrin™, anti-NOGO antibody, glial scar degradation, Rho-ROCK inhibition, stem cell-based approaches, and bioengineered biomaterials [5,20]. Unfortunately, no single technique stands head and shoulders above the others for the treatment of traumatic spinal cord injuries. [28].

Stem cell transplantation may offer hope of functional improvement to patients living with consequences of TSCI. But, for the present time, they remain purely investigational [24]. Animal studies demonstrate that stem cell therapy holds promise to treat spinal cord injuries. Multiple types of stem cells are being utilized, embryonic stem cells, induced pluripotent stem cells, neural stem cells, neural stem cells derived from induced pluripotent stem cells, mesenchymal stem cells, medicinal signaling cells, and neural progenitor stem cells. Neural progenitor stem cells may be preferred choice because they have clear capacity to only form neurons or glial cells after transplantation into spinal cord. To protect against demyelination, directed differentiation of neural progenitor stem cells into oligodendrocytes is being explored. It has been postulated that combinatorial strategies using scaffolds, genes promoting regeneration, and directed differentiation of neuronal progenitor stem cells may be key to repairing TSCI [26,27,29,30].

Alternative avenues of approach use scaffolds, e.g., hydrogels, to span the gap of the spinal cord lesion with stem cells, seems a promising approach to repairing spinal cord damage. There appears to be a synergistic effect between adhesiveness of stem cells and porosity and surface modification of hydrogels for in vitro and in vivo survival during treatment of spinal cord injury [31,32]. A hydrogel seeded with stem cells that were labeled with iron oxide nanoparticles could be tracked with MRI to show migration of the stem cells into the lesion site, as an animal model of neuroregeneration following spinal cord injury [33,34].

Combined application of NeuroRegen, a collagen scaffold hydrogel to promote axonal outgrowth and inhibit glial scar formation after TSCI, and stem cells appears safe and feasible for clinical therapy in some patients [35].

Multiple types of stem cells are being utilized for regenerative medicine therapies, e.g., embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), neural stem cells (NSCs), induced neural stem cells (iNSCs), hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs), medicinal signaling cells (MSCs), Schwann cells, and olfactory ensheathing cells, have been shown as potential sources for regenerative therapies. Despite the risks for tumorigenicity, graft versus host disease response, and ethical concerns, most results of animal studies utilizing embryonic stem cells demonstrate efficacious therapeutic effects of these stem cells in the treatment of nervous system diseases [6,7,36-40].

The use of patient-specific iPSCs holds great potential for an unlimited source of HLA-matched pluripotent stem cells for regenerative medicine therapeutics. The work of Yamanaka and colleagues demonstrating that viral transfection of reprogramming factors, Oct-4, SOX-2, Klf-4, and c-Myc, would transform differentiated somatic cells into telomerase positive pluripotent stem cells was a pioneering discovery in the field of regenerative medicine. Prior to iPSCs, neural stem cells were generated from embryonic stem cells, with their inherent tumorigenicity, graft versus host disease response, and ethical concerns. Unfortunately, artificial induction of iPSCs created alternative issues including genetic and epigenetic abnormalities, and tumorigenicity after transplantation. Indeed, the incidence of malignant transformation in both cell lines (embryonic stem cells and induced pluripotent stem cells) is nearly the same. Overcoming these problems necessitates use of integration-free systems during transfection of reprogramming factors and induction into the neural stem cell phenotype. Additionally, one must thoroughly investigate differentiation potential of the resulting altered cells and validate the purification process of the neural stem cells before transplanting into the individual.

Some published studies have described the successful formation of neural stem cells from induced pluripotent stem cells and their subsequent engraftment into animal models of TSCI. This was followed by functional recovery in the animals. [41-45].

Alternative studies have used iPSCs that had not been induced to form neural stem cell derivative, but rather transplanted in the naïve state. Grafted animals, receiving contusion injury and naïve iPSCs showed significantly better functional recovery than control animals receiving contusion injury alone. The data suggested that grafted undifferentiated iPSCs were able to induce morphological and functional recovery after spinal cord contusion injury [46]. Schwann cells derived from iPSCs have been used for peripheral nerve regeneration and repair by supporting myelination and axonal growth and proposed for personalized regenerative medicine. [47,48].

MSC (mesenchymal stem cell / medicinal signaling cell) therapy can reduce injured volume and promote axonal regeneration [4]. Mesenchymal stem cells (MSCs) can stimulate neural stem cell proliferation to rebuild damaged nerve tissue [49]. Populations of MSCs (mesenchymal stem/stromal cells) adhere to plastic, express cluster of differentiation markers CD105, CD117, CD123, and CD166, and can differentiate into adipogenic, chondrogenic, and osteogenic lineages in vitro. Isolated populations of MSCs have been reported to vary in their potency and self-renewal potential. As a result, mesenchymal stem cells used for clinical therapies often lead to variable and conflicting results. [50-52].

Medicinal signaling cells (MSCs) are self-renewing, non-specialized cells used clinically in regenerative medicine and sourced from bone marrow, adipose tissue, umbilical cord blood, and umbilical Wharton's jelly. Populations of these MSCs are screened for CD73, CD90, CD105 and Cadherin-11 and absence of CD34, CD45, and HLA-DR antigens [53,54]. Medicinal signaling cell-derived secretomes contain soluble proteins, nucleic acids, and lipids (e.g., exosomes, extracellular vesicles) have shown therapeutic effects similar to transplanted MSCs. Medicinal signaling cell-sourced secretomes may bypass many side effects inherent to MSCs themselves, including unwanted differentiation, graft versus host disease response, necessity to expand in culture before therapeutic application, etc. In contrast, a MSC-secretome could be generated commercially from existing MSC cell lines for cell-free off-the-shelf use for acute conditions, enabling activation of anti-apoptotic and pro-survival pathways leading to tissue regeneration and/or repair. [55].

