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·基础研究·

过表达SAP缓解小鼠牙周炎的机制研究

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【摘要】　目的　研究血清淀粉样P物质（serum amyloid P component，SAP）缓解小鼠牙周炎的机理，为确立SAP作为治疗牙周炎的新药物提供实验依据。**方法**　获得单位实验动物伦理委员会批准，建立野生型（wild type，WT）小鼠、SAP转基因（SAP -transgenic，SAP-Tg）小鼠的牙周炎模型，将小鼠分为WT对照组（WT组）、WT牙周炎组（W+P组）、SAP-Tg对照组（Tg组）和SAP-Tg牙周炎组（Tg+P组）。7 d后处死，取材（牙周组织、牙齿和牙槽骨）。酶联免疫吸附试验（enzyme-linked immunosorbent assay，ELISA）检测各组牙周组织中SAP蛋白表达，Micro-CT检测和HE染色检测分析牙槽骨吸收情况（釉牙骨质界到牙槽嵴顶的距离），抗酒石酸酸性磷酸酶（tartrate resistant acid phosphatase，TRAP）染色观察破骨细胞数量，免疫组化（immunohistochemistry，IHC）染色观察巨噬细胞数量，qRT-PCR检测各组牙周组织炎症因子白细胞介素-1β（interleukin-1beta，IL-1β）、白细胞介素-6（interleukin-6，IL-6）和肿瘤坏死因子-α（tumor necrosis factor-α，TNF-α）的表达水平；16S核糖体核糖核酸（16S ribosomal ribonucleic acid，16S rRNA）基因测序技术进行口腔微生物测序。提取WT小鼠、SAP-Tg小鼠的巨噬细胞建立体外炎症模型，分为WT+LPS组和Tg+LPS组，qRT-PCR检测2组巨噬细胞极化相关基因诱导型一氧化氮合酶（inducible nitric oxide synthase，iNOS）、CD86、CD163、CD206的表达，破骨细胞分化诱导后进行TRAP染色。**结果**　ELISA结果显示，相较于WT+P组小鼠，Tg+P组牙周组织中具有更高水平的SAP表达；Micro-CT和HE染色结果显示，相较于WT+P组小鼠，Tg+P组小鼠的牙槽骨吸收减少，釉牙骨质界到牙槽嵴顶的距离缩短；TRAP染色结果显示，相较于WT+P组，Tg+P组破骨细胞数量减少；IHC染色和qRT-PCR结果显示，相较于WT+P组，Tg+P组牙周组织中巨噬细胞聚集明显减少，炎症因子IL-1β、IL-6和TNF-α表达下降。口腔微生物测序结果显示，WT+P组和Tg+P组小鼠牙周炎相关致病菌无明显差异。体外实验结果显示，相较于WT+LPS组，Tg+LPS组的M1型巨噬细胞标志物iNOS和CD86表达下调，M2型巨噬细胞标志物CD163和CD206均表达上调；TRAP染色结果显示，与WT+LPS组相比，Tg+LPS组破骨细胞数量减少。**结论**　牙周炎时，SAP过表达能有效缓解小鼠牙周炎的严重程度，抑制巨噬细胞向M1型极化，并且减少相关炎症因子表达，减少向破骨细胞分化，从而减缓牙槽骨的吸收。

【关键词】　血清淀粉样P物质；　牙周炎；　炎症因子；　巨噬细胞极化；　16S rRNA基因测序；　微生物；　破骨细胞；　牙槽骨吸收
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The mechanism of SAP overexpression in alleviating periodontitis in mice　HUANG Yinyin, LIANG Dongliang, ZOU Yaokun, HAN Jingru, GE Qing, LIU Xueyan, GUO Yadong, HUANG Xinli, YANG Lan.　Department of Implantology, Affiliated Stomatological Hospital of Guangzhou Medical University, Guangdong Provincial Engineering Technology Research Center for Oral Tissue Repair and Reconstruction, Guangzhou Key Laboratory of Basic and Applied Research of Oral Regenerative Medicine, Guangzhou 510182, China

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【Abstract】 Objective To investigate the mechanism by which serum amyloid P component (SAP) alleviates periodontitis in mice, providing an experimental basis to establish SAP as a novel therapeutic agent for periodontitis. **Methods** Ethical approval was obtained from the Institutional Animal Ethics Committee. Periodontitis models were established in wild-type (WT) mice and SAP-transgenic (SAP-Tg) mice, divided into four groups: WT control (WT group), WT periodontitis (WT+P group), SAP-Tg control (Tg group), and SAP-Tg periodontitis (Tg+P group). On day 7, the mice were euthanized, and periodontal tissues, teeth, and alveolar bone were collected. SAP protein expression was detected by enzyme-linked immunosorbent assay (ELISA). Micro-CT and HE staining were used to measure alveolar bone resorption (distance from the cementoenamel junction to the alveolar bone crest). Tartrate-resistant acid phosphatase (TRAP) staining was performed to assess osteoclast number, and immunohistochemistry (IHC) was employed to evaluate macrophage infiltration. The expression levels of inflammatory cytokines including interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) were measured by qRT-PCR. Oral microorganism composition was analyzed using 16S ribosomal RNA (16S rRNA) gene sequencing. Additionally, macrophages from WT and SAP-Tg mice were isolated to establish an *in vitro* inflammation model, divided into WT+LPS and Tg+LPS groups. The expression of macrophage polarization-related genes including inducible nitric oxide synthase (iNOS), CD86, CD163, and CD206) were assessed by qRT-PCR. After the induction of osteoclast differentiation, TRAP staining was performed. **Results** ELISA results demonstrated that periodontal tissues from Tg+P group mice exhibited higher levels of SAP expression compared to the WT+P group. Micro-CT and HE staining analyses revealed that the Tg+P group showed reduced alveolar bone resorption, indicated by a shorter distance between the cementoenamel junction and alveolar bone crest, compared to the WT+P group. Furthermore, TRAP staining results indicated a decrease in osteoclast numbers in the Tg+P group compared to the WT+P group. IHC and qRT-PCR results indicated reduced macrophage infiltration and decreased expression of IL-1β, IL-6, and TNF-α in the Tg+P group. Oral microorganism sequencing showed no significant difference in periodontitis-associated pathogenic bacteria between WT+P and Tg+P groups. *In vitro* experiments demonstrated that compared to the WT+LPS group, the Tg+LPS group exhibited downregulated M1 macrophage markers (iNOS and CD86) and upregulated M2 macrophage markers (CD163 and CD206). TRAP staining confirmed fewer osteoclasts in the Tg+LPS group. **Conclusion** SAP overexpression effectively alleviates periodontitis severity in mice by inhibiting M1 macrophage polarization, reducing pro-inflammatory cytokine expression, and suppressing osteoclast differentiation, thereby attenuating alveolar bone resorption.

