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LRP_5 Affects Homeostasis of the Periodontal Complex

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Abstract

Purpose: The signals responsible for homeostasis in the periodontal complex are unclear. The purpose of this study was to evaluate the role of Low-density lipoprotein receptor-related protein 5(LRP_5) in this process by removing LRP_5, and observing the effects of LRP_5 depletion on cells of the periodontal structures. **Material and Methods:** The function of this LRP_5 was evaluated by conditional elimination of the LRP_5 gene using an Osteocalcin Cre driver. The *OCN-Cre*; $LRP_5^{fl/fl}$ mice were examined using micro-CT and histology, immunohistochemistry to evaluate the periodontal complex. **Results:** Elimination of LRP_5 in the periodontal complex of *OCN-Cre*; $LRP_5^{fl/fl}$ mice results in a different expression of Fibromodulin in the periodontal ligament space. A decrease in osteoclastic activity was found in the periodontal ligament. **Conclusion:** Osteoclastic activities are decreased and expression of fibromodulin is decreased, which implies the involvement of LRP_5 in homeostasis of the periodontal ligament.

Keywords

LRP_5, Periodontal Ligament, Homeostasis

1. Introduction

Low-density lipoprotein receptor-related protein 5 (LRP_5) is known to be a key component in Wnt signaling pathway. Mutations in LRP_5 cause alteration in bone mass. During bone development, a deletion of LRP_5 leads to a decrease in bone mass [1]. On the other hand, a gain in LRP_5 causes an increase in bone mass [2] [3]. Thus, LRP_5 is involved in disease related to bone. It has been known that homeostasis of bone mass is controlled by LRP_5 through osteocytes [4] [5]. However, a controlling process of bone mass by LRP_5 is not well estab-

lished. It is believed that high bone mass mutation occurs in either limb or osteoblast [6]. For treatment of osteoporosis, an osteocyte-specific protein binding to LRP_5 has been used to block Wnt signaling pathway [7].

Mice that carry the same G171V substitution (e.g., Lrp5G171V mice) show an increase in bone mass and bone density [8]. In addition, Lrp5G171V mice exhibited a decrease in the width of periodontal ligament, which is concomitant with an increase in alveolar bone mass [9]. To create a loss of LRP_5 function phenotype, we used *OCN-Cre*; $LRP_5^{fl/fl}$ mice [10]. Analyses of the loss of LRP_5 function animal models provided new information regarding homeostasis of periodontal complex.

2. Material and Methods

2.1. Generation of Mouse Strains

The generation of *OCN-Cre*; $LRP_5^{fl/fl}$ mice has been previously described. Ten 3 month-old mice were analyzed; 5 were *OCN-Cre*; $LRP_5^{fl/fl}$ mice and 5 were wild-type littermates.

2.2. Micro-CT Analysis

Micro-CT was taken using MicroXCT-200 (SkyScan, Belgium) at 60 kV and 7.98 Watt and a resolution of 2 microns. CT slices were reconstructed using MicroXCT7.0 reconstruction software (SkyScan, Belgium). Inveon Research Workplace (IRW) (Erlangen, Germany) was used for analysis.

2.3. Sample Preparation, Processing and Histology

Harvested maxillae from the wild type and *OCN-Cre*; $LRP_5^{fl/fl}$ mice were fixed in 4% paraformaldehyde for one night at 4˚C and then decalcified in a heat-controlled microwave in 19% EDTA for 14 days. After this process, specimens were dehydrated using ethanol series and then embedded with paraffin. Eight-micron-thick sections were cut and collected for analyses.

2.4. Histology

Pentachrome staining was performed [11].

2.5. Cellular Assays and Immunohistochemistry

Alkaline phosphatase staining was performed to investigate osteogenic factors. For immunostaining analyses, tissue sections were deparaffinized and endogenous peroxidase activity was smothered using 3% hydrogen peroxide then washed with PBS. Slides were blocked out using 5% goat serum (Vector S-1000) for 1 hour. The relevant primary antibody was attached and cultured for one night at 4˚C, then washed with PBS. Samples were cultured using relevant biotinylated secondary antibodies (Vector BA-x) for half an hour, and washed in PBS. An advidin/biotinylated enzyme complex (Kit ABC Peroxidase Standard Vectastain PK-4000) was attached and cultured for 30 minutes and a DAB substrate kit (Kit

Vector Peroxidase subtrate DAB SK-4100) was utilized to detect the color reaction. Used antibodies include osteocalcin (Origene, dilution 1:100), Osterix (NIH LF 175, dilution 1:4000), Fibromodulin (Santa Cruz Biotech, dilution 1:1000), dentin sialoprotein (DSP, Millipore, dilution 1:2000), CD 68 (Thermo Fisher Scineticif, dilution 1:100) and Receptor activator of nuclear factor kappa-B ligand (RANKL, (Lab Vision, dilution 1:100)).

