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CONCISE REVIEW

STEM CELLS TRANSLATIONAL MEDICINE

Neural crest-like stem cells for tissue regeneration

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Abstract

Neural crest stem cells (NCSCs) are a transient population of cells that arise during early vertebrate development and harbor stem cell properties, such as self-renewal and multipotency. These cells form at the interface of non-neuronal ectoderm and neural tube and undergo extensive migration whereupon they contribute to a diverse array of cell and tissue derivatives, ranging from craniofacial tissues to cells of the peripheral nervous system. Neural crest-like stem cells (NCLSCs) can be derived from pluripotent stem cells, placental tissues, adult tissues, and somatic cell reprogramming. NCLSCs have a differentiation capability similar to NCSCs, and possess great potential for regenerative medicine applications. In this review, we present recent developments on the various approaches to derive NCLSCs and the therapeutic application of these cells for tissue regeneration.

KEYWORDS
adult stem cells, disease modeling, neural crest stem cells, placental stem cells, regenerative medicine

1 | INTRODUCTION

Neural crest stem cells (NCSCs) are a transient, multipotent cell population that originates along the border of the neural plate during early vertebrate development.[1,2] Various signaling molecules, including Wnt, fibroblast growth factor (FGF), bone morphogenic protein (BMP), Notch and retinoic acid (RA), derived from the non-neural ectoderm, neuroepithelium and underlying mesoderm, activate a cascade of transcription factors that dictate where these cells will form and further develop.[3] These cells undergo an epithelial-to-mesenchymal transition, which allows them to acquire a mesenchymal phenotype upon detaching from their neighboring cells and delaminating from the dorsal neuroepithelium.[4] Such an event induces

these cells to migrate extensively within the developing embryo whereupon they experience various environmental cues. Accumulative evidence indicates that some cells lose plasticity and confer a fate during or even before migration, although a subpopulation of migratory cells appear to retain their multipotency.[5-8] After settling in their final sites of differentiation, these cells proceed to give rise to wide array of cell types and tissues, including bone, cartilage, melanocytes, fibroblasts, and smooth muscle cells (SMCs), in addition to neurons and glial cells.[2-4] As NCSCs are responsible for generating a diverse population of cells and tissues, dysregulation of neural crest (NC) development, cell migration, and differentiation can lead to a broad spectrum of human congenital disorders, including cardiovascular defects, melanoma, craniofacial defects, and neuroblastoma, collectively known as neurocristopathies.[9,10] Therefore, NCSCs can be used to generate a variety of specific cell types for disease modeling. Furthermore, the multipotency of NCSC differentiation makes NCSCs a valuable cell source for tissue regeneration.

In the past, NCSC isolation was limited to embryonic tissues. NCSCs generally express markers such as Sox10, Slug, Snail, Twist, AP-2α, $p75^{NTR}$, and HNK1. Sox10 is critical for neurogenesis and maintenance of multipotency,[11-13] whereas Sox17 functions in the development of definitive endoderm and vasculogenesis.[14,15] Sox9,[16-18] Slug,[19,20] Snail,[21-23] and Twist[24,25] are all found closely related to NC and neural tube development. AP-2α is required for NC induction and expressed among actively migrating NCSCs.[26-28] $p75^{NTR}$ and HNK1 can be utilized to identify migratory NCSCs, albeit HNK1 only labels a small proportion of migrating human NCSCs.[28,29] Recently, the development of pluripotent stem cells (PSCs), especially induced pluripotent stem cells (iPSCs) has enabled the derivation of human neural crest-like stem cells (NCLSCs), providing an opportunity to not only better understand human development, but also an unlimited cell source for patient-specific disease modeling and therapy. In addition, significant progress has been made in identifying and isolating NCLSCs from fetal, perinatal, and adult tissues that can potentially serve as viable cell sources for tissue regeneration. In this review, we will present recent findings on available sources of NCLSCs, and discuss their potential application for tissue engineering and regenerative medicine (Figure 1).

Generally speaking, NCSCs are a cell population in NC-derived tissues during embryonic development, whereas all other sources of NCSCs, including those derived from PSCs in vitro and those isolated from placental and adult tissues, should be defined as NCLSCs. However, previous studies have interchangeably utilized NCSC to describe NCLSC derived from PSCs and adult tissues, and some NCLSCs have been named differently based on their tissue origin. Furthermore, there is also an argument that some adult NCLSCs are remnants of NCSCs arising during the development. To clearly distinguish all of these NCSCs and NCLSCs, single cell RNA sequencing, in addition to lineage tracing, needs to be performed to classify the cells based on genetic profile, which has not been done in a vast majority of previous studies. Therefore, when presenting results from specific studies, we generally use NCSC or NCLSC as we define it, but sometimes utilize the term as defined by the authors of the study to accommodate their hypothesis and viewpoint and to avoid confusion and controversy.

Significance statement

Neural crest stem cells (NCSCs) are a transient population of cells that arise during early vertebrate development and harbor stem cell-like properties. The multipotency of NCSCs enables the generation of a diverse population of cells, thereby making NCSCs a valuable cell source for tissue regeneration, disease modeling, and drug discovery. Although NCSC isolation was initially limited to embryonic tissues, neural crest-like stem cells can be derived from pluripotent stem cells, placental tissues, adult tissues, and somatic cell reprogramming, providing viable cell sources for tissue engineering and regenerative medicine.