Meta-analysis studies demonstrate that combination of two or more types of modalities, e.g., stem cells and scaffolds or a combination of different types of stem cells, are better than either alone for improving motor dysfunction in TSCI [56-58]. Combination therapies, such as MSCs (mesenchymal stem cells/medicinal signaling cells) and NSCs (neural stem cells), may offer a better approach than each one singly for treatment of spinal cord injury. Neural stem cells provide the basis for regeneration, while MSCs (mesenchymal stem cells/medicinal signaling cells) circumvent neurodegeneration and infiltrating immune cells, thereby giving a more robust response in animal models of spinal cord injury. [59]. The mechanism of action for these combinatorial therapies is based on tissue repair and replacement, neurotrophs, regeneration, promotion of angiogenesis, anti-apoptosis, and anti-inflammation [6,60]. Unfortunately, there are safety issues that need to be addressed with these combinatorial therapies, such as thrombosis, embolism, tumorigenicity and instability, infection, high fever, and death [6].

While the above strategies for regenerative medicine have proved safe and minimally efficacious in clinical trials, their use has been almost exclusively in the subacute phase of spinal cord injury. In contrast, for those individuals in the chronic phase of traumatic spinal cord injury, no pharmacological, regenerative medicine, and/or combinatorial therapies have proved efficacious for

regenerating tissues and restoring functions lost due to subsequent comorbidities of traumatic spinal cord injury. These comorbidities include increased pain intensity, higher levels of depression, higher levels of anxiety, loss of bladder/bowel function, loss of ambulation, and low self-efficiency, and lead people with TSCI to have a lower quality of life than the general population.

Based on our previous clinical studies of individuals suffering from long term comorbidities due to various disease states, e.g., Systemic Lupus Erythematosus [61], Idiopathic Pulmonary Fibrosis [62], Chronic Obstructive Pulmonary Disease [63], Celiac Disease [64], Age-Related Dry macular Degeneration [65], Alzheimer's disease [66], Parkinson Disease [67], and total bilateral visual impairment of 13-years duration, [68], adult-derived telomerase positive stem cells repaired the damaged tissues and restored function to the individuals. Therefore, we would propose using telomerase positive endogenous adult-derived stem cells for the treatment of traumatic spinal cord injuries. In the therapy described herein, adult derived telomerase positive totipotent stem cells (TSCs), pluripotent stem cells (PSCs), and mesodermal stem cells (MesoSCs) were used to treat a paraplegic individual of 12-years duration (chronic phase of spinal cord injury) having lost cutaneous sensation below her umbilicus, bladder/rectum function, and lost the ability to move without the use of a wheelchair.

Materials and Methods

A 36-year-old paraplegic female presented to the clinic for bi-monthly pain management below T12 thoracic level. She was absent of cutaneous sensory input below her umbilicus, bladder/rectum function, absent genital function, and could not move around without the use of a wheelchair. She displayed anxiety, depression, and decreased feeling of self-worth. Her intense pain was due to a traumatic spinal cord crush injury from a car accident 12 years previously. Bi-monthly pain remediation utilized lidocaine injected into intervertebral foramina bilaterally from T12 to S4 with C-arm fluoroscopic guidance for pain reduction management.

She was presented with opportunity to join a clinical trial using adult-derived telomerase positive stem cells for amelioration of her symptoms. She agreed to the trial because she wanted to walk. She fit the criteria for an IRB-approved study protocol for neurodegenerative disease, i.e., any male or female, age 18 to 120, with diagnosed traumatic central nervous system injury. Autologous telomerase positive totipotent stem cells, pluripotent stem cells, mesodermal stem cells were to be utilized for her treatment of paraplegia of 12-years duration resulting from a traumatic spinal cord crush injury.

Participant was instructed to follow informed consent guidelines [69] and to ingest combinatorial nutraceutical (CN, Dragonfly Foundation for Research and Development, DFRD, Macon, GA) for 30 days prior to her first harvest and then every day thereafter, to increase the number of telomerase positive stem cells in her body prior to harvest. She was given glacial caps (GC, DFRD) to

ingest 18 hours before stem cell harvest to mobilize her telomerase positive stem cells into her blood stream.

Harvesting the telomerase positive stem cells occurred using venipuncture to withdraw 210-310cc of peripheral blood, based on her body weight. The telomerase positive stem cells were separated from the blood elements using FDA-mandated minimal manipulative procedures, zeta potential/gravity separation, and differential density gradient centrifugation using serum, saline, and sterile distilled water. The TSCs, PSCs, and MesoSCs were further segregated into individual populations and activated. Processing time required approximately 72 hours to complete before administration of the stem cells.

Her first transplant consisted of pooling the TSCs, PSCs, and MesoSCs, mixing the telomerase positive stem cells with the lidocaine anesthetic, and injecting the mixture bilaterally into the intervertebral foramina of T12 to S4 under C-arm fluoroscopic guidance. Results from the first transplant showed pain reduction, but no effect of the telomerase positive stem cells on any sensory-motor function at or below the umbilicus.

We theorized that the lidocaine might have interfered with the activity of the telomerase positive stem cells. A series of experiments were performed, first with lidocaine alone and then comparing lidocaine to other anesthetics, e.g., novocaine, procaine, marcaine, bupivacaine, and sterile saline (0% kill ratio control), for their effect on telomerase positive stem cells [70]. As noted, lidocaine had a 100% kill ratio for the telomerase positive stem cells; novocaine and procaine demonstrated a 50% kill ratio; and marcaine, bupivacaine, and sterile saline had a 0% kill ratio for telomerase positive stem cells.

The clinician next tested bupivacaine as a possible substitute for lidocaine for pain management in the participant, paralleling the same protocol used for lidocaine injections. The participant reported the same to better pain relief with bupivacaine versus lidocaine. The clinician utilized bupivacaine as a substitute for lidocaine as the anesthetic of choice for her ongoing pain management treatments.

