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DNA methylation in spermatogenesis and male infertility (Review)

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Abstract. Infertility is a significant problem for human reproduction, with males and females equally affected. However, the molecular mechanisms underlying male infertility remain unclear. Spermatogenesis is a highly complex process involving mitotic cell division, meiosis cell division and spermiogenesis; during this period, unique and extensive chromatin and epigenetic modifications occur to bring about specific epigenetic profiles in spermatozoa. It has recently been suggested that the dysregulation of epigenetic modifications, in particular the methylation of sperm genomic DNA, may serve an important role in the development of numerous diseases. The present study is a comprehensive review on the topic of male infertility, aiming to elucidate the association between sperm genomic DNA methylation and poor semen quality in male infertility. In addition, the current status of the genetic and epigenetic determinants of spermatogenesis in humans is discussed.

Contents

1. Introduction
2. DNA methylation
3. DNA methylation and spermatogenesis
4. DNA methylation and genomic imprinting
5. DNA methylation and male infertility
6. Conclusion

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1. Introduction

Epigenetics is the study of genomic structural modifications that affect gene expression without altering the underlying nucleotide sequence (1-3). Epigenetic mechanisms involved in the regulation of gene expression include the regulation of non-coding RNA, chromatin remodeling, DNA methylation and histone modifications (4,5). Of these mechanisms, DNA methylation has been implicated in numerous biological functions, such as the development of spermatozoa and early embryos, and the repression of endogenous retrotransposons, while it also has a wide range of effects in gene expression (2,3,6). The dysregulation of DNA methylation has previously been associated with various human disorders, and has been shown to increase the risk of fertilization failure, dysfunction in embryogenesis, perinatal mortality, congenital abnormalities, preterm birth and low birth weight (7-11). The present review assesses the significance of DNA methylation in spermatogenesis in order to elucidate the association between the dysregulation of DNA methylation and male infertility. This may provide a basis for the prevention and treatment of male infertility, as well as permit the evaluation of the epigenetic quality of sperm in order to reduce the risk of epigenetic diseases in cases where conception is performed by assisted reproductive technology (ART).

2. DNA methylation

Epigenetic mechanisms are critical regulators of gene expression during spermatogenesis that may influence male fertility (12-14). Cytosine, a key DNA base, is methylated at the position, typically in the context of CpG dinucleotides. The methylation of constitutive heterochromatic and promoter regions is generally associated with reduced gene transcription (Fig. 1A) (15-18). Therefore, DNA methylation is a type of epigenetic modification that can effectively promote gene silencing (Fig. 1B). The methyl group for this chemical modification of the DNA is donated by S-adenosyl-L-methionine (SAM) (17-22), and the methylation reaction is catalyzed by members of the DNA methyltransferases (DNMT) family, which are classified into the following three types in mammals: DNMT1, DNMT2 and DNMT3. It was demonstrated that DNMT1, DNMT3a and DNMT3b are robustly expressed in the early embryonic stage; however, the biological functions of DNMT2 remain elusive (23-25).

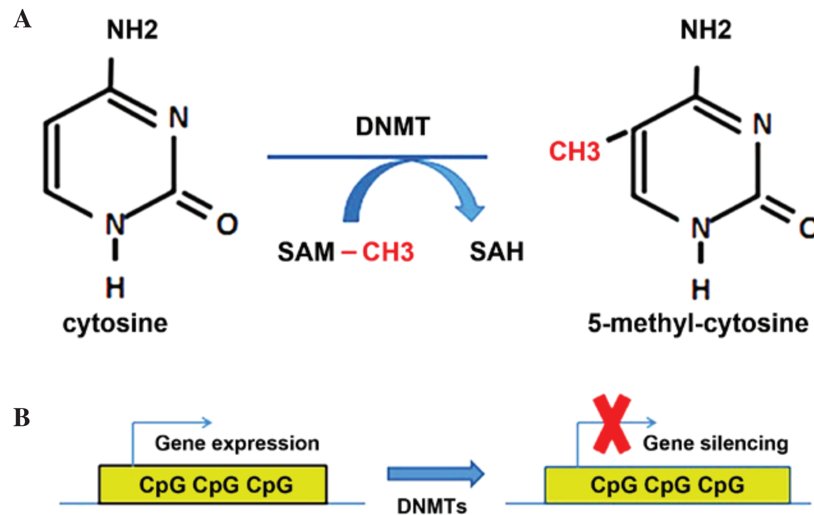

Figure 1. Characteristics of DNA methylation (Nasu,2011). (A) Cytosine is converted to 5'-methylcytosine by the action of DNMTs. (B) Cytosine is typically methylated in the context of CpG dinucleotides, and the methylation of constitutive heterochromatic and promoter regions is generally associated with gene silencing. SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine; DNMT, DNA methyltransferase.

