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文献综述 REVIEW

microRNAs与幽门螺杆菌相关性胃疾病的研究进展

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Research progress of microRNAs and *Helicobacter pylori*-associated gastric diseases

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Abstract

Helicobacter pylori (*H. pylori*) is a major risk factor for gastritis, gastric mucosa-associated lymphoid tissue (MALT) lymphoma and gastric cancer. The role of *H. pylori* in the pathogenesis of gastric diseases has been proposed, but the detailed molecular mechanism remains unclear. MicroRNAs (miRNAs) are a class of very conserved and endogenous non-coding RNAs consisting of 19-24 nucleotides in length that regulate the expression of target genes at the post-transcriptional level. They are involved in important biological processes related to proliferation, apoptosis, differentiation, metastasis, angiogenesis and immune response. Recently, many studies found that miRNAs are aberrantly expressed and participated in the pathogenesis of the *H. pylori*-associated gastric diseases. This paper reviews the recent progress in understanding the relationship between miRNAs and *H. pylori*-associated gastric diseases.

Key Words: *Helicobacter pylori*; MicroRNAs; Gastric disease

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摘要

幽门螺杆菌(*Helicobacter pylori*, *H. pylori*)作为一种环境致病因素, 与胃炎、胃黏膜相关淋巴组织(MALT)淋巴瘤、胃癌等胃部疾病有着密切的病因学联系. 尽管*H. pylori*在胃疾病中的作用早已被阐述, 但其具体的分子机制尚不清楚. 微小RNA(microRNAs, miRNAs)是近年来新发现的一类高度保守、长度约为19-24个核苷酸(nucleotide, nt)的内源性非编码单链RNA, 在转录后水平调控基因的表达, 可能参与增殖、凋亡、分化、转移、血管形成、免疫应答等重要的生物学过程. 最近的研究发现在*H. pylori*相关性胃疾病中, 一些miRNAs的表达发生变化并参与疾病发生、发展过程. 本文对近年来与*H. pylori*相关性胃疾病发病相关的miRNAs的研究进展予以综述.

关键词: 幽门螺杆菌; microRNAs; 胃疾病

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0 引言

幽门螺杆菌(*Helicobacter pylori*, *H. pylori*)是一种环境致病因素, 与胃炎、消化性溃疡、胃黏膜相关淋巴组织淋巴瘤、胃癌等胃部疾病都有着密切的病因学联系, 1994年世界卫生组织国际癌症研究机构(International Agency for Research on Cancer, IARC)正式将其列为Ⅰ类致癌物. 尽管*H. pylori*在胃疾病中的作用早已

■背景资料
幽门螺杆菌(*Helicobacter pylori, H. pylori*)作为环境致病因素, 与胃炎、消化性溃疡、胃黏膜相关淋巴组织淋巴瘤、胃癌等多种胃部疾病有着密切的病因学联系, 被世界卫生组织国际癌症研究机构列为Ⅰ类致癌物, 其致病机制是一个综合的较为复杂的过程, 是多因素作用的结果.

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■研发前沿
*H. pylori*致病机制涉及炎症、免疫、泌酸、氧化等多个方面, 有毒力因子、细胞因子、自由基、毒力基因等多种致病因子参与, 其具体的分子生物学机制尚未完全阐明.

被阐述, 但其具体的分子机制尚不清楚. 微小RNA(microRNAs, miRNAs)是近来新发现的一类高度保守、长度约为19-24 nt的内源性非编码单链RNA, 通过与靶基因mRNA的3'端非翻译区(3'-UTR)互补结合, 促进靶基因mRNA的降解或抑制其翻译, 从而在转录后水平调控靶基因的表达, 可能参与增殖、凋亡、分化、转移、血管形成、免疫应答等重要的生物学过程[1,2]. *H. pylori*感染可引起胃黏膜组织中一系列miRNAs的表达水平变化, 进而通过影响胃黏膜的免疫和炎症反应、氧化损伤强度和异常调节某些癌基因或抑癌基因的表达, 从而参与*H. pylori*相关性胃疾病的发生、发展过程. 本文综述近年来与*H. pylori*相关性胃疾病发病相关的miRNAs的研究进展.

