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ABSTRACT

Periostin, a matricellular adapter protein highly expressed by periodontal ligament fibroblasts, is implicated in the maintenance of periodontal integrity, which is compromised during periodontal diseases. The aim of this study was to explore the influence of chronic periodontal inflammation on tissue periostin levels. Periodontal breakdown was induced in a pre-clinical ligature periodontal inflammatory disease model. Periodontal tissue specimens were harvested at baseline, 2 weeks, and 4 weeks and prepared for histologic, immunofluorescence, and micro-CT examination. Statistical analyses were conducted by Kruskal-Wallis, Mann-Whitney, and Spearman's tests. Periostin detection levels were reduced over time in response to the inflammatory process (1 ± 0.05 ; 0.67 ± 0.03 ; 0.31 ± 0.02 ; $p < 0.001$; baseline, 2, and 4 weeks, respectively). Simultaneously, alveolar bone loss increased from baseline to the 2- and 4-week time-points (0.40 ± 0.02 mm; 1.39 ± 0.08 mm; 1.33 ± 0.15 mm; $p < 0.001$), which was inversely correlated with the levels of periostin ($\rho = -0.545$; $p < 0.001$). In conclusion, periostin PDL tissue levels significantly decrease under chronic inflammatory response and correlate with the detrimental changes to the periodontium over time.

KEY WORDS: periostin, periodontal ligament, periodontium, periodontal disease/periodontitis, bone resorption, extracellular matrix.

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Periostin is Down-regulated during Periodontal Inflammation

INTRODUCTION

Loss of tooth-supporting structures affects up to 90% of the general worldwide adult population experiencing different types of periodontal diseases (Papapanou, 1996) and constitutes the primary cause of tooth loss in adults aged 35 yrs and over (Niessen and Weyant, 1989). Periodontal diseases are induced by bacteria acting in a susceptible host (Page and Kornman, 1997). Different molecules act in this scenario, reflecting periodontal bone loss activity and susceptibility. However, because this is a highly complex disease, it is unlikely that a single biomarker for disease detection and prediction can be found (Kinney *et al.*, 2011).

During periodontal homeostasis and healing, cell turnover, recruitment, and differentiation are tightly regulated processes. In this context, matricellular molecules within the periodontal ligament (PDL) aim to stabilize the extracellular matrix (ECM) for supporting mechanical and biological challenges. They act by providing signals to the constituent cell populations to modulate their phenotype to maintain tissue homeostasis. In that sense, periostin (gene POSTN), a secreted adhesion molecule highly expressed in the PDL, is essential for periodontal tissue integrity. It also plays an important role in tooth development and eruption processes (Kruzynska-Frejtag *et al.*, 2004; Rios *et al.*, 2005, 2008). Furthermore, periostin expression is increased in response to occlusal loading and orthodontic movement (Wilde *et al.*, 2003; Afanador *et al.*, 2005). Its presence in the ECM is induced by biomechanical stimuli via TGF- β 1 signaling (Rios *et al.*, 2008; Wen *et al.*, 2010). Once secreted, it modulates the matrix-cell interactions relevant to connective tissue repair and remodeling, fibroblast attachment and spreading (Hamilton, 2008; Hoersch and Andrade-Navarro, 2010), and activation of cell survival signaling pathways (Bao *et al.*, 2004). Periostin influences mechanical stability and tissue strength by regulating collagen fibrillogenesis (Norris *et al.*, 2007). Moreover, it has been recently used as a marker to investigate the functional integrity of a regenerated PDL interface (Park *et al.*, 2012). In contrast, reduction or absence of POSTN in KO mice leads to periodontal breakdown in a periodontal-disease-like process once occlusal loading starts after teeth eruption (Rios *et al.*, 2005, 2008). These properties highlight its key role during periodontal homeostasis, maintaining a healthy tooth-supporting apparatus against biomechanical challenges.