3. Results

3.1. Bone Volume Is Maintained in OCN-Cre; $LRP_5^{fl/fl}$ Mice

Micro-CT examination of the craniofacial skeleton of *OCN-Cre*; $LRP_5^{fl/fl}$ mice revealed that similar bone volume of skeletal elements (Figure 1). Deletion of LRP_5 did not affect the size of the skeletal elements (Figure 1). The dentition of *OCN-Cre*; $LRP_5^{fl/fl}$ mice was similar compared to wild-type mice. The overall size, shape, and position of the teeth were the same between wild-type and *OCN-Cre*; $LRP_5^{fl/fl}$ mice (Figure 1). The gross morphology of the molars and incisors was similar in wild-type and mutant mice.

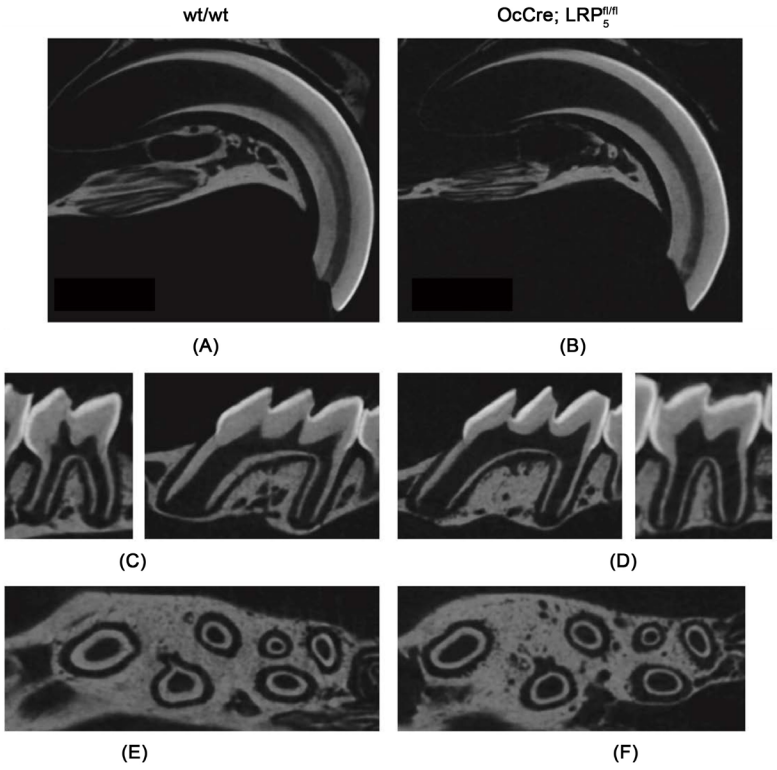

Figure 1. Bone volume is maintained in *OCN-Cre*; $LRP_5^{fl/fl}$ mice. ((A), (B)) Wild-type and *OCN-Cre*; $LRP_5^{fl/fl}$ mice incisors appear to have equivalent size and appearance. Bone volume around incisor area appears similar in wild-type and *OCN-Cre*; $LRP_5^{fl/fl}$ mice. ((C), (D)) The same finding was observed in molars of wild-type and *OCN-Cre*; $LRP_5^{fl/fl}$ mice. ((E), (F)) Cross section of molar areas showed the same finding in the wild-type and *OCN-Cre*; $LRP_5^{fl/fl}$ mice.

3.2. Fibromodulin Expression Is Altered in OCN-Cre; $LRP_5^{fl/fl}$ Mice

Histologic examination of the maxillary periodontal complex confirmed the finding in micro-CT examination (Figure 2(A) and Figure 2(B)). In wild-type mice (Figure 2(C)), the periodontal ligament consisted of numerous cells and