1.1 | Embryonic, fetal, and placental tissue-derived NCLSCs for tissue regeneration

NCSCs have been identified and isolated from various embryonic and fetal tissues,[30] including the trunk neural tube, sciatic nerve, dorsal root ganglia (DRG), gut, heart, and branchial arches. These NCSCs can self-renew and differentiate in vitro and in vivo into distinct NC derivatives,[30] such as neurons, glia, myofibroblasts, osteocytes, and SMCs. Recently, a new method to culture premigratory NCSCs as "crestospheres" was developed, which enabled the maintenance of these cells in vitro for an extended period of time without the loss of their stem-cell characteristics.[31,32] Early studies using postmigratory NCSCs isolated from distinct regions within the embryo revealed that spatially distinct NCSCs displayed cell intrinsic differences that regulated their developmental potential in vivo,[33,34] suggesting that for tissue regeneration strategies, postmigratory NCSCs should be isolated from locations that match where these cells will be utilized therapeutically.

The boundary cap located at the dorsal root entry zone has also served as a source of embryonic NCSCs,[35] which have shown potential as a therapy for pancreatic and neurodegenerative disorders. Boundary cap NCSCs (bNCSCs) cotransplanted with mouse or human pancreatic islets were found to enhance beta cell proliferation and improve islet graft reinnervation and revascularization in diabetic mice, possibly restoring neural-islet interactions that consequently improved islet engraftment and function after transplantation.[36-38] Furthermore, in an in vitro coculture system, bNCSCs partially prevented the cell death of human insulin producing cells in response to pro-inflammatory cytokines, suggesting a potential mechanism by which bNCSCs offer a protective effect that improves islet transplantation and thus, in combination with pancreatic islets, may serve as attractive cell source to treat patients with type 1 diabetes.[39] Additionally, bNCSCs have demonstrated beneficial effects on nerve regeneration. Transplantation of bNCSCs to the site of a dorsal root avulsion injury resulted in cell migration and neuronal differentiation in the host spinal cord as well as differentiation into glia that associated with regenerating sensory axons in the peripheral nervous

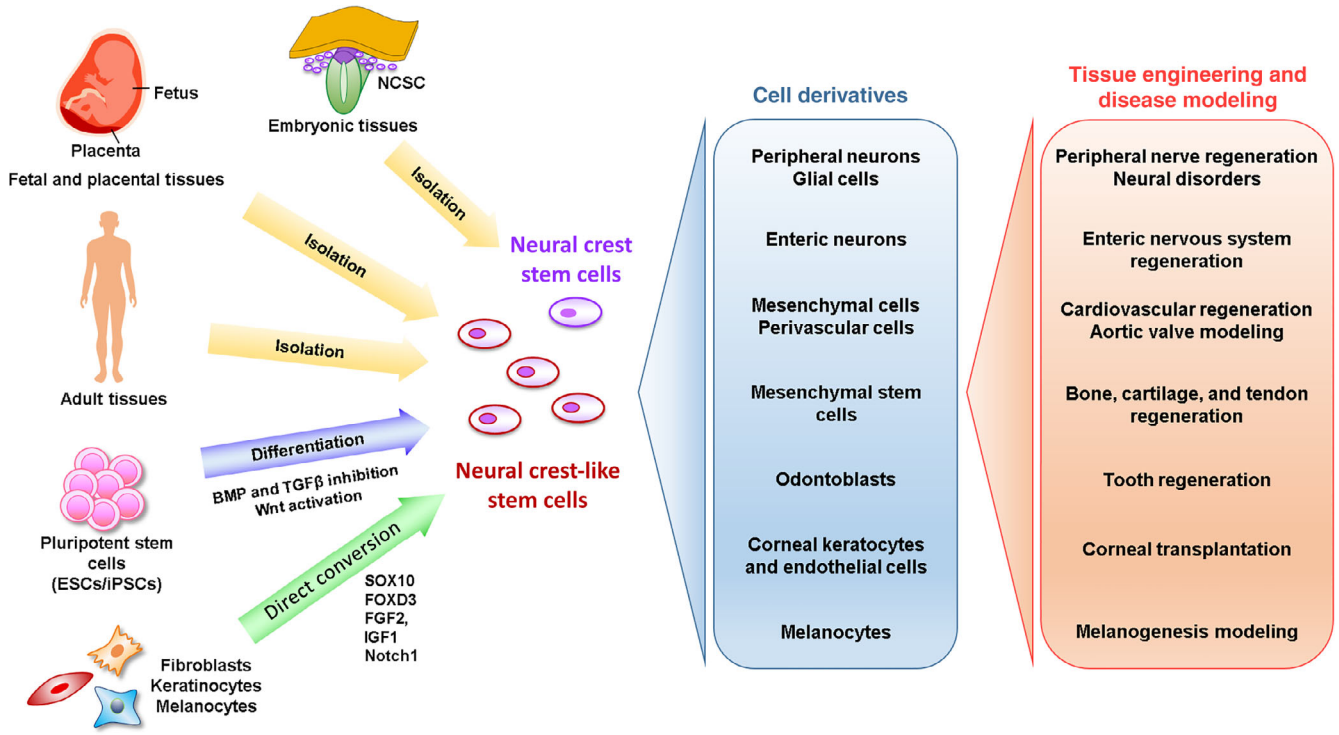

FIGURE 1 Sources and potential applications of neural crest stem cells (NCSCs) and neural crest-like stem cells (NCLSCs). NCSCs and NCLSCs can be isolated from embryonic, fetal, placental, and adult tissues. NCLSCs can also be derived in vitro from pluripotent stem cells and mature cells through differentiation and reprogramming strategies, respectively. NCSCs and NCLSCs can be differentiated to yield distinct cell derivatives that are highly valuable for tissue engineering applications and disease modeling

