Comparison of the Odontogenic Differentiation Potential of Dental Follicle, Dental Papilla, and Cranial Neural Crest Cells

Gang Chen, DDS, PhD,[*†‡] *Qince Sun, MS,*[*†] *Li Xie, PhD,*[*†] *Zongting Jiang, BS,*[*†‡] *Lian Feng, BS,*[*†‡] *Mei Yu, PhD,*[*†] *Weihua Guo, DDS, PhD,*[*†§] *and Weidong Tian, DDS, PhD*[*†‡]

Abstract

Introduction: During tooth development, cells originating from the neural crest serve as precursors to the cells in the dental follicle and dental papilla. Therefore, the current study aimed to understand the associations of cranial neural crest cells (CNCCs), dental follicle cells (DFCs), and dental papilla cells (DPCs) by performing a parallel comparison to evaluate their odontogenic differentiation capacities. **Methods:** In this study, we harvested the 3 cells from C57/green fluorescent protein–positive mice or embryos and compared the cell morphology, surface antigens, microstructures, and gene and protein expression. Under the odontogenic microenvironments provided by treated dentin matrix, the odontogenic differentiations of the 3 cells were further compared *in vitro* and *in vivo*. **Results:** The gene levels of DFCs in neurofilament, tubulin, and nestin were close to the DPCs, and in alkaline phosphatase, osteopontin, dentin matrix protein 1, and dentin sialophosphoprotein were the lowest in the 3 cells. However, Western blot results showed that DFCs possessed more similar protein profiles to CNCCs than DPCs, including collagen 1, transforming growth factor beta 1, osteopontin, neurofilament, and dentin matrix protein 1. Meanwhile, DFCs as 1 source of dental stem cells possessed high potency in odontogenic differentiation *in vitro*. Moreover, similar dentinlike tissues were observed in all 3 groups *in vivo*. **Conclusions:** CNCCs, DFCs, and DPCs possessed different biological characteristics in odontogenic differentiation. *(J Endod 2015;41:1091–1099)*

Key Words

Cranial neural crest cells, dental follicle cells, dental pulp cells, tooth development, tooth regeneration

The cranial neural crest is a transient cell population derived from the crest of the closing neural fold during the early stages of vertebrate embryogenesis (1). Cranial neural crest cells (CNCCs) migrate extensively and give rise to a wide variety of differentiated cell types such as peripheral neurons, glial cells, melanocytes, endocrine cells, and mesenchymal precursor cells (2). During craniofacial development, CNCCs migrate ventrally, accumulating and proliferating on the lateral and ventral side of the head, and produce a series of swellings known as the branchial arches (3). The teeth develop on the frontonasal process, which forms the maxilla or distal part of the upper jaw, and the first branchial arch, which gives rise to the entire lower jaw or mandible and the proximal part of the upper jaw (3).

Teeth are ectodermal organs developing from sequential reciprocal interactions between oral epithelial cells and cranial neural crest–derived mesenchymal cells (4, 5). Oral epithelial cells differentiate into odontogenic epithelial cells that give rise to enamel-forming ameloblasts, whereas mesenchymal cells differentiate into 2 distinct odontogenic cell lineages, dental papilla cells (DPCs) and dental follicle cells (DFCs) (6). DFCs are responsible for the formation of cementum, periodontal ligament, and alveolar bone (7), whereas DPCs are responsible for the formation of dentin and dental pulp tissues (8, 9).

Currently, bioengineering has been suggested as the most effective approach to solve issues related to the loss of dental pulp tissues (10, 11), periodontal tissues (12), and even whole tooth (13, 14). So far, the generation of adult stem cells has offered an alternative source for pluripotent cells and can be used as seed cells in tissue engineering (15). Previous reports imply that DFCs not only express the stem cell markers such as Notch-1, Stro-1, and nestin (16) but also possess high telomerase activity (17, 18). More importantly, the implantation of DFCs with treated dentin matrix (TDM) *in vivo* led to the formation of complete dentin-pulp–like tissues and cementum/periodontal ligament–like complexes (19). Thus, DFCs may represent a population of early stem/progenitor cells compared with other adult dental stem cells such as periodontal ligament stem cells.

