PIWI/piRNA介导表观遗传学调控在生殖细胞发育中的作用

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摘要 piRNA(PIWI-interacting RNA)是主要在生殖细胞系中表达的一类由21~33个核苷酸组成的单链非编码RNA, 与PIWI蛋白结合而发挥调控作用。研究显示, PIWI/piRNA不仅可以在细胞质里对mRNA直接进行剪切和降解, 还能招募大量的表观遗传学修饰蛋白介导基因组和组蛋白的表观遗传学调控关闭基因, 促进配子发育安全有序地进行。该文综述了PIWI/piRNA介导的表观遗传学调控在生殖细胞发育中的作用, 以促进PIWI/piRNA在生命科学领域的研究和应用。

关键词 PIWI/piRNA; 表观遗传学调控; 生殖发育

Effect of PIWI/piRNA Mediated Epigenetic Regulation in Reproductive Development

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Abstract piRNA (PIWI-interacting RNA) is a kind of single strand non-coding RNA composed of 21-33 nucleotides, mainly expresses in germ cells, and plays a regulatory role by binding with PIWI protein. It shows that PIWI/piRNA can not only cleave and degrade mRNA directly in the cytoplasm, but also recruit a large number of epigenetic modified proteins to shut down gene expression by DNA and histone modification, so as to promote the safe and orderly development of gametes. This review summarizes the role of PIWI/piRNA mediated epigenetic regulation in reproductive development in order to promote the research and application of PIWI/piRNA in the field of life science in the future.

Keywords PIWI/piRNA; epigenetic regulation; reproductive development

piRNA(PIWI-interacting RNA)是2006年在小鼠睾丸精原细胞中被发现的一类小非编码RNA[1-2], 对于维持真核细胞基因组稳定起到了重要作用[3-4]。piRNA在非编码RNA中的数量最多, 不同于miRNA和siRNA, piRNA的前体为单链RNA, 加工过程不需要Dicer酶, 而是依赖ZUC(zucchini)核酸酶剪切完成的, 通过初级途径和“乒乓”循环途径大量产生[5], 根据piRNA的核苷酸长度和生成途径的差异, piRNA可以选择与不同的PIWI蛋白结合而发挥调控作用[6]。PIWI招募的表观遗传修饰酶可促进异染色质组装和DNA甲基化, 维持生殖细胞正常发育[7]。

piRNA最早在生殖细胞中被发现, 主要通过沉

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默转座子的作用来维持基因组的稳定, 保证配子发育的安全进行。研究发现, 转座子在哺乳动物基因组中占比非常大, 如在人的基因组中占48%; 其中45%属于反转录转座子, 可在基因组中跳跃而造成基因组不稳定[8]。哺乳动物的进化和繁殖活动受到了严格的调控和监视, 转座子活化会引起配子发育阻滞, 所以配子发育的有序进行是在严格抑制转座子活性的基础上建立的[9]。研究显示, PIWI/piRNA介导mRNA剪切和表观遗传修饰(DNA甲基化和组蛋白修饰等), 高效地抑制了转座子活性并关闭一系列基因, 促进配子和胚胎发育安全有序地进行[10]。PIWI/piRNA介导的表观遗传学调控出现异常, 会造成配子和胚胎发育的终止[11-12], 本文将重点综述PIWI/piRNA介导表观遗传学调控在生殖细胞发育中的作用。

1 PIWI/piRNA的生物学特征

1.1 PIWI蛋白的发现

1997年, LIN和SPRADLING[13]首次在雌性黑腹果蝇中发现PIWI蛋白, 其主要介导转座子沉默、转录沉默和翻译抑制等功能, 维持和促进了卵母细胞的发育。人类中, PIWI有HIWI、HILI、PIWIL3和PIWIL4 4种, 小鼠中包括MIWI、MILI和MIWI2 3种, 果蝇中包括PIWI、AUB(aubergine)和AGO3(argonaute3)等, 它们具有类似的生物学功能[8,14-16]。PIWI含有N-端结构域、PAZ、PIWI和MID 4个结构域, 其中N-端结构域富含精氨酸, 存在蛋白催化的甲基化位点; PAZ结构域可以与piRNA的3′端结合; MID结构域可以与piRNA的5′端结合; PIWI结构域具有类似于核酸内切酶的活性, 可以切割互补的mRNA[17-18]。研究表明, PIWI蛋白N-端缺失26个氨基酸, 该突变体蛋白失去入核的能力[19]。PIWI以piRNA为导向发挥调控作用, 通过抑制转录和降解mRNA发挥基因表达调控作用[20]。

