The role of epigenetics in spermatogenesis

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ABSTRACT

Male germ cells have a unique morphology and function to facilitate fertilization. Sperm deoxyribonucleic acid (DNA) is highly condensed to protect the paternal genome during transfer from male to oocyte. Sperm cells undergo extensive epigenetic modifications during differentiation to become a mature spermatozoon. Epigenetic modifications, including DNA methylation, histone modifications, and chromatin remodeling are substantial regulators of spermatogenesis. DNA hypermethylation is associated with gene silencing. Meanwhile, hypomethylation is associated with gene expression. In sperm cells, promoters of developmental genes are highly hypomethylated. Proper DNA methylation is essential for embryo development. Histone modifications are chemical modifications that change the DNA-binding capacity of histones and the accessibility of regulatory factors to the DNA, thereby altering gene expression. Phosphorylation, methylation, acetylation, and ubiquitination are primary modifications of lysine and serine residues on histone tails. In addition to somatic histones, testis-specific histone variants are expressed, including histone H2B in mature sperm. The replacement of histones with protamines is a crucial step in spermatogenesis. Histone hyperacetylation induces a loose chromatin structure and facilitates topoisomerase-induced DNA strand breaks. As a result, histones are replaced with transition proteins. Next, the transition proteins are replaced with protamines that induce compaction of sperm DNA. This review provides an overview of epigenetic changes during spermatogenesis.

Key words: Chromatin remodeling; DNA methylation; epigenetics; histone modification; spermatogenesis.

Epigenetics is defined as stable changes which can be inherited through mitotic or meiotic division, but alter gene expression without modifying DNA sequences.^[1] In antique Greek epi-, prefix means over, above, and beyond. Therefore it means over, and beyond genetics.

Epigenetic mechanisms mediate interactions between DNA, and miscellaneous proteins which have regulatory roles in replication, and gene expression processes at various stages of development during the life of an organism, with DNA. Each cell type has a specific epigenetic signature. This signature reflects developmental history, and environmental effects in the phenotype of the cell, and the organism.^[2]

Epigenetic changes involve histone proteins which function in the packaging of DNA, and also a series of DNA modifications. DNA encircles histone proteins, and constitutes fundamental structural unit of the chromatin, namely nucleosome. It regulates DNA methylation, and also via modifications of histone, and chromatin it also controls gene expression under varying temporal, and environmental conditions.^[3,4] During development epigenetic profile of the germ cells changes.^[5,6] During post-implantation embryonic phase, pluripotent cells of epiblasts ensure development of primordial germ cells (PGCs). In women PGCs are suppressed during prophase of meiosis I, in men it enters into the phase of mitotic arrest. Epigenetic profiles of germ cells undergo transformations during different phases of meiotic division.^[7]

In this article we will review methylation of sperm DNA, sperm histone, and chromatin modifications occurring during spermatogenesis.

1. Sperm DNA methylation

DNA methylation is a biochemical procedure where a methyl group is added to the 5. carbon of the pyrimidine ring of the cytosine nucleotide located in the CpG islands.^[8] CpG islands

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are 500 bp long-genomic regions which contain very condensed CG dinucleotides (CG/GC >55%).^[9] These sequences are found in the promoter regions of nearly 40% of mammalian genes. DNA methylation seen in CpG islands in the promoter region leads to inheritable silencing at the transcription level. DNA methylation seen in CpG islands plays an important role in the regulation of gene expression. In the regulation of gene expression, methylation especially in the promoter regions of genes, induces changes in the recognition regions of transcription factors with resultant prevention of these factors. This process plays a role in the suppression of gene expression.^[10]

DNA methylation occurs under the impact of DNA methyltransferase (DNMT) enzyme. One of DNA methyltransferases [one DNMT1, one DNMT2, three DNMT3 (DNMT3a/3b and DNMT3L)] catalyze transfer of one methyl group from S-adenosyl methionine to cytosine.^[10] DNMT1 is responsible from maintenance of methylation motif during DNA replication, and termed as maintenance methyltransferase. DNMT3s mediate *de novo* methylation of unmethylated cytosines, and methylates genomic DNA during early phase of embryonic development. Though the role of DNMT2 has not been fully elucidated, as suggested by researchers, it might exert an impact as a tRNA methyltransferase.^[10-12]

