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Methylation Patterns of Brahma during Spermatogenesis and Oogenesis: Potential Implications

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Abstract

Patterns of differential methylation of Brahma (*Smarca2*) correlate with its expression during spermatogenesis and oogenesis. The extent of methylation of Brahma decreases during spermatogenesis, whereas it increases with oocyte maturation.

Keywords

Smarca2; *Sma2*; Brahma; Brm; methylation; CpG island; gametogenesis; chromatin remodeling; SWI/SNF complex; ART

The switch from cellular proliferation to differentiation occurs as a function of the accurate regulation of gene transcription and subsequent translational processes. Transcriptional regulation controls gene expression at cellular stages during spermatogenesis and oogenesis. One transcriptional regulatory mechanism, DNA methylation, attaches methyl groups to cytosine nucleotides within CpG islands. Methylation of CpG sites across the promoter affects transcription of genes and is characteristic at various stages of germ cell differentiation. This regulation is partially responsible for the differential transcription of various genes throughout gametogenesis (1).

The SWI/SNF (Switch/Sucrose NonFermentable) complex, which is silenced by CpG methylation, uses energy from ATP hydrolysis to modulate nucleosome position and density across promoters. This shift in histone position within chromatin is essential for normal cellular differentiation and growth (4). Brahma (Brm, *Smarca2*) provides ATPase activity for the SWI/SNF complex. Repression of Brahma triggers abnormal chromatin organization,

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failure to differentiate, and asymmetric cell division (5,6). There is minimal data regarding altered expression of Brahma in human cell lines, but data from mice characterize the implications of Brahma in mammalian development. In previous studies, disruption of certain genes in mice and their mutant homologues in humans both yielded infertile phenotypes (7,8). Disruption of Brahma activity interferes with embryonic viability, and knockout mouse models display increased growth rate and infertility or subfertility. Aberrant CpG methylation within the Brahma promoter leads to formation of neoplastic cells (9). These data imply that the regulation of expression levels of Brahma has important implications for normal cellular proliferation and differentiation during gametogenesis and subsequent development.

We investigated the relationship of methylation of the promoter and expression of Brahma during gametogenesis. Numerous studies demonstrated that CpG methylation at or around the promoter region of various genes affect their expression (10); minimal studies show the effect of methylation of CpG islands further downstream at other loci. Methylation of the promoter of Brahma or other genes during gametogenesis and embryogenesis may impact cellular proliferation and differentiation and thus may shed light on the perplexing clinical pathophysiologies seen with assisted reproductive technology (ART) (11,12).

The National Institutes of Health Animal Care and Use Committee approved protocols for the study of mice. Male germ cell isolation (1 mouse) was performed as previously described (13). Mouse spermatogonia, sertoli cells, leydig cells, and pachytene spermatocytes were assessed as components of spermatogenesis. Mouse primary oocytes, cumulus cells, and cells at the meiosis I and meiosis II stages (Charles River, Germantown, MD) were obtained for analysis of oogenesis. After collection, DNA was bisulfite treated (14) using EZ DNA Direct Methylation Kit (Zymo Research, Orange, CA). Due to the small number of oocytes, treated DNA was amplified using Whole Genome Amplification Kit (SIGMA-ALDRICH, St Louis, MO). This generated an accurate amplification of the genome, according to previous methylation studies (15), for further analysis.

The CpG island associated with Brahma covers 3405bp and 259 sites (UCSC Genome Browser, CA). Primers were designed to amplify a 280bp region including the promoter, exon1, and intron1. Exon1 and intron1 were included to generate a manageable fragment size. This fragment of chromosome 19 contains 18 CpG sites.

The cloning process is required to obtain sufficient quantities of the gene for analysis in the sequencer we employed. PCR amplification of Brahma was accomplished using High-Fidelity Platinum *Taq* Polymerase (Invitrogen, Carlsbad, CA). The PCR fragment ligated with TOPO cloning vector was transformed into electrocompetent TOP10 cells (Invitrogen, Carlsbad, CA) (16). DNA from colonies was amplified using M13 primers and labeled using BigDye V3.0 (Applied Biosystems, Foster City, CA). Samples were processed in a 3730 DNA Analyzer (Applied Biosystems, Foster City, CA) (17). The methylation status of CpG sites from each clone was analyzed against a Brahma reference sequence (NM_011416) using CpG Viewer (Leeds Institute of Molecular Medicine, Leeds, England).

Methylation of each CpG site in the Brahma promoter was determined for each cell type (Fig. 1). Methylation decreased during development from spermatogonia to pachytene spermatocytes. The 3' end of spermatogonia fragments exhibited hypermethylation at sites 10 through 15, while pachytene fragments showed hypermethylation at sites 2, 14, and 15. Somatic cells of the testis showed an even distribution of methylation across the region, with sertoli cells displaying slightly higher methylation than leydig cells.

In contrast, methylation increased during oogenesis. GV and MI oocytes have methylation at sites 2, 14, and 15, while MII oocytes present an even distribution of methylation across the

region. Cumulus cells exhibit a moderate and even distribution of methylation similar to the somatic cells of the testis.

Previous studies in our laboratory using serial analysis of gene expression (SAGE) demonstrated an increase in expression of Brahma during spermatogenesis (18). This increase correlates with the decreased methylation of the promoter, which facilitates transcription of Brahma, as development proceeds towards the spermatid. If promoter methylation parallels its expression level, expression of Brahma during oogenesis may be deduced from the differential methylation observed.* The increase in methylation suggests decreased expression of Brahma during oogenesis. Previous studies confirm decreased expression of Brahma during the transition from GV oocytes to MII oocytes (19). These changes are opposite the pattern observed during spermatogenesis, and illustrate biological differences between oogenesis and spermatogenesis.

The opposing changes in expression of Brahma correspondingly affect ATPase activity of the SWI/SNF complex. Transcriptional regulation of Brahma may alter the ability of the SWI/SNF complex to control cellular proliferation and differentiation and to impact germ cell and embryonic development. In vitro fertilization with variations in culture conditions or in the biological status of either oocytes or sperm may lead to epigenetic changes resulting in altered methylation and changes in the SWI/SNF complex that may lead to faulty embryo development.

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*Oocytes were difficult to isolate and limited for use. Expression levels were not measured.

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Figure 1.

Methylation status of CpG islands (black = methylated, white = non-methylated) within the promoter region of SMARCA2. CpG islands are represented by scaled lollipops obtained from using CpG Viewer software. Graph portrays percent methylation of all samples from different stages during oogenesis (a) and spermatogenesis (b) (Yellow = Germinal vesicle cells, Orange = Meiosis I stage cells, Red = Meiosis II stage cells, Aqua = Cumulus cells; Pink = Spermatogonia, Purple = Primary spermatocytes at the pachytene stage, Blue = Sertoli cells, Green = Leydig cells).