哺乳动物基因组印记的擦除、建立和维持机理

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摘要 基因组印记主要依靠印记基因DNA甲基化方式调控, 这种表观遗传修饰让多种哺乳动物出现基因单等位表达现象。印记的擦除发生在原始生殖细胞(primordial germ cells, PGCs)时期, 其主要途径为活化诱导的胞苷脱氨酶(activation-induced cytidine deaminase, AID)、TET(ten-eleven translocation)蛋白介导的去甲基化。印记的建立发生在配子发生期, 雌雄有明显的不同。印记的维持在多种因子的共同作用下完成, 主要参与的蛋白有Dnmt1、Dppa3、KAP1和ZFP57等。印记的维持贯穿整个发育阶段, 并通过细胞分裂遗传给子代。机体正常生长发育有赖于印记基因的正常表达。随着第一个印记基因*IGF2R*的发现, 对于印记机制的研究不断推进。该文将概述基因组印记的建立、维持、擦除机理以及克隆动物中存在的印记基因异常重编程。

关键词 基因组印记; 甲基化擦除; 建立; 维持; 克隆动物

Mechanism of Erasure, Establishment and Maintenance of Mammalian Genomic Imprinting

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Abstract Epigenetics modification of genomic imprinting results in single allelic gene expression in many mammals, which are mainly regulated by DNA methylation of imprinted genes. Imprint erasure occurs in the PGCs stage, and its main pathway is demethylation mediated by AID and TET. The imprinting is established during gametogenesis, with marked differences between males and females. Maintenance of imprinting is accomplished under the combined action of various ffactors, including Dnmt1, Dppa3, KAP1, ZFP57, etc. Maintenance of imprinting runs through the whole development stage and is inherited to offspring through cell division. The normal growth and development of organism depends on the normal expression of imprinted genes. With the discovery of *IGF2R*, the first imprinted gene, the study of imprinting mechanism has been advancing. The paper will summarize the mechanism of establishment, maintenance and erasure of genomic imprinting and the abnormal reprogramming of methylation in cloned animals.

Keywords genomic imprinting; imprints erasure; imprints establishment; imprints maintenance; cloned animals

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孟德尔遗传定律认为, 动物体细胞基因的两个拷贝分别来源于父母, 被称为父源拷贝和母源拷贝, 且通常情况下两个拷贝均被表达, 即双等位表达。20世纪80年代早期, 在小鼠胚胎上的研究发现了亲本基因组并不都是双等位表达, 在雄性或雌性配子发生过程中, 部分基因发生了修饰[1]。1991年确定的第一个单等位表达基因*IGF2R*(insulin-like growth factor 2 receptor)仅在母源拷贝表达[2], 这种单等位表达的基因定义为印记基因, 随后又发现了lncRNA H19、*IGF2*(insulin-like growth factor 2)等多个印记基因。到目前为止, 已经在小鼠中识别出近200个印记基因, 在人类中识别出165个[3]。印记基因通常只表达来自父源或母源的拷贝, 并成簇出现。在印记基因的调控中, 主要通过位于基因簇中的印记基因调控区(imprinting control region, ICR)也被称作差异甲基化区域(differentially DNA methylated region, DMR)来实现[4]。近年来的研究发现, 印记基因在配子发生、胚胎发育、神经发育以及出生后机体代谢等方面都发挥重要作用, 印记基因的异常或缺失会导致很多疾病, 例如贝威二氏综合征、帕威二氏综合征、Angelman综合征和鲁塞尔综合征等的发生[5-9]。

机体中所有细胞(个别例外)都携带相同的基因组, 细胞的特化功能是由于不同时空转录翻译的差异, 并不是其遗传信息不同。表观遗传调控可通过DNA共价化学修饰、组蛋白翻译后修饰和RNA介导的基因沉默等途径进行, 从亲本配子传递到受精卵。这些因子通常被称为“表观遗传”因子[10]。其中DNA甲基化是印记基因的主要调控方式, 也是最稳定的表观遗传修饰方式之一。这种DNA甲基化修饰贯穿机体整个发育阶段, 在原始生殖细胞(primordial germ cells, PGCs)进入生殖嵴时被擦除, 雄性生殖细胞在出生前又重新建立甲基化, 而雌性生殖细胞要在出生后才开始建立, 性成熟前完成[4,11]。印记基因甲基化的擦除、建立和维持对于动物的发育是至关重要的。

