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Monitoring and Analysis of Matrix Metalloproteinases in Saliva of Chronic Periodontitis Patients Based on Internet of Things Technology

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Abstract: Periodontitis not only leads to premature tooth loss in adults, but also induces various systemic diseases, which seriously affects the physical and mental health of patients. The purpose of this study was to understand the glycosylation level of EMMPRIN and the expression of GnT-V and MMP-2, 9 in healthy and inflamed periodontal tissues based on the Internet of Things. In vitro experiments, this paper recognizes the glycosylation level of EMMPRIN in cells cultured in vitro. In this study, 10 clinically healthy gingival tissues and 12 patients with severe periodontitis were collected, and the subjects' age. HGFs and HIOECs were cultured in vitro, the expression of EMMPRIN in primary HGFs and HIOECs was detected by Western Blot, and HIOECs were stimulated with different concentrations of Pg.LPS for different time periods. The expression of MMP-2, 9 was significantly increased in gingival connective tissue. In vitro experiments, Western Blot found that the glycosylation level of EMMPRIN increased in a concentration-dependent manner after HIOECs were stimulated by Pg. LPS ($P<0.01$). When 10μg/ml of Pg.LPS was stimulated for 12h, the glycosylation level of EMMPRIN was the highest.

1. Introduction

Due to the high prevalence and high incidence of chronic oral diseases, it has become one of the public health problems of concern to countries around the world. Among the chronic oral diseases, chronic periodontitis is the most common. Chronic periodontitis is a periodontal disease caused by a combination of environmental, genetic, bacterial and other factors. The pathogenesis of chronic periodontitis is mainly the destruction of periodontal support tissue, and its clinical manifestations are relatively slow. The pathogenesis of chronic periodontitis is still unclear. However, various

studies have shown that it is closely related to oral bacteria, living habits, oral hygiene, genetics, host differences, systemic diseases such as diabetes, heart disease, hypertension and other factors. Therefore, exploring the mechanism of the occurrence and development of periodontal disease and its response process with the host has been paid attention to.

A very important feature of EMMPRIN is the ability of high glycosylation, which can obtain biological activity and activate downstream signaling pathways through the modification of post-transcriptional N-terminal glycosylation. EMMPRIN is involved in the progression of various diseases and tumor metastasis through glycosylation. It is closely related to the development and prognosis of the disease. This article reviews the research progress of EMMPRIN glycosylation in diseases. This article introduces the structural characteristics of EMMPRIN and the characteristics of N-terminal glycosylation of EMMPRIN. The role of EMMPRIN glycosylation and its mechanism of action in diseases were explored to explore its status and potential clinical significance in the occurrence and development of periodontitis.

The innovations of this paper are: (1) It is found that there are changes in the glycosylation of EMMPRIN and the expression level of GnT-V in periodontal tissue. The expression of the two is increased in periodontitis and co-localized in the gingival epithelium. In this paper, the relationship between the two and their effects on MMPs were confirmed from the cellular and histological levels. (2) In this paper, a direct co-culture model of HIOEC/HGFs was successfully constructed to simulate the periodontal tissue microenvironment in vivo. In this paper, the relationship between EMMPRIN glycosylation and MMPs was studied through the interaction between cells, and more objective and credible results were obtained.

2. Related Work

F Vohra studied the effects of clinical periodontal and immunological parameters in patients with chronic periodontitis[1]. Moudi B analyzed 210 patients with chronic periodontitis (CP) using the polymerase chain reaction-restriction fragment length polymorphism method [2]. Moeintaghavi A found that the IL-1βgene expression in smokers with chronic periodontitis was lower than that in non-smokers with chronic periodontitis[3]. The Alamelu S found differences in salivary and serum TNF-α levels between healthy and periodontitis subjects. The mean serum TNF-α concentration of healthy subjects in group I was 23.12 pg/mL, and the mean serum TNF-α concentration of periodontitis patients in group II was 24.06 pg/mL [4]. Despite its important role in controlling periodontal disease, mechanical plaque removal is not properly implemented by most people. The goal of YAMAWAKI was to study the potential of membranes containing yolk antibody (IgY) in the control of periodontal bacteria, and the membrane "periguard IV" containing yolk antibody was evaluated [5]. Examination of blood samples and gingival crevicular fluid from patients with chronic periodontitis by Radvar M showed that smoking increases cytokine production [6]. Dong-Lin discussed the clinical effect of continuous oral health education on dual-wavelength laser treatment of chronic periodontitis in the elderly. In the experiment, the proportion of patients who brushed their teeth correctly, had regular oral inspection and maintenance, and maintained good oral habits was 97.33%, 93.33% and 92.00%, respectively, significantly higher than the control group [7]. The Linhartova P B showed a bidirectional relationship between chronic periodontitis (CP) and diabetes (DM) [8].

3. Monitoring Method of Matrix Metalloproteinases by Iot Technology

3.1. Matrix Metalloproteinases

Matrix metalloproteinases (MMPs) are the most direct and critical proteases that cause

extracellular matrix (ECM) degradation. It plays an important role in the tissue destruction process of periodontitis [9]. During periodontitis, multiple host immune inflammatory response pathways can cause excessive secretion of MMPs. It leads to an imbalance between the synthesis and degradation of periodontal ECM, which affects the severity of periodontal tissue destruction. MMPs also play an important role in periodontitis, destroying collagen, gelatin, and various matrix components [10]. In periodontitis, MMPs can be secreted by periodontal host cells and inflammatory infiltrating cells. Studies have found that MMPs can be induced by multiple upstream regulatory factors, and the role of different upstream regulatory factors reflects the body's different responses to pathogenic stimuli [11]. Cytokines, EMMPRIN, CypA, etc. all affect the synthesis, secretion and effect of MMPs, and their respective self-regulation and interaction form a network regulation mechanism.

