—Review—

DNA methylation dynamics during early human development

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Abstract: DNA methylation is essential for normal mammalian development and plays critical roles in various biological processes, including genomic imprinting, X-chromosome inactivation and repression of transposable elements. Although DNA methylation patterns are relatively stable in somatic cells, global reprogramming of DNA methylation occurs during mammalian preimplantation development. Advances in DNA methylation profiling techniques have been revealing the DNA methylation dynamics in mammalian embryos. Recently, we and other groups reported genome-scale DNA methylation analyses of human oocytes and preimplantation embryos, highlighting both the similarities and differences in the DNA methylation dynamics between humans and mice. In this review, we introduce the current knowledge of DNA methylation dynamics during early mammalian development. We also discuss the possibility of the application of genome-scale DNA methylation analysis techniques to human gametes and embryos for diagnostic purposes.

Key words: DNA methylation, Whole genome bisulfite sequencing, Genomic imprinting, Preimplantation epigenetic diagnosis

Introduction

In mammals, DNA methylation predominantly occurs at the 5′ position of the cytosine in CpG dinucleotides, and is mediated by DNA methyltransferase enzymes (DNMTs) [1]. The maintenance methyltransferase, DNMT1, preferentially methylates hemimethylated CpGs during DNA replication, while DNMT3A and DNMT3B are responsible for *de novo* methylation of unmethylated CpGs. DNMT3L lacks catalytic activity but can recruit DNMT3A and DNMT3B to their target regions. Studies using DNMT-deficient mice have demonstrated the essential role of DNA methylation in normal mammalian development and growth [2–4].

The best-documented role of DNA methylation is long-lasting transcriptional silencing [1]. DNA methylation of transcriptional regulatory elements such as promoters, enhancers and insulators, affects the binding of some transcription factors and can lead to the formation of heterochromatin [5]. Remarkably, allele-specific DNA methylation in imprinting control regions (ICRs) is acquired in the germline and regulates parent-of-origin-dependent gene expression throughout life [6]. DNA methylation also plays critical roles in the repression of transposable elements and X-chromosome inactivation.

In somatic cells, DNA methylation patterns are stably maintained through mitotic division and function to ensure epigenetic memory in cell-type-specific gene expression. However, genome-wide reprogramming of DNA methylation occurs during early mammalian development [7–9]. Advances in high-throughput sequencing of bisulfite-treated DNA (bisulfite-seq) have enabled us to obtain genome-scale DNA methylation (DNA methylome) maps of germ cells and preimplantation embryos. In this review, we briefly summarize the current knowledge regarding DNA methylation dynamics during early human and mouse development. We also discuss the possibility of preimplantation epigenetic diagnosis.

DNA Methylation Dynamics in Early Mouse Embryos

Genomic CpG sites are predominantly hypermethylated, except for CpG islands (CGIs) in mouse sperm [10]. Similar patterns are also observed in most somatic cells [11]. In contrast, the oocyte methylome is quite unique and is characterized by large hyper- and hypomethylated domains [10, 12]. Transcription is likely to be involved in the establishment of the unique methylome because

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transcribed regions are preferentially hypermethylated in mouse oocytes. *In vitro* experiments have demonstrated that *de novo* DNMTs preferentially interact with histone H3 lysine 36 trimethylation (a marker of actively transcribed regions) [13, 14], which might play a role in the establishment of the oocyte methylome.

