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牙龈上皮细胞间连接与牙周致病菌关系的研究进展*

黄培勍[1]，贾小玥[2]，赵 蕾[3]，周学东[1]，徐 欣[1△]

1. 口腔疾病研究国家重点实验室 国家口腔疾病临床医学研究中心 四川大学华西口腔医院 牙体牙髓病科（成都 610041）；
2. 口腔疾病研究国家重点实验室 国家口腔疾病临床医学研究中心 四川大学华西口腔医院 儿童口腔科（成都 610041）；
3. 口腔疾病研究国家重点实验室 国家口腔疾病临床医学研究中心 四川大学华西口腔医院 牙周病科（成都 610041）

【摘要】 牙龈上皮屏障是抵御致病菌侵入牙周组织的第一道防线，与牙周疾病的发生发展密切相关。牙周致病菌及其感染炎症微环境可通过下调粒状头样蛋白家族蛋白表达，以及上调上皮连接蛋白编码基因启动子甲基化水平等相关分子机制，抑制牙龈上皮连接蛋白表达，破坏牙龈上皮屏障功能，促进牙周炎的发生发展。本文就细菌及其感染后诱导产生的炎症因子对牙龈上皮细胞间连接的影响以及相关机制等方面对近年来牙龈上皮细胞间连接与牙周致病菌关系的研究进展作一综述。当前研究多集中于单一细菌感染的体外细胞学实验与动物模型研究。我们建议，建立牙龈上皮类器官研究模型，采用多组学研究技术与高分辨三维电镜成像，有望进一步锁定驱动牙周微生态失衡、导致牙龈上皮屏障功能破坏的核心微生物及其关键致病毒力因子，揭示参与牙龈上皮屏障功能维持和破坏的关键细胞分子机制，为牙周炎的发病机制与临床防治提供新的思路。

【关键词】 牙周炎 牙龈上皮屏障 细胞间连接 牙周致病菌

Research Updates: Relationship between Gingival Epithelial Intercellular Junctions and Periodontal Pathogenic Bacteria

HUANG Pei-qing[1], *JIA Xiao-yue*[2], *ZHAO Lei*[3], *ZHOU Xue-dong*[1], *XU Xin*[1△]. 1. *State Key Laboratory of Oral Diseases*, *National Clinical Research Center for Oral Diseases*, *Department of Cariology and Endodontics*, *West China Hospital of Stomatology*, *Sichuan University*, Chengdu 610041, China; 2. *State Key Laboratory of Oral Diseases*, *National Clinical Research Center for Oral Diseases*, *Department of Pediatric Dentistry*, *West China Hospital of Stomatology*, *Sichuan University*, Chengdu 610041, China; 3. *State Key Laboratory of Oral Diseases*, *National Clinical Research Center for Oral Diseases*, *Department of Periodontology*, *West China Hospital of Stomatology*, *Sichuan University*, Chengdu 610041, China
△ Corresponding author, E-mail: nixux1982@hotmail.com

【**Abstract**】 Gingival epithelial barrier is the first line of defense of periodontal tissues against the invasion of pathogenic bacteria. The destruction of gingival epithelial barrier is closely related to the development of periodontal disease. Studies have shown that periodontal pathogenic bacteria and their inflammatory microenvironment can inhibit the expression of gingival epithelial junctional proteins via molecular mechanisms such as the downregulation of the expression of grainyhead-like protein family and the upregulation of the methylation level of gene promoter of epithelial connexin, and thus cause damage to the gingival epithelial barrier and the development of periodontitis. We herein reviewed the effects of bacteria and inflammatory factors induced by bacterial infection on gingival epithelial intercellular junctions and related mechanisms, and summarized the research progress on the relationship between gingival epithelial intercellular junctions and periodontal pathogenic bacteria in recent years. Most recent studies were focused on *in vitro* cytological experiments and animal models of infections caused by a single kind of bacterium. We have suggested that building gingival epithelial organoid model and combining multi-omics approaches with high resolution three-dimensional electron microscopy are expected to help pinpoint the key microorganisms and their most important virulence factors that trigger periodontal microecologcal imbalance and cause functional damage to the gingival epithelial barrier, to reveal the key molecular mechanisms involved in the maintenance and destruction of gingival epithelial barrier function, and to provide new perspectives on the pathogenesis and the clinical prevention and treatment of periodontitis.