3.2. Regulatory Network and Self-regulatory Mechanism of the Host Response to Periodontitis

The pathways play their respective roles in the pathogenesis and progression of periodontitis, but none of these pathways work alone. There is mutual regulation between them, forming a synergistic network, and the clinical manifestations of periodontal tissue destruction are the final result of their joint action [12-13]. The differences in regulation among individuals form differences in host responsiveness, resulting in different degrees of periodontal tissue destruction among individuals, and host susceptibility is explained from host responsiveness. The network regulation of the host response to periodontitis is shown in Figure 1.

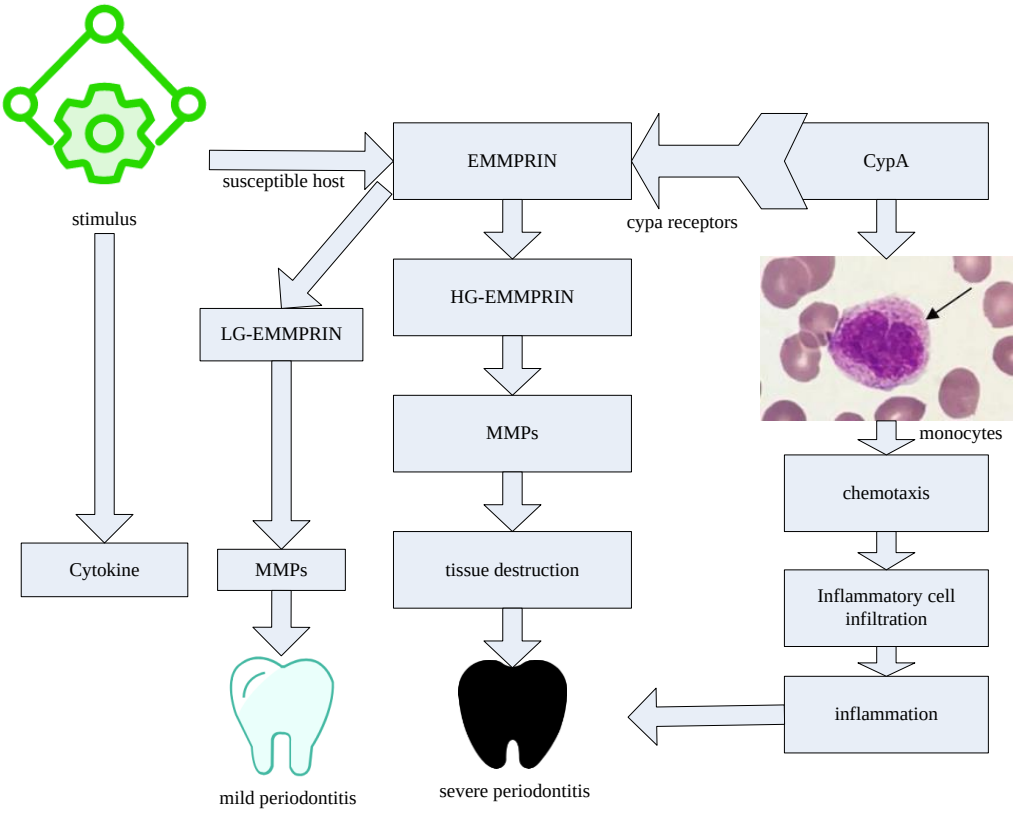

Figure 1. Network regulation of host response to periodontitis

As shown in Figure 1, host factors have multiple network-like regulatory effects on the severity

of periodontitis, affecting the progression of inflammation and the severity of periodontal tissue destruction [14]. The proposal of the regulation network reflects the idea of comprehensive understanding, which can explain the problems that cannot be explained by the previous partial and one-sided research results.

3.3. Type Conversion of MMPS, a Sign of Changes in the Body's Response

In the process of periodontitis, there is a conversion of MMPs types [15-16]. In the early stage of inflammation, MMP-8 is mainly found in the local reaction stage. It is mainly secreted by neutrophils, the number of involved tissue cells is small, and its effect is limited and short-lived. Inflammation continues to progress, and the production of MMP-1/13 indicates the progress of inflammation to the body's response [17]. MMP1/13 is mainly secreted by fibroblasts in tissue cells, but also expressed in endothelial cells and mononuclear macrophages. The switching of MMPs types is a sign of changes in the development of local responses to body responses, as shown in Figure 2.