1.2 piRNA的结构特点

piRNA是由21~33个核苷酸组成的单链小非编码RNA, 大部分由成簇分布的基因组重复序列区域的piRNA簇转录产生, 单向转录的piRNA簇产生的正义链piRNA, 可以靶向转座子序列的反义链; 双向转录的piRNA簇产物可以靶向结合转座子序列的正义链和反义链, 参与胞浆“乒乓”循环过程[21]。PIWI蛋白亚家族和piRNA结合是依据其转录来源的DNA链进行选择的, AUB和PIWI可以和反义链产生的piRNA结合, 5′端富含碱基U; 正义链产生的piRNA在5′端第10个碱基具有A的保守性, 可以与AGO3结合发挥调控作用[22]。正义链和反义链产生的piRNA 3′端具有HEN1甲基转移酶(HEN1 methyltransferase)催化2-*O*-methyl甲基化修饰位点, 促使piRNA稳定存在[23-24]。

1.3 piRNA的生成过程

piRNA主要来源于转座子序列、mRNA的3′-UTR区域和长链非编码RNA[11,25], 其主要由初级途径和“乒乓”循环途径产生[5]。果蝇体细胞中, AUB和AGO3表达水平很低, 主要由PIWI参与初级途径产生piRNA(图1), 小鼠和斑马鱼等动物的初级piRNA途径与之类似。首先, 果蝇中双链piRNA簇转录产生piRNA前体, 然后在转运蛋白NXT1(ntf2-like export factor 1)和NXF1(nuclear RNA export factor 1)的作用下转运到细胞质Yb结构域内进行加工, piRNA的5′端被ZUC和处于线粒体膜上的辅助因子MINO(minotaur)和GASZ(germ cell specific ankyrin, SAM and basic leucine zipper domain-containing protein)识别切割, 产生具有5′U的短链piRNA中间体。然后, 在SHU和HSP83(heat shock protein 83)蛋白的作用下与PIWI结合。接下来, piRNA 3′端被核酸外切酶TRIMMER和PAPI剪切以及被HEN1甲基化酶催化, 产生成熟的piRNA, 最后, 以PIWI/piRNA复合物的形式转运到细胞核内, 招募表观遗传学修饰蛋白进行表观遗传学调控, 也可以在细胞质中进行mRNA切割和降解调控, 促进配子发育[5]。

piRNA在果蝇、斑马鱼和大部分哺乳动物生殖细胞中, 以“乒乓”循环方式扩增(图1), 在小鼠中略有不同, 以异型“乒乓”循环方式扩增, 由MILI/piRNA复合物参与小鼠睾丸中的异型“乒乓”循环, 而MIWI不参与扩增循环, 这与减数分裂粗线期piRNA相关; MIWI2与初级piRNA结合转运至核内, 也不参与扩增循环过程[23]。果蝇生殖细胞中piRNA在一个电子密集和非晶体的核周结构区(nuage)进行加工, 由AUB和AGO3不断切割产生。首先, 由初级途径产生的成熟piRNA与AUB在Nuage结构区中结合形成复合物, 并与靶向互补mRNA相结合并进行剪切, 形成新的piRNA 5′端[10,23], 然后, AGO3与AUB剪切后的mRNA结合, 剪切形成新的piRNA 3′端, 在HEN1甲基化酶的修饰作用下, 形成成熟的piRNA[15]。接