system.[40,41] These bNCSCs not only improved the survival of motor neuron precursors following transplantation into the spinal cord of adult mice,[42] but also prevented the loss of spinal cord neurons and glial activation in acutely injured spinal cord slice cultures through the secretion of brain-derived neurotrophic factor (BDNF),[43] suggesting these cells exhibit neuroprotective, anti-apoptotic, glia-inhibitory, and neurotrophic effects that may aid in neuroregenerative therapies.

Extraembryonic placental tissues, including early or later gestation chorionic villus tissues, are a unique cell source, yielding robust placental mesenchymal stem/stromal cells (PMSCs) well-suited for autologous or allogeneic cell therapy and tissue engineering. Interestingly, PMSCs express NCSC transcription factor markers including Sox9, Sox10, Sox17, Slug, Snail, and Twist,[44,45] suggesting PMSCs may be NC-derived and a type of NCLSC. The uniformity of expression of these transcription factors in PMSC cultures may reflect the developmental origins of these cells and could serve as predictors of some related functional properties. PMSCs were found to also express stem cell-related intracellular structural proteins Nestin, neurofilament medium, and S100β that are often associated with neural lineage phenotypes. Methods have been established to expand PMSCs from various placental tissues.[44-48] The in vitro characteristics of PMSCs are also analogous to those of MSCs isolated from various source tissues[49] in terms of surface marker expression and multipotency.[44-48]

It has been shown that PMSCs display notable immunomodulatory capabilities,[50,51] exhibit wound healing capacity,[52] demonstrate neuroprotective effects,[45,53-56] and may exhibit greater immunomodulatory properties and ex vivo expansion potential compared with adult BM-MSCs.[46,51] As quantified by ELISAs, PMSCs secreted significantly higher amounts of BDNF and hepatocyte growth factor (HGF) than adult BM-MSCs. Both BDNF and HGF are growth-promoting and chemoattractant for young embryonic cranial motor axons.[57] BDNF is a powerful neurotrophin for neuronal regeneration after injury.[58] HGF is also a potent immunoregulatory[59] and angiogenic factor that has been shown to activate endothelial cell migration and proliferation and may contribute to wound healing in vivo by promoting rapid vascularization.[60] Preclinical studies have shown that PMSCs secrete significant amounts of neuroprotective growth factors and cytokines in vitro,[44,45,56] and can protect neurons from damage in vivo,[45,61-63] suggesting that PMSCs are a potent therapy for developmental or perinatal neurological diseases. Specifically, PMSCs have been used to treat a neurodevelopmental disease, myelomeningocele (MMC), commonly known as spina bifida, which is caused by incomplete neural tube closure during development of the spinal cord. Our preliminary animal research in the rodent model[64] as well as in the well-established fetal sheep model[45,61-63] of MMC has shown that in utero treatment with human PMSCs can functionally cure the paralysis associated with MMC in a dramatic and consistent manner. Treatment of MMC with PMSCs in conjunction with an extracellular matrix (ECM) scaffold as a delivery vehicle (PMSC-ECM) drastically and significantly improved the locomotor function compared with control animals treated with delivery vehicle alone, and histological analysis demonstrated that PMSC-ECM consistently and

significantly increased neuron survival in the diseased spinal cord.[45] No adverse effects were observed in any of the lambs treated with human PMSC-ECM. Currently, clinical grade PMSCs produced under current good manufacturing practice[65] are being evaluated in pivotal safety studies and IND-enabling studies and moving toward a clinical trial for the treatment of MMC in human patients.

Enteric neural crest cell (ENCC) is another type of NCLSC that has been utilized for the treatment of enteric neuropathies. ENCCs have been isolated from embryonic and postnatal murine intestine[66,67] and more recently, from fetal and postnatal human gut.[68-71] In murine studies, ENCCs were capable of colonizing the gut upon migrating and differentiating into enteric neurons and glia. Furthermore, these cells formed neural networks and were able to functionally integrate within the host bowel in vivo without any long-term safety issues.[72,73] A recent study investigated the functional viability of ENCCs derived from fetal human gut following in vivo transplantation into postnatal murine colon.[71] It was demonstrated that these cells displayed engraftment, differentiated into neurons and glia of the enteric nervous system (ENS), and moreover, established functional connectivity with the endogenous ENS. The successful engraftment of transplanted ENCCs provides support for the development and use of ENCC as a cell replacement therapy in enteric neuropathies. Apart from ENCCs, NCLSCs from postnatal DRG were also shown to survive, colonize the appropriate gut layers, and generate functional enteric neurons that could integrate with the endogenous ENS following transplantation into the distal colon of postnatal mice,[74] suggesting that NCLSCs from postnatal tissues besides the gut can undergo ENS differentiation and, therefore, may be another potential candidate for the replacement of ENS cells.

