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ABSTRACT

Cementum has been shown to contain unique polypeptides that participate in cell recruitment and differentiation during cementum formation. We report the isolation of a cDNA variant for protein-tyrosine phosphatase-like (proline instead of catalytic arginine) member-a (PTPLA) from cementum. A cementifying fibroma-derived λ -ZAP expression library was screened by panning with a monoclonal antibody to cementum attachment protein (CAP), and 1435 bp cDNA (gb AC093525.3) was isolated. This cDNA encodes a 140-amino-acid polypeptide, and its N-terminal 125 amino acids are identical to those of PTPLA. This isoform, designated as PTPLA-CAP, results from a read-through of the PTPLA exon 2 splice donor site, truncating after the second putative transmembrane domain. It contains 15 amino acids encoded within the intron between PTPLA exons 2 and 3, which replace the active site for PTPLA phosphatase activity. The recombinant protein, rhPTPLA-CAP, has Mr 19 kDa and cross-reacts with anti-CAP antibody. Anti-rhPTPLA-CAP antibody immunostained cementum cells, cementum, heart, and liver. Quantitative RT-PCR showed that PTPLA was expressed in all periodontal cells; however, PTPLA-CAP expression was limited to cementum cells. The rhPTPLA-CAP promoted gingival fibroblast attachment. We conclude that PTPLA-CAP is a splice variant of PTPLA, and that, in the periodontium, cementum and cementum cells express this variant.

KEY WORDS: cementum attachment protein, cementum, protein tyrosine phosphatase-like (proline instead of catalytic arginine), PTPLA, 3-hydroxyacyl-CoA dehydratase 1, periodontal ligament.

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Isolation of Protein-Tyrosine Phosphatase-like Member-a Variant from Cementum

INTRODUCTION

The cementum has been shown to contain polypeptides which have limited expression in cementum, cementoblasts, and putative cementoblasts. These polypeptides, which include cementum attachment protein (CAP) and cementum Protein 1 (CEMP1), are believed to regulate the biological activities of periodontal cells (Grzesik and Narayanan, 2002; Alvarez-Pérez *et al.*, 2006). Characterization of the gene expression and structure of these molecules is essential to an understanding of the molecular mechanisms that control cementum formation and identification of specific markers for cementum (Bartold *et al.*, 2000; Grzesik and Narayanan, 2002).

The CAP has been shown to play a role in cell recruitment and differentiation during cementum formation (Arzate *et al.*, 1992a; Pitaru *et al.*, 1995; Wu *et al.*, 1996; Saito *et al.*, 2001; Grzesik and Narayanan, 2002). It is expressed by dental follicle cells and promotes their adhesion and differentiation (Saito *et al.*, 2001; Handa *et al.*, 2002a,b; Kémoun *et al.*, 2007), and periodontal cells that strongly bind CAP are able to form cementum-like mineralized tissue in culture (Liu *et al.*, 1997; BarKana *et al.*, 2000). Here we report the isolation of a cDNA using a monoclonal antibody raised against the CAP. This cDNA appears to be an alternative splice variant of protein tyrosine phosphatase-like (proline instead of catalytic arginine) member-a (PTPLA; Uwanogho *et al.*, 1999; Li *et al.*, 2000). The protein, designated as PTPLA-CAP, was expressed in bacteria. We show that the recombinant protein has properties similar to those of the CAP isolated from cementum tissue, that its expression is limited to a few tissues, and that, in the periodontium, it is expressed by cementoblastic cells.

MATERIALS & METHODS

Isolation of CAP cDNA and Expression of Recombinant Protein

A λ -ZAP Express EcoRI/XhoI (Stratagene, La Jolla, CA, USA) cDNA library was constructed with mRNA from human cementum tumor-derived cells (Arzate *et al.*, 1992b). After transient expression in cos-7 cells, it was screened by panning with monoclonal anti-CAP antibody 3G9 (Saito *et al.*, 2001). Panning plates were prepared as described elsewhere (Alvarez-Pérez *et al.*, 2006), and positives were analyzed by PCR with T3 and T7 primers. Recombinant clones were constructed with a pENTR/SD/D-TOPO vector for directional cloning of a blunt-end PCR product (Invitrogen, Carlsbad, CA, USA). For protein expression, PCR product was obtained with sense primer 5' CACC ATG GGG CGC CTG ACG GAA GCG 3', and antisense primer 5' CAT AAA TAT TAC AGC AAT AGA 3'; we designed this sequence by eliminating the native stop codon to fuse the PCR product in frame with the

COOH terminal tag of the entry clone in a Gateway pET-DEST 42 vector (Invitrogen) for inducible expression. Protein was expressed in *Escherichia coli* BL21 strain (Invitrogen) in medium containing 100 µg/mL ampicillin (Alvarez-Pérez *et al.*, 2006). Recombinant protein (rhPTPLA-CAP) was purified from bacterial cell lysates by Ni²⁺ affinity chromatography HisPrep FF 16/10 column (GE Healthcare Bio-Sciences Corp., Piscataway, NJ, USA), followed by hydroxyapatite (HA)-Ultragel affinity chromatography (Sigma, St. Louis, MO, USA).

