microRNA与靶基因相互调控机制的研究进展

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摘要 microRNA(miRNA)是一类由内源基因编码的长度约22个核苷酸的单链非编码RNA分子, 其通过剪切信使RNA或非编码RNA、沉默或激活转录、primary miRNA(pri-miRNA)加工及mRNA翻译, 调控几乎所有细胞增殖分化、个体生长发育及内环境稳态。大量研究已对miRNA的生物发生和调控机制进行了清晰阐述。然而, 关于miRNA的转换机制, 尤其是miRNA在特定条件下的快速变化, 仍尚未解决。近年来研究发现, 靶基因能以序列依赖性方式调控miRNA的生成和降解, 表明miRNA和靶基因之间的调控不是单向的, 而是相互调控。本文详细概述靶基因调控miRNA的最新进展, 归纳二者相互作用的条件和机制, 提出miRNA转换的研究方向, 以期为深入研究机体内miRNA与靶基因的相互作用及开发miRNA靶向治疗药物提供理论基础。

关键词 microRNA, 靶基因诱导的miRNA降解, 拖尾, 修剪, 靶基因介导的miRNA保护

microRNA(miRNA)是一类由内源基因编码的长度约22个核苷酸(nucleotide, nt)的单链非编码RNA分子(non-coding RNA, ncRNA), 通过结合Argonaute(AGO)蛋白形成miRNA诱导沉默复合体(miRNA-induced silencing complex, miRISC)作用于靶基因, 从而在转录和转录后水平发挥调控作用^[1]。

miRNA作为机体的重要调控分子, 几乎参与每一个细胞的发生、分化和信号传递等生物学过程^[2~5]。例如, miRNA生成因子Dicer(RNase III)和Drosha(RNase III)的缺失在小鼠胚胎中是致命的^[6]。miRNA首次发现于秀丽隐杆线虫, 在幼虫阶段, *lin-4*基因产生的小RNA与*lin-14* mRNA的3'UTR(3' untranslated region)存在序列互补, 通过RNA-RNA之间的相互作用抑制*lin-14* mRNA的翻译, 使LIN-14蛋白水平下降^[7]。最初, 普遍认为这种小RNA是线虫特有的, 直到其在不同物种中被陆续发现, 才将这类新的调控分子命名为

miRNA^[8]。目前, miRNA数据库miRBase已经收录超过2500种人类的miRNA信息^[9], 经检测它们在人类基因组中保守的靶基因位点>45000个, 且超过60%的编码基因都能与miRNA结合, 这足以证明miRNA调控的广泛性^[10,11]。

近几十年来, 有关miRNA的生物发生、作用机制及生物学作用的研究报道逐年攀升。除了mRNA, 研究发现, pri-miRNA(primary miRNA)、ncRNA和DNA均能作为miRNA的下游靶基因, 并且这些作用方式已被详细阐述。遗憾的是, miRNA转换的研究很少被报道, 转换机制也尚待系统阐明。本文首先描述miRNA的生物发生及其对各种类型靶基因的作用形式; 随后从上调和下调两个角度出发, 重点指出现阶段发现的靶基因反作用于miRNA的条件和机制; 最后探讨miRNA与靶基因的双向调控, 对机体维持稳态或疾病发生发展有何意义。

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1 miRNA生物发生

1.1 miRNA生物发生的经典途径

miRNA生物发生的经典途径可划分为3个阶段,分别是pri-miRNA生成、pre-miRNA(precursor miRNA)生成及出核、成熟miRNA生成(图1(a)). 第1阶段, pri-miRNA生成: 基因外显子或内含子借由RNA聚合酶II(RNA polymerase II, Pol II)转录生成pri-miRNA, pri-miRNA包含帽子结构、多聚腺苷酸化尾和一至数个发夹茎环结构^[12]. Pol II参与pri-miRNA生成,但并不是唯一的生成酶^[13]. 第2阶段, pre-miRNA生成及出核: pri-miRNA被Microprocessor切割生成长度约60~70 nt的发卡结构(pre-miRNA),其特点是3'端有两个突出nt(3' overhang)未与5'端结合^[14]. Microprocessor主要由核酸内切酶Drosha(RNase III)和辅助蛋白DGCR8(DGCR8 microprocessor complex subunit)组成, Drosha负责识别并切割单链RNA和双链RNA衔接区域的碱基,而DGCR8蛋白结合pri-miRNA茎并确保准确切割^[15]. pre-miRNA的3' overhang被XPO5(Exportin 5)识别并转运到细胞质^[16],而敲除XPO5降低但不能废除pre-miRNA的核输出,表明XPO5并不是转运pre-miRNA的唯一蛋白^[17]. 第3阶段,成熟miRNA生成: 核酸内切酶Dicer(RNase III)识别pre-miRNA的3' overhang和5'端磷酸并对茎环进行切割,产生一条含3' overhang的miRNA双链体(miRNA duplex)^[18,19]. 在人类中, Dicer的切割受TAR RNA结合蛋白(TAR RNA-binding protein, TRBP)和干扰素诱导蛋白激酶(PKR activating protein, PACT)的调控;在果蝇中,受Dicer伴侣蛋白Loqs(loquacious)-PB的调控^[20,21]. miRNA duplex通过伴侣蛋白Hsc70(heat shock cognate 70)/Hsp90(Hsp90 chaperone)使AGO蛋白处于易接受外源RNA的状态,随后miRNA duplex加载到AGO蛋白中. 当AGO恢复起始状态则促使一条单链排出,排出链随后被降解,而保留在AGO中的另一条单链即为成熟miRNA,其与AGO形成miRISC在体内发挥作用. 成熟miRNA称为引导链(guide strand),排出链称为乘客链(passenger strand)^[22,23]. 研究表明,AGO2的MID(middle)结构域优先结合miRNA duplex中5'末端为A或U的一条链,所以这种类型单链更易成为引导链^[24].

miRNA在生物发生过程中,由于Dicer或Microprocessor的选择性剪切,可以生成多种异构体miRNA(isomiR). 根据成熟miRNA的来源, isomiR可以分为5'

isomiR、3' isomiR和多态性isomiR^[25]. 其中, 5' isomiR通过种子序列转移作用于不同的靶基因,而3' isomiR的功能尚不清楚,但越来越多的证据表明它们具有不同的功能^[26~28]. 此外, RNA特异性腺苷脱氨酶(adenosine deaminases acting on RNA, ADAR)能够把pri-miRNA的A编辑成I,从而影响miRNA的生成和功能^[29]. 种子序列是指从miRNA的5'端开始第2~8 nt,主要负责靶基因的识别结合,将种子序列相似的miRNA划分为同一家族^[30].