【**Key words**】 Periodontitis Gingival epithelial barrier Intercellular junctions Periodontal pathogen

上皮屏障是人体重要的生物屏障，是多个组织和器官，如呼吸道、胃肠道、口腔等与外部环境的分界线，其功能破坏是导致组织和器官功能失调及疾病发生的重要危险因素[1]。牙龈上皮是牙周组织抵御致病菌入侵的首道屏障，牙龈上皮细胞间连接是牙龈上皮屏障的重要组成部分[2]。本文就近年来牙龈上皮细胞间连接及其与牙周致病菌关系的相关研究进行回顾，以期为牙周炎发生发展相关机制的阐明提供新的思路。

1 牙龈上皮细胞间连接

牙龈上皮由上皮层和固有层两部分组成，健康的牙

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△ 通信作者，E-mail：nixux1982@hotmail.com

龈上皮可保护牙周组织抵抗咀嚼时的摩擦力，抵御组织外部的刺激及病原微生物的入侵[3]。牙龈上皮细胞间连接是牙龈上皮屏障的重要组成部分，主要包括紧密连接、黏着连接、缝隙连接和桥粒(图1)。

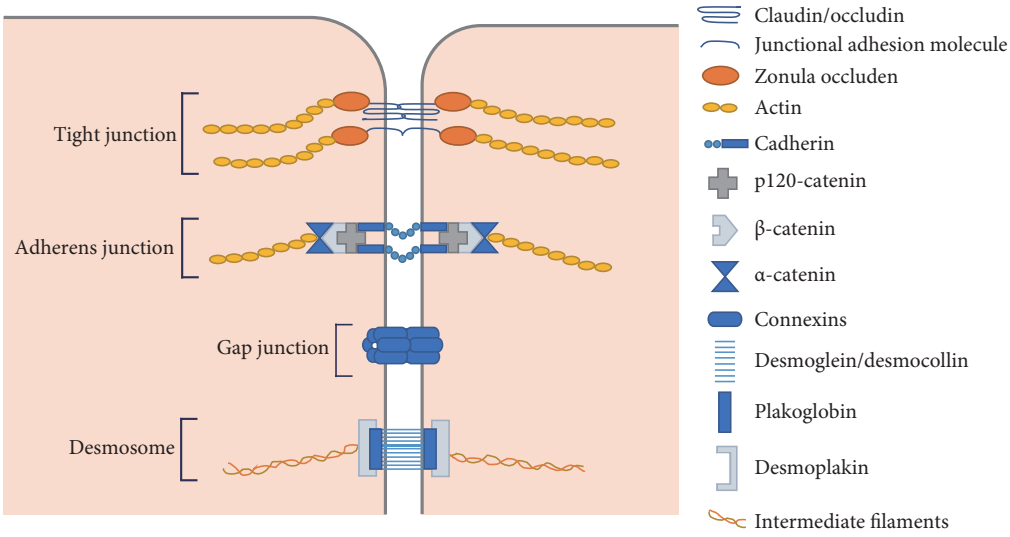

图 1　牙龈上皮细胞间连接及相关蛋白

Fig 1　Gingival epithelial intercellular junctions and the relevant proteins

紧密连接(tight junction)广泛分布于消化道上皮、泌尿道上皮、脑毛细血管内皮等组织中，作为半透性屏障参与维护组织内环境稳定[4]。牙龈上皮细胞间的紧密连接主要由密封蛋白(claudin)、闭合蛋白(occludin)、连接黏附分子(junctional adhesion molecule, JAM)、闭合小环蛋白(zonula occluden, ZO)等组成[5]。claudin是维持紧密连接结构和功能的重要组成之一，在牙龈上皮中以claudin-1表达为多。闭合蛋白多表达于牙龈上皮表层，牙龈上皮闭合蛋白表达减少常伴随牙龈上皮跨上皮电阻降低[6]。ZO在紧密连接中起“骨架”作用，可将肌动蛋白与多种连接蛋白相互连接形成致密的连接结构[7]。JAM隶属免疫球蛋白超家族，人牙龈上皮细胞主要表达JAM-1，可与闭合蛋白、密封蛋白等蛋白共同参与牙龈上皮细胞间连接的调节[8-9]。黏附连接(adherens junction)主要由骨架蛋白、接头蛋白和黏附受体构成。骨架蛋白是细胞内微丝、微管和中间纤维丝的主要成分，起支撑细胞形态、传导细胞内外信息的作用[10]。