These acquired changes are perpetual, and permanent.^[4,13] Hypo-, and hypermethylation can occur spontaneously in different regions of the genome.^[14]

In a recent study, sperm methylation maps of human beings, and chimpanzees have been determined. In this study, it was reported that promoters of the genes responsible from development were hypomethylated, and manifested stronger hypomethylation when compared with promoters of the somatic cells. Besides, recurrent regions of the sperm genome reportedly demonstrate high degrees of methylation, while transposons manifest weaker methylation.^[15]

2. Sperm Histone Modification

Histones are basic proteins rich in lysine, and arginine. They are located in the nuclei of eukaryotic cells, and induce packaging of DNA within lysosomes. H2A, H2B, H3, and H4 histone proteins are found in the nuclei of nucleosomes. Nucleosomes contain octameric proteins formed by combination of two H3-H4 dimers, and two H2A-H2B dimers, and they are bound by H1 histone proteins.^[16] Via simple chemical modifications, histones change DNA-binding capacities, and interaction of other regulatory factors with DNA, and thus lead to alterations in gene activation. Histone modifications are linked to specific enzymes.^[17] Histone modifications are realized by acetylation,

methylation, phosphorylation, and ubiquitination. Generally, acetylation of histone H3, and H4 leads to open chromatin configuration, and active transcription which facilitates binding of transcription factors.^[18] However deacetylation is related to inactivation of transcription, and generally correlates with methylation. Generally, histone acetylation is seen in regions of active transcription, hypoacetylated histones are localized in euchromatic, and heterochromatic regions.^[19] Another regulatory mechanism is methylation of lysine found in histone tails. Chemical changes as conversion of lysine in histone tails to serine are realized through methylation, acetylation and ubiquitination.^[20,21] H3K9, and H3K27 histones are generally associated with inactivation.^[22] However methylation of histones take place both in active, and inactive chromatin sites. Methylation of 9. lysine on amino terminal of histone (H3-9K) leads to DNA silencing, and also involves heterochromatic regions. On the other hand, methylation of the 4. lysine of H3 protein (H3-4K) is associated with activation, and mainly it takes place in promoter regions of active genes. During spermatogenesis, methylation of histone tails is achieved by H3-K4, and H3-K9 methyltransferases.^[23]

Modifications between H3 and H4 histone protein tails lead to various interactions. Some of them regulate transitions between active, and inactive chromatin states through a reverse mechanism called histone code.^[24]

Premeiotic PGCs, and spermatogonia demonstrate specific histone H3K9me3 modification model.^[25,26] However, with the onset of meiotic process in male germ cells these models change (Figure 1).^[27] Histone modification, and changes in its composition play important roles in chromatin modifications required for normal meiotic process, and later maturation of gametes.^[6] Both female, and male gamete cells undergo developmental changes following termination of meiotic division. Some histones on X chromosome, label, and retain H3K9me2, global remodelling in haploid spermatids occur at a lower rate.^[28,29] Variants of H2A, H2B, H3, and H1 are expressed in testis. Compared with standard histones, histone variants have a lower degrees of stability.^[30] In testis, in addition to testis-specific histone variants, somatic histone variants are also expressed. In mature sperm, the most frequently testis-specific histone H2B is found.^[31] Ion channels, and genes involving spermatogenesis are rich in testis-specific histone H2B contrary to genes responsible from development. Testis-specific H1T2-binding histone variant has an important role in the condensation of chromatin during spermatogenesis.^[32] As demonstrated in immunohistochemical studies, histone variant H2AZ which is found in some certain cell types condenses in pericentromeric regions of heterochromatins.^[33,34] H3K4me3 condenses in genes involving

in spermatogenesis, while H3K4me2 is found in regions rich in developmental genes. Hyperacetylation of histone H4 is responsible from replacement of histones with protamines in haploid spermatids. H1s1-binding histone variant (histone-1-like protein in spermatids 1) is expressed in elongated spermatids.^[35,36] As a consequence, histone packaging is an evolutionary, and developmental procedure for spermatogenesis.^[33]

