REVIEW

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Dental pulp as a stem cell reservoir

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Abstract

Currently, much information is provided regarding the presence and the roles in tissue regeneration of stem cell niches residing in post-natal dental pulp. So far, three types of adult stem cells have been isolated from dental pulp. Correct evaluation of these cells is important in order to determine their potential use in clinical fields. The present study aims to review the origins and immunophenotype of these cells. The particularities of interstitial cells of the stem cell niches are also debated.

Keywords: stem niches, dental pulp, exfoliated deciduous teeth, apical papilla, interstitial cells, telocytes.

Stem cell niches

The stem cell niche concept was first proposed as a specialized microenvironment needed for cells to retain their stemness [1]. The niche is a fixed compartment of a structure containing elements that participate in the regulation of stem cell proliferation, control the fate of stem cell progeny, and prevent the stem cells from exhaustion or death [2–4]. Distribution of stem cells in postnatal tissues and organs is related to their existence in a perivascular niche [5].

During human tooth fetal morphogenesis, stem cell niche formation is related to the interrelationship between transcription factors, such as Oct4, Sox-2, Nanog and Stat-3 [6].

Two main types of stem cells have been described: embryonic stem cells (ES cells) and postnatal or adult stem cells (AS cells). ES cells are stem cells derived from the blastocyst. AS cells are tissue-resident self-renewable multipotential cells. AS cells are thought to migrate to the area of injury and differentiate into specific cell types to facilitate tissue repair [7, 8]. Autologous AS cells are an appealing, and practical source for cell-based regenerative therapies that hold realistic clinical potential. The identification of the regenerative potential of AS cells has encouraged intense research during the last years [9–11].

The dental pulp is soft tissue of ectomesenchymal and mesenchymal origin, developing from the dental papilla [12]. Several types of AS cells have been isolated from dental pulp [13]: (a) dental pulp stem cells (DPSCs) [14], (b) stem cells from human exfoliated deciduous teeth (SHEDs) [15], (c) stem cells from apical papilla (SCAPs) [16]. The precise relationship among these different stem cell populations is still unclear [10].

The DPSC niche

Dental pulp stem cells (DPSCs) are multipotent stem cells [13] which have advantages for clinical applications compared with other mesenchymal stem cells (MSCs) derived from bone marrow, adipose tissue, peripheral blood, and umbilical cord blood. The sources are easily available from discarded teeth after extraction with very low morbidity and little ethical issues. DPSCs can be cryopreserved, retaining their multipotential differentiation ability [17, 18].

The presence of DPSCs was proposed by Fitzgerald M *et al.* [19]. So far, at least two different stem cell populations were identified, neural crest-derived and mesenchymal-derived [20]. Both clonal stem cell subpopulations express STRO-1 and classical adult stem cell markers, as well as of Msx-1 and CD31. All cells are positive for vimentin [20, 21].

The DPSC niche in human dental pulp was identified by antibodies against STRO-1, CD146 and pericyte associated antigen 3G5, and was found to be localized in the perivascular and perineural sheath regions [22].

Recent studies monitoring *ex vivo* BrdU uptake by proliferative progenitor cells responding to injury have suggested that the progenitor/stem cell niches reside in the perivascular regions of the pulp, from where they migrate to the site of injury [23]. However, identification of stem/progenitor cell niches, as indicated by raised Notch expression following pulpal injury *in vivo*, has proposed niches in the pulpal stroma, odontoblast-subodontoblast layers, in addition to perivascular structures [24].

DPSCs have the ability to regenerate dentin, at shown in experimental animals [25, 26] and therefore have a high potential for tooth regeneration as odontoblast progenitors [14], being so a promising cell population

in regenerative endodontics [27]. However, ameloblast and odontoblast differentiation depends on interaction between the inner enamel epithelium and dental papillae mesenchyme [28]. DPSCs differentiate into adipogenic, chondrogenic and osteogenic lineages, express epithelial markers (such as cytokeratins-18 and -19) and share common characteristics with neural stem cells; they are also able to differentiate, *in vitro*, into neural or vascular endothelial cells [13, 29, 30]. DPSCs and endothelial cells have a synergistic effect in co-cultures, enhancing differentiation to osteogenic, odontogenic and angiogenic phenotypes [27]. Flk-1 (VEGF-R2) exerts a pivotal role in DPSCs differentiation into osteoblasts and endothelial cells [13]. The differentiation potential of DPSCs from natal teeth to adipogenic, osteogenic, chondrogenic, myogenic and neurono-glial cell lines was also shown, and it was demonstrated an intrinsic tendency of these cells of differentiation towards osteoblasts [31].

