Pulp Stem Cells: Implication in Reparative Dentin Formation

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Abstract

Many dental pulp stem cells are neural crest derivatives essential for lifelong maintenance of tooth functions and homeostasis as well as tooth repair. These cells may be directly implicated in the healing process or indirectly involved in cell-to-cell diffusion of paracrine messages to resident (pulpoblasts) or nonresident cells (migrating mesenchymal cells). The identity of the pulp progenitors and the mechanisms sustaining their regenerative capacity remain largely unknown. Taking advantage of the A4 cell line, a multipotent stem cell derived from the molar pulp of mouse embryo, we investigated the capacity of these pulp-derived precursors to induce *in vivo* the formation of a reparative dentin-like structure upon implantation within the pulp of a rodent incisor or a first maxillary molar after surgical exposure. One month after the pulp injury alone, a nonmineralized fibrous matrix filled the mesial part of the coronal pulp chamber. Upon A4 cell implantation, a mineralized osteodentin was formed in the implantation site without affecting the structure and vitality of the residual pulp in the central and distal parts of the pulp chamber. These results show that dental pulp stem cells can induce the formation of reparative dentin and therefore constitute a useful tool for pulp therapies. Finally, reparative dentin was also built up when A4 progenitors were performed by alginate beads, suggesting that alginate is a suitable carrier for cell implantation in teeth. *(J Endod 2014;40:S13–S18)*

Key Words

Cell niches, dentin repair, osteodentin, pulpoblasts, pulp stem cells

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Postnatal dental pulp contains heterogeneous cell populations responsible for its maintenance, defense, and capacity of repair. The resident cells include stromal fibroblasts also named pulpoblasts by Baume (1), odonto-osteoprogenitors, neuronal and vascular cells, and inflammatory and immune system cells (2). The pulp responds to a variety of pathological injuries by a deposition of reparative dentin by pulp "progenitors" (2, 3). However, the origin, localization, and precise identity of odontogenic stem cells remain largely unknown. Identifying stem cells mobilized in response to pulp injury is a prerequisite to design alternative strategies for pulp healing and regeneration and/or for endodontic treatment (3, 4).

Mitotic divisions and apoptosis constantly renew the pulp cell population, in contrast with odontoblasts and cells of the Hoehl's layer, which are postmitotic cells. Pulpoblasts are implicated in the synthesis and secretion of an extracellular matrix (ECM). Enzymatic cleavages followed by the reuptake of ECM remnants are prerequisite to promote dentin and/or pulp mineralization.

A few pulp cells are stem cells, probably less than 1% of the total cell population. According to Kenmotsu et al (5), approximately 0.40% of the pulp cells may be stem cells (also referred to as the side population) when they are found in young rats, whereas only 0.11% is found in adult rats.

Variations in cell number and the respective distribution and functions have been recorded between the central part of the pulp and the periphery and also between the radicular and coronal pulp (2). This article highlights the pulp complexity and how it is difficult to select genuine stem cell–based and cell-free approaches for pulp therapies.

Dental Pulp Stem Cells

Postnatal dental pulp stem cells (DPSCs) were firstly isolated from human teeth (6). They are largely neural crest–derived cells expressing genes that are also present in embryonic stem cells but lacking expression of mesodermal genes. It is difficult to have a clear idea of the outcome of stem cells because of the fact that their origin has not yet been elucidated although cell membrane markers and receptors specifically identify them. Depending on the culture medium, they become odontoblasts, osteoblasts, chondrocytes, adipocytes, neurons, and smooth muscles. Implanted with Hap/tricalcium phosphate, the clones differentiate into odontoblast-like cells producing a dentin-like structure, which are widely used to produce reparative dentin. In the presence of bone morphogenetic protein 2 and 4 (BMP2 and BMP4), DPSCs differentiate into dentin-forming odontoblasts. This hypothesis is strengthened by the fact that the BMP antagonist Noggin inhibits the capacity of DPSCs to differentiate into odontogenic cells as suggested by the lack of expression of dentin sialophosphoproteins.

Populations of DPSCs exhibit generic mesenchymal stem cell (MSC)-like properties; display the ability to form colonies; and express *in vitro* osteoblastic, adipogenic, chondrogenic, and even neuronal markers (6–9). DPSCs share many similarities with MSCs of the bone marrow (BMSCs), which are the most studied stromal stem cell populations. More than 4,000 human genes are expressed either by BMSCs or DPSCs (9). Dental stem cell populations also express different panels of stem cell surface markers used to characterize hematopoietic stem cells of the bone marrow (BMSCs) (10). However, it is important to note that DPSCs and BMSCs do not have the same embryonic origin. Cells derived from human or animal dental pulps have not been able to support hematopoiesis in transplantation assays (10, 11). DPSCs are thought to contribute to reparative dentin formation, and it appears that they

may correspond to heterogeneous populations of precursor cells or represent distinct differentiation stages along the odontoblastic lineage.

Pulp stem cells are implicated in pulp repair (8). Nevertheless, the formal demonstration that pulpal resident stem cells are actually the reparative dentin-forming cells recruited in response to injury is still lacking. The hypothesis that a subset of stem cells carried by the vasculature replenishes the pulp after a lesion cannot be totally excluded. Moreover, the responsiveness of the pulp provides a dynamic system for tissue repair that may imply migration of stem cells from their resting places to the site of injury. Undifferentiated mesenchymal/mesectodermal cells present in the stroma and perivascular cells, such as Rouget's pericytes, have been proposed as potential progenitors mediating pulp repair after destruction of the odontoblasts and the Hoehl's subodontoblastic cell layer (12, 13). Advances in the identification of stem cell markers are still needed to visualize stem/precursor cells *in situ*.

