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The Effect of Phosphatase and Tension Homolog (PTEN) on Homeostasis of the Periodontal Ligament

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Abstract

Aim: Phosphatase and tension homolog (PTEN) has been known to maintain homeostatic control over the body. The roles of PTEN in periodontal complex are unknown. The purpose of this study was to investigate the role of PTEN in periodontal structures by removing PTEN from osteoblasts and odontoblasts. Materials and Methods: The function of this endogenous PTEN was evaluated by conditionally eliminating the PTEN gene using an Osteocalcin (OCN) Cre driver. The resulting OCN-Cre$^{tg/+}$; Pten$^{fl/fl}$ mice were examined using micro-CT and histology, immunohistochemical analyses for osteogenic markers in the periodontal ligament (PDL) and bone turnover. Results: Bone apposition was increased around molar areas accompanying deposition of cementum in micro CT. Osteoprogenitor markers except for OCN in the PDL maintained their expression in both wild-type and OCN-Cre$^{tg/+}$; Pten$^{fl/fl}$ mice. Both alkaline phosphatase activity and osteoclast activity increased in the PDL of OCN-Cre$^{tg/+}$; Pten$^{fl/fl}$ mice compared to those in wild-type mice. Conclusions: Loss of PTEN causes an increase of bone turnover in the periodontal surrounding tissues with an increase of cementogenesis. These findings underscore the effect of PTEN on homeostasis of the periodontal ligament.

Keywords

Phosphatase and Tension Homolog (PTEN), Periodontal Ligament (PDL)

1. Introduction

The phosphatase and tension homologue (PTEN) plays as a tumor suppressor, which helps control cell division through phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) signaling pathway [1]. PTEN contributes to homeostasis by

regulation of cell dividing and apoptosis [2]. Mutation of the PTEN gene has been reported to a cause of cancer, non-cancerous neoplasia and brain disorder [3]. Defects of the PTEN gene are related to Cowden syndrome and hamartomas in multiple organs [4].

Loss of PTEN in mice osteoblasts showed an accumulation of bone volume and density at all skeleton during a life time, although they were a normal size at a birth [5]. Loss of PTEN was also associated with a decrease of osteoblast apoptosis [5]. Deficiency of PTEN caused an accumulation of collagen in the liver [6] and lung fibrosis [7]. Knockdown of PTEN in fibroblasts resulted in interruption of apoptosis in collagen matrices [8].

Teeth erupt and function well in loss PTEN. It assumed that regulation of development and function in periodontal tissues may be controlled by other regulators to maintain homeostasis when PTEN does not function in the cellular level. However, patients with mutations in PTEN exhibit adenoid facies, high-arched palate, hypoplasia of the soft palate and uvula, papillomatosis of the lips and oropharynx, scrotal tongue, gingival nodule, oral mucosal papillomatosis, periodontal disease, fissured tongue and adenoid face [9]. Reduced level of PTEN caused gingival overgrowth with an increase of proliferating cell nuclear antigen (PCNA) [10]. Loss of PTEN is related to abnormal structure and function. Much less is known about the functions of PTEN in homeostasis of periodontal structures. Thus, these symptoms implicate PTEN in homeostasis of periodontium but leave open many questions regarding its actual role in the development and function of this periodontal tissue. Using mice deficient in PTEN in osteoblasts, we investigate how loss of PTEN affected homeostasis and function of periodontium.

2. Materials and Methods

2.1. Generation of Osteocalcin (OCN)-Cre$^{tg/+}$; Pten$^{fl/fl}$ Mice

The generation of mice lacking Pten in osteoblasts (OCN-Cre$^{tg/+}$; Pten$^{fl/fl}$) and wild-type controls was performed after review and approval by the Institutional Animal Care and Use Committee (IACUC) of the Van Andel Research Institute [5]. Loss of PTEN in mice was made using OCN-Cre mice and homozygous conditional mutants with PTEN alleles. To generate OCN-Cre$^{tg/+}$; Pten$^{fl/+}$ mice, mice with floxed PTEN alleles were crossed with homozygous animals. To generate OCN-Cre$^{tg/+}$; Pten$^{fl/fl}$ mice, OCN-Cre$^{tg/+}$; Pten$^{fl/+}$ mice were crossed with Pten$^{fl/fl}$ mice.

