Porphyromonas gingivalis protease Rgp induces M1-type polarization and pyroptosis in BV-2 cells by inhibiting SIRT1 expression

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Abstract

Background

Periodontitis and Alzheimer’s disease (AD) are age-related diseases that reciprocally act as risk factors. It has been reported that periodontal pathogen *Porphyromonas gingivalis* and its gingipains contribute to neuroinflammation mediated by microglial cells, playing a crucial role in the onset of AD. However, it remains unclear whether gingipains play a pro-inflammatory role by inducing senescent phenotypic changes in microglial cells.

Methods

BV-2 cells were cultured and stimulated with gingival protease (Rgp), in combination with or not SRT1720, an inhibitor of SIRT1. SA-βgal staining was used to observe the altered cellular senescent phenotype. Immunoprotein blotting and real-time fluorescence quantitative polymerase chain reaction (RT-qPCR) were used to confirm the results of SIRT1, NLRP3, Caspase-1 and GSDMD expression. ELISA and flow cytometry were used to detect IL-1β and IL-18 levels in supernatants and altered M1 polarization in BV-2 cells.

Results

Rgp induced BV-2 cells to present a senescent phenotype and downregulated the expression of senescence-related protein SIRT1. BV-2 cells with the senescent phenotype showed a concentration-dependent upregulation of NLRP3 upon Rgp stimulation, accompanied by a significant increase in the M1-type polarization phenotype. Simultaneously, the expression of pyroptosis-related proteins Caspase-1 and GSDMD increased, and flow cytometry analysis indicated an increase in pyroptosis in BV-2 cells. Further restoration verification using the SIRT1 activator SRT1720 showed that, compared to the Rgp stimulation group, the SRT1720 intervention group exhibited increased SIRT1 protein expression in BV-2 cells, decreased NLRP3 expression, and a significant reduction in M1-type polarization. Additionally, the expression of Caspase-1 and GSDMD proteins decreased, the levels of IL-1β and IL-18 in the supernatants decreased, and cell pyroptosis was significantly reduced.

Conclusions

*Porphyromonas gingivalis* protease Rgp induced a senescent phenotype in BV-2 cells and promoted M1-type polarization and pyroptosis of cells by inhibiting SIRT1 expression, thereby exacerbating the inflammatory response.

INTRODUCTION
Periodontitis is a chronic infectious disease affecting the supporting tissues of the teeth, with bacteria in dental plaque biofilm serving as the initiating factor for its onset. *Porphyromonas gingivalis* is currently recognized as the primary pathogenic bacterium in periodontitis. This bacterium, along with its metabolic byproducts, triggers inflammation through pathways such as complement activation and Toll-like receptors, contributing significantly to the pathological mechanisms leading to damage in periodontal tissues\(^{[1-3]}\). Alzheimer’s disease (AD) is an age-related disorder associated with aging of the cortex and hippocampus. Its pathological changes include the deposition of amyloid β (Aβ), the formation of neurofibrillary tangles, and neuroinflammation\(^{[4]}\). Epidemiological studies indicate that periodontitis is a potential risk factor for individuals susceptible to Alzheimer’s disease. The risk of cognitive impairment and dementia increases by 1% for every lost tooth\(^{[5]}\). In addition, infection with *Porphyromonas gingivalis* can lead to increased Aβ deposition in the brains of mice, accelerating the progression of AD\(^{[6]}\). Furthermore, compared to healthy individuals, AD patients show higher concentrations of *Porphyromonas gingivalis* and its gingipains in the cerebrospinal fluid\(^{[7]}\). Gingipains, including arginine-specific proteinases (Arggingipains or gingipain R, Rgp) and lysine-specific proteinases (Lys-gingipain or gingipain K, Kgp), are components of gingipain. Rgp has been confirmed to induce cellular inflammation and apoptosis\(^{[8]}\).