1.2 | Adult tissue-derived NCLSCs for tissue regeneration

As NCSCs proceed through embryonic development, their developmental potential becomes limited and there is a loss of multipotency, although we cannot completely exclude the possibility of NCSC remnants in postnatal tissue. To date, NCLSCs have been discovered in multiple adult tissues, including DRG,[75] skin,[76,77] gut,[66] heart,[78,79] carotid body,[80] nasal passageways[81] and cavity,[82] adipose tissue,[83-85] bone marrow (BM),[86,87] iris,[88] cornea,[89] oral mucosa,[90] palate,[91,92] dental pulp,[93] and periodontal ligament.[94,95] Adult tissue-derived NCLSCs express NCSC markers such as $p75^{NTR}$, Sox10, Sox9, and Snail1/2, and exhibit self-renewal and multilineage differentiation into various cell types,[30,96,97] including neurons, glia, cardiomyocytes, adipocytes, smooth muscle, and chondrocytes, although self-renewal capacity appears to decline with age and these cells demonstrate reduced differentiation potential compared with fetal NCSCs.[66] Despite this, NCLSCs from adult tissues possess stem cell-like qualities, which make them great potential candidates for tissue regeneration.[97] Most of the studies carried out, thus far, using adult tissue-derived NCLSCs have primarily focused on blood vessel, nerve, and bone regeneration.

Vascular stem cells (VSCs) play an important role in vascular remodeling and regeneration.[98] NCLSCs, as a type of VSCs, were found in the media and adventitia layers.[99] These $Sox10^+$ NCLSCs not only differentiated into SMCs in the neointima, but also contributed to chondrogenic and osteogenic cell types in the atherosclerotic lesion, providing a novel perspective on the development of vascular diseases. NCLSCs were also identified in the perivascular cells around microvessels throughout the body.[100,101] Whether NCSLCs isolated from a variety of vascularized tissues are actually vascular NCLSCs remains to be investigated. When $Sox10^+$ NCLSCs were injected into ischemic limb, these cells formed perivascular cells and promoted angiogenesis.[100]

Peripheral nerve injuries (PNIs) are some of the most common types of traumatic lesions affecting the nervous system, which can result in reduced quality of life in affected patients and be a huge social burden.[102] PNI continues to be a major challenge in reconstructive neurosurgery. Owing to huge clinical demand, peripheral nerve regeneration, particularly larger gap injuries, has become a prime focus of basic and clinical research. Accelerating axonal regeneration to promote reinnervation and improve functional recovery after PNI is a clinical necessity and an experimental challenge. Numerous studies have demonstrated the potential utilization of adult tissue-derived NCLSCs for peripheral nerve regeneration.[102-106] Vascular NCLSCs transplanted into nerve conduits enhanced sciatic nerve regeneration.[107] These cells differentiated into perineural cells around the bundles of regenerated myelinated axons, but did not differentiate into Schwann cells. In a recent study, Zhang et al examined the effects of cell cotransplantation on PNI using epidermal NCSC (EPI-NCSC) and olfactory ensheathing cells (OEC) in a rat sciatic nerve defect model.[105] Their findings indicated that EPI-NCSC and OEC cotransplantation promoted sciatic nerve regeneration and improved nerve function. Moreover, the mechanism of PNI improvement by EPI-NCSC and OEC cotransplantation was likely due to an upregulation in the expression of BDNF and nerve growth factor (NGF). Similar findings were observed in the application of BM-derived NC precursors (BM-NCPs) for the repair of sciatic nerve defects in adult rats. These BM-NCPs were capable of repairing nerve defects by promoting axonal regrowth and myelination and preventing muscle atrophy, thereby restoring motor and sensory neuron function, possibly through the secretion of various trophic factors that were distinct from those of BM-MSCs.[106] In addition to trophic support, it has been suggested that NCLSCs may promote tissue repair through the modulation of the immune system,[104,108] suggesting these cells may alter pro- and anti-inflammatory factors that could therefore improve tissue regeneration. The potential mechanisms by which transplanted NCLSCs regulate peripheral nerve repair require further elucidation. Additionally, the application of adult tissue-derived NCLSCs transplanted alone or with other support cells in preclinical large animal studies will provide further insights into clinical outcomes and the potential of this new therapy for PNI.