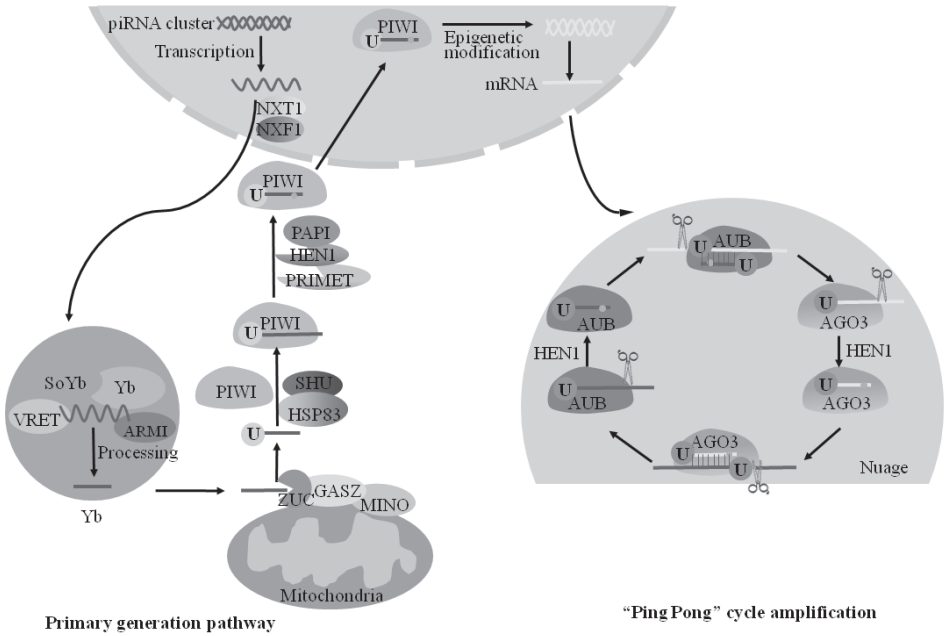

图1 piRNA的生物发生

Fig.1 The biogenesis of piRNA

下来, AGO3携带的piRNA通过碱基互补配对识别互补的mRNA序列并剪切形成piRNA 5′端; 随后, 剪切后的mRNA又与AUB结合形成复合物进行3′端剪切, 并被甲基化修饰, 形成成熟的piRNA[26], AUB携带piRNA可以靶向切割新的mRNA, 不断往复循环, 构成了整个"乒乓"循环过程[22]。其中, AUB和AGO3不断剪切转座子或基因的mRNA, 驱动持续的"乒乓"循环, 并高效抑制转座子和其他基因的表达[25,27]。"乒乓"循环扩增出大量PIWI/piRNA复合体, 可以进入细胞核, 介导基因组和组蛋白表观遗传学调控, 直接关闭相关基因和终止转座子的转录。

2 PIWI/piRNA对生殖细胞发育的表观遗传调控作用

PIWI/piRNA介导的DNA甲基化、组蛋白甲基化、乙酰化和泛素化等调控在配子发育中发挥着极其重要的作用。PIWI蛋白突变引发配子发育阻滞, 果蝇体内*Ago3*缺失后, 导致雌性不育[28], *Piwi*和*Aub*缺失导致雌雄不育[29-30]; 小鼠中*Mili*和*Miwi2*缺失致使雄性不育[6]; 也有很多证据显示, piRNA表达异常引起基因组表观遗传学修饰改变, 转座子被激活, 导致发育阻滞[31]。

2.1 PIWI/piRNA对生殖细胞发育的DNA甲基化调控作用

PIWI/piRNA介导DNA甲基化调控, 主要通过招募DNA甲基转移酶3a/3b(DNA methyltransferase 3a/3b, DNMT3a/3b)[32], 促使基因启动子区和结构区的CpG岛进行甲基化修饰(图2), 关闭一些功能基因和转座子, 促进精子和卵子发育的安全、稳定和有序进行[33]。

配子发育过程是基因表达有序关闭和开启的过程, 通过细胞基因组和组蛋白表观遗传学修饰完成基因表达的精准调控过程, 最终形成高度甲基化和螺旋化的配子基因组, 表达配子发育过程中所需要的蛋白。PIWI/piRNA介导DNA甲基化调控是配子发育调控的基础[20], 从而促进配子发育进行有序的甲基化[34]。研究显示, 小鼠中PIWI/piRNA介导DNA甲基化抑制*Astrin*表达, 促进小鼠卵母细胞有丝分裂和减数分裂过程中的纺锤体解体[35], 通过抑制配子特异性因子1(gametocyte specific factor 1, GTSF1)等, 促进了卵子发育[36]。在果蝇中, PIWI/piRNA介导DNA甲基化抑制Y染色体上Stellate的表达, 抑制转座子Gypsy的活性[31], 促进果蝇卵子的发育[37]。