These two epigenetic regulatory mechanisms, ie. DNA methylation, and histone modification are closely related to each other in the process of gene expression. Successful regulation, and control of gene expression requires close cooperation, and interaction of both of these mechanisms.^[1]

3. Modification of chromatin during spermatogenesis

Fertilization requires realization of many physiological events including movement of sperm cells all along the female reproductive system, their attachment to zona pellucida, and penetration into oocyte.^[37] For the accomplishment of all these phases, a regulatory mechanism involving striking modifications which require replacement of 90-95% histones by protamines becomes effective.^[38] Protamines are small molecules rich in arginine. They are located in nuclei, and synthesized during advanced stages of spermatogenesis.^[39] Protamination of sperm chromatin facilitates compaction of nucleus required for sperm motility, and also protects sperm genome from oxidation, and harmful molecules within the female reproductive system. Extensive packaging of DNA following protamination prevent transcription. Protamination is an epigenetic regulatory process specific to sperm cells.^[38] Before fertilization, paternal haploid genome carried by mature sperm is packaged tightly by protamines, maternal genome which is suppressed in metaphase II is packaged by histone proteins. After fertilization, protamines are rapidly translocated with histones, and oocyte passes through metaphase II, and polar body is thrown out. H3, and H4 histones found in paternal chromatin are more effectively acetylated when compared with those contained in maternal chromatin.^[40,41]

Translocation mechanism taking place between histone proteins, and protamines which ensures packaging of sperm DNA is still a partially understood multistep process. Early modification processes involve replacement of histone variants by selected histones which are expressed during spermatogenesis. In mature sperm mostly testis-specific histone 2B protein variant is found which attracts attention because of its unique expression characteristic. Characteristic in that it does not undergo polyadenylation at its 3' terminal, and it is localized in telomeres of sperm chromosomes.^[42] Acetylation of some histones accelerates due to their replacement by their variants.

Figure 1. Spermatogenesis, and epigenetic changes during spermatogenesis

Acetylation is regulated by acetylase, and deacetylase enzymes. Hyperacetylation of histones leads to loosening of chromatin structure. Loose protein structure stimulates DNA strand breaks caused by topoisomerase enzyme, and facilitates separation of histones, and their replacement by transition proteins (TPs).^[43,44] Hyperacetylation triggers chain of events with resultant replacement of histones by protamines.^[45] Transition proteins 1, and 2 demonstrate moderately basic non-histone proteins. They involve in 12., and 13. stages of spermatogenesis, but leave the process at 14. stage.^[30] These proteins stick to DNA, and thus play critical roles in separation of histone variants, and later condensation by protamine.^[46] Then TPs are totally replaced by protamines. Two protamines (P1, and P2) are nearly equally expressed in human beings. TP, and protamine expressed genes are considerably overlap (Figure 2).^[47] These also have a unique translational regulatory mechanism.^[48] This important posttranslational mechanism involves phosphorylation required for appropriate chromatin condensation.^[49] In mice where one of these transition proteins was intentionally mutated (knockout mice), despite DNA-packaging, though inadequate, could be made with the aid of the other transition protein. and protamine with resultant infertile mice.^[50] Histones break after their replacement by protamine. Incorporation of protamines into sperm chromatin induces tighter DNA-packaging. Thus during configuration, and transfer of spermatozoa, safe conditions are created for the genome. Oakes et al.^[51] reported that the characteristic features of DNA methylation change only a little bit during evolution of genome after pachytene stage of spermatocytes. In fertile men, ratio of P1/P2 protamines equal to one. Erroneous processing of protamine transcripts leads to increase in the production of immature P2 precursors associated with subfertility.^[52,53] Subfertility is also associated with derangement of P1/P2 ratio.^[38,54] Despite controversial publica-

