Chapter 13
Do DNA methylation patterns change during spermatogenesis? What is the role of imprinting of male germ cells?

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Epigenetics refers to heritable mechanisms of modulating gene expression that do not involve alterations in DNA sequence. DNA methylation, histone modifications and small non-coding RNAs are the three main types of molecular information that have been associated with epigenetic regulation of genes. These three mechanisms interact and stabilize each other and disruptions of one or more can lead to inappropriate expression or silencing of genes, resulting in diseases such as cancer and imprinting disorders.

DNA methylation is the most extensively characterized epigenetic mechanism. DNA methylation is found most frequently at the 5'-position of cytosine residues within CpG dinucleotides (where cytosine is 5' to guanine) at 20-30 million sites throughout the mammalian genome; about 70-80% of cytosines within CpGs are methylated. The functions of DNA methylation include roles in the transcriptional repression of retrotransposons and single copy genes (at their CpG-rich promoters), the differential 'marking' of imprinted genes to ensure parent-of-origin specific expression, X chromosome inactivation and modulating the access of transcription factors to gene regulatory regions.

Two types of DNA methylation occur: i) *de novo* methylation or the acquisition of methylation on unmethylated cytosines, or ii) maintenance methylation, which takes place at the time of DNA replication to ensure the propagation of DNA methylation from parent to daughter cells. The majority of *de novo* methylation is targeted to transposons and their remnants and to repeats such as pericentric satellite sequences with smaller amounts at single-copy sequences and the differentially methylated regions (DMRs) of imprinted genes. Methylation of DNA is catalyzed by a family of DNA (cytosine-5)-methyltransferases (DNMT enzymes or DNMTs). DNMT3A and DNMT3B are the main DNMTs involved in the establishment (*de novo* methylation) of DNA methylation patterns whereas DNMT1 plays the

key role in DNA methylation maintenance. Although it lacks enzymatic activity, DNMT3L (DNMT3-like) is related to and works with DNMT3A and DNMT3B. A recently identified rodent-specific DNMT, DNMT3C, has been shown to methylate only a very specific subset of sequences in the genome, young transposable elements (TEs) in the male germ line. DNA methylation can be erased by either passive or active demethylation mechanisms. Passive DNA demethylation is achieved by replication coupled dilution of methylated DNA and sequestration of the maintenance and *de novo* DNA methylation machinery. Ten-eleven translocation (TET) methylcytosine dioxygenases (TET1, TET2 and TET3 enzymes) actively demethylate DNA.

DNA methylation patterns are very dynamic during embryonic development and the establishment of the germ cell lineages (Fig. 1). Following fertilization, the paternal genome goes through a rapid erasure involving active and passive demethylation. In contrast, the maternal genome loses DNA methylation by passive demethylation, more gradually during preimplantation development. Imprinted gene DMRs and some other loci are resistant to this global wave of demethylation. Imprinted gene DMR methylation is maintained during preimplantation development since it is only in the germline (male or female depending on the gene) that a subset of imprinted genes acquire the allele-specific methylation that will result in monoallelic expression in the embryo and postnatal individual.

After implantation, lineage-specific DNA methylation patterns are established. Precursors of eggs or sperm (primordial germ cells – PGCs) go through another round of demethylation to reset marks coming from the parents, to reach a genomic average of <10% DNA methylation, the lowest level of CpG methylation occurring in the genome of a cell (Fig. 1). This erasure is particularly important for imprinted gene DMRs as maternal 'marks' on imprinted genes inherited from the mother must be erased and new paternal 'marks' introduced. Although erasure of DNA methylation at the PGC stage is the most extensive DNA demethylation to occur in any cell, not all of the genome is demethylated. A subset of sequences such as young retrotransposons (marked by a histone modification – H3K9me3) is resistant to demethylation in PGCs.