Her second and third telomerase positive stem cell transplants differed in protocol from her first treatment. In these two treatments segregated populations of TSCs, PSCs, and MesoSCs were each divided into two equal aliquots. One aliquot of TSCs was further subdivided and concentrated into two 0.25cc aliquots for intranasal delivery [68], one aliquot each of PSCs and MesoSCs were pooled from intravenous infusion in 250cc's of sterile heparin/saline into the median cubital vein [71], and one aliquot each of TSCs, PSCs, and MesoSCs were pooled and mixed with bupivacaine for injections into the intervertebral foramina of T-12 to S4 using C-arm fluoroscopic guidance.

Results

The participant's first telomerase positive stem cell transplant

with lidocaine elicited pain relief, but no repair of sensory or motor function below the level of the T12 thoracic vertebrae. As described in the Materials and Methods sections, this was probably due to the anesthetic (lidocaine) killing the telomerase positive stem cells before they had a chance to exert their activity on the tissues.

The participant's second telomerase positive stem cell treatment with bupivacaine elicited pain relief as well as a return of cutaneous skin sensation from her umbilicus to the bottom of her trunk along the inferior border of her inguinal canals, suggesting a return of sensory function. There was no discernible return of motor function in any location below the umbilicus.

The participant's third telomerase positive stem cell transplant with bupivacaine elicited pain relief as well as continued cutaneous skin sensation from umbilicus to inguinal canals, and also included return of cutaneous skin sensations over the anterior, medial, and posterior compartments of her thighs extending to her popliteal fossae (knee joints) bilaterally. Motor function had returned to her abdominal musculature below the umbilicus AND a return of both autonomic and voluntary control of bladder, uterus, and rectal functions. There was no discernible motor activity in either of her lower extremities. The participant dropped out of the study because three treatments with telomerase positive stem cells did not return to her a state where she could walk unassisted.

Discussion

Spinal cord injury is a destructive neurological state that causes motor, sensory, and autonomic dysfunctions. Its pathophysiology encompasses both acute and chronic states and incorporates a cascade of destructive events, including ischemia, oxidative stress, immune cell infiltration, cytokine release, inflammation, induced apoptosis of damaged tissues, and locomotor dysfunctions. Many therapeutic strategies have been proposed to overcome these destructive events and reduce secondary neuronal damage. Strategies have also been developed to promote neuroprotective, immunomodulatory, and neuroregenerative activities to promote neuronal recovery and restoration of neuronal function [72].

To date, no effective pharmacological or regenerative treatment has been developed. Advances in stem cell technologies, biomaterials, immune engineering, and nanotechnologies have been applied to regenerative therapies following spinal cord injuries. Although these therapies have proven safe, their efficacy has not been demonstrated. The pathophysiology of TSCI is complex, multifaceted, and not yet fully understood. Therefore, combinatorial therapies that leverage multiple approaches may be the best approach to achieve efficacious outcomes. Potentially, those strategies would use a combination of pharmacological agents, stem cells, and biomatrices, using minimally invasive procedures to engender neuroprotection and neuroregeneration during regeneration/repair following spinal cord injury [4,6,72].

For cell-based therapies, treatment must be phase-specific to the injury incurred and site of the lesion. Cell-based therapies must

also be personalized to each patient, taking into account their age, gender, neuroinflammatory status, neuroimmune status, endocrine status, and expressed interleukins and TNF- α to optimize chance for successful treatment [73,74].

Embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), neural stem cells (NSCs), induced neural stem cells (iNSCs), hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs), medicinal signaling cells (MSCs), Schwann cells, and olfactory ensheathing cells have been shown as a potential sources for regenerative therapies. The use of patient-specific iPSCs holds great potential for an unlimited source of HLA-matched pluripotent stem cells for regenerative medicine therapeutics. In spite of the risks for tumorigenicity, graft versus host disease response, and ethical concerns, most results of animal studies demonstrate efficacious therapeutic effects of these embryonic stem cells in the treatment of nervous system diseases [6,7,36-40].

The working definition of a stem cell is a cell that can divide to renew itself, to be expanded from a single cell, its degree of potency, and its ability to differentiate (mature) into two or more cell types. Stem cells can be derived from early embryos, both before and after formation of the blastocyst, or from fetal, postnatal, and adult sources. Examples of such stem cells are totipotent stem cells, pluripotent stem cells, ectodermal stem cells, mesoderm stem cells, endodermal stem cells, neural stem cells, hematopoietic stem cells, mesenchymal stem cells, etc. [75-77].

Embryonic stem cells derived from the morula are totipotent and will form all cells of the embryo and the extraembryonic membranes. Embryonic stem cells from the blastocyst are pluripotent and will form all cells of the embryo. The inner cell mass of the forming embryo will divide into three embryonic layers, the ectoderm, mesoderm, and endoderm. The ectoderm will further divide into the surface ectoderm, forming the epidermis and associated structures, and neural ectoderm, forming cells associated with the central nervous system, peripheral nervous system, and neural crest-derived tissues. The mesoderm will divide into structures of the axial skeleton, intermediate mesoderm, and lateral plate splanchnic and lateral plate somatic mesoderm. The endoderm will form the lining cells for the lungs and gastrointestinal system (Figure 1) [78].

The work of Yamanaka and colleagues demonstrating that viral transfection of reprogramming factors, Oct-4, SOX-2, Klf-4, and c-Myc, would transform differentiated somatic cells into telomerase positive induced pluripotent stem cells was a pioneering discovery in the field of regenerative medicine. Prior to iPSCs, neural stem cells were generated from embryonic stem cells, with their inherent tumorigenicity, graft versus host disease response, and ethical concerns. Unfortunately, artificial induction of iPSCs created alternative issues including genetic and epigenetic abnormalities, and tumorigenicity after transplantation. Indeed the incidence of malignant transformation is nearly the same in both cell lines. Overcoming these problems necessitates use of an integration-free system during transfection of reprogramming factors and induction