However, little is known of the association between CNCCs, DFCs, and DPCs. To date, CNCCs can be isolated only from embryonic tissues. As shown in our previous reports, functional proteins and factors that existed in natural dentin could be preserved in TDM, thus providing the inducing microenvironment (20). Therefore, to explore the biological characteristics of CNCCs, DFCs, and DPCs and evaluate their potential in odontogenic differentiation, we harvested the 3 cell types from C57 mice embryos and teeth germs and performed a comparative investigation under the effects of TDM *in vitro* and *in vivo*. This article expands our understanding of the association of CNCCs, DFCs, and DPCs in tooth development.

From the *State Key Laboratory of Oral Diseases, †National Engineering Laboratory for Oral Regenerative Medicine, ‡Department of Oral and Maxillofacial Surgery, and §Department of Pedodontics, West China School of Stomatology, Sichuan University, Chengdu, People's Republic of China.

Address requests for reprints to Weihua Guo, Department of Pedodontics, West China School of Stomatology, Sichuan University, No 14, 3rd Section, Renmin South Road, Chengdu 610041, PR China. E-mail address: guoweihua943019@163.com or Weidong Tian, State Key Laboratory of Oral Diseases, West China Hospital of Stomatology, Sichuan University, No.14, 3rd Section, Renmin South Road, Chengdu 610041, PR China. E-mail address: drtwd@sina.com

0099-2399/$ - see front matter

http://dx.doi.org/10.1016/j.joen.2015.03.003

TABLE 1. Oligonucleotide Primer Sequences

Target cDNA	Sequence	Annealing temperature (°C)	Product size (bp)	Gene bank no.
ALP	5′- CACCTTGACTGTGGTTACTGCTG-3′ 5′- CTTCTTGTCCGTGTCGCTCAC-3′	56	115	NM_007431.2
OPN	5′- TTCACTCCAATCGTCCCTACAG-3′ 5′- CTCCTTAGACTCACCGCTCTTCA-3′	57	165	NM_009263.2
Nestin	5′- AGAGTTTGGTCGTGGGGAGAT-3′ 5′- AGGCAGGAGACTTCAGGTAGAGG-3′	55	134	NM_016701.3
Tubulin	5′- CATAAGTGAATTTGGGGAAGCTG-3′ 5′- ATGATCTTTGTCTTCTGCCTCCA-3′	57	142	NM_001080971.1
NF	5′- AGCACCCCACAGACATCAGAC-3′ 5′- GGCTTTTACCTCCTCCTTCACAG-3′	56	152	NM_010904.3
DMP-1	5′- CAGAGGGACAGGCAAATAGTGAC-3′ 5′- CATCGCCAAAGGTATCATCTCC-3′	57	168	NM_016779.2
DSPP	5′- GTGGGGTTGCTACACATGAAAC-3′ 5′- CCATCACCAGAGCCTGTATCTTC-3′	56	169	NM_010080.2
GAPDH	5′- TCAACGGCACAGTCAAGG-3′ 5′- ACCAGTGGATGCAGGGAT-3′	57	170	NM_008084

ALP, alkaline phosphatase; cDNA, complementary DNA; DMP-1, dentin matrix protein 1; DSPP, dentin sialophosphoprotein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; NF, neurofilament; OPN, osteopontin.