1.2 miRNA生物发生的非经典途径

1.2.1 shRNA生成miRNA途径

除了经典的miRNA生物发生途径,小部分miRNA也能够非经典途径中生成,同样具有沉默靶基因的能力. 在分子实验中,通常使用RNA聚合酶III(RNA polymerase III, pol III)启动子驱动的shRNA表达载体引入外源siRNA,对靶基因进行敲减. shRNA结构上与pre-miRNA相似,因此绕过Microprocessor并由Dicer直接加工^[31]. 最具特色的shRNA由Pol II驱动,转录起始位点即是Dicer底物的5'位点,其5'位点的7-甲基鸟苷(m⁷G)帽子结构可阻止miRNA duplex的5'链(5p)进入沉默复合体,因此成熟的miRNA仅由3'链(3p)组成(图1(b))^[32].

1.2.2 mirtron生成miRNA途径

mirtron是一类pre-miRNA样的内含子发卡,其加工不依赖Microprocessor,由剪接体剪接和内含子套索去分支生成. 在黑腹果蝇和秀丽隐杆线虫中,对mirtron的加工途径有着详细的阐述. mirtron最初以内含子套索的形式存在,随后被去分支酶DBR1(debranching RNA lariats 1)线性化,使内含子形成pre-miRNA样的mirtron. 随后, mirtron被XPO5输出到细胞质,并被Dicer复合物识别和加工,产生成熟的miRNA(图1(d))^[33~35]. 深度测序结果证明, mirtron产生途径广泛分布于哺乳动物、鸟类、植物和无脊椎动物中,并可用作基因治疗^[36~39].

将一类合成不依赖剪接的mirtron,命名为simtron(splicing-independent mirtron-like miRNA),例如, miR-1225和miR-1228. 它们的产生依赖于Drosha,但不需要DGCR8、XPO5和Dicer的参与. 尽管Drosha存在双链RNA结合结构域(double-stranded RNA binding domain, DSB),但Drosha可能会需要一个新的辅助分子,类似于DGCR8指导正确定位和精确切割. 此外, Drosha缺陷并不会影响所有经simtron途径生成的miRNA,一些miR-

图1 miRNA生物发生的经典和非经典途径. (a) miRNA生物发生的经典途径. (b) shRNA生成miRNA途径. (c) miR-451生成途径. (d) mirtron生成miRNA途径. (e) MHV68生成miRNA途径

Figure 1 Canonical and non-canonical miRNA biogenesis pathways. (a) Canonical miRNA biogenesis pathway. (b) miRNA biogenesis by shRNA pathway. (c) miRNA biogenesis by miR-451 pathway. (d) miRNA biogenesis by mirtron pathway. (e) miRNA biogenesis by MHV68 pathway

NA能够转换到mirtron途径生成^[40].

1.2.3 miRNA生物发生的其他途径

除了上述途径, miRNA生成还存在其他的非经典途径. 在哺乳动物中, miR-451是非经典途径中功能最明确的miRNA之一, 不同于其他非经典途径生成的miRNA, miR-451的生成需要Drosha但不依赖Dicer的加工^[41,42]. 在Drosha切割之后, 由于pre-miR-451发夹太短, 导致其不能被Dicer识别而直接进入AGO2中, 其3'端被AGO2切割, 然后再由核酸外切酶PARN(poly(A)-specific ribonuclease)进一步加工, 生成成熟的miRNA(图1(c))^[43~45]. 鼠类疱疹病毒68(murine γ -herpesvirus 68, MHV68)来源的miRNA由Pol III转录, 生成的pri-miRNA被tRNAse Z而非Drosha加工(图1(e))^[46]. 此外, 还发现miRNA来源于small nucleolar RNA (snoRNA)或tRNA, Dicer对于其生成必不可少, 这突出了miRNA

加工途径的多样性^[47,48]. 实际上, 一些研究通过大规模测序或微阵列分析观察到某些miRNA的丰度在Dicer、Drosha或DGCR8基因敲除的细胞中并未改变, 暗示着还存在其他miRNA生物发生途径^[11,31].

2 miRNA的人核机制

2.1 模体介导的miRNA入核

尽管miRNA成熟于在细胞质中, 但其在细胞核和细胞质中均有分布. miR-21是第一种在细胞核中发现的miRNA, 它在核中的占比约为20%^[25]. 随后, Robb等人^[49]发现AGO蛋白定位于胞核, 并首次证明miRISC在细胞核中发挥靶基因的抑制作用. Liao等人^[50]通过二代测序技术系统分析了人类细胞中的miRNA定位, 令人惊讶的是, 大多数miRNA分布于细胞核, 且已超过

细胞质, 这表明多数miRNA在细胞核内发挥作用。

miRNA的入核具有选择性, 虽然miRNA的长度较短, 但仍有特定的序列, 能够控制其定位。miR-29b的3'端存在一个引导其入核的特有模体(AGNGUN, N是任意nt), 而不具有这段序列的miR-29a在细胞核中的含量很少, 这可以解释miRNA家族成员的不同功能^[51]。与之相似, miR-373-3p的入核也取决于该模体^[52]。目前仅有这两个例子证明AGNGUN介导miRNA入核, 该模体如何控制成熟miRNA从细胞质向细胞核穿梭仍不清楚。

2.2 转运蛋白介导的miRNA入核

转运蛋白是膜蛋白的一大类, 介导生物膜内外的化学物质以及信号交换。Wei等人^[53]发现, 转运蛋白karyopherin β 家族成员——IPO8(importin 8)对miRNA的入核至关重要, IPO8通过结合AGO2诱导miR-122、miR-148a、miR-221、miR-30a-3p、miR-29b和miR-709的入核。此外, 介导pre-miRNA出核的karyopherin家族成员——XPO1和XPO5, 与AGO1和AGO2存在共定位^[53], 提示miRNA在细胞核内外的穿梭可能是转运蛋白与AGO互作所介导的。