Antibody Preparation

Hydroxyapatite affinity-purified recombinant rhPTPLA-CAP was separated on 12% SDS-PAGE gel, and Mr 19,000 band was used to immunize New Zealand rabbits (Alvarez *et al.*, 2003).

Cells and Determination of Attachment Activity

Human gingival fibroblasts, cementoblasts, periodontal ligament cells, and alveolar bone cells were obtained as described previously (Arzate *et al.*, 1992b; Pitaru *et al.*, 1995). Attachment activity was determined with human gingival fibroblasts in plates coated with rhPTPLA-CAP (Pitaru *et al.*, 1995; Wu *et al.*, 1996). Wells coated with fibronectin served as positive control, and serum-free medium was negative control.

SDS-Polyacrylamide Gel Electrophoresis and Western Blot

Proteins were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE, 12% gels; for protein extraction from tissues and other details, please see the Appendix). Western blot analyses were performed with monoclonal anti-His (C-term) antibody (Invitrogen), monoclonal anti-bovine CAP antibody 3G9 (Saito *et al.*, 2001; Santa Cruz Biotech., Santa Cruz, CA, USA), and polyclonal anti-rhPTPLA-CAP antibody, as described previously (Wu *et al.*, 1996; Saito and Narayanan, 1999).

Immunostaining

Human tissues used were obtained from the Department of Pathology, School of Dentistry, UNAM, in conformity with the policy of the Research Review Board, Facultad de Odontología, UNAM. Formalin-fixed 5-µ sections were incubated with antigen retrieval solution (Shi *et al.*, 1992) and immunostained (Arzate *et al.*, 1998). Cells were plated at a density of 0.5 x 10³ cells/well in 8-well Lab-Tek chamber slides, cultured for 3 days, and immunostained as described previously (Arzate *et al.*, 1992a; Alvarez-Pérez *et al.*, 2006). Negative controls were sections immunostained with pre-immune rabbit serum or without primary antibody.

Quantitative Real-time PCR (qRT-PCR)

Total RNA was isolated with the Trizol Reagent (Invitrogen) and amplified by PCR with SuperScriptTM III Platinum[®] SYBR[®] Green One-Step qRT-PCR Kit with ROX (Invitrogen). Sense and antisense primers for PTPLA gene, respectively, were: 5'

ACTG GCT CAC CTT CTA CGA 3' and 5' GCA AGG CAA ATG TCT GGA 3'. PTPLA-CAP specific primers were: 5' TCC AGA CAT TTG CCT TGC TT 3' (sense); and 5' TTA CAG CAA TAG AAA AAC AGC ATG A 3' (antisense). Glycerolaldehyde-3-phosphate (GAPDH) gene expression was used for normalization. A 25-µL reaction was set up with the following PCR conditions: (cDNA synthesis) 50°C for 3 min, denaturation at 95°C for 5 min, followed by 40 cycles of 95°C for 15 sec, 60°C for 30 sec, and finally 40°C, 1 min. Amplifications were performed in a Corbett Rotor-Gene 6000 (Qiagen, Valencia, CA, USA). All experiments were performed in triplicate and normalized for GAPDH expression.

Statistical Analysis

Assays were performed in triplicate and repeated twice. Statistical significance was determined by Student's *t* test with Sigma Stat V 3.1 software. Significance level was set at alpha = 0.05.