The pathogenic mechanisms of Alzheimer’s disease (AD) are complex and involve various theoretical frameworks such as the Aβ cascade hypothesis, Tau protein hyperphosphorylation hypothesis, oxidative stress hypothesis, and the neuroinflammation theory. The relationship between neuroinflammation caused by microglial cells and AD is particularly close and well-established. It has been reported that a significant accumulation of senescent microglial cells is present in the brain tissues of both AD patients and animal models\(^{[9,10]}\). These senescent cells exhibit a pathogenic senescence-associated secretory phenotype (SASP), secreting not only inflammatory chemokines that induce chronic inflammation but also enhancing the activity of NOD-like receptor thermal protein domain associated protein 3 (NLRP3)\(^{[11]}\). This leads to increased M1-type polarization and pyroptosis of microglial cells, thereby exacerbating neuroinflammation and pathological damage. Conversely, inhibiting NLRP3 expression can induce M2 polarization of microglial cells, reduce pyroptosis, and provide neuroprotective effects. Further research has revealed that senescent cells often exhibit downregulation of anti-aging gene expression\(^{[12]}\). Silent mating type information regulation 2 homolog-1 (SIRT1), as an anti-aging gene, plays a crucial role in delaying cellular senescence and improving cellular dysfunction caused by aging. Experimental evidence has confirmed that senescent cell expression of SIRT1 is reduced. In AD models, the activation of SIRT1 is closely related to the activation of NLRP3, and it shows SIRT1-dependent inhibition of NLRP3 activation\(^{[13-16]}\). This suggests that SIRT1 may potentially impact the inflammatory response by inhibiting NLRP3 activation, thereby participating in the pathological mechanisms contributing to the development of AD and alleviating the pathological progression of AD\(^{[17,18]}\). Quercetin, as an activator of SIRT1, maintains mitochondrial function in microglial cells, thus improving cellular senescence, controlling inflammation, and slowing down the pathological progression of AD.
Porphyromonas gingivalis is the major pathogenic bacterium in chronic periodontitis, and this bacterium, along with its virulence factors such as gingipains, can enter brain tissues through the blood-brain barrier. This process induces increased expression and activity of NLRP3, leading to pathological changes associated with Alzheimer’s disease (AD). Researches have indicated that gingipains play a crucial role in neuroinflammation mediated by microglial cells[19]. Inhibitors of gingipains can effectively alleviate neuroinflammation and exert neuroprotective effects[20]. Furthermore, Porphyromonas gingivalis and its virulence factors can inhibit the activity and expression of SIRT1 in human neuroblastoma cells, inducing cellular senescent inflammatory responses. Therefore, in view of the influence of the microglial cells senescent phenotype on the progression of AD and the reparative effects of upregulated SIRT1 expression on pathological changes in AD, this experiment utilized Rgp as a stimulating factor for BV-2 cells. The senescence-associated β-galactosidase (SA-β-Gal) staining was employed to assess the changes in the senescent phenotype of BV-2 cells. Western blot (WB), real-time quantitative polymerase chain reaction (RT-qPCR), and flow cytometry were used to measure the expression of SIRT1 and NLRP3, as well as the M1-type polarization phenotype and pyroptosis status of BV-2 cells. The SIRT1 activator SRT1720 was applied to verify the restoration of NLRP3 expression and polarization phenotype in BV-2 cells induced by Rgp. Our findings initially elucidated that the potential mechanism by which Rgp induced senescent inflammatory responses in BV-2 cells was partially attributed to the downregulation of SIRT1, whose subsequent negative regulation effect on NLRP3. This provided a new research perspective for a profound understanding of the correlation between periodontitis and AD.

MATERIALS AND METHODS

BV-2 Cells Culture and Rgp Stimulation

BV-2 cells were purchased from Wuhan Procell Company and cultured in Minimum Essential Medium (MEM, Punuo Sai) containing 10% fetal bovine serum and 1% antibiotics (ThermoFisher). The cells used for experiments were from the third generation. Different concentrations of Rgp (AP73867, SAB) were introduced into the culture medium as cellular stimuli. SRT1720 (S1129, Selleck) was used as an activator in the cellular response validation experiment.