2.3 miRNA在细胞核成熟的假说

Brameier等人^[54]发现, 人类细胞内大部分H/ACA box snoRNA和C/D box snoRNA具有miRNA样功能, 并把它们命名为sno-miRNA。这表明miRNA很可能在核仁中加工成熟。例如, 以前体形式存在于核仁内的miR-664, 与snoRNA SNORA36B/ACA36b高度同源, 表明其很有可能来自细胞核snoRNA的加工^[55]。随后, Gagnon等人^[56]发现, 原本认为定位于细胞质内的miRNA加工蛋白——Dicer和TRBP, 也能够定位于细胞核, 这为miRNA在细胞核成熟的假说提供了有力证据。尽管miRNA有可能在核仁中生成, 但是关于snoRNA生成miRNA的机制尚不清楚, 亟需大量的研究与探索。

3 miRNA调控靶基因的作用方式

3.1 miRNA调控mRNA的稳定性及翻译

3.1.1 miRNA介导mRNA剪切和翻译抑制

miRNA通过加载到AGO形成miRISC来执行其调控功能。AGO包含4个特殊结构域, 分别是氨基(N)-末端结构域、PAZ(Piwi-Argonaute-Zwille)结构域、MID结构域和PIWI(P element-induced wimpy testis domain)

结构域; 还包含两个连接域L1和L2, L1连接氨基(N)-末端结构域和PAZ结构域, L2连接PAZ和MID结构域。氨基(N)-末端结构域和PAZ结构域形成一个叶, PIWI结构域和MID结构域组成另一个叶(图2(a))。其中, PIWI结构域具有RNase H样功能, 催化引导链介导靶mRNA的切割。MID和PIWI结构域结合miRNA的5'端, 而PAZ结构域结合3'端^[57]。哺乳动物基因组可编码4种AGO蛋白(AGO1~4), 其中AGO2是表达量最高的蛋白, 也是唯一一种能够切割靶基因的蛋白^[58,59]。

mRNA的3'UTR含有miRNA靶点, 与种子序列具有很强的互补性, 因此种子序列是预测靶基因的主要标准^[60,61]。结合能力最强的靶mRNA具有miRNA种子序列结合位点和对应miRNA 5'端第1 nt的腺苷酸(t1A), 其次是仅含种子序列结合位点, 最后是含t1A和第2~7 nt结合位点^[60]。t1A不能被引导链识别, 但能被AGO识别, 从而加强miRNA对mRNA的识别和沉默^[62,63]。结构和单分子研究表明, 靶基因识别由两步机制实现, 种子序列第2~6 nt首先由MID和PIWI结构域预先堆叠成螺旋结构, 使第2~4 nt指向螺旋外侧, 以便与靶mRNA形成不牢固的初始配对; 第7~8 nt随后与靶mRNA结合, 形成牢固的miRNA:mRNA配对。否则, AGO就会脱离该位点或沿着mRNA横向扩散, miRNA:mRNA配对行为类似于RNA结合蛋白(RNA binding protein, RBP)^[64~67]。除了种子区域, miRNA的3'端同样贡献于靶基因的识别, 尤其是称为补充区域的第13~16 nt, 引导具有相同种子序列的miRNA家族成员到不同的靶点(图2(a))^[60,68]。

miRNA抑制mRNA表达, 分为诱导其降解和抑制其翻译两种形式。miRNA诱导靶mRNA降解存在两种模式: 一种是miRNA和mRNA的完全互补配对, AGO2直接催化切割靶mRNA(图2(f)), 这种模式在植物中普遍存在, 也是RNAi技术应用于临床的基础^[69,70]。另一种是miRNA和mRNA的不完全互补配对, 这种模式在人和哺乳动物中普遍存在, 先去除mRNA 3'尾和5'帽子, 再由5'-3'核酸外切酶XRN1(5'-3' exoribonuclease 1)降解(图2(b))^[71]。具体过程如下: 首先, AGO通过募集GW182蛋白(在人类中称为TNRC6, 并有3种同系物TNRC6A/B/C)与多腺苷酸结合蛋白(cytoplasmic poly(A)-binding protein, PABPC)相互作用并促进其在mRNA 3'端解离, GW182还募集poly(A)-核酸酶去腺苷酸化复合物亚基2(poly(A)-nuclease deadenylation complex subunit 2, PAN2)-PAN3和碳分解代谢物阻遏蛋白4

图 2 miRNA靶向mRNA的调控机制。(a)成熟miRNA以miRISC作为基本功能单位。上图:成熟miRNA结构。下图:miRNA靶向mRNA模式图。(b~d)miRNA对mRNA的调控作用依赖于部分互补配对。(b)miRNA介导的mRNA降解;(c)miRNA介导的翻译起始抑制;(d)miRNA介导的翻译延长抑制;(e)miRNA介导的翻译激活;(f)miRNA与mRNA完全互补配对,直接介导AGO2对mRNA进行降解

Figure 2 Mechanisms of miRNAs targeting mRNAs. (a) miRISC is the basic functional unit of mature miRNAs. Top panel: Structure of mature miRNAs. Bottom panel: Schematic diagram of miRNA targeting mRNA. (b~d) Regulation of mRNA by miRNA in the partial complementary pairing manner. (b) Stimulation of mRNA decay; (c) inhibition of translation initiation; (d) inhibition of translation elongation; (e) stimulation of translation; (f) regulation of mRNA by miRNA in the complementary pairing manner. Induction of mRNA degradation

(carbon catabolite repressor protein 4, CCR4)-NOT去腺苷酸酶复合物^[72,73]。随后, PAN2-PAN3和CCR4-NOT降解mRNA的poly尾。最后, CCR4-NOT通过募集DCP1 (decapping enzyme subunit 1)-DCP2脱帽酶复合物去除mRNA的帽子结构,脱帽和脱尾的mRNA被外切酶XRN1所降解^[74~76]。

在miRNA抑制mRNA翻译过程中,抑制可以发生在翻译起始、延长以及新生肽水解等过程。翻译起始的抑制包括miRNA干扰起始因子与5'帽子结合(图2(c))、促进起始因子与5'帽子解离和招募抑制因子等多

