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Research article

c-kit positive cells and networks in tooth germs of human midterm fetuses

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SUMMARY

Numerous studies have attempted to characterize the dental pulp stem cells. However, studies performed on prenatal human tissues have not been performed to evaluate the *in situ* characterization and topography of progenitor cells. We aimed to perform such a study using of antibodies for CD117/c-kit and multiplex antibody for Ki67+ caspase 3. Antibodies were applied on samples dissected from five human midterm fetuses. Positive CD117/c-kit labeling was found in mesenchymal derived tissues, such as the dental follicle and the dental papilla. The epithelial tissues, that is, dental lamina, enamel organ and oral epithelia, also displayed isolated progenitor cells which were CD117/c-kit positive. Interestingly, CD117/c-kit positive cells of mesenchymal derived tissues extended multiple prolongations building networks; the most consistent of such networks were those of the dental follicle and the perivascular networks of the dental papilla. However, the mantle of the dental papilla was also positive for CD117/c-kit positive stromal networks. The CD117/c-kit cell populations building networks appeared mostly with a Ki67 negative phenotype. The results suggest that CD117/c-kit progenitor cells of the prenatal tooth germ tissues might be involved in intercellular signaling.

1. Introduction

Although the tooth is a unique organ from structural and functional points of view, the principles that guide its development are shared in common with other organs. The most important developmental events are those guiding epithelial-mesenchymal interactions, which involve a molecular crosstalk between the ectoderm and mesenchyme, two tissues with different origins (D'Souza, 2002). 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 ectoderm origin, has molecular commonality with pharyngeal endodermal cells (Ohazama et al., 2010).

Constitutive activated tyrosine kinases (TKs) stimulate multiple signaling pathways responsible for DNA repair, apoptosis, and cell proliferation (Pytel et al., 2009). Stem cell factor (SCF) is the pleiotropic ligand for the TK receptor, which is c-kit (CD117). Binding of SCF to c-kit promotes cell proliferation, differentiation, and recruitment of progenitor cells in various biologic systems (Gagari et al., 2006). CD117/c-kit expression was demonstrated in human adult dental pulp cells (Gagari et al., 2006; Graziano et al., 2008; Laino et al., 2005), which are mesenchymal derivatives (Laino et al., 2005).

There are no studies available to evaluate the specific prenatal expression of CD117/c-kit during tooth development, neither in (ecto)mesenchymal-, nor in epithelial-derived tissues. Therefore, we aimed to perform such a study, targeting (ecto)mesenchymal-derived tissues; *i.e.* the dental follicle and dental papilla, and epithelial-derived tissues; *i.e.* the dental lamina, enamel organ and primitive oral epithelium.

2. Materials and methods

Autopsy samples of tooth germs were dissected out in blocks from four human midterm fetuses with ages varying from 4 to 6 gestational months (g.m.). Samples were drawn immediately postabortion. The pregnancy interruption was due to miscarriage. No history of pathology that would adversely affect development was recorded. Approval for the present study was granted by the

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Ethics Committee of the "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania.

Samples were fixed 24 h in buffered formalin (8%) and were processed with an automatic histoprocessor (Diapath, Martinengo, BG, Italy) with paraffin embedding. Sections were cut manually at 3 micrometers, and were mounted on SuperFrost® electrostatic slides for immunohistochemistry (Thermo Scientific, Menzel-Gläser, Braunschweig, Germany).

The following primary antibodies were used: (1) CD117/c-kit (clone Y145, Biocare Medical-PME 296 AA, Concord, CA, USA, prediluted RTU); (2) Ki 67+ caspase 3 (clone DVB-2, Biocare Medical-PPM 240 DS AA, Concord, CA, USA, prediluted RTU).

For CD117/c-kit labeling, sections were incubated for 30 min at room temperature (RT) with a polymer, and then were incubated for 5 min at RT with Biocare's DAB, and counterstained with hematoxylin.

For Ki67+ caspase, sections were retrieved under pressure using Biocare's Decloaking Chamber, followed by a wash in distilled water. Sections were incubated with the primary antibody for 60 min at RT; for double stain detection they were then incubated for 30 min at RT using Biocare's MACH 2 Double Stain 2. Chromogen: sections were incubated for 5 min at RT with Biocare's Betazoid DAB. Counterstaining was done with hematoxylin.

Positive controls: samples of gastrointestinal stromal tumors and colon cancer were used for CD117/c-kit, respectively Ki67+ caspase 3 antibodies. Sections incubated with non-immune serum were used as negative controls.

The microscopic slides were analyzed and micrographs were taken and scaled using a Zeiss work station described elsewhere (Rusu et al., 2013a).

3. Results

All samples presented the same features, as described below. CD117/c-kit labeling of dental papilla identified periendothelial immune positive cells (Fig. 1) sending processes that were coating the endothelial tubes; these cells were finally diagnosed as being pericytes, according to their topography, histological appearance and the periendothelial distribution.

CD117/c-kit diffuse interstitial positive labeling was found in the dental papilla. Also, CD117/c-kit positive cells were found in the proliferative and ameloblast layers of the enamel organ (Fig. 2).

