病毒miRNA与免疫逃逸

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摘要 微小RNA(microRNA, miRNA)是一种非编码的小分子RNA, 长度一般在22 nt左右, 通过与mRNA 3'UTR的特异性结合介导转录后调控过程。现已鉴定出的miRNA涵盖了从植物到人类的多个物种, 并参与了调节生长、免疫、凋亡等多种生命活动。最近发现, DNA病毒感染宿主时也能编码产生miRNA, 并在病毒免疫逃逸中扮演着重要角色。病毒感染是一个复杂的过程, 病毒需要逃脱免疫系统才能对宿主产生持续性感染, 而病毒miRNA能调控宿主和自身基因表达, 帮助病毒感染宿主, 且因其本身没有免疫原性, 而成为病毒逃避免疫应答的重要工具, 但其中的分子机制尚不十分清楚。该文就病毒miRNA如何调控病毒自身与宿主基因进行免疫逃逸的近期研究作一综述。

关键词 病毒miRNA; 基因调控; 免疫逃逸

微小RNA(microRNA, miRNA)是一种长约22个核苷酸的非编码小分子RNA, 通过形成RNA诱导沉默复合物(RNA-induced silencing complex, RISC)与mRNA的3'UTR互补结合, 进行转录后调控: 若能与mRNA的3'UTR互补区完全匹配则降解; 部分匹配则使翻译受到抑制[1-2]。现在已经有专门的miRNA数据库miRBase(www.mirbase.org), 目前miRBase中已注释了近2万条miRNA序列, 涵盖了160余个物种。miRNA作为一种重要的调控分子参与了多种基因的转录后调控, 影响细胞的生长、凋亡与增殖、免疫等生命活动, 已预测大部分人类基因都可受miRNA的调控[3-5]。

1 病毒miRNA与机体免疫

宿主抗病毒感染的途径主要有固有免疫与适应性免疫, 其中固有免疫是抗病毒的第一道防线, 病毒入侵首先刺激吞噬细胞分泌细胞因子, 吞噬病毒。之后, 自然杀伤细胞(natural killer cell, NK cell)与靶细胞结合而被激活, 活化的NK细胞直接杀伤感染细胞。同时, NK细胞能释放细胞因子干扰病毒复制、发挥免疫调理作用, 还能激活其他免疫细胞发挥协同抗病毒作用[6]。宿主还通过抗原递呈作用激活适应性免疫, 产生相应的浆细胞和细胞毒T淋巴细胞(cytotoxic T lymphocyte, CTL), 浆细胞可产生病毒特异性抗体而中和病毒颗粒, CTL细胞可识别感染细胞表面的抗原肽(MHC I类分子复合物)并与之结合, 通过释放穿孔素、颗粒酶及高表达FasL(Fas ligand)等致靶细胞溶解或凋亡[7]。

在病毒与宿主的进化博弈中, 产生了病毒编码的miRNA(viral miRNA), 自2004年从疱疹病毒γ亚科的艾伯斯坦–巴尔病毒(Epstein-Barr virus, EBV)中发现了第一条病毒miRNA[8]以来, 到目前为止, 在miRBase中已收录了近300条病毒miRNA。病毒入侵宿主细胞后, 病毒miRNA的合成借助了宿主细胞的合成系统, 其miRNA的形成与功能发挥基本上是在宿主中完成的。现已发现的病毒miRNA中有90%的序列来自三个疱疹病毒亚科(α、β、γ)。由于miRNA的形成起始于细胞核, 目前在细胞质中进行复制的DNA病毒尚未发现存在病毒miRNA。在RNA病毒中, 只在1型人类免疫缺陷病毒(human immunodeficiency virus type-1, HIV-1)[9-11]、西尼罗河病毒(West Nile virus, WNV)[12]和牛白血病病毒(bovine leukemia virus, BLV)中发现了miRNA[13]。

病毒可通过miRNA干扰宿主细胞的病毒抗原递呈、免疫细胞激活和对感染细胞的识别等正常免疫过程, 影响细胞因子的水平, 帮助病毒实现免疫逃逸, 形成潜伏感染[14-16]。