RESULTS

Sequence Analysis

The human cementifying fibroma λ-Zap expression cDNA library was transiently expressed in cos-7 cells. After 3 rounds of "panning" with anti-bovine CAP IgG antibody (3G9), a 1435-bp cDNA was isolated. The nucleotide and deduced amino acid sequences of this cDNA are shown in Fig. 1. It has a consensus Kozak initiation sequence (CXXATGG), and the translation start site (ATG) is at nucleotide 37. The stop codon TGA is at base 457, and this is followed by a 3' untranslated region of 979 nucleotides. The cDNA, designated tentatively as PTPLA-CAP, encodes a polypeptide of 140 amino acids (Fig. 1; GenEMBL AY455942; gb AC093525.3). BLAST analysis of the non-redundant NCBI protein database revealed that the N-terminal 125 amino acids of PTPLA-CAP and 3-hydroxyacyl-CoA dehydratase 1/protein tyrosine phosphatase-like (proline instead of catalytic arginine) gene encoded on chromosome 10p13-p14 (NP_055056) are identical. A T-BLASTN search of the human genome demonstrated that the C-terminal 15 amino acids of the PTPLA-CAP are not present in PTPLA sequence (isoform 1, Fig. 1), and it is encoded by a read-through of the splice donor site of exon 2 that is utilized for expression of the full-length PTPLA with 288 amino acids (Uwanogho *et al.*, 1999; Li *et al.*, 2000). Thus, the PTPLA-CAP represents a second isoform of the 3-hydroxyacyl-CoA dehydratase 1/PTPLA. The PTPLA (isoform 1; Fig. 1) is predicted to contain 4 to 5 transmembrane spanning domains, and the second putative transmembrane domain is encoded by exon 2 (residues 121-125) and exon 3 (residues 126-143). Residues 126-136 of the second transmembrane domain contain the PTPLA active site IVHCLIGIVPT and the catalytic cysteinyl residue. In PTPLA-CAP, the exon 3 residues are replaced by the 15-amino-acid sequence VSFPSCCFSIAVIFM encoded by read-through into the intron downstream of exon 2 (Fig. 1). These intron-encoded 15 residues are hydrophobic and provide the remainder of the residues necessary for the second transmembrane domain initiated within exon 2. The PTPLA-CAP protein truncates immediately after

the second transmembrane domain and lacks the active site for phosphatase activity of PTPLA. Sequence analysis (SignalP 3.0 server) shows only a low probability of the presence of signal sequence, with probability of cleavage between positions 38 and 39.

The predicted translation product has a calculated molecular weight 14.9 kDa and pI 7.72. Analysis of predicted post-translational modifications (ExPASy) revealed that it does not contain N-glycosylation sites, while potential O-glycosylation motifs (S/T) are located at positions 5, 22, 25, 30, 32, 33, 39, 42, 43, and 48. Theoretical potential phosphorylation motifs, including a cdc2 (position 12, 22, 32), cdk5 (position 22, 30, 32), GSK3 (position 30), p38 MAPK (position 30, 33), CKII (position 42, 43, 53), and PKC (position 114), are present.

Purification of Recombinant Human PTPLA-CAP

The protein was expressed in *E. coli* and purified with Ni²⁺ and hydroxyapatite affinity chromatography. The purified protein migrated with ~19 kDa in SDS-polyacrylamide gels (lanes 1 and 2, Fig. 2A). It was recognized by monoclonal anti-His (C-term) (lane 3) and polyclonal anti-rhPTPLA-CAP (lane 5) antibodies, and by anti-CAP monoclonal antibody 3G9 (lane 4, Fig. 2A).

Cell Attachment Activity

The rhPTPLA-CAP promoted attachment of human gingival fibroblasts, and the attachment was dependent upon protein concentration (Fig. 2B). The attachment activity was comparable with that of fibronectin.

Immunostaining and Localization of CAP

Immunostaining with anti-rhPTPLA-CAP antibody showed staining of cementoblasts facing cementum and the matrix (Fig. 3a). Collagen-like fibers of periodontal ligament and periodontal ligament cell population were stained positively (Figs. 3b, 3c). The staining was similar to that of anti-CAP monoclonal antibody 3G9, which cross-reacts with cementoblasts and the periodontal ligament cell population (Fig. 3d). Cementoblastoma-derived cells were strongly positive, while periodontal ligament cells were moderately positive, and alveo-