许多生殖发育相关的蛋白通过与PIWI/piRNA相互作用调控精子发育(表1), 其中TDRD(tudor domain containing protein)家族是进化保守的蛋白家族, 在PIWI蛋白N-端的精氨酸甲基化修饰作用下, 可与PIWI蛋白特异性结合, 在piRNA的生成和生殖细胞发育中必不可少[23,38]。其中, *Tdrd1*、*Tdrd9*和*Tdrd12*等突变, 导致转座子甲基化异常, 影响精子发

育[39-42]。MVH(mouse vasa homolog)是小鼠配子发育中重要的调控蛋白, 参与配子细胞特异表达, 其突变阻碍piRNA与MIWI2结合, 减少了基因组甲基化和转座子重新甲基化, 阻碍piRNA的循环过程, 导致精母细胞阻滞在减数分裂期, 而导致小鼠不育[43-44], *Tdrkh*突变体引起了转座子的低甲基化, 繁殖过程阻滞在合子时期, 影响小鼠的精子发育[45]; 其他关键生殖细胞特异性蛋白GASZ、MOV101(moloney leukemia virus 10-like 1)和MAEL(maelstrom)等在生殖干细胞中特异性表达, 与PIWI蛋白相互作用, 共同调控精子细胞基因组甲基化, 促进精子发育有序进行[46-48]。

2.2 PIWI/piRNA对生殖细胞发育的组蛋白甲基化调控作用

组蛋白甲基化修饰引起基因结构变化, 影响

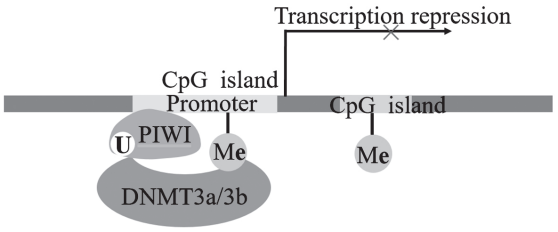

图2 PIWI/piRNA介导DNA甲基化调控作用

Fig.2 PIWI/piRNA mediates DNA methylation regulation

表1 PIWI/piRNA介导甲基化调控在精子发生中的调控作用

Table1 The role of PIWI/piRNA mediates methylation regulation in spermatogenesis

蛋白亚类 Protein subclass	互作蛋白 Interacting protein	PIWI相关蛋白突变引起精子发育阻滞时期 Sperm development is arrested caused by PIWI mutation				调控机制 Regulation mechanism
		细线期 Leptotene	偶线期 Zygotene	粗线期 Pachytene	圆形精子 Round sperm	
MILI	MAEL, MOV10L1, TDRD12, TDRD6, TDRD1 and MVH	—	—			DNA methylation regulation of MILI could turn off related genes and transposons
MIWI	MOV10L1, TDRD6, TDRD2, MVH, CAF1, GASZ, MAEL and TDRD8	—	—	—	—	DNA methylation and ubiquitination regulation of MIWI could promote sperm development
MIWI2	TDRD9, TDRD2	—				DNA methylation of MIWI2 promotes embryonic developmen
TDRD1	TDRD12, MILI and MIWI	—	—	—	—	DNA methylation regulation of TDRD1 could turn off related genes and transposons
TDRD9	MIWI2	—	—			DNA methylation regulation of TDRD9 could turn off related genes and transposons
MVH	MAEL, MIWI and MILI	—	—			DNA methylation regulation of MVH promotes sperm development
MOV10L1	MIWI and MILI	—	—			DNA methylation regulation of MOV10L1 promotes sperm development and maintains spermatogenesis
GTSF1	PIWI	—				DNA methylation regulation of GTSF1 promotes ovum development
GASZ	MIWI and MILI	—	—	—		DNA methylation regulation of GASZ promotes sperm development
MAEL	MIWI, MILI and MVH	—	—	—	—	DNA methylation regulation of MAEL promotes the development of acrosome and flagellum

RNA聚合酶与启动子结合, 进而调控基因表达水平。其中H3K9、H3K27和H4K20甲基化修饰, 导致该区域结构更紧密, 作为基因表达抑制的标志; H3K4和H3K36甲基化修饰引起基因组结构更开放, 作为促进基因表达的标记[49-52]。在小鼠配子发育过程中, PIWI/piRNA偶联无芒相关同源框蛋白(aristaless-related homeobox, ARX)和泛连接蛋白(pannexins, PANX), 招募赖氨酸特异性去甲基转移酶(lysine-specific demethylase 1, LSD1)与之结合, 引起H3K4me2向H3K4me转变, 特定基因转录活性也随之降低; 此外, 小鼠中PIWI/piRNA也吸附EGG(eggless)、H3K9甲基转移酶辅助因子WDE(windei)和异染色质蛋白1a(heterochromatin protein 1a, HP1a)等, 增加H3K-9me3修饰, 关闭特定基因和转座子, 维持和促进配子发育[53-55](图3)。