【Key words】 serum amyloid P component; periodontitis; inflammatory factors; macrophages polarization; 16S ribosomal RNA gene sequencing; microorganism; osteoclast; alveolar bone resorption

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牙周炎是发生在牙周组织的慢性炎症性疾病，不仅会导致牙齿的松动移位和脱落，还与机体的动脉粥样硬化、类风湿性关节炎等多种全身性疾病的发生发展息息相关[1]，严重影响人们的身体健康和生活质量。微生物是牙周炎的始动因素[2]，而微生物侵入后引发宿主一系列的免疫反应才是导致牙周炎发展的决定因素[3-4]。在牙周炎早期，巨噬细胞通过对牙周生物膜致病性的控制、适应性免疫的激活等，对牙周炎的免疫反应起重要作用[5]，进而决定牙周炎的发展方向。因此，探索微生物与巨噬细胞在牙周炎发生发展中的关键中间分子，有助于进一步揭示微生物通过宿主的巨噬细胞，调控牙周炎进展的机制，为牙周炎的靶向治疗提供新思路。

正五聚体（pentraxin）家族是一类在进化中保守的蛋白质，主要分为短臂和长臂两个类别，最早被发现、最重要的作用之一便是充当先天性免疫中可溶性模式识别分子（pattern recognition molecules，PRMs），调节针对病原菌的宿主防御[6]。血清淀粉样P物质（serum amyloid P component，SAP）是经典的短臂正五聚体蛋白，是一种在进化上高度保守的外分泌糖蛋白，它主要作为循环中的模

式识别受体，在先天免疫和炎症性疾病中发挥作用[7]。研究发现，SAP能与多种细菌结合，促进补体激活、调理作用、吞噬作用和增强对感染的抵抗力，对宿主起到保护作用，但是也有研究表明SAP对细菌具有保护作用，这说明SAP参与微生物的调节[8]。除此之外，SAP在不同的哺乳动物疾病模型中可以调节巨噬细胞向不同的表型极化，从而发挥促进炎症或者抑制炎症的作用。例如，在小鼠肾纤维化模型中，SAP可降低M1型标记物的表达，增加肾巨噬细胞的M2型标记物的表达，而在肺纤维化疾病模型中，SAP给药可促进肺巨噬细胞M1型的极化[9-11]。由此可见，SAP显示出其在先天免疫中的重要调节作用。研究发现，SAP基因缺失导致小鼠牙周炎时牙龈卟啉单胞菌（*Porphyromonas gingivalis*，*Pg*）数量增加，M1型巨噬细胞增多，最终牙周炎加重[12]。然而，过表达SAP对牙周炎的影响，SAP是否可以作为牙周炎的治疗靶点尚不清楚，本文探讨过表达SAP对牙周炎的影响，为确立SAP作为牙周炎治疗的新药物提供实验依据。

1　材料和方法

本项研究经广州医科大学实验动物伦理委员会审查批准（编号：GY2020-003）。实验在广州医科大学实验动物中心屏障环境动物房完成，实验结束后，动物尸体按照动物研究伦理准则交由动物管理中心妥善处理。

1.1　材料

C57BL/6J背景的SAP基因过表达（SAP-Tg）小鼠（广东药科大学血管生物学研究所馈赠）（动物合格证号：SYXK（粤）2021-0122）、C57BL/6J（WT）小鼠（动物合格证号：SCXK2018-0002，购自广东省医学动物实验中心，中国广东）、戊巴比妥钠（4390-16-3，Merck Millipore，美国）、PBS缓冲液（10010072，Gibco，美国）、5-0医用真丝编织线（A312，上海浦东金环医疗用品股份有限公司，中国）、SAP蛋白ELISA试剂盒（ab235639，abcam，英国）、BCA蛋白测定试剂盒（BB-3401，贝博生物，中国）、细胞培养基（8122059，Gibco，美国）、胎牛血清（FSD500，ExCell，中国）、PCR荧光探针试剂盒（A0012，EZBioscience，英国）、牛血清蛋白（A8020，Solarbio，中国）、生理盐水（IN9000，Solarbio，中国）、抗酒石酸酸性磷酸酶（tartrate resistant acid phosphatase，TRAP）染色试剂盒（G1492，Servicebio，中国）、Anti-F4/80兔单克隆抗体（D2S9R，CST，美国）、羊抗兔IgG多克隆抗体（ZB-2306，中杉金桥，中国）、内源性过氧化酶阻断液（P0100A，Beyotime，中国）、苏木素染色液（C0107，Beyotime，中国）、伊红染色液（C0109，Beyotime，中国）、返蓝液（G1866，Solarbio，中国）、分化液（G1862，Solarbio，中国）、盖玻片（80340-0130，世泰，中国）、高速离心机（5425R，Eppendorf，德国）、RNA提取试剂盒（12183018A，赛默飞，美国）、分光光度计（NanoDrop2000，赛默飞，美国）、PCR扩增仪（S1000，Bio-Rad，美国）、qRT-PCR系统（12011319，LC-480 II，瑞士罗氏）、酶标仪（A51119700DPC，赛默飞，美国）、生物安全柜（BSC-1100IIA2-X，Biobase，中国）、生物显微镜（DM4000B-LED/DFC450，Leica，德国）、石蜡切片机（TM HM 325，赛默飞，美国）、微型计算机断层扫描（micro-computed tomography，Micro-CT）（Sky-Scan1176，Kontich，比利时）。

1.2　动物分组

实验选用8 w雄性小鼠，体重22～25 g。所有实验小鼠均为SPF级，将小鼠分为WT对照组（WT组）、WT牙周炎组（WT+P组）、SAP-Tg对照组（Tg组）和SAP-Tg牙周炎组（Tg+P组）。所有小鼠均饲养在温度（24 ℃±2 ℃）和湿度（60%±5%）的条件下，保持光/暗环境各12 h循环；小鼠所用饲料及垫料均购买自广东省医学动物实验中心，饮用水及垫料均经过高温高压灭菌后用于小鼠。

1.3　牙周炎模型

分别将WT+P组和Tg+P组的小鼠麻醉后俯卧固定在小鼠专用固定板上。采用丝线结扎法建立牙周炎模型[13-14]。5-0丝线结扎缠绕左侧上颌第二磨牙。将缝线轻轻地塞进牙间隙，在腭侧打结。在整个实验期间，都保持所有小鼠的结扎线无掉落。

