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ORIGINAL ARTICLE

Preliminary investigation on the molecular mechanisms underlying the correlation between *VDR-FokI* genotype and periodontitis

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Abstract

Background: The only polymorphism that could change the protein structure in vitamin D receptor (VDR) is the *FokI* polymorphism (rs2228570). The FF genotype has the strongest transcriptional activity of VDR and is correlated with higher susceptibility to periodontitis. To reveal the possible molecular mechanisms for the correlation preliminarily, the influence of *VDR-FokI* genotype on the expression of *osteoprotegerin (OPG)* and *receptor activator of nuclear factor kappa B ligand (RANKL)* in human gingival fibroblasts (hGFs) and human periodontal ligament cells (hPDLCs) was investigated in this study.

Methods: hGFs and hPDLCs from 15 donors (five FF, seven Ff, and three ff) were treated with $1,25OH_2D_3$, with or without the specific knockdown of VDR using siRNA. The mRNA and protein expression of *OPG* and *RANKL* were detected using real-time PCR and enzyme-linked immunosorbent assay, respectively.

Results: Both in hGFs and hPDLCs, $1,25OH_2D_3$ could significantly induce the mRNA and protein expression of *RANKL*, and FF genotype had significantly higher induction than the other genotypes, however, neither $1,25OH_2D_3$ nor *VDR-FokI* had significant influence on the *OPG* expression. As a result, the RANKL/OPG ratio was significantly elevated under $1,25OH_2D_3$ stimulation and FF genotype had the most remarkable elevation. When VDR was knocked down, all the differences among the three genotypes disappeared.

Conclusion: The strongest transcriptional activity of FF genotype might contribute to the strongest enhancement of *RANKL* expression and RANKL/OPG ratio in hGFs and hPDLCs stimulated by $1,25OH_2D_3$, which might help to reveal the mechanisms of the correlation between FF genotype and susceptibility to periodontitis.

KEYWORDS

calcitriol, DNA modification methylase *FokI*, osteoprotegerin, periodontitis, polymorphism, RANK ligand, receptors, single nucleotide

1 | INTRODUCTION

Vitamin D is of great importance in the modulation of calcium homeostasis and immune function,[1] and has been verified to be involved in periodontal immune defense.[2–4] The biological function of vitamin D depends on its binding with vitamin D receptor (VDR),[1] and the relationship between *VDR* gene polymorphisms and periodontitis aroused researchers' attention.[5–8] Of all the single nucleotide polymorphisms in *VDR*, only one specific polymorphism can result in differences in the amino acid sequence of the VDR protein,[9] and has no linkage with any other polymorphisms in *VDR*.[10] This specific polymorphism is rs2228570 in exon 2. If the transition of C to T in rs2228570 exists, a new initiation codon ATG will appear and different transcription will occur, eventually leading to the formation of a longer VDR protein with 427 amino acids, otherwise, a shorter VDR protein with 424 amino acids will be generated.[9] Rs2228570 is also called *VDR-FokI* polymorphism because the transition of C to T could be recognized by the *FokI* restriction endonuclease (the f genotype). Without the transition, there is no recognition site of *FokI* restriction endonuclease and the genotype is designated F.

In 2003, a Japanese group studied the relationship between rs2228570 and periodontitis for the first time; however, no statistically significant association was detected in 74 patients with chronic periodontitis (CP) and 94 controls.[11] Similarly, in a Chinese study of 107 severe CP patients and 121 controls, no association between rs2228570 and severe CP was reported.[12] In a Libyan study of 99 CP patients and 97 controls, the lack of association between rs2228570 and CP was reported again.[13] On the contrary, in a Thai study with a large sample size of 1460 participants, both FF and Ff genotypes were reported to be more susceptible to severe CP.[14] Recently, it was revealed by a Romanian study including 53 CP patients and 47 controls that both FF and Ff genotypes were more likely to develop CP than ff genotype,[15] which is in line with the results of the Thai study. Although there were different views about the association between rs2228570 and CP, the study with larger sample size indicated the existence of correlation. In addition, the correlation between the shorter VDR and CP was revealed in a recent meta-analysis.[6] The relationship between rs2228570 and aggressive periodontitis (AgP) has been only studied in Asians and the existence of correlation was relatively uncontroversial. In 2006, Park et al.[16] enrolled 93 patients diagnosed with AgP and 143 controls in South Korea, and discovered the correlation between FF genotype and increased susceptibility to AgP (odds ratio = 1.83). A Chinese group, who enrolled 51 AgP patients and 53 controls, revealed that both the FF genotype (OR = 2.90) and the F allele (OR = 2.02) were associated with higher risk of AgP.[17] In our own study including 66 AgP patients, 52 CP patients and 60 controls, it was also indicated FF genotype had a higher prevalence of AgP than the other genotypes.[18] It was also revealed by a meta-analysis that the allele F might be an indicator for higher susceptibility of AgP in Asians.[5] According to the new classification of periodontal diseases,[19–22] there is no evidence that AgP and CP are two different diseases. Thus, it could be concluded that people with the shorter VDR were more likely to develop periodontitis; however, the mechanism is unknown and has never been reported.