DNMT1, which is the most abundant DNMT in mammalian cells (26-30), predominantly methylates hemimethylated CpG dinucleotides in the genome, and is considered to be the key maintenance methyltransferase during cell division (29-33). The DNMT2 gene is the most highly conserved of the methyltransferases in eukaryotes, and has been identified in organisms that exhibit DNA methylation, as well as in those that do not (26-30). DNMT3a and DNMT3b primarily perform *de novo* methyl transfer reactions via interactions with transcriptional repressors. They are considered to differ mechanistically due to inherent differences in their catalytic domains, and it has been suggested that DNMT3a is distributive while DNMT3b is processive (31-36). The third significant member in the DNMT3 family is DNMT3L, which is considered to be required for the establishment of maternal imprints in oocytes (37-41), and has also been shown to be expressed during spermatogenesis (37,42-44). SAM, as the methyl donor for DNMTs, is formed through the addition of adenosine triphosphate to methionine, which is catalyzed by the methionine adenosyltransferase enzyme. Subsequent to the methyl transfer reaction, SAM is converted into S-adenosyl-L-homocysteine, which acts as a potent inhibitor of DNMTs and histone methyltransferases (45-49).

3. DNA methylation and spermatogenesis

Germ cell development is a highly ordered process initiated during the growth of a fetus and is completed in adults. The epigenetic modifications occurring in germ cells are crucial for the function of germ cells and for embryonic development after fertilization (50,51). Previous studies have demonstrated that male germ cells in adult mice have a highly distinct epigenetic pattern, characterized by a unique genome-wide pattern of DNA methylation (42,52-55). The methylation status of testicular DNA is highly distinct, displaying an eight times higher number of hypomethylated loci, as compared with somatic tissues (51,56-58). Alterations in DNA methylation serve a crucial role in establishing an epigenetic state during the early stages in the development of germ cells, allowing for transcription to occur at the later stages (Fig. 2) (59-61).

Genome-wide methylation studies have demonstrated that the epigenomes of sperm cells and of somatic cells are significantly different; however, the sperm epigenome is very similar to that of embryonic stem cells (61-65). Using restriction landmark genomic scanning technology, DNA methylation was detected for chromosomes 6, 20 and 22 in spermatozoa, with numerous sites in sperm and somatic cells exhibiting varying degrees of methylation (66-68). The extent of methylation in sperm cells was significantly increased, as compared with fibroblasts (>20%), liver cells (10%), and $CD4^+$ and $CD8^+$ lymphocytes (5%) (68,69). DNA hypermethylation is associated with gene silencing. By contrast, hypomethylation is associated with gene expression. In sperm cells, promoters of developmental genes are highly hypomethylated. A previous gene ontology analysis demonstrated that hypomethylation in mature sperm cells promoted developmental transcription and signaling, which is bound by self-renewal mesh transcription factor of human embryonic stem cells, including OCT4, SOX2, NANOG, KLF4 and FOXD3 protein. Furthermore, recurrent regions of the sperm genome reportedly demonstrate high degrees of methylation, while transposons manifest weaker methylation. Whether other mechanisms exist requires further analysis (65,69,70).

The paternally expressed human gene, MEST/PEG1, is demethylated in the fetus and remains unmethylated throughout all stages of sperm development in the adult life (37,71). By contrast, in male germ cells, the H19 gene is methylated prior to meiosis at the spermatogonial stage of development (37,72). Reinitiation of mitotic division of male germ cells during puberty coincides with an upregulation of DNMT1 within the spermatocytes. During the early stage of meiosis, DNMT1 levels in spermatocytes are increased; however, the level of the DNMT1 enzyme is reduced in pachytene stage spermatocytes (37,73). In previous studies, the knockout of DNMT1 resulted in a lack of DNA methylation in the fetus, which, due to a lack of genomic imprinting, led to apoptosis