PIWI/piRNA介导的组蛋白甲基化调控作用对生殖细胞发育起到重要作用。研究显示, 人类PIWIL4/piRNA在胚胎干细胞和卵母细胞中介导组蛋白H3K4甲基化修饰, 对维持其正常生长发育及功能具有重要作用[56]。研究还发现, PIWIL4/piRNA引导赖氨酸去甲基酶1A/5B(lysine demethylases 1A/5B, KDM1A/5B)减少H3K4me2修饰, 关闭特定的基因和转座子, 促进生殖发育有序进行[56]。最近研究发现, 一种泛素样蛋白(ubiquitin-like containing PHD and RING finger domains 1, UHRF1)与II型精氨酸甲基转移酶5(protein arginine methyltransferase 5, PRMT5)共同在小鼠PIWI/piRNA通路中调控配子发育[57]。UHRF1结合PRMT5在原始生殖细胞介导H2A和H4去甲基化导致转座子沉默, 维持生殖细胞正常发育; 此外, UHRF1在DNA复制过程中, 会与半价甲基化DNA结合, 招募DNMT1在H3K9me3修饰位点结合, 进行DNA甲基化修饰, 从而促进配子发育[58-60]。该研究还发现, UHRF1的缺失会导致DNA低甲基化、转座子活性上调和染色质形态改变, 造成小鼠不育[57]。

2.3 PIWI/piRNA对生殖细胞发育中组蛋白乙酰化调控作用

组蛋白乙酰化受组蛋白乙酰转移酶(histone acetyltransferase, HAT)和组蛋白去乙酰化酶(histone deacetylases, HDAC)共同调控[61], 是重要的基因表观遗传调控方式之一。HAT在组蛋白N-端保守的赖氨酸残基上进行乙酰化修饰, 可将乙酰辅酶A的乙酰基转移到组蛋白上, 导致组蛋白与DNA结合的电荷作用力减弱, 染色质结构疏松, 促进基因转录; 而HDAC则会促进DNA与组蛋白的紧密结合, 不利于转录的进行[61]。精子的发生和卵母细胞成熟等生殖过程受到组蛋白乙酰化的精确调控, 乙酰化水平异常导致生殖细胞生成障碍[62]。研究发现, PIWI/piRNA在生殖细胞发育中介导组蛋白乙酰化调控作用, 实验表明, 人类中的PIWI/piRNA介导组蛋白乙酰化调控中显示PIWIL2的表达减少, 细胞分裂停在G_2/M期[63], PIWIL2/piRNA招募HAT, 进行H3K9乙酰化调控, 促使周期蛋白依赖性激酶2(cyclin-dependent kinases 2, CDK2)和cyclin A的转录增加, 最终促进胚胎干细胞增殖, 显示出其在胚胎阶段的调控作用[64]。但目前对于PIWI/piRNA在生殖细胞发育中组蛋白乙酰化调控作用研究还不透彻, 需要更多实验来探究组蛋白乙酰化调控作用, 丰富表观遗传学机制。