In a reporter construct model, the transcriptional activity of the shorter VDR was verified to be significantly higher than that of the longer VDR for the first time.[23] After that, although there was one study in which no significant differences were detected between the transcriptional activities of the shorter and the longer VDR using reporter plasmids,[24] the data indicating that the shorter VDR had a higher transcriptional activity than the longer one were obtained in most of the researches using similar expression vectors.[25–28] In our previous study,[18] the endogenous different *VDR-FokI* genotypes in human gingival fibroblasts (hGFs) and human periodontal ligament cells (hPDLCs) were investigated and it was demonstrated that FF-VDR could induce more active transcription of vitamin D-responsive genes (*alkaline phosphatase* and *osteocalcin*) than the other kinds of VDR in hPDLCs, also supporting the mainstream results. In another study of our goup,[29] the influence of endogenous *VDR-FokI* genotypes on another vitamin D-responsive gene *CYP24A1* was studied in hGFs and hPDLCs and FF-VDR also had the most remarkable transcriptional activities in both kinds of cells.

Based on these mentioned scientific data, it was hypothesized that the higher transcriptional activity of the FF-VDR might contribute to the higher susceptibility of people with FF-VDR to periodontitis. In the present study, the influence of rs2228570 on the expression of *osteoprotegerin (OPG)* and *receptor activator of nuclear factor kappa B ligand (RANKL)* in hGFs and hPDLCs will be investigated to provide some molecular evidence for the hypothesis.

2 | MATERIALS AND METHODS

2.1 | Cell culture

The study protocol was approved by the institutional review board of Peking University School and Hospital of Stomatology, Beijing, PR China (PKUSSIRB-2011007), and was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2013. Written informed consent was obtained from each participant.

Fifteen volunteers' hGFs and hPDLCs obtained in our previous study[18] were used and there were five FF genotypes, seven Ff genotypes, and three ff genotypes, which were determined in the previous study.[18]

In all the following parts of the present study, hGFs and hPDLCs of passages four to six as well as dextran-coated

charcoal-stripped FBS (DCC-FBS)* were used. All the experiments were completed in triplicate.

2.2 | 1,25OH$_2$D$_3$ treatment

All the donors' cells were seeded into six-well plates at a density of 10,000/cm^2 in Dulbecco modified Eagle medium without phenol red† supplemented with 10% DCC-FBS. When the cells reached 80% confluence, 10 nM 1,25OH$_2$D$_3$‡ was added in the treatment group, and only ethanol as the vehicle of 1,25OH$_2$D$_3$ was used in the control group. After 72 hours, the supernatants were centrifuged for 5 minutes at 1200 rpm. Cells in the same wells were digested by 0.25% (wt/vol) trypsin and 0.02% (wt/vol) EDTA, and the number of cells was counted before lysis in Trizol.§ All the samples were chilled at −80°C for the subsequent experiments.

2.3 | Detection of OPG and RANKL mRNA expression

A reverse transcription kit¶ was used to reversely transcribe the RNA to cDNA. Real-time PCR was finished using a SYBR Green kit# in a real-time thermocycler.‖ Data were analyzed using SDS software and glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) was internal control. The sequences of the primers were as follows: OPG forward primer, 5′-ACAACTTGCTGTGCTGCGC-3′; OPG reverse primer, 5′-TCCTGGGTGGTCCACTTAATG-3′; RANKL forward primer, 5′-GACATCCCATCTGGTTCCCA-3′; RANKL reverse primer, 5′-CCCAACCCCGATCATGGTA-3′; GAPDH forward primer, 5′-GAAGGTGAAGGTCGGAGTC-3′; GAPDH reverse primer, 5′-GAAGATGGTGATGGGA TTTC-3′. Quantification of *OPG* and *RANKL* mRNA expression levels were presented as relative mRNA levels calculated by the $2^{-\Delta\Delta Ct}$ method.[30]

2.4 | Measurement of OPG and RANKL protein expression

OPG and RANKL concentrations in the cell culture supernatants were measured by enzyme-linked immunosorbent assay as reported previously.[31] Commercially available enzyme-linked immunosorbent assay kits were used for quantifying the amounts of OPG** and RANKL,†† which were divided by the quantities of cells before statistical analysis.

2.5 | RNA interference of VDR

In each well of 6-well plates, 5×10^5 cells were seeded in Opti-MEM without phenol red‡‡ supplemented with 5% DCC-FBS. When the cells were about 70% confluent, 20 nM VDR siRNA§§ or 20 nM non-silencing control siRNA¶¶ were added into the medium with siRNA Transfection Reagent.##

12 hours after transfection, some of the cells were collected for the confirmation of the effect of RNA interference (RNAi) using real-time PCR, which was carried out as described earlier. The sequence of the forward VDR primer was 5′-GGTGGAGGGAGCCATCCTT-3′ and the reverse VDR primer was 5′-TGGGACAGCTCTAGGGTCACA-3′. The other cells were treated with 10 nM 1,25OH$_2$D$_3$ for another 72 hours. Then, the expression of OPG and RANKL was measured with the same methods previously mentioned.

2.6 | Statistical analyses

The distribution of the variants was determined by the Shapiro-Wilk test. ANOVA was used for the comparison of the expression levels of OPG and RANKL, as well as RANKL/OPG ratios in different genotype groups. Post hoc comparison was conducted with the least significant difference method. Comparison of OPG and RANKL expression, as well as the RANKL/OPG ratios, in the two kinds of cells was accomplished using the paired-samples t test.