7 d后，吸入麻醉后颈椎脱位处死小鼠。采集上颌骨，包括牙周组织、牙齿和牙槽骨，进行酶联免疫吸附试验（enzyme-linked immunosorbent assay，ELISA）、Micro-CT检查、苏木精-伊红染色法（hematoxylin-eosin staining，HE）和免疫组化技术（immunohistochemistry，IHC）、TRAP染色、实时荧光定量PCR（quantitative real-time polymerase chain reaction，qRT-PCR）、16S核糖体核糖核酸（16S ribosomal ribonucleic acid，16S rRNA）基因测序技术进行口腔微生物基因测序，分析牙周炎的病理生理变化，测量釉牙骨质界到牙槽嵴顶的距离，计算牙槽骨的吸收高度。

1.4　ELISA检测牙周组织中SAP蛋白

将牙周组织样本在PBS中冲洗，以彻底除去多余的血液，并称重。将牙周组织切成小块，倒入匀浆玻璃管中，按照组织重量加入适量ELISA试剂盒配套的裂解缓冲液，然后手工匀浆。将所得悬浮液用超声细胞破碎仪超声处理，直至溶液澄清。然后，将匀浆以10 000 ×g离心15 min，收集上清液并按照ELISA试剂盒相应步骤进行检测4组牙周组织中SAP蛋白的表达。

1.5　Micro-CT和HE染色检测牙槽骨吸收

对4组样本进行Micro-CT扫描（60 kV，100 μA），扫描体积的体素分辨率为10 μm。扫描结束后，运用该系统支持的NRECON及CTVOX软件进行三维重建。测量各组小鼠上颌牙周组织6个位点，第一磨牙测量远中腭沟和远中尖，第二磨牙测量近中尖、远中尖和中央沟，第三磨牙测量牙尖，每个位置重复测量3次。所有测量结果的平均值作为该样本的牙槽骨高度吸收值（釉牙骨质界到牙槽嵴顶的距离）。分别从2D和3D层面进行分析，并统计各组小鼠的牙槽骨高度吸收值。

将4%多聚甲醛固定后的牙周组织置于EDTA脱钙溶液中脱钙约30 d。脱钙期间，每隔4 d更换新的脱钙液，30 d后使用注射器针头轻刺样本的非实验观察区域的骨质弹性，判断脱钙程度，并将组织置于自动脱水机行脱水处理。石蜡包埋后，进行组织切片。各切片经脱蜡，进行HE染色，再次测量釉牙骨质界到牙槽嵴顶的距离。

1.6　TRAP染色检测牙周组织中破骨细胞情况

取50 μL副品红溶液与50 μL亚硝酸钠溶液在洁净离心管中混匀，得到六偶氮副品红溶液；向第1步的100 μL六偶氮副品红溶液中加入100 μL的AS-BI磷酸盐底物溶液，吹吸数次充分；吸取1.8 mL反应缓冲液加入到第2步的混合液中充分混匀；第3步的混合液经针式滤器过滤（0.45 μm水系滤膜）即得到TRAP工作液。WT+P组及Tg+P组样本切片孵育完成后倾去纯水，每个组织点滴加过滤好的200～300 μL TRAP工作液覆盖组织，37 ℃避光反应20～30 min。复染细胞核后脱水，透明，封片。光学显微镜拍摄图像，视野范围内计数破骨细胞的数量。

1.7　IHC染色检测牙周组织中巨噬细胞情况

抗F4/80巨噬细胞抗体在4 ℃下孵育过夜。然后将4组组织切片与相应的辣根过氧化物酶标记的抗兔/抗鼠二抗在37 ℃下孵育45 min。用PBS冲洗3次后，使用二氨基联苯胺作为显色剂观察辣根过氧化物酶活性，随后用苏木精复染。最后，将切片脱水并封片。组织切片在光学显微镜下观察，并使用Image J软件计算每张图像中阳性免疫染色的面积。

1.8　qRT-PCR检测炎症基因

选取WT+P组、Tg+P组小鼠的左侧上颌第二磨牙区域牙槽骨，包括上颌组织，用PBS清洗，保存在干燥的冰冻管中。样品浸泡在液氮中，储存在−80 ℃下。提取组织RNA时，冷冻样品放入液氮预冷的研钵中，在研钵中加入1 mL含有1% β-硫代乙醇的裂解液，并快速研磨成裂解液，使用PureLink RNA Mini Kit，在NanoDrop分光光度计中测量RNA浓度和质量。在PCR扩增仪中，使用Evo M-MLV反转录试剂盒法逆转录总RNA。使用Takara TB-Green PreMix Ex Taq试剂进行qRT-PCR，检测特定相关炎症基因白细胞介素-1β（interleukin-1beta，IL-1β）、白细胞介素-6（interleukin-6，IL-6）和肿瘤坏死因子-α（tumor necrosis factor-α，TNF-α）的表达。所用引物序列见表1。

表1　炎症相关基因引物序列

Table 1　Primer sequences of inflammation-related genes

Genes	Sequence（5'-3'）
GAPDH	F: GTGAAGGTCGGTGTGAACGG
	R: TCCTGGAAGATGGTGATGGG
IL-6	F: CTGCAAGAGACTTCCATCCAG
	R: AGTGGTATAGACAGGTCTGTTGG
TNF-α	F: TGTCTCAGCCTCTTCTCATT
	R: TGATCTGAGTGTGAGGGTCT
IL-1β	F: GAAATGCCACCTTTTGACAGTG
	R: TGGATGCTCTCATCAGGACAG

IL-6: interleukin-6; TNF-α: tumor necrosis factor-α; IL-1β: interleukin-1 beta

1.9　16S rRNA基因测序技术进行口腔微生物基因测序

采集了WT+P组、Tg+P组小鼠唾液、牙龈组织和结扎线，使用对应的DNA提取试剂盒进行基因组DNA抽提后，利用Thermo NanoDrop One检测DNA的纯度和浓度。以基因组DNA为模板，根据测序区域的选择，使用带barcode的特异引物进行PCR扩增。本实验选择16S V4区测序，引物区域为515F和806R，电泳后按照NEBNext® Ultra™ II DNA Library Prep Kit for Illumina®（New England Bio-

labs,美国）标准流程进行建库操作,使用 Illumina Nova 6000平台对构建的扩增子文库进行PE250测序(广东美格基因,中国)。

1.10　巨噬细胞体外实验

全骨髓法提取WT小鼠和SAP-Tg小鼠的骨髓间充质干细胞(mesenchymal stem cells derived from bone marrow,BMSCs),用巨噬细胞集落刺激因子-1诱导成巨噬细胞后,用*Pg*来源的脂多糖(lipopolysaccharides,LPS)诱导炎症环境,分为WT+LPS组和Tg+LPS组,分别进行qRT-PCR,检测巨噬细胞向M1型极化相关基因诱导型一氧化氮合酶(inducible nitric oxide synthase,iNOS)、CD86和巨噬细胞向M2型极化相关基因CD163、CD206的表达,并进行TRAP染色检测破骨分化情况。qRT-PCR方法与上文相同,所用引物如表2所示。TRAP染色时将细胞2×10^4个/孔接种于48孔板中。培养24 h后,换入破骨诱导培养基,继续培养,之后每隔1 d更换1次培养基。5 d后,显微镜下观察破骨细胞,并按照说明书进行染色。