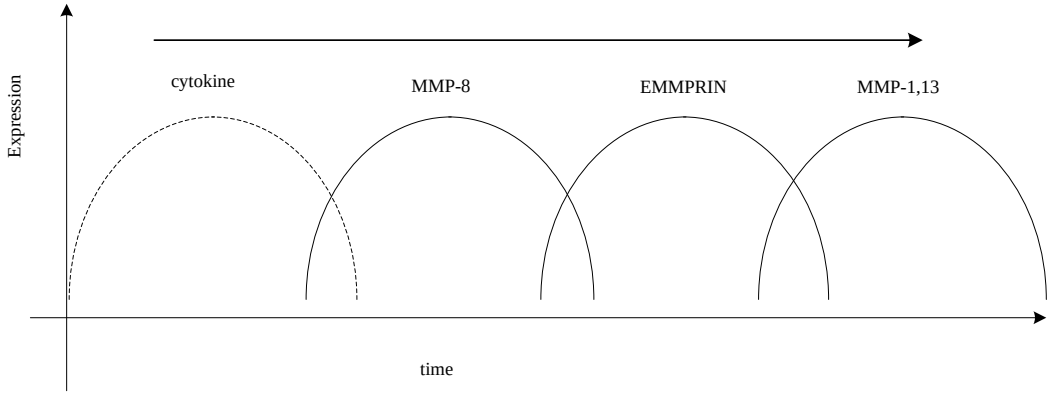

Figure 2. The relationship between the two paths and the type conversion of MMPs

As shown in Figure 2, periodontitis will also progress from mild to severe. However, clinically, when the body responds obviously and MMP-1 increases, some patients still do not develop severe periodontitis. It suggested that although the pathway of EMMPRIN-MMPs-periodontitis was closely related to the occurrence of severe periodontitis, there should be other factors involved that led to individual differences.

3.4. Algorithms Related to Iot Services

(1) Service probability topic clustering

Due to the widespread use and increase in the number of IoT services, a challenge in the related research work on the IoT service discovery process is the large-scale search space formed by the IoT. Cluster tagging of services is crucial to facilitate efficient service discovery [18]. Where Dirichlet(a) is shown by formula (1)(2), B(a) is the Beta function, and Γ(a) is the Gamma function.

$$Diriichlet(X \mid a) = \frac{1}{B(a)} \prod_{i=1}^{K} x_i^{a^{i-1}} \quad (1)$$

$$B(a)=\frac{\prod_{i=1}^{k}\Gamma(a^{i})}{\Gamma(\sum_{i=1}^{k}a^{i})} \tag{2}$$

The topic vectors are generated from the Dirichlet(a) distribution, where a is the prior parameter and θ_c is the topic probability given the concept c, which together determine the topic t . But the topics contain different feature words, and the distribution of all t can yield the probability of word w[19]. The Dirichlet (β) distribution with the prior parameter β generates the word distribution through the topic-domain association parameter φ, given a topic r with a conceptual distribution probability of φ_r. The topic can be obtained after the concept word distribution probability is given, then the conditional probability of each concept word in the service after obtaining Θ , φ, C_s passes through the variable s, and the calculation formula of t is (3):

$$P(w\mid\Theta,\phi,C)=p(\theta\mid a)=\frac{1}{C_s}\sum_{c\in C_s}\sum_{r=1}^{R}\varphi_{wsir}\theta_{tc} \tag{3}$$

Service vector dimensionality reduction is performed by calculating the probability of topics contained in each service by Gibbs sampling. According to formula (4), the service S_i is clustered into the topic cluster with the largest corresponding topic probability.

$$TC(S_i)=T_k\cap\forall j(j\neq k)\rightarrow P(S_i,T_j)<P(S_i,T_k) \tag{4}$$

(2) Similar matching of IoT services

The key step in the IoT service discovery process is to match the services required by users in the massive service library [20]. The matching here includes similarity matching between service topics and functional logic. First, matching services are initially screened according to the similarity of the service topic probability, and then the service logic functions are similar matching. The similarity between the request service topic and the service cluster topic is calculated as shown in (5), and the service subset similar to SR_Topic is obtained, where k is the number of topics.

$$Sim_{topic}(sr,ss)=\frac{\sum_{i=1}^{k}sr_t^{i}*ss_t^{i}}{\sqrt{\sum_{i=1}^{k}(sr_t^{i})^{2}*\sum_{i=1}^{k}(ss_t^{i})^{2}}} \tag{5}$$

In order to eliminate the situation that the semantic relationship between concept levels is not considered based on the distance ontology similarity, this paper proposes to use the information of the ontology concept to calculate the similarity between two concepts by formulas (6) and (7).

$$Csim(c_i,c_j)=\begin{cases}3*IC(coman(c_i,c_j))-IC(c_i)-IC(c_j), c_i\neq c_j\\ 1, otherwise\end{cases} \tag{6}$$

$$IC(c_i)=1-\frac{\log(wubwd(c_i)+1)}{\log(C)} \tag{7}$$

The matching of service function logic mainly includes the matching of service name, input and

output parameter description, and service semantic description uses ontology concept to mark service description elements to associate them with ontology concept [21]. Therefore, for the service set after subject similarity screening, the similarity between the service request SR and the candidate service CS based on the ontology concept is calculated by formula (8), and the service function logic matching is performed.

$$sim_{funclog}(sr,cs) = w_{s*n} * Csim(rs.anm, sc.name) + w_{s.op} * Csim(sr.op, cs.op)(w_{s.n} + w_{s.op} = 1), w_{s.n}, w_{s.op} \in [0,1] \tag{8}$$

Where $w_{s.n}$ and $w_{s.op}$ are the similarity weights of service names and service operations. The similarity $Csim(sr.op, cs.op)$ of service operands is calculated by formula (9), where $w_{s.in}$ and $w_{s.out}$ are the similarity weights of service operation input parameters and output results.

$$Csim(sr.op, cs.op) = w_{s.in} * \frac{1}{N}\sum_{i=1}^{N} Csim(s.op.in_i, cs.op.in_i) + w_{s.in} * \sum_{i=1}^{N} Csim(s.op.out, cs.op.out)(w_{s.n} + w_{s.op} = 1) \tag{9}$$

This step is mainly to further determine the similar service set that matches the requested service by calculating the functional logic of the service request and the functional logic similarity of each service in the candidate service set. Finally, synthesizing the topic similarity and functional logic similarity, the final similarity of service request and service is calculated by formula (10), where γ is the weight of the similarity of service topics with a value between 0 and 1.