接头蛋白的主要成分是连环蛋白，主要包括α-连环蛋白(α-catenin)、β-连环蛋白(β-catenin)和p120-连环蛋白(p120-catenin)等[11]。黏附受体的主要成分为钙黏素，可介导Ca^{2+}依赖的细胞间黏附，牙龈上皮中主要表达上皮钙黏蛋白(epithelium cadherin, E-cadherin)[12]。缝隙连接(gap junction)是由相邻细胞细胞质膜上连接子相互对接形成的允许离子和小分子代谢物通过的通道[13-14]。连接子由六个相同或相似的跨膜蛋白——连接蛋白(connexins, Cxs)环绕而成，可与细胞骨架蛋白、黏附连接蛋白和紧密连接蛋白等相互作用[13]。在口腔上皮组织中，Cxs主要分布于在复层鳞状上皮细胞之间[15]。桥粒(desmosome)主要分布于复层鳞状上皮，主要由两类蛋白质构成：一类为跨膜蛋白，主要包括桥粒芯糖蛋白(desmoglein)和桥粒芯胶蛋白(desmocollin)；另一类为胞质内的接合蛋白，主要包括桥粒斑蛋白(desmoplakin)及桥粒斑珠蛋白(plakoglobin)等[16]。研究显示，桥粒与天疱疮、多形性红斑、慢性牙周炎等口腔疾病密切相关[17]。

牙龈上皮是牙周组织抵御外界微生物入侵和有害性外源性刺激的第一道屏障，在牙周炎的发生、发展过程中扮演重要角色。牙龈上皮细胞通过紧密连接、黏附连接、缝隙连接、桥粒等结构相互连接，共同形成的物理性屏障是牙龈上皮屏障的基础[18]。微生物入侵牙龈上皮组织主要包括两大途径，其一为直接破坏细胞结构的细胞内途径，其二为破坏牙龈上皮细胞间连接的细胞旁途径[19]。探究牙周致病菌及其诱导的炎症微环境对牙龈上皮细胞间连接的影响有望为牙周炎的防治提供新的思路。

2　细菌及其产物对牙龈上皮细胞间连接的影响

牙龈卟啉单胞菌(*Porphyromonas gingivalis*, *P. gingivalis*)、齿垢密螺旋体(*Treponema denticola*, *T. denticola*)、伴放线聚集杆菌(*Aggregatibacter actinomycetemcomitans*, *A. actinomycetemcomitans*)、具核梭杆菌(*Fusobacterium nucleatum*, *F. nucleatum*)、福塞斯

坦纳菌(*Tannerella forsythia*, *T. forsythia*)等是重要的牙周致病菌[20]。近期研究发现,牙周致病菌可通过影响牙龈上皮细胞间连接,影响牙周疾病的发生发展[21]。