1 microRNAs的生物起源和功能

miRNAs基因转录是由RNA多聚酶Ⅱ催化完成的, 首先形成miRNA初级转录产物(primary miRNA, pri-miRNA), 然后在细胞核中酶-蛋白质复合体(Drosha酶-DGCR8蛋白)的作用下, pri-miRNA产生大约70 nt大小的发卡状前体miRNA(precursor miRNA, pre-miRNA), 由核转运受体exportin-5转运到胞浆中, 经Dicer酶剪切产生大约19-24 nt的双链miRNA. 其中只有一条单链可以选择性结合到RNA诱导的基因沉默复合物(RNA-induced silencing complex, RISC)中而成为成熟的miRNA, 通过与靶基因mRNA碱基配对引导RISC降解靶mRNA或阻碍翻译过程[3-7]. 同一miRNA可以调控多个mRNA分子, 不同的miRNA也可以协同调控同一mRNA. 迄今, 在153个物种中已发现19 724个成熟的miRNA(数据来源于miRBase Sequence Database). 根据预测, 人类细胞中约1/3的蛋白编码基因受miRNAs的调控[8].

编码miRNAs的基因位于基因间区或内含子中, 一些成簇存在, 可能彼此之间在功能上存在协同作用. Calin等[9]研究发现, 半数以上的miRNAs基因定位于与肿瘤发生相关的染色体区域和脆性位点, 如杂合性缺失区(LOH)、纯合性缺失区(HD)、扩增区、断裂点区、靠近癌基因或抑癌基因的部位. miRNAs的遗传变异、扩增、缺失或基因沉默都可能引起肿瘤的发生或提高个体的肿瘤易感性. miRNAs对肿瘤发生发展的作用首次于慢性淋巴性白血病(chronic lymphoblastic leukemia, CLL)中被证实. 研究发现miR-15a, miR-16-1在CLL中下调, 进一步发现, 这2个miRNA分子的下调与抗凋亡蛋白BCL-2的高表达有关, BCL-2是miR-15a和miR-16-1的下游作用靶点[10]. 之后大量研究证实, miRNAs在肿瘤的发生、发展中扮演着重要角色, 通过调控重要的肿瘤相关基因, 参与肿瘤细胞增殖、凋亡、侵袭或血管形成等过程[11,12]. microRNAs的致癌作用主要通过2种方式实现: (1)作为抑癌因子在肿瘤组织中低表达. 如let-7家族在肺癌、乳腺癌、结肠癌等多种肿瘤中低表达, 通过靶向调节Ras、c-Myc、HMGA2的表达起到抑癌基因的作用[13-18]; (2)作为原癌基因下调肿瘤抑制因子或抑癌基因的表达从而产生致癌作用. 如miR-21在恶性胶质瘤中表达水平升高, 通过抑制凋亡关键性基因caspases的表达而促进肿瘤的形成[19].

除了与肿瘤发生发展密切相关, miRNAs还可以促进免疫细胞的产生和分化、参与天然免疫和获得性免疫应答反应[20,21]. Chen等[22]和Shivdasani[23]报道, 小鼠体内过表达miR-181a, 不仅可以促进B淋巴细胞的分化, 还可使循环中的T淋巴细胞减少50%, 而CD8+亚群的数量减少90%. 另外, miR-181a还可以抑制胸腺成熟基因如Bcl-2、CD69和T细胞受体的表达. miR-155在天然免疫反应中发挥着重要的作用. Bic/miR-155基因敲除小鼠发生了免疫缺陷和肺气道重塑[24]. 另外, 在IFN-α/β的刺激下, 或通过Toll样受体(Toll-like receptor, TLR)配基, 如多聚次黄嘌呤胞嘧啶核苷酸(polyriboinosinic polyribocytidylic acid, poly I: C)或脂多糖(lipopolysaccharide, LPS)处理后, 巨噬细胞中miR-155的表达上调[25]. Li等[26]研究表明miR-181a通过对T细胞受体(T cell receptor, TCR)信号通路的调节影响T细胞对抗原的敏感性.