$$Sim(sr,ss) = \gamma * Sim_{topic}(sr,ss) + (1-\gamma) * sim_{funclog}(sr,ss) \tag{10}$$

The algorithm of the distribution graph method is as follows:

$$S_1, S_2, \ldots S_K \tag{11}$$

Where S_1 is called the lower limit of the measurement and S_K is called the upper limit. The median value is shown in formulas (12) and (13):

$$S_A = S_{\frac{K+1}{2}} \qquad K = 2i+1 \tag{12}$$

$$S_A = \frac{S_{\frac{K+1}{2}} + S_{\frac{K}{2}}}{2} \qquad K = 2i \tag{13}$$

$$dZ = Z_\mu - Z_l \tag{14}$$

Then the judgment interval of valid data is [c1, c2], as shown in formulas (15) and (16):

$$C_1 = Z_l - \frac{\alpha}{2} dZ \tag{15}$$

$$C_2 = Z_\mu + \frac{\alpha}{2} dZ \tag{16}$$

The final fusion value of multi-sensor data should be shown in formula (17):

$$\hat{Y} = \sum_{k=1}^{n} W_k X_k \tag{17}$$

So the total mean square error is as shown in formula (18):

$$\sigma^2 = \sum_{k=1}^{n} W_k \sigma_k^2 \tag{18}$$

$$W_k = \frac{1}{\sigma_k^2 \sum_{k=1}^{n} \frac{1}{\sigma_k^2}} \tag{19}$$

At this time, σ^2 is the minimum value, and can be expressed as formula (20):

$$\sigma_{\min}^2 = \frac{1}{\sum_{k=1}^{n} \sigma_k^2} \tag{20}$$

4. Monitoring Experiments of MMPS

4.1. Experimental Method

(1) Human gum sample collection and ethics statement

A total of 22 subjects were included in this part of the experiment, and all the subjects were patients in the periodontal department of the Stomatological Hospital. The relevant operations of the specimens were in accordance with the requirements of the Declaration of Helsinki of the World Association of Physicians.

The age of 10 patients with chronic periodontitis in this experiment was 26-55 years old (mean age 40+9.626 years old, 5 females, 5 males). Inclusion criteria for this article were based on a consensus report from an international symposium in 1999, as follows: 1) Scope: involving $\leqslant$30% of sites; 2) Severity (based on clinical attachment loss level): clinical attachment loss $\geqslant$5 mm. The 12 patients with crown lengthening had no periodontitis on average, and their age ranged from 19 to 38 years (mean age, 28.08±6.052 years, 7 women, 5 men). Clinical parameters of 6 sites were recorded for each patient. The clinical indicators of all samples in this experiment are shown in Table 1.

Table 1. Clinical indicators of all samples in this experiment

	Healthy group	Chronicperiodontitis group
Number of patients	12	10
Age: years	28.08±6.052	40±9.626
Plaque index	1.76±0.305	3.20±0.535*
Gingival index	0.60±0.63	2.96±0.77*
Probing depths: mm	3.53±0.56	8.00±1.34*
Clinical attachment loss: mm	0.0	6.05±0.97*

The number, age, and periodontal clinical examination indicators of patients with periodontal health and periodontitis are recorded in Table 1. Statistical analysis was carried out in this paper.

The gingival tissues were used for immunohistochemical detection and immunofluorescence

detection. After the tissue was obtained, it was immediately fixed in 4% paraformaldehyde for 24 h, rinsed with running water for 24 h, dehydrated and embedded in paraffin.

(2) Establishment and material collection of rat periodontitis model ligated with silk thread

In this paper, the bilateral mandibular first molars of SD rats were selected for ligation. Ligation method: it uses 2 needle holders to clamp three 0 nylon silk threads, leaving a distance of 1 cm between the 2 needle holders to prevent the silk thread from being pressed between the first molar and the second molar. After crossing the interproximal contact point, the wire is carefully pressed into the gingival sulcus in the distal first molar, and the wire is knotted at the neck of the mesial first molar. Note: The silk thread should be all located in the gingival sulcus to be considered as successful modeling. The modeling is completed by the same person within one day. Seven days after silk ligation, the rats were sacrificed (under anesthesia) using cervical dislocation. The bilateral mandibles were separated, and the central incisors were cut. The unilateral mandibles were placed in a 4% paraformaldehyde solution for 48 hours and rinsed for 48 hours for subsequent experiments.

(3) Preparation and sectioning of paraffin specimens

1) In this paper, human gingival tissue samples are cut into small pieces about 1 cm3 in size. For rat samples, the mesial to distal third molars were collected from the first molars and placed in a marked plastic embedding box. Here, tissue dehydration was performed in graded alcohol in a fume hood according to the following alcohol concentrations and dehydration times:

2) After the tissue samples were dehydrated, they were placed in n-butanol for clarification overnight.

3) It was embedded in paraffin I (n-butanol:wax=1:1) for 2 hours and in paraffin I (pure wax) for 2 hours. Embedding at ease: The epithelial layer and the connective tissue are in the same plane, to ensure that the epithelium and the connective tissue are on one slice when sectioning. Wax blocks can be stored for a long time until use.

4) Slicing and baking: Before slicing, it freezes the cut surface of the wax block on the freezer for a few minutes, then fixes the wax block and slices it. Each sample was serially sectioned with a thickness of 4 um, and qualified sections were only obtained when the epithelium and connective tissue were on the same piece. The tissue section requirements of rat mandible are as follows: only when the crowns and roots of the first molar and the second molar of the rat are on one slice are qualified slices. Slices can be stored at 4 ℃ for a long time after being baked overnight at 60 ℃.