*P. gingivalis*属革兰阴性厌氧菌,大量研究显示,*P. gingivalis*对牙龈上皮细胞间连接有显著影响。*P. gingivalis*感染后的口腔上皮细胞ZO-1、闭合蛋白、JAM-1等表达下调,上皮渗透性升高,跨上皮电阻降低,上皮完整性破坏[22-23]。*P. gingivalis*可下调牙龈上皮细胞E-cadherin表达,导致细菌侵入增加,上皮结构破坏[24]。*P. gingivalis*感染后人牙龈上皮细胞桥粒斑菲素蛋白(plakophilin)表达显著降低,牙龈上皮渗透性升高[25]。上述研究提示,*P. gingivalis*与牙龈上皮紧密连接、黏附连接、桥粒等细胞间连接均密切相关,然而关键致病毒力因子目前尚未完全阐明。牙龈素(gingipains)、脂多糖(lipopolysaccharide, LPS)、菌毛(fimbriae)等是*P. gingivalis*的主要毒力因子[26]。近年来,*P. gingivalis*相关毒力因子对牙龈上皮细胞间连接的影响逐渐成为研究热点。牙龈素是*P. gingivalis*产生的胰蛋白酶样半胱氨酸蛋白酶,研究发现,牙龈素处理后的牙龈上皮细胞闭合蛋白和E-cadherin表达下调[27]。使用牙龈素特异性抑制剂或敲除*P. gingivalis*的牙龈素表达相关基因后,牙龈上皮细胞间连接蛋白表达水平回升,提示牙龈素或与牙龈上皮屏障功能破坏密切相关[28]。LPS是*P. gingivalis*外膜的重要组成成分,研究发现其可降低牙龈上皮细胞E-cadherin、闭合蛋白、claudin-1、claudin-4、claudin-15等蛋白的表达;上述细胞经马来酸伊索拉定和阿奇霉素处理后,E-cadherin表达回升,同时伴有牙龈上皮渗透性降低,上皮屏障功能恢复,提示LPS或可破坏牙龈上皮细胞间连接,影响牙龈上皮屏障功能[29-31]。菌毛是自细菌细胞外膜延伸出的薄层蛋白附属物,是促进*P. gingivalis*侵入宿主组织的重要成分[32]。研究发现,具有Ⅱ型菌毛的*P. gingivalis*可降解细胞间的黏附连接相关蛋白,提示*P. gingivalis*的菌毛或可影响牙龈上皮屏障功能[33]。

*F. nucleatum*属革兰阴性厌氧菌,可表达多种黏附素连接牙周早期定植菌及晚期定植菌,与牙周菌斑的形成密切相关,是龈上菌斑、龈下菌斑、牙周袋内感染部位的优势菌[34-35]。*F. nucleatum*感染后的肠上皮细胞occludin及ZO-1表达下调,单层上皮细胞跨上皮电阻降低,渗透性升高,提示*F. nucleatum*或可影响上皮细胞间连接,破坏上皮屏障功能[36]。共培养4 h后*F. nucleatum*可黏附于口腔角化细胞表面,5 h后可侵入单层口腔角化上皮细胞间[37]。*F. nucleatum*感染后的口腔上皮细胞跨上皮电阻降低[38]。*F. nucleatum*处理5 d后,口腔上皮细胞E-cadherin表达下调[39]。上述研究提示,*F. nucleatum*及其产物或与牙龈上皮细胞间连接及牙龈上皮屏障功能的破坏密切相关。

*A. actinomycetemcomitans*属革兰阴性兼性厌氧菌,与侵袭性牙周炎密切相关[40]。*A. actinomycetemcomitans*可抑制牙龈上皮细胞ZO-1、E-cadherin、β-catenin等蛋白的表达[41-42]。*A. actinomycetemcomitans*的外膜蛋白可抑制人牙龈上皮细胞表达Cx43蛋白[43]。上述研究提示,*A. actinomycetemcomitans*或对牙龈上皮紧密连接、黏附连接和缝隙连接均存在影响,与牙龈上皮屏障功能的破坏密切相关。

