doi: 10.13241/j.cnki.pmb.2014.30.006

纤粘连蛋白在实验性牙周炎鼠牙龈组织中的表达 *

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摘要 目的:观察分析大鼠实验性牙周炎牙龈组织中纤粘连蛋白(fibronectin,FN)的表达及意义。**方法:**26只8周龄雄性Wistar大鼠,随机分为局部丝线结扎高糖软食4周和8周两组,每组实验动物10只,空白对照组3只。运用免疫组化方法,观察分析FN在健康牙龈组织中、牙周炎牙龈组织中的表达及意义。**结果:**健康牙龈组织中,FN相对均匀弥漫表达于整个牙龈结缔组织基质内;牙周炎牙龈组织中,FN表达具有部位特异性,即上皮下结缔组织最上部基质内FN表达明显下调;结合上皮根端基底细胞基底面下FN表达明显上调,表达强度和范围随结合上皮根向增生程度的增加而增强。**结论:**炎性刺激下调炎症中心区牙龈结缔组织基质内FN表达;炎症刺激上调结合上皮根端基底细胞基底面下FN表达,其表达强度和范围随结合上皮根向增生程度的增加而增强扩大,暗示FN参与牙龈结合上皮根向增生迁移活动。

关键词:纤粘连蛋白;牙周炎;组织形态;免疫组化

中图分类号:R781.4+2 **文献标识码:**A **文章编号:**1673-6273(2014)30-5821-03

Expression of Fibronectin in Gingival Tissues of Experimental Periodontitis*

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ABSTRACT Objective: To observe the expression of fibronectin in gingival tissues of experimental periodontitis. **Methods:** A total of 26 Wistar rats, male, eight-week-old, were used, 20 of which were randomly chosen as experimental objects and 6 as control ones. The experimental objects were randomly divided into 2 groups with equal numbers, then ligated with silk suture and fed with high glucose diet for 4 weeks and 8 weeks respectively. By using immunohistochemistry, the expression of fibronectin healthy gingival tissues and periodontitis gingival tissues was observed. **Results:** In healthy gingival tissues, fibronectin showed a relative diffuse distribution in the gingival connective tissue matrix. In periodontitis gingival tissues, the expression of fibronectin indicated a regional specificity. Fibronectin expressed decreasingly in the upmost subepithelium gingival connective tissue matrix, while increasely in the basal surface of base cell beneath the junctional epithelium root apex. The expression intensity and circumscription was enhanced with junctional epithelium root apex proliferation. **Conclusions:** Inflammation stimulus decreased the expression of fibronectin in the centr-inflammation's gingival connective tissue matrix, and breakdown collagen-matrix wire frame texture; Inflammation stimulus increased the expression of fibronectin in the basal surface of base cell of the junctional epithelium root apex. The expression intensity and circumscription were enhanced with junctional epithelium root apex proliferation; Fibronectin possibly participates in epithelium cells proliferationand transference of junctional epithelium for root apex direction.

Key words: Fibronectin; Periodontitis; Tissue morphous; Immunohistochemistry

Chinese Library Classification: R781.4+2 Document code: A

Article ID:1673-6273(2014)30-5821-03

前言

纤粘连蛋白(fibronectin, FN)是一种重要的细胞外基质成分之一[1-3],为具有高度粘附活性的糖蛋白[4-6],广泛弥漫地表达于人和多种动物健康牙龈结缔组织基质内[7]。随着个体年龄的增长,FN在结合上皮基底面下形成线形染色,此染色线随着结合上皮根向迁移的不断延伸而不断延长,推测牙龈结合上皮根向迁移与FN有关[8]。FN在非健康牙周组织内的表达形式、部位和强度因牙周疾病性质的不同而存在着明显的差异[9],其影响机制及其在疾病发生发展中的作用尚缺乏系统研究。

本实验拟通过动物实验方法、免疫组化技术和光镜手段,观察FN在大鼠牙周炎牙龈组织中的表达,探讨其在牙周组织

* 基金项目:黑龙江省科技厅基金项目(D2004-43)

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(收稿日期:2014-03-04 接受日期:2014-03-30)

炎性破坏及结合上皮根向迁移中的作用。

1 材料与方法

1.1 材料

26 只 8 周龄雄性 Wistar 大鼠，体重 200-250g。所选大鼠牙列完整[2(I 1/1，C 0/0，P 0/0，M 3/3)]，牙体无缺损及畸形，牙周无发育异常及炎症。随机分为局部丝线结扎高糖软食 4 周组和局部丝线结扎高糖软食 8 周组两大组；每一小组大鼠 13 只，其中施以实验因素 10 只，空白对照 3 只。

1.2 方法

1.2.1 大鼠牙周炎实验模型的建立 1%戊巴比妥钠腹腔注射麻醉实验组大鼠，3/0 丝线于双侧上颌第二磨牙牙颈部龈缘水平结扎，并沿根尖方向压入龈沟内（以不损伤结合上皮为原则）。定期检查丝线结扎情况。实验组大鼠配以高糖软食（100g/L 高糖水代替饮水，常规鼠料浸泡成软食代替颗粒饲料）。对照组大鼠饮水及饲料正常。