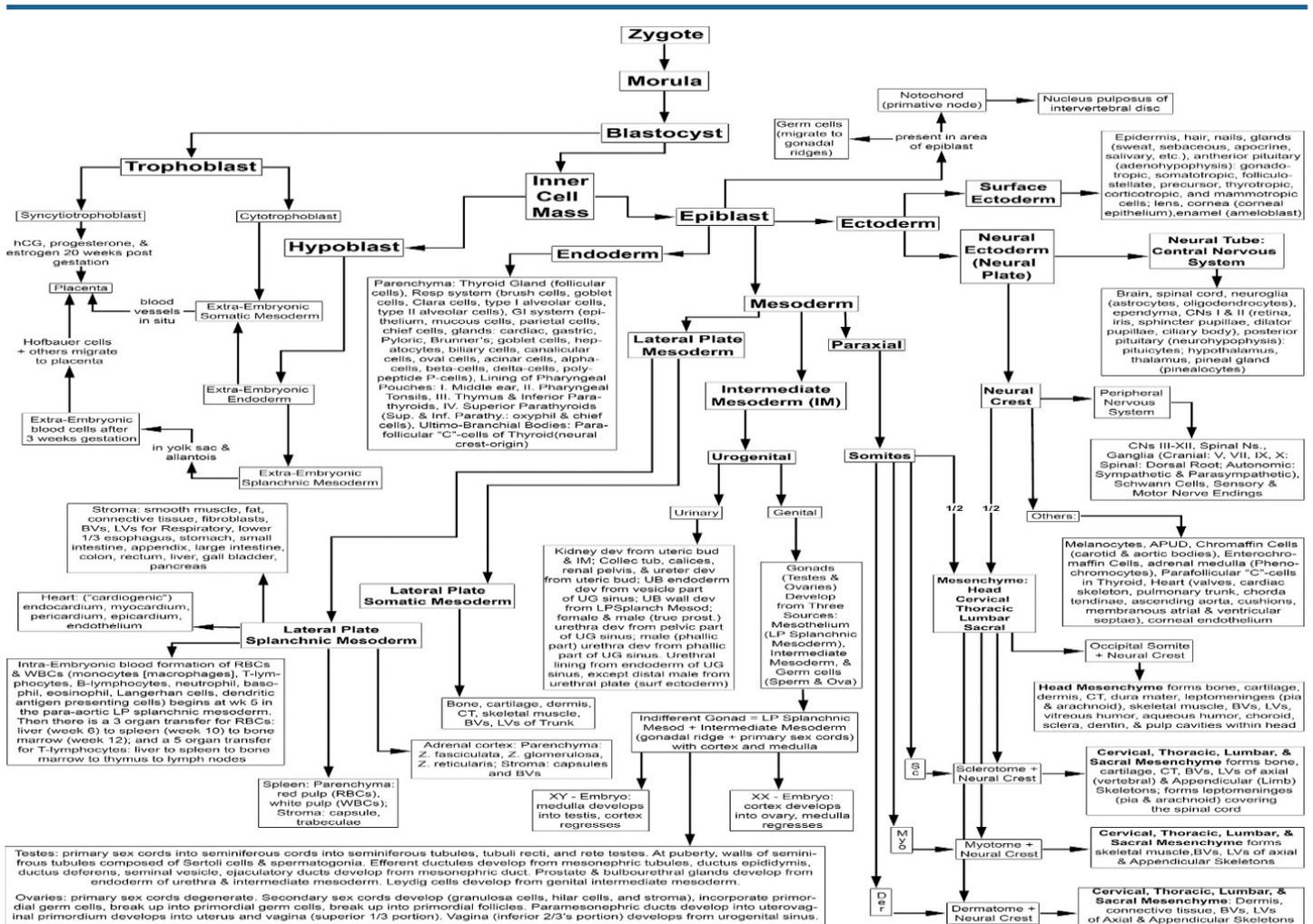


Figure 1: Lineage Map of Embryonic Development. Reprinted with permission from Young HE, Black Jr AC. Adult stem cells. *Anat Rec* 2004; 276A:75-102 [78].

into the neural stem cell phenotype, a thorough investigation of differentiation potential in the resulting altered cells, and assessment of the purified end product is mandatory before transplanting into the individual. Some published studies have described the successful formation of neural stem cells from induced pluripotent stem cells and their subsequent engraftment into animal models of TSCI. This was followed by functional recovery in the animals. [41-45]. Alternative studies have used iPSCs that had not been induced to form neural stem cell derivative, but rather transplanted in the naïve state. Grafted animals, receiving contusion injury and naïve iPSCs showed significantly better functional recovery than control animals receiving contusion injury alone. The data suggested that grafted undifferentiated iPSCs were able to induce morphological and functional recovery after spinal cord contusion injury [46]. Neural stem cells arise from embryonic neural ectoderm that forms neuroepithelial cells. The neuroepithelial cells produce radial glial cells that in turn form fetal and adult neurons, astrocytes, oligodendrocytes, and ependymal cells within the central nervous system [77,78].

There are two schools of thought as to the best source tissue to generate neural stem cells. One school believes that embryonic

stem cells, with their ability for self-renewal, clonal capacity, normal karyotype, and potential to form neural stem cells, may be the best source for NSCs for stem cell therapeutics [77]. In contrast, the other school of thought believes that induced pluripotent stem cells, with their ability for self-renewal, clonal capacity, normal karyotype, matched HLA-markers to the recipient, lack of ethical concerns, and potential to form neural stem cells, make them the best choice to derive neural stem cells for regenerative therapies [79].

There are four potential avenues for neural stem cells to be used for treatment of spinal cord injury, i.e., reactivation adult neural stem cells (NSCs), induction of neural stem cells from somatic cells via induced pluripotent stem cells (iPSC-iNSCs), induction of neural stem cells from embryonic stem cells (ESC-iNSCs), and cell transplantation therapies. Adult NSCs reside in G0 phase of the cell cycle in the dentate gyrus of the hippocampus and the subventricular zone of the lateral ventricles. Their reactivation will necessitate interactions between extrinsic stimuli from various niches and intrinsic factors, such as transcription factors, signaling pathway, epigenetics, and metabolism to jump start the neural stem cells to exit the G0 phase and enter the cell cycle. Alternatively, iPSC- iNSCs from somatic cells or ESC-iNSCs

offers another approach. To overcome the limitation of viral-based reprogramming of iNSCs from iPSCs, bioactive small molecules are being explored as a replacement for transcription factors, making iNSCs from iPSCs more amenable to clinical application than iNSCs from ESCs [26, 80,81].