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2　病毒miRNA参与病毒免疫逃逸的分子机制

2.1 病毒miRNA干扰免疫识别

NK细胞与CTL对病毒感染细胞的杀伤是宿主对病毒的重要防御模式[17], 在长期的进化过程中, 病毒已演化出了相应的对策。研究发现, 猴病毒40(simian virus 40, SV40)在感染后期会累积SV miRNA, 其靶mRNA是病毒早期基因编码的T抗原, 而T抗原是CTL受体识别的配体。SV miRNA使T抗原水平下调, 从而降低了CTL对感染细胞的敏感性。用SV miRNA缺失的病毒突变体感染细胞发现: CTL对感染细胞的敏感性没有降低, 即SV40病毒利用SV miRNA逃避免疫系统[18]。研究还发现, 另外两种多瘤病毒——JC人多瘤病毒(JC human polyomavirus, JCV)和BK人多瘤病毒(BK human polyomavirus, BKV)编码的miRNA也有类似的功能[19]。此外, 人巨细胞病毒(human cytomegalovirus, HCMV)编码的miRNA(miR-US4-1)能下调氨肽酶ERAP1的水平[20], 影响了MHC I(major histocompatibility complex class I)递呈肽段, 降低了CTL的敏感性, 使病毒感染细胞逃过免疫监视。

NKG2D(natural-killer group 2, member D)蛋白在多种免疫细胞中表达, 参与适应性免疫应答和固有性免疫应答, 调节NK细胞、T细胞、巨噬细胞、树突状细胞的功能, 是一种重要的激活型受体, 在免疫细胞激活中有重要作用[21]。JCV和BKV可产生miRNA抑制NKG2D的配体ULBP3(UL16 binding protein 3)的表达水平, 从而逃过NKG2D依赖性的细胞杀伤[22]。而EB病毒、卡波济肉瘤相关疱疹病毒(Kaposi's sarcoma herpesvirus, KSHV)、人巨细胞病毒编码的miRNA可作用于NKG2D的另一个配体MICB, KSHV和HCMV的病毒miRNA可使MICB含量下调40%, EBV则能使MICB下调60%。病毒miRNA对MICB的下调抑制了免疫细胞活化, 使得病毒逃过免疫监视[23]。同样作为NKG2D配体的MICA(MHC class I chain-related A)与MICB有90% 的ORF同源, 但病毒miRNA对MICA却无明显作用。EBV、HCMV编码的miRNA可能与MICA有两个结合位点, 这两个结合位点所处的位置与miRNA和MICB的结合位点相似。然而, MICA上的对应结合位点与MICB相比并不完全匹配, 将MICA 3'UTR的对应位点改变成EBV miRNA结合位点时, 出现了MICA的下调, 但不如MICB明显。MICB有7倍于MICA的3'UTR(1 213 nt与174 nt), 较短的3'UTR也可能起着一定的作用。病毒miRNA对MICB和MICA调控的差异, 提示MICB可能在疱疹科病毒感染中起重要作用[23]。改变mRNA与miRNA的保守配对区, 可能是宿主细胞对病毒miRNA的一种应对方式, 但作用相似的病毒miRNA其种子序列并不相同, 这使宿主细胞通过改变3'UTR与miRNA配对的保守序列不能同时对多种病毒起作用。