All the statistical analyses were performed using SPSS 11.5‖‖ and statistical significance was only accepted when $P < 0.05$.

3 | RESULTS

In hGFs, the mRNA expression of OPG was not significantly different among the different genotypes after 1,25OH$_2$D$_3$ treatment (Figure 1A), whereas the mRNA expression of RANKL was induced in the 1,25OH$_2$D$_3$ treatment group, and the induction of the FF genotype was over two times of those of the other two genotypes (Figure 1B). Still in hGFs, the RANKL/OPG ratios were also elevated in

* TBD, Tianjin, China.
† Sigma-Aldrich, St. Louis, MO.
‡ Sigma-Aldrich, St. Louis, MO.
§ Invitrogen, Thermo Fisher Scientific, Waltham, MA.
¶ Fermentas, Thermo Fisher Scientific, Waltham, MA.
# Roche, Basel, Switzerland.
‖ Applied Biosystems, Thermo Fisher Scientific, Waltham, MA.
** R&D, Minneapolis, MN.
†† R&D, Minneapolis, MN.
‡‡ Invitrogen, Thermo Fisher Scientific, Waltham, MA.
§§ sc-106692, Santa Cruz Biotechnology, Santa Cruz, CA.
¶¶ sc-37007, Santa Cruz Biotechnology, Santa Cruz, CA.
Santa Cruz Biotechnology, Santa Cruz, CA.
‖‖ SPSS Inc., Chicago, IL.

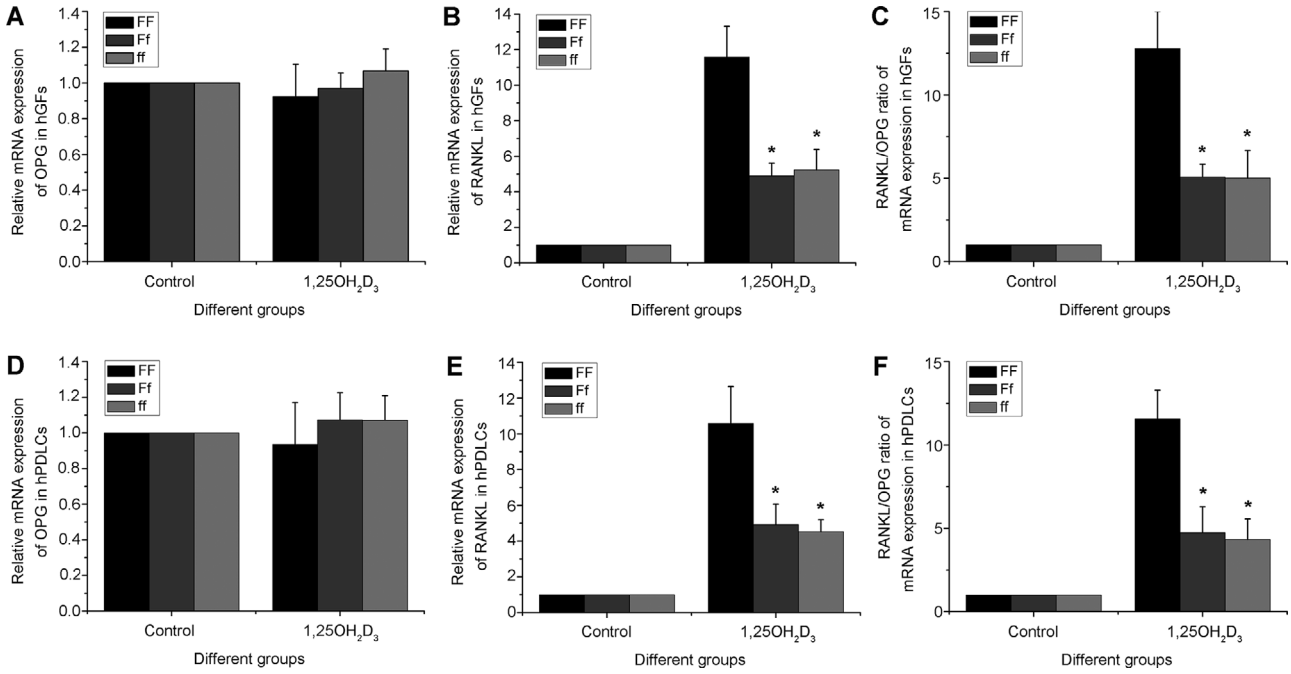

FIGURE 1 Influence of *VDR-FokI* genotype on the mRNA expression of OPG (**A**), RANKL (**B**), and RANKL/OPG ratio (**C**) in hGFs. Influence of *VDR-FokI* genotype on the mRNA expression of OPG (**D**), RANKL (**E**), and RANKL/OPG ratio (**F**) in hPDLCs. Data are presented as mean ± SE. *Significantly different from FF genotype in the same group

the $1,25OH_2D_3$ treatment group, and the FF genotype had the most remarkable elevation (Figure 1C). In hPDLCs (Figure 1D through 1F), the trends were similar with those in hGFs, and the induction of RANKL mRNA expression by $1,25OH_2D_3$ in FF-hPDLCs was also over two times of those in the other hPDLCs (Figure 1E). As were the RANKL/OPG ratios of mRNA expression in hPDLCs treated with $1,25OH_2D_3$ (Figure 1F).