种形式^[77~80]。抑制翻译延长主要是通过促进核糖体在mRNA上的解离来实现,miRNA对内在核糖体进入位点(internal ribosome enter site, IRES)驱动的翻译抑制主要就是通过这种形式(图2(d))^[81,82]。miRNA的结合位点大多数位于mRNA的3'UTR,但少数结合位点也存在于5'UTR和ORF中,同样可以介导翻译抑制^[83~85]。例如,miR-17/20a的结合位点定位于p53活化激酶DAPK3 (death associated protein kinase 3)基因的外显子2,通过互补配对下调DAPK3的蛋白水平,从而减少p53的激活并促进癌症发生^[86]。

miRISC同时诱导靶基因的降解和翻译抑制。如GW182蛋白和CCR4-NOT复合体不但涉及靶基因降解,而且参与翻译抑制^[87-89]。在这两种模式中,miRNA诱导靶基因降解占主导地位:在培养的哺乳动物细胞稳态中,66%~90%的靶基因抑制效应来源于miRNA诱导靶基因降解^[90,91]。miRNA对靶基因的抑制效应存在多元化合作机制,如靶mRNA上的非种子序列结合位点可以加强种子序列结合位点募集miRISC。该机制对秀丽隐杆线虫是必要的,位点之间的合作也是miRNA执行其功能的一大特点^[92]。Selbach等人^[93]通过基因芯片和蛋白质组学分析,阐述单种miRNA可以通过miRISC来影响数百种蛋白质合成和数千种基因表达。除了单种miRNA能够调节多种靶基因之外,一种靶基因也能够受多种不同的miRNA共同调节,多种miRNA起到合作调节作用。这种合作既可以是协同效应也可以是拮抗效应,有助于解释miRNA在含有相同结合位点的不同mRNA中的作用不同^[94,95]。

3.1.2 miRNA介导翻译激活

除沉默效应外,miRNA还能够激活mRNA翻译。Ørom等人^[96]发现,miR-10a可以减弱氨基酸饥饿诱导的蛋白表达下降,这对茴香毒素处理的翻译诱导必不可少。miR-10a结合核糖体蛋白转录本5'UTR内的5'TOP模体,进而促进核糖体转录本的翻译、提高总蛋白水平并诱导细胞转化。随后的研究发现,miRNA激活靶基因翻译独立于GW182蛋白,依赖于AGO2和碱基互补配对。在非洲爪蟾未成熟卵母细胞的G0期,表达升高的xlmiR16通过非种子序列与细胞状态调节剂Myt1(myelin transcription factor 1)的3'UTR结合,依赖AGO2和FXR1(fragile X mental retardation-related protein 1)上调其表达,进而诱导促成熟因子CDC2(cyclin-dependent kinase 1 S homeolog)磷酸化并抑制卵母细胞成熟(图2(e))^[97]。Zhang等人^[98]发现了一种重要的miRNA miR-1对肌肉生成有着重要意义。肌肉生成可以诱导miR-1的表达,miR-1能有效进入线粒体中,以依赖于AGO2和miRNA:mRNA配对的方式来促进多种线粒体特异性转录本翻译,从而为肌肉的生成提供能量。这种作用不依赖于GW182蛋白,且该蛋白不能进入线粒体。细胞质中的miR-1一方面通过靶向促增殖因子Hand2(heart and neural crest derivatives expressed 2)抑制增殖,另一方面通过靶向分化抑制因子HDAC4(histone deacetylase 4)促进肌肉细胞分化。因此,miR-1介导的细胞质翻译抑制和

线粒体翻译增强可能构成肌肉分化和功能高度协调的生肌程序。

3.2 miRNA介导circRNA剪切

环状RNA(circular RNA, circRNA)作为一种新兴分子,通常由pre-mRNA转录子的非顺序反向剪接或线性RNA的反向融合产生。circRNA的3'和5'端共价连接形成环状结构,没有游离端,不受核酸外切酶降解的影响,这赋予它们比线性转录本更高的稳定性^[99,100]。circRNA经典功能是作为特定miRNA的分子海绵,调节mRNA的稳定和翻译,即通过与miRNA的靶基因竞争miRNA,从而抑制miRNA对靶基因的调控^[101]。miRNA同样涉及circRNA的调控。如,miR-671通过广泛互补配对结合circRNA CDR1as,并通过AGO2将其降解。miR-7可以加强这种作用,可能是将miR-671沉默复合物募集到CDR1as或通过尚未确定的机制将其保留在CDR1as^[102,103]。与之相似,miRNA-1224可以结合circRNA-Filip1l(filamin A interacting protein 1-like)前体的5'环化区域,通过AGO2对其进行剪切,进而抑制circRNA-Filip1l的表达,阻止其对疼痛相关基因Ubr5(ubiquitin protein ligase E3 component n-recognin 5)的转录激活。这足以解释,miRNA-1224在完全弗氏佐剂(complete Freund's adjuvant, CFA)诱导的慢性炎症性疼痛中低表达^[104]。

3.3 miRNA介导lncRNA剪切

长链非编码RNA(long non-coding RNA, lncRNA)是长度超过200 nt的ncRNA,在肿瘤、炎症反应以及心血管等多种疾病中发挥重要作用^[105]。miRNA与lncRNA同样可以互相调节,miRNA海绵机制是lncRNA的主要功能之一,同时lncRNA也可以被miRNA所调控^[106]。如,miRNA let-7可以与lncRNA HOTAIR(HOX transcript antisense RNA)互作,以AGO2依赖性方式介导其降解。RBP HuR(antigen R)可以促进let-7对HOTAIR的剪切,进而增强HOTAIR降解。因此,在低表达HuR的细胞(如衰老细胞)中,HOTAIR作为泛素介导蛋白水解的调节剂,表达稳定,并充当E3泛素连接酶Mex3b(mex-3 RNA binding family member B)和Dzip3(DAZ interacting zinc finger protein 3),及其各自的泛素化底物Snurportin-1和Ataxin-1的支架,促进Snurportin-1和Ataxin-1的泛素化,使其快速降解,以防过早衰老^[107]。let-7同样可以依赖HuR和AGO2对

lincRNA(long intergenic noncoding RNA)-p21(cyclin-dependent kinase inhibitor 1A)进行剪切,从而减弱lincRNA-p21对JUNB(JunB proto-oncogene)和CTNNB1(catenin beta 1)mRNA的靶向抑制作用^[108]。