After fertilization, the methylation patterns of oocytes and sperm are globally erased, except for ICRs and some transposable elements (e.g., intracisternal A-particles). The global demethylation of parental genomes has attracted much attention because immunostaining studies have indicated that both DNA replication-dependent (passive) and -independent (active) demethylation are involved in this process [15]. DNA demethylases remained unidentified in mammals for a long time, but the oxidation of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) by ten-eleven translocation (TET) proteins was discovered as a mechanism of active demethylation [16, 17]. Among the three mouse TET proteins, TET3 is involved in the demethylation of the paternal genome [18]. 5hmC is poorly recognized by DNMT1 [19] and the paternal 5hmC is predominantly diluted through DNA replication [20]. Although some other pathways have been proposed [17], their contributions to the removal of the paternal 5hmC may be limited [21]. Unlike the paternal genome, the demethylation of the maternal genome was thought to depend on the passive dilution of 5mC [15]. However, recent bisulfite-seq analyses in combination with knockout mouse models of DNA methyltransferases and demethylases suggest that the demethylation process, which was proposed based on immunostaining studies, requires substantial modifications. TET3 is now considered to be involved in the demethylation of both maternal and paternal genomes [21–23]. Most recently, the paternal genome was reported to be subjected to TET3-independent active demethylation and *de novo* methylation in zygotes [24]. This TET3-independent active demethylation might be mediated by the base excision repair pathway [25, 26]. Interestingly, the TET3 protein in oocytes has recently been shown to be dispensable for mouse development [27, 28]. Therefore, further studies are required to elucidate the underlying mechanisms and the functional significance of the global demethylation after fertilization.

It should be noted that bisulfite-seq cannot distinguish 5mC from 5hmC. Furthermore, fully methylated DNA (DNA methylated on both strands) and hemimethylated DNA (DNA methylated on one strand) are also difficult to distinguish. Both 5hmC and hemimethylated DNA can act as intermediates in DNA demethylation. Thus, for better understanding of the global demethylation after fertilization, bisulfite-seq data will need to be interpreted in combination with data obtained from other techniques that can distinguish 5mC from 5hmC (e.g., TET-assisted bisulfite-seq [29]) and fully-methylated DNA from hemimethylated DNA (e.g., hairpin-bisulfite-seq [30]).

DNA Methylation Dynamics in Early Human Embryos

To understand the DNA methylation dynamics in early human embryos, we recently conducted whole genome bisulfite-seq (WGBS) analyses of human oocytes and early embryos [31]. For the construction of WGBS libraries, we utilized the amplification-free post-bisulfite adaptor tagging (PBAT) method, which can be applied to a small number of cells (less than 1,000 cells) [32]. Two other groups have also conducted similar studies using reduced representation bisulfite sequencing (RRBS), which is less comprehensive than WGBS and suitable for the analysis of CpG rich regions [33, 34]. These DNA methylome analyses revealed similarities and differences in the DNA methylation dynamics between human and mouse embryos.

The human sperm genome is predominantly hypermethylated, except for CGIs [35], as is the mouse sperm genome. The human paternal genome is globally demethylated after fertilization, which is at least partially due to TET-dependent demethylation [22]. However, given the complexity of the demethylation process in mouse early embryos, some additional mechanisms may be involved. While the underlying mechanism is not fully elucidated, the paternal genome is nearly completely demethylated in both human and mouse blastocysts (Figs. 1A and B).

Similar to mouse oocytes, human oocytes have large hyper- and hypomethylated domains associated with transcription [31, 33]. However, the methylated regions are substantially different in human and mouse oocytes, which may reflect their divergent transcriptome profiles [36]. Furthermore, we found that the maternal genome is demethylated to a much lesser extent in human blastocysts than in mouse blastocysts (Figs. 1A and B). Remarkably, oocyte-specific methylated CGIs (CGIs hypermethylated in oocytes and hypomethylated in sperm) show methylation levels very similar to known ICRs in human blastocysts (Fig. 1C). ICRs are resistant to demethylation after fertilization, and this suggests that many oocyte-specific methylated CGIs may remain maternally methylated in human blastocysts. Recent studies have consistently demonstrated that many of the oocyte-specific methylated CGIs actually serve as ICRs in the human placenta [37, 38]. Most of these novel ICRs