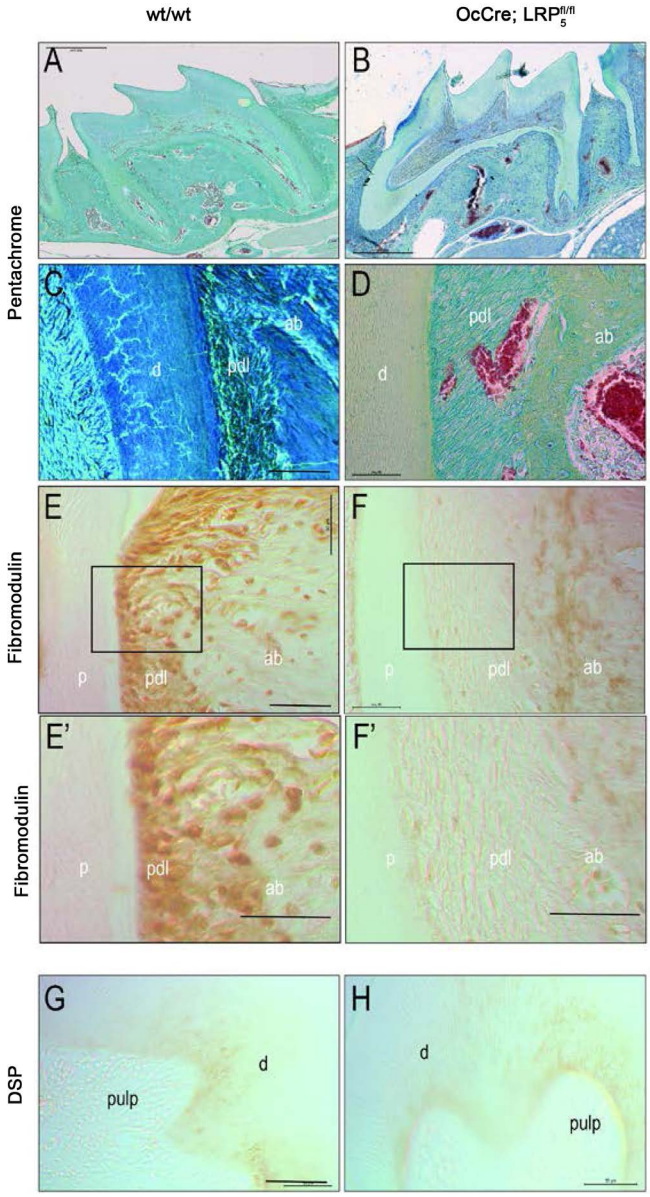

Figure 2. Fibromodulin expression is altered in *OCN-Cre*; $LRP_5^{fl/fl}$ mice. ((A), (B)) Pentachrome staining showed that intact tooth structure and periodontal complex in both the wild-type and *OCN-Cre*; $LRP_5^{fl/fl}$ mice. ((C), (D)) Higher magnification revealed that intact cementum and well organized periodontal ligament fibers in the wild-type and mutant mice. ((E), (F)) Expression of Fibromodulin in *OCN-Cre*; $LRP_5^{fl/fl}$ mice were reduced compared to the wild-type mice. Black arrows inidicated that expression of Fibromodulin in the periodontal ligament space was significantly reduced. ((G), (H)) Expression of DSP was found in the wild-type and *OCN-Cre*; $LRP_5^{fl/fl}$ mice. Scale bar: 50 μm.

collagen fiber bundles. Histological examination of the periodontal complex demonstrated that, compared to wild-type mice, there was no alteration in the fibrillar structure of the PDL in *OCN-Cre*; $LRP_5^{fl/fl}$ mice (**Figure 2(D)**). In addition, the width of periodontal ligament showed no difference between wild-type and *OCN-Cre*; $LRP_5^{fl/fl}$ mice. Higher magnification revealed that there was no difference in the alveolar bone and root surfaces in *OCN-Cre*; $LRP_5^{fl/fl}$ mice compared to wild-type mice.

Using Fibromodulin immunostaining [12], we found variations in expression. In wild-type mice, Fibromodulin was uniformly dispensed in the PDL space (**Figure 2(E)**). In *OCN-Cre*; $LRP_5^{fl/fl}$ mice, however, Fibromodulin expression was very low in the PDL space (**Figure 2(F)**).

There was no difference in expression of DSP between wild-type and mutant mice (**Figure 2(G)** and **Figure 2(H)**).

3.3. Alteration of Osteogenic Markers in Periodontal Ligament Space of *OCN-Cre*; $LRP_5^{fl/fl}$ Mice

In wild-type mice, *osteocalcin* was expressed throughout the PDL space (**Figure 3(A)**). In *OCN-Cre*; $LRP_5^{fl/fl}$ mice, *osteocalcin* was minimally expressed in the periodontal ligament (**Figure 3(B)**). We also found that *Osterix* was strongly expressed in the wild-type and mutant periodontal ligament (**Figure 3(C)** and **Figure 3(D)**).

