Received: 2 September 2017 | Revised: 29 October 2017 | Accepted: 27 November 2017

DOI: 10.1002/JPER.17-0530

JOURNAL OF Periodontology

ORIGINAL ARTICLE

# Preliminary investigation of the vitamin D pathway in periodontal connective tissue cells

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## Abstract

**Background:** The vitamin D pathway, from toll-like receptor activation to human cationic antimicrobial protein (hCAP-18/LL-37) generation, has been identified in monocytes and keratinocytes. This study aimed to investigate the vitamin D pathway in human gingival fibroblasts (hGFs) and human periodontal ligament cells (hPDLCs) and to provide preliminary evidence of its role in periodontal immune defense.

**Methods:** Primary cultures of hGFs and hPDLCs were stimulated with 1,25-dihydroxy vitamin $D_3$ and 25-hydroxy vitamin $D_3$, with or without *Porphyromonas gingivalis* lipopolysaccharide. CYP27B1 RNA interference and vitamin D receptor (VDR) antagonism were also used for reverse proof. The mRNA expression of hCAP-18/LL-37, VDR, interleukin (IL)-6, IL-8, and monocyte chemotactic protein-1 were detected using real-time polymerase chain reaction. The LL-37 concentrations were measured using enzyme-linked immunosorbent assay.

**Results:** In hGFs and hPDLCs, 25-hydroxy vitamin $D_3$ and 1,25-dihydroxy vitamin $D_3$ induced hCAP-18/LL-37 expression, which was further increased by *Porphyromonas gingivalis* lipopolysaccharide. If the function of CYP27B1 or VDR was blocked, the induction was significantly weakened. IL-8 and monocyte chemotactic protein-1 mRNA expression could be suppressed by the vitamin D pathway.

**Conclusion:** These findings suggest that the vitamin D pathway exists in hGFs and hPDLCs and plays an important role in immune defense in periodontal soft tissues.

KEYWORDS

CAP18 lipopolysaccharide-binding protein, gingiva, periodontal ligament, vitamin D

As an important fat-soluble vitamin in vivo, vitamin $D_3$ plays an indispensable role in the regulation of calcium homeostasis and immune function.[1,2] Vitamin $D_3$ is transformed to 25-hydroxy vitamin $D_3$ (25-[OH]VD) by the 25-hydroxylase CYP27A1 which is abundant in liver, and then to 1,25-dihydroxy vitamin $D_3$ (1,25-[OH]VD) by the 1,$\alpha$-hydroxylase CYP27B1 which is abundant in kidney.[1] These synthetic reactions have been found to exist not only in liver and kidney, but also in other organs and cells including skin,[3] prostate,[4] and macrophages.[5] In human gingival fibroblasts (hGFs) and human periodontal ligament cells (hPDLCs), the synthesis from vitamin $D_3$ to 25-(OH)VD, then to 1,25-(OH)VD, by detection of CYP27A1 and CYP27B1 activity,[6,7] was verified.

Liu et al.[8] first revealed the 25-(OH)VD-mediated induction of the human cationic antimicrobial protein of 18 kDa (hCAP-18) after toll-like receptor 2 (TLR2) activation by the synthetic 19-kD *Mycobacterium tuberculosis*–derived lipopeptide (TLR2/1L) in monocytes and named the connected reactions from TLR activation to hCAP-18 induction the vitamin D pathway, with CYP27B1 being the important factor. A similar vitamin D pathway was further investigated

in the epidermis, and Schauber et al. observed an increase in hCAP-18 expression in keratinocytes stimulated by 25-(OH)VD together with transforming growth factor (TGF)-$\beta$1 and CYP27B1, which was also verified to be a key factor in the vitamin D pathway in keratinocytes.[9] hCAP-18 is a vital antimicrobial peptide and is composed of a conserved cathelin-like domain and a C-terminal peptide of 37 amino acid residues called LL-37.[10] hCAP-18 can be cleaved to release the mature peptide LL-37, with another name as cathelicidin, which exhibits a broad antimicrobial spectrum extracellularly.[11,12] In addition, hCAP-18/LL-37 possesses an immuno-inflammatory response regulatory role by affecting cytokine and chemokine expression or secretion.[13–17]

Periodontal expression of hCAP-18/LL-37 has been found mainly in gingival epithelial cells[18–20] or infiltrated neutrophils,[21] and is positively correlated with inflammatory status. hCAP-18/LL-37 has also been detected in human gingival crevicular fluids (GCF), with patients with aggressive or chronic periodontitis exhibiting higher LL-37 levels in GCF than controls.[22,23] In in vitro studies, stimulation of gingival/oral epithelial cells using 25-(OH)VD, 1,25-(OH)VD, or its analog resulted in an elevation of hCAP-18/LL-37 expression.[24,25] The question was raised whether the vitamin D induction of hCAP-18/LL-37 may also exist in periodontal soft tissue cells such as hGFs and hPDLCs. It has been reported that vitamin D can inhibit the expression of interleukin (IL)-8 in hPDLCs stimulated with *Porphyromonas gingivalis* (*Pg*),[26] while hCAP-18/LL-37 could also exert a similar biologic function.[17] Therefore, it is reasonable to hypothesize that the vitamin D pathway also exists in hGFs and hPDLCs, and may play a role in periodontal immune defense. In the present in vitro study, the relationship between hCAP-18/LL-37 expression and vitamin D with or without inflammatory stimulus was investigated to test this hypothesis.