SA-βgal Staining

BV-2 cells in logarithmic growth phase were seeded at a density of 3×10^5 cells/well in a 6-well cell culture plate. The cells were treated with Rgp for 4 hours, while BV-2 cells cultured in MEM without Rgp served as the negative control group. After the incubation, the supernatant was removed, and the cells were washed with 1ml PBS. Subsequently, 1ml of fixative solution was added to each well and incubated at room temperature for 15 minutes. After removing the fixative solution, the cells were washed three times with 1ml PBS. Then, 1ml of staining working solution was added to each well, sealed with sealing film, and incubated overnight at 37°C. Using a fluorescence inverted microscope, four images from different fields were captured for each sample. The Image J software was used to quantify the number
of senescent phenotype cells. This was achieved by calculating the ratio of blue-stained cells (senescent phenotype cells) to the total numbers of cells, which represented the percentage of β-galactosidase-positive cells.

**RT-qPCR**

Total RNA was isolated from BV-2 cells using the Eastep® Super Total RNA Extraction Kit (Promega) following the manufacturer's instructions. 1µg of total RNA was used for complementary DNA (cDNA) synthesis. Quantitative reverse transcription polymerase chain reaction (RT-qPCR) was performed using sequence-specific primers and SYBR Green Master Mix, with β-actin used as an internal reference. The relative quantity was determined using the $2^{\Delta\Delta CT}$ method. Primer sequences are listed in Table 1.

<table>
<thead>
<tr>
<th>Genetics</th>
<th>Primer Sequences (5'-3')</th>
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<tr>
<td>NLRP3</td>
<td>F: GACTGGCAAAAGGCTGTG</td>
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<tr>
<td></td>
<td>R: AGTTTCTCAAGGCTACC</td>
</tr>
<tr>
<td>SIRT1</td>
<td>F: ACTGGAGCTGGGGTTTCT</td>
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<tr>
<td></td>
<td>R: CTTGAGGGTCTGGGAGGT</td>
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<td>Caspase-1</td>
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<td></td>
<td>R: ATCTGGCTGCTCAAATGAA</td>
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<td>GSDMD</td>
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<tr>
<td></td>
<td>R: CACTCAGCGAGTACACATTTCATT</td>
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<tr>
<td>β-actin</td>
<td>F: ATGCTCTCCCTCAGCCCATCCT</td>
</tr>
<tr>
<td></td>
<td>R: ATCGGAACCGCTCGTTGCAAAT</td>
</tr>
</tbody>
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**Western Blot (WB)**

Total protein was extracted from collected cells using the BCA Protein Assay Kit (P0012S, Beyotime). Equal amounts of protein samples were subjected to 4–20% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto a PVDF membrane. The membrane was incubated overnight at 4°C with antibodies against NLRP3 (ab190479, Abcam, 1:1000), SIRT1 (ab263899, Abcam, 1:1000), GSDMD (20770-1-AP, Proteintech, 1:1000), β-actin (81115-1-RR, Wuhan Sanying, 1:1000), and Caspase-1 (22915-1-AP, Proteintech, 1:1000). This was followed by incubation with rabbit IgG secondary antibody (A0208, Beyotime, 1:1000) at 37°C for 1 hour. Protein bands were detected using enhanced chemiluminescence reagent (Biosharp).

**ELISA Experiment**
The standard gradient was diluted, and the test supernatant was added to the enzyme-labeled plate and incubated at 37°C for 1 hour. The plate was washed four times with wash buffer. The corresponding detection antibody solution was incubated at 37°C for 1 hour, followed by four washes with wash buffer. The HRP solution was incubated at 37°C for 15 minutes. At this point, the solution in the enzyme-labeled plate turned blue. Stop Solution was added to terminate the reaction, changing the liquid in the plate from blue to yellow. Finally, readings were taken using an enzyme label reader.