Schwann cells derived from iPSCs have been used for peripheral nerve regeneration and repair, support myelination and axonal growth and are proposed for personalized regenerative medicine [47,48]. MSCs have been reported to stimulate neural stem cell proliferation to rebuild damaged nerve tissue [49]. Populations of mesenchymal stem/stromal cells (MSCs) adhere to plastic, express cluster of differentiation markers CD105, CD117, CD123, and CD166, and can differentiate into adipogenic, chondrogenic, and osteogenic lineages in vitro. Isolated populations of MSCs have been reported to vary in their potency and self-renewal potential. As a result, mesenchymal stem cells used for clinical therapies often lead to variable and conflicting results. [51,52]. Medicinal signaling cells (MSCs) are self-renewing, non-specialized cells used clinically in regenerative medicine and sourced from bone marrow, adipose tissue, umbilical cord blood, and umbilical Wharton's jelly. Populations of these MSCs were screened for CD73, CD90, CD105 and Cadherin-11 and absence of CD34, CD45, and HLA-DR antigens [53,54]. Medicinal signaling cell-derived secretomes containing soluble proteins, nucleic acids, and lipids (e.g., exosomes, extracellular vesicles) have shown therapeutic effects similar to transplanted MSCs. MSC-sourced secretomes may bypass many side effects inherent to MSCs themselves, including unwanted differentiation, graft versus host disease response, necessity to expand in culture before therapeutic application, etc. In contrast, MSC-secretomes could be generated commercially from existing MSC cell lines for cell-free off-the-shelf use for acute conditions, enabling activation of anti-apoptotic and pro-survival pathways leading to tissue regeneration and/or repair. [55]. Exosomes, derived from medicinal signaling cells, have great potential in treatment of acute SCI. Such mechanisms include preventing cell apoptosis, modulating the inflammatory response, and promoting endogenous repair mechanisms, promoting angiogenesis, and promoting neurogenesis [4,82]. MicroRNAs (miRNA) are endogenous non-coding RNAs that regulate gene expression by mediating translational inhibition or mRNA degradation. Exosomes are the vehicle for transporting miRNAs that regulate signaling pathways, including the oxidative stress response. Exosomes containing miRNAs are involved in CNS disorders, such as traumatic brain injury, traumatic spinal cord injury, vascular ischemia, epilepsy, Alzheimer's disease, Parkinson's disease, and gliomas. In the future, exosomes containing certain miRNAs may facilitate novel therapeutic approaches for regenerative medicine. [83,84]. Extracellular vesicles (exosomes, secretomes) are viewed as a safe, non-immunogenic delivery system with high target specificity after systemic administration to deliver therapeutic payloads for regenerative medicine and drug delivery [85].

While the above strategies for regenerative medicine have proved safe and minimally efficacious in clinical trials, their use has been

almost exclusively in the subacute phase of spinal cord injury. Based on our previous clinical studies of individuals suffering from long term comorbidities due to various neurodegenerative disease states, e.g., Age-Related Dry macular Degeneration [65], Alzheimer's disease [66], Parkinson Disease [67,71], and total bilateral visual impairment of 13-years duration [68], adult-derived telomerase positive stem cells repaired the damaged tissues and restored function to the individuals. Therefore, we would propose using telomerase positive endogenous adult-derived stem cells for the treatment of traumatic spinal cord injuries, in both the subacute and chronic phases of this disorder. In the therapy described herein, adult derived telomerase positive totipotent stem cells (TSCs), pluripotent stem cells (PSCs), and mesodermal stem cells (MesoSCs) were used to treat a paraplegic individual of 12-years duration (chronic phase of spinal cord injury) having lost cutaneous sensation below the umbilicus, lost bladder and rectal function, and lost the ability to ambulate without the use of a wheelchair.

Three routes of introduction of the telomerase positive stem cells were utilized in this study, e.g., intranasal, intravenous, and bilateral injections into the intervertebral foramina between T12 and S4 vertebrae. Intranasal delivery offers many advantages over standard delivery methods, such as bypassing the blood brain barrier, being non-invasive, a fast onset of action, and in many cases reduced side effects due to more targeted delivery [86-90].

Previous studies utilized medicinal signaling cells that secrete beneficial agents in situ as a promising strategy to restore myelination following diffuse white matter injury and improve neurodevelopmental outcome of extreme preterm infants [91]. However, MSCs have not been used successfully in adults when multiple treatments are necessary to cause an effect. This has been due to a logistical problem with their intranasal delivery. Mesenchymal stem cells are far larger than the spaces between olfactory epithelial cells. To increase the diameter of the spaces between the olfactory epithelial cells, a high osmolarity substance, e.g., mannitol, is used to shrink the olfactory epithelial cells. This allows the mesenchymal stem cells to migrate between the cells, along the olfactory nerve rootlets, through the cribriform plate, past the blood brain barrier to gain entrance to the CNS. In post-puberal adults, application of mannitol one time will not cause any undue harm. However, multiple treatments with mannitol can cause permanent channels to be created between the olfactory epithelial cells. While this may seem ideal for multiple treatments with mesenchymal stem cells, these permanent channels can become conduits for bacterial and viral access to the CNS causing an inflammation of the meninges, that may prove lethal to the individual [65-67].