1.2.2 牙周组织标本制备 按实验设计，颈椎脱位法分组处死实验动物，解剖分离出双侧磨牙区牙体 - 牙周联合组织块，10%中性福尔马林液常温下固定 24 小时，10%EDTA 液微波脱钙 3-4 周，常规石蜡包埋，近远中向连续切片。每个组织块，选择颊舌向中点处组织切片 10 张，每组 5 张。

1.2.3 FN 免疫组织化学染色 片厚 0.5-1 μm。用 APES 处理过的载玻片捞片，烤片；脱蜡、脱苯；蒸馏水水洗 2 遍，3% H_2O_2 阻断 10 分钟，蒸馏水水洗 2 遍；滴加胃蛋白酶，37℃ 温箱内孵育 20 分钟；PBS 漂洗 3 次（每次 5 分钟），滴加兔抗鼠 FN 多克隆抗体稀释液 4℃ 过夜；吹掉一抗，PBS 漂洗 3 次（每次 5 分钟），滴加二抗工作液，置保湿盒内 20 分钟；吹掉二抗，PBS 漂洗 3 次（每次 5 分钟），DAB 显色，光镜下控制显色时间；流水冲洗 3-5 分钟；苏木素复染；脱水、透明，中性树胶封固，烘片。光镜下观察，Motic Med 6.0 数码医学图像分析系统摄片。

2 结果

2.1 对照组 FN 的表达

结合上皮根方附着于釉牙骨质界之牙骨质端。胶原纤维束边缘清晰，染色均匀，排列规则有序。FN 阳性表达为棕黄色颗粒，定位于牙龈固有层和牙周膜血管内。FN 相对均匀弥漫表达于整个牙龈固有层结缔组织基质内；在结合上皮基底面，FN 粗线形密集表达；在沟内上皮下毛细血管密集区毛细血管周围基质内 FN 凝集纤维网样表达，(图 1)。

2.2 牙周炎模型组 FN 的表达

局部丝线结扎高糖饮食使大鼠牙周组织产生炎症反应：结合上皮增生、根向迁移、钉突形成；结合上皮冠方与牙根表面分离形成牙周袋，牙周袋壁上皮增生溃疡；牙龈固有层中血管增生、扩张、充血、血栓形成，炎性细胞浸润；牙龈上皮下和牙周膜冠方胶原纤维束水肿、变性、溶解、排列紊乱。

FN 的表达形式和表达强度具有明显部位特异性变化，表达强度和范围随结合上皮根向增生程度的增加而增强。在牙周袋上皮下和冠方结合上皮下结缔组织基质内，FN 棕黄色颗粒表达明显减少或消失；在根方结合上皮基底面，FN 棕黄色颗粒细线形表达；结合上皮浅层结缔组织内 FN 深棕黄色凝集团块面积实验性牙周炎 8 周组比实验性牙周炎 4 周组大；结合上皮下深层结缔组织基质内，FN 棕黄色纤维网状表达(图 2-3)。

图 1 对照组大鼠牙龈乳头 FN 免疫组化染色(× 400)

Fig.1 Immunohistochemical staining for the fibronectin in rat gingival papilla in control group(× 400)

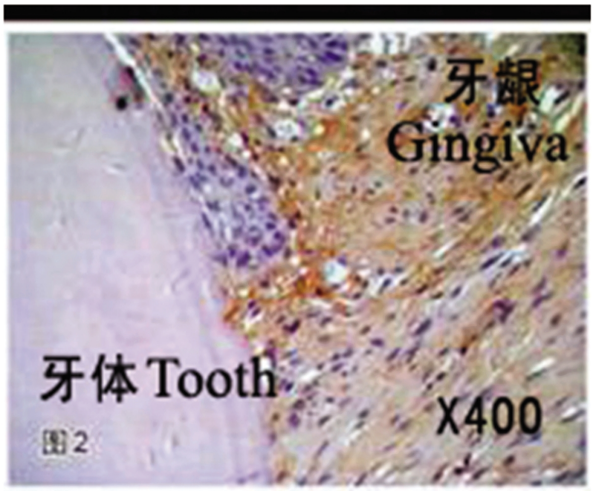

图 2 实验性牙周炎 4 周组大鼠牙龈乳头 FN 免疫组化染色(× 400)

Fig.2 Immunohistochemical staining for the fibronectin in rat gingival papilla in experimental periodontitis for four weeks(× 400)

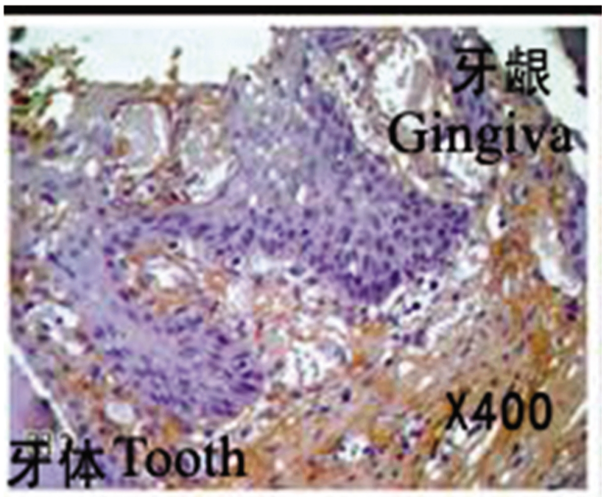

图 3 实验性牙周炎 8 周组大鼠牙龈乳头 FN 免疫组化染色(× 400)