Spinal cord injury (SCI) has many distinct factorial aspects including primary mechanical damage, reactive gliosis, secondary cell apoptosis, and the inability of axons to regenerate.[109,110] Axon regeneration does not occur due to the nonresponsive environment

of the injured spinal cord. Emerging evidence suggests that NCLSCs derived from various adult tissues may serve a promising strategy for SCI.[111-113] Recently, human dental pulp (hDP)-NCSCs were used to evaluate the effect of hDP-NCSC delivery on the lesion site and functional recovery after SCI.[111] The data provided a theoretical and experimental basis for hDP-NCSC transplantation for the treatment of SCI as it was shown that these cells could significant improve motor recovery following spinal cord trauma. Compared with other stem cells, hDP-NCSCs offer several advantages such as good primitiveness, strong amplification ability, simple acquisition, and weak in vivo rejection, without any damage to the donor. Therefore, hDP-NCSCs can provide a new cell source and therapy for SCI.[111] Apart from hDP-NCSCs, transplanted EPI-NCSCs were shown to not only provide neurotrophic support in an ex vivo SCI contusion model,[112] but also improve motor function after SCI in rats, in particular demonstrating beneficial synergistic effects when delivered in combination with the potent antioxidant Astaxanthin.[113] These findings suggest that further research into combinatorial strategies with EPI-NCSCs and pharmaceutical agents may prove to be valuable for the treatment of SCI. Indeed, valproic acid has been proposed as a potential candidate as it can enhance the expression level of various trophic factors, such as BDNF and glial cell line-derived neurotrophic factor (GDNF), and promote SCI recovery.[112,114] The purpose of neuroregenerative medicine is to replace, regenerate, or arrest the loss of cells and tissues due to neurodegenerative and neurological disorders. Fortino et al successfully induced periodontal ligament stem cells (PDLSCs) derived from the NC into neural-like cells using a combination of basic FGF and epidermal growth factor.[115] The ease of sourcing and expansion, their embryologic NC origin, and the lack of ethical implications in their use make PDLSCs an attractive cell source for neuroregenerative medicine.

Previous studies have shown that adult tissue-derived NCLSCs are multipotent and, thus, can differentiate into various NC derivatives, including bone cells. For instance, Ono et al used double transgenic (P0-Cre/CAG-CAT-EGFP) mice to investigate the precise distribution and properties of neural crest-derived stem cells (NCDCs) in adult oral tissues. They found that these NCDCs widely reside throughout different adult oral tissues, such as the buccal mucosa, gingiva, tongue, and palate.[116] In addition, NCDCs were found to proliferate and differentiate into osteoblastic cells in vitro. In another study, Wnt pathway activator lithium chloride (LiCl) was used to investigate whether it could promote odontoblast differentiation of hair follicle neural crest cells (hfNCCs). The results showed that LiCl activated canonical Wnt signaling and promoted the proliferation and odontogenic differentiation of hfNCCs, suggesting that hfNCCs may be a good candidate for tooth regeneration.[117] As adult tissue-derived NCLSCs can undergo osteogenic differentiation in vitro, they are a promising cell source for bone regeneration.[118,119] NCLSCs isolated from excised human oral mucosa could generate spheres that were multipotent and capable of self-renewal in vitro.[118] Subcutaneous implantation of composites of osteogenic-induced NCLSCs and multiporous polylactic scaffolds into immunocompromised mice for 10 weeks revealed these cells were capable of generating ectopic bone tissue in vivo. Similarly, human palate-derived NCLSCs incorporated into an allogen bone substitute were found to contribute to the formation of new bone tissue during peri-implant bone repair and induced extended peri-implant bone remodeling with good biocompability,[119] indicating these stem cell-supported allogen bone scaffolds may be beneficial for bone regeneration, although the long-term effects of these implants has yet to be fully evaluated. A recent study demonstrated that adult manidular skeletal stem cells activated an embryonic NC cell-like gene regulatory program that is dependent on the focal adhesion kinase pathway during distraction osteogenesis,[120] enabling these cells to acquire a more plastic, developmental state that aids in jaw bone regeneration. In addition, alveolar bone-derived MSCs[121] display high osteogenic potential and promote ectopic bone formation in vivo,[122–125] providing a more accessible MSC source compared with the iliac crest.

Dental pulp stem cells (DPSCs) are also a source of NCLSCs that have multilineage differentiation potential, immunomodulatory properties, and high regenerative capability.[126,127] As a result, DPSCS can be utilized to treat a broad spectrum of disorders,[126,127] including PNI,[128,129] retinal injury, diabetes, cerebral ischemia, myocardial infarction, muscular dystrophy, and neurological diseases.[128,130,131] Indeed, in several case studies and preliminary clinical trials, the transplantation of DPSCs has proven to be a safe and effective therapeutic strategy,[127] indicating that DPSCs are a promising cell source for tissue regeneration and the treatment of various diseases.

1.3 | PSC-derived NCLSCs for tissue regeneration

PSCs, including embryonic stem cells (ESCs) and iPSCs, offer the advantage that they can proliferate extensively and give rise to cells from all three germ layers, thus making them powerful and instrumental for regenerative cell therapy, disease modeling, and drug discovery.[132-135] Utilizing existing knowledge of NCSC specification during development, researchers have created protocols to generate NCLSCs from mouse and human PSCs in vitro.[30] Although initial protocols relied on deriving NCLSCs through a neural progenitor cell population or coculture with stromal cells, direct and specific NCSC induction protocols have been developed that are based on using small molecule activators of Wnt signaling and inhibitors of BMP and Activin/Nodal signaling.[136-138] This direct induction approach was further modified and refined into a completely defined system utilizing inhibition of transforming growth factor beta and glycogen synthase kinase 3 to generate NCLSCs, thereby improving the potential translation of these cells for clinical application.[139] Moreover, inclusion of additional factors, such as BMP-4, RA, and FGF-2, and modulation of Wnt signal[140] has enabled researchers to induce NCLSCs with distinct regional identity (eg, cranial,[141] trunk,[142] and vagal[138,143,144] NCLSCs), and as a result provides an approach to not only study regional identity in vitro, but also the possibility to generate NC derivatives that may closely resemble the in vivo counterparts and be utilized for tissue regeneration. As these NCLSCs can be differentiated in vitro into various cell types,[30] including peripheral neurons, glial cells, chondrocytes, osteocytes, myofibroblasts, melanocytes, SMCs, and

adipocytes, this had led researchers to investigate the in vivo regenerative potential of NCLSCs derived from mouse and human PSCs.