The OPG (Figure 2A) and RNAKL (Figure 2B) protein generation, as well as the RANKL/OPG ratio of protein expression (Figure 2C), in FF-hGFs in the control group and the OPG protein generation in FF-hGFs in the $1,25OH_2D_3$ treatment group were not significantly different from those in the other hGFs. $1,25OH_2D_3$ treatment significantly upregulated RANKL expression, and the expression in FF-hGFs (856.50 ± 70.82 fmol/10^5 cells) was significantly higher than those in Ff-hGFs (380.08 ± 41.54 fmol/10^5 cells) and ff-hGFs (404.60 ± 19.18 fmol/10^5 cells) (Figure 2B). As were the RANKL/OPG ratios of protein expression in hGFs in the $1,25OH_2D_3$ treatment group (447.77 ± 47.10 versus 183.68 ± 22.72 versus 216.59 ± 25.52, respectively, $P < 0.05$) (Figure 2C). In hPDLCs, no significant differences were detected in the control groups (Figure 2D through 2F) and in the OPG protein expression in the $1,25OH_2D_3$ treatment group (Figure 2D). The protein expression of RANKL in FF-hPDLCs was significantly higher than those in the other hPDLCs in the $1,25OH_2D_3$ treatment group (898.90 ± 69.704 versus 409.00 ± 53.90 versus 409.43 ± 42.53 fmol/10^5 cells, respectively, $P < 0.05$) (Figure 2E). Similarly, under $1,25OH_2D_3$ stimulation, the RANKL/OPG ratio of protein expression in FF-hPDLCs (1071.10 ± 124.14) was significantly higher than those in Ff-hPDLCs (508.23 ± 130.88) and ff-hPDLCs (475.64 ± 85.58) (Figure 2F).

The effect of RNAi against VDR in hGFs and hPDLCs was 83.21% ± 2.74% and 83.89% ± 2.76%, respectively, which was evaluated using real-time PCR. As manifested in Figure 3A through 3F, the mRNA expressions of OPG and RANKL, as well as the RANKL/OPG ratio of mRNA expression, in the effective RNAi group was calculated as 1. Significant differences were detected in the control RNAi group when the mRNA expression of RANKL and the RANKL/OPG ratio of mRNA expression were compared among different genotypes, and the FF genotype had the highest RANKL expression and RANKL/OPG ratio both in hGFs (Figure 3B, 3C) and in hPDLCs (Figure 3E, 3F).

As presented in Figure 4, there were no significant differences detected in the effective RNAi groups in hGFs (Figure 4A through 4C) and hPDLCs (Figure 4D through 4F). In the control RNAi groups, the protein expressions of OPG in hGFs (Figure 4A) and hPDLCs (Figure 4D) were not significantly different among different genotypes, either. The generation of RANKL protein by FF-hGFs (872.48 ± 89.83 fmol/10^5 cells) was significantly higher than those by Ff-hGFs (376.94 ± 31.39 fmol/10^5 cells) and ff-hGFs (403.83 ± 27.98 fmol/10^5 cells) in the control RNAi group (Figure 4B). Similarly, FF genotype had the