1 基因组印记的擦除

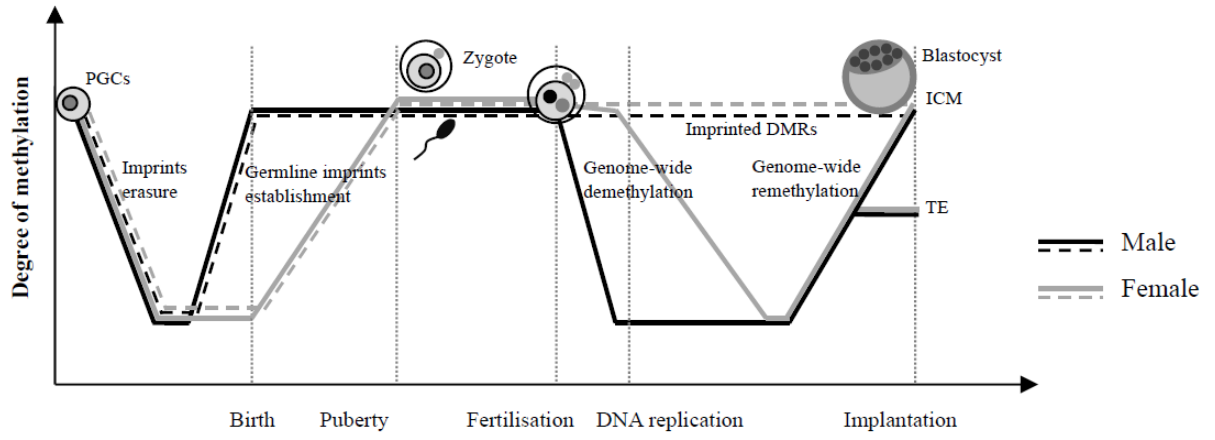
表观重编程包括DNA甲基化、组蛋白修饰和X染色体失活等, 这些多发生在PGCs恢复多能性的阶段[11-12]。精卵受精后7~8 h内, 雄原核在第一次分裂前发生主动去甲基化, 而雌原核则要在分裂数次之后才进行被动去甲基化。在去甲基化表观重编程的大潮中, 印记基因能保持其表观修饰。

印记的擦除发生在PGCs时期。对于PGCs印记擦除的机制还未完全了解, 就目前研究所知, 印记是逐步被擦除的。以小鼠为例, 第一阶段, 大约在胚胎发育的第8.5天(E8.5)开始, 基因组甲基化几乎全部丧失。第二阶段发生在E9.5至E13.5, 特定基因座发生去甲基化[13]。PGCs中的阶段特异性去甲基化可以防止早熟分化, 这是亲代将DNA传递给后代必不可少的[14]。

近年来的研究对DNA去甲基化的机理提出了多种假设。活化诱导的胞苷脱氨酶(activation-induced cytidine deaminase, AID)参与PGCs去甲基化, 其能对5-胞嘧啶(5-mC)进行脱氨基作用使之变为T, 在DNA去甲基化的组织中表达。但AID缺乏的细胞, 仍然表现为去甲基化, 可见还有其他的去甲基化的机制存在。5-羟甲基胞嘧啶(5-hmC)是一种重要的DNA去甲基化中间产物[15], 它可在主动去甲基化途径中充当中介, 或者通过排斥DNA甲基转移酶1(DNA methyltransferase 1, Dnmt1)进行被动去甲基化[16-17], 再或者通过抑制主动去甲基化的中介物[16]发挥去甲基化的作用。另外, TET(ten-eleven translocation)蛋白家族在去甲基化中也发挥着重要作用。在哺乳动物中, TET蛋白家族介导5-mC转换为5-hmC, 连续氧化可将5-hmC转化为5-甲酰基胞嘧啶(5-fC)和5-羧基胞嘧啶(5-caC)[18], TET1和TET2在E11.5和E12.5在PGCs表达[19]。5-fC和5-caC能在小鼠的胚胎干细胞基因组中被检测到[20]。同样, 敲除了*TET1*的小鼠胚胎干细胞中, 5-fC和5-caC的水平显著降低, 小鼠表现印记基因表达水平异常和ICR甲基化[21]。在HEK293细胞中过表达TET2蛋白后, 能检测到相对稳定水平的5-fC和5-caC。TET3则更像去甲基化活动的守卫, 抑制TET3活性或TET3缺失会造成合子5-hmC的量减少, 但是父本5-mC擦除不受影响。该研究表明, TET3在低甲基化基因组位点中起保护作用, 而不是作为去甲基化事件的起始者[22]。