2 *H. pylori*相关胃炎microRNAs的变化

胃黏膜上皮细胞的固有免疫是宿主抵御*H. pylori*入侵的第一道防线. *H. pylori*感染胃黏膜, 其菌体成分(如LPS, 热休克蛋白60)可被胃黏膜上皮表面受体TLR4识别, 激活TLR4/AP1/NF-κB信号通路, 从而启动一系列的免疫及炎症级联反应, 引起免疫相关基因的表达和多种炎症分子的“瀑布式”释放, 造成胃黏膜的损伤和炎症. 在此过程中, 一系列miRNAs的表达发生异常改变并参与调节*H. pylori*相关的免疫和炎症反应.

2.1 miR-223 Matsushima等[27]在日本人群中应用

芯片和实时荧光定量PCR进行*H. pylori*感染后胃黏膜组织miRNAs表达谱的筛选和鉴定, 研究发现*H. pylori*感染者miR-223的表达是非感染者的5.18倍, 且其表达水平与胃黏膜固有层中性粒细胞浸润程度呈正相关, 经过4 wk *H. pylori*根除治疗且胃黏膜中性粒细胞消失后, miR-223的表达水平恢复正常. 他们还证实用miR-223表达水平诊断*H. pylori*感染的敏感性和特异性可高达100%. 另有研究表明miR-223可特异表达于髓系细胞, 敲除其编码基因可导致中性粒细胞高度成熟和对刺激高度敏感, 表现为细胞核分裂和泡状核增多, 变异的中性粒细胞谱系标志的表达异常, 杀伤真菌的活性增强等. 由于中性粒细胞功能亢进, miR-223突变的小鼠易自发产生炎症性肺病, 受内毒素作用后机体组织严重破坏. 一种促进髓系细胞分化的转录因子Mef2c是miR-223的靶点, 且Mef2c基因敲除后可以纠正miR-223缺陷小鼠的表型[28]. 研究提示miR-223作为一种内源性分子, 可以调控粒细胞的发育和炎症反应.

2.2 miR-196a2 miR-196a2主要在*H. pylori*相关慢性炎症中发挥作用. Okubo等[29]的研究表明miR-196a2存在rs11614913(C>T)单核苷酸多态性(single nucleotide polymorphism, SNP), 携带TT基因型的个体*H. pylori*感染后胃黏膜单核细胞浸润程度更高, 炎症反应更重(调整后OR = 1.62, 95%CI 1.05-2.49, $P = 0.03$). 其机制尚不明确, 推测rs11614913可能与某个相邻基因的遗传变异存在连锁不平衡, 而该基因与*H. pylori*感染导致的慢性炎症有关, 或miR-196a2通过靶向调节某个炎症相关基因来发挥作用. 另外, Peng等[30]研究发现, miR-196a2基因多态性(rs11614913)与胃癌发病风险性有关. 与野生纯合子TT和杂合子CT的携带者比较, 中国人群中携带CC基因型的个体胃癌发病风险显著增加.

2.3 miR-146a Taganov等[31]发现*H. pylori* LPS可通过TLR4信号通路刺激单核细胞, 使miR-146a的表达增加, 且miR-146a的高表达只能被位于胃黏膜上皮细胞表面的TLR2, 4, 5受体活化后所诱导, 通过下调宿主的炎症反应, 与胃黏膜*H. pylori*的持续和慢性感染有关. 有研究[32,33]证实*H. pylori*感染胃黏膜后, 促炎因子IL-8(interleukin-8), TNF-α(tumor necrosis factor-α), IL-1β(interleukin-1β)以NF-κB依赖的方式诱导miR-146a的表达, miR-146a的表达增加可抑制其TLR4信号通路下游分子IRAK1(interleukin-1 receptor-associated kinase 1)和TRAF6(TNF receptor-associated factor 6)的表达, 从而下调NF-κB的活化信号, 减少促炎因子IL-8, GRO-α(growth-related oncogene-α), MIP-3α(macrophage inflammatory protein-3α)等的释放, 下调炎症反应并削弱宿主消除细菌的能力, 促使*H. pylori*感染慢性化的发生. 另有研究报道miR-146a基因存在rs2910164 G/C单核苷酸多态性, 长期感染*H. pylori*后, 携带CC基因型个体可能通过影响TLR4/NF-κB介导的炎症反应使罹患胃癌的风险显著增高(调整后OR = 1.30, 95%CI 1.02-1.66, $P = 0.03$)[29].