4.2. Cell Culture, Protein Extraction and Concentration Determination

(1) Primary gingival fibroblasts HGFs were collected from 10 patients with orthodontic tooth extraction who visited the Oral and Maxillofacial Surgery Clinic of the Stomatological Hospital. Their age is 16-24 years old, non-smoking, free from systemic diseases and periodontitis. All tissue collections were approved by the Ethics Committee of the Dental Hospital. And after the consent of each patient and their family members and signed informed consent, the relevant operations of the specimen are in line with the requirements of the World Medical Association Declaration of Helsinki. After passage, cultures were grown in a-MEM containing 10% FBS, and HGFs were used for experiments at passages 3-6.

(2) Cell cryopreservation: After cell digestion, HIOECs were cryopreserved with K-SFM:DMSO=9:1 cryopreservation solution, and the ratio of HGFs cryopreservation solution was a-MEM:FBS:DMSO=7:2:1. In this paper, the programmed cooling box was used in the -80 ℃ refrigerator for 48 hours, and then transferred to the liquid nitrogen tank.

4.3. Preparation of Protein Samples

To detect the changes of EMMPRIN glycosylation level in HIOECs, we seeded HIOECs in 6-well plates at a density of 8*105 per well. After 48 hours, Pg.LPS was added to the culture medium to prepare stimulation solutions of different concentrations to stimulate HIOEC cells for different time periods. The preparation of SDS-PAGE gel is shown in Table 2.

Table 2. SDS-PAGE gel preparation method

Element	8% separating gel	10% separating gel	5% stacking gel
ddH2O/ml	4.63	4	3
30% acrylamide/ml	2.67	3.3	0.75
1.5M Tris-HCl(pH6.8)/ml	2.5	2.5	0
1.0M Tris-HCl(pH6.8)/ml	0	0	0.75
10%SDS/ul	100	100	60
AP/ul	100	100	45
TEMED/μl	5	5	6
Total volume/ml	10	10	4.5

As shown in Table 2, the glass plate was washed, dried naturally, and fixed on a glue-making rack. The separation glue was first prepared, poured to an appropriate height, and then sealed with ddH2O. After about 30 min, the separating gel was completely polymerized, pour out the ddH2O, and dry it with clean filter paper. To prepare stacking gel, insert comb teeth immediately after pouring. It was left standing at room temperature for 2h or 4℃ overnight, and the comb teeth were pulled out after the concentrated gel was fully polymerized, and the sample was loaded. The preparation of electrophoresis buffer and transfer buffer is shown in Table 3.

Table 3. SDS-PAGE gel preparation method

Element	electrophoresis fluid	Transfer fluid
Tris-base/g	3.04	3
Glycine/g	18.8	14.4
SDS/g	1	0
Methanol/ml	0	200

As shown in Table 3, after transfer to the membrane, 5% nonfat milk powder prepared with TBST was used for blocking at room temperature on a shaker for 1 h. It uses the primary antibody diluent to prepare the corresponding concentration of primary antibody, soaks the PVDF membrane in the -anti-incubation solution,

4.4. Results Evaluation and Statistical Analysis

In this paper, GraphPad Prism 6.0 (GraphPad Software, San Diego, CA, USA) software was used for statistical analysis of the experimental data. All data were repeated 3 times.

5. Experimental Analysis of Periodontitis Patients and SD Rats

5.1. Gingival Tissue and SD Rats in Periodontally Healthy Patients

In the gingival tissue of periodontal healthy patients and the interproximal gingival tissue of mandibular first and second molars of SD rats, HE staining showed no obvious abnormality in the structure of epithelial spikes. The cells were arranged in an orderly manner, there was no inflammatory cell infiltration, and there were few vascular structures. Immunohistochemical staining (IHC) showed that EMMPRIN was mainly expressed in the cytoplasm of the basal cells of the gingival epithelium, with less expression in the connective tissue. Figure 3 shows the expression of glycosylated EMMPRIN in periodontal healthy patients and rats.

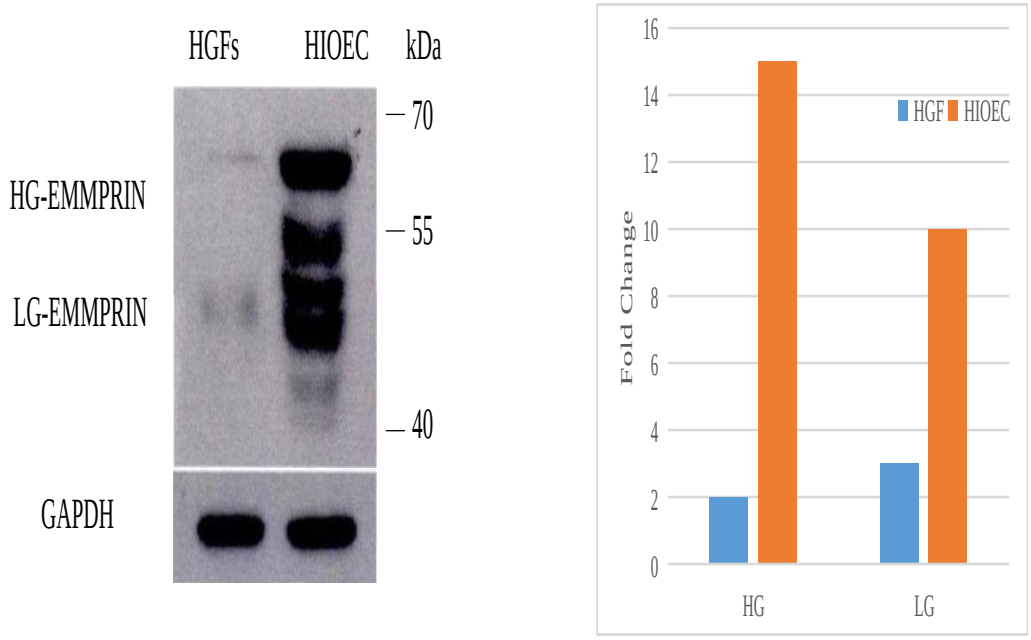

Figure 3. Expression of glycosylated EMMPRIN in periodontal healthy patients and rats