2 基因组印记的建立

亲本的印记基因通过细胞分裂遗传给子代体细胞, 但必须经过重新建立这一过程。在卵母细胞和精子发生过程开始, DNA甲基化标记ICRs, 使双亲等位基因出现差异化表达[24]。在基因组印记的建立过程中, 雌性和雄性有明显的差别(图1)。在雌性生殖细胞中, ICR重新甲基化发生在减数分裂前期I



当PGCs进入生殖嵴, 全基因组发生去甲基化擦除父源和母源基因组印记。随后在配子发生中, 性别特异性甲基化的建立包括新的印记的建立都有赖于从头甲基化。受精后, 全基因组发生广泛的去甲基化。父系基因组进行主动去甲基化, 母系基因组进行被动去甲基化。雄原核进行主动去甲基化在DNA第一次复制前完成, 而母源基因在DNA复制几次后进行被动去甲基化。一旦甲基化重新建立, 就一直维持。在胚胎附植期, 将会发生全基因组再次甲基化, 将这些甲基化标志遗传到子代细胞贯穿整个个体发育。

When PGCs enter the genital ridge, global demethylation of the whole genome occurs to erase the paternal and maternal chromosomal imprintings. At later stages of germ cell development (before birth in male and after birth in female), *de novo* methylation results in the establishment of sex-specific germ cell methylation patterns, including methylation marks at imprinted loci. After fertilization, the methylation marks inherited from the gametes are erased again, with the paternal genome undergoing active demethylation before the first replication of DNA and the maternal genome undergoing passive demethylation after several copies of the DNA. Once the methylation is re-established, it will be maintained. Upon implantation, a wave of *de novo* methylation establishes the initial embryonic methylation pattern. These methylation markers are inherited to the progeny cells throughout the individual development.

图1 小鼠配子发生和早期胚胎发育过程中全基因组和印记的甲基化重编程过程(根据参考文献[23]修改)

Fig.1 Genome-wide imprint methylation programming during gametogenesis and early embryonic development in mice (modified from reference [23])

的双线期, 不同的ICR不同步建立甲基化。母源印记建立开始于动物出生后, 在卵母细胞成熟时全部完成[17,25-26]。而父源印记的建立在动物出生前完成, 例如在小鼠雄性生殖细胞中, 父源印记的建立在E13.5之后不久开始并在出生前完成[27]。

近期研究发现了小鼠详细的印记建立机制。哺乳动物体内有3种有活力的从头甲基化转移酶Dnmt3a、Dnmt3b和Dnmt3c。雄性和雌性生殖细胞建立印记都需要Dnmt3a[28]。除*Rasgrf1* ICR外, Dnmt3b不参与其他基因甲基化建立[28-29], 所以印记建立并不完全需要Dnmt3b, 去除Dnmt3b的卵母细胞或精子都没有出现明显的ICR低甲基化。Dnmt3c最近被鉴定为从头甲基转移酶, 能够对转座子进行甲基化和沉默, 该酶对于维持小鼠生育力是必需的[30]。另外还有1个联合因子Dnmt3L, 与Dnmt3a、Dnmt3b同源, 在生殖细胞中大量表达。Dnmt3L没有甲基化转移酶催化域, 但能提高甲基化转移酶活性, 参与维持Dnmt3a的稳定性[31], 是从头甲基化转移酶的辅助因子[32]。在囊胚阶段前, 雌性和雄性生殖细胞都高表达Dnmt3a和3L[33-35]。编码Dnmt3a和Dnmt3L的基因分别被敲除后, 母源印记无法建立, 故而它们在卵母细胞发育中、母源印记建立中是必需的[33,36-38]。另外研究表明, Dnmt3L ADD(ATRX-Dnmt3-Dnmt3L)结构域能控制精子发生过程中胞嘧啶甲基化的建立[32]。

