Differentiation 77 (2009) 29–37

Contents lists available at ScienceDirect

Differentiation

journal homepage: www.elsevier.com/locate/diff

Differentiation and regenerative capacities of human odontoma-derived mesenchymal cells

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ARTICLE INFO

Article history:
Received 10 March 2008
Received in revised form
1 July 2008
Accepted 15 July 2008

Keywords:
Odontoma
Dentin
Tooth regeneration
Odontoblasts
Mesenchymal

ABSTRACT

Regenerating human tooth *ex vivo* and biological repair of dental caries are hampered by non-viable odontogenic stem cells that can regenerate different tooth components. Odontoma is a developmental dental anomaly that may contain putative post-natal stem cells with the ability to differentiate and regenerate *in vivo* new dental structures that may include enamel, dentin, cementum and pulp tissues. We evaluated odontoma tissues from 14 patients and further isolated and characterized human odontoma-derived mesenchymal cells (HODCs) with neural stem cell and hard tissue regenerative properties from a group of complex odontoma tissues from 1 of 14 patients. Complex odontoma was more common (9 of 14) than compound type and females (9 of 14) were more affected than males in our set of patients. HODCs were highly proliferative like dental pulp stem cells (DPSCs) but demonstrated stronger neural immunophenotype than both DPSCs and mandible bone marrow stromal cells (BMSCs) by expressing higher levels of nestin, Sox 2 and βIII-tubulin. When transplanted with hydroxyapatite/tricalcium phosphate into immunocompromised mice, HODCs differentiated and regenerated calcified hard tissues *in vivo* that were morphologically and quantitatively comparable to those generated by DPSCs and BMSCs. When transplanted with polycaprolactone (biodegradable carrier), HODCs differentiated to form new predentin on the surface of a dentin platform. Newly formed predentin contained numerous distinct dentinal tubules and an apparent dentin–pulp arrangement. HODCs represent unique odontogenic progenitors that readily commit to formation of dental hard tissues.

1. Introduction

Post-natal stem cells have been isolated from different dental tissues including dental pulp (Gronthos et al., 2000), periodontal ligament (Seo et al., 2004), Hertwig's root sheath (Sonoyama et al., 2007), exfoliated deciduous teeth (Miura et al., 2003) and cementum (Grzesik et al., 1998). Each of these only regenerated one of the different tooth components that include enamel, dentin, cementum, periodontal ligament and dental pulp. Identifying dental stem cells capable of regenerating majority, if not all, of the tooth components is still ongoing. Odontoma, a developmental malformation composed of disorganized normal dental tissues, may contain stem cell populations with the unique ability to regenerate different tooth components. An odontoma that contains multiple well-formed miniature teeth is referred to as a compound odontoma, while one with a heterogeneous mixture of irregular tooth-like structures such as enamel, dentin, cementum, pulp tissue and odontogenic epithelium is referred to as a complex odontoma (Sapp et al., 2004). Within an odontoma is a rich network of undifferentiated cells that have not been clearly defined. The ability of these cells to form complex tooth structures and direct spatial relationships of enamel, dentin and cementum is still unclear. Despite association of odontomas with various developmental disorders of neuroectodermal origin (el-Saggan et al., 1998), odontomas are generally regarded as hamartomas (abnormal mixture of normal tissue elements) and not neoplastic proliferations (Sapp et al., 2004). Although dental lamina that initiates tooth development is formed from epithelial–mesenchymal interactions (Thesleff et al., 1991; Thesleff and Hurmerinta, 1981), the human odontogenic ectomesenchyme is derived from neural crest (Sharpe, 2001; Slavkin et al., 1988). Cell-fate determination and neural crest induction are also mediated by Wnt protein (Garcia-Castro et al., 2002). Specifically, Wnt/β-catenin signaling initiates '*de novo*' formation of teeth (Thesleff et al., 1995; Yamashiro et al., 2007). Several neural crest-derived tissues like dental pulp, periodontal ligament and maxilla/mandible

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0301-4681/$ - see front matter © 2008 Published by Elsevier Ltd. on behalf of International Society of Differentiation
doi:10.1016/j.diff.2008.09.005

have unique site-specific post-natal stem cells (Gronthos et al., 2000; Seo et al., 2004; Akintoye et al., 2006). So it is possible that odontoma originating from neural crest also contain multipotent post-natal stem cells that can be induced *in vitro* to differentiate into dental tissues.

Putative post-natal stem cells isolated from adult and deciduous teeth have limited regenerative capacity and form hard tissues that are still far from an anatomically or histologically precise tooth structure (Gronthos et al., 2000). Stem cells derived from the apical dental papilla exhibited greater capacity for dentin regeneration than those derived from dental pulp. Moreover, transplantation of a composite of apical papilla stem cells with periodontal ligament stem cells regenerated *in vivo* a well-formed root complex that was able to support a fixed porcelain crown (Sonoyama et al., 2006). While these results are promising, regenerating all the tooth components will require recapitulation of the embryonic tooth environment using highly pluripotent stem cells. Odontomas may be a viable source of multipotent odontogenic stem cells that readily differentiate into dental tissues. It is therefore conceivable that human odontoma contains post-natal stem cells that readily commit to dental differentiation and are apparently able to form majority of the dental tissues including enamel, dentin, cementum and pulp.