2.2. Micro-Computed Tomography (CT) Analyses

Micro CT analyses of the teeth in 10 mice (5 wild-type, 5 OCN-Cre$^{tg/+}$; Pten$^{fl/fl}$) were taken using MicroXCT-200 (SkyScan, Belgium) at 60 KV, 7.98 W, and a resolution of 2 μm. Scans were acquired with 800 CT slices and evaluated in the molar area using 8 μm^3 isotropic voxel size. For analyses, individual CT slices were reconstructed with MicroXCT 7.0 reconstruction software (SkyScan, Bel-

gium), and data were analyzed with Inveon Research Workplace (IRW, Erlangen, Germany).

2.3. Sample Preparation, Processing

Maxillae from 3-month-old mice (5 wild-type, 5 OCN-Cre$^{tg/+}$; Pten$^{fl/fl}$) were harvested and fixed in 4% paraformaldehyde overnight at 4˚C. Samples were decalcified in a heat-controlled microwave in 19% EDTA for 2 weeks. After demineralization, specimens were dehydrated through an ascending ethanol series prior to paraffin embedding. Then 8 μm thick longitudinal sections were cut and collected on Superfrost-plus slides for histology.

2.4. Histology

Movat's pentachrome staining was performed [11]. For alkaline phosphatase (ALP) staining, slides were preincubated overnight at 4˚C in alkaline phosphatase buffer containing 100 mM Tris (pH 9.5), 50 mM $MgCl^2$, 100 mM NaCl, and 0.1% Tween 20. Slides were then incubated in BM-purple solution (Roche Diagnostic Corporation, Indianapolis, IN) overnight at 4˚C until a dark purple color reaction appeared. Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) was used to detect cell death.

2.5. Immunohistochemistry

For immunostaining, tissue sections were deparaffinized, endogenous peroxidase activity was quenched by 3% hydrogen peroxide for 5 minutes, and then washed in phosphate-buffered saline (PBS). Slides were blocked with 5% goat serum (cat. no. S-1000; Vector, Burlingame, CA, USA) for 1 hour at room temperature. The appropriate primary antibody was added and incubated overnight at 4˚C before washing in PBS. The slides were incubated with the appropriate biotinylated secondary antibodies (BA-x; Vector) for 30 minutes, and then washed in PBS. Anavidin/biotinylated enzyme complex (Kit ABC Peroxidase Standard Vectastain PK-4000; Vector) was added and incubated for 30 minutes, and a 3,3'-diaminobenzidine (DAB) substrate kit (Kit Vector Peroxidase substrate DAB SK-4100; Vector) was used to develop the color reaction. The antibodies used include cluster of differentiation 68 (CD68+) (LSBio, dilution 1:20), Osteocalcin (OCN, Abcam dilution 1:7000), Osterix (Abcam dilution 1:200), PTEN (Abcam, dilution 1:100), Runt-related transcription factor (Runx2) (GeneTex, dilution 1:20).

3. Results

3.1. Deficiency of PTEN Caused Anomalies of Periodontal Structures

Using a micro-CT, removal of PTEN caused a significant increase in mineralized tissue formation. In molar of OCN-Cre$^{tg/+}$; Pten$^{fl/fl}$ mice, cementum was largely deposited, which caused hypercementosis (**Figures 1(A)-(D)**). Bone volume