# 1 | MATERIALS AND METHODS

## 1.1 | Cell culture

The research plan was approved by the institutional review board of Peking University School and Hospital of Stomatology (PKUSSIRB-2011007) and written informed consent was obtained from each participant.

Primary cell culture of hGFs and hPDLCs from 30 different donors (15 each) was obtained. Culture was carried out according to previously described methods.[6,26] Briefly, hGFs and hPDLCs were obtained from periodontally healthy, extracted third molars of 30 young healthy volunteers (13 males, 17 females, aged 20 to 30 years; mean age: 23.6 years). Periodontal ligament tissues around the middle third of the root were gently curetted and minced. Gingival tissues attached to the crown were also minced, and isolated cells were inoculated into 24-well plates. Both cell types were cultured in phenol red–free Dulbecco's modified Eagle's medium* supplemented with 10% fetal bovine serum,† 100 U/mL penicillin, and 100 mg/mL streptomycin. Cultures of both cell types were maintained in an atmosphere of 5% $CO_2$ at 37°C. After reaching 80% confluence, hGFs and hPDLCs were digested with 0.25% phenol red–free trypsin and 0.02% EDTA, after which they were subcultured at a 1:3 ratio. Cells of passage 4 or 5 were used in all subsequent experiments.

## 1.2 | Detection of hCAP-18/LL-37 expression

Cells from 10 donors (5 hGFs and 5 hPDLCs) were seeded into six-well plates at a density of 5,000/cm$^2$. Four days later, cells were treated separately with 1,000 nM 25-(OH)VD,‡ 10 nM 1,25-(OH)VD,§ or 0.1% ethanol for 48 hours. Cell lysates were collected using a reagent¶ and supernatants were obtained. RNA was extracted from cell lysates according to the manufacturer's instructions. Reverse transcription-polymerase chain reaction (PCR) was then performed to obtain cDNA using a reverse transcription kit# after which real-time PCR was conducted using a kit‖ in a real-time thermocycler.** *GAPDH* was used as the internal control. The sequences of the primers were as follows: *hCAP-18* forward primer, 5′-AGGATTGTGACTTCAAGAAGGACG-3′; *hCAP-18* reverse primer, 5′-GTTTATTTCTCAGAGCCC AGAAGC-3′; *GAPDH* forward primer, 5′-GAAGGTGAA GGTCGGAGTC-3′; *GAPDH* reverse primer, 5′-GAAGATG GTGATGGGATTTC-3′. Quantification of hCAP-18/LL-37 expression levels is presented as relative mRNA levels calculated by the equation $2^{-}\triangle\triangle$Ct.[27]

## 1.3 | RNA interference of CYP27B1

Five hGFs (from donors A, B, C, D, E) and five hPDLCs (from donors F, G, H, I, J) were seeded at a density of 15,000/cm$^2$ in six-well plates. When they reached 70% to 80% confluence, cells were transfected with small interfering (si)RNA (20 nM) specifically to silence CYP27B1, or non-silencing NC (negative control). Both siRNAs were used with a reagent†† according to the manufacturer's

* Sigma Aldrich, St. Louis, MO.

† PAA, Coelbe, Germany.

‡ Sigma Aldrich.

§ Sigma Aldrich.

¶ TRIzol, Thermo Fisher Scientific, Waltham, MA.

# Thermo Fisher Scientific.

‖ Roche, Indianapolis, IN.

** Thermo Fisher Scientific.

†† GenePharma, Jiangsu, China.

instructions. The sequence of CYP27B1 siRNA was CTGGTTTACGGTTTCTTATAA, as used in our previous study.[7] At 24 hours after siRNA transfection, cells were treated with 1,000 nM 25-(OH)VD or 10 nM 1,25-(OH)VD for 48 hours. Cells were then harvested and CYP27B1 mRNA was detected using real-time PCR. *CYP27B1* forward primer was 5′-GAAGTGCTAAGACTGTACCCTGT-3′ and reverse primer was 5′-CCTTGAAGTGGCATAGTGACAC-3′.