This study tested the hypothesis that a niche of neural crest-associated post-natal stem cells with dental regenerative capacity reside in odontomas. We identified highly proliferative human odontoma-derived mesenchymal cells (HODCs) that shared stem cell characteristics with dental pulp stem cells (DPSCs) and bone marrow stromal cells (BMSCs). When transplanted into immuno-compromised mice, HODCs regenerated highly differentiated dentin, cementum and pulp-like tissues *in vivo*, making them a unique population of odontogenic progenitor cells.

2. Methods

2.1. Patients and tissue sampling

Fourteen patients with radiographically diagnosed odontoma were enrolled in a tissue-collection clinical protocol (#417200) approved by the University of Pennsylvania after written informed consent. Cells were harvested from one 7-year-old female patient with a group of complex odontomas in the right maxilla (Fig. 1). Half of the odontoma samples were fixed in 4% PFA for histological analysis, while the other half were immediately placed in cold α-MEM for cell culture and further analysis.

2.2. Cell culture

Isolation of HODCs was done using an established protocol for isolation of human trabecular bone cells (Robey, 1995) described briefly. The pieces of odontoma tissues were carefully cleaned with sterile surgical blade to remove any contaminating soft tissues. The samples were broken into smaller fragments with sharp surgical scissors in a reaction vial containing enzyme medium made of Dulbecco's modified Eagle's medium (DMEM; plus high glucose and glutamine) and Ham's F-12 medium (Invitrogen, Life Technologies, Carlsbad, CA) at 1:1 ratio, 100 IU/ml penicillin, 67 mmol/l streptomycin sulfate, 0.13 mmol/l sodium L-ascorbate and 1 mmol/l calcium. Repeated fragmentation in several changes of enzyme medium was carried out until the samples became granular. The odontoma granules were digested with 250 IU/ml Collagenase P (Cat #11213873001, Roche Applied Science, Indianapolis, IN) in enzyme medium for 2 h at 37 °C. After repeated washes in enzyme medium, the odontoma granules were seeded in 150 mm culture dish containing growth medium made of DMEM and Ham's F-12 medium (1:1), 100 IU/ml penicillin, 67 mmol/l streptomycin sulfate, 0.13 mmol/l sodium L-ascorbate and 10% fetal bovine serum (Equitech Bio Inc., Kerville, TX) incubated at 37 °C in a humidified atmosphere of 6% CO_2 and air. The medium was changed twice per week and HODCs that emerged from the odontoma granules were maintained in culture until 75% confluence. Sub-confluent primary HODCs were released with trypsin–EDTA (Invitrogen, Life Technologies, Carlsbad, CA) and stored in liquid nitrogen until tested. Control post-natal stem cells compared with HODCs were dental pulp stem cells (DPSCs) and mandible bone marrow stromal cells (BMSCs) from a gender-matched 20-year-old normal volunteer enrolled in an institutionally approved protocol at the University of Pennsylvania (protocol #709137). These were isolated and cultured as described previously (Akintoye et al., 2006; Gronthos et al., 2000). Primary cultures of DPSCs and BMSCs were established in α-Minimum Essential Medium (α-MEM) supplemented with 100 IU/ml penicillin, 67 mmol/l streptomycin sulfate, 0.13 mmol/l sodium

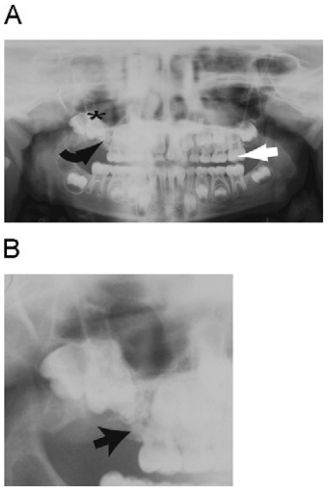

Fig. 1. Representative panoramic radiograph demonstrating location of odontoma. A complex odontoma is present in the right maxilla of this 7-year-old female patient (A). The location of the odontoma (curved black arrow) blocked eruption of the fully developed right maxillary first molar (black star). Note the left maxillary first molar (straight white arrow) is fully erupted and in occlusion with the opposing mandibular first molar. More detailed evaluation of odontoma (B) shows there are multiple odontomas blocking the normal eruption pattern of the permanent maxillary molar.

Table 1
Patient demography, location and histological characteristics of odontomas

Patient #	Age (years)	Sex	Jaw location	Histological variety
1	6	F	Maxilla	Complex
2	6	F	Mandible*	Complex
3	7	F	Maxilla	Complex
4	9	M	Maxilla*	Compound
5	10	F	Maxilla	Complex
6	11	M	Maxilla	Compound
7	16	M	Mandible	Compound
8	19	F	Maxilla	Complex
9	21	F	Mandible	Complex
10	25	M	Mandible	Complex
11	25	F	Mandible	Compound
12	27	F	Mandible	Complex
13	35	F	Maxilla	Compound
14	49	M	Mandible	Complex

M = male; F = female; * = anterior jaw.