As NCSCs give rise to peripheral nervous tissue in humans, it comes as no surprise that human ESC/iPSC-derived NCLSCs can be differentiated into peripheral neurons and Schwann cells[30] and as such, have shown great promise for the treatment of PNI. Results from several studies where these derived NCLSCs were suspended in different types of hydrogels and transplanted in conjunction with biomaterials, such as nanofibrous tubular scaffolds and polymeric tubular conduits, to generate tissue-engineered nerve conduits for peripheral nerve repair have been encouraging.[145-148] In rat and mice sciatic nerve injury models, grafted NCLSCs were not only able to survive, but also promoted axonal regrowth and myelination,[145-147] enhanced angiogenesis,[147] and secreted neurotrophic factors[146,148] (eg, BDNF and NGF), thereby providing trophic support to stimulate peripheral nerve regeneration. More importantly, NCLSCs accelerated functional recovery as assessed through electrophysiological and behavioral analysis.[146,147] Interestingly, the application of physical stimulation, such as low-intensity pulsed ultrasound[149,150] and electrical stimulation,[151] after NCLSC transplantation, can also further improve nerve regeneration and functional recovery following PNI. Apart from PNI, these PSC-derived NCLSCs can also be utilized to treat spinal cord damage in patients with spina bifida.[152] Findings from these studies suggest that PSC-derived NCLSCs are a potent therapy for neural regeneration and repair.

Cumulative evidence has also shown that ENCCs and enteric-like neurons can be generated from ESC/iPSC-derived NCLSCs and, thus, potentially serve as a therapeutic cell source for ENS disorders and regeneration.[143,144,153-155] Grafted ENCC precursors were able to repopulate the adult mouse colon and capable of targeted migration into the gut region.[143] In addition, these ENCCs rescued disease-related mortality in an $Ednrb^{s\text{-}1/s\text{-}1}$ Hirschsprung (HSCR) mice model,[143] demonstrating these cells were functional in vivo. Moreover, iPSC-derived NCLSCs transplanted into the hindgut of severe combined immunodeficiency (SCID) mice were not only capable of migrating towards myenteric and submucosal regions, but also differentiated into glial cells and mature enteric neurons in vivo.[155] Similar findings were observed in human intestinal organoids after the inclusion of human PSC-derived NCLSCs,[144,154] enabling the development of human tissue-engineered intestines that are potentially useful for the treatment of enteric neuropathies and the study of human gastrointestinal tract mobility disorders.

PSC-derived NCLSCs have also been differentiated into various cell types, including MSCs, osteocytes, and chondrocytes, that are beneficial for bone, cartilage, and tendon regeneration.[30,156-160] Xu et al examined whether iPSC-derived NCLSCs could repair a rat patellar tendon window defect. Transplantation of these NCLSCs in a fibrin gel promoted the host endogenous repair process, resulting in a significant improvement in tendon healing and repair.[156] Moreover, MSC-like cells generated from iPSC-derived NCLSCs have been utilized to repair rat femoral osteochondral defects and regenerate mouse craniofacial bone in vivo, respectively. In comparison to human BM-MSCs, tissue engineered constructs of MSCs from iPSC-derived NCLSCs did not undergo chondrogenesis in vivo nor did they effectively repair osteochondral defects.[158] On the other hand, mouse iPSC-NCLSC-MSCs transplanted into calvarial defects differentiated into osteoblasts, produced no tumors, and contributed to bone regeneration.[159] These studies highlight how these derived cells may behave differently in vivo. However, a direct comparison cannot be made as the cells used in these studies were from different species and distinct defect models were implemented. Further investigation of craniofacial bone repair using MSCs from human iPSC-derived NCLSCs would reveal whether these cells could be a potential cell source for clinical application.

Melanocytes, which produce melanin and play a critical role in skin homeostasis and protection, have also been derived from ESCs and iPSCs after proceeding through a NC stage.[138,161-163] Notably, human ESC-derived melanocytes were able to localize to the appropriate layer after being introduced into a human skin xenograft model. Engraftment of these skin reconstructs into SCID mice revealed that these cells survived and were functional for over 4 weeks.[161] Furthermore, these melanocytes were capable of producing melanin and functionally integrated into a reconstituted pluristratified epidermis in vitro.[162] Altogether, this in vitro system provides an opportunity to study melanocyte developmental biology and may serve as a potential cellular therapy for hypopigmentation disorders.