表2　极化相关基因引物序列

Table 2　Primer sequences of polarization-related genes

Genes	Sequence(5'-3')
GAPDH	F: GTGAAGGTCGGTGTGAACGG
	R: TCCTGGAAGATGGTGATGGG
CD86	F: CTGGACTCTACGACTTCACAATG
	R: AGTTGGCGATCACTGACAGTT
CD163	F: GGTGGACACAGAATGGTTCTTC
	R: CCAGGAGCGTTAGTGACAGC
CD206	F: CTCTGTTCAGCTATTGGACGC
	R: TGGCACTCCCAAACATAATTTGA
iNOS	F: GGAGTGACGGCAAACATGACT
	R: TCGATGCACAACTGGGTGAAC

iNOS: inducible nitric oxide synthase

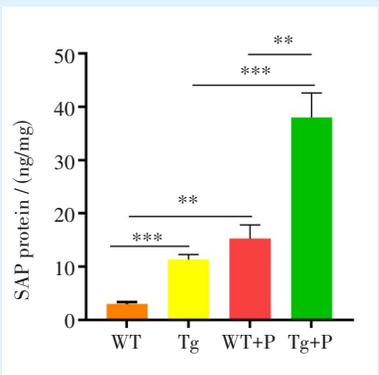
1.11　统计学分析

采用SPSS 25软件进行统计分析,采用GraphPad Prism统计软件(v.8.0.2)作统计图,结果描述采用均数±标准差表示。两独立样本的均数比较,若数据符合正态分布、方差齐,则采用两独立样本*t*检验;若方差不齐,则采用*t*'检验;若不满足正态分布,则采用Mann-Whitney *U*非参数检验。多个独立样本的均数比较,若数据方差齐、符合正态分布则采用单因素方差分析(One-way ANOVA),若方差不齐,采用Welch检验;若数据不满足正态分布,则采用Kruskal-Wallis的非参数检验。$P<0.05$为差异具有统计学意义。

2　结　果

2.1　过表达SAP基因减少小鼠牙周炎时牙槽骨的吸收

ELISA结果显示,相较于WT组,Tg组牙周组织中过表达SAP;相较于WT组和Tg组,WT+P组和Tg+P组小鼠牙周组织中SAP表达均有所上升;而且,相较于WT+P组,Tg+P组牙周组织中具有更高水平的SAP表达(图1)。



When periodontitis was not established, the expression of SAP in the periodontal tissues of the Tg group was higher than that of the WT group. When periodontitis was established, the WT+P group had a higher level of SAP expression than the WT group, Tg+P group had a higher level of SAP expression than Tg group, and Tg+P group had a higher level of SAP expression than WT+P group (n=3). ** P<0.01, *** P<0.001. WT: WT control group; WT+P: WT periodontitis group; Tg: SAP-Tg control group; Tg+P: SAP-Tg periodontitis group. WT: wild type. SAP-Tg: serum amyloid P component-transgenic

Figure 1　The expression of SAP in periodontal tissue of mice

图1　小鼠牙周组织中SAP的表达

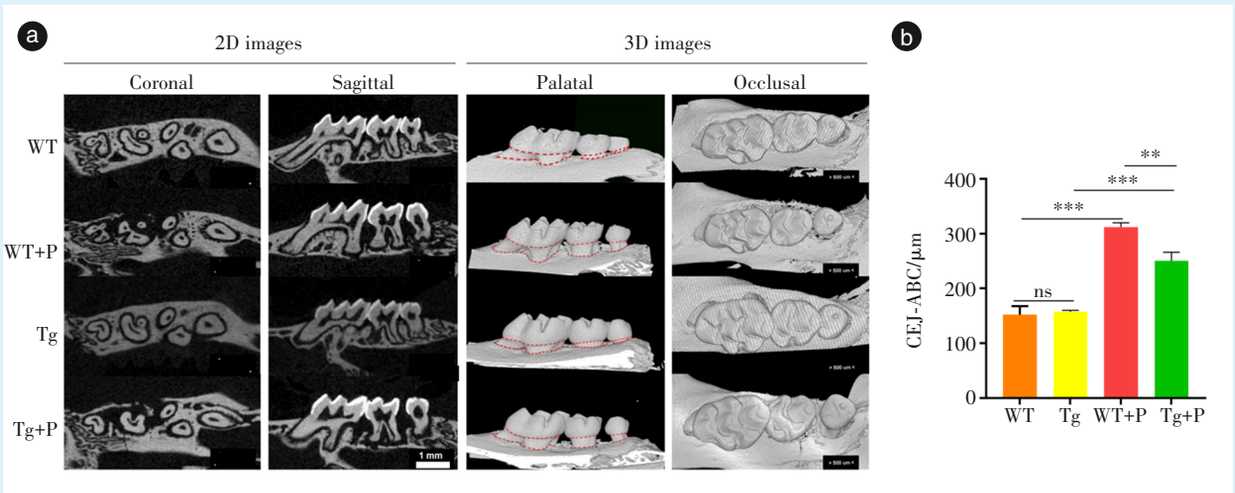
Micro-CT结果表明,WT组和Tg组的牙槽骨无明显吸收;但Tg+P组比WT+P组表现出更少的牙槽骨吸收(图2)。HE染色结果显示,相较于WT+P组,Tg+P组小鼠釉牙骨质界到牙槽嵴顶的距离明显缩短(图3),与Micro-CT的结果一致。