### 1.4 | Antagonism of vitamin D receptor (VDR)

Five hGFs and five hPDLCs from the same donors used in the RNA interference experiment were seeded into six-well plates at a density of 5000/cm$^2$. Four days later, cells were treated separately with either VAZ (vitamin D receptor [VDR] antagonist ZK159222[28,29] *) for 1 hour, then 25-(OH)VD for 48 hours or 25-(OH)VD alone for 48 hours. ..... Cells were then harvested. The subsequent steps for detection of gene expression of hCAP-18/LL-37 were the same as described previously.

### 1.5 | Co-Stimulation with *Pg*-LPS

Five cultures of hGFs (from donors K, L, M, N, O) and five hPDLCs (from donors P, Q, R, S, T) were used. Cells were stimulated with 1,000 nM 25-(OH)VD plus 10 $\mu$g/mL *Pg*-LPS or 10 nM 1,25-(OH)VD plus 10 $\mu$g/mL *Pg*-LPS or 10 $\mu$g/mL *Pg*-LPS alone for 48 hours. The *Pg*-LPS used was purchased† and according to the product information, acted as a ligand of both TLR2 and 4. The mRNA expression of CYP27B1, VDR, and hCAP-18/LL-37 were analyzed using real-time PCR. IL-6, IL-8 and monocyte chemotactic protein (MCP)-1 mRNA expression levels were also analyzed using real-time PCR. The sequences of the primers were as follows: *VDR* forward primer 5′-GGTGGAGGGAGCCATCCTT-3′; *VDR* reverse primer 5′-TGGGACAGCTCTAGGGTCACA-3; *IL-6* forward primer 5′-GTGAGGAACAAGCCAGAGC-3′; *IL-6* reverse primer 5′-TACATTTGCCGAAGAGCC-3′; *IL-8* forward primer 5′-TTTTGCCAAGGAGTGCTAAAGA-3′; *IL-8* reverse primer 5′-AACCCTCTGCACCCAGTTTTC-3′; *MCP-1* forward primer 5′-CAGCCAGATGCAATCAATGCC-3′; *MCP-1* reverse primer 5′-TGGAATCCTGAACCCACTTCT-3′.

CYP27B1 RNA interference and VDR antagonism were also conducted, after which 25-(OH)VD and *Pg*-LPS were added to the culture medium for 48 hours. hCAP-18/LL-37 mRNA expression levels were analyzed using real-time PCR.

### 1.6 | Measurement of LL-37 in supernatants

LL-37 levels were measured using an enzyme-linked immunosorbent assay kit.‡ The LL-37 concentrations in the supernatants of each group mentioned previously were calculated according to the manufacturer's instructions.

### 1.7 | Statistical analysis

The normality of the data was determined by Shapiro-Wilk test. Paired sample t-tests and one-way analysis of variance were used to determine the difference in hCAP-18/LL-37 expression between groups. The Wilcoxon test and Kruskal-Wallis test were adopted instead if the data were not normally distributed. Statistical analysis was performed using a statistical software package.§

## 2 | RESULTS

### 2.1 | 1,25-(OH)VD and 25-(OH)VD induction of hCAP-18/LL-37

mRNAs of hCAP-18/LL-37, VDR, and the 1,$\alpha$-hydroxylase CYP27B1 were detected in all the hGF and hPDLC samples. The relative expression level of hCAP-18/LL-37 was found to be upregulated significantly after 48 hours exposure to 10 nM 1,25-(OH)VD or 1,000 nM 25-(OH)VD, reaching levels approximately three to four times that of the negative control (Figure 1A) at the mRNA level and twice that of the negative control at the protein level (Figure 1B). The hCAP-18/LL-37 protein level of hGFs reached 96.1 ± 17.8 pg/mL in the 1,25-(OH)VD group and 91.3 ± 35.6 pg/mL in the 25-(OH)VD group, while in hPDLCs the concentrations were 62.5 ± 23.3 pg/mL and 70.5 ± 31.9 pg/mL, respectively.

### 2.2 | Effect of CYP27B1 RNA interference on hCAP-18/LL-37

After confirming the induction of hCAP-18/LL-37 expression by vitamin D, the intermediate steps were investigated by depressing CYP27B1 and VDR and observing whether the downstream product hCAP-18/LL-37 was also depressed. After effectively interfering with the expression of CYP27B1, the relative expression level of hCAP-18/LL-37 decreased 71% in hGFs and 73% in hPDLCs (Figures 2A and 2B). At the protein level, the concentration of hCAP-18/LL-37 in the

* Bayer Pharma AG, Berlin, Germany

† Invivogen, San Diego, CA.

‡ Qiyi Bio Tech, Shanghai, China.

§ IBM Corp., Armonk, NY.