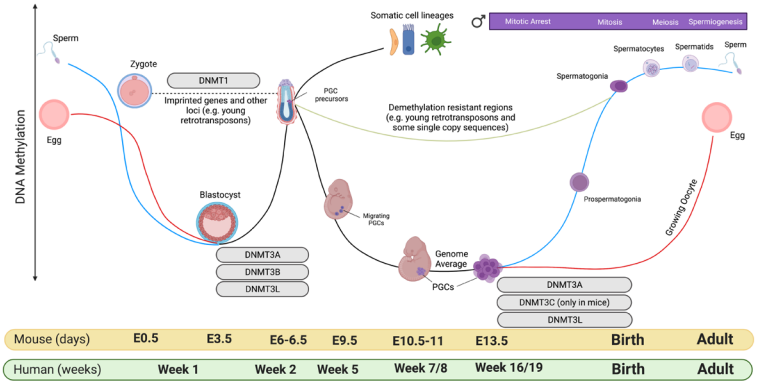

Figure 1: Epigenetic reprogramming of DNA methylation patterns during embryonic and male germ cell development. PGC, primordial germ cell. Created with BioRender.com.

Germ cell-specific DNA methylation patterns are established at different time points for males and females. Male germ cells acquire male-specific DNA methylation marks for the most part premeiotically in the prenatal prospermatogonia in the period between midgestation and birth (Fig. 1). DNA methylation is nearly complete (~80%) by the pachytene phase of meiosis. Mitotic spermatogonia must also maintain the DNA methylation patterns acquired in the prenatal period. Further minor remodeling of DNA methylation occurs throughout meiosis and spermiogenesis. As most sperm-derived DNA methylation will be erased in the early embryo, it is postulated that germline DNA methylation, at sequences other than imprinted genes and repeats, may play specific roles in germ cell development, gene expression or chromatin structure during spermatogenesis. DNMT3A and DNMT3L are the major enzymes directing *de novo* DNA methylation in male germ cells; in mouse studies, mutations in the genes for these two DNMTs lead to male sterility. In rodents, DNMT3C targets ~1% of the genome (at young retrotransposons).

Transposable elements are parasitic sequences making up a significant part of the genome in higher eukaryotes (˜37% in mice and ˜45% in humans). The majority of these sequences have lost their ability to transpose except for the evolutionarily young ones. The young TEs need to be silenced by several pathways including DNA methylation in order to protect the genomic integrity of the organism. One of the important mechanisms leading to *de novo* DNA

methylation at these young TEs is the piwi-interacting RNA (piRNA) pathway. piRNAs are a class of non-coding RNAs highly expressed in male germ cells, and with their PIWI protein partners (e.g. MILI, MIWI2 and PLD6 in mice) they can recruit the *de novo* DNA methylation machinery to evolutionarily young retrotransposons in male germ cells. piRNA mutants and *Dnmt3C* mutant mice show a similar phenotype and demethylation of young retrotransposons along with the *Rasgrf1* imprinted locus indicating possible cooperation between them to keep these highly mobile TEs in check. It is not clear how humans are able to repress these young retrotransposons in the male germline since they lack DNMT3C.

DNA methylation plays a key role in the monoallelic expression of imprinted genes. Studies in mice and humans have shown that imprinted genes are not only crucial for prenatal development of the embryo and placenta but they are also required for the regulation of neurodevelopment, metabolism and physiological adaptations in the postnatal period. About 200 imprinted genes have been identified; changes in their expression and function can cause congenital diseases and, in some cases, increased cancer risk. Of the known imprinted genes, there are only three paternally (sperm) derived methylation imprints and over 20 loci that acquire methylation in the maternal germline (unmethylated in sperm). Imprinted genes provide an example of loci subject to epigenetic inheritance since their gamete-derived DNA methylation patterns can evade preimplantation demethylation and be passed on to the embryo and persist throughout development and/or life.

There is much current interest in the effects of infertility, the environment, and aging on human sperm DNA methylation and the potential for transmission of altered DNA methylation (epimutations) to the next generation. Until recently, most studies of DNA methylation in human sperm have targeted individual loci or a limited number of CpGs. Somatic cell contamination and genetic heterogeneity have emerged as key confounders in human sperm DNA methylation studies. Genome-wide DNA methylation analysis techniques such as whole genome bisulfite sequencing (WGBS), now allow DNA methylation to be assessed accurately at all CpG sites in the genome. WGBS profiles have been produced for the normal human sperm methylome and are starting to be used to assess alterations due to age and environmental exposures. Importantly, genome-wide DNA methylation analysis techniques such as WGBS probe methylation at genic, intergenic (including retrotransposons) and key regulatory sequences. High resolution sperm DNA methylation profiling with

genome-wide approaches and careful attention to confounders will be useful for prospective studies interested in connecting fathers' infertility/exposures to adverse outcomes in his children.

Suggested reading

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