**Flow Cytometry to Detect BV-2 Cells Pyroptosis**

The collected cell suspension was transferred to a centrifuge tube and centrifuged at 1000 rpm for 5 minutes to pellet the cells. The supernatant was removed, and 1µl of PBS was added to the pellet and mixed. Cell counting was performed using a counting plate to calculate the number of cells to be transferred to a new EP tube, which was then labeled. The pellet in the EP tube was centrifuged at 1000 rpm for 10 minutes in a low-temperature tabletop high-speed centrifuge to remove the supernatant, leaving the pellet. To the pellet in each EP tube, 300µl of 1× Binding buffer was added, followed by the addition of 5 µl of Annexin V and PE to each tube. Gentle mixing was performed in each EP tube, and they were then incubated in a light-protected, low-temperature refrigerator at 4°C for 15 minutes, as per the product instructions. Afterward, 200µl of 1× Binding buffer was added for machine detection. The data obtained were analyzed using FlowJo software.

**Flow Cytometry to Assess BV-2 Cells M1-type Polarization**

The collected cell suspension was transferred to a centrifuge tube and centrifuged at 1000 rpm for 5 minutes to pellet the cells. The supernatant was removed, and 1ml of PBS was added to the pellet for mixing. Cell counting was performed using a counting plate to calculate the number of cells to be transferred to a new EP tube, which was then labeled. The pellet in the EP tube was centrifuged at 1000 rpm for 10 minutes in a low-temperature tabletop high-speed centrifuge to remove the supernatant, leaving the pellet. To the pellet in each EP tube, 100 µl of PBS was added, followed by the addition of 10 µl of flow cytometry antibodies (FITC-CD206 antibody and PE-CD16/32 antibody) to each tube. Gentle mixing was performed in each EP tube, and they were then incubated in a light-protected, low-temperature refrigerator at 4°C for 40 minutes, as per the product instructions. The tubes were centrifuged at 1500 rpm for 10 minutes in the low-temperature tabletop high-speed centrifuge, the supernatant was removed, and the pellet was retained. This process of adding 100µl of PBS, centrifuging, and discarding the supernatant was repeated twice. Finally, 500µl of PBS was added to the EP tubes, stored in a light-protected low-temperature area, and promptly analyzed using flow cytometry. The data obtained were analyzed using FlowJo software.

**Statistical Analysis**

The experimental data were analyzed using SPSS 20.0 software. Measurements are expressed as $\bar{x} \pm s$, the intergroup differences were analyzed using t-tests, while one-way analysis of variance (ANOVA) was used for multiple group comparisons. A statistical significance level was set at $P < 0.05$ to indicate significant differences.
RESULTS

Effect of Rgp on Senescent Phenotype and SIRT1 Expression in BV-2 Cells

In order to preliminarily clarify whether the action of Rgp on BV-2 cells induced the cells to show senescent phenotypic changes, BV-2 cells were treated with $5 \times 10^{-4}$ mg/ml Rgp for 4 hours, which did not significantly affect BV-2 activity. Staining with the senescence-associated β-galactosidase (SA-β-Gal) revealed a notable increase in blue-stained cells in the Rgp experimental group, which indicated that there were more BV-2 cells showing a senescent phenotype ($P < 0.05$, Fig. 1 (a, c)). Simultaneously, to assess the impact of Rgp on the expression of the anti-aging protein SIRT1 in BV-2 cells, different concentrations ($5 \times 10^{-5}$ mg/ml, $5 \times 10^{-4}$ mg/ml, and $5 \times 10^{-3}$ mg/ml) of Rgp were used to stimulate BV-2 cells for 4 hours. The results of WB and qPCR indicated that with the increase in Rgp stimulation concentration, the expression of SIRT1 in BV-2 cells decreased significantly ($P < 0.05$, Fig. 1(b, d, e)). Thus, it was evident that Rgp downregulated the expression of BV-2 cells anti-aging protein SIRT1 and induced a senescent phenotype in these cells.