2.4 miR-155 miR-155可负性调节机体的免疫和炎症反应, 与多种病毒感染、炎症性疾病、恶性肿瘤的发病有关[34-38], *H. pylori* LPS的直接刺激, 或IFN-β(interferon-β), IFN-γ(interferon-γ)等细胞因子通过TNF-α自分泌/旁分泌信号途径的间接诱导, 均可促使胃黏膜中miR-155的表达增加[39]. *H. pylori*感染后, 胃黏膜上皮细胞表面TLR4受体识别LPS, 激活TLR4/AP-1(activator protein 1)/NF-κB信号通路, 上调miR-155的表达, 而miR-155的高表达可抑制该信号通路的下游重要分子IKK-ε(IkB kinase ε), SMAD2(Sma- and Mad-related protein 2), FADD(Fas-associated death domain protein)和MyD88(myeloid differentiation protein 88, MyD88)的表达, 减少IL-8, GRO-α的释放[40,41], 下调炎症或免疫反应, 削弱宿主消除*H. pylori*的能力, 使*H. pylori*感染持续存在.

miR-146a, miR-155作为2种负性炎症调控因子, 在*H. pylori*感染的胃黏膜中表达水平均增加且呈正相关[32], 说明两者在*H. pylori*相关胃炎发病中可能存在协同作用.

3 *H. pylori*相关胃癌microRNAs的变化

慢性*H. pylori*感染可以引起胃黏膜组织中某些miRNAs的异常表达并参与调节某些原癌、抑癌基因和信号传导通路, 影响细胞的增殖、凋亡等过程, 进而参与胃癌的发生发展.

3.1 miR-21 miRNAs可负性调节某些抑癌基因来促进肿瘤的发生、发展, 产生类似“癌基因”的作用. miR-21在包括胃癌在内的许多肿瘤中表达增加[42]. RECK(reversion-inducing cyste ine-rich protein with Kazal motifs)是胃癌的抑制基因, 具有抑制基质金属蛋白酶(matrix metalloproteases, MMPs)表达与活性的功能, 因此, RECK基因的表达可抑制胃癌细胞浸润转移及血管生成[43,44].

■相关报道

近年研究发现, 微小RNA(microRNAs, miRNAs)可能参与增殖、凋亡、分化、转移、血管形成、免疫应答等重要的生物学过程. 在*H. pylori*相关性胃疾病中, 一些miRNAs的表达发生变化并与疾病发生发展过程相关.

■应用要点
本文研究miRNA在*H. pylori*相关性胃疾病中的重要调控作用为更好地理解该疾病的分子生物学机制提供了新的思路, 为疾病的治疗提供了新的策略.

Zhang等[45]通过应用miRNA靶基因数据库和荧光素酶实验证明了RECK是miR-21的一个靶基因. 该研究发现miR-21在*H. pylori*感染的胃黏膜和与*H. pylori*共培养的胃黏膜细胞中表达增高, 过表达miR-21可以促进细胞的增殖、侵袭、迁移, 抑制细胞的凋亡. *H. pylori*感染胃黏膜上皮细胞后, 引起NF-κB信号通路的激活和白介素-6(interleukin-6, IL-6)的分泌, 进而激活AP-1和STAT3, 引起miR-21的过表达[46,47]并下调RECK, 促进胃癌细胞的增殖、浸润转移及血管生成.