However, reductions in periostin levels as a co-factor for periodontal destruction and breakdown have not been yet studied *in vivo*. Therefore, the aim of this study was to explore the influence of spatial and temporal periodontal inflammation on the levels of the matricellular adapter protein

periostin over time in an inflammatory-induced periodontal disease animal model. Accordingly, disease progression in terms of linear and volumetric bone changes, periostin levels, and inflammatory process was analyzed.

MATERIALS & METHODS

Experimental Design

All animal procedures were approved by the University of Michigan Committee of Use and Care of Animals. Thirty male Sprague-Dawley rats (weight, 250-300 g each) were assigned to 3 equal experimental groups: control group at the time of ligature placement (baseline); animals subjected to disease induction for 2 wks; and animals subjected to disease induction for 4 wks.

Ligature-induced Periodontal Disease

Sprague-Dawley rats were anesthetized *via* intraperitoneal injections of a ketamine and xylazine cocktail (Rios and Giannobile, 2011). Silk sutures (4/0) were secured around the cervical parts of the right maxillary first and second molar teeth and placed in the gingival sulci. The ligatures were evaluated twice weekly, gently displaced apically into the gingival sulci to ensure a subgingival position, and replaced when dislodged or lost.

Quantitative Micro-CT Analysis

Block biopsies were harvested at each time-point. Rat mandible specimens were fixed in a 10% buffered formalin phosphate solution for 1 day and scanned by micro-CT (eXplore Locus SP, GE Healthcare Inc., London, ON, Canada) with $18 \times 18 \times 18 \mu\text{m}^3$ voxel size. Linear alveolar bone loss (ABL; mm) was determined in the interproximal area between the first and second molars by measurement of the distance from the cemento-enamel junction (CEJ) to the alveolar bone crest (ABC). After the teeth were digitally removed, regions of interest (ROI) for the assessment of mineralized tissue volume were created with the same size in the interproximal area between the first and second molars (Park *et al.*, 2007), extending from the apical end of the roots to the CEJ (Fig. 1A). Based on the HU grayscale level, bone volume (BV; mm^3), and bone mineral density [BMD; mg (HyAp)/cc] were quantified. MicroView Analysis+ 2.1.2 software (GE Healthcare) was used to perform these analyses. All measurements were repeated 2 times *per* site, and the mean value was recorded.

Histologic and Immunofluorescence Analyses

After the μCT scanning, the harvested mandibles were decalcified with 10% ethylene diaminetetraacetic acid (EDTA; Sigma-Aldrich Co. LLC, St. Louis, MO, USA) for 4 wks and embedded in paraffin blocks for histological sectioning. Hematoxylin-eosin staining was used to evaluate bone destruction and soft-tissue changes.

Polymorphonuclear neutrophils (PMNs) and T-lymphocytes in the area were evaluated by immunohistochemical staining for

myeloperoxidase and CD3, respectively. Briefly, after an antigen retrieval pre-treatment (citrate buffer, pH 6.0, boiled for 10 min), CD3 (1:200 dilution; ab5690, Abcam, Inc., Cambridge, MA, USA) and myeloperoxidase (1:250 dilution; ab45977, Abcam) primary antibodies were incubated for 30 min, followed by the addition of secondary antibodies (30 min), DAB staining (5 min), and hematoxylin counterstain (1 min) in an autostainer (EnVision+ protocol; Dako North America, Inc., Carpinteria, CA, USA). Sections were then dehydrated and covered with a glass coverslip. The ApopTag Kit (S7100; EMD Millipore Corporation, Billerica, MA, USA) was used to detect apoptotic cells. Three blinded examiners analyzed a total of 8 digital sections of each group and each staining (original magnification of 20X). Regions of interest measuring $200 \times 300 \mu\text{m}$ were established in an apical direction from the most coronal contact between the soft tissue and the tooth surface, and positive cells were counted.