As shown in Figure 3, Western Blot was used to detect the expression of EMMPRIN in oral epithelial cells (HIOEC) and human gingival fibroblasts (HGFs). EMMPRIN was mainly expressed in HIOEC, and the expression level of EMMPRIN in HGFs was significantly lower than that in HIOEC. The expression levels of both hyperglycosylated and hypoglycosylated EMMPRIN in HIOEC were significantly higher than HGFs by more than 20 times ($P<0.001$).

5.2. Experimental Analysis of the Gums of Patients with Periodontitis and SD Rats

The epithelial nail process is elongated, the cell arrangement is disordered, a large number of inflammatory cells are infiltrated, and the number of blood vessels is increased, which can be seen by IHC. EMMPRIN was expressed in the full thickness of epithelium and connective tissue, and EMMPRIN was expressed in the cytoplasm and cell membrane. It shows that the degree of glycosylation of EMMPRIN is higher. In patients and rats with periodontitis, the expression of glycosylated EMMPRIN is shown in Figure 4.

As shown in Figure 4, among them, Figure A1 and Figure A2 are the HE staining of gingival tissue of patients with periodontitis. Figures A3 and A4 are representative immunohistochemical (IHC) staining pictures of EMMPRIN glycosylation expression in gingival tissue of periodontitis

patients. Figures B1 and B2 are HE staining of gingival tissue of SD rats with periodontitis. Figures B3 and B4 are representative immunohistochemical (IHC) staining pictures of EMMPRIN glycosylation expression in gingival tissue of periodontitis rats. In the interproximal gingival tissue of the mandibular first molars and second molars of the experimental periodontitis rats ligated with silk thread, HE staining was seen, and the epithelial layer was obviously destroyed. In the connective tissue, the cell arrangement is disordered, the inflammatory cells infiltrate a lot, the number of blood vessels increases significantly, and the diameter increases.

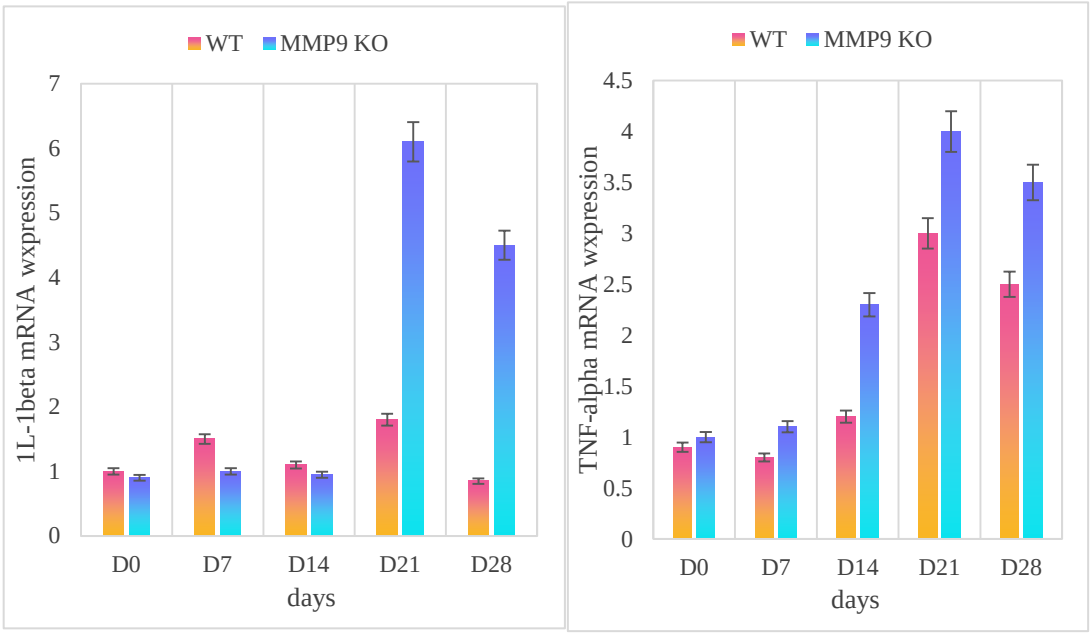

Figure 4. Expression of glycosylated EMMPRIN in patients and rats with periodontitis

5.3. EMMPRIN Glycosylation, Expression of GnT-V and MMP-2,9

Through double immunofluorescence labeling with EMMPRIN and GnT-V, we found that both EMMPRIN and GnT-V were expressed in the cells of gingival epithelial tissue in the gingival tissue of periodontal healthy and periodontitis patients. GnT-V co-localized with EMMPRIN in basal epithelial cells. Immunofluorescence staining of N-acetylglucosamine transferase (GnT)-V and EMMPRIN was performed on gingival tissues of healthy control group and periodontitis group. EMMPRIN co-localized with GnT-V in gingival epithelial cells as shown in Figure 5.

