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Review article

Multifaceted neuro-regenerative activities of human dental pulp stem cells for functional recovery after spinal cord injury

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ABSTRACT

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

Contents

1. Dental pulp stem cells

Humans have two sets of teeth; 20 are deciduous, and 32 are permanent. The center of each tooth includes a cavity pulp chamber, which is filled with soft connective tissue referred to as dental pulp (Nanci and Ten Cate, 2003) (Fig. 1). The major components of dental pulp include odontoblasts, fibroblasts, immune cells, extracellular matrix, blood vessels and nerve fibers. The pulp tissues are connected by a systemic network through the apical foramen, which provides nutrition and sensation in response to external stimuli.

Human adult dental pulp stem cells (DPSCs) and stem cells from human exfoliated deciduous teeth (SHEDs) are self-renewing stem cells in the perivascular niche of the dental pulp (Gronthos et al., 2002b). Such cells likely originate from the cranial neural crest during the embryonic period, and they simultaneously express early mesenchymal, neuroectodermal stem/progenitor cell markers and certain embryonic stem cell markers (Gronthos et al., 2000; Miura et al., 2003; Kerkis et al., 2006; Sakai et al., 2012).

Most SHEDs and DPSCs express 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). Because naturally exfoliated

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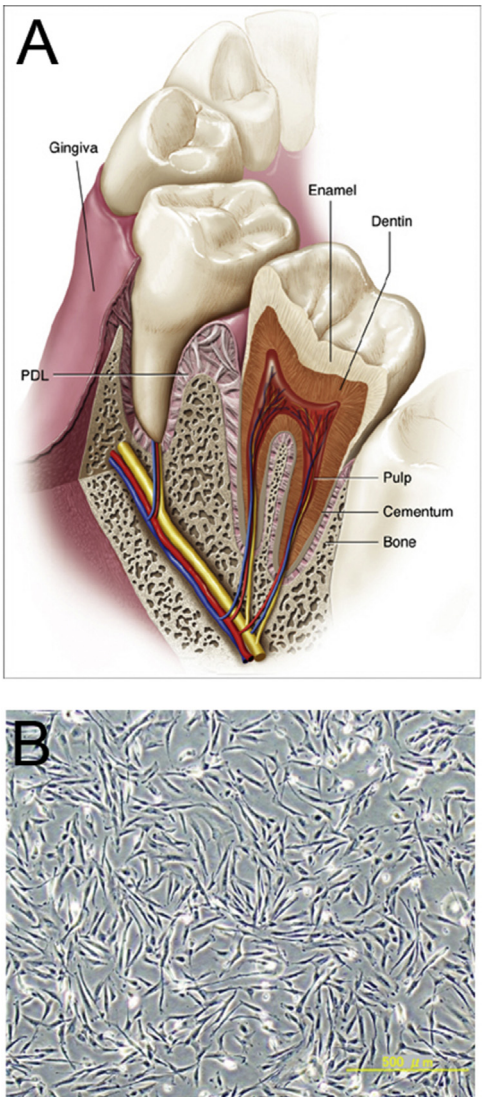

Fig. 1. Diagram of tooth and pulp stem cells. (A) The tooth and its supporting structure (from Ten Cate's Oral Histology, Nanci and Ten Cate, 2008). PDL, periodontal ligament. (B) Morphology of pulp stem cells. The cells exhibit a fibroblast-like morphology with a bipolar spindle shape. Scale bar in (B): 500 μm.

deciduous and impacted adult wisdom teeth are dispensable, DPSCs and SHEDs can be easily collected using 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 SHED proliferation rate is 1.5 times greater than that of DPSCs (Miura et al., 2003). Similar to BMSCs, SHEDs are multipotent cells that can differentiate in vitro into multiple cell types, including odontoblasts, osteoblasts, chondrocytes, adipocytes, endothelial cells, myocytes, and functionally active neurons (Gronthos et al., 2000, 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 a 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 that encode extracellular and cell-surface proteins at levels at least two-fold higher than in BMSCs (Sakai et al., 2012). The array of trophic factors produced by engrafted DPSCs and SHEDs provides significant therapeutic benefits for treating 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, such studies collectively show that pulp stem cells compose a highly proliferative, multi-potent, and self-renewing ecto-mesenchymal stem cell-like population that actively secretes a broad range of trophic and immunomodulatory factors.

2. Brief overview of SCI pathophysiology

The development of effective treatments for SCI has been difficult due to this injury's complicated pathophysiology. During the acute phase, a primary mechanical insult disrupts tissue homeostasis. Such disruption 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 necrotic and apoptotic death in 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 oligodendrocytes near the injured spinal cord (SC) site respectively generate chondroitin sulfate proteoglycans (CSPGs) and myelin proteins (including myelin-associated glycoprotein (MAG), nogo, OMG, netrin, semaphorin, and ephrin). Such extracellular molecules function as axon growth inhibitors (AGIs) and act through the intracellular Rho GTPase signaling cascade (Silver and Miller, 2004; Yiu and He, 2006). Thus, multiple pathogenic signals synergistically accelerate progressive neuronal deterioration after SCI. Therefore, therapeutic strategies for functional recovery from SCI must have multiple reparative effects that target various pathogenic mechanisms (Schwab et al., 2006).

3. Multifaceted neuro-regenerative activities of pulp stem cells

3.1. Anti-inflammatory activity

Under various pathogenic conditions, macrophages differentiate into polarized pro-inflammatory (M1) or anti-inflammatory (M2) states and have either detrimental or beneficial effects on tissue healing (Gordon, 2003; Mosser and Edwards, 2008). In the acute phase for SCI, most of the accumulating microglia/macrophages are of the M1 type, and few M2 macrophages are observed during this period (Kigerl et al., 2009; David and Kroner, 2011). 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). Such 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 may be used to determine the level of inflammation and resulting prognosis due to SCI.

Recent studies have demonstrated that M2 macrophage polarization is induced following SCI, and certain underlying mechanisms have been elucidated. CSPGs is a major glial scar component