The available results may be developed through work on isolated different stem cell populations, or lines, of dental pulp. Lack of specific cell surface markers and of unitary concept may raise difficulties [32].

The mechanisms controlling the development of teeth are largely unknown. It has been suggested that incisors are derived from cells having ectodermal characteristics whereas the presumptive molar epithelium, despite being of ectodermal origin, has molecular commonality with pharyngeal endodermal cells [33].

The SHED niche

SHEDs have been isolated from dental pulp derived from exfoliated deciduous teeth. SHEDs present a higher proliferation rate than DPSCs, increased cell-population doublings, sphere-like cell-cluster formation, osteoinductive capacity *in vivo*, and failure to reconstitute a complete dentin pulp-like complex [15, 34]. Regarding odontogenic differentiation and osteogenic induction, SHEDs are distinctly different from DPSCs [35]. It has been suggested that dental neural crest-derived progenitor cells from the apical papilla of retained human third molars are precursors for primary odontoblasts, whilst SHED differentiate into replacement odontoblasts [36]. The potential of SHEDs to differentiate into osteogenic and adipogenic cells has been proven, as well as their mesenchymal surface molecule expressing STRO-1, CD146, SSEA4, CD73, CD105, CD166 and activating multiple signaling pathways, including TGFβ, ERK, Akt, Wnt, and PDGF [37].

SHED cells may represent a population of multipotent stem cells that are more immature than the DPSCs [35].

The SCAP niche

During tooth development, after crown formation is completed, the apical mesenchyme forms the developing periodontium; the inner and outer enamel epithelia fuse below the cervical level of the crown, forming an epithelial sheath with an important role in root formation [38].

The apical papilla of human immature permanent teeth may represent a source of new type of stem cells [16, 39]. It is possible that SCAPs are derived from neural crest cells or at least associated with neural crest cells, analogous to dental stem cells such as DPSCs and SHED [15, 40]. Morphogenetic dependent compartmentalization of neural crest derived cells into periodontal mesenchyme and dental papilla may be responsible for potential cell differentiation [41].

In order to differentiate the sources of odontoblasts' origin, it has been suggested that primary odontoblasts are those derived from the developing dental papilla, which form primary and secondary dentin, whereas replacement odontoblasts derive from dental pulp and replace primary odontoblasts, forming tertiary or reparative dentin [39]. With this regard, SCAPs seem to be the source of primary odontoblasts that account for the formation of root dentin, whilst DPSCs are probably the source of replacement odontoblasts that form reparative dentin [10, 42].

As long as the root develops, the dental papilla's location moves in an apical direction [39]. A layer of densely populated cell rich zone has been observed between the pulp and the apical papilla. The latter tissue can be easily detached from the apex due to a loose physical connection [39].

Mesenchymal markers, including CD13, CD24, CD29, CD44, CD73, CD90, CD105, CD 106 and CD146 are expressed in SCAPs [10, 43]. In addition, these cells show a positive staining for neural markers, such as βIII tubulin, GAD, NeuN, nestin, GFAP, neurofilament M, NSE, and CNPase [39]. It has been demonstrated that VEGF-C, VEGF-D, and VEGFR-3 are involved in the differentiation and maturation of odontoblasts and formation of the periodontal ligament and root [44]. In regenerative endodontics it is important to use fully characterized cell lines; in a cell line population of SCAPs coexpression of CD73, CD90 and CD105 was maintained in all passages but there were dramatic changes of gene expression when cells were maintained in culture with the up-regulation of different markers [45].

To conclude, SCAPs are derived from a developing tissue that may represent a population of early stem/ progenitor cells, which may be a superior cell source for tissue regeneration. Therefore, it can be hypothesized that developing tissues may contain stem cells distinctive from those of mature tissues [10].