To circumvent the potential size problem and use of mannitol with respect to intranasal delivery of the stem cells, we opted to use the smallest telomerase positive stem cells, i.e., the totipotent stem cells (TSCs). Previous characterization studies of TSCs demonstrated that these very small cells (0.1 to 2.0 microns in size) would form all cell types of the body, including neural progenitor stem cells, and differentiated cell types of the neural

ectodermal lineage, including dopaminergic neurons, pyramidal neurons, interneurons, astrocytes, oligodendrocytes, radial glial cells; ganglion cells, melanocytes, and other derivatives of neural crest (Figure 2) [92-94]. To keep the TSCs from being trapped in nasal mucus secretions, the participants washed the mucus from their nostrils with 0.65% saline prior to TSC application to the olfactory epithelium [65-67].

Telomerase positive PSCs and MesoSCs were pooled and delivered by intravenous infusion into the systemic vasculature to allow the body to dictate the final location of some of the telomerase positive stem cells for repair. In previous studies for systemic lupus erythematosus [61], age-related dry macular degeneration [65], and Parkinson disease [95], it was noted that the participant's body redirected the telomerase positive stem cells to sites that were deemed more life threatening to the individual than the originally intended placement sites.

Lastly, pooled TSCs, PSCs, and MesoSCs were mixed with bupivacaine and injected bilaterally into the intervertebral foramina of vertebrae T12 to S4 to elicit pain relief and help restore functionality to her peripheral nerves, i.e., regeneration of Schwann cell covering; endoneurial, perineurial, and epineurial coverings; synapses; motor endplates, etc., to support axonal

outgrowth to peripheral tissues (Figure 3). This was especially noticed with increase in sensation (sensory input) from below umbilicus to knee joint; as well as autonomic control of bladder and rectum function; and voluntary motor control of abdominal musculature, and voluntary urinary bladder and rectum emptying.

The participant did, however, exhibit an unrealistic expectation of being able to be free of her wheelchair after only two stem cell treatments that demonstrated positive results. Decreased skeletal muscle activity in her lower extremities may result from either disuse atrophy, such as casting a leg due to a fracture which results in decreased muscle mass, or neurogenic atrophy, in which one or more nerves are compromised (e.g., transected, demyelinated, etc.) to selected muscles. In the participant described herein, a crush injury to her spinal cord at the level of T12 caused neurogenic atrophy to all structures below the point of injury, and then was perpetuated as disuse atrophy because of inability to voluntarily use those muscles, resulting in her lower extremity flaccid musculature. This can be ascribed to a process called Wallerian degeneration-regeneration [96]. When nerve processes are crushed, their axons and overlying myelin sheaths degenerate distally from the lesion because they rely on their cell bodies for their maintenance and survival. While the crushing injury causes irreparable damage to the nerve processes, the nerve's connective