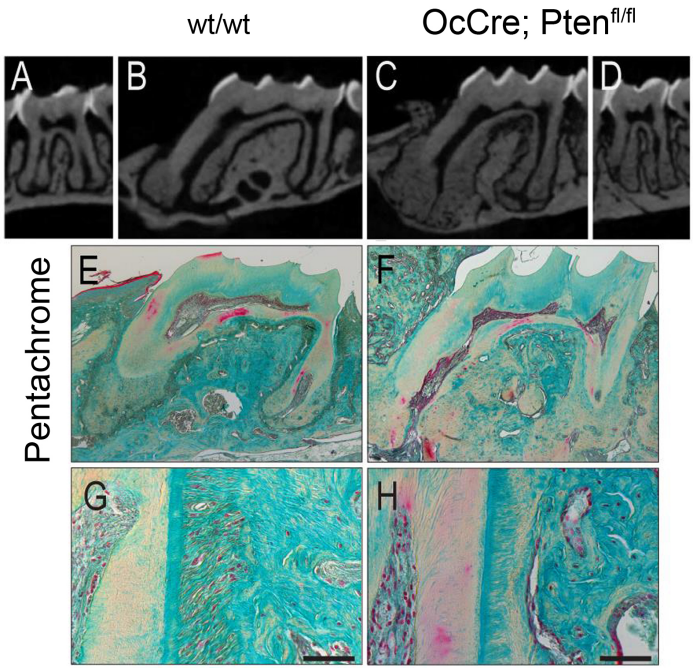

Figure 1. Down regulation of PTEN caused anomalies of periodontal structures. (A)-(D) Micro-CT examination revealed hypercementosis in the maxillary first and second molars of OCN-Cre$^{tg/+}$; Pten$^{fl/fl}$ mice compared to the root surface in the wild-type mice. (E), (F) Histology supported this finding: a significant cementum was deposited in the root of the maxillary first molar of OCN-Cre$^{tg/+}$; Pten$^{fl/fl}$ mice and normal root surface in wild-type mice. (G), (H) Higher magnification of mesial root of the maxillary first molars in OCN-Cre$^{tg/+}$; Pten$^{fl/fl}$ mice demonstrated disorganized periodontal fibers.

surrounding molars was largely increased. Pentachrome staining demonstrated the same findings observed in micro-CT (**Figure 1(E)** and **Figure 1(F)**). Higher magnification image showed abundance of cementum and a highly disorganized PDL in OCN-Cre$^{tg/+}$; Pten$^{fl/fl}$ mice (**Figure 1(G)** and **Figure 1(H)**).

3.2. Adult Osteoblast Maintained Their Dependency on Endogenous PTEN Signaling

We examined the mineralizing dental tissues and confirmed the PTEN-responsive status of osteoblast (**Figure 2(A)**) and odontoblast (**Figure 2(B)**) in wild-type mice. Thus, mineralizing tissues in periodontal complex maintain their PTEN-responsive status into adulthood.

3.3. Osteoprogenitor Marker Except for OCN in the PDL Was Not Affected by PTEN

Expression and distribution of Osterix and Runx2 were relatively unaffected in the periodontal ligament space (PDL) of OCN-Cre$^{tg/+}$; Pten$^{fl/fl}$ mice (**Figures 3(A)-(D)**). OCN was densely expressed in the PDL of OCN-Cre$^{tg/+}$; Pten$^{fl/fl}$ mice (**Figure 3(E)** and **Figure 3(F)**).

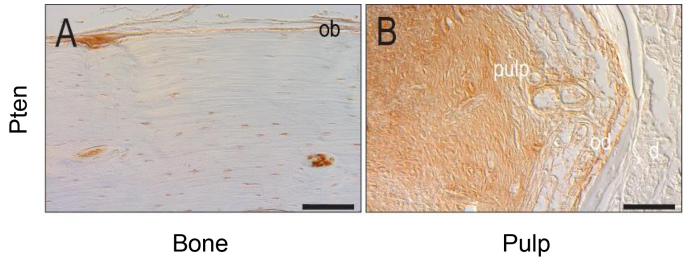

Figure 2. PTEN responsiveness was maintained until adulthood. (A) Osteoblasts of 3-month-old wild-type mice showed PTEN expression in alveolar bone. (B) Odontoblasts of 3-month-old wild-type mice showed PTEN expression in pulp. Abbreviations: d, dentin; ob, osteoblast; od, odontoblast. All scale bars are 500 μm.