We also performed immunofluorescence staining to periostin on paraffin sections by using an affinity-purified rabbit polyclonal antibody (1:400 dilution; ab14041 Abcam). Immunological reaction was visualized by the use of a sheep polyclonal secondary antibody to rabbit conjugated with Texas Red™ (1:200 dilution; ab6793-1, Abcam). The sections were then treated with an anti-fade agent containing 4',6-diamidino-2-phenylindole (DAPI) (ProLong® Gold anti-fade reagent with DAPI; Invitrogen, Life Technologies Corporation, Grand Island, NY, USA) and covered with a glass coverslip. The stained slides were imaged with an OLYMPUS Fluoview 500 confocal microscope (Olympus America Inc., Center Valley, PA, USA) at constant settings. ImageJ (National Institutes of Health, Bethesda, MD, USA; <http://rsbweb.nih.gov/ij/>) was used to analyze the periostin fluorescence intensity. Briefly, areas to be analyzed extended from the ABC to the apex of the root, non-PDL areas were digitally subtracted, the entire figure was quantitatively mapped, and a mean fluorescent light intensity value was obtained and adjusted to total PDL area *per* image. All values were normalized to baseline. ImageJ was also used to represent the periostin intensity in a pseudo-3D figure (heat-map).

Statistical Analysis

PASW Statistics 18.0 (SPSS Inc., Chicago, IL, USA) was used for the statistical analyses. All data are presented as the mean value $\pm$ SEM (standard error of the mean). The significance of the differences among groups was determined by the Kruskal-Wallis test ($\alpha = 0.05$), since the population was not normally distributed ($p < 0.05$, Shapiro-Wilk test). The Mann-Whitney U test was used to define pairwise statistical differences with Bonferroni correction ($\alpha = 0.0167$). Spearman's rank coefficient ($\alpha = 0.05$) was used to demonstrate any correlations between variables.

RESULTS

Linear and Volumetric Analyses

ABL (mm) was significantly different among groups ($p < 0.001$; Kruskal-Wallis). ABL in the baseline group (0.40 ± 0.02) was

Figure 1. μ CT analyses. (A) Volumetric and linear μ CT images depicting ROIs (yellow cubes), used for volumetric measurements, and CEJ (green arrows) and ABC (red arrows) anatomical landmarks, used for ABL measurements. Scale bar = 1 mm. (B) ABL (mm) was significantly lower at baseline than at 2 and 4 wks (a, $p < 0.001$; Mann-Whitney). (C) BMD (mg/cc) was significantly lower at 2 (b, $p < 0.003$; Mann-Whitney) and 4 wks (c, $p < 0.002$; Mann-Whitney) after the ligature placement. Error bars represent SEM.

significantly lower than that in the diseased groups at both 2 (1.39 ± 0.08 ; $p < 0.001$; Mann-Whitney) and 4 wks (1.33 ± 0.15 ; $p < 0.001$; Mann-Whitney). No significant difference was found between the 2 diseased groups ($p = 0.364$) (Figs. 1A, 1B).

Consistently, volumetric analysis (BV, mm^3) revealed a significant difference among groups ($p < 0.001$; Kruskal-Wallis). Baseline levels (0.77 ± 0.03) were significantly higher than those in disease at 2 (0.39 ± 0.04 ; $p < 0.001$; Mann-Whitney) and 4 wks (0.37 ± 0.04 ; $p < 0.001$; Mann-Whitney). No significant difference was found between the 2 diseased groups ($p = 0.762$; Mann-Whitney). BMD (mg/cc) was also significantly different among groups ($p < 0.002$; Kruskal-Wallis), being significantly higher at baseline (198.61 ± 24.69) compared with that in disease at 2 (75.07 ± 21.31 ; $p < 0.003$; Mann-Whitney)

and 4 wks (97.58 ± 11.64 ; $p < 0.002$; Mann-Whitney). No significant difference was found between the 2 diseased groups ($p = 0.199$; Mann-Whitney) (Figs. 1A, 1C).

Evaluation of the Inflammatory Process

Histological analysis demonstrated a high impact on PDL tissue structure over time (Figs. 2A, 3A). In the baseline group, a well-organized PDL tissue was observed. ABC looked normal, without major Howship's lacunae. PDL and gingival fibers showed a regular orientation from bone to tooth. In contrast, the 2-week group showed a disorganized PDL fiber orientation, and inflammatory infiltrated and reduced ABC, with those detrimental effects being even more evident for the 4-week group.