3.4 miRNA调控pri-miRNA加工

miRNA由pri-miRNA加工生成,对自身及其他miRNA的pri-miRNA又存在调控作用,这种调控性miRNA定位于细胞核。Tang等人^[109]首次证明了细胞核miRNA通过结合pri-miRNA,来阻碍其他miRNA的生物发生。miR-709主要定位于多种小鼠细胞的细胞核,特异性结合pri-miR-15a/16-1基底区上一段19 nt的识别元件(距离3'端茎0.8 kb),抑制pre-miR-15a/16-1生成,进而在转录后水平减少其表达。凋亡状态下,miR-709快速出核,促进pre-miR-15a/16-1加工为成熟miRNA,通过靶向抗凋亡分子Bcl-2(BCL2 apoptosis regulator)来促进细胞凋亡。随后的研究也证实,miR-709作为pre-miR-15a/16-1的抑制剂,可以起到抗无血清和TNF- α 诱导凋亡的作用。Zisoulis等人^[110]发现,在秀丽隐杆线虫中,胞核let-7可以特异性结合自身初级转录本pri-let-7的3'端,进而促进pri-let-7的加工成熟。这种作用依赖于秀丽隐杆线虫的AGO蛋白——ALG-1(Argonaute like gene 1),如果破坏ALG的核定位,则会减弱这种效应。Wang等人^[111]发现,过表达miR-122可以抑制肝癌的发生发展。这是由于抑癌分子miR-122可以通过转录后水平抑制癌基因miR-21的表达,阻碍其对肿瘤抑制蛋白PDCD4(programmed cell death 4)的沉默作用,进而抑制肝癌。在机制上,miR-122通过结合pri-miR-21基底区中19 nt的识别元件(距离5'端茎13 nt)阻碍Microprocessor将pri-miR-21加工为pre-miR-21,进而抑制miR-21成熟。

3.5 miRNA调控转录

3.5.1 miRNA介导的转录激活

调控DNA的miRNA主要分布于细胞核,能够诱导转录激活(transcriptional gene activating, TGA)。例如,来源于细胞核的小活化RNA miR-320可以结合CD36的启动子区域,再通过AGO2募集Pol II来促进脂肪酸转位酶CD36的转录,进而引发心脏脂毒性^[112]。在HEK293T细胞中,细胞核miR-24-1可以作为增强子的驱动剂,通过序列互补配对来直接结合靶基因的增强子,进而激活增强子RNA(enhancer RNA, eRNA)的表达,改变组蛋白修饰,并增加p300(E1A binding protein

p300)和Pol II在增强子位点的富集。miR-24-1对靶基因增强子的促进作用,并不是一对一的关系,它可以激活多种靶基因转录。此时,细胞质miR-24-1仍然可以通过传统的作用方式发挥功能,即miR-24-1抑制靶基因的表达。这表明一种miRNA可以在细胞核和细胞质内发挥双重作用^[113]。

3.5.2 miRNA介导的转录沉默

现阶段,报道的miRNA介导的转录沉默(transcriptional gene silencing, TGS)均依赖于启动子区域结合。例如,细胞核miR-552通过非种子序列与CYP2E1(cytochrome P450 2E1)启动子区域中十字形结构的环状发夹形成杂交体,进而抑制SMARCE1(SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily E member 1)和RNA聚合酶II与启动子的结合。此时,胞质miR-552通过种子序列与CYP2E1转录本的3' UTR结合沉默其表达,表明miR-552可以在转录和转录后水平双重抑制CYP2E1表达^[114]。Younger和Corey^[115]发现,miR-423-5p可以靶向人类孕激素受体(progesterone receptor, PR)启动子中的高度保守区域,从而减弱Pol II并加强H3K9me2(histone H3 lysine 9 dimethylation)与启动子的结合。miRNA诱导的TGS不是一对一关系,一种miRNA可以调节多种DNA转录,一种靶基因的转录也同时受到多种miRNA调控。如,miR-423-5p通过结合DNA启动子,以依赖AGO2的形式抑制PR和免疫球蛋白超家族成员1(immunoglobulin superfamily member 1, IGSF1)的转录。与之相似,PR可以同时受到miR-423-5p、miR-373和miR-520c-3p的抑制,提示基因启动子序列的识别可能是miRNA的一般特征。

4 靶基因调控miRNA的作用方式

4.1 靶基因下调miRNA表达

4.1.1 靶基因抑制miRNA生成

靶基因对miRNA的抑制作用依赖于序列互补结合,但是并没有发现诱发miRNA下调的靶基因与miRNA互补结合的一般规律。例如, circATRNL1可以下调miR-23a-3p的表达,而这种作用依赖于miRNA 5'端第2~8 nt与circRNA的结合,表明仅仅种子序列的结合就可以诱导miRNA下调^[116]。Wu等人^[117]发现, circCORO1对let-7c-5p的下调同样依赖于种子序列的结合。lncRNA Sox2OT-V7通过抑制肿瘤细胞miR-142/miR-22的生成,来促进阿霉素(doxorubicin, DOX)诱导的骨肉

瘤自噬和化学抗药性. Sox2OT-V7与miR-142/miR-22的结合,即不是在miRNA的5'区域,也不是在3'区域,而是发生在中间区域^[118].虽然,很多研究报道了靶基因对miRNA的抑制作用及二者的结合位点,却很少有研究阐述靶基因下调miRNA的作用机制.现有的研究表明,靶基因下调miRNA存在两种调控方式:靶基因抑制miRNA生成、靶基因诱导miRNA降解.