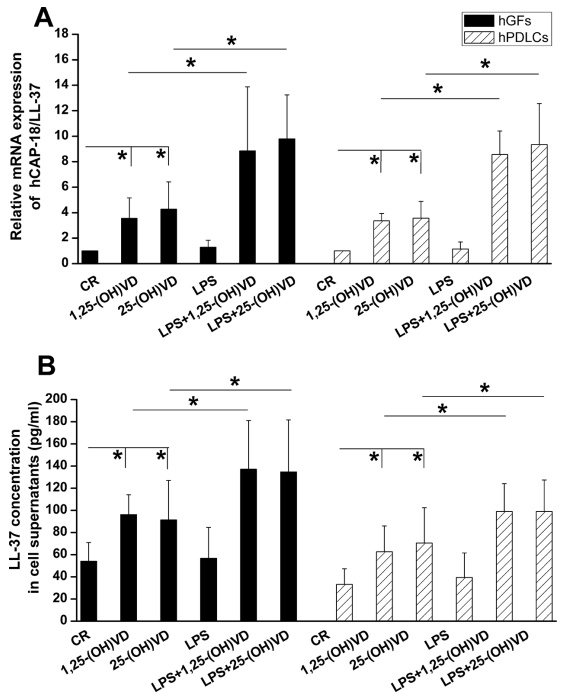


**FIGURE 1** Induction of hCAP-18/LL-37 expression and hCAP-18/LL-37 secretion by 1,25-(OH)VD and 25-(OH)VD treatment alone or in combination with *Pg*-LPS **A)** Relative expression level of hCAP-18/LL-37 in hGFs and hPDLCs. Both cell types were treated with ethanol; 1,25-(OH)VD; 25-(OH)VD; *Pg*-LPS; *Pg*-LPS plus 1,25-(OH)VD; or *Pg*-LPS & 25-(OH)VD. **B)** hCAP-18/LL-37 concentrations in the supernatants of the six groups of hGFs and hPDLCs. Because cells were from five different donors, data are presented as mean + SE. * Statistically significant difference between certain groups under the same line

RNAi group remained below 40 pg/mL in both hGFs and hPDLCs (Figures 2C and 2D).

## 2.3 | Effect of VDR antagonism on hCAP-18/LL-37

Antagonism of VDR also resulted in a significant reduction of mRNA expression of hCAP-18/LL-37, at both the mRNA and protein level. The relative expression of hCAP-18/LL-37 mRNA decreased 77% in hGFs and 73% in hPDLCs (Figures 2E and 2F). Meanwhile the hCAP-18/LL-37 protein level in the VAZ group reached between 30 and 40 pg/mL (Figures 2G and 2H).

## 2.4 | Effect of *Pg*-LPS on the vitamin D pathway

Compared with stimulation with 25-(OH)VD/1,25-(OH)VD only, stimulation with 25-(OH)VD/1,25-(OH)VD plus *Pg*-LPS resulted in a significant increase in hCAP-18/LL-37 expression (Figures 1A and 1B) in both hGFs and hPDLCs. Either CYP27B1 RNA interference or VDR antagonism significantly reduced this elevation (Figure 3)

Specifically, stimulation with 25-(OH)VD/1,25-(OH)VD plus *Pg*-LPS led to a dramatic elevation in hCAP-18/LL-37 expression by as much as 8- to 10-fold that in the control group, and both were significantly higher than in the groups stimulated with 25-(OH)VD/1,25-(OH)VD alone. Concentrations of hCAP-18/LL-37 in the co-stimulation groups also reached an average of 137 pg/mL in hGFs and 98 pg/mL in hPDLCs. hCAP-18/LL-37 expression in the CYP27B1