2.2　过表达SAP基因小鼠牙周炎时破骨细胞减少

TRAP染色结果显示,相较于WT+P组,Tg+P组牙周组织内的破骨细胞数量明显减少(图4)。

2.3　过表达SAP基因小鼠牙周炎时巨噬细胞聚集减少

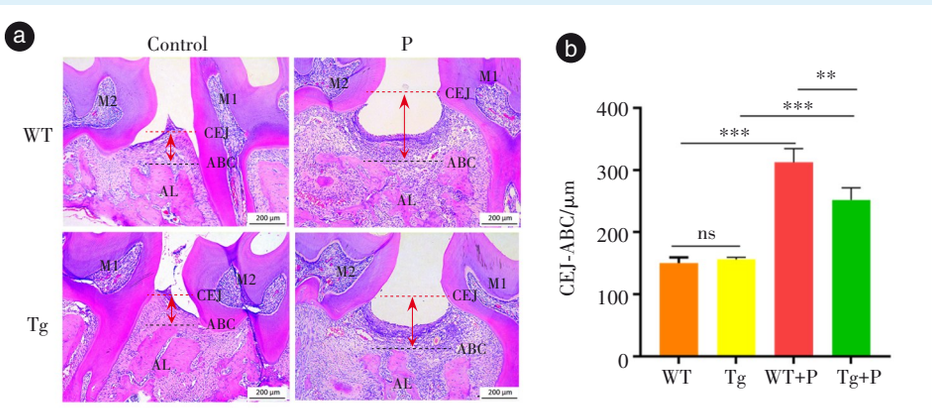
IHC结果显示,相较于WT+P组,Tg+P组牙周



a: two-dimensional and three-dimensional Micro-CT reconstruction images of the maxilla in WT and SAP-Tg mice. b: quantitative analysis of the CEJ-ABC distance from Micro-CT images. When periodontitis was not established, there was no significant difference in alveolar bone height between the Tg group and the WT group. When periodontitis was established, mice in the Tg+P group had less alveolar bone resorption than those in the WT+P group (n=4). ns: no statistical difference, ** P<0.01, *** P<0.001. WT: WT control group; WT+P: WT periodontitis group; Tg: SAP-Tg control group; Tg+P: SAP-Tg periodontitis group. CEJ: cementoenamel junction; ABC: alveolar bone crest.The red line above: CEJ; the red line below: ABC. WT: wild type. SAP-Tg: serum amyloid P component-transgenic

Figure 2　Micro-CT image of alveolar bone in mice

图 2　小鼠牙槽骨 Micro-CT 图像



a: HE staining images of periodontal tissue from the left second molar of WT and SAP-Tg mice. b: quantitative analysis of the CEJ-ABC distance from HE staining. When periodontitis was not established, there was no significant difference in alveolar bone height between the Tg group and the WT group. When periodontitis was established, mice in the Tg+P group had less alveolar bone resorption than those in the WT+P group(n=4). ns: no statistical difference, ** P<0.01, *** P<0.001. WT: WT control group ;WT+P: WT periodontitis group; Tg: SAP-Tg control group; Tg+P: SAP-Tg periodontitis group. CEJ: cementoenamel junction; ABC: alveolar bone crest. WT: wild type. SAP-Tg: serum amyloid P component-transgenic

Figure 3　HE staining image of periodontal tissue in mice

图 3　小鼠牙周组织 HE 染色图像

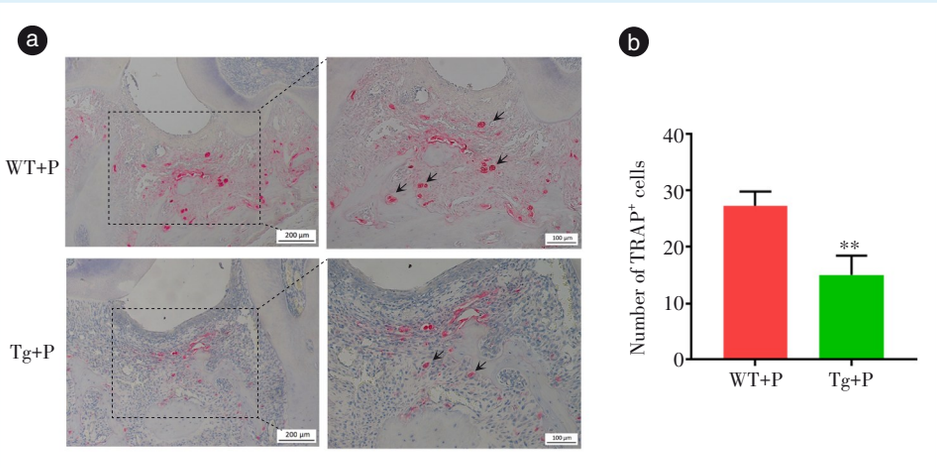
组织中巨噬细胞聚集明显减少(图5)。

2.4　过表达SAP基因小鼠牙周炎时炎症因子表达降低

qRT-PCR结果显示,相较于WT+P组,Tg+P组小鼠牙周组织中炎症因子IL-1β、IL-6和TNF-α的表达明显降低(图6)。

2.5　过表达SAP基因小鼠牙周炎时口腔微生物发生变化

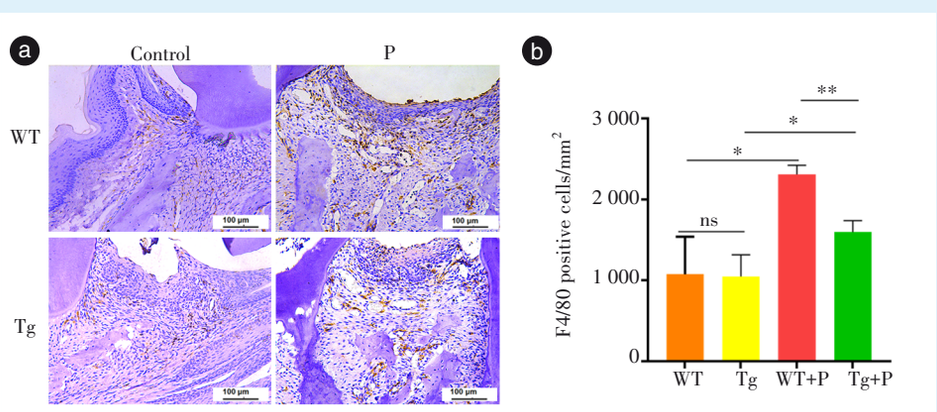
16S rRNA微生物测序菌落组成柱状图分析显示,Tg+P组和WT+P组小鼠最丰富的细菌是厚壁



a: TRAP staining images of periodontal tissue from the left second molar of the WT+P and Tg+P groups, with osteoclasts indicated by the arrows. b: statistical chart of osteoclast quantity. There were fewer osteoclasts in the Tg+P group than in the WT+P group (n=4). ** P<0.01. WT+P: WT periodontitis group; Tg+P: SAP-Tg periodontitis group. WT: wild type. SAP-Tg: serum amyloid P component-transgenic. TRAP: tartrate resistant acid phosphatase