Corneal scarring or blindness resulting from damage to the corneal stroma or corneal endothelial (CE) dysfunction is currently being treated with surgical corneal transplantation.[164] However, limitations, such as graft failure and shortage of donor cornea, still exist, prompting researchers to search for more suitable alternatives, such as an NCLSC-based cell therapy. Using a two-step induction process, several studies have derived corneal endothelial cells (CECs)[165-167] and corneal keratocytes[168,169] from ESCs and iPSCs after first generating an NCLSC population. Transcriptomic analysis of CEC-like cells from human ESC-derived NCLSCs using RNA sequencing has revealed that the transcriptome of these cells closely resembles that of adult CECs.[167] Yet, whether these cells would integrate and aid in tissue regeneration in vivo remains unknown. Preliminary studies using CECs generated from human ESCs that proceeded through a periocular mesenchymal phase, rather than an NCSC stage, have shown promising results in improving CE dysfunction in rabbit models,[170] suggesting the possibility that keratocytes and CECs generated from ESC/iPSC-derived NCLSCs may yield similar beneficial effects. Although further investigation into the functionality of these cells in vivo is still required, these NCLSC-derived corneal cells may serve a promising alternative for corneal repair.

Mouse iPSC-derived NCLSCs can also undergo differentiation into odontoblast-like cells upon cotransfection of Pax9 and BMP4 expression plasmids.[171] Interestingly, transplantation of control and transfected NCLSCs did not give rise to teratomas after they were subcutaneously injected into mice, suggesting these cells were not tumorigenic and, thus, a safe cell source for tooth regeneration. Another study also showed that mouse iPSC-derived NCLSCs could differentiate into odontogenic mesenchymal cells.[172] Culturing these NCLSCs with conditioned medium from mouse dental epithelium

further promoted their differentiation into odontoblasts. However, whether PSC-derived NCLSCs can be differentiated into other dental cell types (eg, dental pulp and dental follicle cells) and aid in dental tissue regeneration has yet to be determined.

In addition to tissue regeneration, numerous studies have demonstrated the potential of using iPSCs from patients affected by neurocristopathies for modeling human disease in vitro. Various diseases have been studied, thus far, including CHARGE syndrome,[173,174] Ewing sarcomas,[175] familial dysautonomia,[176,177] pigmentation disorders (eg, Hermansky-Pudlak and Chediak-Higashi syndromes),[138] HSCR disease,[143,178] Bardet-Biedl syndrome,[179] Treacher Collins syndrome,[180] and cardiovascular malformations, such as bicuspid aortic valves.[181] Findings from these studies have not only provided demonstration of disease-related phenotypes, but also evidence on the origin of certain neurocristopathies, which appear to arise from defects in NCSCs, rather than MSCs.[174,175,181] Additionally, this technology has served as a platform to identify potential drug candidates that are capable of restoring impaired function and possibly serve as therapeutic agents.[143,176,182]

Apart from deriving NCLSCs from PSCs through directed differentiation, it has also been shown the NCLSCs can be generated using direct reprogramming, a process that bypasses the iPSC stage during the conversion of somatic cells into distantly related cell types.[183,184] In contrast to directed differentiation, which can take up to several weeks,[137] reprogramming can yield NCLSCs in 10 to 14 days.[177,185] Therefore, this approach provides a rapid method of obtaining NCLSCs that can be potentially administered clinically. To date, NCLSCs have been derived from human and mouse fibroblasts,[177,186-188] keratinocytes,[189,190] and melanocytes[191] via the introduction of transcription factors, such as *SOX10* and *FOXD3*, specific growth factors, or forced expression of Notch1 signaling, respectively. It has been reported that the derived NCLSCs are functional in vivo when they were investigated for neural repair in a zebrafish model[186] and migration capability in a chick embryo model system[177,191]; however, more detailed and comprehensive analysis of in vivo functional outcomes is still necessary. Furthermore, this reprogramming approach has been applied to generate NCLSCs from fibroblasts isolated from patients with familial autonomic dystrophy, enabling the production of patient-specific cells, which holds great promise for personalized medicine and disease modeling.[177] Further elucidation on whether reprogrammed-derived NCLSCs can be used for the various aforementioned tissue regeneration applications will greatly expand their therapeutic utility and clinical applicability.

2 | CONCLUSIONS AND FUTURE DIRECTIONS

Current progress in the derivation and therapeutic application of NCLSCs suggests that these cells have great potential for regenerative cell therapy, disease modeling, and drug discovery. NCLSCs isolated from various fetal and adult tissues have demonstrated beneficial effects in several tissue engineering paradigms. However, acquisition and isolation of cells from fetal tissue are still somewhat controversial and adult NCLSCs may display limited multipotentiality.[30] As such, PSCs may serve as a promising alternative for the derivation of NCLSCs. Although ESC-based cell therapies are currently undergoing clinical trials,[134,135] the discovery of iPSCs has generated new excitement in the field of personalized regenerative medicine as these cells lack the ethical controversy associated with ESCs and are easier to obtain from the primary source of tissue. The source of iPSCs is an important factor to consider when generating NCLSCs as it has been shown that NCLSCs derived from iPSCs generated from NC tissue, such as periodontal ligament, are more equivalent to their in vivo counterparts compared with fibroblast-derived iPSC-NCLSCs,[192] suggesting these cells may have improved therapeutic efficacy. Nonetheless, several challenges still exist and need to be overcome before there is effective clinical translation of iPSC-based cell therapies.[193]

