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Functional Multipotency of Stem Cells and Recovery Neurobiology of Injured Spinal Cords

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Abstract

This invited concise review was written for the special issue of *Cell Transplantation* to celebrate the 25th anniversary of the American Society for Neural Therapy and Repair (ASNTR). I aimed to present a succinct summary of two interweaved lines of research work carried out by my team members and collaborators over the past decade. Since the middle of the 20th century, biomedical research has been driven overwhelmingly by molecular technology-based focal endeavors. Our investigative undertakings, however, were orchestrated to define and propose novel theoretical frameworks to enhance the field's ability to overcome complex neurological disorders. The effort has engendered two important academic concepts: Functional Multipotency of Stem Cells, and Recovery Neurobiology of Injured Spinal Cords. Establishing these theories was facilitated by academic insight gleaned from stem cell-based multimodal cross-examination studies using tactics of material science, systems neurobiology, glial biology, and neural oncology. It should be emphasized that the collegial environment cultivated by the mission of the ASNTR greatly promoted the efficacy of inter-laboratory collaborations. Notably, our findings have shed new light on fundamentals of stem cell biology and adult mammalian spinal cord neurobiology. Moreover, the novel academic leads have enabled determination of potential therapeutic targets to restore function for spinal cord injury and neurodegenerative diseases.

Keywords

functional multipotency, recovery neurobiology, spinal cord injury, neural oncology, neural stem cell, mesenchymal stromal stem cell, induced pluripotent stem cell, polymer, locomotion, central pattern generation, serotonin

Introduction

More than 4500 years ago, humans recorded, for the first time, medical encounters of traumatic spinal cord injury (SCI) in a Surgical Papyrus that was purchased by Edwin Smith, an American Egyptologist, at Luxor, Egypt in 1862[1]. The scroll was translated 68 years later by James Henry Breasted, and published in 1930[2]. The document presented 48 trauma cases, among which 6 appeared to involve the cervical spine, with two persons sustaining injuries directly to the spinal cord parenchyma[2]. Based on the knowledge of the author's era, interventions described were "packing the wounds with fresh meat" or not giving treatment at all to SCI patients.

Modern approaches to understanding anatomical and functional perspectives of the central nervous system (CNS) can be dated to the late 16th century and early 17th century[3]. In those days, anatomists, as pioneers of neurobiology research, markedly advanced the scope of characterization for the physical structure of the brain, and, to a lesser degree, the spinal cord. In contrast, very limited progress was made in understanding the function of the CNS. Anatomists still debated if the spinal cord was a simple outgrowth of the brain or vice versa, while philosophers continuously speculated whether the human soul was housed in the brain, heart

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or spinal cord—lingering topics since the 4th or 5th centuries B.C. during the classical period of ancient Greece[4,5].

Accordingly, the initial therapeutic nihilism towards spinal cord trauma persisted through the following millennia until the early 20th century, when surgical techniques and instruments became available to improve alignment and stabilization of the spine[6,7]. Around the same time, contemporary research activities to understand the injured mammalian spinal cord were launched systematically by Dr. Alfred R. Allen, an American neuropathologist. On faculty at the University of Pennsylvania, Dr. Allen, during 1908 to 1914, published three papers that showed clinical and experimental evidence of continuous evolvement of tissue damage around the lesion epicenter following primary mechanical insults[8–10]. He postulated that, besides the direct destructive impact of the primary injury, "edematous and hemorrhagic outpouring into the cord tissue, which by its pressure and chemical activity inhibits temporarily all conduction function or destroys permanently the spinal cord"[10]. The hypothesis was then tested by performing longitudinal myelotomy, trying to alleviate the aforementioned assaults in an original weight-drop model of spinal cord contusion designed and assembled in the Allen laboratory[8]. The observation, rationale and experimental findings laid down the foundations of the *Secondary Injury Theory* that started drawing intensive research investment particularly since the 80s of the 20th century[11,12].