Figure 4　TRAP staining image of periodontal tissue in mice with periodontitis

图4　小鼠牙周炎时牙周组织TRAP染色图像



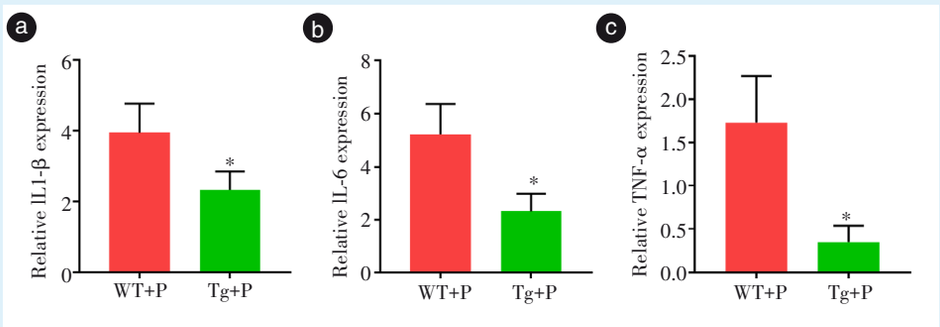
a: IHC staining images of macrophages in periodontal tissues of the WT and Tg groups. b: statistical chart of the macrophage staining results. When periodontitis was not established, there was no significant difference in the number of macrophages in the periodontal tissues between the WT and Tg groups. When periodontitis was established, the number of macrophages in the WT+P group was higher than that in the WT group, and the number of macrophages in the Tg+P group was higher than that in the Tg group. Compared with the WT+P group, the number of macrophages in the Tg+P group decreased (n=4). ns: no statistical difference, * P<0.05, ** P<0.01. WT: WT control group; WT+P: WT periodontitis group; Tg: SAP-Tg control group; Tg+P: SAP-Tg periodontitis group. IHC: immunohistochemistry. WT: wild type. SAP-Tg: serum amyloid P component-transgenic

Figure 5　IHC staining image of periodontal tissue in mice

图5　小鼠牙周组织IHC染色图像

菌门、变形菌门、拟杆菌门和放线菌门，2组细菌群落在门水平和属水平上存在差异（图7a），相较于WT+P组，Tg+P组小鼠牙周炎期间在门水平口腔微生物群落的变化表现为厚壁菌门和放线菌门减少，变形菌门和拟杆菌门增加（图7a左）；相较于WT+P组，Tg+P组小鼠牙周炎时在属水平上口腔微生物群的变化表现为孪生球菌属和双歧杆菌属的减少，乳酸菌属和链球菌属的增多（图7a右）。

基于主坐标分析（PCoA）的样本β多样性测定结果也表明了各组的显著变异性：第一轴（PC1）显



a-c: the mRNA expression of IL-1β, IL-6 and TNF-α. The expression of inflammatory factors in the periodontal tissues of mice in the Tg+P group decreased compared with that in the WT+P group. * P<0.05. WT+P: WT periodontitis group; Tg+P: SAP-Tg periodontitis group. IL-1β: interleukin-1 beta; IL-6: interleukin-6; TNF-α: tumor necrosis factor-α. WT: wild type. SAP-Tg: serum amyloid P component-transgenic

Figure 6　Expression of inflammatory factors in mice with periodontitis

图6　小鼠牙周炎时炎症因子的表达

示了41.7%的变异，第二轴（PC2）反映了采样点内的变异，WT+P组和Tg+P组小鼠在菌种组成上存在显著差异（图7b）。

线性判别分析（LDA）效应大小分析结果显示，WT+P组和Tg+P组菌群组成有显著差异，WT+P组差异菌群为孪生球菌属、链球菌属、双歧杆菌属和乳球菌属，Tg+P组差异菌群为斯氏菌属和红蝽菌属，但未发现与牙周炎相关的病原体有显著性差异（图7c）。

2.6　过表达SAP基因在体外抑制巨噬细胞M1型极化并减少向破骨细胞分化

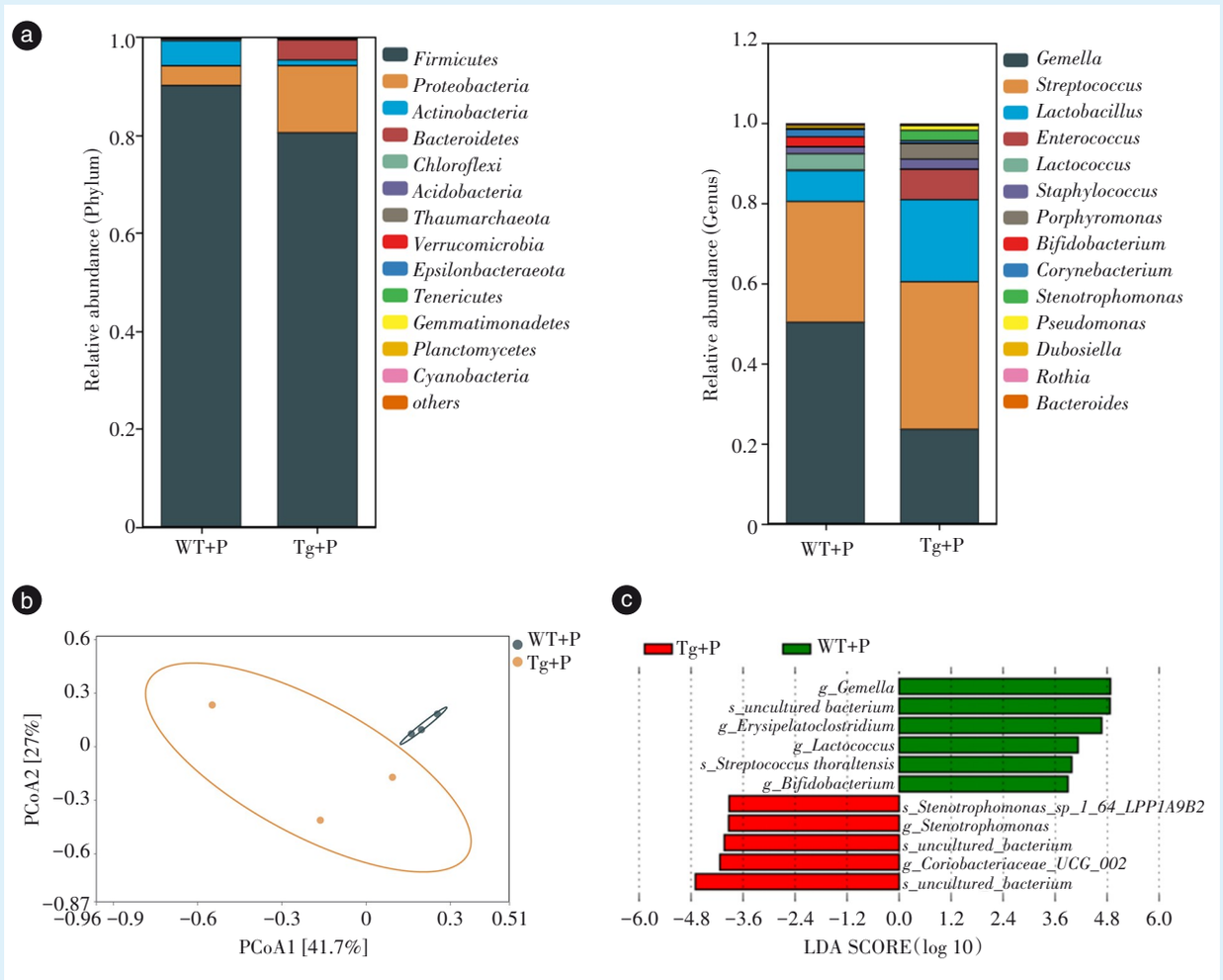
qRT-PCR结果显示，相较于WT+LPS组，Tg+LPS组的M1型巨噬细胞标志物iNOS和CD86表达下调，M2型巨噬细胞标志物CD163和CD206表达上调（图8a、8b）。TRAP染色结果显示，与WT+LPS组相比，Tg+LPS组破骨细胞数量减少（图8c）。