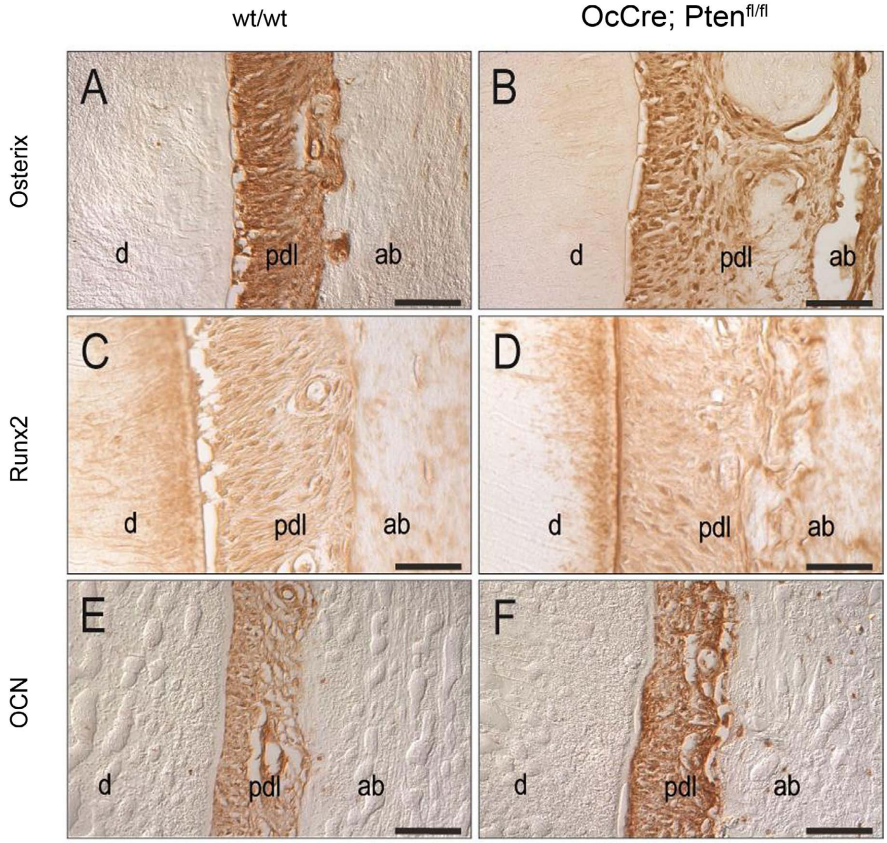

Figure 3. Osteogenic markers were expressed in the PDLOCN-Cre$^{tg/+}$; Pten$^{fl/fl}$ mice. (A), (B) Osterix and (C), (D) Runx2 expression in the PDL showed no difference between wild-type and OCN-Cre$^{tg/+}$; Pten$^{fl/fl}$ mice. (E), (F) significantly increased OCN expression was observed in the PDL space of OCN-Cre$^{tg/+}$; Pten$^{fl/fl}$ mice. Abbreviations: d, dentin; ab, alveolar bone; pdl, periodontal ligament. All scale bars are 500 μm.

3.4. Bone Turnover Was Altered with Loss of PTEN

With a significant increase of bone apposition around molars in micro CT (**Figure 1**), ALP activity, a marker of mineralization, in the PDL of OCN-Cre$^{tg/+}$; Pten$^{fl/fl}$ mice appeared to be increased compared to that of the wild-type mice (**Figure 4(A)** and **Figure 4(B)**). Coupled with this increased osteogenesis, we

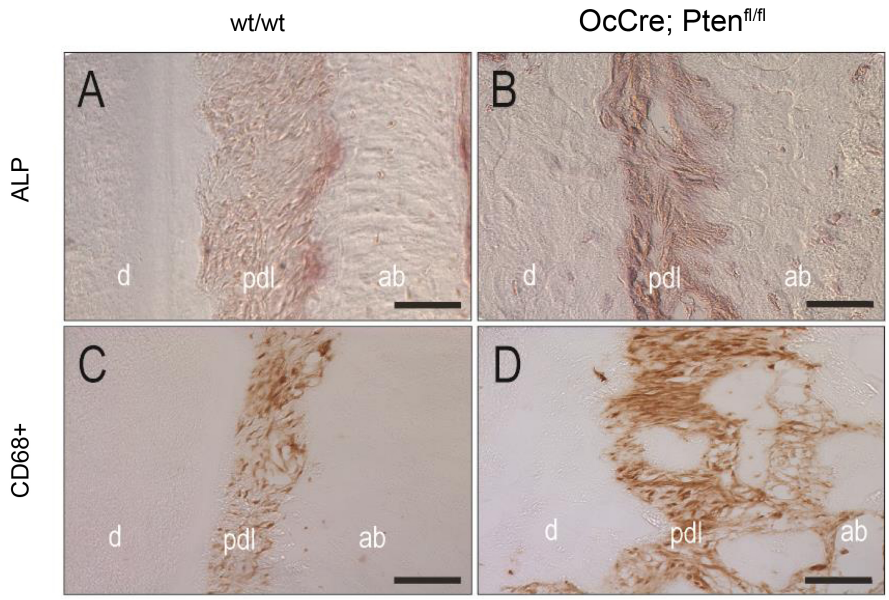

Figure 4. Bone turnover was altered in OCN-Cre$^{tg/+}$; Pten$^{fl/fl}$ mice. (A), (B) Alkaline phosphatase (ALP) showed more dense expression in the PDL space of OCN-Cre$^{tg/+}$; Pten$^{fl/fl}$ mice compared to that in wild type mice. (C), (D) Expression of CD68+ was increased in the PDL space of OCN-Cre$^{tg/+}$; Pten$^{fl/fl}$ mice compared to that in wild-type mice. Abbreviations: d, dentin; ab, alveolar bone; pdl, periodontal ligament. All scale bars are 500 μm.

found increased bone resorption in OCN-Cre$^{tg/+}$; Pten$^{fl/fl}$ mice. Using CD 68+ staining, we visualized a high level of osteoclast activity in OCN-Cre$^{tg/+}$; Pten$^{fl/fl}$ mice (**Figure 4(C)** and **Figure 4(D)**).