As shown in Figure 5, to evaluate EMMPRIN glycosylation and MMP-2, 9 expression in gingival tissue, we used IHC to compare the expression levels and locations of the indicators in the gingival tissue of periodontal health and periodontitis patients. EMMPRIN has different glycosylation forms, different levels of glycosylation, and different biological functions. In this part of the experiment, we observed that the expression of EMMPRIN was significantly increased in the gingival epithelial tissue of periodontitis patients compared with the gingival tissue of periodontally healthy individuals. It extends from the basal layer to the full thickness of the epithelium. The comparison of the expression levels of MMP-2 and MMP-8 is shown in Figure 6 .

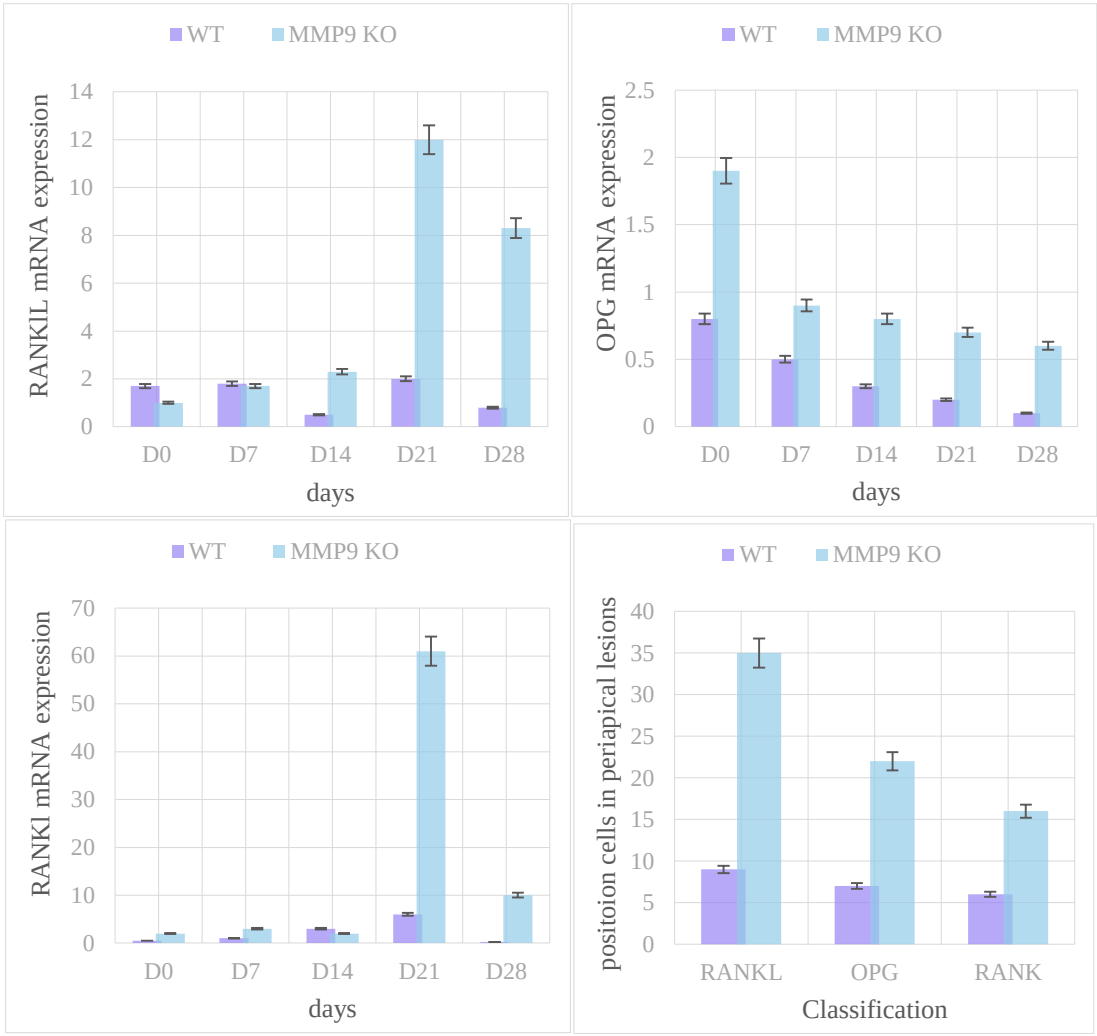

Figure 5. EMMPRIN co-localizes with GnT-V in gingival epithelial cells

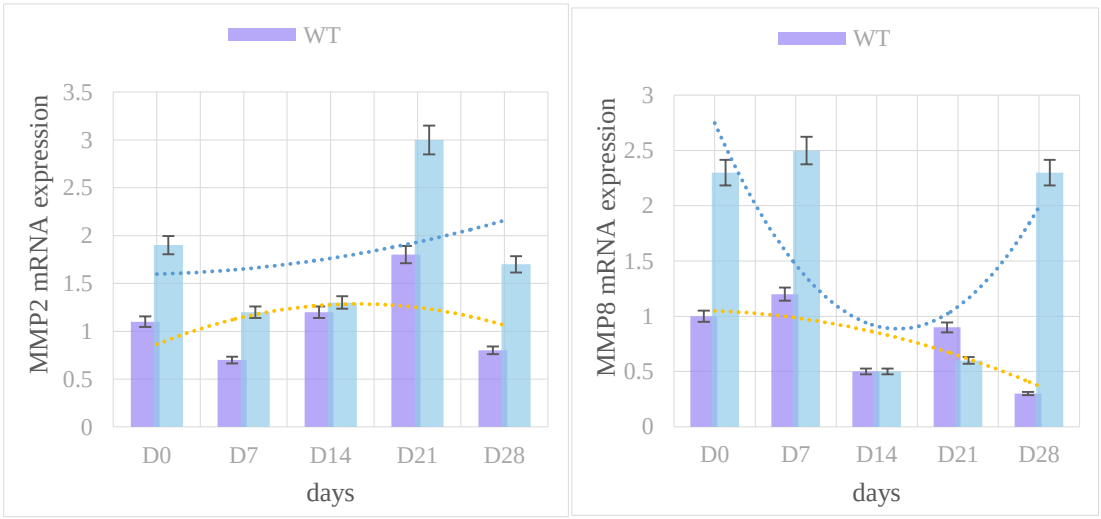

Figure 6. Comparison of expression levels of MMP-2 and MMP-8

As shown in Figure 6, there were more MMP-2 positive cells in the MMP-9 knockout mice, which were mainly distributed in the concentrated area of inflammatory cells. The expression of 9

was significantly enhanced, and MMP-2,8 expression was also seen in inflammatory cells and tissue cells.

5.4. Changes of EMMPRIN Glycosylation Level in HIOECs Stimulated by Pg.LPS

We used Pg.LPS to stimulate HIOEC to mimic an inflammatory environment to observe its effect on EMMPRIN glycosylation. We stimulated HIOECs with different concentrations of Pg.LPS. From the Western Blot analysis, it was found that Pg.LPS stimulation can increase the level of EMMPRIN glycosylation in HIOEC as shown in Figure 7.

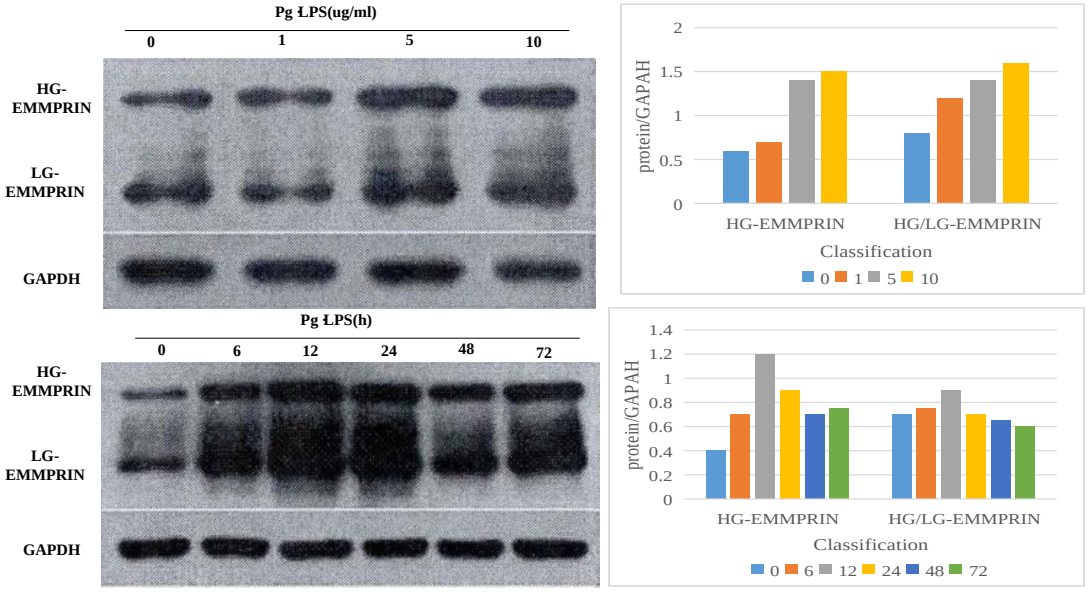

Figure 7. Pg. LPS stimulation can increase the level of EMMPRIN glycosylation in HIOEC