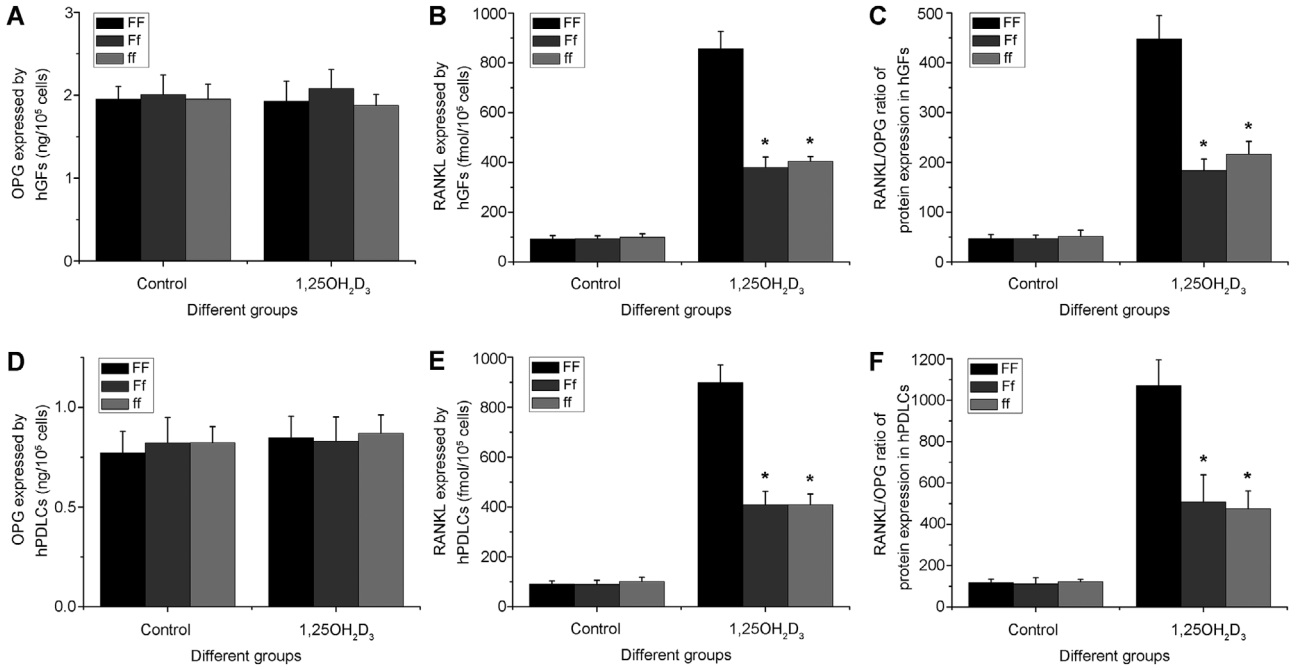

FIGURE 2 Influence of *VDR-FokI* genotype on the protein expression of OPG (**A**), RANKL (**B**), and RANKL/OPG ratio (**C**) in hGFs. Influence of *VDR-FokI* genotype on the protein expression of OPG (**D**), RANKL (**E**), and RANKL/OPG ratio (**F**) in hPDLCs. Data are presented as mean ± SE. *Significantly different from FF genotype in the same group

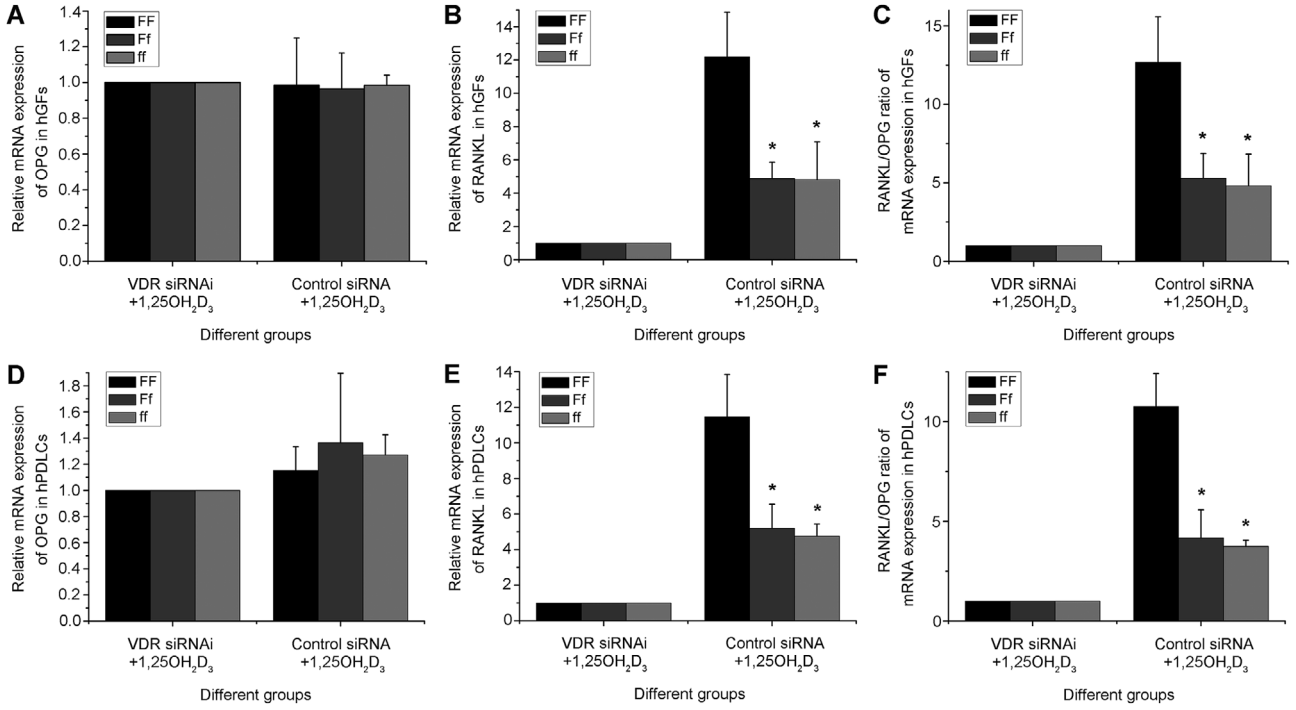

FIGURE 3 Influence of *VDR-FokI* genotype on the mRNA expression of OPG (**A**), RANKL (**B**), and RANKL/OPG ratio (**C**) in hGFs treated with interfering RNA before $1,25OH_2D_3$ stimulation. Influence of *VDR-FokI* genotype on the mRNA expression of OPG (**D**), RANKL (**E**), and RANKL/OPG ratio (**F**) in hPDLCs treated with interfering RNA before $1,25OH_2D_3$ stimulation. Data are presented as mean ± SE. *Significantly different from FF genotype in the same group