3.2 let-7家族 另有一些miRNAs通过抑制癌基因来抑制肿瘤的发生、发展, 如let-7在翻译水平上抑制ras和c-myc的表达[13], 被看作“抑癌基因”. Matsushima等[27]分别用含完整细胞毒素相关基因(cytotoxin associated gene, cagA)致病岛和cagA致病岛/cagA基因缺失的*H. pylori*菌株与胃上皮细胞作用后, 发现与含完整cagA致病岛菌株共培养的胃上皮细胞中let-7a, let-7d, let-7f表达水平明显降低, 说明cagA可能参与调节let-7家族一些miRNAs的表达. *H. pylori* cagA蛋白经Ⅳ型分泌系统进入胃上皮细胞后, 通过激活丝裂原活化蛋白激酶(mitogen-activated protein kinase, MAPK)[48]和NF-κB信号通路[49]下调let-7家族某些miRNAs, 从而上调ras等致癌基因的表达, 引起胃上皮细胞的恶性转化.

3.3 miR-218 miR-218可以抑制细胞增殖, 促进凋亡, 作为一种“抑癌基因”在胃癌中表达降低[50]. Gao等[51]研究发现miR-218在*H. pylori*感染的胃黏膜与*H. pylori*共培养的AGS细胞和胃癌组织中表达均降低. miR-218的过表达能抑制细胞增殖并促进凋亡. 表皮生长因子受体和过度表达蛋白(epidermal growth factor receptor-coamplified and overexpressed protein, ECOP)是miR-218的直接作用靶点. miR-218的过表达可以抑制ECOP所介导的NF-κB的转录活性和由NF-κB调节的增殖基因环氧化酶-2(COX-2)的转录. *H. pylori*感染导致胃黏膜组织和胃癌AGS细胞中miR-218表达下降, 通过上调其靶基因ECOP, 激活NF-κB信号通路, 增加COX-2的表达, 进而促进胃癌细胞的增殖, 抑制凋亡, 促进胃癌的发生.

3.4 miR-124a Ando等[52]研究发现*H. pylori*感染的正常人和原发性胃癌患者3种“抑癌基因”(miR-124a-1, miR-124a-2和miR-124a-3)甲基化水平升高, 表明*H. pylori*感染可以引起一些抑癌miRNAs编码基因启动子区CpG岛甲基化异常, 使其表达沉默, 进而参与胃癌的发生. 与近期感染相比, 曾经感染*H. pylori*的个体上述3种miRNAs基因的甲基化水平并未降低. 另有研究表明, 胃癌组织中miR-124a基因的甲基化水平与Rb和CDK6(cyclin dependent kinase 6)表达水平呈正相关, 并与肿瘤大小、分化程度、淋巴结转移和浸润程度相关[53], 提示*H. pylori*感染胃黏膜后引起miR-124a基因启动子区超甲基化, 使miR-124a表达下降, 引起Rb和CDK6的上调, 进而参与胃癌的发生发展.

3.5 miR-200家族 侵袭和转移是导致胃癌患者死亡的最主要原因, 而上皮-间质转化(epithelial-mesenchymal transition, EMT)可能是其重要机制之一. EMT是指上皮细胞在特定的生理和病理条件下向间充质细胞转化的现象, 被看成导致肿瘤进展的重要病理过程.

H. pylori CagA蛋白可经Ⅳ型分泌系统转运到宿主胃黏膜上皮细胞中, 破坏细胞间紧密连接或者改变细胞极性[54], 使上皮细胞获得迁移, 运动能力, 诱导EMT的产生, 一些miRNAs在这一过程中发挥调节作用.

H. pylori CagA与E-钙粘蛋白(E-cadherin)相互作用, 激活Wnt/β-连环蛋白(β-catenin)信号通路[55,56], 引起EMT. 胃癌组织中低表达的miR-200家族(包括miR-200a, miR-200b, miR-200c, miR-141, miR-429), 通过活化转录抑制因子锌指E-盒结合同源异形盒(zinc-finger E-box binding homeobox 1, ZEB1)和ZEB2/SIP1, 下调E-cadherin, 促进EMT过程, 而ZEB1/2也可负性调节miR-200家族, 两者形成负反馈回路, 协同调节胃癌的侵袭和转移[57-61]. miR-103/107通过下调miR-200水平, 诱导EMT的发生[62], 这表明不同miRNAs之间存在相互作用, 共同调节EMT过程.