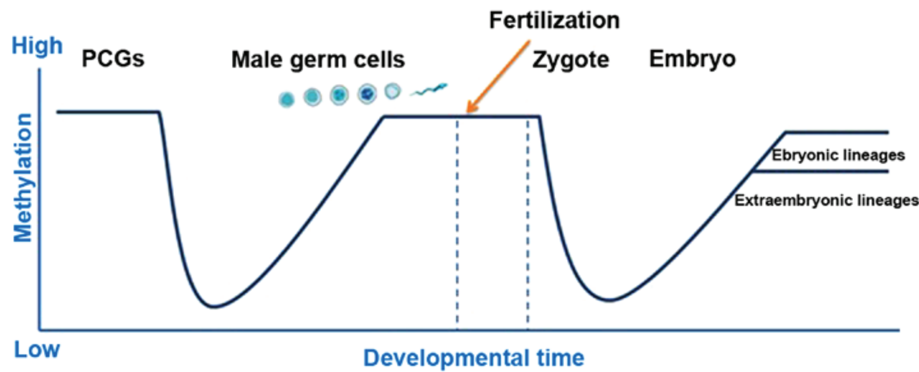

Figure 2. Methylation reprogramming in male germ cells (Dean, 2005). PGCs in mice become demethylated early in development. Re-methylation is initiated in pro-spermatogonia in male germ cells, and fertilization signals the second stage of methylation reprogramming. Thereafter, the paternal genome is demethylated by an active mechanism immediately following fertilization. PCGs, primordial germ cells.

of spermatagonial cells (65,67,70,74). However, DNMT1 heterozygous mice had a normal reproductive ability, thus suggesting that DNMT1 in the heterozygous state is sufficient to maintain the required DNA methylation pattern (30,75,76). DNMT3a expression in the testis is upregulated prior to birth and during early postnatal life, whereas DNMT3b expression is downregulated during embryonic development and upregulated postnatally (15-20). Notably, mice lacking DNMT3L have smaller testes, and a negligible number of spermatozoa by adulthood, resulting in sterile animals. In a previous study, DNMT3L was expressed only in germ cells, and the expression pattern was observed in males and females (77). However, in males, DNMT3L was initially detected at 12.5 days after fertilization of the egg, and the levels peaked at 15.5 days and remained upregulated until birth (77-79). However, the expression of DNMT3L was significantly 79 following birth and was found to be low in mature germ cells (36-38), suggesting an association between DNMT3L and DNA methylation. In addition, the inactivation of DNMT3L was shown to cause mitotic delays, chromosome synapsis errors and the ceasing of spermatogenesis at the zygotene stage of mitosis, thus inhibiting the maturation of germ cells (46-48).

In certain individuals with deletion mutations in DNMT genes, a proportion of the paternal differentially methylated regions (DMRs) were normal, which suggested that DNMTs have some functional duplication (77-79). Notably, oocytes lacking DNMT3L were able to undergo normal meiosis and exhibited methylation of repeat sequences; however, a significant lack of methylation-mediated imprinting existed only in the female imprinting positions (48-52). This difference in the effects of gene knockout between males and females raises the question of the divergent regulatory mechanisms between genders (65,69).

4. DNA methylation and genomic imprinting

Genomic imprinting is an epigenetic mechanism resulting in parental expression of certain genes resulting in the alteration of gene transcription, while the actual gene sequence remains unchanged (56,80,81). DNA methylation regulates genomic imprinting, thus resulting in only one inherited copy of the relevant imprinted gene being expressed in an embryo. The majority of imprinted genes contain DMRs, in which methylation differs between the paternal and maternal alleles. DNA methylation-mediated genomic imprinting is established during gametogenesis, prior to fertilization (59,60).