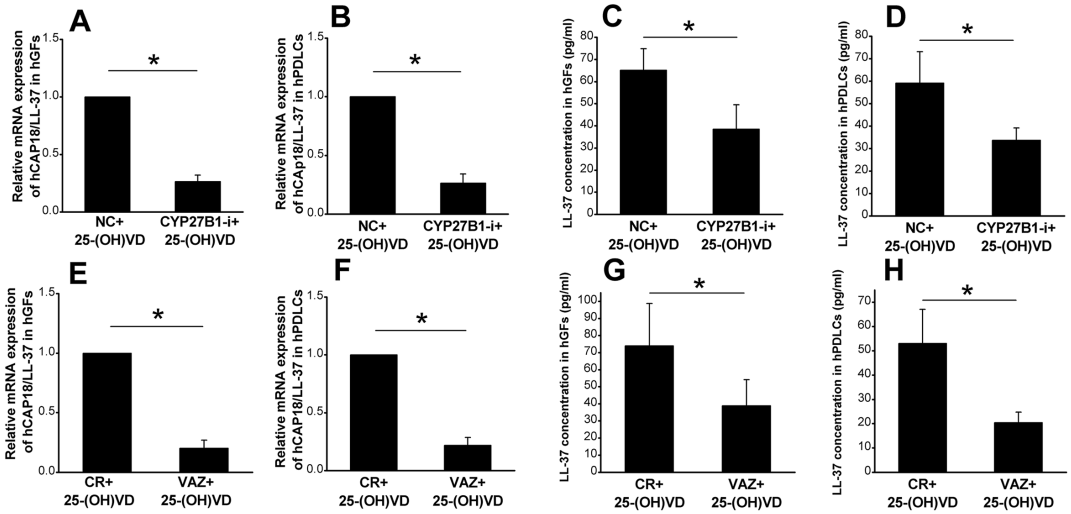


**FIGURE 2** Effective depression of hCAP-18/LL-37 expression levels and hCAP-18/LL-37 concentrations by CYP27B1 mRNA interference or VDR antagonism before 25-(OH)VD treatment. **A** and **B)** Relative expression levels of hCAP-18/LL-37 in hGFs and hPDLCs treated with CYP27B1 interfering RNA before 1,000 nM 25-(OH)VD stimulation. **C** and **D)** Relative expression levels of hCAP-18/LL-37 in hGFs and hPDLCs treated with the VDR antagonist ZK159222 (VAZ) before 1,000 nM 25-(OH)VD stimulation. **E** and **F)** hCAP-18/LL-37 concentrations in the cell supernatants of hGFs and hPDLCs in the CYP27B1 mRNA interference group. **G** and **H)** hCAP-18/LL-37 concentrations in the cell supernatant of hGFs and hPDLCs in the VDR antagonism group. * Statistically significant difference between group and the control group

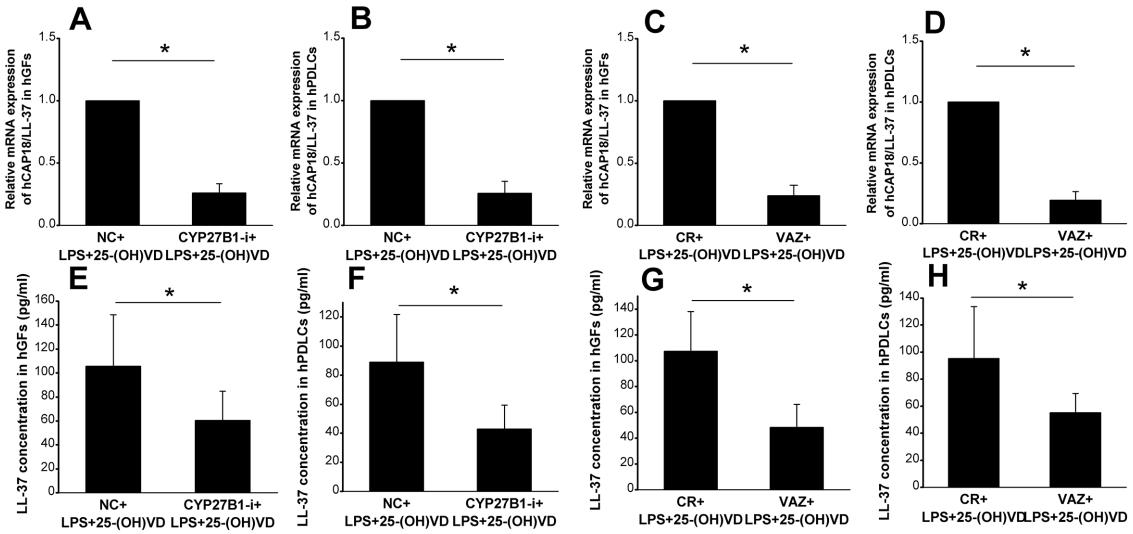


**FIGURE 3** hCAP-18/LL-37 expression levels and hCAP-18/LL-37 concentrations were effectively depressed by CYP27B1 mRNA interference or VDR antagonism before *Pg*-LPS plus 25-(OH)VD treatment. **A** and **B)** Relative expression levels of hCAP-18/LL-37 in hGFs and hPDLCs treated with CYP27B1 interfering mRNA before 10 $\mu$g/mL *Pg*-LPS plus 1,000 nM 25-(OH)VD stimulation. **C** and **D)** Relative expression levels of hCAP-18/LL-37 in hGFs and hPDLCs treated with the VDR antagonist ZK159222 (VAZ) before 10 $\mu$g/mL *Pg*-LPS & 1,000 nM 25-(OH)VD stimulation. **E** and **F)** hCAP-18/LL-37 concentrations in cell supernatants of hGFs and hPDLCs in the CYP27B1 mRNA interference group. **G** and **H)** hCAP18/LL-37 concentrations in the cell supernatants of hGFs and hPDLCs in the VDR antagonism group. * Statistically significant difference between group and the control group

interference group was reduced by 74.9% in hGFs and 78.4% in hPDLCs, while the reductions in the VDR antagonism group were 73% in hGFs and 83.3% in hPDLCs.

