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Comparative analysis of human mitochondrial methylomes shows distinct patterns of epigenetic regulation in mitochondria

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ABSTRACT

DNA methylation and histone modifications across the nuclear genome have been extensively analyzed, but the epigenetic modifications associated with the mitochondrial genome have not yet been analyzed at high resolutions. In the present work, we analyzed methyl-cytosine profiles from methylated DNA immunoprecipitation datasets from 39 different human cell and tissue types from the NIH Roadmap Epigenomics project and validated the data using an orthologous bisulfite sequencing dataset. We observe a distinct distribution of zmethyl-cytosine in mitochondrial genomes which are conserved across all cell and tissue types. This study thus describes the first comprehensive map of methyl cytosines across the human mitochondrial genome.

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1. Introduction

The availability of high-throughput sequencing technology has led to a phenomenal improvement in the understanding of the epigenetic regulation of the genome (Yuan, 2012). Whole genome methylomes are currently available for human (Lee et al., 2012) and other model organisms like mouse (Stadler et al., 2011), rat (Sati et al., 2012b), zebrafish and plants (Feng et al., 2010). In addition, extensive collaborative initiatives worldwide, including the Human Epigenome Project (2008) (Jones and Martienssen, 2005), aims to decipher the role of DNA methylation in maintaining the structure and function of the nuclear genome. The regulation of the genome through DNA methylation has been one of the recent and active areas of study (Esteller, 2007). Our group has recently shown that DNA methylation patterns could mark protein-coding exons and could have differential roles in regulating long noncoding RNA genes (Sati et al., 2012a). Methylation changes have been extensively analyzed with respect to a number of disease states (Esteller, 2007).

Although DNA methylation in the context of nuclear DNA has been well studied, similar studies on mitochondrial DNA methylation are lacking. Seminal contributions from Mushkambarov et al. (1976) and Pollack et al. (1984) led to the initial characterization of mitochondrial DNA methylation. It has been suggested that methylation in mitochondrial DNA almost exclusively occurs at CpG sites (Pollack et al., 1984) and it is estimated that 1.5–5% of the cytosines are methylated in mitochondrial DNA (Pollack et al., 1984; Vanyushin and Kirnos, 1977). Earlier studies have analyzed mitochondrial genomes derived from multiple species and suggested that a CpG dinucleotide could have roles in the organization and regulation of the mitochondrial DNA. The specificity of mitochondrial methyltransferases has also been one of the areas of interest. Earlier reports by Vanyushin et al. have characterized the specificities of the nuclear and mitochondrial methyltransferase enzymes (Vanyushin and Kirnos, 1977; Vanyushin and Kirnos, 1976; Kudriashova et al., 1976) on heterologous DNA and suggested that the nuclear enzyme has a di-pyrimidine and tri-pyrimidine specificity while the mitochondrial enzyme methylates mono-pyrimidine.

Epigenetic regulation of the mitochondrial genome has been an enigma (Manev et al., 2012). Recent evidence points to a larger role of mitochondrial epigenetics through its regulatory cross-talk with other biological pathways (Minocherhomji et al., 2012). In addition to the mitochondrial DNA methyltransferase, recent reports have shown that mammalian nuclear encoded DNMT1 could also translocate to the mitochondria (Shock et al., 2011) and modulate the methylation of cytosine residues in the mitochondrial DNA. Further, it has been suggested that mitochondrial genetic variations, including ones that

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potentially define mitochondrial haplogroups could modulate nuclear DNA methylation levels and thereby the expression and regulation of genes encoded by the nuclear genome (Chinnery et al., 2012). Recent studies have also proposed mitochondrial DNA methylation as a potential biomarker (Iacobazzi et al., 2013).

The association of mitochondrial DNA methylation with diseases has also been recently explored. It has been shown that the hypermethylation of polymerase γ 1 binding sites in the mitochondrial genome in hyperglycemic states could potentially modulate the epigenetic memory in diabetic retinopathy (Tewari et al., 2012), one of the serious complications of uncontrolled hyperglycemia in diabetes mellitus. Mitochondrial DNA methylation changes have also been studied in association with aging. Evidence suggests that aging affects 5-hydroxymethyl-cytosine levels in the brain frontal cortex in mice, but not 5-methyl-cytosine, which correlates well with the differential changes in mitochondrial DNMT1 and Ten-Eleven-Translocation enzyme (TET) levels in different areas of the brain (Dzitoyeva et al., 2012). Differences in mitochondrial DNA methylation have also been studied in association with prolonged exposure to pharmacological agents including valproate (Chen et al., 2012), a popular anticonvulsant.

The availability of genome-scale epigenome datasets in public domain provides a starting point towards systematically understanding the mitochondrial epigenome profile. In the present report, we describe the first and comprehensive genome-scale analysis of DNA methylation in the human mitochondrial genome encompassing datasets for 39 cell lines including primary cell lines. We show that the pattern and distribution of methyl cytosines are relatively constant across the mitochondria, with the exception of a few loci that are differentially methylated in different tissues and time-points.