Interstitial cells of the stem cell niches; the c-kit receptor

Stem/progenitor cells must migrate through the interstitial space to concentrate at the site of damage. However, information is lacking to what extent the interstitial interface is influencing the development of stem/progenitor cells [46]. It was shown that contacts between stem/progenitor cells with the interstitial interface influence further development [46]. The interstitial cells of Cajal (ICCs) are pacemakers of the gastrointestinal tract, and are also involved in signal transmission within tissues; usually, but not mandatory, ICCs label with antibodies against CD117 (c-kit) [47–53]. C-kit positive networks of interstitial cells were identified in cardiac

tissues, and were presumed to build a stromal network of non-neural transmission [54]. It was shown that CD44 positive cells correspond to ICCs, thus immunolocalization of CD44 can be used as a special marker, in addition to Kit, to identify ICCs [55].

Telocytes (TCs) represent a new interstitial cell type recently described [56]. TCs have a specific morphology and phenotype, both *in situ* and *in vitro* [57]. TCs are interstitial cells with specific prolongations named telopodes [58]; they contact different interstitial cells, nerve fibers, blood capillaries and "nurse" resident stem cells in niches [56, 59–64]. However, a specific immune phenotype of TCs is still in debate; only transmission electron microscopy (TEM) is a reliable tool to diagnose TCs *in situ* [58, 62, 65]. Before being distinguished as a distinctive cell type on morphological basis, TCs were consistently considered as being interstitial Cajal-like cells (ICLCs) [66–76], before being reconsidered as being "cell with telopodes" [77]. It was suggested that ICLCs might act as supporting nurse cells of the cardiac niches and may be responsible for activation, commitment and migration of the stem cells out of the niches [59, 75, 78]. This is reasonable, as time as stromal cells with telopodes are usually encountered in perivascular locations (Figure 1), and, on the other hand, stem cells are located in the microvasculature of the tissue they reside [22].

CD117/c-kit, and its ligand, the Stem Cell Factor (SCF), are involved, in normal tissues, in cell proliferation and differentiation, and in recruitment of progenitor cells in biologic systems [79]. CD117/c-kit labeling should be approached with caution, and in panels of markers, as it is known as also labeling neurons, glia, mast cells, retinal cells, and even pericytes [63, 80–82]. Stem/progenitor cells in murine dental pulp have a CD117-positive phenotype [83, 84]. DPSCs isolated by different methods were proven to have a similar phenotype, CD29+/CD44+/CD90+/CD105+/CD117+/CD146+ [85]. As compared to DPSCs, SHEDs have a significantly higher positivity for CD71, CD105, CD117 and CD166 [86]. Osteoblasts derived from human DPSCs were CD117+/CD34+/STRO-1+/CD45- [87]. Human dental pulp fibroblasts, as well as human gingival fibroblasts, were shown to express SCF and/or CD117/c-kit, but additional stem cell markers were not investigated in that study [79]. Human natal DPSCs were isolated, characterized, and compared to bone marrow-derived mesenchymal stem cells [31]. Both cell types expressed CD13, CD44, CD73, CD90, CD146, and CD166, but did not express CD34, CD45, CD117, and other markers, phenotypes which were considered consistent for an undifferentiated state [31]. DPSCs are more developed and metabolically active than the bone marrow-derived mesenchymal stem cells [29].

DPSCs undergoing osteogenic differentiation are CD117+/CD34+/CD45- [31, 88]. During osteogenic differentiation, stromal DPSCs change their surface antigen phenotype, lose the stem cell markers (such as CD34 or CD117), and gain differentiation markers (such as CD44) [13]. The nuclear factor c-Myc regulates the balance between hematopoietic stem cells renewal and differentiation, by influencing the stem cell niche interaction with the stem cells [89].