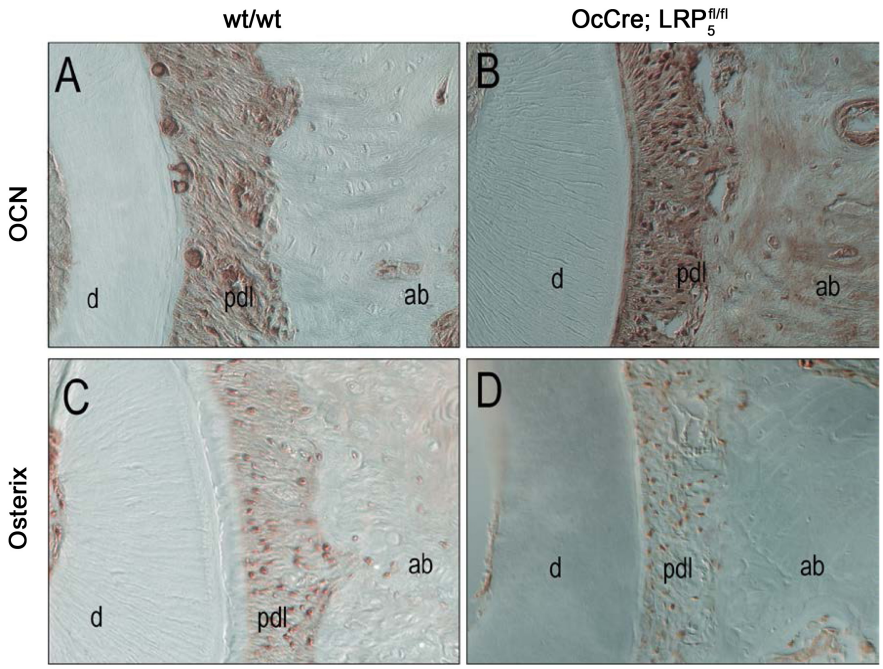

Figure 3. Osteogenic markers in periodontal ligament space of *OCN-Cre*; $LRP_5^{fl/fl}$ mice. ((A), (B)) *Osteocalcin* was clearly expressed throughout the PDL space in wild-type mice while a decrease in *Osteocalcin* expression was found in *OCN-Cre*; $LRP_5^{fl/fl}$ mice. ((C), (D)) *Osterix* expression was found in both the wild-type and *OCN-Cre*; $LRP_5^{fl/fl}$ mice. Scale bar: 50 μm.

3.4. Alteration of Osteoclastic Activity in *OCN-Cre*; $LRP_5^{fl/fl}$ Mice

In wild-type mice, CD 68 was expressed throughout the PDL space (**Figure 4(A)**), while expression of CD 68 was altered in the periodontal ligament of *OCN-Cre*; $LRP_5^{fl/fl}$ mice (**Figure 4(B)**). RANKL expression in wild-type mice found throughout the PDL space (**Figure 4(C)**) while expression of RANKL was reduced in the periodontal ligament of *OCN-Cre*; $LRP_5^{fl/fl}$ mice (**Figure 4(D)**). No difference in ALP activity was found between the wild-type and *OCN-Cre*; $LRP_5^{fl/fl}$ mice periodontal ligament (**Figure 4(C)** and **Figure 4(D)**).

4. Discussion

Wnt signaling pathway is involved in homeostasis of periodontal complex [9] [13] [14] [15]. Using gain- and loss-of-Wnt function animal models, reduced Wnt

