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Multifaceted Neuro-Regenerative Activities of Human Dental Pulp Stem Cells for Functional Recovery after Spinal Cord Injury

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http://dx.doi.org/10.5772/58906

1. Introduction

Spinal cord injury (SCI) often leads to persistent functional deficits, due to loss of neurons and glia and to limited axonal regeneration after injury. Recently, three independent groups have reported that transplantation of human adult dental pulp stem cells (DPSCs) and stem cells from human exfoliated deciduous teeth (SHEDs), into the acute, sub-acute or chronic phase of rat or mouse SCI resulted in marked recovery of hindlimb locomotor functions. This review summarizes the primary characteristics of human dental pulp stem cells and their therapeutic benefits for SCI treatment. Experimental data from a number of preclinical studies suggests that pulp stem cells may promote functional recovery after SCI through multifaceted neuro-regenerative activities.

2. Dental pulp stem cells

Humans have two sets of teeth, 20 deciduous and 32 permanent ones. In the center of each tooth, there is a cavity pulp chamber, which is filled with soft connective tissue called dental pulp (Nanci and Ten Cate, 2003) (Fig.1). The major components of dental pulp are odontoblasts, fibroblasts, immune cells, extracellular matrix, blood vessels and nerve fibers. The pulp tissues are connected with systemic network through the apical foramen; this provides nutrition and sensation for responding to the external stimuli. Human adult dental pulp stem cells (DPSCs) and stem cells from human exfoliated deciduous teeth (SHEDs) are self-renewing stem cells

residing within the perivascular niche of the dental pulp (Gronthos et al., 2002b). They are thought to originate from the cranial neural crest, of embryonic period and they simultaneously express early markers for both mesenchymal, neuroectodermal stem/progenitor cells and some of embryonic stem cells markers (Gronthos et al., 2000, Miura et al., 2003, Kerkis et al., 2006, Sakai et al., 2012).
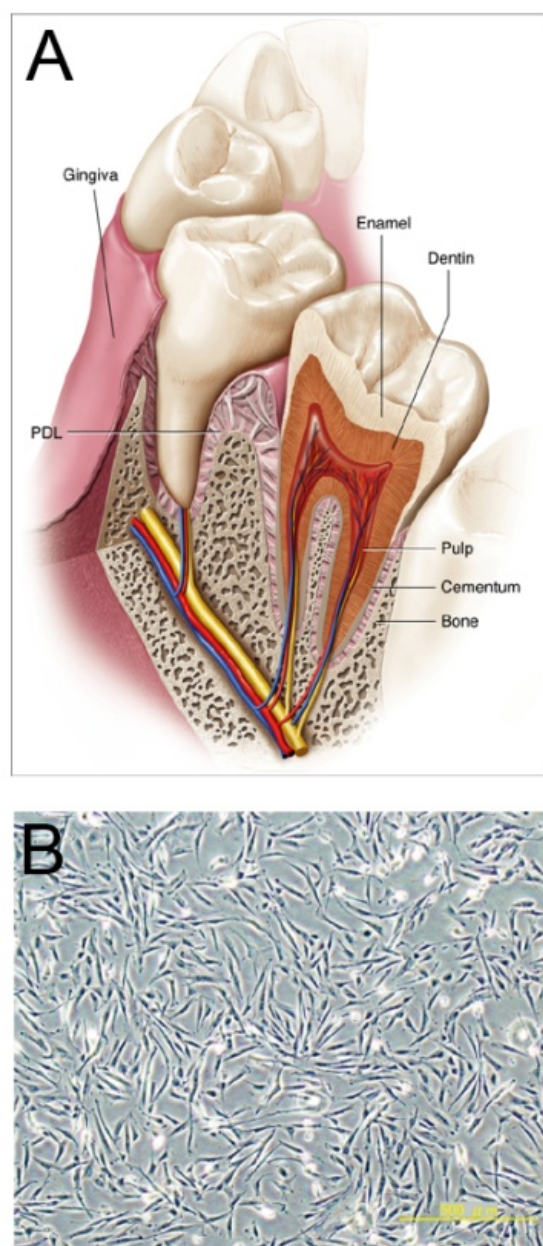
Figure 1. Diagram of tooth and pulp stem cells. (A) The tooth and its supporting structure (from Ten Cate's Oral Histlogy, Nanci and Ten Cate, 2008). PDL, Periodontal ligament. (B) Morphology of pulp stem cells. They exhibit a fibroblastic morphology with a bipolar spindle shape. Scale bar in (B): 500 μm.