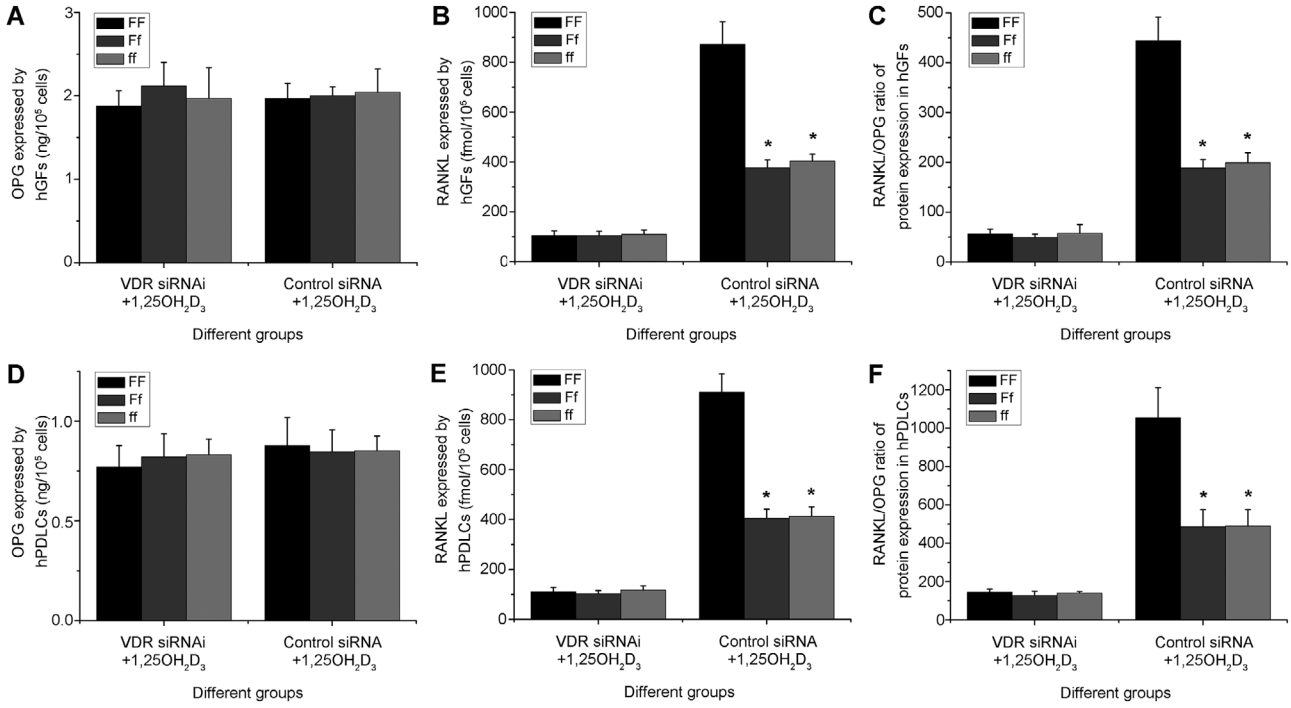

FIGURE 4 Influence of *VDR-FokI* genotype on the protein expression of OPG (**A**), RANKL (**B**), and RANKL/OPG ratio (**C**) in hGFs treated with interfering RNA before $1,25OH_2D_3$ stimulation. Influence of *VDR-FokI* genotype on the protein expression of OPG (**D**), RANKL (**E**), and RANKL/OPG ratio (**F**) in hPDLCs treated with interfering RNA before $1,25OH_2D_3$ stimulation. Data are presented as mean ± SE. *Significantly different from FF genotype in the same group

highest RANKL/OPG ratio of protein expression in hGFs (444.56 ± 46.84 versus 188.72 ± 16.80 versus 199.25 ± 20.04, respectively, $P < 0.05$) (Figure 4C). FF-hPDLCs also had higher RANKL protein expression (911.08 ± 73.26 fmol/10^5 cells) than Ff-hPDLCs (404.66 ± 36.70 fmol/10^5 cells) and ff-hPDLCs (413.23 ± 37.34 fmol/10^5 cells) in the control RNAi group (Figure 4E). As were the RANKL/OPG ratios of protein expression (1054.64 ± 156.18 versus 486.06 ± 88.39 versus 489.41 ± 84.97, respectively, $P < 0.05$) (Figure 4F).

Paired comparison of the two kinds of cells (Figure 5) showed that the expression of OPG mRNA in hGFs was significantly higher than that in hPDLCs in both the two groups (Figure 5A). As were the expressions of OPG protein in the control group (1.98 ± 0.19 versus 0.81 ± 0.11 ng/10^5 cells, $P < 0.05$) and in the $1,25OH_2D_3$ treatment group (1.99 ± 0.22 versus 0.84 ± 0.11 ng/10^5 cells, $P < 0.05$) (Figure 5B). No significant differences were found in the expression of RANKL in hGFs and hPDLCs (Figure 5C, 5D). hPDLCs had significantly higher RANKL/OPG ratios of mRNA expression in both the two groups (Figure 5E). The RANKL/OPG ratios of protein expression in hPDLCs were also significantly higher than those in hGFs both in the control group (47.96 ± 7.95 versus 116.16 ± 22.23, $P < 0.05$) and in the $1,25OH_2D_3$ treatment group (278.29 ± 128.44 versus 689.34 ± 301.71, $P < 0.05$) (Figure 5F).

