Are there epigenetic events associated with male germ cell formation? What is the role of genomic imprinting in male germ cells?

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Epigenetics refers to heritable mechanisms of modulating gene expression that do not involve alterations in DNA sequence. Thus epigenetics affects the phenotype of a cell without affecting its genotype and is governed by processes that permit heritability from the parental to the daughter cells but are at the same time potentially modifiable or even reversible. DNA methylation, histone modifications and small RNAs are the three main molecular mechanisms 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 “epigenetic diseases” such as cancer and imprinting disorders. Epigenetic mechanisms are conserved across higher eukaryotes including humans suggesting that they act in combination to regulate higher order chromatin and ultimately genome integrity.

Epigenetic patterning begins in the germline and is essential for normal embryo and postnatal development. Epigenetic modifications occurring during germ cell development are postulated to play roles in gene expression, meiosis, genomic integrity and genomic imprinting. Male and female germ cell development is a particularly critical time for the acquisition of the differential ‘marking’ of imprinted genes to ensure parent-of-origin specific expression. Genomic imprinting involves about 100 genes to date and refers to variation in the expression of these genes according to their maternal or paternal origin; imprinted genes play key roles in growth, placental function and many aspects of development including neurobehavioral processes. Many imprinted genes have distinct sequences known as differentially methylated regions or DMRs where DNA methylation differs between the maternal and paternal alleles. Since DNA methylation is the most extensively characterized epigenetic mechanism, it will be used here to illustrate the importance of epigenetics to male germ cell biology.

DNA methylation is found 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 60-80% of cytosines within CpGs are methylated. Methylation of DNA is invariably associated with transcriptional silencing. Two types of DNA methylation occur, either *de novo* methylation or the acquisition of methylation on unmethylated cytosines, or maintenance methylation, that takes place at the time of DNA replication to ensure the propagation of genomic 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 DMRs of imprinted genes. Methylation of DNA is catalyzed by a family of DNA (cytosine-5)-methyltransferases (DNMT enzymes or DNMTs). The main DNMTs involved in the establishment and maintenance of methylation patterns are DNMT1, DNMT3A and DNMT3B. Although it lacks enzymatic activity, DNMT3L (DNMT3-like) is related to and works with DNMT3A and DNMT3B. Demethylation occurs passively when methylation is not maintained following DNA replication or actively by an as yet poorly understood process/enzyme.

Epigenetic modifications including DNA methylation are for the most part erased in primordial germ cells and then reacquired in a sex-specific manner during germ cell development (Fig. 1). The erasure is particularly important for imprinted genes as maternal ‘marks’ on imprinted genes inherited from the mother must be erased and new paternal ‘marks’ introduced. A second period of erasure occurs in the preimplantation embryo and is thought to affect most sequences with the exception of imprinted genes and some repeat sequences. Imprinted gene methylation is maintained during preimplantation development since it is only in the germline (male or female depending on the gene) that imprinted genes acquire the allele-specific methylation that will result in monoallelic expression in the embryo and postnatal individual. As most methylation will be erased in the early embryo, it is postulated that germline DNA methylation, at sequences other than imprinted genes and repeats, plays specific roles in germ cell development, gene expression or chromatin structure during gametogenesis.

Following erasure in primordial germ cells (at approximately mid-gestation in the fetus), most male germline DNA methylation is acquired premeiotically in the prenatal gonocytes or pro-spermatogonial cells in the period between mid-gestation and birth (Fig. 1). DNA methylation acquisition in male germ cells continues after birth in the mitotic and meiotic germ cells and is complete by the pachytene phase of meiosis. Mitotic spermatogonia must also maintain the DNA methylation patterns acquired in the prenatal period. The precise signals that target DNA methylation to specific sequences in male germ cells are unclear but may include RNA-directed events. Potential candidates for RNA-based targeting are the recently described germ cell-specific small RNAs, the piRNAs. Gene-targeting experiments in mice have identified DNMT3a and DNMT3L as the predominant enzymes involved in the methylation of repetitive and imprinted sequences in the male germline. Absence of these enzymes results in the failure of spermatogenesis and infertility. For example, in male mice lacking DNMT3L, male germ cells over-express retrotransposons, and there is

asynapsis and non-homologous synapsis during meiotic prophase and subsequent apoptosis of germ cells prior to pachytene.

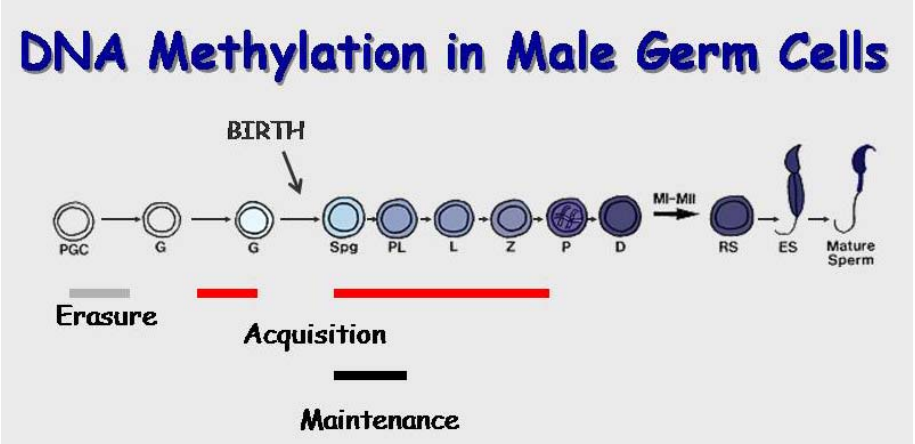

FIG. 1. Timing of the erasure (gray bar), acquisition (red bar) and maintenance (black bar) of DNA methylation patterns in the prenatal and postnatal periods of male germ cell development. PGC, primordial germ cell; G, gonocyte or prospermatogonia; Spg, spermatogonia; PL, preleptotene spermatocyte; L, leptotene spermatocyte; Z, zygotene spermatocyte; P, pachytene spermatocyte; D, diplotene spermatocyte; RS, round spermatid; ES, elongating spermatid.

Evidence is accumulating that errors in the establishment or maintenance of germ cell DNA methylation patterns can cause human diseases such as imprinting disorders and cancer. Sperm samples from oligospermic patients have been reported to contain DNA methylation defects at imprinted loci. It has been suggested that paternal age effects that are seen for schizophrenia and other human conditions may involve errors in the maintenance of genomic methylation in the spermatogonia of older men. Furthermore, there is also concern for more than one generation, since while most epigenetic errors will be erased in the germline of the progeny, there are several examples of the potential for inheritance of epigenetic defects. Ongoing studies are assessing the effects of environmental conditions, diet and drugs on DNA methylation patterns in human sperm.

Suggested reading

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