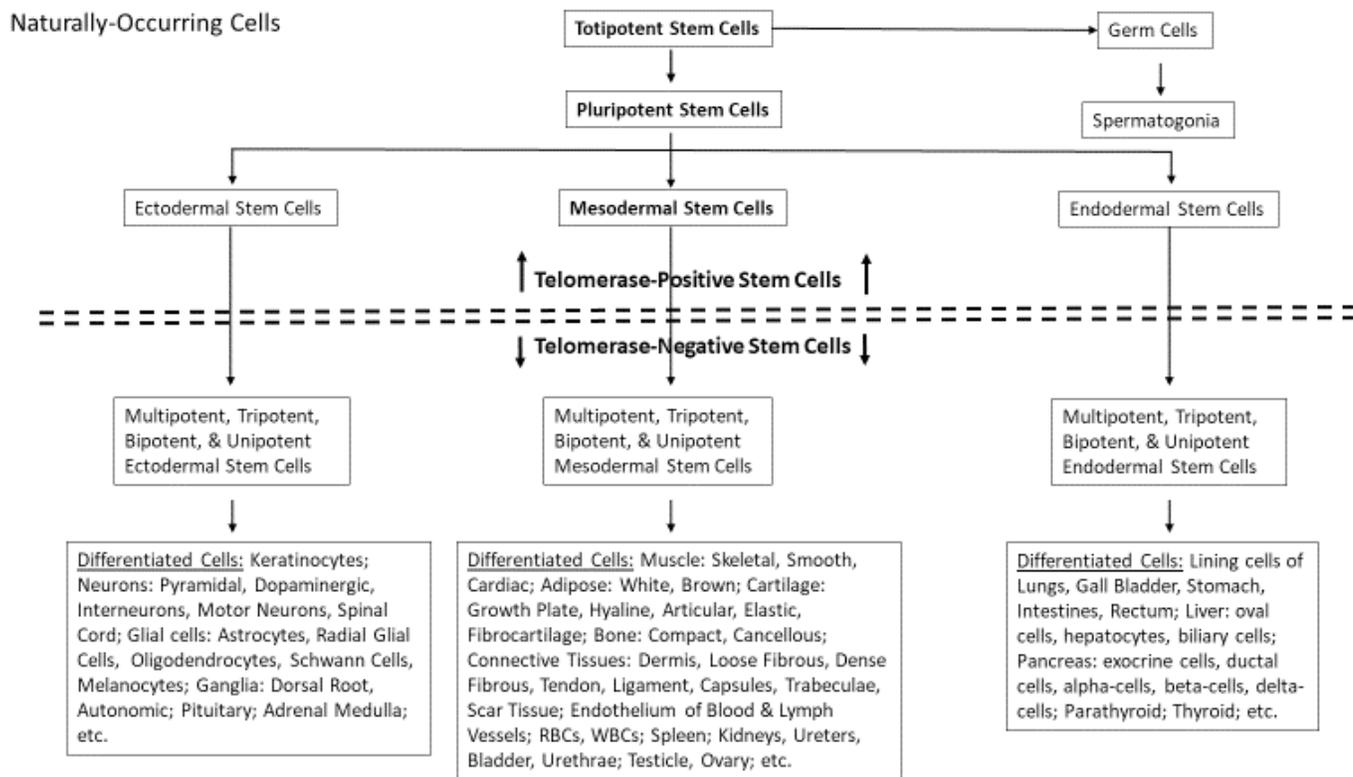


Figure 2: Diagram of respective downstream differentiation potentials of telomerase-positive stem cells with essentially unlimited proliferation potential (above dotted line) and telomerase-negative progenitor stem cells and differentiated cells, which conform to Hayflick's limit of 50-70 population doublings before senescence and cell death (below dotted line). Telomerase positive stem cells, telomerase negative progenitor stem cells, and differentiated cell are found within the body. Scl-4 β , and Scl-9 β , and Scl-44 β were three individual clones of telomerase positive totipotent stem cells (TSCs) derived by repetitive serial dilution single cell clonogenic analysis, demonstrated the potential to form all cells of the body from ectodermal, mesodermal, and endodermal germ layer lineages, including their respective progenitor stem cells and differentiated cell types, the gametes, and the nucleus pulposus of intervertebral disc (the only adult structure of the notochord, the primary individual). Note the differentiation into neurons, astrocytes, oligodendrocytes, Schwann cells, dorsal root ganglia, autonomic ganglia, etc. Reprinted with permission from Young HE, Speight MO. Characterization of endogenous telomerase-positive stem cells for regenerative medicine, a review. *Stem Cell Regen Med* 2020; 4(2):1-14 [92].

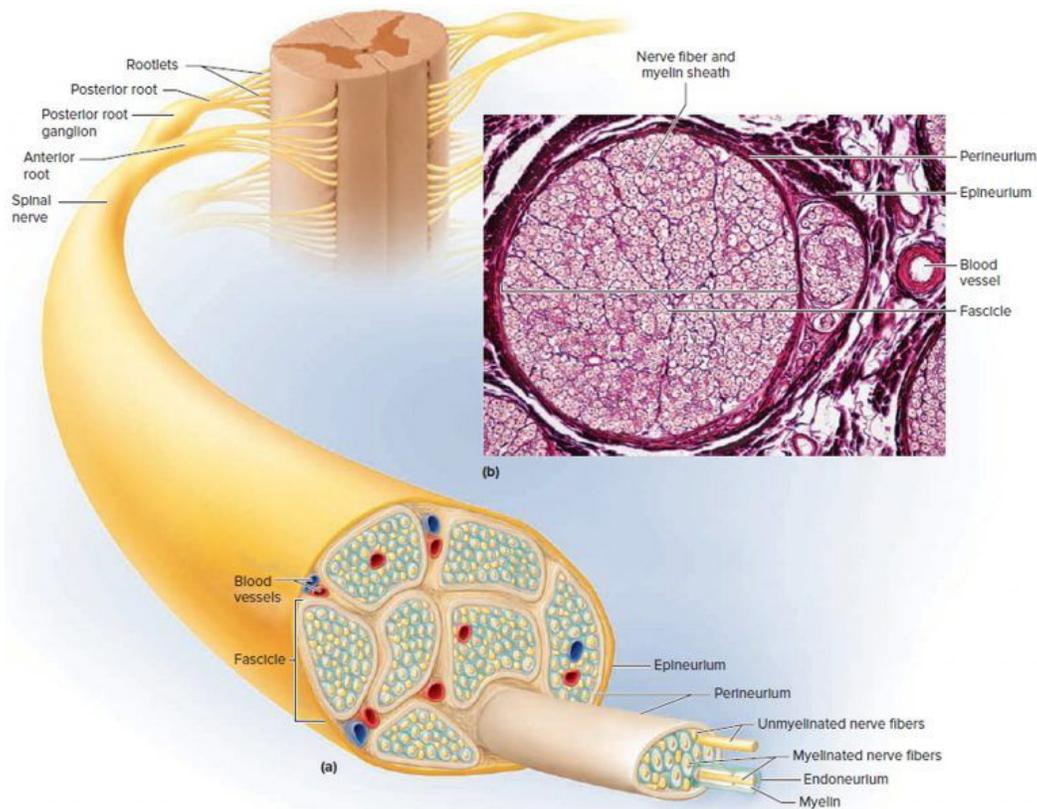


Figure 3: Mixed nerve containing both myelinated motor fibers and unmyelinated sensory fibers. Each neural fiber (myelinated or unmyelinated) is surrounded by a connective tissue covering termed the endoneurium. Collections of these endoneurial-encased neural fibers are within a bundle, or fascicle, and surrounded by a connective tissue covering termed the perineurium. Collections of fascicles are encased with an epineurial sheath, forming a mixed nerve. Reprinted with permission from <http://www.healthjade.net> pinterest.com/pin/840413980457041529/.