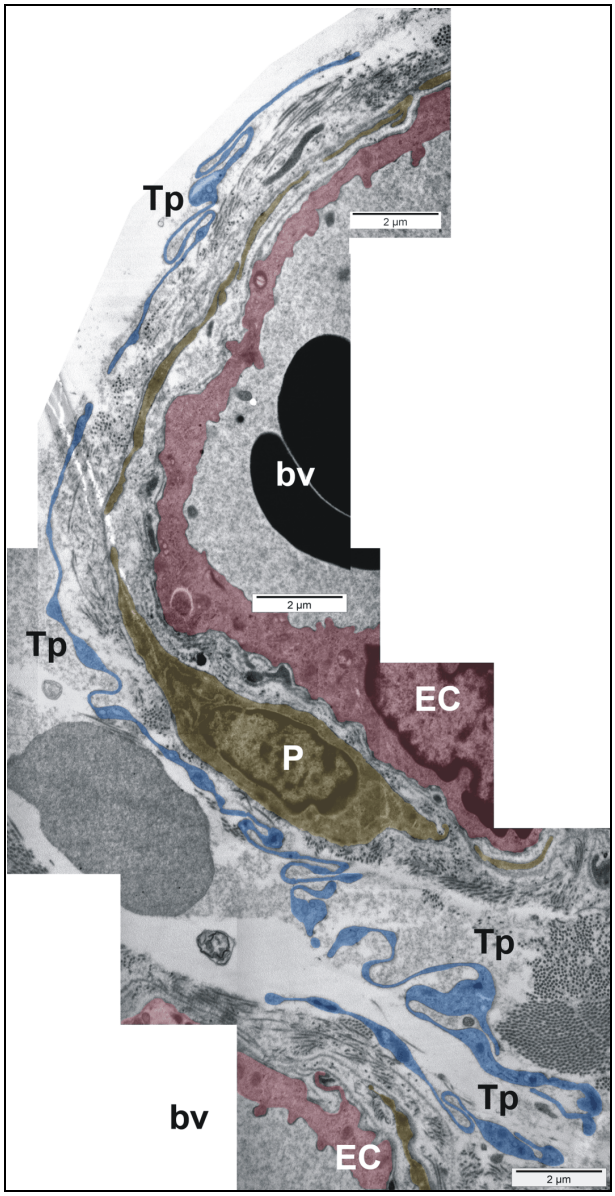

Figure 1 – ***Ultrathin section of rat trachea. Two-dimensional sequenced concatenation of nine serial electron micrographs. Perivascular telopodes (Tp, digitally colored in blue) are identified. EC (digitally colored in red): Endothelial cells; P (digitally colored in yellow): Pericytes; bv: Blood vessel.***

The phenotype of dental pulp stem cells: MSCs or MMSCs?

In 2006, the *Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy* established minimal criteria to define human MSCs, which include the following: adhesion to plastic, expression of specific surface antigens and capacity for *in vitro* multipotential (chondrogenic, adipogenic and osteogenic) differentiation, which is demonstrated by staining for cell type-specific markers. The immuno-phenotypic definition of MSCs requires that more than 95% of the population expresses the CD105, CD73 and CD90 surface antigens by flow cytometry analysis and that <2% of the population expresses the pan-leukocyte marker CD45, the primitive hematopoietic progenitor and endothelial cell marker CD34, the monocyte and

macrophage markers CD14 and CD11, the B-cell markers CD79 and CD19, or HLA class II [90, 91]. It was proposed that the plastic-adherent cells currently described as MSC to be termed multipotent mesenchymal stromal cells (MMSCs), while the term MSC should be reserved for a subset of cells that prove stem cell activity by clearly stated criteria. Thus, it is imperative that investigators unequivocally define the acronym. The immunophenotype of MMSCs still supports clarifications.

Based on these criteria, DPSCs and SHEDs fulfill all the requirements to be considered MSCs [92]. However, in SCAPs, although mesenchymal markers are expressed, the chondrogenic differentiation has not been demonstrated [10].

Concluding considerations and future perspectives

In the present review, the properties of DPSCs, SCAPs, SHEDs and interstitial cells have been emphasized, with focus on their origin and immune phenotype. However, there are still unknown answers to several questions that we may address below:

1. Do the frontal teeth have a different pattern of development from the posterior teeth? If so, the plasticity of DPSCs might differ, depending on the tooth type.

2. Which is the origin of SHEDs? They may originate from a perivascular microenvironment. STRO-1- and CD146-positive cells were found to be located around blood vessels of the pulp [93].

3. With regard to the special properties of the apical area, which are the relationships between cells in the apical papilla and those in the apical cell rich zone, and the vascular density of the apical papilla as compared with pulp's one?

4. Strong pieces of evidence support the presence of ICLCs/TCs as resident cells of the stem cell niches and the role of these cells in interstitial signaling within tissues. However, they were not previously documented in dental pulp tissue. That is, the dental pulp stem niche has not been yet evaluated by mirroring, for example, the cardiac stem cell niche.

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