Although NCLSC therapy shows great promise for tissue regeneration, the safety and potential risks associated with these cells, such as tumorigenicity, need to be overcome before these cells can serve as a viable therapeutic option. In several studies where the therapeutic effects of human PSC-derived NCLSCs were investigated in animal models, no tumors were present, even up to 1 year after transplantation.[145,158,171] However, transplantation of a specific subset of NCLSCs derived from PSCs resulted in tumors and unwanted grafts.[194] Thus, the differentiation stage of the transplanted cells is an important factor to be considered. In addition to the formation of teratomas or tumors of NC origin, including neuroblastoma and melanomas, the immunogenic response of transplanted NCLSCs is an important parameter that should also be considered and closely monitored. A recent study has shown that iPSC-derived NCLSCs exhibit a nonimmunogenic phenotype, rather than an immunosuppressive one, as demonstrated by low levels of immune-related antigens in a noninflammatory environment and no induction of T-cell proliferation or pro-inflammatory cytokine production.[195] In contrast, another study proposed that PSC-derived NCLSCs exhibited immunosuppressive properties.[196] More work on the immunogenic properties of these cells will aid to provide more clarity on the issue. Overall, careful consideration of these concerns will ensure these cells are clinically safe and effective upon transplantation.

To facilitate the therapeutic applications of NCLSCs in the clinical setting, current NCLSC isolation, expansion, and differentiation protocols may require further optimization to consider good manufacturing practices.[197] Furthermore, during the development of favorable scale-up procedures to achieve large numbers for cell transplantation, certain aspects, such as passage number, should be taken into account as increased cell passaging can potentially diminish the therapeutic effects of these cells[198] and result in chromosomal defects that may lead to tumorigenesis.[199] In stem cell-based therapies, often times materials are used in combination with stem cells.[200,201] These materials can provide a scaffold that not only promotes cell survivability and the regeneration process, but also greatly influences cell fate and function in vivo. Future developments of next-generation biomaterials that are biocompatible and able to further improve NCSC expansion and differentiation in vitro and in vivo will be highly desirable for regenerative therapies.

Although NCSC and NCLSC appear to share common characteristics, such as multipotency, NC marker expression (eg, Sox10, $p75^{NTR}$, AP-2, and Nestin) and pluripotent gene expression, and a molecular signature representative of EPI-NCSC and embryonic NCSC has been defined,[202] there also are some apparent differences. For instance, long SAGE-transcriptome profiling revealed that human NCSCs exhibited a unique NC molecular signature, expressed pluripotent genes (*Nanog*, *POU5F1*. and *Sox2*), and were found to have global molecular profile similar to ESCs.[203] On the other hand, mouse epidermal NCLSCs only partially share the gene expression pattern of PSCs in that they express Myc, Klf4, and Sox2 but significantly lower levels of Nanog and Oct-4 when compared with mouse ESCs,[204] an attribute that can potentially reduce their tumorigenicity potential. A recent study performed transcriptome profiling at different time points during the induction of cranial neural crest cells (cNCCs) from mouse iPSCs and found that cNCCs exhibited gene expression profiles that were only partially similar to those previously reported.[205] Interestingly, they observed that these cNCCs did not express certain NC specifier genes (eg, *FoxD3*, *Gbx2*, *Msx1*, *Dlx3*, *Zic2*, and *Zic3*) during the derivation process and other markers, such as Sox10, were only expressed at day 14, indicating that cNCCs take longer to acquire a migratory phenotype in vitro in comparison to mouse embryos in vivo.[205] These findings suggest that the molecular network that governs gene expression during iPSC-derived cNCC induction may potentially differ to the in vivo NCSC gene regulatory network. Moreover, although there is substantial overlap among human, mouse, and avian NCSC transcriptomes, there exists a specific subset of genes that are only expressed by human cells,[203] highlighting the importance of human NCLSCs derivation and their translational potential for regenerative medicine. Further genome and epigenome profiling of NCSCs and NCLSCs from various sources will reveal new molecular insights into the unique attributes of each cell type and the similarities they share.

Advancements in high-throughput omics technologies and drug screening platforms will provide new mechanistic insights into the signaling pathways and gene expression profiles of NCLSCs during development[206-208] and tissue repair, and furthermore, facilitate the development of therapeutics that can be used to treat patients with neurocristopathies. Moreover, iPSC technology in conjunction with gene-editing platforms, such as CRISPR-Cas9,[209-211] will aid to broaden our understanding of disease pathogenesis and provide an approach to correct genetic mutations in patient-derived NCLSCs, thereby enabling the therapeutic utilization of these cells upon restoring normal cell function. Additionally, the systemic delivery of gene editing components provides an opportunity to modulate disease-causing alleles in vivo without the need for cell isolation.[210,211] Taken together, NCLSCs, which can be isolated and derived from multiple sources, are a promising cell source for regenerative medicine.

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CONFLICT OF INTEREST

The authors declared no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

J.S., X.D., A.W.: wrote and edited the manuscript; S.L.: conceived overall review content and edited the manuscript.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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