RESEARCH REPORTS
Biological

Y. Zhang[1], Y. Song[1], S. Ravindran[1], Q. Gao[1], C.C. Huang[1], A. Ramachandran[1], A. Kulkarni[2], and A. George[1]*

[1]Brodie Tooth Development Genetics & Regenerative Medicine Research Laboratory, Department of Oral Biology, University of Illinois at Chicago, Chicago, IL 60612, USA; and [2]NIDCR, NIH, Bethesda, MD, USA; *corresponding author, anneg@uic.edu

J Dent Res DOI: 10.1177/0022034513516631

DSPP Contains an IRES Element Responsible for the Translation of Dentin Phosphophoryn

APPENDIX

MATERIALS & METHODS

Exosome Isolation from Culture Medium

Supernatant was collected from confluent T4-4 cells and centrifuged at 400 *g* at 4ºC for 5 min to remove debris. The cleared supernatants were processed with an Exosome extraction kit (Life Technologies, Grand Island, NY, USA) according to the manufacturer's protocol. The final pellet was re-suspended in 200 µL of PBS for immune transmission electron microscopy (TEM) and Western blot analysis.

Exosome Staining

Exosomes were mounted on carbon-/formvar-coated nickel grids (Electron Microscopy Sciences, Hartfield, PA, USA) fixed with 4% neutral formalin in PBS for 5 min and washed. They were permeabilized with 0.1% Triton X 100 in PBS and blocked with 5% BSA. Exosomes were labeled with rabbit anti-dentin phosphophoryn (DPP) or anti-CD-63 (Santa Cruz Biotechnology, Santa Cruz, CA, USA). They were washed and incubated with 20 nm gold-conjugated goat anti-rabbit antibody at a 1:100 dilution. The grids were washed and negatively stained with 1% aqueous uranyl acetate and viewed with a JEOL TEM at 80 kV.

Kidney Capsule Protocol

Mandibular molars were isolated from E13.5 dentin sialophosphoprotein (DSPP) null mouse embryos (a kind gift from Dr. Ashok Kulkarni). They were then enzyme-digested and dissociated into single-cell suspensions. Isolated cells were cultured as single layers in dishes with 10% FBS DMEM media (Song *et al*., 2006). These cells were transfected with replication-incompetent lentivirus media containing DPP. The transfected cells were pelleted and recombined with the dental epithelium isolated from DSPP knockout E10.5 mouse embryos.

Immunohistochemistry

Extracellular matrix (ECM) was isolated as previously published, and immunohistochemical analysis was performed for the localization of DSP and DPP (Ravindran *et al.,* 2012). The experimental protocol used was according to published protocols with anti-DPP (1:200) (rabbit polyclonal) and anti-dentin sialoprotein (DSP), (1:1,000) (monoclonal antibody, a kind gift from Dr. Chunlin Qin).

APPENDIX REFERENCES

Ravindran S, Gao Q, Kotecha M, Magin RL, Karol S, Bedran-Russo A, *et al.* (2012). Biomimetic extracellular matrix-incorporated scaffold induces osteogenic gene expression in human marrow stromal cells. *Tiss Eng Part A* 18:295-309.

Song Y, Zhang Z, Yu X, Yan M, Zhang X, Gu S, *et al.* (2006). Application of lentivirus-mediated RNAi in studying gene function in mammalian tooth development. *Devel Dyn* 235:1334-1344.

Appendix Table. Mouse DSPP Primers for Subcloning into pDsRED-GFP and pRF

Primer	Sequence
Region 1 72 bp of DSP and 24 bp of DGP	-forward ATACTAGTGACACCCGAGATGCAGAG
	-reverse ACGAATTCGTTGGGACCTTCAGTTTC
Region 2 126 bp of DSP and 90 bp of DGP	-forward GCACTAGTGAAGAAGGCGACAGTACC
	-reverse ACGAATTCTCCATTGCTATCTTTACT
Region 3 126 bp of DSP	-forward GCACTAGTGAAGAAGGCGACAGTACC
	-reverse ACGAATTCCTGATCTTGGCTCTTCCCA
Region 4 267 bp of DGP	-forward CACTAGTGGAATAGAAACTGAAGGTCCCAAC
	-reverse ACGAATTCTCCTTGCATGGACTCGTC
DSP+DGP (without signal peptide)	-forward ACTAGTATTCCGGTTCCCCAGTTAGTACCA
	-reverse ACGAATTCTCCTTGCATGGACTCGTC
DSPP (without signal peptide and stop codon)	-forward ACTAGTATTCCGGTTCCCCAGTTAGTACCA
	-reverse ACTAGTATCATCACTGGTTGAGTGGTTACTGTC

DSSP=dentin sialophosphoprotein; DGP= dentin glycoprotein; DSP=dentin sialoprotein.