2.4 PIWI/piRNA对生殖细胞发育的组蛋白泛素化调控作用

PIWI/piRNA介导组蛋白泛素化是重要的表观遗传调控机制之一, 对生殖细胞的发育具有重要意义。泛素化与泛素/蛋白酶体降解途径联系在一起: 首先, 泛素蛋白以ATP提供的能量参与泛素酶的交酯反应, 并与泛素结合酶结合, 然后, 通过转运或氨解反应与泛素连接酶和靶蛋白结合成一个多聚泛素化复合物, 识别并降解靶蛋白[65]。泛素化在蛋白质的代谢、定位、降解和调节等方面都起着至关重要的作用, 精子发育特定阶段可以降解PIWI, 促进精子发育[66]。精子形成过程中, 首先, APC/C与泛素组成的复合物和一种E3泛素连接酶RNF8(ring finger protein 8)在小鼠细胞质中与MIWI/piRNA复合物结合以复合体形式存在, 在泛素化途径的降解作用下使MIWI发生降解, 泛素和APC/C组成的复合物解聚; 而RNF8游离并进入细胞核中, 大多数组蛋白在RNF8介导的泛素化作用下被鱼精蛋白替代, 形成染色质高度浓缩状态的DNA-鱼精蛋白复合体, 促使精子特定阶段的发育; 若MIWI与RNF8结合, RNF8则无法进入细胞核介导H2A和H2B组蛋白泛素化修饰, 使得精子发育受阻[66-67](图4)。该研究还表明, RNF8末端的一段短肽(RNF8-N)可以缓解MIWI对RNF8的抑制作用, 完成精子发育过程中的组蛋白泛素化修饰。精子发生、卵母细胞成熟和胚胎发育等生殖过程受到组蛋白泛素化的精确调控[63], PIWI/piRNA在精子中的组蛋白泛素化调控作用, 维持精子正常发育进程。

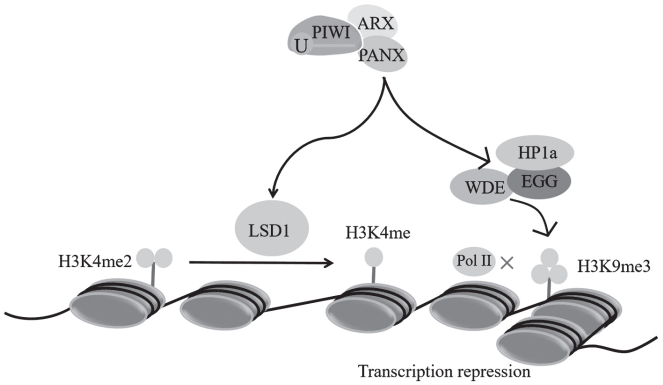

图3 PIWI/piRNA介导组蛋白甲基化调控作用

Fig.3 PIWI/piRNA mediates histone methylation regulation

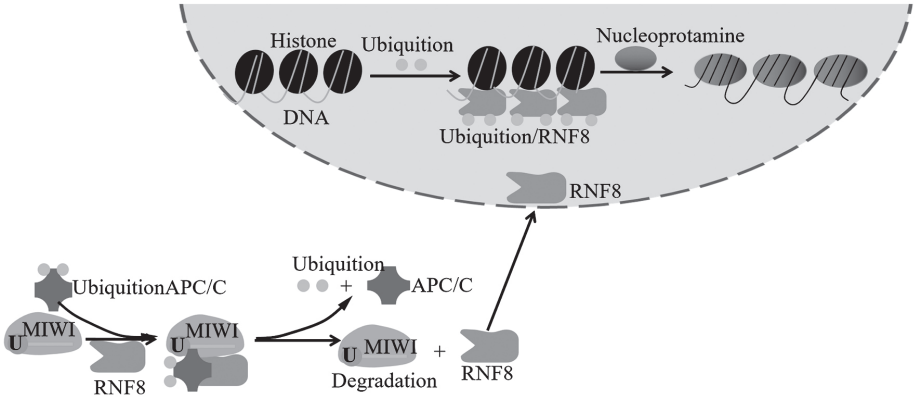

图4 PIWI/piRNA介导组蛋白泛素化调控作用

Fig.4 PIWI/piRNA mediates histone ubiquitination regulation

3 总结与展望

PIWI/piRNA介导表观遗传学调控在生殖细胞发育过程中发挥着重要的作用, PIWI和piRNA表达异常均会引起配子发育阻滞。沉默转座子以维持基因组稳定, 是配子发育的基础, PIWI/piRNA介导表观遗传学调控, 包括DNA甲基化、组蛋白甲基化、组蛋白乙酰化和组蛋白泛素化等, 不仅在转录水平上发挥了重要调控作用, 而PIWI家族蛋白可以在piRNA指导下切割mRNA, 也显示了转录后调控作用, 对卵母细胞和精子的发育有重要意义。但目前组蛋白乙酰化相关研究还较少, 需要更多的研究来解析PIWI/piRNA介导组蛋白乙酰化在生殖细胞发育过程中的作用, 深入研究表观遗传学机制有助于完善生殖细胞发育理论, 丰富表观遗传学调控理论。随着研究深入, PIWI/piRNA有可能成为生殖类疾病诊断的小分子标记物, 促进生殖医学诊断以及治疗的发展。

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