	M G R L T E A A	
GAGAGCTGGCTGCGCCGAGCCCCCTGCGCGCTGCACATGGGGCGCCTGACGGAAGCGGGC		60
A A G S G S R A A G W A G S P P T L L P		
GCAGCGGGGAGCGGCTCTCGGGTGCAGGCTGGGCAGGGTCCCTCCACGCTCCTGCCC		120
L S P T S P R C A A T M A S S D E D G T		
CTGTCTCCACGTCCTCCAGGTGCGCGCCACCATGGCGTCCAGCGACGAGGACGGCACC		180
N G G A S E A G E D R E A P G E R R R L		
AACGGCGGCCCTCGGAGGCCGGCAGGACGGGAGGCTCCCGCGAGCGGAGCGCCCTG		240
G V L A T A W L T F Y D I A M T A G W L		
GGGGTCTTGGCCACCGCTGGCTCACCTTCTACGACATCGCCATGACCGGGGGTGGTTG		300
V L A I A M V R F Y M E K G T H R G L Y		
GTTCTAGCTATTGCCATGGTACGTTTTTTATATGGAAAAAGAACACACAGAGGTTTATAT		360
K S I Q K T L K F F Q T F A L L E V S F		
AAAAGTATTTCAGAACACTTAAATTTTTCCAGACATTTGCCTTGCTTGGTAAAGTTTT		420
P S C C F S I A V I F M *		
CCATCATGCTGTTTTCTATTGCTGTAATATTTATGTGAACATTTTTTGTGTGCAAAAT		480
CAATATGATTTTTGCTGATTACATACCACCTATGGATTGCTGAAAGCCCAATGTTGACAGT		540
GGTTTTATTAATGTTTTCTATTATTGACGTAATGATTTTTTTCTATTGCTTACAGATAG		600
TTACACTGTTTAAATGGTGGAGTTTTTGGCTCAATTTTATACTTTTATATAAAATGGCCTTT		660
AGGGCAATAGTTCACTTGTTTTTCTTTTTAAGGAATTGTACCTACTTCTGTGATTTGTGAC		720
TGGGGTCCAAGTGAATCAAGAATCTTTATGGTGGGCTCATCTACAGTATAAAACC		780
AGTAAGTGACACAAACATGTTGCTACTTGAAGCTGCAGGAAGCTTCCATTCATAGGA		840
ATCTAAAATATTAACATTTGTATATTTTCAGGAGGTGGAAGTGTGGACAAAAGGAGTTG		900
GGTTTGAATTTAGATGTTGTGCATTGACTTAGTGCTTAGCAGAATGACTTTGATACAC		960
AAACAAACCCAAATATTGGCATCTTAGATCCGTAATATATTTCTCCTTTGATATTTTCCAG		1020
TTTTTGAATCAGTATTTCAGGTTCTGTCAAATAACCTGCAGAAAATGTTCTTTTCTATAG		1080
AAGTTTCTTTTCAATCCAAGTTGTCCTCTTTGATATGTAATCGAAGTTGTCAGTATACA		1140
ATTTATTTTCTGGCCAGCCGAGTGGCCACACTGTAATCCAGCACTTTGGGAGGC		1200
CAAGGCGGGTGGATCACCTGAGGTGAGGAGTTTGAGACCAGCTGGCCCAACATGGTGAAA		1260
CCCTGTCTCTACTAAAATAACAAAATTAACAGGCGTGGGGCGGAGCACCTGGAATCCC		1320
AGCTACTCAGGAGGCCGAGGACAAGAATCGCTTGAACCCGAGAGGCAAGGTTGCATTG		1380
AGCTGAGATTTGTCGCCACTGCACCTGGGGCAGACAGCGAGACTCCGCTG		1435
Isoform2	MGRLTEAAAAGSGSRAAGWAGSPPTLLPLSPTSPRCAATMASSDEDGTNGGASEAGEDRE	60
Isoform1	MGRLTEAAAAGSGSRAAGWAGSPPTLLPLSPTSPRCAATMASSDEDGTNGGASEAGEDRE	60
Isoform2	APGKRRRLGFLATAWLTFFYDIAMTAGWLVLAIAMVRFYMEKGTTHRGLYKSIQKTLKFFQT	120
Isoform1	APGKRRRLGFLATAWLTFFYDIAMTAGWLVLAIAMVRFYMEKGTTHRGLYKSIQKTLKFFQT	120
Isoform2	FALLEV SPSCCFSIIVFM	140
Isoform1	FALLEI VHCLIGIVPTSVIVTGVQVSSRIFMVWLITHSIKPIQNEESVVLFLVAWTVTEI	180
Isoform1	TRYSFYTFSLDHLPIFYKWARYNFFIILYPVGVAGELLTIYAALPYVKKTMGFSIRLPN	240
Isoform1	KYNVSFDYYYFLLITMASYIPLFPQLYFHMRLRQRKVLHGEVIVEKDD	288

Figure 1. The cDNA and deduced amino acid sequence of PTPLA-CAP (AY455942). In PTPLA-CAP, the sequence VSFSPSCCFSIIVFM, which replaces IVHCLIGIVPT in PTPLA (isoform 1; AF114494), is highlighted in black. For a schematic drawing showing mRNA sequence and exon positions of PTPLA-CAP and PTPLA, see Schild *et al.* (2009). Isoform 2 encoding the PTPLA-CAP protein is encoded by an alternate spliced mRNA that does not have a splice between exons 2 and 3; instead, the C-terminal end of the protein terminates after 14 residues encoded within the intron between exons 2 and 3. The non-functional splice donor site "AGGT" of exon 2 is underlined. The predicted transmembrane spanning domains in PTPLA (see isoform 1) are highlighted in gray. The 14 intron encoded amino acids (boxed) constitute the C-terminal half of a second putative transmembrane domain within the PTPLA-CAP protein; thus, the alternately spliced isoform 2 (PTPLA-CAP protein) lacks the additional transmembrane domains in the longer isoform 1 PTPLA. Sequence analysis shows only a low probability of a signal sequence, with cleavage probability between positions 38 and 39.

lar-bone-derived cells and human gingival fibroblasts were negative (Appendix Fig. 1A). Heart, liver, and kidney showed positive staining, and other tissues had weak or no reactivity (Appendix Fig. 1B). Western blot analysis detected a ~39 kDa protein in extracts of cementum and periodontal ligament tissues, but not gingiva and alveolar bone (Fig. 3B).