Effects of Rgp on NLRP3 Expression and M1-type Polarization Phenotype in BV-2 Cells

To investigate the inflammatory and polarization phenotype of BV-2 after Rgp-induced changes in the senescent phenotype, this experiment employed WB, qPCR and Elisa to assess the expression and activation of the NLRP3. The results of WB and qPCR indicated a concentration-dependent increase in NLRP3 expression with escalating Rgp concentrations ($P < 0.05$, Fig. 2(a-c)). Moreover, the levels of Interleukin-1β (Human IL-1 beta protein, IL-1β) and Interleukin-18 (Human IL-18 protein, IL-18) in the supernatant gradually elevated with Rgp concentration ($P < 0.05$, Fig. 2(d-e)). Simultaneously, flow cytometry was utilized to examine polarization phenotype changes in BV-2 cells. The findings demonstrated an elevation in CD16/32 expression with increasing stimulus concentration, indicating an increase in M1-type polarization of the cells ($P < 0.05$, Fig. 2(f-g)). The evidence demonstrated a significant increase in NLRP3 expression and M1-type polarization after Rgp induced BV-2 cells to show a senescent phenotype.

Induction of BV-2 Cells Pyroptosis by Rgp

To investigate the effect of Rgp on the pyroptosis of BV-2 cells presenting a senescent phenotype, this experiment assessed the expression of pyroptosis-related proteins Gasdermin D (GSDMD) and Caspase-1. The WB and qPCR results demonstrated that with increasing Rgp concentrations, there was an elevation in GSDMD and Caspase-1 expression ($P < 0.05$, Fig. 3(a-e)). Additionally, flow cytometry was utilized to detect pyroptosis in BV-2 cells. The results revealed an increase in BV-2 cell pyroptosis with increasing stimulus concentrations ($P < 0.05$, Fig. 3(f-g)), indicating that stimulation with Rgp led to an elevation in GSDMD and Caspase-1 expression, inducing an increase in cell pyroptosis.
The Restorative Validation of SIRT1 Activator on Senescent Inflammatory Changes in BV-2 Cells

SRT1720, a specific activator of SIRT1, was utilized in a recovery validation experiment in this study. Treatment with 5uM SRT1720 for 2 hours was applied to BV-2 cells with no significant effects on cell viability. Results demonstrated that after SRT1720 intervention, compared to the inflammatory group, there was a significant increase in SIRT1 protein expression and a decrease in the expression of NLRP3, Caspase-1, and GSDMD ($P < 0.05$, Fig. 4(a-i)). Moreover, levels of IL-1β and IL-18 in the supernatant decreased ($P < 0.05$, Fig. 4(j-k)). Additionally, both cells pyroptosis and M1-type polarization decreased ($P < 0.05$, Fig. 4(l-m)). Therefore, this experiment initially suggested that SRT1720 had a restorative effect on the senescent inflammatory response induced by Rgp in BV-2 cells.

**DISCUSSION**

Periodontitis is an age-related disease caused by bacterial infection$^{[21]}$. The main pathogenic bacterium, *Porphyromonas gingivalis*, promotes oral ecological imbalance and alters host defense mechanisms, which are crucial pathological mechanisms in periodontitis$^{[22]}$. Studies have shown that *Porphyromonas gingivalis*, particularly its gingipain, a major virulence factor, is present in the periodontal pockets of periodontitis patients. Higher concentrations of Rgp, a specific gingipain, can be detected in deep periodontal pockets$^{[23]}$. Epidemiological reports suggest a coexistence of periodontitis and Alzheimer's disease (AD), indicating a potential bidirectional relationship between the two$^{[24]}$. Ilievski et al. found that after 22 weeks of oral administration of *Porphyromonas gingivalis* to mice, immunofluorescence (IF) and confocal microscopy revealed the localization of gingipains within microglial cells$^{[25]}$. Similarly, Stephen et al. detected the presence of gingipains Kgp and Rgp in brain tissues of Alzheimer's disease patients and a mouse Alzheimer's disease model$^{[7]}$. Due to the inseparable association of AD's progression and pathological damage with cellular senescence and inflammation, although research findings on gingipains inducing neuroinflammation are relatively conclusive$^{[4, 24]}$, there are few reports on whether they can exert pro-inflammatory effects by inducing changes in cellular senescent phenotype. This study aims to explore the preliminary impact of gingipain Rgp on the senescent phenotype of BV-2 cells and the expression of SIRT1, an anti-aging-related protein. Moreover, it seeks to uncover the resultant changes in cell phenotypes and cell pyroptosis mediated by these effects, thereby offering a novel research perspective on exploring the correlation between periodontitis and AD.