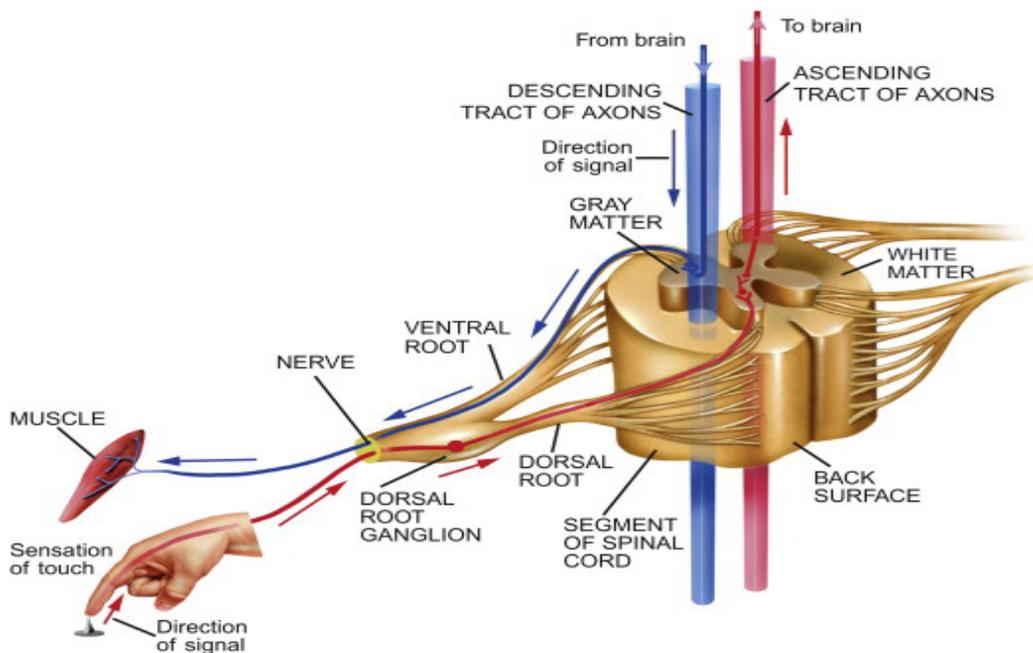


Figure 4: Segment of spinal cord demonstrating sensory input via dorsal root ganglion to anterior/dorsal horn of spinal cord, to an interneuron, and then to an ascending tract to the brain; descending tract from brain to posterior/ventral horn of spinal cord, synapsing in gray matter and sending a ventral motor root to an end point, a motor end plate on skeletal muscle. Reprinted with permission from Segul G, Watson C. *The Human Nervous System*, 3rd Ed, 2012.

tissue coverings, e.g., endoneurial, perineurial, and epineurial connective tissue coverings remain intact (Figure 3). In addition, compromising the vascular supply to the nerve (vasa nervorum) can also cause nerve degeneration.

In addition, there was a logistical problem in regenerating axonal processes to reach the sensory endings and motor endplates to the level of the feet, to allow walking to occur in a time frame of essentially four months of time or 120 days.

During Wallerian regeneration, neuronal cell bodies and their associated processes regenerate at a rate of about 2-mm per day (= 240mm = 24cm = 9.4"). The portions of the nervous system that need to be repaired to restore functionality include mixed motor/sensory peripheral nerves, dorsal root ganglia, sensory tracts up to the brain, descending motor tracts from the brain to the ventral horn, motor nerve rootlet outgrowths to mixed nerve. While 24cm and four months is a sufficient distance and time frame for reinnervation of her bladder, uterus, and rectum, it is an insufficient distance and time frame to regenerate sensory and motor constituents (e.g., sensory nerve endings, motor end plates, nerve processes, myelin sheaths, etc.) to the terminal portions of her lower extremities, i.e., feet.

To restore function to all structures below the level of the crush injury, e.g., sensory input to dorsal root ganglion cells, which conveys sensory input to anterior horn of spinal cord, interneurons, ascending sensory tracks, descending motor tracks, interneurons, ventral horn motor neurons, motor axons, would necessitate restoration of all structures involved (Figure 4). This might have been accomplished by intranasal infusion of TSCs (totipotent stem cells), intravenous infusion of PSCs (pluripotent stem cells), and intervertebral foramina injections of TSCs and PSCs. Both TSCs and PSCs will differentiate into all cell types associated with neural ectoderm (Figure 3) [92]. The MesoSCs (mesodermal stem cells), delivered by intravenous infusion and direct injection into the intervertebral foramina, may have contributed to restoring vasculature (nutrient supply and waste removal) to the regenerating tissues since they will differentiate into vascular tissues and have shown that ability in animal model systems of induced disease, e.g., Parkinson disease [97] and cardiovascular disease [98]. This is in contrast to MSCs (mesenchymal stem cells) that will only form fat, cartilage, and bone [50] and will not grow new blood vessels, or MSCs (medicinal signaling cells) that release exosomes to modulate the immune system [53].

Indeed, restoration of bladder and rectum function as well as signs of cutaneous sensory restoration from below her umbilicus to anterior, medial, and posterior compartments of her thighs just proximal to her knee joints suggest that TSCs, PSCs, and MesoSCs might have been involved in this restorative process. It is envisioned that if continued stem cell treatments would have occurred then it would have been possible to restore ambulation in her lower extremities.

Conclusion

A 36-year-old paraplegic female presented to the clinic for bi-monthly pain management below T12 thoracic level. She was absent of cutaneous sensation below level of T12, absent of bladder/rectum function, absent genital function, could not move around without the use of a wheelchair. She displayed anxiety, depression, and decreased feeling of self-worth. Her intense pain was due to a traumatic spinal cord crush injury from a car accident 12 years previously. She was presented with opportunity to join a clinical trial using autologous adult-derived telomerase positive stem cells for amelioration of her neurogenic problems. She agreed to the trial because she wanted to walk. Her first autologous stem cell transplant did not ameliorate any of her symptoms. In retrospect, this was due to the anesthetic having a 100% kill ratio for telomerase positive stem cells. Switching to an anesthetic with a 0% kill ratio allowed the telomerase positive stem cells the potential to restore neurogenic function as previously noted for Parkinson disease, age-related dry macular degeneration, and Alzheimer's disease. Following two telomerase positive stem cell treatments there was restoration of sensation from below her umbilicus to just proximal to her knee joints and restoration of function of her urinary bladder and rectum. Due to the limited time frame following her treatments (e.g., four months), no sustained voluntary control was seen in the musculature of her lower extremities. Her inability to walk following two telomerase positive stem cell treatments prompted her to drop out of the study. Due to the ability of telomerase positive totipotent stem cells and pluripotent stem cells to form all structures derived from neural ectoderm and the ability of the mesodermal stem cells to form vasculature, the restoration of bladder and rectum function, and signs of sensory restoration from below her umbilicus to along the anterior, medial, and posterior compartments of her thighs just proximal to her knee joints suggest that TSCs, PSCs, and MesoSCs might have been involved in this restorative process. It is envisioned that if continued telomerase positive stem cell treatments had occurred then it would have been possible to restore ambulation to her lower extremities. Since no adverse events were reported during her study, telomerase positive stem cells appeared to be safe for administration. And with restoration of the neurogenic activities during the limited time frame of treatment, administration of telomerase positive stem cells appears to be efficacious in their abilities to restore neurogenic function to the tissues absent of those activities for 12 years.

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