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Human neural progenitors differentiate into astrocytes and protect motor neurons in aging rats

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ABSTRACT

Age-associated health decline presents a significant challenge to healthcare, although there are few animal models that can be used to test potential treatments. Here, we show that there is a significant reduction in both spinal cord motor neurons and motor function over time in the aging rat. One explanation for this motor neuron loss could be reduced support from surrounding aging astrocytes. Indeed, we have previously shown using *in vitro* models that aging rat astrocytes are less supportive to rat motor neuron function and survival over time. Here, we test whether rejuvenating the astrocyte niche can improve the survival of motor neurons in an aging spinal cord. We transplanted fetal-derived human neural progenitor cells (hNPCs) into the aging rat spinal cord and found that the cells survive and differentiate into astrocytes with a much higher efficiency than when transplanted into younger animals, suggesting that the aging environment stimulates astrocyte maturation. Importantly, the engrafted astrocytes were able to protect against motor neuron loss associated with aging, although this did not result in an increase in motor function based on behavioral assays. We also transplanted hNPCs genetically modified to secrete glial cell line-derived neurotrophic factor (GDNF) into the aging rat spinal cord, as this combination of cell and protein delivery can protect motor neurons in animal models of ALS. During aging, GDNF-expressing hNPCs protected motor neurons, though to the same extent as hNPCs alone, and again had no effect on motor function. We conclude that hNPCs can survive well in the aging spinal cord, protect motor neurons and mature faster into astrocytes when compared to transplantation into the young spinal cord. While there was no functional improvement, there were no functional deficits either, further supporting a good safety profile of hNPC transplantation even into the older patient population.

1. Introduction

Sarcopenia, the loss of skeletal muscle mass and function, is a common symptom of aging (Miljkovic et al., 2015). The elderly undergo a steady decline in muscle strength, which can lead to reduced motor function and increased frailty. Furthermore, with age there is an exponential increase in the prevalence of amyotrophic lateral sclerosis (ALS), a neurodegenerative disease characterized by motor neuron death, neuromuscular junction (NMJ) denervation, muscle wasting and motor function loss (Gordon, 2013). As such, novel methods to alleviate or regenerate age-related motor function decline could potentially both enhance the overall health of the elderly and slow age-associated diseases like ALS.

The underlying cause of sarcopenia is unknown but there are likely multiple contributory factors such as a reduction in satellite cell activation (Ryall et al., 2008) and NMJ degeneration (Gonzalez-Freire et al., 2014). In ALS, massive loss of motor neurons is a critical source of ensuing muscle atrophy (Gordon, 2013), suggesting that perhaps a similar phenomenon could occur in normal aging. Indeed, in humans, spinal motor neuron numbers undergo a steady decline around 60 years of age, dipping to 30–50% total loss of motor neurons in the following two decades (Tomlinson and Irving, 1977; Lexell, 1997; Kawamura et al., 1977). Interestingly, despite motor neuron loss beginning at age 60, a significant decline of actual motor function does not typically occur until later (Rygiel et al., 2014). The fact that substantial loss of motor neurons is required before an observable decline of motor function appears, suggests that there is a time window during which motor neuron protection may be able to delay ensuing motor function demise.

Stem cell transplantation is a promising therapeutic method for the replacement or protection of motor neurons that degenerate during aging and in neurodegenerative diseases. One difficulty with replacing dying motor neurons with stem cell-derived neurons is that transplanted neurons lack the temporal and spatial control for proper

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circuit rewiring (Gowing and Svendsen, 2011). An alternative approach to replacing dying neurons is to protect these cells. One method of protection could be the introduction of astrocytes, the main support cells of the central nervous system (Nicaise et al., 2015; Lund et al., 1993). Importantly, we have recently shown *in vitro* that young astrocytes are significantly more supportive to motor neurons than old astrocytes (Das and Svendsen, 2015). Neural progenitor cells (NPCs) can differentiate into astrocytes, which makes them an ideal source for young astrocytes that can be transplanted to protect host neurons (Thomsen et al., 2014b; Haidet-Phillips and Maragakis, 2015). Indeed, *in vivo* work from our group has shown that fetal-derived human neural progenitor cells (hNPCs) transplanted into the $SOD1^{G93A}$ transgenic ALS rat spinal cord differentiate into astrocytes and promote motor neuron survival (Klein et al., 2005; Nichols et al., 2013; Suzuki et al., 2007a). Therefore, we hypothesized that the transplantation of hNPCs into the aged spinal cord may create a niche of "young" astrocytes that could promote the survival of aging motor neurons. Furthermore, as the preservation of motor neurons may preserve age-associated reductions in muscle mass, strength and function, we postulated that motor neuron survival may rescue an age-related decline in motor function.

Glial cell line-derived neurotrophic factor (GDNF) is a potent pro-survival factor for motor neurons (Zurn et al., 1994; Zhao et al., 2004; Henderson et al., 1994). Our group has extensively shown that hNPCs genetically modified to secrete GDNF can protect degenerating motor neurons in the lumbar spinal cord of $SOD1^{G93A}$ transgenic ALS rat (Klein et al., 2005; Suzuki et al., 2007a). Additionally, adding GDNF to aged astrocyte cultures was able to improve the astrocytes' ability to offer neuronal support (Das and Svendsen, 2015). As such, we hypothesized that the transplantation of GDNF-secreting hNPCs into the aging spinal cord could protect aged motor neurons through the synergistic presence of both young astrocytes and growth factor.