The mRNA expression levels of CYP27B1 in hGFs (Figure 4A) and hPDLCs (Figure 4B) stimulated with 25-(OH)VD plus *Pg*-LPS were significantly higher than those in cells stimulated with 25-(OH)VD alone, as were the mRNA expression levels of VDR (Figures 4C and 4D). In addition, significant differences in the VDR mRNA expression levels were also found between the 1,25-(OH)VD plus *Pg*-LPS group and the 1,25-(OH)VD group (Figures 4C and 4D).

The mRNA expression levels of the chemotactic cytokines IL-8 and MCP-1 in the 25-(OH)VD plus *Pg*-LPS group and the 1,25-(OH)VD plus *Pg*-LPS group were both significantly lower than those in the *Pg*-LPS group (Figure 5).

## 3 | DISCUSSION

In the present study, 25-(OH)VD-mediated induction of hCAP-18/LL-37 was detected after TLR2/4 activation by *Pg*-LPS and the existence of the vitamin D pathway in hGFs and hPDLCs was demonstrated (Figure 6). Tang previously identified that 1,25-(OH)VD was able to suppress IL-8 expression in hPDLCs stimulated with *Pg*.[26] In the present study, similar phenomena were observed and our findings suggested that activation of the vitamin D pathway could be the underlying mechanism. Interfering with CYP27B1 expression and VDR function both showed more than 70% reduction in relative expression of hCAP-18/LL-37, which indicated that CYP27B1 and VDR are both key factors in the pathway. However, it should be pointed out that 25-(OH)VD alone, without any other activator, could also induce hCAP-18/LL-37 in hGFs and hPDLCs, but not in monocytes or keratinocytes.[8,9] This was in line with a previous study in human gingival epithelial cells,[24] in which stimulation of primary cultures of human gingival epithelial cells with 25-(OH)VD alone resulted in significantly higher hCAP-18/LL-37 expression. Thus, the vitamin D pathway in periodontal soft tissues might share more similarities.

To the current authors' knowledge, this is the first report of the vitamin D pathway in connective tissue–derived cells. In three previous studies,[18,19,21] little or no staining of hCAP-18/LL-37 was detected in periodontal connective tissues using immunohistochemistry. Additionally, in the study[21] by Turkoglu et al., hCAP-18/LL-37 mRNA could be detected using real-time PCR only in fewer than half of the gingival tissue samples containing epithelia and connective tissues. According to our data, the expression of hCAP-18/LL-37 by hGFs and hPDLCs was relatively low when no vitamin D was present, while much higher expression in both cell types was detected upon stimulation with 25-(OH)VD or 1,25-(OH)VD, and the expression level was further enhanced when *Pg*-LPS and 25-(OH)VD/1,25-(OH)VD were added simultaneously. Thus, lack of vitamin D stimulation might be the reason for the absence of detection of hCAP-18/LL-37 in previous studies.