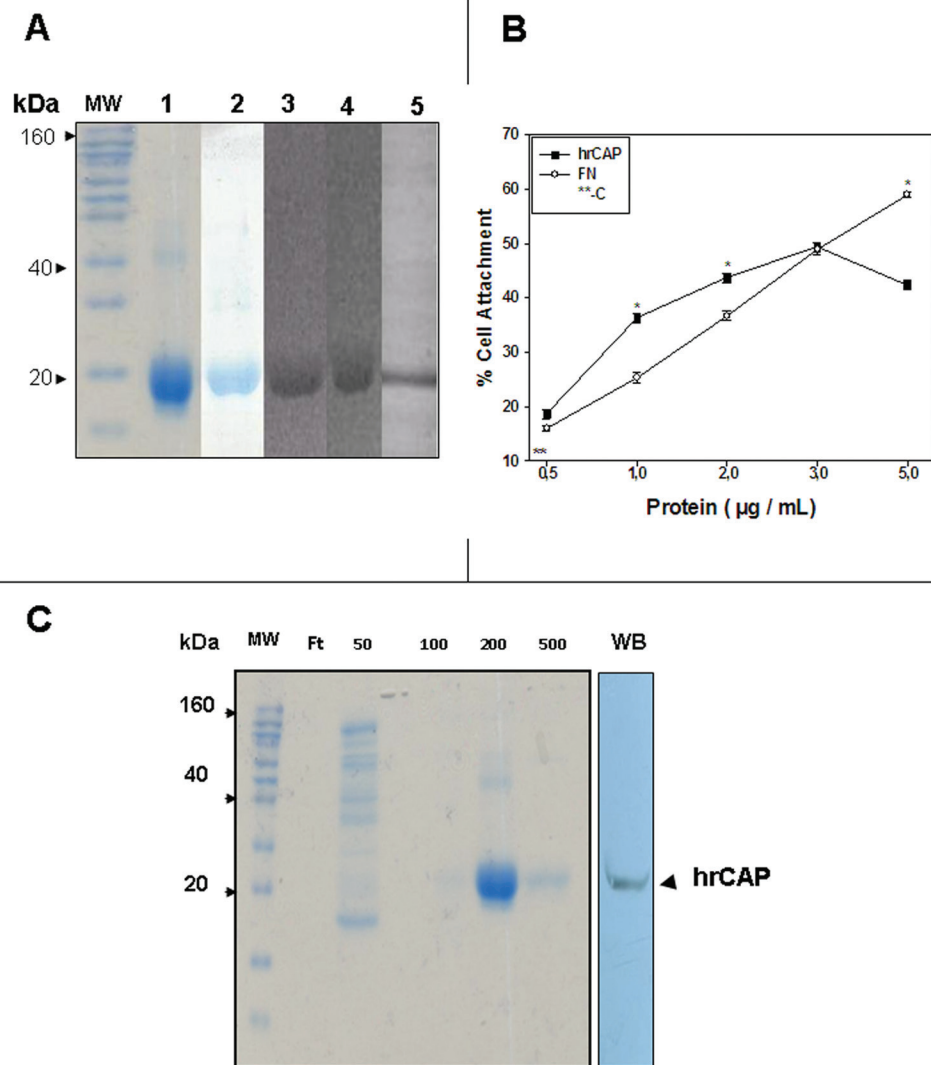


Figure 2. Properties of rhPTPLA-CAP. **(A)** SDS-polyacrylamide gel electrophoresis of proteins expressed by bacteria carrying the expression construct. Lane 1: Novex Sharp (Invitrogen) protein markers, Coomassie-blue-stained. Lane 2: expressed protein, before purification, Coomassie-blue-stained. Lane 3: expressed protein, after Ni²⁺ affinity chromatography. Lane 4: Western blot, anti-6xHis antibody. Lane 5: Western blot, anti-CAP antibody 3G9. Lane 6: Western blot, anti-rhPTPLA-CAP polyclonal antibody. **(B)** Attachment assay with purified rhPTPLA-CAP. Human gingival fibroblasts were plated at 2×10^4 cells/well in 24-multiwell Costar plates not treated for tissue culture and previously coated with 0.5, 1.0, 2.0, and 5.0 µg/mL of hrPTPLA-CAP. Cell attachment was measured after 4 hrs. Wells coated with 5 µg/mL of fibronectin served as positive control (FN), and serum-free medium was negative control (**C). Values plotted represent mean \pm SD of triplicates. * indicates statistically significant differences ($p < 0.05$). **(C)** Binding of rhPTPLA-CAP to hydroxyapatite (HA). The recombinant protein was loaded on a HA Ultrogel column and eluted with 50, 100, 200, and 500 mM Na₂PO₄, pH 7.2. Coomassie blue staining shows that the rhPTPLA-CAP is eluted by 200-500 mM Na₂PO₄. Ft: flow through. Western blot (WB) shows that the HA-binding protein (hrCAP) cross-reacts with anti-bovine CAP monoclonal antibody (3G9; arrow).