Figure 2. Immunohistochemical analyses of the inflammatory process. (A) H&E images showing inflammatory infiltrate and periodontal fiber disorganization over time. Original magnification: 40X. Scale bar: 50 μ m. (B) Myeloperoxidase- and (C) CD3-immunopositive and (D) apoptotic cell detection (black arrows). Original magnification: 60X. Scale bar: 40 μ m. (E) Myeloperoxidase- and (F) CD3-immunopositive and (G) apoptotic cell quantification showing a generalized increase of all markers compared with baseline (a, $p < 0.001$, Mann-Whitney). Additionally, CD3-immunopositive and apoptotic cells significantly increased from 2 to 4 wks (b, $p < 0.011$ and c, $p < 0.008$, Mann-Whitney, respectively). Error bars represent SEM.

Specific immunohistochemical analyses showed a statistically significant increase of myeloperoxidase- (2.63 ± 0.65 , 27.57 ± 4.69 , 38.17 ± 9.74) (Figs. 2B, 2E) and CD3-positive cells (4.5 ± 1.02 , 19.71 ± 3.08 , 33.86 ± 4.06) (Figs. 2C, 2F) as well as apoptotic cells (2.0 ± 0.57 , 30.86 ± 5.26 , 64.5 ± 8.05) (Figs. 2D, 2G) from baseline to 2 and 4 wks ($p < 0.001$, Mann-Whitney). From 2 to 4 wks, CD3 and apoptotic positive cells increased significantly ($p < 0.011$ and $p < 0.008$, Mann-Whitney, respectively), but not myeloperoxidase ($p = 0.534$, Mann-Whitney).

Periostin Levels

Periostin fluorescence intensity levels were significantly reduced over time by the effect of the inflammatory process induced by

the ligature ($p < 0.001$; Kruskal-Wallis) (Figs. 3B, 3C). At baseline (1.0 ± 0.05), relative periostin levels were significantly higher than those in diseased groups at both 2 (0.67 ± 0.03 ; $p < 0.001$; Mann-Whitney) and 4 wks (0.31 ± 0.02 ; $p < 0.001$; Mann-Whitney). The difference between the 2- and 4-week groups was also statistically significant ($p < 0.001$; Mann-Whitney) (Fig. 3D).

Correlation between Levels of Periostin and Bone Destruction

Ligature-induced periodontal inflammation resulted in clear correlation of periostin levels and detrimental changes to the periodontium over time, at linear (ABL-periostin, r^2 linear = 0.681; $\rho = -0.545$, $p < 0.001$, Spearman) (Fig. 4A), volumetric

Figure 3. Histological and immunofluorescence analyses. (A) H&E histological images showing alveolar bone reduction, inflammatory infiltrate, and periodontal fiber disorganization in the ligated interproximal region between the first and second molars over time. Original magnification: 20X. Scale bar: 100 μ m. (B) Immunofluorescence images showing reduction of periostin immunolocalization (in red) over time. DAPI in blue. Original magnification: 20X. Scale bar: 100 μ m. (C) Heat-map 3D representation of periostin immunofluorescence intensity from images presented in (B). (D) Significantly lower relative periostin levels in the PDL area at 2 and 4 wks compared with baseline (a, $p < 0.001$; Mann-Whitney). Levels at 4 wks were also significantly lower than those at 2 wks (b, $p < 0.001$; Mann-Whitney). Error bars represent SEM.

Figure 4. Correlation between levels of periostin and bone destruction. (A) Correlation between mean levels of periostin and ABL (r^2 linear = 0.681; $\rho = -0.545$, $p < 0.001$, Spearman). (B) Correlation between periostin levels and BMD (r^2 linear = 0.565; $\rho = 0.411$, $p < 0.012$, Spearman). Error bars represent SEM for both the horizontal (periostin) and the vertical (ABL or BMD) axes. Orange dotted line: Confidence interval = 80%.