Most SHEDs and DPSCs express a set of adult bone marrow stromal stem cell (BMSC) markers (CD90, CD73, and CD105), neural stem/progenitor cell markers (Doublecortin, GFAP, and Nestin), and early neuronal and oligodendrocyte markers (βIII-tubulin, A2B5 and CNPase), but not markers for mature oligodendrocytes (MBP and APC) (Sakai et al., 2012). Since naturally exfoliated deciduous and impacted adult wisdom teeth are dispensable, DPSCs and SHEDs can be easily obtained by utilizing a simple protocol (Liu et al., 2006). DPSCs and SHEDs exhibit a faster rate of proliferation and a higher number of population doublings in vitro, compared with BMSCs. Furthermore, the rate SHEDs is 1.5 times faster than that of DPSCs (Miura et al., 2003). Like BMSCs, they are multipotent cells that can differentiate in vitro into a variety of cell types including odontoblasts, osteoblasts, chondrocytes, adipocytes, endothelial cells, myocytes, and functionally active neurons (Gronthos et al., 2000, Gronthos et al., 2002a, Batouli et al., 2003, Miura et al., 2003, Nosrat et al., 2004, Kerkis et al., 2006, d'Aquino et al., 2007, Arthur et al., 2008, Arminan et al., 2009, Wang et al., 2010). Furthermore, when transplanted into the transected spinal cord (SC), they specifically differentiate toward mature oligodendrocyte lineages (Sakai et al., 2012: see below).

A cDNA microarray analysis showed that SHEDs express many genes encoding extracellular and cell-surface proteins at levels at least two-fold higher than are expressed in BMSCs (Sakai et al., 2012). It has been shown that the array of trophic factors produced by engrafted DPSCs and SHEDs provide significant therapeutic benefits for the treatment of preclinical animal disease models, including myocardial infarction, systemic lupus erythematosus (SLE), ischemic brain injury, SCI, and colitis (Gandia et al., 2008, Nakashima et al., 2009, Yamaza et al., 2010, de Almeida et al., 2011, Leong et al., 2012, Ma et al., 2012, Sakai et al., 2012, Taghipour et al., 2012, Zhao et al., 2012, Inoue et al., 2013, Yamagata et al., 2013). Thus, these studies collectively show that tooth-derived stem cells are a highly proliferative, multi-potent, and self-renewing ecto-mesenchymal stem cell-like population that actively secretes a broad repertoire of trophic and immunomodulatory factors.

3. Brief overview of the pathophysiology of SCI

The development of effective treatments for SCI has been stifled by this injury's complicated pathophysiology. During the acute phase, a primary mechanical insult disrupts tissue homeostasis. This triggers a secondary response, in which activated resident microglia and infiltrating blood-derived macrophages initiate severe inflammation by releasing high levels of multiple neurotoxic factors that induce the necrotic and apoptotic death of neurons, astrocytes, and oligodendrocytes. This response spreads beyond the initial injury site, and leads to irreversible axonal damage and demyelination (Schwab et al., 2006, Popovich and Longbrake, 2008, Rowland et al., 2008). Subsequently, reactive astrocytes and oligodendro-cytes near the site of the injured spinal cord (SC) respectively produce chondroitin sulfate proteoglycans (CSPG) and myelin proteins (including myelin-associated glycoprotein (MAG), Nogo, OMG, Netrin, Semaphorin, and Ephrin). These extracellular molecules function as axon growth inhibitors (AGIs), acting through the intracellular Rho GTPase signaling cascade (Silver and Miller, 2004, Yiu and He, 2006). Thus, multiple pathogenic signals act to synergis-

tically accelerate the progressive neuronal deterioration following SCI. Therefore, therapeutic strategies for functional recovery from SCI must exert multifaceted reparative effects targeting a variety of pathogenic mechanisms (Schwab et al., 2006).

4. Multifaceted neuro-regenerative activities of pulp stem cells

4.1. Anti-inflammatory activity

Under various pathogenic conditions, macrophages differentiate into polarized pro-inflammatory (M1) or anti-inflammatory (M2) states, and direct either detrimental or beneficial effects on tissue healing (Gordon, 2003, Mosser and Edwards, 2008). In the acute phase of SCI, the majority of accumulating microglia/macrophages are of the M1 type, and few M2 macrophages are seen throughout this period (Kigerl et al., 2009, David and Kroner, 2011). The activated M1 macrophages secrete high levels of pro-inflammatory cytokines and neurotoxic factors, including tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, glutamate, and reactive oxygen species (Hausmann, 2003, Donnelly and Popovich, 2008). These neurotoxic factors accelerate glial scar formation (Popovich and Longbrake, 2008), and they induce neuronal cell death (Takeuchi et al., 2006, Block et al., 2007) and the retraction of damaged dystrophic axons (Horn et al., 2008, Busch et al., 2009). In contrast, M2 cells counteract the pro-inflammatory M1 effects and promote tissue remodeling by secreting anti-inflammatory cytokines (e.g. IL-10 and TGF-β), and scavenging cellular debris (Gordon, 2003, Mosser and Edwards, 2008, David and Kroner, 2011). Thus, macrophage polarity has the potential to determine the level of inflammation and the resultant prognosis following SCI.

