RNAIII抑制肽抑制葡萄球菌对Hela细胞的黏附

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文章亮点：

1 RNAIII抑制肽是最近发现的葡萄球菌群体感应的有效抑制剂，具有预防和治疗葡萄球菌感染的潜在价值。

2 课题组前期试验证实，RNAIII抑制肽可以有效减少葡萄球菌对骨科内植物表面的黏附。本次实验拟探索RNAIII抑制肽能否抑制细菌对宿主细胞的黏附。

关键词：

植入物；人工假体；表皮葡萄球菌；RNAIII抑制肽；黏附；HeLa细胞；群体感应；生物被膜；实验研究；国家自然科学基金

主题词：

组织工程；细菌；感染；核糖核酸；葡萄球菌

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摘要

背景：葡萄球菌导致的细菌感染和生物被膜形成可在骨科植入物或创口愈合时发生。受细菌群体感应机制调控，葡萄球菌RNAIII抑制肽可以干预葡萄球菌的群体感应系统，阻断葡萄球菌细胞间的信号传导通路，从而抑制葡萄球菌的生物被膜形成，防止葡萄球菌感染。

目的：观察RNAIII抑制肽抑制表皮葡萄球菌对人宫颈癌上皮细胞黏附的效果。

方法：体外培养人宫颈癌上皮细胞，实验分4组，空白组每孔加入DMSO的生理盐水，RNAIII抑制肽组加含RNAIII抑制肽的DMSO溶液，左氧组加入左氧氟沙星的水溶液，联合组用药剂量参照上述两组联合干预。通过组间对照的方法，对比研究表皮葡萄球菌在生理盐水、RNAIII抑制肽、左氧氟沙星及两药联合作用下对Hela细胞的黏附情况。

结果与结论：空白组 Hela 细胞层表面有大量细菌黏附，而各用药组细菌黏附的数量均显著低于空白组($P < 0.001$)，左氧组光点计数明显低于RNAIII抑制肽组($P < 0.05$)，而联合组Hela细胞层表面黏附的细菌数量进一步降低($P < 0.01$)。结果证实，RNAIII抑制肽可以有效抑制表皮葡萄球菌对宿主细胞表面的黏附，并且与抗生素有协同作用。

邢庆昌，郝立波，王继芳. RNAIII抑制肽抑制葡萄球菌对 Hela 细胞的黏附[J].中国组织工程研究，2014，18(44):7183-7187.

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RNAIII inhibiting peptide suppresses the adhesion of *staphylococcus epidermis* on the Hela cells

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Abstract

BACKGROUND: Staphylococcal infections and its biofilm formation can occur when orthopedic implants or wound is healing, and are regulated by bacterial population sensing mechanism. RNAIII inhibiting peptide intervenes the quorum-sensing system of staphylococcal and blocks the signal transduction among staphylococcal cells, and inhibits staphylococcal biofilm formation, and then prevents staphylococcal infections.

OBJECTIVE: To investigate the influence of RNAIII inhibiting peptide on the adhesion of *staphylococcus epidermis* to the Hela cells.

METHODS: The Hela cells were cultured *in vitro*. There were four groups in this study. In the blank group, saline with dimethyl sulfoxide was added in each well. In the RNAIII inhibiting peptide group, dimethyl sulfoxide solution containing RNAIII inhibiting peptide was added. In the levofloxacin group, levofloxacin was added. In the combination group, the dose was in accordance with above methods. Using intergroup control method, the adhesion of *staphylococcus epidermis* to the Hela cells was compared under the effects of saline, RNAIII inhibiting peptide and levofloxacin and their combination.

RESULTS AND CONCLUSION: In the blank group, abundant bacterial adhered to Hela cells. The number of adhered bacteria was significantly lower in each medicine group than in the blank group ($P < 0.001$). The spot count was significantly lower in the levofloxacin group than in the RNAIII inhibiting peptide group ($P < 0.05$). In the combination group, the number of bacteria adhered to Hela cells was decreased ($P < 0.01$). Results verified that

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RNAIII inhibiting peptide effectively suppressed the adhesion of *staphylococcus epidermis* to the host cells, and showed synergistic effects on antibiotics.