(BV-periostin, r^2 linear = 0.762; $\rho = 0.642$, $p < 0.001$, Spearman), and mineral density levels (BMD-periostin, r^2 linear = 0.565; $\rho = 0.411$, $p < 0.012$, Spearman) (Fig. 4B). Areas with lower levels of periostin corresponded to the areas where more coronal-apical detrimental tissue changes were noticeable (higher ABL, lower BV and BMD).

DISCUSSION

New insights into the pathogenesis of periodontitis incorporate gene, protein, and metabolite data into dynamic biological networks that include disease-initiating and progression mechanisms. Periostin as a modulator of critical matrix-cellular interactions is relevant in those processes.

Of the known proteins expressed in the PDL, periostin shows the greatest specificity (Saito *et al.*, 2002). It is localized between the cytoplasmic processes of periodontal fibroblasts and cementoblasts and the adjacent collagen fibrils (Suzuki *et al.*, 2004). In those areas, periostin is closely related to the PDL fiber bundles from the root-surface cementum to the alveolar bone engaging the tooth-supporting apparatus and reflecting a mature and stable tissue (Park *et al.*, 2012).

In contrast, periostin deletion induces a reduction in collagen fiber diameter that results in a decreased tissue modulus of elasticity (Norris *et al.*, 2007). In the case of periostin KO mice, the periodontal ligament is unable to sustain the normal physiological

occlusal load, which results in a rapid deterioration of the functional and structural integrity of the periodontium: advanced alveolar bone loss, severe clinical attachment loss, and significant widening of the PDL region (Rios *et al.*, 2005, 2008; Ma *et al.*, 2011). In the present study, the inflammatory process initiated by the ligature placement was able to induce a periostin reduction that was correlated with bone loss. This collateral effect of inflammation on periostin expression directly compromised the integrity of the PDL, favoring an inappropriate response to traumatic or even normal occlusal forces, ultimately leading to bone destruction and periodontal breakdown.

No significant differences in ABL, BV, or BMD were observed within the 2- and 4-week time-points, which is consistent with results of previous studies (Jin *et al.*, 2007; Pellegrini *et al.*, 2009; Rios and Giannobile, 2011). However, the levels of periostin were reduced from 2 to 4 wks. This observation may lead to subsequent bursts of periodontal destruction due to changes in the PDL tissue strength and ability to respond to mechanical challenges (Norris *et al.*, 2007). Periostin is primarily expressed within the PDL region by PDL fibroblasts (Saito *et al.*, 2002). Therefore, the decrease in periostin levels reflects the loss of PDL tissue integrity, which might be due to a switch from a well-organized PDL to an inflammatory infiltrated tissue. Analysis of our data shows a significant increase in T-lymphocytes (CD3-positive cells) and apoptotic cells at 4 wks vs. baseline and 2 wks. An initial significant increase in PMNs (myeloperoxidase-positive cells) was observed but not maintained from 2 to 4 wks. This scenario reflects an inflammation profile toward a more stable, yet chronic, inflammatory infiltrated tissue. Some studies have suggested an increase of periostin detection in other chronic pathological conditions, leading to a fibrotic situation (Hakuno *et al.*, 2010; Daines *et al.*, 2011; Uchida *et al.*, 2012). Our results may indicate a different periostin expression pattern in periodontal diseases. When those differences are analyzed, the specific periodontal environment (bacterial contamination, mechanical requirements, etc.) must be considered. Based on our *in vitro* studies, the expression of periostin is specifically reduced in human PDL fibroblasts by the effects of inflammatory cytokines and bacterial by-products (Padial-Molina *et al.*, Epub ahead of print). However, it is important to highlight that two overlapping scenarios could support the altered levels of periostin in the chronically inflamed periodontium: (1) Bacterial/inflammatory challenge could lead to decreased periostin produced by PDL fibroblasts, and (2) a decrease in the number of PDL fibroblasts could explain the lower periostin levels. Both scenarios represent an important observation with significant clinical implications that compromise the function and structure of the periodontium. When periostin is reduced or completely depleted, the stability of the PDL is compromised, and it is more susceptible to injury and the subsequent inflammatory process, since the structural and biomechanical capacities are reduced (Rios *et al.*, 2005, 2008). Therefore, the *in vivo* reduction of periostin in our periodontal inflammatory model occurs not only due to the changes in the PDL tissue (*i.e.*, from an organized, specialized, and competent tissue to a chronic inflammatory infiltrated tissue, which contains less competent PDL fibroblasts capable of expressing periostin), but also due to a direct effect