Ki67 positive cells were scarcely observed within the dental papilla, but not in periendothelial locations (Fig. 3). Ki67 labeling identified proliferative stages of cells of the enamel organ.

CD117/c-kit positive cells of the dental follicle were found, being Ki67 negative (Fig. 4). These were bipolar cells with prolongations (Figs. 4 and 5), serially linked, configuring a multilayered network coating the outer adamantine epithelium (Fig. 5).

Multipolar progenitor cells, CD117/c-kit positive, were found within the secondary dental lamina, and were scarcely distributed beneath the basal epithelial layer (Fig. 6).

Ki67 positive, proliferative cells of the enamel organ were found in the stellate reticulum and in the outer adamantine epithelium. Scarce Ki67 positive cells were also present in the dental follicle. A higher density of Ki67 positive cells was observed in the secondary dental lamina, mostly in the basal epithelial layer (Fig. 7).

In the fetal oral epithelium CD117/c-kit positive cells were in basal or suprabasal locations, the later sending off prolongations passing between the basal cells, toward the basal epithelial lamina (Fig. 8).

4. Discussion

During the sixth week of human embryogenesis, the ectoderm covering the stomodeum begins to proliferate, giving rise to the dental laminae. Ectodermal–mesodermal interactions then lead to distinct stages, recognizable at the microscopic level. Once the tooth germ has developed, the neural crest cells differentiate into the dental organ, dental papilla and dental follicle. Thus, dental

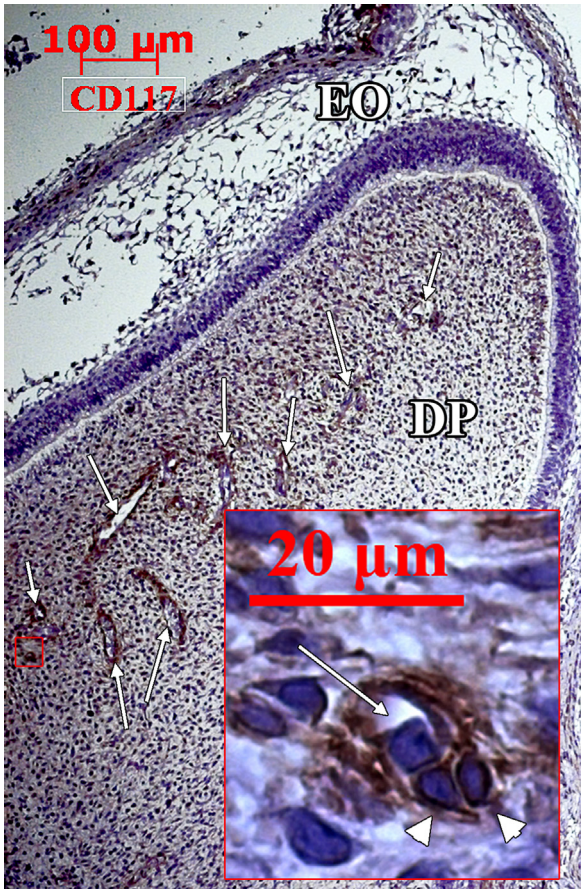

Fig. 1. Tooth germ of a 4.4 g.m. human fetus, immunolabeling with CD 117/c-kit. The enamel organ (EO) and dental papilla (DP) are indicated. Numerous blood vessels of the DP are identified (arrows) and present intense perivascular immune reactions. Inset: perivascular CD 117/c-kit positive cells (arrowheads) send prolongations with uneven caliber.

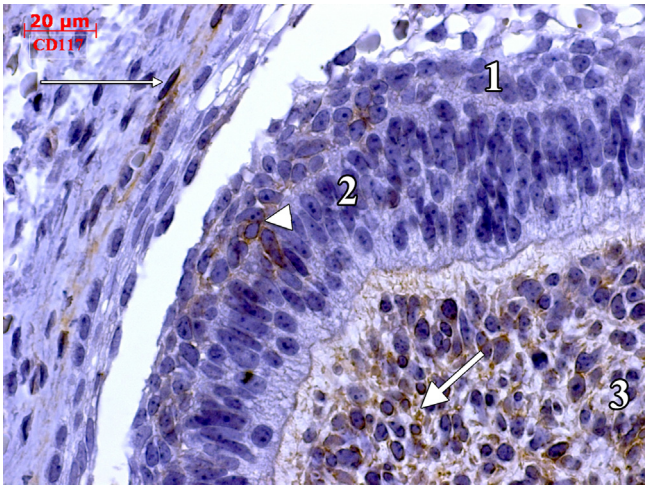

Fig. 2. Tooth germ of a 4.4 g.m. human fetus, immunolabeling with CD117/c-kit. Positive cells of the dental follicle are indicated (thin arrow). Positive progenitor cells (arrowhead) are identified at the border between the proliferative (1) and ameloblasts (2) layers of the enamel organ. Within the dental papilla (3) immune positive cells send processes and configure an interstitial network (thick arrow).