Expression in Periodontal Cells

Immunostaining showed the presence of PTPLA-CAP in cementum and cementum cells (Fig. 3); in contrast, PTPLA is expressed in many tissues (Uwanogho *et al.*, 1999; Li *et al.*, 2000), but not in rat teeth (Schild *et al.*, 2009). Therefore, we compared the expression of PTPLA-CAP and PTPLA in human periodontal cells. We performed Quantitative PCR using primer pairs for

PTPLA and for the sequence flanking the alternatively spliced domain in PTPLA-CAP (Fig. 1). The results showed that PTPLA was expressed in gingival fibroblasts, periodontal ligament cells, and osteoblasts, and it was expressed relatively less (~10% relative to gingival fibroblasts) in cementum cells (Fig. 4A). In contrast, the alternatively spliced PTPLA-CAP sequence was expressed only in cementoblasts (Fig. 4B).

DISCUSSION

Our study has identified an alternatively spliced mRNA that encodes a truncated isoform of 3-hydroxyacyl-CoA dehydratase 1/PTPLA. This mRNA, PTPLA-CAP, encodes a 140-amino-acid protein that is identical to the first 125 N-terminal amino acids of PTPLA, which is 288 amino acids long (isoform 1, Fig. 1). The remainder of the C-terminus of PTPLA-CAP is encoded by a read-through of the splice donor site in exon 2 of the PTPLA isoform into the adjacent intron. This protein is predicted to have 2 transmembrane spanning domains, and it truncates immediately after the second transmembrane domain. The truncation eliminates the PTPLA sequence IVHCLIGIVPT, which has the signature phosphatase active site motif (I/VHCX XGXXP(S/T) (Uwanogho *et al.*, 1999; Li *et al.*, 2000), and additional C-terminal transmembrane spanning domains predicted for the PTPLA isoform. We have examined the NCBI EST database for the presence of corresponding EST sequences and detected 1 human and 1 rat EST showing the same alternate splice.

The human EST (CN305861) was derived from undifferentiated human embryonic stem cell lines maintained on a feeder-free layer.

The PTPLA mRNA is widely expressed in many tissues (Uwanogho *et al.*, 1999; Li *et al.*, 2000; Schild *et al.*, 2009). In the periodontium, PTPLA is expressed in gingival fibroblasts, periodontal ligament cells, and osteoblasts, and only marginally in cementum cells (Fig. 4); in contrast, qRT-PCR revealed that

the PTPLA-CAP is expressed in cementum cells, not in other cells (Figs.3A, Figs.4). It is also expressed in the heart. This result is consistent with a recent report demonstrating expression in rat heart and muscle (Schild *et al.*, 2009). This report demonstrates no expression in rat teeth, and our results showed a low level of expression in cementum cells (Fig. 4); the reasons for this difference are not clear, although one possibility is the human *vs.* rat species difference. It is likely that the low level of PTPLA expressed in cementum cells (Fig. 4A) represents the PTPLA-CAP in these cells.

The rhPTPLA-CAP migrates with ~19 kDa, while the anti-rhPTPLA-CAP antibody recognizes a 39-kDa protein in cementum and periodontal ligament extracts. We believe that the most likely explanation for the size difference between the recombinant and cell/tissue proteins is post-translational modification; we have not performed experiments to determine this possibility. Cell lysates of gingival fibroblasts, periodontal ligament cells, bone cells, and cementum cells contain ~40 and 38 kDa species; these are absent in blots with antibody pre-incubated with rhPTPLA-CAP (Appendix Fig. 2), indicating that these represent PTPLA-CAP. The PTPLA-CAP is not detected in gingiva and bone (Fig. 3A), while it is detectable in cells of these tissues; one possible reason for this difference is that *in vitro* culture conditions facilitate the expression and persistence of PTPLA-CAP in culture.