4 | DISCUSSION

There have already been numerous studies on the association between single nucleotide polymorphisms and periodontitis, in which periodontitis groups and control groups were enrolled, the genotypes were detected, and the association was analyzed using statistical methods. However, such kinds of descriptive researches could only supply rather limited scientific values because the results were likely to be influenced by sample size, race, etc., and it was of great difficulty to obtain enough scientific evidence for identifying a genetic biomarker of periodontitis. Therefore, investigation on the molecular mechanisms underlying the association between single nucleotide polymorphisms and periodontitis is needed, and as far as it was concerned, the present study is the first attempt in this field.

Because *RANKL* is a vitamin D responsive-gene,[32] the expression of RANKL could be greatly induced by $1,25OH_2D_3$ in hGFs and hPDLCs. Because FF-VDR had the strongest transcriptional activity in the three *VDR-FokI* genotypes, which was verified in our previous studies,[18,29] it was not surprising to find that FF-hGFs and FF-hPDLCs had the most remarkable upregulations of RANKL when VDR was stimulated. To obtain further evidence, RNAi against VDR was accomplished, and it was demonstrated that VDR was the reason for the differences in the upregulations of

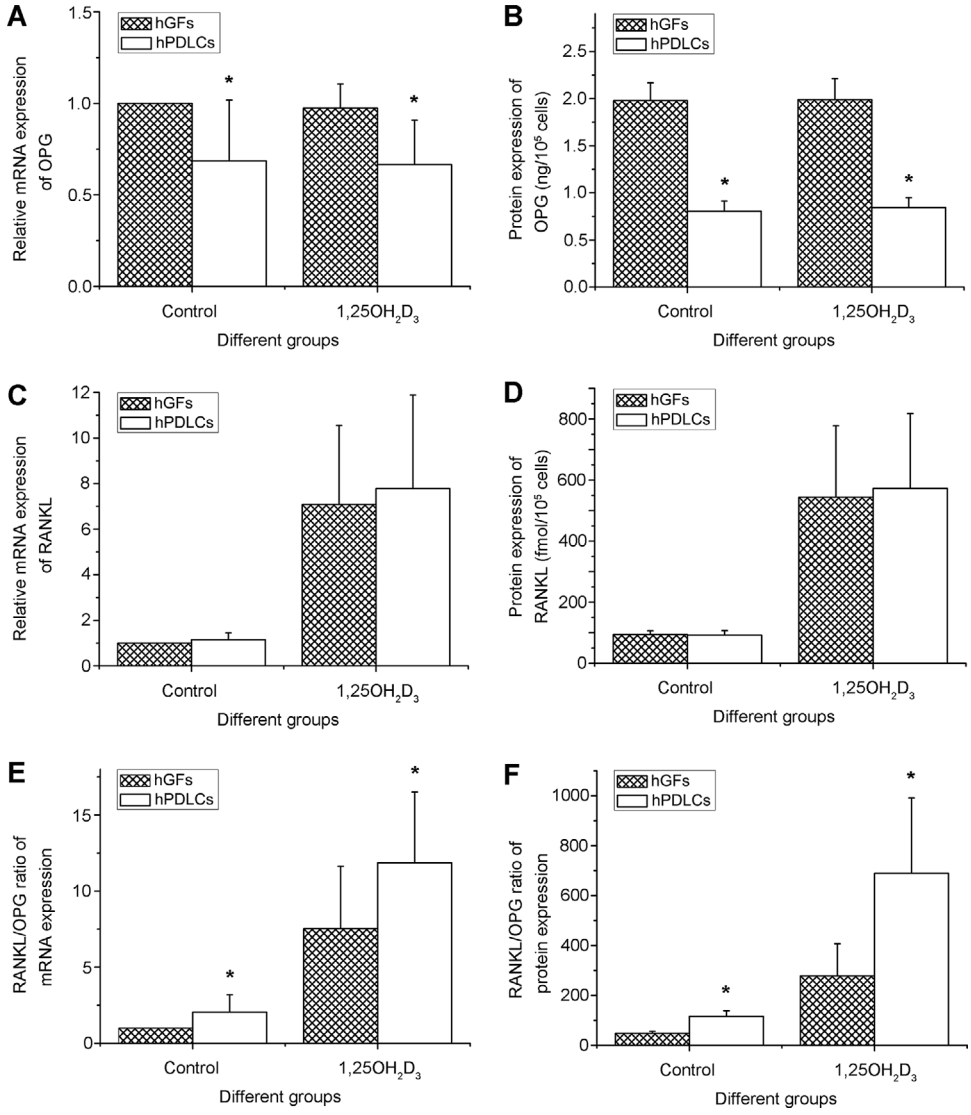

FIGURE 5 Paired comparison of hGFs and hPDLCs. (**A**) mRNA expression of OPG. (**B**) Protein expression of OPG. (**C**) mRNA expression of RANKL. (**D**) Protein expression of RANKL. (**E**) RANKL/OPG ratio of mRNA expression. (**F**) RANKL/OPG ratio of protein expression. Data are presented as mean ± SE. *Significantly different from hGFs in the same group

RANKL expression. Additionally, because there was plenty of 25-hydroxy vitamin D_3 in periodontium with vitamin D hydroxylase activity to transform 25-hydroxy vitamin D_3 to $1,25OH_2D_3$,[3,33,34] the periodontal supply of VDR ligand in vivo was sufficient. Therefore, it is probable for FF-hGFs and FF-hPDLCs to have higher RANKL expression and higher RANKL/OPG ratios in vivo. Because OPG and RANKL are key factors in the regulation of osteoclastogenesis and bone resorption, and higher RANKL/OPG ratio is an indicator of higher possibility of bone resorption,[32,35] greater potential of FF genotype to bone resorption around these cells could be reasonable, which might provide insight for the higher susceptibility of FF-genotype to periodontitis.