Subject headings: tissue engineering; bacteria; infection; RNA; staphylococcus
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0 引言 Introduction

细菌能够形成生物被膜，使其能够抵御人体免疫系统和抗生素的杀灭作用，细菌对宿主细胞或骨科内植物的黏附是生物被膜形成的关键[1]。细菌对宿主细胞或内植物的黏附、聚集、播散，乃至形成生物被膜的整个过程受“群体感应(quorum sensing，QS)”机制的调节[2]。通过影响这一细菌间的信号调节机制以抑制细菌的黏附、生物被膜形成和毒素分泌，进而预防和控制感染，是一条有别于传统抗生素的感染治疗新途径[3]。RNAIII抑制肽(RNAIII inhibiting peptide，RIP)是最近发现的葡萄球菌群体感应机制的有效抑制剂，具有预防和治疗葡萄球菌感染的潜在价值[4-6]。

文章通过体外实验对RNAIII抑制肽能否影响表皮葡萄球菌在HeLa表面的黏附进行了探索，对其防治葡萄球菌感染的可行性进行了初步论证。

1 材料和方法 Materials and methods

设计：细胞学实验。

时间及地点：于2007年1至6月在解放军总医院药理研究所细菌实验室完成。

材料：

菌株：菌株表皮葡萄球菌ATCC35984(上海复旦大学范长胜教授馈赠)。

细胞：HeLa细胞购自协和医科大学细胞室。

RNAIII抑制肽抑制葡萄球菌对Hela细胞的黏附实验相关主要试剂及仪器：

试剂及仪器	来源
RNAIII抑制肽	本室自主合成，批号：20061010
DMEM培养基，特级胎牛血清，0.25%胰蛋白酶，	美国Gibco公司
10×PBS溶液	美国Hyclone公司
胰酶大豆肉汤TSB	法国BioMerieux公司
Muller-Hinton培养基(MHA)	美国DIFCO公司
FITC	美国Sigma公司
左氧氟沙星	丽珠集团丽珠制药厂
碳酸氢钠	北京化工厂
5400系列CO_2孵箱	美国NAPCO公司
IMT-2型Olympus倒置显微镜，DP700荧光显微镜	日本Olympus公司
比浊仪	法国bioMerieux公司
TDZ5-WS型离心机	湖南湘仪离心机厂

方法:

细菌培养与染色：将表皮葡萄球菌ATCC35984接种到50 mL TSB培养基，在37 ℃、150 r/min培养箱中摇晃过夜。取20 mL菌液。通过离心从上清液收集细菌，再悬浮于含有10 mg FITC的由1.5 mL PBS和10.5 mL碳酸氢钠(0.5 Mp，pH 9.5)组成的混合溶液中，菌液大幅搅拌4 ℃过夜培养。菌液用0.5 mol/L的碳酸氢钠溶液离心洗涤3次，用比浊仪调配成0.5麦氏单位(McFarland，Mc)的PBS菌液备用。

Hela细胞的培养与爬片制备：将冻存的Hela细胞放入37–42 ℃水浴中快速解冻；将其全部吸出到离心管内，并加入等体积的培养液稀释(含体积分数10%胎牛血清的DMEM培养液)，1 000 r/min离心5 min；弃去上清液，加入适量的培养液并轻轻吹散细胞，然后将其全部吸入到25 cm^2的培养瓶内并使其内含有 5 mL的培养液，放入37 ℃、体积分数5% CO_2孵箱培养。待细胞生长铺满80%的培养瓶底面积或有较多的浮游细胞时，将培养瓶内的培养液吸出；加入体积分数0.25%胰蛋白酶1 mL消化1 min，显微镜下观察，当细胞皱缩变圆甚至脱落时，加入含体积分数10%胎牛血清的DMEM培养液终止消化；用弯头吸管吹打培养瓶底使细胞脱落，并吸出细胞悬液到离心管中，1 500 r/min离心5 min；弃去上清液，加入1.0–1.5 mL的冻存液(含体积分数10%的二甲亚砜及50%的胎牛血清的DMEM)轻轻吹散细胞使其成细胞悬液。取无菌24孔板，每孔加入1×10^7 L^{-1}细胞悬液(以含体积分数10%胎牛血清的DMEM配制)，于37 ℃、体积分数5% CO_2孵箱培养18 h，培养第2天开始进行细菌黏附实验，以保证Hela细胞能够在暴露到细菌前结合到培养板表面。