From then on, well-organized academic, research, and medical efforts have been given to developing potential therapies for SCI, mainly through mitigating secondary injury events (e.g., norepinephrine, ionic imbalance, excitotoxicity, inflammation, oxidative damage, etc.) and/or augmenting endogenous recovery mechanisms (e.g., wound healing, axon regeneration, neurotrophic factor production, stem cell activation, etc.), seeking to tilt the balance in favor of functional improvement[13–16]. The need for a cure is obvious, but effective therapy for SCI is still far from reality[11,17–19]. What has been proposed is that there are five prevalent barriers to adult spinal cord repair: (a) lengthy secondary injury processes, (b) inhibitory environment for neurogenic activities, (c) insufficient trophic factors, (d) inadequate regeneration, and (e) lack of spontaneous activation of compensatory neural circuits for evoking locomotor pattern generation[19–21].

Hope for truly repairing lesioned spinal cord was greatly lifted by the discovery and ability to experimentally manipulate neural stem cells (NSCs)[14,15,20,21]. Considered as the most ideal candidate for reconstructing the injured or diseased CNS, NSCs, by their innate biology, have capacity for self-renewal, and can give rise to phenotypes of all three neural lineages (i.e., neurons, oligodendrocytes, and astrocytes) through asymmetric cell division, proliferation, and differentiation[22,23]. Thereby, early attention of devising NSC-based therapy for SCI was centered on engrafting donor NSCs to the injury zone in order to regenerate neural cells to replace those that had died, and to induce long distance regeneration of the corticospinal (CST) and rubrospinal tracts (RST) of motor axons[17,24]. However, these attempts have not been able to produce truly tangible results[17,21].

Since the middle of the 20th century, biomedical research has been driven overwhelmingly by molecular technology-based focal endeavors. Considering that CNS function relies on the integrity of circuitry consortiums, our own investigative undertakings were orchestrated to define novel theoretical frameworks to better understand neural circuits pivotal for post-neurotrauma recovery. The outcomes reviewed here have indeed engraved a neural repair path that is much different from the established roadmap and logical reasoning for treating SCI. The findings to date support our *central hypothesis* that a main barrier preventing development of effective treatments for SCI may have been formed by conventional neurobiological principles that have been used to guide therapeutic development for the injured adult spinal cord. The paper thereby describes how some of these deficits were overcome by applying cross-disciplinary multimodal research strategies. The approach led to discoveries that have enabled us to determine alternative neural and neuromuscular circuitry to restore function post SCI, and to define two new academic concepts (see below)[18,25–29].

Functional Multipotency of Stem Cells Revealed in NSC-based Multimodal Investigations of SCI

Until the early 2000s, experimental strategies for treating acute injury to post-developmental mammalian spinal cord revolved mainly around promoting long distance axon regeneration, neuronal protection, preservation of residual axons and myelin by sparing oligodendrocyte (ODC), and neuronal or ODC regeneration. Several tactics were proposed and applied clinically, most notably anti-secondary injury therapy using high dose methylprednisolone (MP, which remains controversial[30]), minocycline[12,31], riluzole[32], granulocyte colony-stimulating factor[32], glibenclamide[32], and cethrin (VX-210)[32] as well as hypothermia[33] (benefits including reducing edema[34]). It was speculated that, following succession treatments for the brain and spinal cord that were presumably under top down management, interventions to promote CST and RST axon regeneration would reconnect the severed neural pathway to make the distal spinal cord function again. However, laboratory investments pursuing this traditional rationale and its related experimental designs to repair the lesioned CNS have so far not been fruitful[35]. As examples, many experiments used neurotrophins, neurotrophic factors, and different signaling pathway manipulation compounds, including those of oncogene activators to increase neuroprotection and axonal growth. In parallel, neutralizing antibodies to Nogo and other myelin, oligodendrocyte or reactive astrocyte-related "inhibitory molecules" were studied widely, albeit yielding contradictory reports[36,37]. Although the conflicting findings may not

totally negate the potential for the approach, they suggest that these strategies, when used in isolation, are not sufficient to promote functional restoration after SCI[21].