Fig.3 Immunohistochemical staining for the fibronectin in rat gingival papilla in experimental periodontitis for eight weeks(× 400)

3 讨论

3.1 FN 在牙龈结缔组织基质中的表达及意义

FN 是牙龈固有层结缔组织基质的主要分子成分之一[10],为糖蛋白[11]。FN 分子结构具有与细胞表面整合素受体和胶原纤维等物质相结合的位点,是细胞间及细胞与结缔组织间的粘附介质[12]。I 型胶原构成牙龈固有层结缔组织的大部分,使牙龈具有一定强度的张力,是重要的机体支持结构之一[10]。

本实验结果显示:FN 在健康对照鼠牙龈固有层内均匀弥漫表达,胶原纤维染色均匀、排列规则;在实验性牙周炎鼠牙龈固有层内表达下调,胶原纤维染色不均匀、断裂、消失,血管扩张充血,组织水肿。表明:牙周炎致病因素破坏牙龈胶原 -FN 基质网架结构,牙龈胶原 - 基质网架结构破坏、血管扩张充血和组织水肿是牙龈松软红肿的病理学基础。

龈牙结合部是龈上、下菌斑积聚处,是宿主防御系统与细菌相互抗争的重要场所[10]。某些牙周致病菌能刺激成纤维细胞、多型核中性粒细胞和巨噬细胞产生细胞外基质降解酶,同时还能产生纤维蛋白溶解酶原激活物,将纤维蛋白酶原转化为纤维蛋白酶,继而激活潜在形式的基质金属蛋白酶,降解细胞外基质[13]。在狗实验性牙周炎组织中,可见到 FN 被部分降解成纤维无定形物质[14]。本实验结果显示,FN 在实验性牙周炎鼠牙龈固有层内的表达下调存在部位性和时间性差异,表明:炎症刺激程度及持续时间影响牙龈固有层结缔组织基质中 FN 表达。

3.2 FN 在牙龈血管周围细胞外基质中的表达及意义

FN 在机体内的存在形式有血浆型 FN(可溶解性)和细胞型 FN(不可溶性)两种,其中血浆型 FN 是一种免疫调理蛋白[15],可促进网状内皮系统和吞噬细胞的吞噬功能,帮助清理细菌、免疫复合物和坏死组织碎片等[16]。在组织损伤修复过程中,伤口周围血管可产生 FN,使其在血管壁表达增加[17]。

本实验结果表明牙周的炎性刺激影响牙龈毛细血管周围基质内 FN 的表达强度和表达类型。牙周炎牙龈组织内毛细血管周围基质 FN 是源于血浆型 FN,还是源于炎症时结缔组织内细胞合成,以及是否参与局部免疫防御反应尚需进一步研究。

3.3 FN 在结合上皮周围细胞外基质中的表达及意义

结合上皮可阻止来自菌斑或龈沟液的对牙周组织的炎性刺激,对维护牙周组织的内外微环境起基础屏障功能[10]。牙周炎重要的病理学特征之一是结合上皮根向增生迁移。目前的研究结果表明,结合上皮根向迁移可能涉及多种因素在内,如牙周组织的炎症,与年龄相关的生理性牙龈退缩或牙齿的被动萌出[18]。但有关于结合上皮根向迁移的具体机制尚不明了。

Sakai T 推测结合上皮根端下方的 FN 可能是细胞迁移的"扳机点"[19]。FN 可通过介导细胞对不同物质的粘附或延展,促进细胞尤其是上皮细胞的迁移[20]。FN 的功能特性可能与上皮细胞的移动性和稳定性有关,从而诱导结合上皮的根向迁移。

本实验中随着实验时间的延长,实验性牙周炎大鼠牙龈结合上皮根向迁移加重,FN 染色也随之沿结合上皮基底部延展,表达进一步上调。暗示:结合上皮根端下胶原纤维束附着丧失为结合上皮根向迁移提供所需空间,基底细胞基底面下 FN 表达上调为结合上皮根向增生迁移提供信号。

综上所述,FN 在牙周炎前后牙龈组织中其表达形式及分布部位都发生了显著的变化。FN 作为牙周组织细胞外基质的重要组成部分,在牙周炎的发生、发展过程中,发挥着重要的作用,但 FN 与组织细胞间的具体作用机制尚有待更多的实验研究加以验证。

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位点的多态性与慢性牙周炎不具有显著相关性,但慢性牙周炎是一种复杂的多基因疾病,TGF-β1 基因其它位点的多态性以及其它细胞因子的基因多态性都可能影响宿主对牙周炎的易感性。因此,探索不同基因的多态性与牙周炎的关系仍是十分必要的,可从基因方面揭示牙周炎的发病机制,为临床工作提供理论依据。

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