以组蛋白H3修饰为代表的组蛋白标记也起着至关重要的作用。研究表明, 组蛋白修饰可调节早期胚胎DNA甲基化谱的建立[39]。例如H3K36me3、H3K9me2和H3K9me3, 与DNA甲基化和转录沉默相关[40]。母体基因组上的H3K27me3可以通过DNA甲基化方式独立调节等位基因特异性表达[41], 并且能限制早期胚胎中增强子的功能[42]。

3 基因组印记的维持

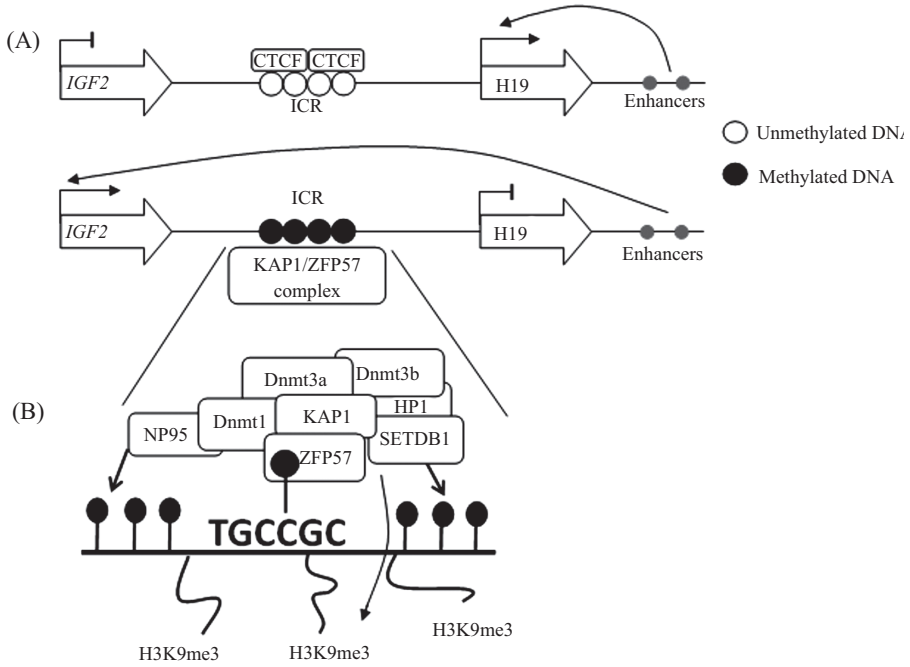
印记一旦建立, 就要在整个胚胎发育过程中维持, 这一过程十分重要, 因为植入前胚胎要经历全基因组表观重编程, 而在ICR发生不同甲基化以保护印记基因免除这种重编程[17,43]。

Dnmt1在维持印记基因过程中起到了重要作

用。在小鼠体内, Dnmt1有2个亚型, 卵母细胞特异型(Dnmt1o)和体细胞特异型(Dnmt1s)。Dnmt1o维持植入前胚胎在一个细胞周期的甲基化状态。因此, 母源Dnmt1o突变体胚胎表现出一系列印记甲基化丢失的现象[44]。

Dnmt1s对植入前胚胎起到维持体细胞甲基化的作用[45]。Dnmt1o和Dnmt1s在S期连同它的联合作用因子NP95被募集到半甲基化位点。NP95通过SRA结构域识别半甲基化CG位点, 并通过E3泛素连接酶泛素化H3K18和H3K23[46], 组蛋白H3的泛素化有助于促进DNA半甲基化到甲基化的转变[47]。*Dnmt1*基因敲除的小鼠表现出广泛的DNA低甲基化, 母源和合子敲除 *Dnmt13a*和*Dnmt13b*后, 并不影响父源基因H19 ICR维持甲基化, 所以*Dnmt1*基因对维持体细胞甲基化有不可替代的作用, 而Dnmt3a和Dnmt3b在维持甲基化方面并不是必需的。近期有研究指出, Dnmt1不仅参与基因组印记的维持, 还参与基因组中部分区域的从头甲基化[48]。