Encouragingly, work launched to introduce stem cell-based multimodal implants as a platform technology to investigate and treat the injured spinal cord, revealed that stem cells, NSCs, and MSCs (mesenchymal stromal stem cells) as pilot examples, could exert multiple biofunctions. These included mitigating secondary injury attacks (e.g., neuroinflammation, reactive gliosis, etc.)[27], promoting neural repair (production of trophic factors and anti-inflammatory cytokines, serotonergic reinnervation, endogenous NSC activation, angiogenesis, etc.)[14,27], and activating alternative neural pathways (e.g., proprioceptive input, propriospinal projection network, locomotion pattern generator, etc.)[26], all being derived from the inducible capabilities of stem cells to maintain homeostasis[38,39]. Apropos of the discoveries, we have come to view NSC as an "anchor" that can bind and integrate multiple therapeutic tactics. Studies, including our own, have demonstrated that NSCs, when transplanted into the injured brain or spinal cord of rodents or non-human primates, migrated preferentially to, and became integrated within, the damaged areas, with some showing differentiation markers matching those of host region-specific cells[25,28,40,41].

The impact of this vital function of NSCs is, in general, greater than any specific neuronal, astrocytic, or oligodendrocytic replacement per se. The precise mechanism by which NSCs exert this homeostatic pressure was originally not entirely clear, though, based on our work, it was attributable, to a large degree, to the intrinsic ability of NSCs to secrete neurotrophic and immunomodulatory factors, and to form gap junctions with host cells and other NSCs in inducible and regulatable manners[25,28,41–44]. Similar findings were independently reported by many other investigators, including Pluchino et al. [45,46], Llado et al. [47], Li et al. [48], Bjugstad et al., [40] and Redmond et al. [49].

An original example of harnessing and exploiting such inherent stem cell programs is presented here to illuminate our reasoning and research process. To direct neural repair more effectively following SCI, our collaborative team pioneered the platform design in which NSCs were cultured on a three-dimensional (3D) biosynthetic scaffold in vitro that mimicked the general structure of a healthy spinal cord[28]. It had an inner section, engineered to emulate the gray matter with an isotropic pore structure of 250–500 μm in diameter, to facilitate seeding of murine NSCs (mNSCs). The outer section of the scaffold, modeled to mimic the white matter, had long, axially oriented, pores for potential axonal growth guidance, and radial porosity to allow fluid transport while inhibiting the ingrowth of meningeal or astroglial scarring tissue by an outer shell layer. Implantation of the scaffolded mNSC unit into an adult rat T9–10 midline hemisection (lesion length: 4 mm) model of SCI resulted in long-term improvement in hindlimb function (persistent for 1 year) relative to control groups. At 70 days and >1 year post injury, animals implanted with scaffolded mNSCs still exhibited coordinated, weight-bearing hindlimb stepping. Histopathological and immunocytochemical analysis suggested that the recovery was not initiated by neuronal replacement or long distance CST axon regeneration despite the pro-neurogenic environment provided by the multimodal implant. Rather, it was attributable predominantly to a reduction in host tissue loss from secondary injury processes as well as neuroinflammation (e.g., diminished scale of chronic reactive gliosis). This work was the first to demonstrate explicitly the so called "chaperone" neuroprotective effects of the NSC in injured spinal cords[28]. The data suggested that donor-derived neuroprotection and promotion of local intraspinal cord neural plastic and other recovery events might have played a main role in inducing functional recovery. The results, besides demonstrating a novel platform technology to investigate and treat SCI, have more broadly served as a prototype for the use of NSCs or other types of stem cells to anchor multidisciplinary strategies in regenerative medicine, including gene therapy, material science-based bioreactor building, growth factor delivery, anti-inflammation treatment, and pharmacological intervention against secondary injury[15,50,51].