Different Dental Stem Cells

In addition to DPSCs, others stem cells have been obtained from human dental tissues (deciduous teeth and the apical part of the papilla) (6, 11, 14) and periodontal tissues (gingiva, cementum, alveolar bone, and periodontal ligament) (15). Stem cells were also isolated from the dental follicle. Other cellular candidates have been identified and implicated as new tools in the formation of mineralized tissues. BMSCs gave rise to osteoblast-like cells that generate a bone-like structure. DPSCs are producing structures resembling dentin, whereas BMSCs create bone condensation at some distance to capillaries (14).

Dental stem cells are considered as a population of MSC-like cells. Therefore, the markers that have been used for identifying MSCs were also instrumental for isolating dental stem cells. Subpopulations of DPSCs or stem cells from human exfoliated deciduous teeth (SHED) expressing c-kit/CD34/cell surface antigen expressed by stromal elements in human bone marrow (STRO-1) are considered as multipotent stem cells although c-kit and CD34 have also been shown to be markers for hematopoietic cell lineages (11–19). Therefore, dental stem cells and non–dental stem cells sharing a high proliferation rate, multidifferentiation ability, and easy accessibility are candidates for the development of tooth regeneration and/or tooth engineering (6, 8, 10).

Can Nonresident Migrating Cells Become Stem Cells?

In addition, 2 other groups of cells may potentially be stem cell candidates. These possibilities have been experimentally addressed.

The Origin and Role(s) of Nonresident Progenitor and Hematopoietic Cells in Pulp Repair

Investigating the potential roles of nonresident progenitors and hematopoietic cells in the contribution of stem cells found in the dental pulp, nonresident progenitors were suggested to contribute to reparative dentinogenesis. Parabiosis was established between transgenic (freen fluorescent protein + [GFP+]) and wild-type (GFP−) mice to ensure cross-circulation between the 2 mice. Pulp exposure and capping of the first maxillary molar were performed. GFP-positive cells were detected in close association with reparative dentin formed at the site of pulp exposure of GFP-negative mice (20).

This observation shows the participation of migrating nonresident progenitor and hematopoietic cells in the formation of reparative dentin. Used for the cell migration the blood circulation and moving throughout the mesenchymal tissues, nonresident progenitor GFP + cells were clearly implicated in the reparative process.

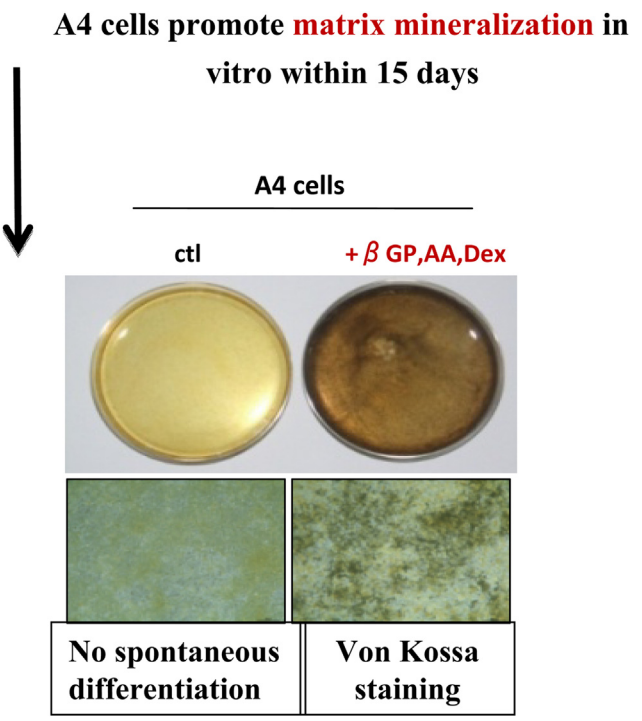

Figure 1. The multipotent pulp-derived A4 cell line cultured in monolayer for 15 days in an odonto-/osteogenic medium (βGP/AA/Dex) forms a mineralized matrix as shown by the von Kossa staining. In absence of an inducer, the A4 cells never differentiate spontaneously.

MSCs and Cell Migration

MSCs are found in bone marrow, cord blood, and dental pulp. They differentiate into osteoblasts, chondroblasts, adipoblasts, fibroblasts, myofibroblasts, and neuroblasts. The cells migrate from bone marrow, transit through the vasculature, and arrive at the affected tissues. Chemotaxis implicates enzymes, phosphorylases, and proteins inducing cell polarization and directional movement. These cells take origin in the bone marrow, and they are found in blood vessels. This implies that they may penetrate through the open apex and colonize the dental pulp.

Some years ago, a limited number of stem cells were identified, and marked differences were detected between embryonic and adult stem cells. Differences in gene expression, transcription factors, and growth factors account for the dissimilarities noticed between embryonic and adult lines. Adult stem cells alone are considered in this review within the frame of a therapeutic armamentarium. The number, origin, and stage of differentiation have increased, and selecting acceptable stem cells among a heterogeneous cell population becomes quite a hard task.

Genomic Stability

Immortalized human stem cells have been obtained from dental pulp. Genomic stability from transformed residing cells of ectomesenchymal origin has the potential to differentiate into odontoblast-like cells. It is actually required that the cells and their derivatives maintain their genomic stability. The presence of mosaicism and the accumulation of karyotypic abnormalities have been reported within cultured cell subpopulations. Gradually, many of the cultured cells (70%) exhibit karyotypic abnormalities after some passages. The heterogeneous spectrum of abnormalities indicates a high frequency of chromosomal mutations that continuously arise upon extended