分组及细菌对Hela细胞黏附试验：FITC标记的细菌按1∶10用PBS稀释，按10^6个细菌/孔(90 μL PBS)将细菌滴入到上述铺好Hela细胞的96孔培养板内。实验随机分4组：空白组、RNAIII抑制肽组、左氧组和联合组。干预5 min后，向空白组每孔加入50 μL含有0.75%DMSO的生理盐水，RNAIII抑制肽组每孔加入含50 μg RNAIII抑制肽的DMSO溶液(体积分数0.75%)，左氧组每孔加入含100 μg左氧氟沙星的水溶液50 μL，联合组组用药剂量参照上述两组。含有细菌和Hela细胞的24孔板在37 ℃下孵育30 min，用PBS冲洗，用荧光显微镜(×400)在400 nm处进行检测、拍照，每组检测6个孔，每孔随机拍摄6张图像，图像采用Image pro plus 6.0软件进行荧光光点计数，取每孔的平均值。

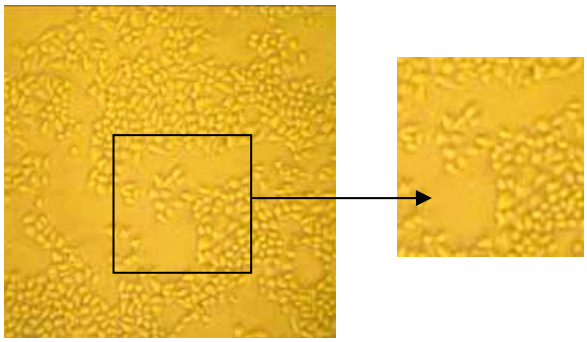

图 1　Hela 细胞层(DAB 染色，倒置光学显微镜，×100)
Figure 1　Hela cell layer (3,3'-diaminobenzidine staining, inverted optical microscope, ×100)
图注：Hela 细胞黏附到培养板底部，并继续增殖。

表 1　RNAIII抑制肽影响表皮葡萄球菌对 Hela 细胞的黏附
Table 1　RNAIII inhibiting peptide affects the adhesion of *staphylococcus epidermis* on the Hela cells　(×400 visual field)

组别	Hela 细胞表面黏附细菌的光点计数(n=6，光点数)						
	孔 1	孔 2	孔 3	孔 4	孔 5	孔 6	合计($\bar{x}$±s)
空白组	436	502	458	473	393	546	468±53
RNAIII抑制肽组	216	234	229	188	301	169	223±46[a]
左氧组	107	125	203	203	217	133	156±44[ab]
联合组	54	38	64	104	78	46	46[acd]

表注：实验分 4 组，空白组每孔加入 DMSO 的生理盐水，RNAIII抑制肽组葡萄球菌 RNAIII抑制肽的 DMSO 溶液，左氧组加入左氧氟沙星的水溶液，联合组组用药剂量参照上述两组联合干预。与空白组比较，[a]P < 0.001；与 RNAIII抑制肽组比较，[b]P < 0.05，[c]P < 0.01；与左氧组比较，[d]P < 0.01。