The CAP promotes the preferential adhesion of putative cementoblasts (Liu *et al.*, 1997; BarKana *et al.*, 2000; Saito *et al.*, 2001). Adhesion to CAP is sufficient to promote growth-factor-induced DNA synthesis, and this is mediated through signaling events participating in cell-cycle events (Saito and Narayanan, 1999; Komaki *et al.*, 2000; Yokokoji and Narayanan, 2001). The action of CAP appears to be mediated through binding to $\alpha 5\beta 1$ integrin (Ivanovski *et al.*, 1999); however, we do not know if the rhPTPLA-CAP also binds to this receptor. Like the CAP, the

rhPTPLA-CAP promotes attachment of periodontal cells and binds to hydroxyapatite (Pitaru *et al.*, 1992) (Fig. 2C). These observations indicate that the PTPLA-CAP and CAP may play a regulatory role during cementum formation (Grzesik and

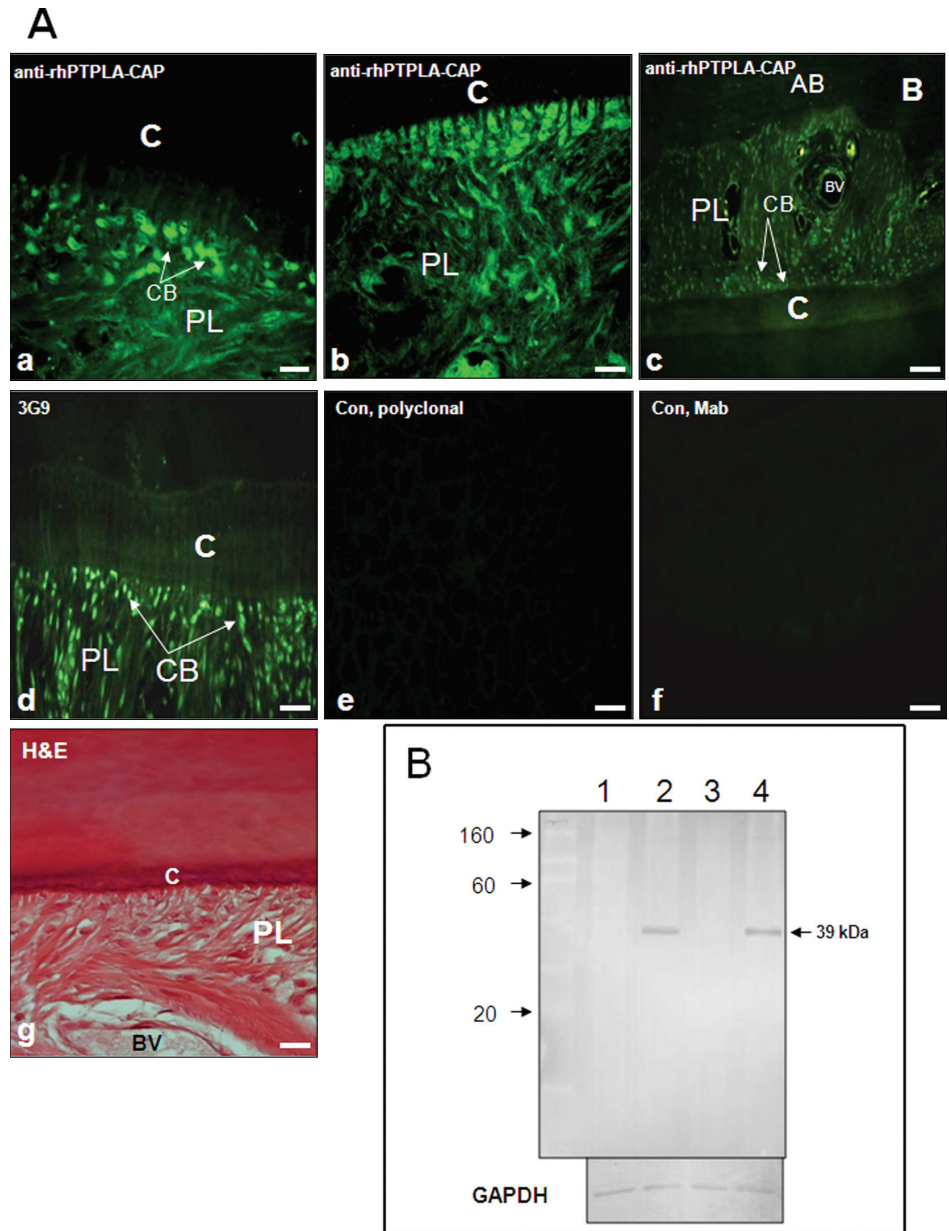


Figure 3. Expression of PTPLA-CAP in human periodontium. (A) Immunostaining of periodontal tissue with anti-rhPTPLA-CAP polyclonal antibody. Staining is observed in cementoblasts facing cementum and in the matrix (a) (arrows). Cementoblasts and collagen-like fibers of the periodontal ligament are stained positively (b) Expression of PTPLA-CAP in human periodontium. Lower view of periodontal tissue shows immunostaining in cementoblasts (arrows) and periodontal ligament cell population (c). Monoclonal antibody against bovine cementum attachment protein (3G9) cross-reacts with cementoblasts (arrows) and periodontal ligament cell populations (d). Controls with pre-immune rabbit (e) or mouse serum (f) were negative. H&E staining shows tissue orientation (g). BV, blood vessel; C, cementum; PL, periodontal ligament. Magnification 20x (Bar = 100 μ m). (B) Western analysis of gingival tissue (lane 1), cementum (lane 2), alveolar bone (lane 3), and periodontal ligament (lane 4) extracts with anti-rhPTPLA-CAP antibody. The antibody recognizes a 39-kDa protein in extracts of gingival and periodontal ligament tissues. The bottom panel shows staining for GAPDH for protein loading.