*T. denticola*属革兰阴性厌氧菌,与急性坏死性溃疡性牙龈炎、侵袭性牙周炎及慢性牙周炎密切相关[44]。经*T. denticola*处理后的上皮细胞细胞间连接疏松,细胞间隙结构塌陷,上皮渗透性升高[45]。*T. denticola*可通过抑制上皮细胞表达ZO-1破坏上皮屏障完整性,侵入上皮组织[46]。经*T. denticola*处理后的牙龈上皮ZO-1和claudin-1表达下调,牙龈上皮跨上皮电阻降低[47]。此外,由*P. gingivalis*、*F. nucleatum* 、*T. denticola* 、*T. forsythia*等10种细菌共同培养形成的龈下生物膜对牙龈上皮紧密连接及桥粒等细胞间连接相关蛋白的表达均有显著抑制作用[48]。上述结果提示,*T. denticola*或可通过破坏牙龈上皮细胞间连接,破坏牙龈上皮屏障完整性。

上述研究表明,牙周致病菌可通过抑制牙龈上皮细胞间多种连接蛋白的表达,破坏上皮屏障功能。然而,在牙周病的发生发展过程中,参与维持牙龈上皮屏障功能的关键连接蛋白尚不清楚,其结构破坏进而导致牙周致病菌入侵的具体分子机制亦未明确。此外,当前研究多集中于单一牙周致病菌,牙周炎作为一种多细菌感染性疾病,有待建立更完善的疾病模型与临床队列,锁定导致上皮屏障功能破坏的牙周关键致病菌,解析其调控细胞间连接蛋白的分子机制。

3 炎症因子对牙龈上皮细胞间连接的影响

牙龈上皮组织中存在着由多种免疫细胞构成的复杂免疫网络,细菌感染诱发牙周组织炎症时,活化的免疫细胞能够分泌多种细胞因子参与炎症反应,如白细胞介素(interleukin, IL)、肿瘤坏死因子(tumor necrosis factor, TNF)、干扰素(interferon, IFN)等,进一步破坏牙龈上皮屏障功能[49]。

IL是一类常见淋巴因子,牙龈上皮细胞IL-8表达升高常伴随牙龈上皮细胞间黏附作用减弱及上皮完整性破坏[50]。经IL-1β处理后的人牙龈成纤维细胞黏附连接及紧密连接相关蛋白β-catenin、E-cadherin、ZO-1等表达显著

降低[51]。动物实验发现, 牙周炎小鼠牙龈组织中IL-31表达升高, 伴随紧密连接相关蛋白claudin-1表达降低[52]。TNF主要由活化的巨噬细胞、NK细胞及T淋巴细胞产生[53]。TNF-α处理后的牙龈上皮细胞claudin-1及E-cadherin表达降低, 牙龈上皮渗透性升高[54]。阿奇霉素、茶多酚等处理后的牙龈上皮细胞TNF-α表达下调, 牙龈上皮屏障功能增强[55-56]。上述研究提示, IL、TNF-α等炎症因子或参与牙龈上皮屏障功能的调节。近年来, IFN-γ、TGF-β、抗微生物肽等也因与牙龈上皮屏障功能的相关性受到广泛重视[57-59]。然而, 在牙周炎症微环境中参与调控的牙龈上皮细胞间连接蛋白的核心因子尚不清楚, 有待通过多组学联合研究锁定关键炎症因子, 解析其调控细胞间连接蛋白表达的分子网络, 阐明其调控牙龈上皮屏障功能的具体分子机制。

4　牙周致病菌破坏牙龈上皮细胞间连接的机制

牙周致病菌与牙龈上皮细胞间连接的破坏密切相关, 但相关分子机制尚不清楚。近期研究显示, 粒状头样(grainyhead-like, GRHL)蛋白家族、DNA甲基化等相关分子机制与牙龈上皮细胞间连接破坏有关。