Dnmt1s维持体细胞系印记, 这个过程不仅有Dnmt参与, 还有很多其他因子的加入, 如Dppa3 (developmental pluripotency associated protein 3)、KAP1(KRAB-association protein 1)、ZFP57(zinc finger protein 57)和MBD3(methyl-CpG-binding domain 3)[17]。Dppa3通过阻止由Dnmt1介导的从头甲基化来保护母源基因免除去甲基化[50](图2)。ZFP57是锌指蛋白KRAB家族中的一员, 能维持父源和母源多位点甲基化, 能识别并结合甲基化的结合模体TGC^{5m}CGC[51]。ZFP57的KRAB结构域能和KAP1(也称作TRIM28或TIF1β)相互作用, 形成ZFP57-KAP1复合物, 继而募集组蛋白去乙酰化酶复合物(nucleosome remodeling



A: H19/*IGF2*的表达由位于中间的ICR是否甲基化修饰来调控。H19和*IGF2*的启动子共用同一增强子, 位于H19下游。母源染色体上, 未甲基化ICR结合CTCF, 阻断增强子对上游*IGF*启动子的作用, 致使*IGF2*不表达而H19表达; 在父源染色体上, CTCF不能结合在甲基化的ICR, 致使ICR的绝缘体功能失效, 从而使得下游的增强子可以激活*IGF2*的表达而沉默了H19的转录。B: 维持印记基因ICR甲基化的因子。首先ZFP57通过“TGCCGC”位点(其中CG是甲基化修饰)识别印记基因ICR, 然后和KAP1结合, 由KAP1募集SETDB1、NuRD复合体、异染色质蛋白1以及Dnmt1等形成KAP1/ZFP57蛋白复合体, 来维持印记基因ICR的甲基化。

A: the expression of H19/*IGF2* is regulated by whether the intermediate ICR is methylated or not. The promoters of H19 and *IGF2* share the same enhancer and are located downstream of H19. On the mother chromosome, unmethylated ICR combined with CTCF, blocks the effect of the enhancer on the upstream *IGF2* promoter, resulting in the non-expression of *IGF2* and the expression of H19; in the parent chromosome, CTCF cannot bind to the methylated ICR, and cause the insulator function of ICR to fail, so that the downstream enhancer can activate the expression of *IGF2* and silence the transcription of H19. B: the KAP1/ZFP57 protein complex maintains ICR methylation of imprinted genes. First, ZFP57 recognizes the imprinted gene ICR, through the “TGCCGC” site and then binds to KAP1. The SETDB1, the NuRD complex, heterochromatin 1 and Dnmt1 was recruited by KAP1, and formed KAP1/ZFP57 protein complex to maintain the methylation of imprinted gene ICR.

图2 KAP1/ZFP57蛋白复合体维持印记基因ICR甲基化(根据参考文献[49]修改)

Fig.2 KAP1/ZFP57 protein complex maintains ICR methylation of imprinted genes (modified from reference [49])

and histone deacetylase, NuRD)、组蛋白H3K9三甲基化酶(SET domain bifurcated 1, SETDB1)和异染色质蛋白(heterochromatin protein 1, HP1)[52-54]。ZFP57-KAP1复合物通过保护基因不受TET介导的去甲基化的影响, 使胚胎干细胞保持异染色质甲基化和DNA甲基化[55]。另外, ZFP57能建立*SNRPN*位点ICR的甲基化, 其具体机制有待研究。除此之外, KAP1也是维持基因组印记的关键调节蛋白之一。有研究表明, 同时敲除小鼠胚胎母源和合子的*KAP1*基因, 会对胚胎印记的维持产生严重影响, 检测后发现, 所有胚胎均发生了印记的丢失, 证明母源及合子的KAP1均为早期胚胎印记维持所必需[56]。