By the time the primitive streak has formed during embryonic development [embryonic day 7.5 (E7.5)], the primordial germ cells (PGCs) carry the paternally and maternally inherited imprinting patterns (82). This DNA methylation pattern is maintained in PGCs, but is rapidly lost around E8, when PGCs begin to migrate towards the developing gonad. Upon their arrival at the genital ridge (between E10.5 and E11.5), global demethylation of mouse PGCs begins in order to remove their inherited imprinting pattern (61,62). At this time, PGCs continue to proliferate and undergo differentiation into distinct male and female germlines (at ~E12.5), while the DNA methylation pattern of somatic cells is maintained. By E13-14, demethylation of the DNA is completed and male gametes are arrested at the prophase stage of mitosis (59,60). It has previously been suggested that mitosis arrest may be required following demethylation, since the replication of unmethylated DNA has been associated with an increased risk of unrepressed retrotransposon transposition, which may lead to mutations. Therefore, in sperm cells, DNA methylation-mediated genomic imprinting is initiated prenatally, prior to meiosis, and is completed by the pachytene phase of postnatal spermatogenesis (61,62,65,69,70,74).

5. DNA methylation and male infertility

Infertility is a major public health concern that has a significant social, psychological and economic impact, with an equal proportion of males and females affected (83-85). Male infertility is associated with genetic, environmental and numerous other factors (86,87). The underlying mechanisms are unknown in 70% of male infertility cases, even in cases where the causes of male infertility are clear. Therefore, further studies are required in order to elucidate the mechanisms underlying male infertility. In male factor infertility, epigenetic modifications may serve a pivotal role by regulating male germ cell development and maintenance (88-92). Thus, abnormal imprinting as a result of DNA methylation dysregulation may be associated with male infertility.

Previous studies have demonstrated that disturbed spermatogenesis is associated with incorrect imprinting (74).

An analysis of semen from infertile men has shown that 14.4% of anomalous patriarchal methylation occurred in H19 and gene trap locus 2 gene (93-95). In spermatozoa from oligozoospermic men, the occurrence of hypermethylation at several maternal DMRs or hypomethylation of H19 and intergenic-DMR was increased, particularly in patients with ejaculation volumes of $<10x10^6$/ml (96-99). In men with oligoasthenoteratozoospermia, methylation was markedly reduced at all CpGs, reaching statistical significance in subgroups with a sperm concentration of $<10x10^6$/ml. These findings suggest that abnormal DNA methylation-mediated genomic imprinting is associated with oligoasthenoteratozoospermia and oligozoospermia (100-107).

In patients with male infertility, paternally and maternally imprinted gene methylation abnormalities have previously been reported. In oligospermic patients, a low methylation or unmethylation pattern at the H19 imprinted gene has been associated with hypermethylation at the MEST imprinted gene, as well as a reduced sperm quality and decreased DNA methylation-mediated imprinting, as compared with fertile men (108,109).

DNA methylation markers have been detected in the spermatogonia stage; therefore, the abnormal DNA methylation patterns observed in infertile men may be due to the failure of re-methylation in spermatogonia or alterations to methylation maintenance in spermatocytes, sperm cells or the mature sperm cell. In addition, abnormal DNA methylation may be associated with the abnormal activation of DNMTs (110-114).

Alterations in DNA methylation-mediated genomic imprinting have been observed more frequently in men with oligoasthenoteratozoospermia and oligozoospermia (115). However, whether methylation defects in imprinted genes will affect the development and growth processes of ART offspring remains to be elucidated. If epigenetic modifications are a key factor in the maturation of sperm cells, alterations in the epigenetic patterns of infertile men may provide a reasonable explanation for complications associated with ART, including low birth weight, premature births, congenital abnormalities, an increased perinatal mortality rate and pregnancy complications (116,117). Embryos obtained from patients with hypospermatogenesis and almost complete hypomethylation at the H19 DMR following ART all exhibited developmental arrest (117,118). However, patients with oligoasthenoteratozoospermia with a partial hypomethylation of H19 presented a reduced fertilization rate following intracytoplasmic sperm injection (ICSI). A previous analysis of the methylation status of ICSI infants with a low birth weight revealed hypermethylation at the MEST gene in one of the infants included in the study (119,120).

6. Conclusion

In conclusion, DNA methylation has been closely associated with male infertility (66,121). Understanding the mechanisms underlying DNA methylation is particularly important in order to develop therapeutic strategies for male genital system diseases caused by abnormal sperm DNA methylation. Studies on the mechanisms underlying the regulation of DNA methylation during spermatogenesis are still in their initial stages. Numerous issues remain, such as transgenerational inheritance of human epigenetic genes and the association between DNA methylation and other epigenetic factors, while it is unclear whether the risk of abnormal methylation-induced embryonic diseases has a threshold or is continuously increasing process.

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