Vitamin D is increasingly considered to be beneficial for periodontal health in the recent studies including our own study,[4,36,37] however, vitamin D seemed harmful to the periodontal health in the present study. Actually, it was revealed by the co-culture model of human peripheral blood mononuclear cells with hGFs or hPDLCs that although osteoclastogenesis could be induced by hGFs and hPDLCs, the induced osteoclast-like cells were not functional and extra activation was needed.[38,39] Thus, $1,25OH_2D_3$ only elevated the RANKL/OPG ratios in hGFs and hPDLCs, which enhanced the number of osteoclast-like cells and increased the potential of bone resorption, but did not result in bone resorption directly. The activation of such osteoclast-like cells in periodontium needs further elucidation.

Although the biological role of vitamin D is dependent on the combining with VDR, VDR has some intrinsic effects independent of vitamin D binding.[40,41] It was reported that FF-VDR could increase immune-related transcription factors (e.g., nuclear factor κB, nuclear factor of activated T-cells,

etc.)-driven transcription in immune cells such as monocytes, dendritic cells and lymphocytes, and FF-immune cells (monocytes and dendritic cells) had higher IL-12 expression than the corresponding ff-cells, and FF-lymphocytes had stronger proliferation than ff-lymphocytes.[40] Vitamin D did not play any role in these results, and the authors considered that the unliganded effects of VDR were responsible for the association between FF-VDR and stronger immune response.[40] Besides, VDR could also function unliganded in the regulation of mammalian hair cycling via regulating genes affecting Wnt signaling in keratinocytes.[41] However, the knowledge about the intrinsic effects of VDR was still not enough, and whether such effects played a role in the association between FF-VDR and periodontitis will be investigated in our future research.

Previously, most studies about the influence of *VDR-FokI* polymorphism on the vitamin D responsive-gene expression were focused on the exogenous VDR expression using engineered vectors with different *VDR-FokI* genotypes.[23,25,26,28] However, there was evidence that the biological functions of exogenous VDR was not reconciled with those of endogenous VDR. In one study,[27] exogenous FF-VDR was verified to have higher transcriptional activity than the other VDRs, but significant differences were not found among different endogenous *FokI* genotypes in primarily cultured fibroblasts. Therefore, to reflect what really happens in vivo as much as possible, the endogenous VDR expression model was chosen in the present study, which had been verified to be effective in our previous studies.[18,29]

There is also advantage of the model in the study of heterozygote. Except for FF and ff, rs2228570 can also generate a heterozygote Ff, which could produce two kinds of VDR proteins.[24,28] In the exogenous VDR expression model, the function of Ff was rarely studied except in one early study,[23] in which both homozygous expression vectors were transfected to construct the heterozygote, and the transcriptional activity of the artificial heterozygote was between those of the two homozygotes. In the present study, the influence of Ff-VDR on OPG and RANKL expression in hGFs and hPDLCs was significantly lower than that of FF-VDR and was similar with that of ff-VDR, which was in line with our previous results.[18,29] Because the natural heterozygote rather than the artificial one was studied in the endogenous VDR expression model, our findings on heterozygotes might be more representative of the actual situation in the body.

Because osteoclastogenesis and bone resorption are mainly regulated by bone marrow stromal cells or osteoblasts,[32,35] and hGFs and hPDLCs both display similarity to osteoblasts,[42,43] it was believed that both hGFs and hPDLCs were involved in the regulation of periodontal bone metabolism, which was supported by the fact that both the cells could secrete OPG and RANKL in the present study. However, according to the present data, hPDLCs might be more active in inducing osteoclastogenesis than hGFs because hPDLCs synthesized less OPG, which was in line with other researchers' data.[38,44,45] Because the alveolar bone is always in the dynamic balance of bone formation and bone resorption,[46] and hPDLCs are nearer to the alveolar bone than hGFs anatomically, it is reasonable that hPDLCs play a more active role than hGFs in alveolar bone remodeling. What should be pointed out that the hGFs and hPDLCs used in the present study were all from the same donors, and paired comparison avoided the potential bias of heterogeneity of cells from different donors, which made the differences between the two kinds of cells more credible.

5 | CONCLUSIONS

In hGFs and hPDLCs under $1,25OH_2D_3$ stimulation, FF-VDR could induce higher RANKL expressions and RANKL/OPG ratios than Ff-VDR and ff-VDR, which might result in more osteoclastogenesis. The higher induction might be attributed to the stronger transcriptional activity of FF-VDR. The present study provided some scientific clues to explain the correlation between *VDR-FokI* genotype and periodontitis; however, more studies on the activation of osteoclast-like cells in periodontium are still needed.

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AUTHOR CONTRIBUTION

Kaining Liu co-developed research plan, finished primary cell culture, real-time PCR, RNAi, statistical analyses, and drafted the manuscript. Bing Han finished ELISA. Jianxia Hou participated in primary cell culture and advised in interpretation of results. Huanxin Meng initiated the idea, co-developed research concept, and revised the manuscript.

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