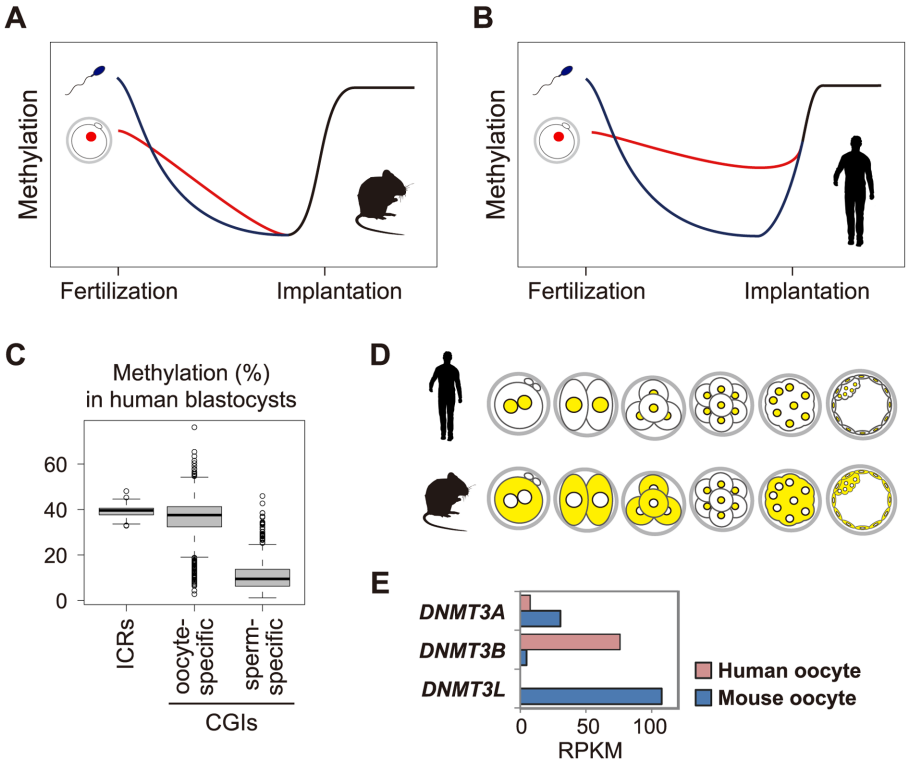

Fig. 1. DNA methylation dynamics in early human and mouse embryos. (A) Schematic illustration of DNA methylation dynamics during early mouse development. After fertilization, both maternal and paternal genomes are demethylated followed by *de novo* methylation after implantation. TET3-independent active demethylation and *de novo* methylation of the paternal genome in zygotes [24] has been omitted. (B) Schematic illustration of DNA methylation dynamics during early human development. The maternal genome is demethylated to a much lesser extent than the paternal genome in human preimplantation embryos. (C) Box plots of mean methylation levels of oocyte- and sperm-specific methylated CGIs in human blastocysts. Oocyte-specific (sperm-specific) methylated CGIs are defined as CGIs with ≥80% methylation in oocytes (sperm) and ≤20% methylation in sperm (oocytes). Methylation levels of ICRs are shown for comparison. Oocyte-specific methylated CGIs showed methylation levels very similar to known ICRs. The original data are from [31]. (D) Spatial and temporal expression patterns of DNMT1o (the oocyte-specific isoform of DNMT1). DNMT1o is the major isoform of DNMT1 in both human and mouse preimplantation embryos. The DNMT1o localization is shown in yellow. DNMT1o is localized in the nuclei of human early embryos. In contrast, it is mainly localized in the cytoplasm of mouse early embryos. It is controversial whether DNMT1o is localized in the nuclei of mouse 8-cell embryos (see [39, 41] for details). (E) Gene expression levels of *DNMT3A*, *DNMT3B* and *DNMT3L* in human and mouse oocytes. The original data are from [10, 57]. RPKM: reads per kilobase per million.

do not maintain maternal methylation in somatic cells. Therefore, the differential regulation of parental genomes during human preimplantation development may be important for genomic imprinting, especially in the human placenta. Intriguingly, few ICRs identified in the human placenta are imprinted in the mouse placenta, which might reflect the fact that both the maternal and paternal genomes are significantly demethylated in mouse preimplantation embryos.