2.2 病毒miRNA调控细胞因子干扰免疫

细胞因子在整个免疫过程中起着重要的作用, 病毒miRNA可以干扰细胞因子的正常表达, 扰乱机体的正常免疫功能, 帮助病毒免疫逃逸。KSHV能在人骨髓单核细胞和小鼠的巨噬细胞中表达miR-K12-3和miR-K12-7, 它们抑制转录因子C/EBPβ(CCAAT/enhancer-binding protein β)的异构体LIP(C/EBPβ p20)的表达水平, 其是白细胞介素-6(interleukin-6, IL-6)和白细胞介素-10(interleukin-10, IL-10)的转录负调控因子, 使得IL-6和IL-10表达上调[24]。IL-6和IL-10能抑制树突状细胞的成熟, IL-10还可以抑制T细胞、NK细胞、巨噬细胞细胞因子的产生[25-29]。此外, KSHV还通过病毒miRNA下调TWEAKR(TNF-like weak inducer of apoptosis receptor)使趋化因子8(interleukin-8/CXCL8)和人单核细胞趋化蛋白-1(monocyte chemoattractant protein-1, MCP-1/CCL2)表达下降[30]。CXCL8有促中性粒细胞活化和趋化中性粒细胞、T细胞的作用[31], 而MCP-1可与CCR2(CD192)结合对单核/巨噬细胞和嗜碱粒细胞有趋化激活作用[32], 病毒miRNA抑制炎症反应而利于感染细胞的存活。EBV编码的病毒miRNA在原发性淋巴瘤中可对干扰素诱导的T细胞α趋化因子(IFN-inducible T-cell attracting chemokine α, I-TAC/CXCL-11)表达产生抑制, 而CXCL11有趋化活化的T细胞、中性粒细胞和单核细胞的功能[33]。miRNA通过调节以上细胞因子的水平帮助病毒免疫逃逸, 但该过程是否还受其他细胞因子的调控, 有待进一步研究。

2.3 病毒miRNA帮助病毒潜伏感染

2.3.1　*病毒miRNA参与细胞周期调控*　为建立潜伏感染, 病毒通过调控细胞周期、促进感染细胞增殖并抑制凋亡, 使病毒自身得到复制, 而又不释放至细胞外, 以此逃过免疫系统监视, 使感染细胞长期存活[14-16]。2007年, Samols等[34]报道了4种KSHV

编码的miRNA可将凝血酶敏感素1(thrombospondin 1, THBS1)下调4倍。THBS1有抑制肿瘤增殖和血管形成的活性, 它的下调能降低TGF-β的活性, 影响TGF-β通路, 对KS肿瘤(Kaposi's sarcoma tumors)的形成和发展起了重要作用。KSHV编码的miRNA(miR-K12-11)还能作用于TGF-β信号通路中重要的蛋白SMAD5, 影响TGF-β信号通路的信号传递, 从而影响细胞增殖凋亡等过程[35]。此外, 细胞周期中重要的负调控因子p21蛋白水平也受KSHV miRNA的调控[36]。已证实, p21能通过抑制CDK1与CDK2而激活多条抑癌途径, 并参与p53途径和TGF-β途径, 影响细胞增殖[36-37]。Cyclin E与早G_1期CDK2结合能使细胞进入S期, 在HMCV感染的细胞中, 其能通过编码miR-US25-1调节细胞周期蛋白Cyclin E2的表达, 使Cyclin E2达到一个合适的水平, 使细胞进入S期[38-39]。病毒可调控细胞周期, 干扰宿主对病毒感染细胞的正常识别, 有助于病毒免疫逃逸。虽然病毒miRNA调控细胞周期与癌变之间的关系还有待研究, 但为该类疾病的治疗提供了一个潜在靶点。