Figure 2. Replacement of histones localized in sperm chromatin by protamines

tions, deviations in protamine ratio has been associated with various phenotypic features including decreased sperm counts, and function, and poor embryonic quality.^[48,55,56] Besides variations were detected in average P1/P2 ratio in ejaculate at different measurement time points.^[57,58] In conclusion, abnormal protamination is seen prevalently in men with abnormal sperm parameters contrary to fertile men with normal sperm parameters.^[53,59] In many studies abnormal replacement of histones by protamines have been associated with reduced semen quality, decreased success rates of IVF, and pregnancy rates.^[56,58,60,61]

Appropriate protamine levels cause firmly packaging of chromatin leading to tighter compaction of sperm nuclei. Mammalian sperm chromatin constitutes of three parts. These components include protamine-bound DNA, histone-bound DNA, and sperm nuclear matrix bound-DNA.^[62] Packaging of DNA with protamine is realized via formation of disulfide bonds between protamines, and toroidal structure of the chromatin subunit.^[63] In each toroidal subunit nearly 50 kb DNA is packaged.^[39] Recently Ward has proposed a specific model of DNA-packaging by protamines. According to this donut loop model, each toroid links, loop domains of DNA and matrix attachment regions.^[62,64] This model demonstrates that protamine-bound DNA is protected from damage, by compressing, and packing toroids, while toroid-linker, and histone-bound DNA regions are exposed to DNA damage induced by endonucleases.^[65]

On the other hand, nearly 5-10% of DNAs of fertile, and a higher percentage of DNAs in infertile men are bound to histones.^[66,67] It has been reported that the presence of somatic cell like-chromatins in the sperm nuclei can induce transmission of divergent genetic information to offsprings. Up to recent times, we were ignorant about the function of histones which translo-

cate with protamines in the sperm nuclei, and mostly inadequate protaminations were thought to be the source of these histones. Two important studies have determined the location of histones which do not replace with protamines, and also retained all along the sperm genome. Arpanahi et al. cut human, and rat sperm chromatin with endonucleases, and determined endonuclease-sensitive regions.^[68] They reported that endonuclease-sensitive regions are mainly bound to histones, and harbour regulatory genomic regions which also contain promoters. Hammoud et al.^[66] used a different method. They used micrococcal nuclease digestion, and gel purification procedures to differentiate chromatin-bound histone from protamine-bound histone. This protein purification is followed by microarray analysis, and sequencing procedure so as to identify histone-bound DNA, and protein-bound DNA. In this study, sperms of fertile, and normospermic men were used. It has been shown that histones concentrate in the promoters of required genes to ensure their development, and this process is not interrupted in miRNA, and genes undergoing imprinting. This study demonstrates that protamines do not concentrate in any gene family. In conclusion, these two studies demonstrate that the retained histones might have a potential epigenetic role in the embryonic development. As reported by Hammoud et al. epigenetic role of the variants of retained histones might be directed to a certain goal. Indeed, their more recent studies have demonstrated that both genes responsible for the development of embryonic stem cells, and H3K4me3 and H3K27me3 in critical genes responsible for the development were bivalently marked. These bivalently marked DNA regions are also methylated. They are also situated between regions which ensure creation of balanced state for genome.^[66,69] Recent studies indicate that histones have an important role in the determination of the epigenetic position of the sperm. They do not distribute in the genome randomly, and specific histone modifications regulate activation, and silencing of the genome.^[69-71] These data have shown that sperm chromatin can transfer inheritable epigenetic characteristics which might exert an effect on post-fertilization transcriptional regulation.^[72]

Despite numerous studies performed on male infertility within last years, still substantial number of cases of infertility remain unexplained. Various studies have demonstrated critical roles of epigenetic factors in spermatogenesis. Thorough understanding of these factors, and their mechanisms of action may contribute to the determination of the causes of male infertility, and also ways of controlling these mechanisms.

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