The differential regulation of the maternal genome between humans and mice may partially be explained by the expression and localization patterns of DNA methyltransferases. In mouse zygotes and preimplantation embryos, DNMT1 is mainly localized in cytoplasm, which may lead to passive demethylation [39]. In contrast, DNMT1 is predominantly localized in the nuclei of human zygotes and preimplantation embryos [40] (Fig. 1D). In addition, the expression patterns of *DNMT3A*, *DNMT3B* and *DNMT3L*, which regulate *de novo* DNA methylation, differ in human and mouse oocytes. In mouse oocytes, *Dnmt3a* and *Dnmt3l* are highly expressed and essential for *de novo* DNA methylation, whereas *Dnmt3b* is

poorly expressed [4, 41]. In contrast, in human oocytes, *DNMT3B* has much higher expression than *DNMT3A*, and *DNMT3L* is not expressed [31, 42] (Fig. 1E). Intriguingly, centromeric satellite repeats are highly methylated in human oocytes, but not in mouse oocytes [31, 43]. DNMT3B contributes to DNA methylation of centromeric satellite repeats in both human and mouse cells [44, 45]. Therefore, the differential expression patterns of *DNMT3B* might explain the human-specific hypermethylation of centromeric satellite repeats in oocytes.

Towards Preimplantation Epigenetic Diagnosis

Many studies have noted an increased risk of rare imprinting disorders such as Beckwith-Wiedemann syndrome in children conceived via artificial reproductive technology (ART) [46, 47]. A recent meta-analysis reported that the odds ratio of any imprinting disorder in children conceived via ART was 3.67 compared to naturally conceived children [48]. ART procedures and/or infertility of patients undergoing ART are assumed to increase the risk of imprinting disorders. It has also been reported that abnormal methylation in ICRs is frequently observed in ART-derived human preimplantation embryos (6–89%) [49], which might potentially increase the risk of imprinting disorders. The results of studies using animal models strongly suggest that some ART procedures can induce methylation errors in ICRs and imprinting disorders. For example, *in vitro* culture of preimplantation embryos causes loss of methylation in the *IGF2R* ICR and results in large offspring syndrome in ruminants [50, 51]. Various ART procedures, including superovulation, *in vitro* embryo culture and *in vitro* fertilization, are also associated with methylation errors in ICRs of mouse embryos [47].

ART-associated methylation changes are not likely to be restricted to imprinted regions [52]. A recent study utilized the Illumina Infinium Methylation array and identified many non-imprinted regions differentially methylated in ART and naturally conceived children [53]. To evaluate the safety of each ART procedure and reduce the incidence of pathological pregnancies associated with preimplantation DNA methylation errors, genome-scale DNA methylation analyses of human gametes and preimplantation embryos would be useful. However, DNA methylation analyses of individual human oocytes and preimplantation embryos are quite challenging. Especially for diagnostic purposes, DNA methylation analysis techniques applicable to a single cell are required. Whereas some studies have reported single-cell bisulfite-seq techniques [54, 55], these techniques are not likely to be sensitive enough to detect DNA methylation errors in a single cell due to their low DNA recovery. Alternatively, a classic method using methylation-sensitive restriction enzymes was successfully used for the simultaneous DNA methylation assessment of several ICRs in a single mouse blastomere [56]. This technique might potentially be applicable to the simultaneous detection of many more loci and be used in combination with preimplantation genetic diagnosis. Although further technical improvements are needed, single-cell methylation analysis techniques might be applied to human embryos for diagnostic purposes in the near future. The WGBS data of human oocytes and early embryos that we have reported will be a useful reference for preimplantation epigenetic diagnosis.

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