2.3.2 *抑制宿主细胞凋亡* 在固有性免疫和适应性免疫中, 诱导感染细胞发生凋亡是宿主避免病毒扩散的一种策略。在EBV和KSHV中发现了凋亡蛋白相关的miRNA基因, 病毒miRNA可调控宿主基因表达以抗细胞凋亡。潜伏膜蛋白1(latent membrane protien 1, LMP1)参与多条信号转导途径, 影响核转录因子NF-κB与p53的表达水平, 在EBV引起的鼻咽癌中有重要作用。LMP1对细胞有双向调控作用: 低水平的LMP1能促进细胞的增殖, 抑制p53诱导的凋亡发生; 高表达的LMP1对上皮细胞具有毒性作用, 可诱导凋亡的发生[40-41]。在EBV感染的细胞中, 发现BART miRNA能下调LMP1的水平[42-43]。LMPl受病毒自身的miRNA调控后, 其下游信号途径受到抑制, 使EBV感染的细胞易向肿瘤发展。此外, EBV编码的miRNA还可下调PUMA(p53 upregulated modulator of apoptosis)的水平。PUMA属于Bcl-2家族BH3-only亚家族, 有四种不同的转录本(α、β、γ、δ), 其中PUMA-α参与各种刺激诱导的p53依赖途径和非依赖途径的细胞凋亡过程, 是凋亡的关键分子之一[44-45]。EBV miRNA还可以对Bcl-2家族BH3-only亚家族的另一个成员Bim蛋白进行负调控。活化的Bim分子可以通过与Bcl-2/Bax相互作用来激活Bax引发线粒体途径的细胞凋亡[46]。此外, 通过芯片(RIP-Chip)分析鉴定出Bax在线粒体上的受体TOM22也受EBV miRNA调控, 能抑制Bax引发的凋亡[47]。以往认为, 在EBV感染后BHRF1蛋白水平提高能直接抑制凋亡和提高Burkitt淋巴瘤(Burkitt's lymphoma, BL)细胞的存活[48-49]; 而最近研究发现, 在EBV感染的Jijoye-BL细胞系中, 抑制miR-17家族、miR-142-3p或miR-BART10-3p能使BHRF1蛋白表达上调, 促进了细胞凋亡[50]。BHRF1蛋白介导的凋亡具体机制还需要进一步研究。

在KSHV感染的人脐静脉内皮细胞中发现了3种病毒miRNA, 它们以Bcl-2相关因子BCLAF1(Bcl-2-associated transcription factor 1)为靶点[51]。BCLAF1有诱导凋亡和转录抑制的作用[52], 研究发现, EBV miRNA(BART17-5p)和宿主的miRNA(miR-142-3p)也作用于BCLAF1[50]。此外, KSHV中还发现了3种miRNA(miR-K12-1, -3, -4-3p)可直接作用于凋亡酶caspase 3从而抑制凋亡, 抑制这三种miRNA可使感染细胞的caspase 3表达上调, 使细胞凋亡率增加[53]。

切除修复交叉互补基因1(excision repair cross complementing group 1, *ERCC1*)编码的蛋白能参与DNA损伤检测和剪切修复, 与p53途径诱导的凋亡有关。即早反应蛋白3(immediate early response 3, IER3)在细胞损伤时会发生上调, 其表达的下调有抗凋亡的作用, 在血清饥饿和DNA损伤诱导的凋亡中有重要作用[54-59]。研究发现, HIV-1反转录中形成了长末端重复序列(long terminal repeat, LTR), 其反式激活反应元件(trans-activation responsive element, TAR element)能够编码miRNA[9], TAR miRNA能抑制ERCC1和IER3的表达水平, 从而抑制了HIV-1整合过程中由DNA损伤引起的凋亡[60]。此外, HIV-1的另一个miRNA(hiv1-mir-H1, miRH1)在血单核细胞中能降低凋亡拮抗转录因子(apoptosis antagonizing transcription factor, AAFT)的表达, 影响Bcl-2、C-myc、Par-4和Dicer酶水平, 促进细胞凋亡[61]。miRH1表现出了与TAR miRNA相反的作用, 两者如何平衡, 是否因感染细胞种类不同而表现出不同的功能仍需进一步研究。

2.3.3 *抑制潜伏期到裂解期转换* 病毒miRNA通过对裂解周期调控使得病毒维持潜伏而不裂解, 逃避免疫系统的监视。BALF5是EBV裂解周期复制必需的DNA聚合酶, 在EBV病毒中发现miR-BART2可下调BALF5的表达量、抑制EBV进入裂解期持续在潜伏期[62]。此外, EBV编码的miR-BART6-5p能抑制Dicer酶表达, 影响细胞和病毒的miRNA表达, 沉默