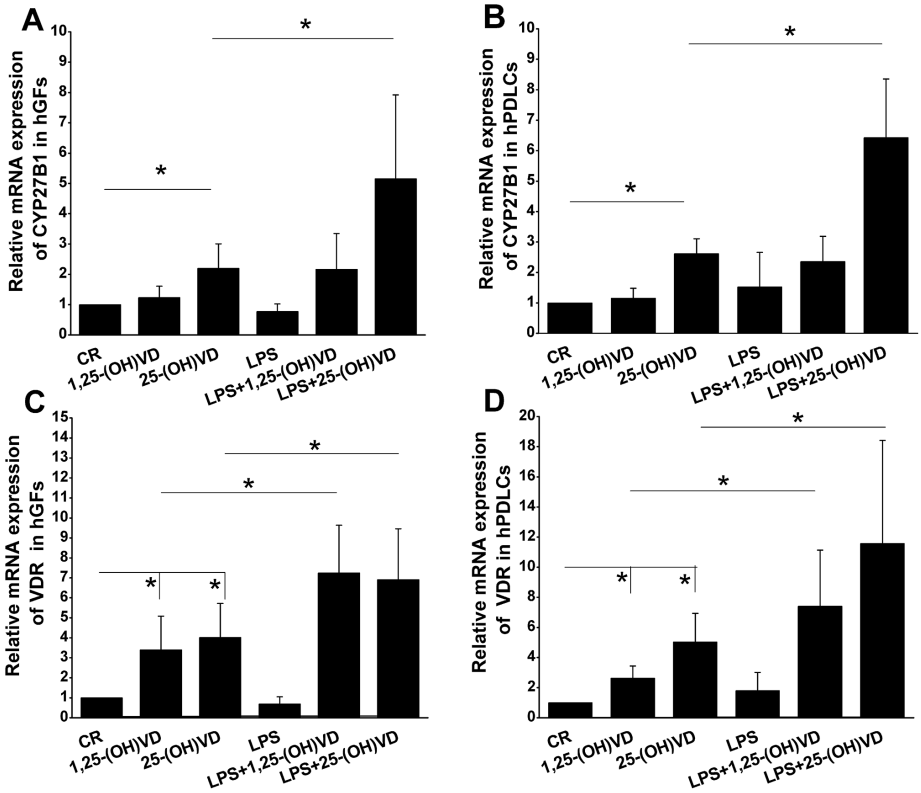


**FIGURE 4** Induction of CYP27B1 and VDR by 1,25-(OH)VD, 25-(OH)VD treatment alone or combined with *Pg*-LPS. **A** and **B)** Relative expression levels of CYP27B1 in hGFs and hPDLCs in the following six groups: ethanol; 1,25-(OH)VD; 25-(OH)VD; *Pg*-LPS; *Pg*-LPS plus 1,25-(OH)VD; and *Pg*-LPS plus 25-(OH)VD. **C** and **D)** Relative expression levels of VDR in hGFs and hPDLCs in the six groups as stated above. * Statistically significant difference between certain groups under the same line

In the study by Tada et al.,[25] a human gingival epithelial cell line (Ca9-22) exhibited increased expression of hCAP-18/LL-37 upon stimulation with either 100 nM 1,25-(OH)VD analog or 100 nM 1,25-(OH)VD. The enhancement was as much as 75-fold compared with that of the negative control in 24 hours and even higher with longer incubation time. In the present study, upregulation of hCAP-18/LL-37 after 25-(OH)VD or 1,25-(OH)VD was only about 3- to 4-fold in hGFs and hPDLCs. In addition, relative expression levels of hCAP-18/LL-37 in hPDLCs were much lower than those in hGFs in the present study, in both the control and the experimental group. Considering the location of the three types of cells *in vivo*, this phenomenon is reasonable and understandable. Gingival epithelial cells were the first line of defense and played the most important role in the immune defense of periodontal soft tissue in the three types of cells. With periodontal inflammation exacerbating from the surface to deeper within periodontal soft tissues, the anti-infective abilities of subepithelial connective tissue cells were activated and functioned through the vitamin D pathway. Thus, the most active vitamin D pathway was detected in gingival epithelial cells. hGFs were nearer to the front line of immune defense of periodontal soft tissue than hPDLCs and had higher generation of hCAP-18/LL-37 than periodontal ligament cells, indicating that hGFs might be of greater importance than hPDLCs in periodontal immune defense. Thus, it could be speculated that the more important the role a cell played in immune defense, the more active the vitamin D pathway was present in the cell, and the more hCAP-18/LL-37 was generated, indicating the role of the vitamin D pathway in immune defense. Other evidence also supported this hypothesis in two studies.[30,31] If either the human keratinocyte cell line (Hacat) or the human monocyte cell line (U937) were treated with 25-(OH)VD for 5 days, the generation of LL-37 by U937 (20 to 60 ng/mg) was about 10 times higher than that by Hacat (3 to 5 ng/mg).[30] In addition, it was reported that the LL-37 concentration was approximately 400 pg/mL in the supernatant of epidermal keratinocytes,[31] which was approximately 5 to 10 times higher than the concentrations of LL-37 in cell supernatants of hGFs and hPDLCs in the present study.

Although the vitamin D pathway in hGFs and hPDLCs was relatively less active than in epithelial or immune cells, the biologic significance of the vitamin D pathway in hGFs and hPDLCs is still worth studying for the following reason. It is known that IL-8 acts as an important chemokine for neutrophils, while MCP-1 is chemotactic to monocytes.[32,33] The influence of the vitamin D pathway in hGFs and hPDLCs on IL-8 and MCP-1 mRNA expression implied the