We first exposed BV-2 cells to Rgp derived from *Porphyromonas gingivalis* to investigate whether the Rgp induced senescent phenotypic changes in BV-2 cells. SA-β-Gal staining revealed an increase in blue-stained cells, indicating SA-β-Gal-positive cells, after Rgp stimulation, thereby preliminarily confirming the induction of a senescent phenotype by Rgp. WB and qPCR analysis showed a reduction in SIRT1 in BV-2 cells, which a protein with anti-aging effects. Previous studies on aged mice with Alzheimer's disease (AD) found a significant increase in senescent microglial cells in brain tissue, often characterized by low expression of SIRT1 and a senescence-associated secretory phenotype (SASP)$^{[26]}$. SASP cells exhibit enhanced expression of nuclear factor kappa-B (NF-κB) and NLRP3, leading to
intensified neuroinflammation and promoting memory impairment in mice. Therefore, following the initially identification of Rgp’s induction of a senescent phenotype in BV-2 cells, this experiment subsequently utilized ELISA and flow cytometry techniques to further assess the NLRP3 activation and changes in polarization in BV-2 cells exhibiting a senescent phenotypes. The results showed a significant upregulation in NLRP3 expression and a noticeable increase in M1-type polarization in BV-2 cells displaying a senescent phenotype. Existing evidence suggests that M1-type polarization of microglial cells, which affects cell migration and phagocytic function, is one of the critical factors promoting the progression of AD. The activation of the NLRP3 is crucial for regulating microglial cells polarization. Studies by Marino using a streptozotocin-induced diabetic rat model of diabetes-associated cognitive impairment demonstrated that cognitive impairment is associated with an increase in M1-type polarization of microglial cells\[^{27, 28}\]. In vitro, changes in the polarization phenotype of microglial cells under high sugar conditions support the results of animal models. It appears that various pathogenic factors, including both periodontitis and diabetes, may induce AD pathological changes by promoting M1-type polarization of microglial cells.

Activation of the NLRP3 is a crucial mediator of pyroptosis, a process executed by gasdermin D (GSDMD). Pyroptosis, triggered by the NLRP3 inflammasome and executed by GSDMD, involves the activation of caspase-1 and the cleavage of the N-terminal domain of GSDMD. This cleavage forms GSDMD pores, promoting the release of IL-1\(\beta\) and IL-18 and leading to cell pyroptosis\[^{29}\]. Upon examination, it was found that with increasing Rgp stimulation concentrations, the expression of pyroptosis-related proteins, Caspase-1 and GSDMD, in BV-2 cells increased. Flow cytometry analysis confirmed an increase in pyroptosis in BV-2 cells. There are two main forms of cell death: pyroptosis and apoptosis. Apoptosis has non-inflammatory consequences, while pyroptosis induces pro-inflammatory reactions\[^{30}\]. Studies have shown that the activation of NLRP3 by stimulating factors can induce an increase in pyroptosis in microglial cells in the mouse brain, exacerbating cognitive impairment in Alzheimer's disease\[^{31}\]. In summary, it could be concluded that gingipain Rgp induced BV-2 cells to show a senescent phenotype, inhibited the expression of the anti-aging protein SIRT1, and affected NLRP3 expression through the negative regulation of SIRT1, consequently promoting cellular M1-type polarization and pyroptosis, thereby exacerbating inflammation.