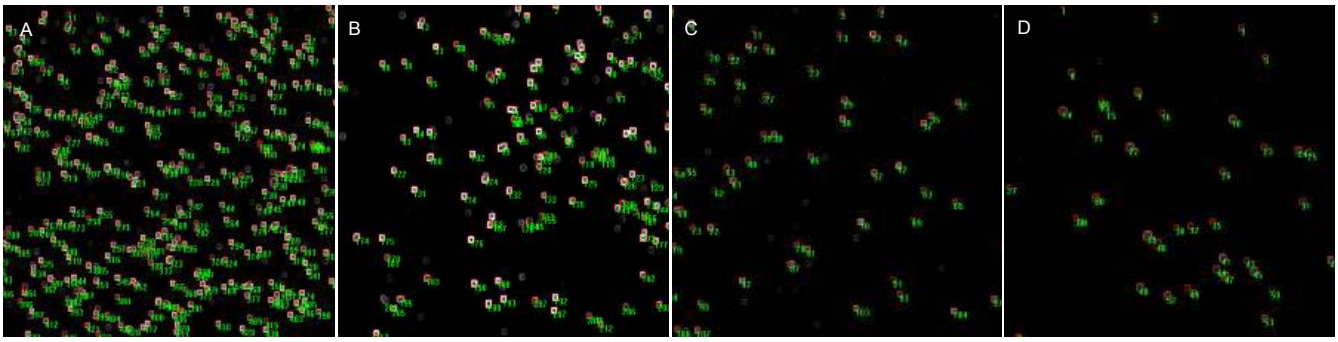

图 2　RNAIII抑制肽抑制表皮葡萄球菌对 Hela 细胞的黏附(FITC 染色，×400，荧光显微镜)
Figure 2　RNAIII inhibiting peptide suppresses the adhesion of *staphylococcus epidermis* on the Hela cells (FITC staining, fluorescence microscope, ×400)
图注：实验分 4 组，空白组每孔加入 DMSO 的生理盐水，RNAIII抑制肽组葡萄球菌 RNAIII抑制肽的 DMSO 溶液，左氧组加入左氧氟沙星的水溶液，联合组用药剂量参照上述两组联合干预。图中 A-D 分别为空白组、RNAIII抑制肽组、左氧组和联合组，荧光光电数分别为 436，216，107，54(400 倍光镜视野)。

主要观察指标：荧光显微镜下图像分析及荧光光点计数。

统计学分析：计量资料以$\bar{x}$±s表示，采用SPSS 13.0软件对组间数据进行两样本t 检验分析，P < 0.05为差异有显著性意义。

2　结果　Results

Hela细胞在24孔培养板上过夜培养后，用倒置显微镜可观察到Hela细胞黏附到培养板底部，并继续增殖(**图1**)。

RNAIII抑制肽影响表皮葡萄球菌对Hela细胞的黏附：空白组Hela细胞层表面有大量细菌黏附，而各用药组细菌黏附数量均显著低于空白组(P < 0.001)，左氧组光点计数明显低于RNAIII抑制肽组(P < 0.05)，而将两药联用后，Hela细胞层表面黏附的细菌数量进一步降低(P < 0.01)。RNAIII抑制肽可抑制表皮葡萄球菌对Hela细胞的黏附，而左氧氟沙星的功效应该与其杀灭细菌减少了菌液(培养液)中的细菌数量有关。尽管两种药物的作用机制不同，但两药联用后对细菌的黏附显示出更强的抑制效果(**表1**和**图2**)。

3　讨论　Discussion

细菌耐药性日益成为一个世界性的难题。葡萄球菌(金黄色葡萄球菌和表皮葡萄球菌)作为人类最常见的致病菌，同时也是院内感染的最为常见的原因[7]。当前，多数葡萄球菌对抗生素具有不同程度的耐药性。葡萄球菌耐药的原因之一在于它们能够黏附在宿主细胞或内植物表面，形成生物被膜，以抵御抗生素的攻击。因此，能够抑制、减少细菌的黏附应是新型生物抑菌、抗菌制剂必备的功效之一。