3　讨　论

第四次全国口腔健康流行病学调查报告显示我国中老年人群牙周健康率均不足10%[15-16]。牙周炎导致牙齿脱落的同时还与动脉粥样硬化、类风湿性关节炎和糖尿病等全身性疾病息息相关，是一个亟需解决的重大公共卫生问题[1]。

SAP和C-反应蛋白是经典的短臂五肽，在先天免疫和炎症性疾病中发挥作用[17-18]。SAP主要由肝脏合成，并进入血液循环到达全身，其表达主要由IL-6诱导[19-20]，作为PRMs可以与微生物病原体和凋亡细胞相互作用，并促进其补体介导的清除作用[21]。在人类和大多数哺乳动物中，血浆中SAP的水平维持在20～50 μg/mL相对恒定的水平，而在小鼠中SAP作为一种急性期蛋白，在急性炎症期血浆水平急剧上升，可升高20倍[22]。一些研究已经描述了C-反应蛋白水平与牙周炎相关[23-25]，前期研究也发现SAP缺失时会加剧牙周炎的情况[11]，但未曾有SAP过表达对牙周炎影响的报导。

基因修饰小鼠的优势是具有遗传稳定性、能模拟人体自然发病阶段、可在体内研究特定分子在疾病中的作用机制和靶向治疗，所以被广泛应用于医学研究[26]。在本研究中，选用基因修饰的SAP-Tg小鼠和WT小鼠，通过结扎其上颌第二磨牙建立牙周炎模型。结果显示，相较于WT组，Tg组牙周组织中过表达SAP；相较于WT组和Tg组，WT+P组和Tg+P组小鼠牙周组织中SAP表达均有所上升，说明SAP与牙周炎的发展存在一定的相关性，与Emsley等[7]发现SAP在先天免疫和炎症性疾病中发挥作用的结果一致。当牙周炎发生时，SAP表达水平升高的可能原因是牙周炎导致牙周组织破坏，细胞损伤后释放PRMs，这些分子可能激活免疫系统，促使SAP生成以参与损伤修复；而且，相较于WT+P组，Tg+P组牙周组织中具有更高水平的SAP表达。健康小鼠中，WT组和Tg组小鼠的牙槽骨吸收无明显区别，但牙周炎时WT+P组和Tg+P组小鼠牙槽骨吸收具有明显差异性，Tg+P组小鼠牙槽骨吸收明显减少，其釉牙骨质界和上皮组织之间的联系更紧密，组织结构相对完整，表明SAP-Tg小鼠具有更轻的病理过程。进一步的研究发现，牙周炎时Tg+P组小鼠负责骨降解的破骨细



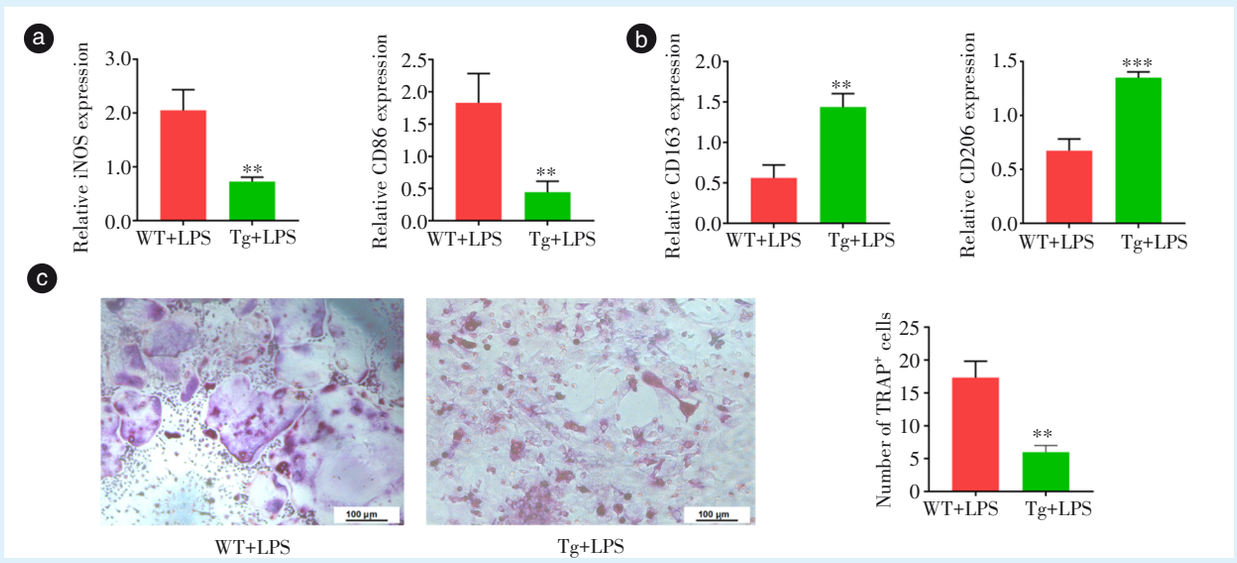
a: colony composition at the phylum and genus levels. The left shows the taxonomic composition of the microbial community at the phylum level. The most abundant bacteria are *Firmicutes*, *Proteobacteria*, *Bacteroidetes*, and *Actinobacteria*. Compared with the WT+P group, the changes in oral microbiota during periodontitis in Tg+P group are characterized by a decrease in *Firmicutes* and *Actinobacteria* and an increase in *Proteobacteria* and *Bacteroidetes*. The right presents the composition of the microbial community at the genus level. The changes in oral microbiota during periodontitis in the Tg+P group are characterized by a decrease in *Gemella* and *Bifidobacterium* and an increase in *Lactobacillus* and *Streptococcus* (n=3). b: the significant variability of each group based on the principal coordinate analysis graph. The first axis (PC1) shows 41.7% of the variation, and the second axis (PC2) reflects the variation within the sampling points, showing that there were significant differences in the composition of bacterial species between the WT+P group and the Tg+P group (n=3). c: LDA effect size graph. The differential microbiota in the periodontitis group of WT+P group were *Gemella*, *Lactococcus*, *Streptococcus*, and *Bifidobacterium*. The differential microbiota in the Tg+P group were *Stenotrophomonas* and *Coriobacteriaceae* (n=3). WT+P: WT periodontitis group; Tg+P: SAP-Tg periodontitis group. LDA: linear discriminant analysis. WT: wild type. SAP-Tg: serum amyloid P component-transgenic