该miRNA使得EBNA2、LMP1等蛋白水平上调, 可使细胞进入潜伏期III或裂解期, 表明Dicer酶的表达抑制使得病毒维持在了潜伏期II[63]。

对HCMV裂解周期的研究发现, 其编码的miRNA-UL112-1的调控作用是多靶点的, 包括MICB、IE转录激活蛋白1(UL123, IE72/IE1)和UL114。其中, IE1在裂解早前期表达, 是进入裂解期的必需蛋白, 其表达下调可抑制病毒进入裂解周期; 而UL114具有尿嘧啶DNA糖基酶活性, 在病毒DNA复制校正中有重要作用, UL114下调可能对病毒生存有利, 这增加了病毒基因组突变的概率却不影响病毒DNA的复制[64-66]。

在HEK293细胞和真皮微血管上皮细胞中发现, KSHV编码的miRNA可使裂解基因中复制转录激活因子(replication and transcription activator, Rta)的水平出现下调[67-69]。Rta是病毒从潜伏期到裂解期转换的关键调控因子, 其下调使下游裂解期基因表达下调, 对病毒进入裂解期产生抑制作用[70]。视网膜母细胞瘤样蛋白2(retinoblastoma-like protein 2, Rbl2)是DNA甲基化酶的负调控因子。在KSHV中发现, miRNA下调Rbl2的水平导致了DNA甲基化酶1、3a、3b的上调, 增加了DNA的甲基化, 抑制了病毒和宿主的DNA转录和复制, 从而抑制病毒进入裂解复制周期[68]。

KSHV编码的miRNA(miR-K1)还参与了NF-κB途径, 通过下调IκBα水平来激活NF-κB, 从而使*vFLIP*(viral FLICE inhibitory protein)基因激活, 抑制裂解复制激活所必需的AP-1途径[67]。KSHV编码的miRNA(miR-K12-11)则抑制了NF-κB途径上游的IKKε表达, 削弱了干扰素通路, 降低了细胞抗病毒免疫反应, 抑制了KSHV裂解期的协同激活作用[71]。

3　病毒miRNA研究展望

从2004年在EBV中首次发现病毒miRNA到目前为止, 已发现了近300条病毒miRNA, 其调节涉及宿主和病毒自身基因的表达过程。病毒miRNA在病毒免疫逃逸中扮演着重要角色, 与蛋白质相比, miRNA作为一种没有免疫原性的小分子, 合成迅速, 还能从感染细胞转运至邻近正常细胞影响其功能[72-73]。病毒miRNA的作用方式多样: 除与3'UTR结合外还能作用于5'UTR[38], 甚至还可以直接作用于宿主miRNA[61]; 能单独作用于宿主mRNA, 也能与其他病毒miRNA协同作用, 且不受空间阻遏效应的影响[74]。此外, 病毒miRNA在不同细胞中表达不同[75], 不同的感染时期表达也不同[76]。宿主细胞本身miRNA调控网络的变化就可能引发多种病变, 其靶基因转录产物参与了多种生理功能, 如B细胞功能、固有免疫、凋亡和细胞周期调节等[77-78]。而研究发现, 部分病毒miRNA与宿主miRNA具有同源性[79]。这些都说明病毒miRNA调控是一个复杂的过程, 随着病毒miRNA对宿主基因转录后调控研究的深入, 以及它与宿主的相互作用的阐明, 将有利于我们进一步了解病毒及相关疾病, 使以病毒miRNA为靶点的疾病治疗成为可能。

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Viral miRNAs and Immune Avasion

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Abstract MicroRNAs (miRNAs) are non-coding RNA molecules with ~22 nucleotides in length that post-transcriptionally regulate gene expression by complementary binding to 3'UTR of the target mRNAs. MiRNAs have been identified in lots of species from plant to human. MiRNAs modulate multiple cellular processes including development, immunity and apoptosis. Recently, DNA viruses were found to express miRNAs which play an important role in immune evasion during host infection. Viral infection is a complex process requiring immune evasion in order to establish persistent infection of the host. During this process, viruses express non-coding miRNAs, which help modulate cellular and viral gene expression making it more favorable for infection. These viral miRNAs are nonimmunogenic and therefore are important tools used to evade immune responses. However, the function of most viral miRNAs are not well understood. We summarized our current knowledge of virus-encoded miRNAs, and how they contribute to immune evasion by targeting viral and host cellular genes.

Key words viral microRNA; gene regulation; immune evasion

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