of the inflammatory molecular environment around the main periostin expressers (*i.e.*, PDL fibroblasts). This inflammatory environment could further increase the pathological changes as tissue mechanical properties decrease. Such mechanisms support our present *in vivo* results and favor the idea of a complex regulatory pathway of periodontal tissue homeostasis where periostin is a key factor for maintaining tissue integrity under biomechanical, bacterial, and inflammatory challenges.

Periostin, a key factor for periodontal stability, is reduced as a consequence of inflammatory-induced periodontal disease. Periostin could represent a novel biological agent with significant potential for diagnostic and susceptibility assessment purposes. It could add supporting scientific information to capture the dynamic nature of the matricellular biochemical events involved in the transition between periodontal health and disease. Further studies of the diagnostic value of periostin as a marker for disease activity and susceptibility in humans, to confirm our *in vitro* and animal studies, are needed and have already been initiated by our group (clinicaltrials.gov Id #: NCT01180920).

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REFERENCES

- Afanador E, Yokozeki M, Oba Y, Kitase Y, Takahashi T, Kudo A, *et al.* (2005). Messenger RNA expression of periostin and Twist transiently decrease by occlusal hypofunction in mouse periodontal ligament. *Arch Oral Biol* 50:1023-1031.
- Bao S, Ouyang G, Bai X, Huang Z, Ma C, Liu M, *et al.* (2004). Periostin potentially promotes metastatic growth of colon cancer by augmenting cell survival via the Akt/PKB pathway. *Cancer Cell* 5:329-339.
- Daines SM, Wang Y, Orlandi RR (2011). Periostin and osteopontin are over-expressed in chronically inflamed sinuses. *Int Forum Allergy Rhinol* 1:101-105.
- Hakuno D, Kimura N, Yoshioka M, Mukai M, Kimura T, Okada Y, *et al.* (2010). Periostin advances atherosclerotic and rheumatic cardiac valve degeneration by inducing angiogenesis and MMP production in humans and rodents. *J Clin Invest* 120:2292-2306.
- Hamilton DW (2008). Functional role of periostin in development and wound repair: implications for connective tissue disease. *J Cell Commun Signal* 2:9-17.
- Hoersch S, Andrade-Navarro MA (2010). Periostin shows increased evolutionary plasticity in its alternatively spliced region. *BMC Evol Biol* 10:30.
- Jin Q, Cirelli JA, Park CH, Sugai JV, Taba M Jr, Kostenuik PJ, *et al.* (2007). RANKL inhibition through osteoprotegerin blocks bone loss in experimental periodontitis. *J Periodontol* 78:1300-1308.
- Kinney JS, Morelli T, Braun T, Ramseier CA, Herr AE, Sugai JV, *et al.* (2011). Saliva/pathogen biomarker signatures and periodontal disease progression. *J Dent Res* 90:752-758.
- Kruzynska-Freitag A, Wang J, Maeda M, Rogers R, Krug E, Hoffman S, *et al.* (2004). Periostin is expressed within the developing teeth at the sites of epithelial-mesenchymal interaction. *Dev Dyn* 229:857-868.