It has since been shown that NSCs hold innate biology traits that involve their default ability to, under proper induction, produce secretomes (i.e., all proteins secreted into the extracellular space, represented by neurotrophic factors and other cytokines) and exosomes, cell-derived vesicles that spread molecules of proteins and various types of nucleic acids (e.g., DNA, RNA, and miRNA), as well as their capability to form gap junctions and undergo cell fusion (Fig. 1). All manifests in a developmental stage- and/or microenvironment-dependent fashion[42,52–55]. The unique multi-functional profile is also possessed by other types of stem cells including MSCs[18,26,56], embryonic stem cells[57], and induced pluripotent stem cells (iPS cells)[58]. This inducible multifunctionality (i.e., functional multipotency) empowers stem cells to interact with the surrounding environment, in a suitable, regulated, stimulus-appropriate manner, seeking to maximize cell survival[15]. These factors, in our assessment, are components of the stem cell's inherent developmental program. It can be literally "called to active duty" by environmental cues via specific signaling transduction or biophysical impact to exert proper homeostatic forces on a dynamically growing organ system which, otherwise, could become dysequilibrated[59]. The result of the inherent "program"—a dividend from developmental biology—is to promote, enable, induce, or catalyze the host to work constructively with stem cells in an attempt to build or reconstitute its own tissue, to minimize hurdles to this process, and to protect endangered cells from cell death or other harmful influences. Methods to optimize this process—i.e., to act in concert with normal developmental propensities, is undoubtedly desirable for augmenting any tissue, organ or system repair[21,41].

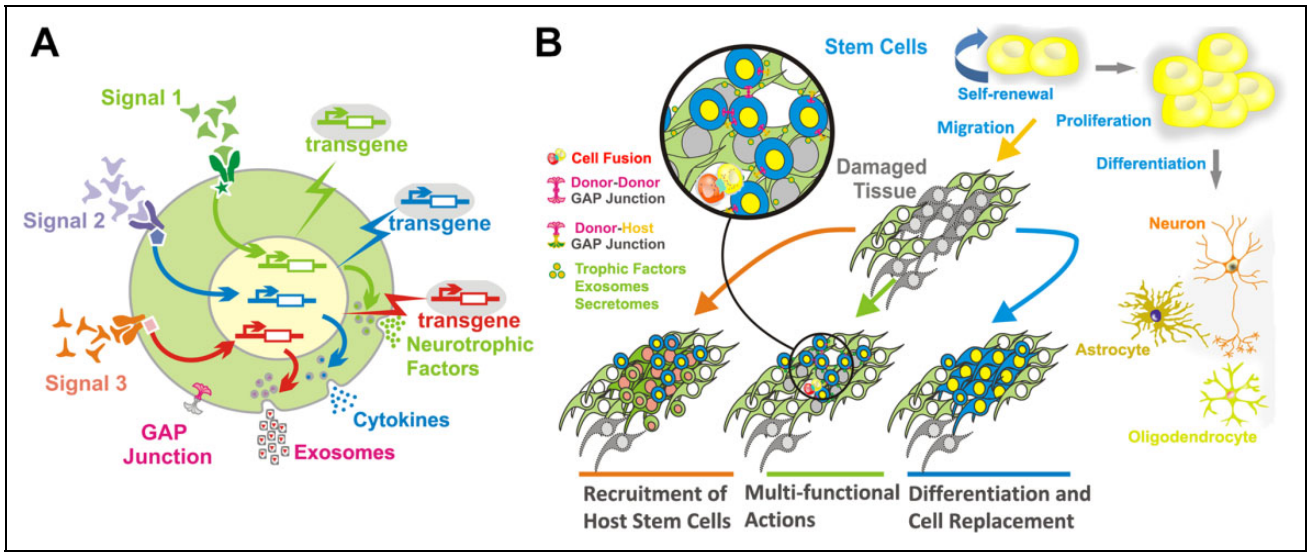

Fig. 1. Schematic summary of functional multipotency of stem cells. (A) Besides lineage development, stem cells possess intrinsic capabilities to respond to environmental signaling stimulation to customize the content profile of secretomes and exosomes to stage homeostasis. This capacity can be further tailored by genetically engineering the cells with extra copies of transgenes of desirable molecules. (B) Donor stem cells, prototype or genetically modified, can provide therapeutic benefits through at least three distinct mechanisms that may cast synergistic impacts: (1) homeostatic regulation through functional multipotency to perform target homing to deliver cytokines in interactive manners that are regulated via specific signaling pathways, to establish gap junctions, and to form cell fusion (upper inset); (2) replacement of the dysfunctional or dead host cells; and (3) recruitment of and nourishment for host endogenous stem cells. Therapeutic mechanism No. 1 apparently carries a wide spectrum of regulatory tactics that can be further explored to refine the trophic factor and/or other molecules (e.g., microRNA) secretion at each developmental stage or neural disorder status as NSCs integrate into and prepare, modify, and guide the surrounding CNS environment towards the homeostatic formation and maintenance of a physiologically functioning adult nervous system.