Figure 7　Analysis of oral microorganisms in mice with periodontitis

图7　小鼠牙周炎时口腔微生物分析

胞数量减少，表明SAP基因过表达可以减轻牙周骨质的流失，可能对与牙周炎相关的骨吸收起保护作用。这些发现提示SAP基因可能具有维持牙周健康的作用，对于牙周炎有潜在的治疗作用。

巨噬细胞是先天免疫系统的重要组成部分，通过识别病原体和招募其他免疫细胞到感染部位来帮助调节免疫反应[27-28]。研究显示牙周炎进展期时，会出现明显的巨噬细胞聚集，并向M1型极化[29]。而在本研究中，相较于WT+P组，Tg+P组小鼠牙周组织中的巨噬细胞明显减少，这表明SAP基因在牙周炎症期间可以调节宿主的免疫反应。SAP过表达可以降低巨噬细胞活性，以此减少牙周组织的损伤。炎症介质如细胞因子（如IL-1β、IL-6、TNF-α）和趋化因子是由免疫细胞和牙龈上皮细



a&b: the expression levels of iNOS, CD86, CD163 and CD206 were detected by qRT-PCR respectively. Compared with the WT+LPS group, the Tg+LPS group expressed higher levels of CD163 and CD206, and lower levels of iNOS and CD86 (n=3). c: TRAP staining images of osteoclast differentiation induced by BMDM and statistical graph of osteoclasts. Compared with the WT+LPS group, there were fewer osteoclasts in the Tg+LPS group (n=3). ** P<0.01, *** P<0.001. WT+LPS: BMDM of WT mice+LPS; Tg+LPS: BMDM of SAP-Tg mice+LPS. BMDM: bone marrow-derived macrophages. iNOS: inducible nitric oxide synthase. LPS: lipopolysaccharides. WT: wild type. SAP-Tg: serum amyloid P component-transgenic. TRAP: tartrate resistant acid phosphatase

Figure 8　*In vitro* polarization and TRAP staining images of mouse BMDM in an inflammatory environment

图 8　炎症环境下小鼠骨髓源巨噬细胞体外极化及TRAP染色图像

胞产生的。这些分子招募并激活其他免疫细胞，放大炎症反应[30]。本研究结果显示，Tg+P组小鼠牙周组织中炎症因子IL-1β、IL-6和TNF-α的表达水平明显下调，这一结果表明，通过表达SAP基因，可以显著降低牙周组织内的炎症因子水平，提示SAP控制牙周组织炎症的潜力。

口腔微生物群落由700多种细菌组成，它们互相保持稳定，在健康人中通常是非致病性的[31-32]。然而，根据“关键病原体假说”，某些低丰度的微生物病原体可以将正常的良性微生物群转变为微生物失调致病菌群[33]。研究发现，SAP能与多种细菌结合[34]，促进补体激活、调理作用、吞噬作用和增强对感染的抵抗力，对宿主起到保护作用[35]，但是也有研究表明SAP对细菌具有保护作用，这说明SAP参与微生物的调节[36]，但SAP对微生物的作用不明确[37]。前期研究发现，SAP基因缺失时，巨噬细胞对*Pg*的吞噬功能出现缺陷，造成*Pg*数量增加，巨噬细胞向M1型极化，进而加重了牙周炎。而本研究表明，过表达SAP基因小鼠在牙周炎时口腔微生物也发生了一系列变化，但与牙周炎相关的特定病原体未有明显变化，进而把关注点放在SAP过表达影响巨噬细胞极化的研究上。

有研究表明，炎症环境下炎症因子可直接或间接激活破骨细胞分化从而加重骨吸收[38]，如在炎症过程中，单核巨噬细胞产生的IL-6、TNF-α等炎症因子可以直接刺激破骨细胞前体细胞的分化和激活[39-40]。炎症因子刺激免疫细胞和成骨细胞分泌RANKL也可间接增强破骨细胞的分化和增殖[41-42]。促炎M1型巨噬细胞可释放并激活Th17细胞以产生IL-6、TNF-α和RANKL等促炎介质，也可进一步促进破骨细胞的形成和活性，从而导致牙周炎期间牙槽嵴吸收增多[43-44]。体外实验显示，过表达SAP基因在体外抑制巨噬细胞M1型极化并减少向破骨细胞分化，进一步明确了过表达SAP基因缓解牙周炎可能是通过抑制巨噬细胞向M1型极化，从而减少牙槽骨吸收。

综上所述，SAP过表达可以缓解小鼠的牙周炎，加上前期研究发现SAP缺失会加重小鼠的牙周炎[11]，这些都提示SAP在牙周炎过程中可能是一种保护性物质，本研究为SAP作为牙周炎治疗的新药物提供了进一步的科学依据。但本研究未收集临床样本进行相关验证，SAP过表达时如何抑

制巨噬细胞向M1型极化的相关分子机制也未进行深入研究，因此后续会结合临床，进一步阐明相关的分子机制，为寻找新的牙周炎治疗方案提供理论依据。

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