In this study, we first asked whether there is a discernible motor neuron loss in the rat cervical spinal cord during aging and whether it faithfully recapitulates the sarcopenia phenotype seen in human aging. To our knowledge there are no studies that have transplanted neural progenitor cells into the aged rat spinal cord. Therefore, we next transplanted either wildtype hNPCs or GDNF-secreting hNPCs into the rat spinal cord over time in order to confirm whether cells can survive and engraft in this environment, a key consideration for the feasibility of using progenitor cell transplants in aging recipients. Finally, we tested whether wildtype and GDNF-secreting hNPCs can protect motor neuron loss and any downstream effects on the aging motor system.

2. Methods

2.1. Animals

Male Sprague–Dawley rats (Taconic, Hudson, NY) were bred with female Sprague–Dawley rats (Harlan, Indianapolis, IN). Aged rats were wildtype Sprague–Dawleys that were bred in house and maintained in our colony. All laboratory animal procedures were approved by the Institutional Animal Care and Use Committee of Cedars-Sinai Medical Center.

2.2. hNPC

Briefly, hNPCs were derived from a human male fetal cortex at 8 weeks of age and propagated in culture as free floating neurospheres (Sareen et al., 2009; Svendsen et al., 1998). Wildtype hNPCs ($hNPC^{WT}$) were infected with lentivirus encoding human GDNF in order to generate hNPCs stably expressing GDNF ($hNPC^{GDNF}$). Research lot vials of long-term (10 weeks) cultures of $hNPC^{WT}$ or $hNPC^{GDNF}$ were generated by dissociating the neurospheres using TrypLE (ThermoFisher Scientific, Canoga Park, CA) and single cells were *re*-suspended in freezing media (Cell Freezing Medium-DMSO Serum Free, 1×, C6295; Sigma-Aldrich, St. Louis, MO) at a concentration of 5×10^6 cells/ml for storage in liquid nitrogen (Shelley et al., 2014).

$hNPC^{WT}$ or $hNPC^{GDNF}$ research lots were thawed, rinsed with 2.6% DNase I (Pulmozyme, Genentech, San Francisco, CA) in Hibernate medium (Gibco, Canoga Park, CA), counted, centrifuged, re-suspended at the final concentration (5×10^4 cells/μl) in Hibernate E media and stored on ice during surgery. Cell viability before surgery was determined using trypan blue exclusion counts and viability after surgery was confirmed by plating cells pre-surgery and post-surgery on laminin-coated coverslips for 24 h before fixation.

2.3. Transplant surgeries

Rats were anesthetized with isoflurane and the cervical vertebrae were exposed and clamped in a spinal stereotaxic frame (David Kopf Instruments, http://www.kopfinstruments.com) to maintain a steady position. Injection holes were drilled in laminae within C3 and C5 spinal cord segments, and the spinal cord was exposed. Cell suspension (2 μl per injection site at a concentration of 5×10^4 cells/μl) was bilaterally injected at 8 injection sites beginning at 1.5 mm ventral from the dorsal dura surface at C3 to C5 using a 10 μl Hamilton syringe outfitted with a 75–100 μm tipped micropipette. For sham surgeries, rats received the identical surgery with 2 μl of Hibernate media per injection site. Cyclosporine (10 mg/kg, Novartis, Cambridge, MA) was administered intraperitoneally daily beginning 3 days before surgery and continuing until sacrifice.

2.4. Behavioral testing

Body weight measurements and all behavioral testing began one week before transplantation and continued weekly until sacrifice.

The Basso–Beattie–Bresnahan (BBB) locomotor rating test was used to measure motor function (Basso et al., 1995; Thomsen et al., 2014a). Rats were allowed to walk freely while hindlimb and forelimb movements were observed for approximately 4 min. Each hindlimb BBB score was based on the 21 point scoring scale from no movement (0) to normal locomotion (21). Scoring takes into account paw rotation, toe clearance, weight support, the frequency of each, and the amount of movement occurring from each joint.

A grip strength assay was used to measure the maximal force of forelimbs before the animal releases the bar. Animals were held around the midsection, facing the bar of the grip strength meter (GSM). The animals were held parallel to the bar so that they do not reach at an angle during the trials. The hindlimbs were not allowed to touch any surface. When the forepaws are brought into contact with the bar of the GSM, the animals reliably grasp the bar and are then gently pulled away from the device. The rat is allowed to grip the bar fully and the way that the grip is established is observed for accuracy of reading before pulling away. Each testing session assessed the forepaws separately four times.

An open field activity test was used to measure activity. Rats were placed in a plexiglass enclosure in which beam breaks were calculated over a period of 10 min.

2.5. Tissue collection and histology

Rats were anesthetized and transcardially perfused with 0.9% NaCl and fixed with 4% paraformaldehyde (PFA) (Sigma-Aldrich). Spinal cord tissue was collected, post-fixed for two hours in 4% PFA, and transferred to 30% sucrose for 48 h before sectioning at 35 μm on a sliding microtome (SM2010R; Leica, Wetzlar, Germany). Every 12th section sample of the cervical spinal cord was immunostained according to standard techniques with the following human-specific antibodies against Nissl (NeuroTrace, 1:10,000), cytoplasm (SC121, 1:2000) and nucleus (SC101, 1:1000) and glial fibrillary acidic protein (GFAP,