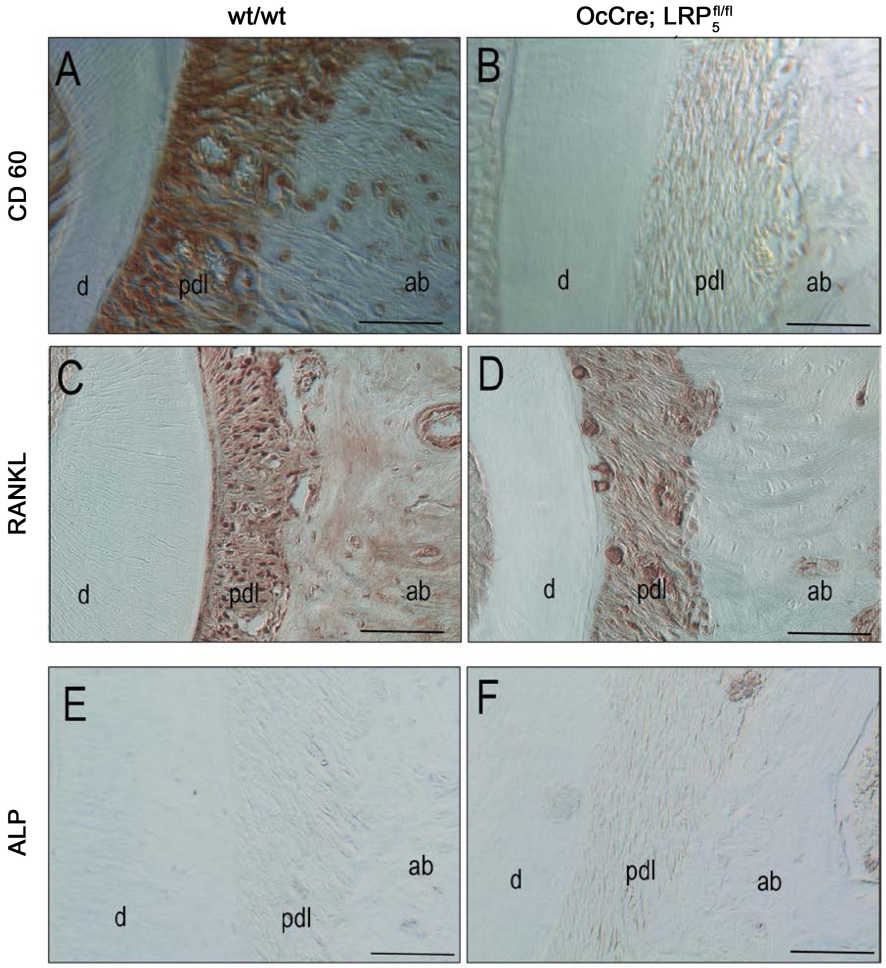

Figure 4. Alteration of osteoclastic activity in *OCN-Cre*; $LRP_5^{fl/fl}$ mice. ((A), (B)) Altered CD 68 expression was observed in periodontal ligament space of *OCN-Cre*; $LRP_5^{fl/fl}$ mice compared to the wild-type mice. ((C), (D)) RANKL expression was decreased in *OCN-Cre*; $LRP_5^{fl/fl}$ mice periodontal ligament space compared to that in the wild-type mice. ((E), (F)) ALP expression was similar between wild-type and *OCN-Cre*; $LRP_5^{fl/fl}$ mice. Scale bar: 50 μm.

signaling exhibits an increase in the width of the PDL while elevated Wnt signaling reduces the width of the PDL. Elevated Wnt signaling by mutations in the Wnt co-receptor Lrp5 caused an increased osteogenic gene expression and decreased bone resorption, which leaded to alveolar bone accumulation. On the other hand, our CT data and histology showed that *OCN-Cre*; $LRP_5^{fl/fl}$ mice exhibited insignificant changes in alveolar bone mass. One possible explanation for this comes from the fact that a reduction in bone mass occurs when LRP_5 is removed only in osteocytes [16].

Periodontal cells are reported to be Wnt responsive [17], so PDL cells are affected by Wnt signaling. Expression of fibromodulin in $Lrp5^{ACT}$ mice is strong in a previous study [9], while expression of fibromodulin in *OCN-Cre*; $LRP_5^{fl/fl}$ mice was dramatically reduced. The reason for this is not known. However, it may implicate that a reduction of fibromodulin leads to a disorganized periodontal collagen and missing its typical extracellular matrix [18].

Bone formation is known to be influenced by LRP_5. However, it is not clear that bone resorption depends on LRP_5. Osteoclast activity is known to be influenced by the coupled action of the Osteoprotegerin and RANKL. Osteoclast activity indicated by TRAP staining was significantly decreased in $Lrp5^{ACT}$ mice, while RANKL expression in $Lrp5^{ACT}$ mice was not altered compared to the wild-type mice [9]. In this study, osteoclast activity indicated by CD 68 expression, and RANKL expression were decreased in *OCN-Cre*; $LRP_5^{fl/fl}$ mice. Here, bone resorption was influenced by LRP_5, although the mechanism appears elusive. On the other hand, Ad-Dkk1 treated mice showed a significant increase in both TRAP activity and expression of RANKL [9]. In the case where Wnt signaling is particularly lower, both TRAP and RANKL activity are influenced. Ongoing work is in progress to explain the mechanism related to the role of LRP_5 during bone resorption.

5. Conclusion

Using loss-of-LRP_5 function animal model, we show that reduced LRP_5 is involved in altered collagen structure in the periodontal ligament and bone resorption.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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