The author, working with his colleagues, proposed and subsequently established an updated concept of the stem cell. The concept of "Functional Multipotency of Stem Cells" describes that in addition to the essential totipotency, pluripotency or multipotency of lineage phenotypic development, stem cells possess transiently inducible biofunctions relative to the fixed spectrum of functions of a terminally differentiated cell, to mediate proper cell division, migration, differentiation, organogenesis, and system function under homeostasis[14,15,27,39,60]. This theory provides a more complete picture of the stem cell biology, rendering phenotypic differentiation of pluripotent or multipotent stem cells (e.g., the ability of NSCs to differentiate into all three types of neural cells) that the conventional study principally touches on only as one part of the entire stemness biology portfolio.

Under this novel conceptual context, investigators can further appreciate and seek the logic and technology behind the wide range of molecular tactics the stem cell appears to operate at each developmental, adult or aging stage as it integrates into and prepares, modifies, guides, and repairs the surrounding micro- and macro-environment towards the formation and self-maintenance of a physiologically functioning tissue, organ, and system (Fig. 1). Evidently, embracing this understanding of the stem or progenitor cell's "functional multipotency" in comparison to specialized functions of other adult cells (e.g., insulin production by a pancreatic beta cell) is crucial. Implementing this guideline will create opportunities for researchers to more correctly and optimally exploit stem cell biology to advance investigational or therapeutic applications that will ultimately include reconstituting and reactivating dysfunctional CNS-Peripheral Effector circuitry.

Recovery Neurobiology of Injured Spinal Cords Defined by Functional Multipotency of Stem Cells

Due to the pathophysiological complexity and limited natural recovery capability of the adult mammalian CNS, efficacious treatment of neurotrauma, stroke, and neurodegenerative diseases remains an unmet clinical demand. Based on a broad spectrum of reports, including our own describing varied therapeutic effects of NSCs, it has become clear that functional multipotency of stem cells can be judiciously used as an investigative tool to evaluate what may be key components to initiate functional recovery of injured adult mammalian spinal cords[14,15]. For this purpose, we focused on human MSCs (hMSCs), which can offer autologous transplantation feasibility[25,61], and have been experimentally and clinically shown to exert therapeutic effects on SCI and brain injury (TBI)[25,62,63]. It is worth noting that studies of neural transdifferentiation possibility of MSCs (i.e., putative differentiations of MSCs into neural cells

without reentering the pluripotency phase) did not show long-term functional improvement in SCI models. The poor outcomes were thought to be caused primarily by suboptimal survival of MSCs, leaving this and other neural therapeutic mechanisms of MSCs undetermined[64]. Most clinical SCI are acutely incomplete and could potentially benefit from our established technology of 3D biodegradable polymer scaffolding for NSC delivery into the injury epicenter to improve donor efficacy. We had an opportunity to reductively verify neurobiological mechanisms underlying motosensory recovery of the injured spinal cord by functional multipotency-mediated effects of hMSCs. However, the study had to be done without interference from donor-derived neural cells. The aim was achieved by scaffolding hMSC (note: not NSCs that have inevitable possibility of becoming neural cells) in specially tailored poly(lactic-*co*-glycolic) acid (PLGA) polymer[18]. Specifically, to deploy hMSC-produced multimodal actions that promote neural protection, beneficial plasticity including endogenous NSC proliferation, anti-inflammation, and angiogenesis but no transdifferentiation[25,51], we designed a unique microtexture PLGA scaffold that maintained the stemness of hMSCs and verified it in an organotypic dorsal root ganglion (DRG) coculture system. Applying pro-inflammatory agents according to different designs in such an in vitro system induced both anti-inflammatory and proneurogenic actions of the scaffolded hMSCs[18]. Next, the multifaceted effects of hMSCs in the scaffold-improved survival and stemness status were comprehensively studied in vivo to probe the host cellular and circuitry components underlying the "Recovery Neurobiology" as a new theoretical framework of injured adult mammalian spinal cords[15,26].