GRHL2蛋白隶属GRHL家族, 参与调控胚胎发育、上皮细胞分化以及表皮损伤修复等一系列生命过程[60]。近期研究发现, GRHL家族和上皮屏障功能密切相关[61]。*P. gingivalis*感染后的人角质形成细胞GRHL2表达降低; 敲除*grhl2*基因后, 人角质形成细胞E-cadherin、claudin-1、ZO-1等蛋白表达显著降低, 上皮渗透性升高, 上皮屏障功能破坏。*grhl2*基因敲除小鼠上述蛋白表达降低, 外周血中*P. gingivalis*增加, 牙槽骨吸收明显[62]。上述研究提示, GRHL或参与调控牙周致病菌导致的牙龈上皮屏障功能破坏, 从而影响牙周炎发生发展。

DNA甲基化是指S-腺苷甲硫氨酸分子上的甲基转移到DNA分子中胞嘧啶的第五位碳原子上, 形成5-甲基胞嘧啶的过程。DNA甲基化可影响基因表达, 与口腔癌、口腔扁平苔藓、慢性牙周炎等口腔疾病密切相关[63]。临床研究发现, 与牙周健康人群相比, 慢性牙周炎患者牙周组织中促炎细胞因子、模式识别受体、细胞外基质蛋白等均存在表达异常及相应基因启动子区甲基化水平改变, 提示牙周炎发生发展的过程中存在异常的DNA甲基化现象[64]。*P. gingivalis*感染后的人牙龈上皮细胞*tjp*1、*cdh*1和*pkp*2基因启动子区DNA甲基化水平上调, 所对应的紧密连接、黏附连接及桥粒相关蛋白ZO-1、E-cadherin及plakophilin-2的基因与蛋白表达下调, 牙龈上皮跨上皮电阻降低; 使用DNA甲基转移酶抑制剂后可显著下调上述基因启动子区DNA甲基化水平, 上调*tjp*1、*cdh*1和*pkp*2基因与蛋白表达以及牙龈上皮跨上皮电阻, 恢复*P. gingivalis*感染后牙龈上皮的屏障功能[65]。上述研究提示, DNA甲基化在牙周致病菌破坏牙龈上皮细胞间连接的过程中发挥了重要作用, 但牙周细菌感染上调细胞间连接蛋白基因甲基化水平的空间位点及其具体的分子机制, 尚有待进一步通过疾病动物模型, 结合单细胞测序与空间转录组学等前沿技术进一步深入分析与验证。

5　总结与展望

牙龈上皮屏障是牙周组织抵御牙周致病菌入侵的第一道防线, 由紧密连接、黏附连接、缝隙连接及桥粒形成的牙龈上皮细胞间连接是牙龈上皮屏障的重要组成部分。近期大量研究结果表明, 牙龈上皮细胞间连接与牙周致病菌及其诱导产生的炎症因子关系密切。然而, 当前研究多集中于单一细菌感染的体外细胞学实验与动物模型研究。牙周炎的发生发展是口腔微生态失衡下多细菌生物膜与宿主免疫交互作用的结果, 介导牙龈上皮屏障功能破坏的核心微生物尚不清楚, 参与牙龈上皮屏障功能维持与破坏的牙周细胞及其相关免疫分子机制也有待进一步研究; 此外, 牙龈屏障功能维持在抵御牙周组织破坏、延缓牙槽骨炎性吸收中的作用与地位也有待进一步验证。亟待通过建立牙周炎临床队列研究以及疾病动物模型、牙龈上皮类器官等研究模型, 结合宏基因组、代谢组及单细胞转录组等多组学联合技术与高分辨三维电镜成像技术, 锁定驱动牙周微生态失衡、导致牙龈上皮屏障功能破坏的核心微生物及其关键致病毒力因子, 揭示参与牙龈上皮屏障功能维持和破坏的关键细胞分子机制与关键连接蛋白, 为牙周炎的临床防治提供新的靶点与思路。

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