CagA还可激活细胞外调节蛋白激酶(extracellular regulated protein kinase, Erk)通路促进EMT的发生[63], miR-17, miR-20a在CagA/Erk-GEF-H1-RhoA-ROCK-c-Myc-microRNA-p21信号通路中发挥作用, 通过下调p21的表达引起胃癌的发生[64].

4 *H. pylori*相关胃黏膜淋巴组织淋巴瘤microRNAs的变化

miRNAs还参与调节其他*H. pylori*相关胃疾病的发病过程, 如胃黏膜相关淋巴组织淋巴瘤. 胃黏膜相关淋巴组织淋巴瘤是起源于胃黏膜或黏膜下层的淋巴样组织的恶性淋巴瘤, 其发生与*H. pylori*感染引起的慢性炎症密切相关, 可以转化

为胃弥漫性大B细胞淋巴瘤(gastric diffuse large B-cell lymphoma, gDLBCL). Craig等[65]和Craig等[66]发现, miR-203启动子区的超甲基化可下调miR-203的表达, 进而上调ABL1, 促使胃炎向胃MALT淋巴瘤的转化. 进一步的研究发现Myc可介导miR-34a表达降低, 通过上调FoxP1促进胃MALT淋巴瘤向gDLBCL的转化. miR-34a有可能成为胃恶性淋巴瘤的替代治疗靶点. Liu等[67]的研究表明E2A+的胃MALT淋巴瘤类浆细胞浸润较少, 记忆性B细胞相关的miR-223表达升高, 易蔓延至胃周淋巴结, 对*H. pylori*根除治疗反应性差.

5 结论

已有研究表明miRNAs在肿瘤发生、进展和转移中起到重要作用, 显示其作为肿瘤诊断、预后标志物以及治疗新策略的可能性. 本文综述了miRNAs与*H. pylori*相关性胃疾病的最新研究进展, 结果显示*H. pylori*感染胃黏膜后, 可以导致一系列miRNAs的表达发生异常改变并参与调节*H. pylori*引起的免疫、炎症反应和肿瘤相关基因的表达. miRNAs可能是联系炎症和肿瘤之间的一个桥梁, 为了解炎症相关肿瘤的发病机制提供了新的线索. 最近的研究发现miRNAs可以在血清等体液中稳定存在, 并且血清miRNAs表达谱的变化与肿瘤和其他疾病的发生、发展具有明确的相关性[68-71], 以其敏感性和特异性高、无创、便捷等优点显示出极大的临床应用前景, 因此, 特异的血清miRNAs有可能成为*H. pylori*相关胃疾病早期诊断的生物标志物. 鉴于miRNAs在*H. pylori*相关胃疾病的重要调控作用, 针对特异的miRNAs可以对其进行干预治疗.

由于miRNAs处在非常复杂的基因调控网络中, 其在*H. pylori*相关性胃疾病中的作用也远远超过了我们的想象. *H. pylori*具有一系列毒力相关蛋白, 包括细胞毒素相关蛋白(CagA)、细胞空泡毒素(VacA)、鞭毛蛋白(Fla)等, 因此应进一步鉴定*H. pylori*的何种毒力基因、毒力蛋白导致miRNAs的异常表达及其致病机制. 同一种miRNA在*H. pylori*相关胃炎和胃癌中的表达情况并不一致, 应鉴定胃癌前疾病和胃癌特异性的miRNAs. 总之, 随着对miRNAs研究的深入, 我们可以更好的理解*H. pylori*相关胃疾病的发病机制, 对其采取更加有效的防治措施, 降低*H. pylori*相关胃恶性疾病的发病率和死亡率.

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■同行评价

本文思路清晰, 结构合理, 较好地阐述了microRNAs的生物起源和功能, *H. pylori*相关胃病microRNAs的变化及其作用机制, 对深入理解*H. pylori*相关胃病的分子机制具有较好的科学意义.

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