The study established that uniquely tailored polymer scaffolding maintained hMSC stemness and enhanced donor engraftment, resulting in robust motosensory improvement, neuropathic pain and tissue damage mitigation, and myelin preservation[26,28]. The scaffolded nondifferentiated hMSCs exerted multimodal effects of neurotrophism, angiogenesis, neurogenesis, antiautoimmunity, and antiinflammation. Hindlimb locomotion was improved by reinstated integrity and activity of submidbrain circuits of serotonergic reticulospinal innervation at lumbar levels, the propriospinal projection network (PSN), neuromuscular junction (NMJ), and central pattern generator (CPG).

The approaches provided both in vitro and in vivo platforms for understanding molecular mechanisms, cellular interaction and neural/neuromuscular circuitry underlying the neural therapeutic impact of hMSCs[18,26]. Our findings derived from inductive and deductive data analyses elucidated that "Recovery Neurobiology", as an academic concept, is the study of the ability of the injured adult spinal cord, under proper treatment, to deploy polysynaptic neural circuits different from normal neurophysiological pathways for postinjury representation of function." Notably, the essential components of the recovery neurobiology (e.g., PSN, serotonergic modulation[65], NMJ, and CPG) can be targeted for development of neurological, neurosurgical and functional rehabilitation therapies to overcome disabilities and complications of clinical SCI and other neurological disorders.

Conclusion

It has been increasingly recognized that malfunctioning CPG circuitry post SCI results from deprivation of descending, ascending, and peripheral input, which is responsible for neuromuscular degeneration[66]. Hypothetically, CPG plasticity can be tuned beneficially by effective interventions such as stem cell-based multimodal treatment to reconstruct a functional neuromuscular network for communication among the limb and trunk muscles, PSN network, lumbar or cervical cord CPG, and intra-spinal cord serotonergic modulation[26,67,68]. This postulation has been confirmed repeatedly by anecdote clinical case reports since 2002[69–71]. Indeed, functional recovery, including over-ground walking, was achieved for patients of varied age groups with subacute and chronic severe SCI, following formulated lumbar stimulation and locomotion training[69–72].

The data suggested that epidural electrical stimulation primarily facilitated propriosensory input. The cases therefore showed that activity, plasticity, and local circuit-dependent CPG recovery in the lesioned spinal cord is clinically feasible if key mechanistic targets of Recovery Neurobiology can be therapeutically tuned. Restoration of lower (or hind) limb locomotion in humans (or rodents) does not require regeneration of CST or RST axons to reinnervate neurons located below the injury site (including neoplastic lesion[73,74]) as long as the injury spares the CPG, its surrounding PSN and related peripheral nerve innervation of the effector muscles[26,69–75].

What has been appreciated is that investigating such Recovery Neurobiology targets for SCI will need to be done comprehensively in a multimodal manner. The strategy should concurrently treats (1) abnormality of NMJs and muscles[76–78], (2) deficiency of descending and ascending neural facilitation (e.g., 5HT modulation and proprioceptive input)[13,26,65], and (3) CPG malfunction[79]. Therefore, the author emphasizes that SCI research has to pay substantial attention to understanding fundamental neurobiology of the adult spinal cord in regards to its altered relationship with the brain after injury. Equally important for devising SCI therapies is to obtain anatomic specifics of neuromuscular connectomes (i.e., the complete map of the connections in the nervous and muscular systems) involved bidirectionally and bilaterally in the spinal cord-reptilian brain motor pattern generation in adult primates[79–81]. The anticipated findings will permit the field to move more effectively towards uncovering how to recouple the adult mammalian sensorimotor cortex with the distal spinal cord post injury.

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