4. Discussion

PTEN is activated in both the tooth and the tongue during mouse development from embryo stage (E) 13.5 to E16.5 [12]. PTEN is highly expressed in proliferative cervical loops and differentiating pre-ameloblasts and pre-odontoblast of human incisor [13]. PTEN is expressed in tooth germ of the human mandibular third molar [14]. Expression of PTEN was observed in odontoblast as well as osteoblast in the periodontal complex of wild-type mice. Similar to accumulation of bone in the skeleton after loss of PTEN [5], volume of hard tissues including cementum and alveolar bone notably increased in OCN-Cre$^{tg/+}$; Pten$^{fl/fl}$ mice (**Figure 1**). It was reported that loss of PTEN caused an increase bone volume in all skeletal sites due to both increase of the number of osteoblasts and decrease of apoptosis, which resulted from continuous stimulation of the PI3K pathway in the molecular level [5]. Less is known of accumulation of dental hard tissues in loss of PTEN. Here, cementum was profoundly increased in the molar root of OCN-Cre$^{tg/+}$; Pten$^{fl/fl}$ mice. Loss of PTEN appears to associate with loss of homeostasis during cementogenesis. Persistent activation of cementogenesis caused hypercementosis in molars of OCN-Cre$^{tg/+}$; Pten$^{fl/fl}$ mice. The underlying mechanism related to hypercementosis needs to be investigated.

Expression of osteogenic factors in the PDL of OCN-Cre$^{tg/+}$; Pten$^{fl/fl}$ mice was similar to that in the PDL of the wild-type mice. However, OCN, a marker of osteoblastic activity was increased in PDL of OCN-Cre$^{tg/+}$; Pten$^{fl/fl}$ mice, which is a similar finding in serum of OCN-Cre$^{tg/+}$; Pten$^{fl/fl}$ mice [5]. Loss of PTEN was associated with an increase of several genes, which were expressed in the differentiation of osteoblast [5]. Expressions of collagen I and osteocalcin were increased in the later stage of osteoblast differentiation [5]. Increased expression of OCN in the PDL space appeared to relate to an increase of bone mass in the periodontal structure.

Bone turnover, expressed by ALP and CD68+ was altered in OCN-Cre$^{tg/+}$; Pten$^{fl/fl}$ mice. During healing process of fracture, bone resorption was significantly increased in OCN-Cre$^{tg/+}$; Pten$^{fl/fl}$ mice [15]. Consistent with this finding, osteoclast activity was increased in OCN-Cre$^{tg/+}$; Pten$^{fl/fl}$ mice. Loss of PTEN increased both bone formation and bone resorption. Thus, appropriate coupling between bone formation and bone resorption has occurred.

Ageing is strongly associated with telomere length and telomerase pathway is involved with PTEN signaling [16]. Alterations of telomere architecture are followed by the loss of PTEN [17]. In aged rat, a decrease in PTEN and following an increase Akt level resulted in the progressive enlargement of neurons of the spinal cord [18]. In aged lungs, low expression PTEN-induced putative kinase promoted fibrosis [19]. On the other hand, a decrease in AKT signaling and following an increase PTEN leaded to a decline of the survival rate of ageing outer hair cells [20]. In aged human endothelial cells, PTEN expression was increased and impaired angiogenesis [21]. Thus, alteration of PTEN expression, either decreased or increased with ageing, impaired homeostasis.

Here, mice with loss of PTEN showed no obvious functional impairments, while structural and molecular changes occurred in periodontal complex of OCN-Cre$^{tg/+}$; Pten$^{fl/fl}$ mice. Loss of PTEN caused disruption of homeostasis, which may be vulnerable to progression of any periodontal disease. By better understanding the link between PTEN and periodontal disease with ageing we will provide insight into how to overcome age-related progression of periodontal disease.

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Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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