Recent studies have demonstrated the induction of M2 macrophage polarization following SCI, and some of the underlying mechanisms are beginning to be elucidated. CSPG, a major component of the glial scar that is mainly known for its ability to inhibit axonal growth, has recently been shown to promote M2 polarization of infiltrating blood-derived macrophages (Rolls et al., 2008, Shechter et al., 2009, Shechter et al., 2011). In addition, recent reports have shown that BMSC transplantation using SCI or brain ischemia models leads to M2 induction (Ohtaki et al., 2008, Nakajima et al., 2012). BMSC-mediated M2 induction requires both the pre-sensitization of BMSCs by pro-inflammatory factors, such as IFN-γ, TNF-α, and LPS, and direct cell-to-cell contact (Nemeth et al., 2009, Singer and Caplan, 2011). Thus, CSPG together with pro-inflammatory factors in the injured SC may be involved in the pre-sensitization of engrafted BMSCs to activate their M2-inducing machinery.

As described in the previous section, SHEDs also exhibit strong immunosuppressive properties that effectively ameliorate several autoimmune diseases, including SLE and colitis (Yamaza et al., 2010, Ma et al., 2012, Zhao et al., 2012). Importantly, intravenously administered SHEDs express Fas-Ligand, which induces T-cell apoptosis, thereby triggering immune tolerance (Zhao et al., 2012). This elevates the ratio of regulatory T cells (Tregs) to pro-inflammatory T cells, resulting in anti-inflammatory conditions (Yamaza et al., 2010). We also found that, in the mouse hypoxic ischemia model, both intracerebral transplantation of SHEDs, and administration of serum-free conditioned media (CM) derived from SHEDs (SHED-CM),

generates anti-inflammatory conditions and promotes functional recovery (Yamagata et al., 2013). Thus, tooth-derived stem cells have strong immunoregulatory properties that promote tissue regeneration in the injured CNS.

4.2. Regeneration of the injured axon

Both axonal regeneration and the re-formation of appropriate neuronal connections are required for functional recovery from SCI. However, multiple AGIs block the inherent regenerative capacities of injured axons (Silver and Miller, 2004, Schwab et al., 2006, Yiu and He, 2006, Rowland et al., 2008). It is well known that AGIs constitute an intricate molecular network in the extracellular space of the injured CNS, where they activate a common intra-cellular signaling mediator, Rho GTPase, and its effector, Rho-associated kinase (ROCK) (Maekawa et al., 1999, Winton et al., 2002, Dubreuil et al., 2003, Monnier et al., 2003, Yamashita and Tohyama, 2003). Activation of the Rho-ROCK cascade induces growth-cone collapse and axonal repulsion (Hall, 1998). In contrast, inactivation of either Rho by C3 transferase, or ROCK by the kinase inhibitor Y-27632 down-regulates AGI signaling and promotes functional recovery after SCI (Lehmann et al., 1999, Dergham et al., 2002, Fournier et al., 2003). Thus, Rho-ROCK signaling is an important target for SCI treatments; however, few studies have investigated the effect of stem-cell transplantation on regulating AGI/Rho-ROCK signaling cascades.

Importantly, engrafted SHEDs were recently shown to promote the regeneration of two major types of descending axons (CST and 5-HT) beyond the lesion epicenter, and to concomitantly inhibit SCI-induced Rho activation. Furthermore, both SHED-CM and DPSC-CM (but not BMSC-CM) promote neurite extension by primary cerebral granular neurons (CGNs) cultured on two different AGIs (CSPG and MAG) (Sakai et al., 2012). Thus, tooth-derived stem cells promote the regeneration of transected axons through the direct inhibition of multiple AGI signals by paracrine mechanisms.

In addition, the engraftment of DPSCs into avian embryos results in the chemoattraction of trigeminal ganglion axons via the chemokine CXCL12 and its receptor, CXCR4 (Arthur et al., 2009). DPSCs and SHEDs express several neurotropic factors that promote neurite extension (de Almeida et al., 2011, Sakai et al., 2012). Our preliminary analysis showed that these trophic factors, when applied individually, failed to promote the neurite extension of CGNs cultured on CSPG-coated dishes; however it is possible that they may promote axonal regeneration in a synergistic manner.