RNAIII抑制肽是一种葡萄球菌感染的全面抑制剂[4]。研究显示它可以预防葡萄球菌生物被膜形成[8]、预防葡萄球菌生物被膜相关感染[9]、抑制金黄色葡萄球菌毒素的产生[10-11]、抑制耐药葡萄球菌感染[10, 12]、预防移植物相关的耐药葡萄球菌感染[13]、增强抗生素治疗葡萄球菌感染的效果[14]。但是，有关RNAIII抑制肽抑制细菌黏附的实验数据比较缺乏。

2006年，实验首次通过固态法合成得到了RNAIII抑制肽[6]，并且证实，RNAIII抑制肽能够抑制葡萄球菌在人工关节材料表面的黏附[15]。为了进一步验证RNAIII抑制肽能否抑制细菌对宿主细胞的黏附，本次实验实验选取表皮葡

萄球菌ATCC35984为实验菌株，以人类宫颈癌上皮细胞(Hela)为黏附对象，以左氧氟沙星为对照药物， 通过荧光显微镜对细菌在细胞培养板上Hela细胞层的黏附情况进行观测、拍照，并采用专业的图像处理软件对获得的数字图像进行量化分析。与既往国外研究相比，实验采用的Hela细胞更加容易培养，体积更大，有利于实验的顺利进行[4]。同时在设计中选取左氧氟沙星为对照药，实验设计更加严密合理，也对RNAIII抑制肽与传统抗生素之间的相互作用进行了探索。

实验结果显示，RNAIII抑制肽确实可以抑制表皮葡萄球菌对Hela细胞的黏附，这与以往的研究结果相同[4]。说明RNAIII抑制肽能够抑制葡萄球菌黏附分子的形成，阻止细菌在宿主细胞表面聚集形成生物被膜。

同时，实验结果也显示，RNAIII抑制肽与抗生素同时存在，更加有效地抑制了细菌对宿主细胞的黏附，说明RNAIII抑制肽与抗生素有协同作用。尽管RNAIII抑制肽对细菌黏附、聚集的抑制作用与抗生素对细菌的杀灭作用机理不同，但RNAIII抑制肽的存在影响了细菌生物被膜的形成，使抗生素能够充分发挥对散在细菌的杀灭作用。这也证明了RNAIII抑制肽，这种葡萄球菌生物被膜抑制剂的潜在药用价值。

天然的RNAIII抑制肽由凝固酶阴性葡萄球菌菌株RN833(ATCC 55619产生，它具有和RAP极为相似的NH2-末端序列(分别是YSPXTNF和YKPITN)[16-47]，能够优先与TRAP结合，阻止其磷酸化，从而阻断葡萄球菌的群体感应(QS)机制[7, 17]。实验中所用的RNAIII抑制肽系实验自主合成[6]，采用的是与国外不同的固态合成的方法，实验的有效性也进一步证明了实验自主合成的RNAIII抑制肽的生物活性。

实验中仍存在偏倚和不足。实验的设计源于对葡萄球菌QS机制的理解，实验结果对RNAIII抑制肽的功效进行了验证，但并没有从分子生物学水平对RNAIII抑制肽在葡萄球菌QS系统中的作用机制进行揭示，缺乏对RNAIII抑制肽抑制细菌黏附作用的细节论证，今后实验将不断完善。

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作者贡献：第一作者完成了大部分实验内容，第二、三作者对课题和实验的实施进行了指导。

利益冲突：文章及内容不涉及相关利益冲突。

伦理要求：实验不涉及与伦理冲突内容。

学术术语：生物被膜-是细菌在特定条件下、在宿主细胞或内植物表面形成的，包含一种或多种细菌、细菌分泌的细胞外多聚物基质等多种成分的膜样结构。生物被膜为非均质结构，有孔道或孔隙贯穿其间，细胞间营养物质可通过孔道进行循环，生物被膜和生存于其内的细菌构成一个稳定的生态微环境，这一微环境可以保护细菌对抗外界的不利因素，从而使细菌得以生存。

作者声明：文章为原创作品，无抄袭剽窃，无泄密及署名和专利争议，无一稿两投，内容及数据真实，文责自负。

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