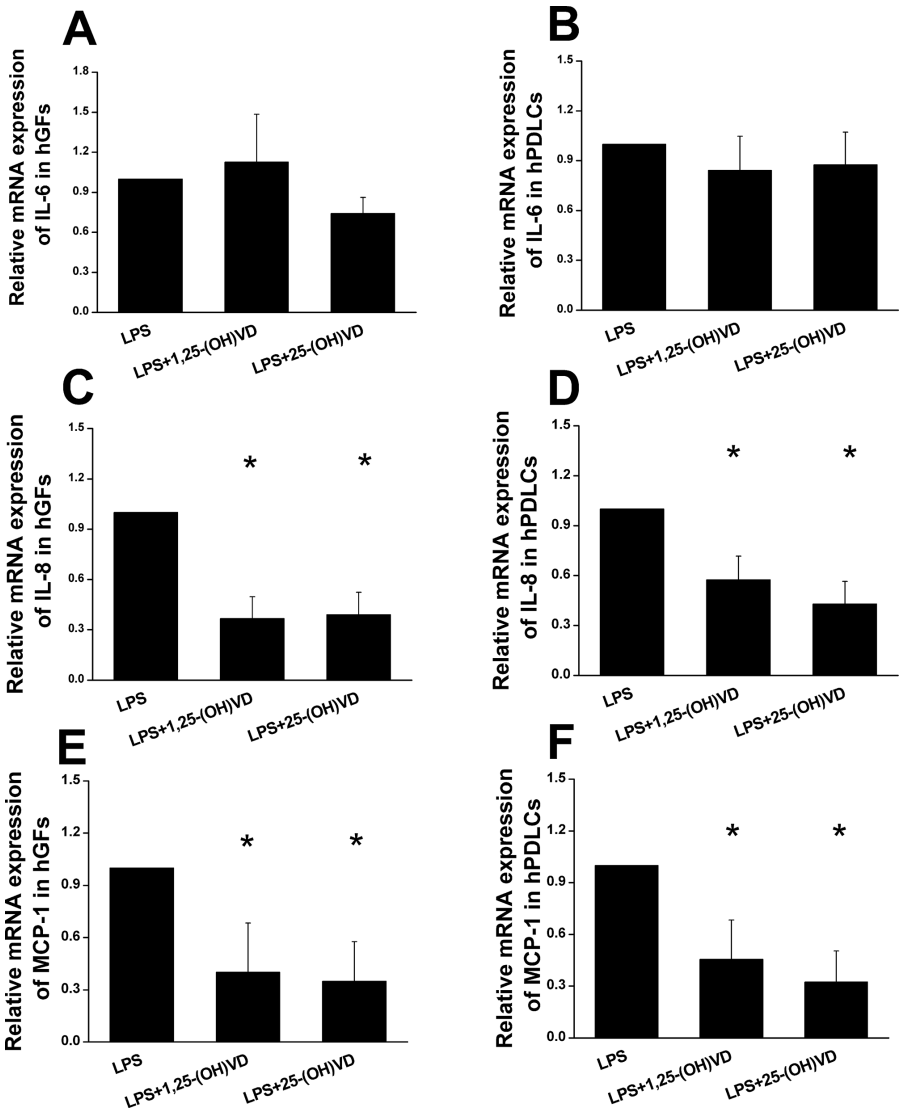


**FIGURE 5** Modulation of IL-6, IL-8, and MCP-1 by *Pg*-LPS plus 25-(OH)VD/1,25-(OH)VD. **A** and **D)** Relative expression levels of IL-6 in hGFs and hPDLCs treated with *Pg*-LPS, *Pg*-LPS plus 1,25-(OH)VD, and *Pg*-LPS plus 25-(OH)VD. **B** and **E)** Relative expression levels of IL-8 in hGFs and hPDLCs in the three groups. **C** and **F)** Relative expression levels of MCP-1 in hGFs and hPDLCs in the three groups. * Statistically significant difference between group and the control group

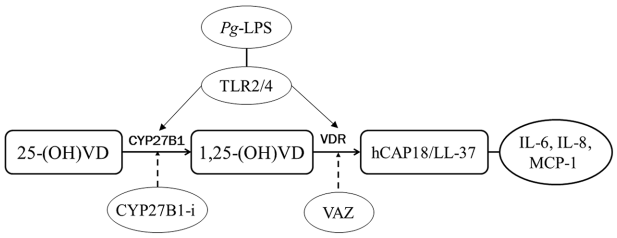


**FIGURE 6** Schematic model for vitamin D pathway in hGFs and hPDLCs

regulatory role played by hGFs and hPDLCs in the inflammatory infiltration of cells with a more active vitamin D pathway. Therefore, the vitamin D pathway could be responsible for the involvement of periodontal connective tissue cells in periodontal immune defense. Considering infiltrating neutrophils were an important source of hCAP-18/LL-37 in periodontal tissues, especially in periodontitis,[21] further investigation of the relationships among vitamin D, periodontal inflammation, and hCAP-18/LL-37 in neutrophils is needed.

In the present study, cells were all from primary cultures of different donors. Although all data were obtained from cells of five donors, the existence of individual differences among the cells from primary culture was the limitation of this study.

## 4 | CONCLUSIONS

hCAP-18/LL-37 expression could be induced by vitamin D and *Pg*-LPS, indicating that the vitamin D pathway is likely

to exist in both hGFs and hPDLCs and might play a role in the immune defense of periodontal soft tissues. However, only a part of the vitamin D pathway has been investigated in the present study, and more studies will be needed to understand the whole pathway and its biologic function.

## ACKNOWLEDGMENTS

This research was funded by National Natural Science Foundations of China (Beijing, 81100749) and GengYun Grant (Beijing). VDR ligand ZK159222 was a generous gift from Dr. Ekkehard May and colleagues in Bayer Pharma AG, Berlin, Germany. The two authors Zhanyi Gao and Kaining Liu contributed equally to this work and were co–first authors. The authors report no conflicts of interest related to this study.

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**How to cite this article:** Gao Z, Liu K, Meng H. Preliminary investigation of the vitamin D pathway in periodontal connective tissue cells. *J Periodontol*. 2018;89:294–302. https://doi.org/10.1002/JPER.17-0530