Materials and Methods

Experimental Design

DFCs and DPCs were isolated and cultured from dental follicles and dental papillae of 7-day-old, C57 mice, respectively. CNCCs were obtained from the first branchial arches of C57 mouse embryos (E 9.5). Human treated dentin matrix (h-TDM) was obtained from premolars, extracted for clinical reasons, and processed as previously described (21). Inactivated dentin matrix (IDM) used as the control was prepared (19) through high temperature and pressure. For further tracking the implanted cells in immunocompromised mice *in vivo*, the green fluorescent protein (GFP)-labeled cells from GFP transgenic C57BL/6J mice (C57/GFP + mice) were obtained and used. C57, C57/GFP + mice, and immunocompromised mice were all provided by the Laboratory Animal Research Centre of Sichuan University.

To investigate the gene and protein expressions of the 3 cells, real-time reverse transcription–polymerase chain reaction (RT-PCR) and Western blot analysis were used in this study. To further compare their responses to odontogenic microenvironment *in vitro*, the 3 cells were cocultured with TDM for 2 weeks. The odontoblast markers, dentin matrix protein 1 (DMP-1), dentin sialophosphoprotein (DSPP), and nestin, and the osteoblast marker osteopontin (OPN) were evaluated

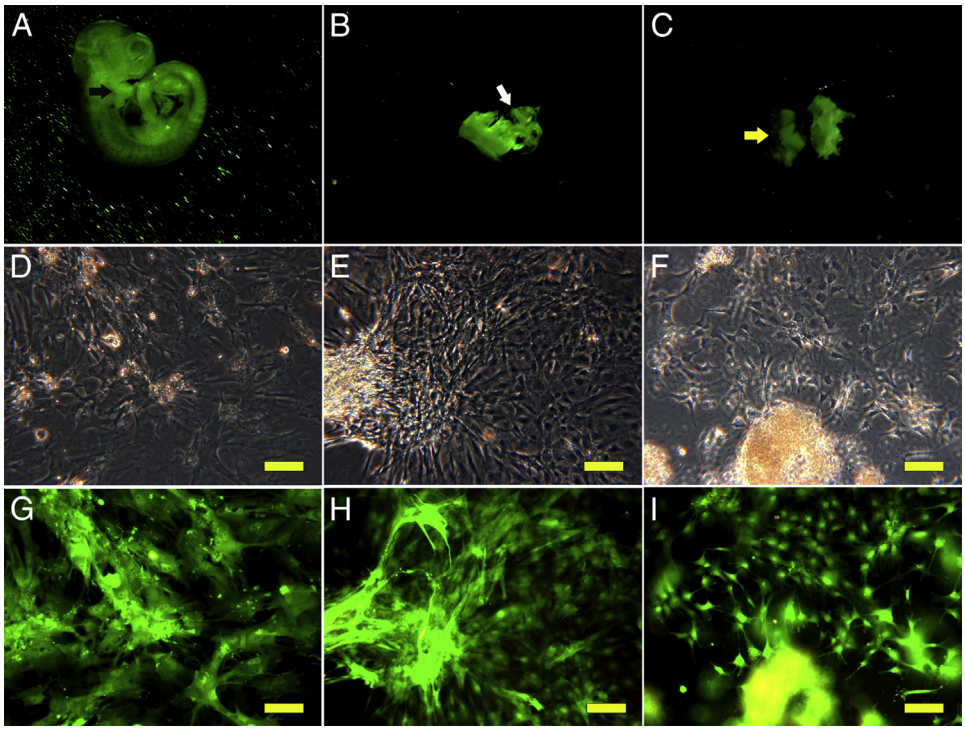

Figure 1. Derivation of cultured CNCCs, DFCs, and DPCs. The first branchial arch (*black arrow*) obtained from (*A*) C57/GFP + mouse embryo aged 9.5 days. (*B*) Dental follicle and (*C*) dental papillae obtained from a 7-day-old C57/GFP + mouse. During primary culture of (*D* and *G*) CNCCs, (*E* and *H*) DFCs, and (*F* and *I*), an obvious fluorescent signal can be observed using fluorescence microscopy. Scale bars = 100 μm. (The cells from common C57 mouse used in the *in vitro* comparisons are not shown here.)