As shown in Figure 7, the changes in the glycosylation level of EMMPRIN increased in a concentration-dependent manner with Pg.LPS. The glycosylation level of EMMPRIN represented by the ratio of hyperglycosylated EMMPRIN to low glycosylated EMMPRIN was significantly increased under the stimulation of different concentrations of Pg.LPS. When the concentration of Pg.LPS was 1 μg/ml, the expression of hyperglycosylated EMMPRIN could be increased. Next, we stimulated HIOECs with 10 ug/ml of Pg.LPS and examined the level of EMMPRIN glycosylation at different time points. From Western Blot analysis, it was found that the level of EMMPRIN glycosylation was the highest at 12 h. The results showed that when 10μg/ml Pg.LPS stimulated HIOEC for 12h, the level of EMMPRIN glycosylation was the highest.

6. Conclusion

During periodontitis, massive inflammatory cell infiltration, collagen fiber degradation, and alveolar bone destruction can be seen in the periodontal tissue, eventually leading to tooth loss. During the pathogenesis of periodontitis, periodontal pathogenic bacteria and their virulence factors can directly act on the periodontal tissue through their own metabolites to cause tissue damage. It can also induce an immune inflammatory response in the host. Among many host factors, matrix metalloproteinases (MMPs) are the most direct and important proteases for host destruction. It is positively correlated with the destruction degree of periodontitis tissue in the pathogenesis of periodontitis. When HIOEC/HGFs were co-cultured, the time of HIOEC adherence was shorter than that when cultured alone. In the HIOEC/HGFs co-culture model cultured in serum-free KGM

medium, HIOEC and HGFs cells did not age significantly.

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Data Availability

Data sharing is not applicable to this article as no new data were created or analysed in this study.

Conflict of Interest

The author states that this article has no conflict of interest.

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