靶基因作用于miRNA抑制其生物发生的研究虽然有很多文献报道,但是它的机制尚未完全阐明,特别是靶基因与成熟miRNA的直接互作.目前,已知靶基因抑制miRNA生成过程均发生在加工水平,例如, Xiao等人^[119]发现,超保守区衍生的lncRNA uc.173,通过降低miR-195水平来调节肠黏膜生长并刺激肠上皮更新.机制上,uc.173直接结合pri-miR-195并降低其稳定性,进而抑制miR-195的成熟. Yu等人^[120]发现,结直肠癌细胞内的lncRNA CCAT2通过抑制pre-miR-145的核输出,阻断其成熟.在舌鳞状细胞癌(TSCC)细胞系中,lncRNA MPRL通过miR-483/FIS1途径促进线粒体分裂并提高癌细胞对顺铂化疗的敏感性.这是由于MPRL可以结合pre-miR-483的环状区域,进而阻断TRBP-DICER复合物对pre-miR-483的识别和加工,导致成熟miR-483水平下降^[121].在星形细胞瘤中,lncRNA CASC2c与miR-101的表达呈显著负相关. CASC2c作为miR-101的靶基因,可以被miR-101所降解.同时, CASC2c也可以抑制Dicer对pre-miR-101的加工,来负向调节miR-101水平,这足以说明,ncRNA与miRNA的互作不是单向的,二者存在双向抑制作用^[122].

4.1.2 靶基因诱导miRNA降解

触发靶基因诱导miRNA降解(target-directed miRNA degradation, TDMD)需要miRNA与靶基因的广泛互补配对,除了种子序列的结合外,miRNA 3'端的互补(C pairing)对TDMD的触发必不可少^[123-125].在种子序列互补和C pairing之间存在非互补区域,人们把这个非互补区域叫做中心裂(central bulge).对不同情况,TDMD所能允许中心裂的大小是不同的,因此并没有研究出触发TDMD最为简短或者最为有效的miRNA与靶基因的结合形式^[126-128].

靶基因诱导miRNA降解分为三步:分别是拖尾(miRNA 3'端添加nt,通常是U或者A)、修剪(miRNA 3'端缩短)和成熟miRNA降解(图3(b)).针对广泛互补结合有一种解释,miRISC中引导链的3'端结合在PAZ结构域内,当miRNA与靶基因广泛互补结合,可以使miRNA的

3'端在PAZ域中脱落并暴露于核酸转移酶和核酸外切酶^[126,129].之后,末端核酸转移酶(terminal nucleotidyl transferase, TNTase)在miRNA的3'端添加非模板nt,通常是U或A. TNTase是DNA聚合酶- β 超家族成员,它参与编码或非编码RNA 3'端nt的添加,通常是在末端添加腺苷或者尿苷,在人类中已经发现12种TNTase^[130,131].迄今为止,在HeLa细胞中,只有末端尿苷转移酶(terminal uridylyl transferase 1, TUT1)与TDMD核蛋白复合物共纯化.然而,敲减TUT1不会影响TDMD效率,这可能是由于其他TNTase冗余造成^[132].在果蝇和植物中拖尾主要是添加尿苷^[126],但在哺乳动物中添加腺苷的频率更高^[124,133].最终,拖尾的miRNA在3'-5'核酸外切酶作用下被降解,可能发生在miRISC中,也可能是在AGO排出miRNA后被降解^[128,134].靶基因对miRNA的降解并不是一对一的关系,一种靶基因可以针对多种miRNA进行降解^[124].在TDMD机制中,DIS3L2(DIS3 like 3'-5' exoribonuclease 2)是目前发现的唯一一种降解miRNA的酶,它属于核酸外切酶RNase II/RNB家族,在很多物种中保守,介导miRNA降解的条件是靶基因诱导尿苷化,而不是miRNA 3'末端的腺苷化^[132,135].

在植物中,植物甲基转移酶HEN1可以修饰miRNA的3'端为2'-O-甲基,该修饰可以阻止miRNA的3'端拖尾,反之HEN1的突变会诱导miRNA降解(图3(c))^[136,137].

最近有研究报道,胞质poly(A)聚合酶Gld2(GLD2 poly(A) polymerase)参与miRNA拖尾,但它的缺失并没有影响miRNA修剪,也就是miRNA水平,这对拖尾是否可以作为TDMD的一个中间步骤提出疑问^[102,138].随后, Han等人^[139]使用甲基化修饰阻断miR-7的拖尾和修剪,仅部分减弱了Cyrano对其降解,显示拖尾和修剪并不是TDMD的一个必要过程,这提示TDMD的重要调控分子仍有待挖掘.为了找出TDMD的调控分子,他们使用了全基因组CRISPR-Cas9技术,发现泛素连接酶可能会涉及Cyrano诱导的miR-7降解.实验证明, ZSWIM8(zinc finger SWIM-type containing 8)泛素连接酶可以识别AGO2表面的K493赖氨酸,进而介导蛋白酶体降解miR-7,且该过程不依赖于拖尾和修剪(图4). ZSWIM8泛素连接酶介导的TDMD过程不仅仅针对miR-7, Shi等人^[140]发现,它的缺失会使哺乳动物、果蝇和线虫中的多种miRNA累积.这表明, ZSWIM8泛素连接酶对TDMD的调控具有广泛性.

TDMD的发生并非偶然,同样具有其生物学作用.

图 3 拖尾和修剪介导的miRNA降解. (a) 哺乳动物大脑内的一个ncRNA调控网络; (b) 拖尾介导的miRNA降解; (c) 植物HEN1保护miRNA免于降解

Figure 3 Tailing and trimming-dependent miRNA degradation. (a) A network of ncRNAs in the mammalian brain; (b) tailing-dependent miRNA degradation; (c) protection of HEN1 for plant miRNAs

图 4 泛素连接酶ZSWIM8介导的miRNA降解

Figure 4 ZSWIM8 ubiquitin ligase-dependent miRNA degradation

如,神经元再生相关蛋白(neuronal regeneration related protein, Nrep)转录物可以诱导小鼠脑中miRNA-29的降解,如果破坏Nrep内miRNA-29的结合位点,会导致小鼠脑中miRNA-29的表达升高,从而损伤小鼠的平衡和运动学习能力,因此维持TDMD水平对动物行为有着重要作用^[141]. 疱疹病毒saimiri(herpesvirus saimiri, HVS)表达的小非编码RNA HSUR,通过降解T细胞中的miR-27,来促进T细胞激活^[142,143]. 溶血性小鼠巨细胞病毒(murine cytomegalovirus, MCMV)编码的转录物m169,以序列依赖性的方式降解宿主细胞内的miR-27a/b,这对MCMV在体内的有效复制非常重要^[144]. 在哺乳动物大脑的神经元细胞中,发现一种调控网络,包括一种lncRNA(Cyrano)、一种circRNA(Cdr1as)和两种miRNA(miR-671和miR-7)(图3(a)). Cyrano通过与miR-7广泛互补配对将其降解,降低了miR-7对靶基因的抑制,并增加Cdr1as的累积,Cdr1as是调控神经活动的环状RNA,它对机体维持正常的脑功能至关重要. 如果没有Cyrano,miR-7会直接靶向Cdr1as或者加强miR-671对Cdr1as的降解,导致Cdr1as在神经元胞质中的表达下降^[145].