- Ma D, Zhang R, Sun Y, Rios HF, Haruyama N, Han X, *et al.* (2011). A novel role of periostin in postnatal tooth formation and mineralization. *J Biol Chem* 286:4302-4309.
- Niessen LC, Weyant RJ (1989). Causes of tooth loss in a veteran population. *J Public Health Dent* 49:19-23.
- Norris RA, Damon B, Mironov V, Kasyanov V, Ramamurthi A, Moreno-Rodriguez R, *et al.* (2007). Periostin regulates collagen fibrillogenesis and the biomechanical properties of connective tissues. *J Cell Biochem* 101:695-711.
- Padial-Molina M, Volk SL, Rodriguez JC, Marchesan JT, Galindo-Moreno P, Rios HF (2012). TNF-alpha and *P. gingivalis* lipopolysaccharides decrease periostin expression in human PDL fibroblasts. *J Periodontol* [Epub ahead of print July 6, 2012] (in press).
- Page RC, Kornman KS (1997). The pathogenesis of human periodontitis: an introduction. *Periodontol* 2000 14:9-11.
- Papapanou PN (1996). Periodontal diseases: epidemiology. *Ann Periodontol* 1:1-36.
- Park CH, Abramson ZR, Taba Jr M, Jin Q, Chang J, Kreider JM, *et al.* (2007). Three-dimensional micro-computed tomographic imaging of alveolar bone in experimental bone loss or repair. *J Periodontol* 78:273-281.
- Park CH, Rios HF, Jin Q, Sugai JV, Padial-Molina M, Taut AD, *et al.* (2012). Tissue engineering bone-ligament complexes using fiber-guiding scaffolds. *Biomaterials* 33:137-145.
- Pellegrini G, Seol YJ, Gruber R, Giannobile WV (2009). Pre-clinical models for oral and periodontal reconstructive therapies. *J Dent Res* 88:1065-1076.
- Rios HF, Giannobile WV (2011). Preclinical protocols for periodontal regeneration (Chapter 7). In: *Osteology guidelines for oral and maxillofacial regeneration: preclinical models for translational research*. Giannobile WV, Nevins M, editors. Berlin: Quintessence Publishing Co., Inc., pp. 77-104.
- Rios H, Koushik SV, Wang H, Wang J, Zhou HM, Lindsley A, *et al.* (2005). Periostin null mice exhibit dwarfism, incisor enamel defects, and an early-onset periodontal disease-like phenotype. *Mol Cell Biol* 25:11131-11144.
- Rios HF, Ma D, Xie Y, Giannobile WV, Bonewald LF, Conway SJ, *et al.* (2008). Periostin is essential for the integrity and function of the periodontal ligament during occlusal loading in mice. *J Periodontol* 79:1480-1490.
- Saito Y, Yoshizawa T, Takizawa F, Ikegame M, Ishibashi O, Okuda K, *et al.* (2002). A cell line with characteristics of the periodontal ligament fibroblasts is negatively regulated for mineralization and Runx2/Cbfa1/Osf2 activity, part of which can be overcome by bone morphogenetic protein-2. *J Cell Sci* 115(Pt 21):4191-4200.
- Suzuki H, Amizuka N, Kii I, Kawano Y, Nozawa-Inoue K, Suzuki A, *et al.* (2004). Immunohistochemical localization of periostin in tooth and its surrounding tissues in mouse mandibles during development. *Anat Rec A Discov Mol Cell Evol Biol* 281:1264-1275.
- Uchida M, Shiraishi H, Ohta S, Arima K, Taniguchi K, Suzuki S, *et al.* (2012). Periostin, a matricellular protein, plays a role in the induction of chemokines in pulmonary fibrosis. *Am J Respir Cell Mol Biol* 46:677-686.
- Wen W, Chau E, Jackson-Boeters L, Elliott C, Daley TD, Hamilton DW (2010). TGF-ss1 and FAK regulate periostin expression in PDL fibroblasts. *J Dent Res* 89:1439-1443.
- Wilde J, Yokozeki M, Terai K, Kudo A, Moriyama K (2003). The divergent expression of periostin mRNA in the periodontal ligament during experimental tooth movement. *Cell Tissue Res* 312:345-351.