Currently, research on drugs related to the SIRT1 pathway in the field of anti-neuroinflammation has been increasingly fruitful. An et al. found that aspirin can upregulate SIRT1, reduce the senescence of microglial cells, decrease the secretion of inflammatory factors such as IL-1\(\beta\) and tumor necrosis factor \(\alpha\) (TNF\(\alpha\)), and improve the phagocytic function of microglial cells\[^{15}\]. Minocycline, in part by upregulating SIRT1, reduces the expression of the pro-inflammatory factor IL-1\(\beta\) and inducible isoform of nitric oxide synthase (iNOS), promotes M2 polarization of microglial cells, and inhibits neuroinflammation\[^{32}\]. To clarify the role of SIRT1 in Rgp-induced BV-2 cells presenting a senescent phenotype and promoting inflammatory responses, we pretreated the cells with the SIRT1 activator SRT1720 and then reexamined the expression of NLRP3, as well as changes in polarization and pyroptosis. The experimental results showed that, compared to the Rgp-stimulated group, the SRT1720 intervention group had increased
SIRT1 protein expression in BV-2 cells, decreased NLRP3 expression, and significantly reduced M1-type polarization. Moreover, the protein expression of Caspase-1 and GSDMD was downregulated, the levels of IL-1β and IL-18 in the supernatant decreased, and cell pyroptosis was also reduced.

The present experimental data suggest that the gingipains Rgp promotes inflammatory changes in BV-2 cells and can act by inducing a cellular senescent phenotype. As periodontitis and AD are both age-related and mutual risk factors, it is highly likely that the activation of the senescent inflammatory pathway by pathogenic factors is an important pathway that links the two diseases closely. We have to admit that our study has certain limitations. For instance, we conducted preliminary assessment of BV-2 cell senescence using SA-β-Gal staining alone, without examining senescence markers. Additionally, the use of the activator SRT1720 in the experiments might affect other members of the SIRT family. Most importantly, the present study is only a one-way study of the inflammatory effects of microglia cells induced by periodontal pathogenic factors. Hence, in future studies, we plan to perform more precise assessments of cellular senescence markers and consider genetic knockout or overexpression of SIRT1. Moreover, conducting animal experiments would further validate our research findings.

Declarations

Declarations of Conflicting Interest: The authors declared no potential conflict of interest with respect to the research, authorship, and/or publication of this article.

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Author Contribution

Tian Yaguang, Li Mengsen and Cai Hongxuan contributed to the experimental ideas, design, data collection and analysis, manuscript writing and revision, while Si Weixing, Zhang Zan, Dai Jingyi, and Wang Zhurui contributed to data analysis and manuscript revision. All authors have given final approval and agreed to be accountable for all aspects of the work.

Data availability statement:

The data are available from the corresponding author on reasonable request.

Ethics declaration: not applicable.
References


**Figures**
Figure 1

Effects of Rgp on Senescent Phenotype and SIRT1 Expression in BV-2 cells

(a, c): SA-β-Gal staining; (b, d): SIRT1 protein expression; (e): SIRT1 mRNA expression, *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001

(Arrows pointing to blue-stained positive cells indicated the senescent phenotype cells)
Figure 2

Effects of Rgp on Inflammatory and M1-type Polarization Phenotypes of BV-2 cells

(a) - (c): NLRP3 protein and mRNA expression; (d) - (e): Content of IL-18 and IL-1β in the supernatant; (f) - (g): M1-type polarization detection of BV-2 cells, *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001
Figure 3

Effects of Rgp on pyroptosis of BV-2 cells presenting a senescent phenotype

(a)-(e): GSDMD, Caspase-1 protein and mRNA expression; (f)-(g): BV-2 cell pyroptosis detection, *P<0.05; **P<0.01, ***P<0.001, **** P<0.0001
Figure 4

Restorative Effect of SRT1720 on the inflammatory changes in BV-2 cells presenting a senescent phenotype

(a)-(i): SIRT1, NLRP3, GSDMD, Caspase-1 protein and mRNA expression; (j)-(k): The content of IL-18 and IL-1β in the supernatant; (l): BV-2 cell pyroptosis detection; (m): M1-type polarization detection of BV-2 cells, * P<0.01; ** P<0.01; *** P<0.001; **** P<0.0001

Supplementary Files

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- SupplementalAppendix.docx