4.3. Anti-apoptotic activity

Pharmacological blockade of neuron and/or oligodendrocyte apoptosis by a number of agents promotes functional recovery after SCI. These agents include the following: erythropoietin (Celik et al., 2002, Gorio et al., 2002), inhibitors of purine receptor P2X7 (OxATP and PPADS) (Wang et al., 2004), a neutralizing antibody against CD95 (FAS) antigen (Demjen et al., 2004), and minocycline (Stirling et al., 2004, Teng et al., 2004). Engrafted SHEDs suppress the apoptosis of neurons and oligodendrocytes, resulting in the remarkable preservation of

neurofilaments and myelin sheaths in the region surrounding the lesion epicenter (Nosrat et al., 2001, de Almeida et al., 2011). Intracerebral transplantation of DPSCs from rhesus macaques promotes proliferation, cell recruitment, and maturation of endogenous stem/progenitor cells by modulating the local microenvironment (Huang et al., 2008). Notably, SHEDs also strongly inhibit the apoptosis of astrocytes recruited to the lesion (Sakai et al., 2012).

Classically, reactive, CSPG-generating astrocytes have been considered an obstacle to axonal regeneration; however, recent genetic studies in mice indicate that the conditional ablation of astrocytes after SCI results in larger lesions, failure of blood-brain-barrier repair, increased inflammation and tissue disruption, severe demyelination, and profound cell death of neurons and oligodendrocytes (Bush et al., 1999, Faulkner et al., 2004, Okada et al., 2006, Herrmann et al., 2008, Rolls et al., 2009). Thus, the collective evidence demonstrates that, in addition to their anti-regenerative activity, astrocytes also play an important role in neuro-protection during the acute phase of SCI. SHEDs can suppress astrocyte apoptosis and minimize secondary injury, as well as inhibit the AGI activity of CSPG derived from astrocytes. Thus, SHEDs have the potential to promote functional recovery after SCI through two distinct mechanisms involving astrocyte regulation.

4.4. Cell-replacement activity

Undifferentiated rat and human pulp stem cells can form neurospheres in vitro (Sasaki et al., 2008, Wang et al., 2010) and simultaneously express multiple neural stem/progenitor markers (Gronthos et al., 2002a, Miura et al., 2003, Sakai et al., 2012). In addition, DPSCs can differentiate in vitro toward functionally active neurons, which express voltage-gated Na+ channels, and in vivo toward neuron-like cells 48 hours after transplantation into the mesencephalon of avian embryos (Arthur et al., 2008). Furthermore, simultaneous PKC and cAMP activation induces the differentiation of DPSCs into functionally active neurons (Kiraly et al., 2009). Thus, pulp stem cells display a capacity for neuronal differentiation both in vivo and in vitro.

Recently, three independent groups reported that pulp stem cells show neuro-regenerative activity in rodent SCI models. Interestingly, engrafted pulp stem cells promoted significant functional recovery in all three studies, but exhibited variable capacities for differentiation. In the first study, DPSCs were transplanted into the compressed mouse SC at day 7 (sub-acute phase) or day 28 (chronic phase) after injury, and the engrafted DPSCs differentiated into glia cells expressing S-100 and GFAP (de Almeida et al., 2011). In the second study, undifferentiated or neural-phenotype induced SHED (iSHED) were transplanted into the contused rat SC at 7 days after injury. Engrafted SHED and iSHED differentiated primarily into MAP2+ mature neurons and GFAP+ astrocytes, and to a lesser extent into MBP- and NG2-expressing oligo-dendrocytes (Taghipour et al., 2012). In the third study, from our group, undifferentiated SHEDs were transplanted into the completely transected rat SC immediately after the surgery. The engrafted SHEDs survived well following SCI: more than 30% of the engrafted SHEDs survived as a cell mass in the injured SC 8 weeks after transplantation and more than 90% of the engrafted SHEDs differentiated toward mature oligodendrocytes, expressing APC and MBP (Sakai et al., 2012).

Taken together, these experimental data suggest that the microenvironment of the transplanted stem cells significantly affects their capacity for differentiation. In the acute phase of SCI, the injured SC contains high levels of pro-inflammatory mediators. Thus these factors may activate the oligodendrocyte-specific differentiation cascade of pulp stem cells.

5. Conclusion

Recent experimental data from a number of studies reveals that engrafted SHEDs provide a number of distinct therapeutic benefits for treatment of SCI: (1) the suppression of the early inflammatory response; (2) inhibition of the SCI-induced apoptosis of neurons, astrocytes, and oligodendrocytes, which promotes the preservation of neural fibers and myelin sheaths; (3) regeneration of the transected axon through the direct inhibition of multiple AGI signals (including CSPG and MAG) by paracrine mechanisms; and (4) cell replacement in the damaged SC through the SHEDs' capacity for differentiation towards oligodendrocytes, neurons and astrocytes. Thus, we propose that tooth-derived stem cells may provide significant therapeutic benefits for treating SCI through both cell-autonomous and paracrine/trophic regenerative activities.

Acknowledgements

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