TDMD在机体微环境中可能会扮演什么样的角色? (1) TDMD通过下调miRNA,来提高miRNA下游靶基因的表达,进而发挥调控作用. 在小鼠成纤维细胞中,内源性RNA(Serpine1)可以靶向降解miR-30b-5p和miR-30c-5p,如果抑制该TDMD效应,则会促进miR-30b/c对下游靶基因的沉默,从而调节细胞周期再进入和凋亡^[124]. (2) miRNA的一个普遍特征是它们紧密地聚集在基因组内,表现为成簇表达. 实际上,预计超过30%的人类miRNA会在多顺反子miRNA簇内编码^[146]. pri-miRNA上有多个茎环结构,最终会生成多种miRNA,由于TDMD的降解机制,可能会使不同细胞类型或不同细胞状态下的miRNA表达存在差异,进而发挥不同功能. 例如,miR-27、miR-23和miR-24来源于同一pri-miRNA,当发生HVS或MCMV感染时,只有miR-27被TDMD机制所降解. 值得注意的是,HCMV感染最初会诱导pri-Mir17-92簇中所有成员的转录,而TDMD仅靶向miR-17家族. 因此,TDMD使HCMV可以同时上调同一簇的4种成员(miR-18a、miR-19a、miR-19b和miR-92a)和下调另两种成员(miR-17和miR-20a)^[147]. (3) 在miRNA竞争有限的AGO情况下,TDMD耗尽某一miRNA可能额外地促进其他miRNA的生成. 因为miRNA半寿期很长,TDMD对于机体微环境内miRNA的快速转化至关重要^[148].

4.2 靶基因上调miRNA表达

4.2.1 靶基因保护miRNA免于降解

在秀丽隐杆线虫中,靶基因通过与let-7互补结合,抑制了XRN-2介导miRNA从miRISC中的解离和降解,从而导致miRNA水平升高,将这种靶基因介导的miRNA保护作用定义为TMMP(target-mediated miRNA protection). 体内和体外研究证实,内源性靶基因同样存在TMMP效应,表明TMMP的发生不是偶然,它与miRNA降解共同维持体内miRNA的稳态. TMMP的发生依赖于靶基因与miRNA的部分互补配对,其中miRNA种子序列突变就会破坏靶基因对miRNA的稳定作用. TMMP效应存在竞争关系,如miR-84和let-7有相似的序列,竞争性结合同一mRNA,miR-84的过表达会增加let-7的降解,反之亦然. 此外,靶基因也可以增加某些不具有热力学不对称性的miRNA随从链在AGO内累积,如miR-241*,导致miR/miR*的比例发生变化,提供一种从现有基因座产生miRNA的多样化途径^[149,150].

circRNA同样具有维持miRNA稳定的作用,Cdr1as在哺乳动物大脑中含量丰富,Cdr1as的敲除会导致miR-7的转录后下调,进而减轻miR-7对许多立早基因(immediate early gene, *IEG*)的抑制作用,如,*c-Fos*(*Fos* proto-oncogene). 已知*IEG*与大脑神经精神障碍的前脉冲抑制(prepulse inhibition, PPI)受损有关,这部分解释了Cdr1as敲除诱导的脑功能异常. 除了*IEG*,敲除Cdr1as还会影响某些昼夜节律基因的表达,例如,*PER1*(period circadian regulator 1)和*Sik1*(salt inducible kinase 1)的上调,它们的变化与小鼠前脑的睡眠抑制和延长清醒相关. Cdr1as在兴奋性神经元组织中尤其丰富,它的保护作用具有组织特异性只存在于脑组织中,这很可能与神经元的功能相关. 在肝癌细胞中,Cdr1as可以负向调控miR-7的表达,表明Cdr1as对miR-7的调控作用可能取决于细胞的类型和功能^[151~153].

4.2.2 靶基因介导的miRNA生成

不同于TMMP,靶基因还能够通过诱导miRNA生成上调其表达. miRNA的生成分为3个阶段,分别是pri-miRNA、pre-miRNA和成熟miRNA的产生^[154],也就是说可以分别从这3个阶段促进miRNA生成. Bose等人^[155]发现,在氨基酸饥饿条件下,高表达的mRNA CAT-1(cationic amino acid transporter-1)企图通过驱动miRNA-122的合成,来恢复细胞稳态. 在机制上,CAT-1与miRNA-122的结合促进了Dicer1对pre-miRNA的加工,从而使成熟miRNA水平增加. miRNA水平与靶基因

的浓度成正比, 这种作用依赖于miRNA与靶基因的结合, 其中种子序列的结合是至关重要的, 靶基因内的结合位点越多促进miRNA的生成能力就越强。大多数内源性mRNA的3'UTR存在多个miRNA结合位点, 如果一种mRNA被miRNA抑制, 则它对下游miRNA成熟的促进作用下降, 最终影响另一种miRNA的水平, 这可以解释miRNA的相互调节。由于CAT-1也是miRNA-122的下游靶基因, 表明mRNA可以通过驱动其上游miRNA的合成来自动调节自身水平。

circLONP2定位于结直肠癌细胞核, 可以直接作用于pri-miR-17, 通过与DDX1(DEAD-box helicase 1)互作间接招募DGCR8, 来促进pri-miR-17的成熟。随后, circLONP2驱动外泌体递送miR-17-5p, 来下调受体细胞中miR-17-5p的下游靶基因PTEN(phosphatase and tensin homolog), 从而增强受体细胞的侵袭和迁移能力。该机制对结直肠癌的转移非常重要, 这也是首次发现circRNA可以诱导miRNA成熟^[145]。