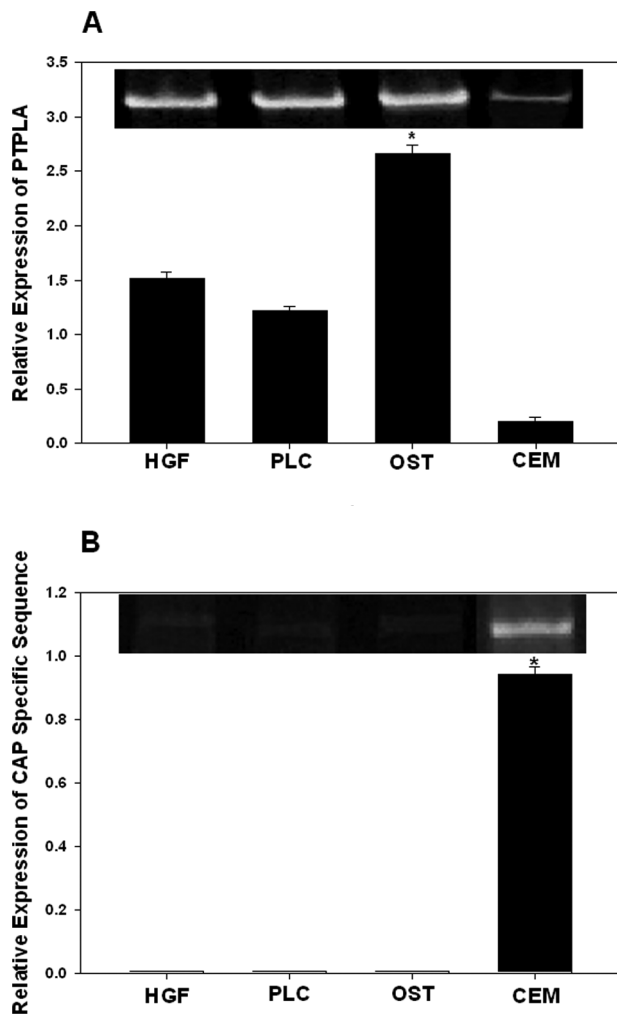


Figure 4. Relative expression of PTPLA and PTPLA-CAP determined by quantitative PCR after normalizing for GAPDH. **(A)** PTPLA is expressed in human gingival fibroblasts (HGF), periodontal ligament cells (PLC), and alveolar-bone-derived cells (OST). Cementoblastoma-derived cells showed relatively weak expression. No bands were visible in controls without reverse transcriptase (data not shown). **(B)** Expression of PTPLA-CAP measured with the primer sequences specific for PTPLA-CAP. Values plotted in A and B represent mean \pm SD of triplicates. *Differences were statistically significant ($p < 0.005$).

Narayanan, 2002). However, the significance of its homology to PTPLA and 3-hydroxyacyl-CoA dehydratase is intriguing. The enzyme 3-hydroxyacyl-CoA dehydratase (HACD1/PTPLA) and 3 other homologues are involved in the third step, catalyzing the dehydration of 3-hydroxyacyl-CoA during the synthesis of very long-chain fatty acids (Ikeda *et al.*, 2008); whether the CAP has a similar function is not clear. The homologue of PTPLA-CAP, PTPLA, is a phosphatase; however, its active site is absent in PTPLA-CAP. Thus, one possibility is that the PTPLA-CAP may regulate the phosphatase activity of PTPLA. Interestingly, cyclosporine A, which inhibits calcineurin, another phosphatase, also induces cementum formation and mineralization

(Ayanoglou, 1998; Arzate *et al.*, 2005). If this is the case, the outcome of PTPLA-CAP action is likely to be different in other tissues, which are not mineralized, where it is expressed. Further work is necessary to investigate these possibilities.

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