4 克隆动物印记基因异常重编程

不同于正常受精的动物, 克隆动物存活率低, 大多数克隆胚胎在植入期死亡, 能出生的克隆动物也通常表现出很多异常, 例如克隆胎儿的胎盘、肺、心脏、肝等器官都表现出过度增长、心肺功能不全等。研究显示, 早期克隆胚胎重编程过程中基因组印记紊乱是影响体细胞克隆效率的重要因素[57]。

KONO[58]首先提出, 克隆动物发育异常可能和印记基因表达异常有关, 因为克隆动物发育异常的症状和印记基因相关疾病以及印记基因突变模型上的症状很相似。近年来发现, 克隆动物都存在不同程度的异常甲基化情况。我们课题组[59-60]在死亡的克隆牛胎盘和内脏等多个组织上检测了多个印记基因的表达和DNA甲基化水平, 发现死亡克隆牛多个组织上存在大量印记基因的表达和甲基化水平在死亡克隆牛多个组织上是异常的, 例如*IGF2R*和*XIST*(X chromosome inactivation)出现严重的去甲基化, 且表达异常。我们进一步通过MeDIP-seq甲基化测序全基因组分析发现了死亡克隆牛上*IFG2*等多个印记基因异常低甲基化的现象[61]。我们还在牛早期胚胎上检测了H19/*IGF2*、*IGF2R*和*XIST*三个ICRs的甲基化水平, 结果显示牛体外受精胚胎的甲基化水平在50%左右, 但3个ICRs区在克隆胚胎上都表现不同程度的去甲基化现象[62]。YU等[63]研究发现, 对猪诱导的多能干细胞和克隆胚胎上印记基因异常沉默。DENG等[64]研究中发现3日龄死亡克隆山羊的耳、肺和脑组织中*XIST*甲基化水平显著升高, *XIST*的高甲基化可能是由于不完全的重新编程所致, 并在死亡的雌性克隆山羊中被保留, 从而可能导致*XIST*的调控失调。CURCHOE等[65]研究H19/*IGF2*印记基因在克隆牛和对照组非体细胞核移植牛上的差异, 发现在克隆动物的胎盘、肝等多个组织中均存在ICR低甲基化问题, 表现为H19的双等位表达。张明月等[66]研究发现, *PEG11*基因(progression elevated gene 11)在自然繁殖牛的7个组织中均为单等位基因表达; 而在体细胞核移植牛肺脏中*PEG11*基因为双等位基因表达, 其余6个组织中为单等位基因表达。WEI等[67]研究发现, 死亡的克隆猪胎盘*IGF2*、H19、*PEG3*和*GRB10*印记基因显著低于存活组。苏建民等[68]发现, *PEG10*在围产期死亡且在有发育缺陷的转基因克隆牛胎盘上的DNA甲基化程度异常高, 而存活组表现出较为正常的DNA甲基化程度。以上的研究表明, 克隆动物上普遍存在印记紊乱的问题, 而供体体细胞核去分化不彻底、重编程不完全, 发育相关的印记基因印记紊乱很可能就是克隆胚胎发育异常以及克隆效率低的主要原因之一[69]。

核移植胚胎上为什么普遍存在基因组印记甲基化丢失的现象? 我们前期高通量测序(未发表数据)发现, 具有保护印记基因甲基化功能的基因ZFP57在克隆胚胎上异常低表达。克隆过程中去核可能使部分母源ZFP57蛋白丢失, 另外ZFP57在核供体细胞上几乎不表达, 核移植后ZFP57没有完全重编程等因素也可能是导致ZFP57在克隆胚胎上异常低表达的原因。另外, 我们在死亡克隆牛组织上和早期克隆胚胎上发现的印记基因异常去甲基化的ICR区域含有ZFP57的特异识别位点。因此我们推测ZFP57的异常表达可能与克隆胚胎印记基因异常去甲基化有关。

5 总结

基因组印记的擦除、建立、维持是亲代遗传物质传递给子代必须经过的过程, 在胚胎发育中起着重要作用, 同时其影响和调控着发育、行为、睡眠和生物钟等生理过程, 印记紊乱是克隆动物发育异常的关键所在。基因组印记的擦除、建立、维持的机理尚不完全清楚。进一步阐明这些机制对动物繁殖育种、治疗相关遗传病有着重要意义。

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