5 展望

miRNA作为一种重要的调控分子, 其异常表达是癌症、肝脏疾病、肾脏疾病及心血管疾病等的病因^[156-160]。因此, 研究miRNA转换机制对治疗和预防疾病至关重要。随着miRNA研究的不断深入, 发现靶基因能够调控miRNA表达, 这对进一步了解miRNA转换机制有着重要意义^[145]。靶基因对miRNA的调控可以分为两个方向: 靶基因抑制miRNA生成或诱导其降解, 进而降低miRNA表达水平^[122,139]; 靶基因促进miRNA生成或保护其免于降解, 进而提高miRNA表达水平^[145,151]。现阶段, 靶基因诱导miRNA降解的研究还处于起步阶段, 涉及的机制并没有被清楚阐明, 不依赖拖尾和修剪过程, 由泛素连接酶介导TDMD的发现, 颠覆了以往的认识, 但同时也提出了新的问题, 泛素连接酶的调控过程都涉及哪些蛋白的参与? 此外, 拖尾和修剪作为TDMD过程的必要步骤是存在争议的, 一方面, 植物甲基转移酶HEN1对miRNA的保护作用早被证实^[136,137],

另一方面, Han等人^[139]发现, Cyrano可以不依赖拖尾和修剪来靶向降解miR-7。当前, 并不能判断拖尾和修剪对TDMD过程的重要程度, 虽然Cyrano可以不依赖这两个过程对miR-7进行降解, 需要更深入的研究来揭示拖尾和修剪的作用, 如, 拖尾和修剪是否有利于miRNA从AGO内的释放或募集3'-5'核酸外切酶对miRNA进行降解。目前, 对靶基因抑制miRNA生成的机制知之甚少, 主要是靶基因与pri-miRNA互作来影响其成熟^[119-121]。

在靶基因上调miRNA的研究中, 分为诱导miRNA表达和稳定miRNA使其免于降解两个部分, 对于二者机制方面的研究甚少。Shenas等人^[161]仅报道了敲减Snail-1会抑制miR-21和miR-29b的表达。Zhang等人^[162]仅报道了敲减H19可以显著下调miR22-5p的表达, 他们并没有进行机制方面的研究, 因此, 无法对这两种过程进行区分。对于TMMP, 它是否通过改变miRISC定位或修饰起到保护作用, 仍有待解决。如果修饰可以维持一段时间, 表示较低的靶基因水平就足以保护miRNA免受降解。此外, 靶基因还可以保护某些miRNA的游客链免于降解, 这种游客链的积聚是否可以改变miRISC中的引导链, 还没有明确的证据证实^[150]。TMMP和TDMD是否共同驱动miRNA的时间和空间特异性也需要进一步研究验证。

虽然, 靶基因与miRNA相互调控方面的研究有限, 但对于机体维持稳态和治疗疾病有着重要意义。生理状况下, miRNA与靶基因存在一种动态平衡, 如果这种平衡被破坏, 就会引发一系列疾病反应。就比如, 正常组织中, lncRNA CASC2c与miR-101的相互抑制维持一个平衡状态。在星形细胞瘤中, 该平衡被破坏(高表达CASC2c或低表达miR-101), 进而加剧癌细胞增殖、侵袭和迁移等恶性进程^[122]。小鼠脑内的Nrep转录物可以诱导miRNA-29的降解, 二者达到一种平衡, 如果破坏这种平衡, 就会损伤小鼠的平衡和运动学习能力^[141]。因此, 深入了解miRNA与靶基因的动态平衡将有利于人们从靶基因入手治疗疾病, 挖掘和开发一些调控靶基因的药物, 从而恢复这种稳态。

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Summary for “microRNA与靶基因相互调控机制的研究进展”

Advances in reciprocal regulation mechanisms of microRNAs and target genes

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MicroRNAs (miRNAs), which are encoded by endogenous genes, are a class of single-stranded non-coding RNAs with approximately 22 nt in length. They act on target genes by binding to Argonaute (AGO) family proteins to form miRNA-induced silencing complexes (miRISCs), thereby functioning at the transcriptional and post-transcriptional levels. With the in-depth study of miRNAs, it has been found that in addition to messenger RNAs (mRNAs), non-coding RNAs (ncRNAs), primary miRNAs (pri-miRNAs), and DNAs can also act as the target genes of miRNAs. By mRNA or ncRNA cleavage, DNA transcription silence or activation, pri-miRNA processing, and mRNA translation, miRNAs regulate almost all cell proliferation/differentiation, individual growth/development, and homeostasis. Since miRNAs are differentially expressed in various cell types and play a role in the whole development process, studies on miRNAs have focused on their origins, biogenesis, and mechanisms. However, the mechanisms of miRNA transformation, especially the rapid change of miRNAs under specific conditions, have not been well addressed. Recently, it has been discovered that target genes can regulate the biogenesis, degradation, and protection of miRNAs in a sequence-dependent manner, indicating that the regulation of miRNAs and target genes is not unidirectional, but reciprocal. The target genes modulate the biogenesis of miRNAs by mediating the processing, stability, and nuclear export of pri-miRNAs or precursor miRNAs (pre-miRNAs). The modulation depends on the binding of miRNAs to the complementary sequence on the target genes. However, no general binding pattern represents the modulation of target genes on miRNA biogenesis. Extensive complementarity between target genes and miRNAs is a crucial requirement for the target-directed miRNA degradation (TDMD). Central bulge with varied length is a mismatched region in the center of an extensive complementarity and is essential to trigger TDMD. The mechanisms of TDMD are controversial. It may be due to the exonuclease-induced miRNA degradation, which is dependent on tailing and trimming; or the ZSWIM8 ubiquitin ligase-triggered miRNA degradation, which is independent of tailing and trimming. Organisms increase miRNA stability through target genes and coordinate with TDMD to maintain miRNA homeostasis. Elevated accumulation of the passenger strand in AGO is found in the process of target genes promoting miRNA stability. However, it is unclear whether the process will trigger a transformation of the guide strand. Although the study on the regulation of miRNAs by target genes is still in infancy, it provides a novel perspective for the regulation of miRNAs. In this review, the latest progress in the regulation of miRNAs by target genes was outlined in detail, the conditions, mechanisms, and functions of their interaction were summarized, and future research directions on miRNAs transformation were proposed in order to provide a theoretical basis for further understanding the interaction between miRNAs and target genes *in vivo* and the development of miRNA-based therapeutic approaches.

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