2. Materials and methods

The alignment files for 39 cell lines and tissues were downloaded from the Human Epigenome Consortium website. The datasets correspond to IDs GSM493615 (breast luminal epithelial cells), GSM456941 (H1 cell line), SM543017 (breast myoepithelial cells), GSM543019 (breast stem cells), GSM543021 (breast vHMEC), GSM543025 (CD4 naive primary cells), GSM543027 (CD8 naive primary cells), GSM543023 (peripheral blood mononuclear primary cells), GSM613843 (breast luminal epithelial cells), GSM613856 (breast luminal epithelial cells), GSM613864 (breast luminal epithelial cells), GSM613846 (breast myoepithelial cells), GSM613857 (breast myoepithelial cells), GSM613847 (breast stem cells), GSM613853 (breast stem cells), GSM613859 (breast stem cells), GSM613862 (CD4 memory primary cells), GSM613914 (CD4 memory primary cells), GSM613913 (CD4 naive primary cells), GSM613917 (CD8 naive primary cells), GSM543016 (H1 cell line), GSM613911 (peripheral blood mononuclear primary cells), GSM669614 (brain fetal week 17 F), GSM669615 (brain fetal week 17 F), GSM669608 (CD4 memory primary cells), GSM669607 (CD4 naive primary cells), GSM669609 (CD8 naive primary cells), GSM669610 (neurosphere cultured cells cortex derived), GSM669612 (neurosphere cultured cells cortex derived), GSM669611 (neurosphere cultured cells ganglionic eminence derived), GSM669613 (neurosphere cultured cells ganglionic eminence derived), GSM669606 (peripheral blood mononuclear primary cells), GSM707019 (brain fetal day 122 M), GSM707023 (brain germinal matrix fetal week 20 M), GSM707021 (penis foreskin fibroblast primary cells), GSM707022 (penis foreskin keratinocyte primary cells), GSM707020 (penis foreskin melanocyte primary cells), GSM817248 (brain fetal day 85 F), and GSM817249 (brain fetal day 96 F).

The mitochondrial reference genome used in the current study was obtained from the UCSC human genome build hg19 (Karolchik et al., 2012; Meyer et al., 2013). All genes were mapped with respect to this co-ordinate system and reference genome.

The alignments corresponding to the reads are available in standard BED file formats. We used custom scripts to retrieve reads mapping to the mitochondrial genome. The position information of the mappings was used to construct a bin-wise map of methylation across the mitochondrial genome. Briefly, the entire mitochondrial genome (16,571 bases) was divided into non-overlapping bins of 50 nucleotides and the number of reads overlapping each of the bins was calculated. This provided the reference spatial distribution of methylation across the mitochondrial genome. This was repeated for each of the 39 samples analyzed in the present study. The read densities were scaled to the number of reads mapping to the mitochondrial genome to derive the methylation coverage.

The read coverage across the mitochondrial genome was assembled and clustered. We used the popular 'gplots' package in statistical tool R to perform clustering. The average read coverage across all the samples was also computed and the z-scores of the methylation density of each of the bins across were computed. The regions with a z-score greater than 3 were considered for analyzing the differential methylation across each of the tissues and time points.

The read coverage across the Gene Start Site (GSS) of 37 annotated genes in the mitochondrial genome was plotted and clustered for each of the tissues under consideration. The plots correspond to 10 base non-overlapping bins, each upstream and downstream of the GSS, and correspond to 200 bases on either side of the GSS (Supplementary Fig. 1).

The mapping of reads to the mitochondrial genome is not without caveats as a significant portion of the mitochondrial genome has homology with the nuclear genome, which could affect the analysis. We have independently simulated overlapping 76 base reads derived from the mitochondria and mapped back to the whole genome (hg19). A count of 1, against the reads is expected and signifies a unique region in the mitochondrial genome. A greater count for mapping would mean non-unique regions.

The bisulfite sequencing dataset was downloaded for the H1 cell line from the NIH Human Epigenome Roadmap project (ID GSM429321). Bisulfite reads were mapped against the human (hg19) mitochondrial genome, using BSMAP and alignment was processed as per previously published protocols (Xi and Li, 2009). A percentage methylation cutoff of 10 and per base read coverage of a minimum of 5 were considered. Further we performed a statistical analysis on the basis of the positional preference (presence or absence) of both MeDIP and bisulfite, throughout the mitochondrial genome in 50 bp non-overlapping windows.

3. Results and discussion

3.1. Datasets and read mapping

We downloaded a total of 39 datasets corresponding to 5 unique tissues that were available and were out of embargo from the NIH Human Epigenome Roadmap project. The tissues included brain, breast, blood (PBMC, CD4 and CD8 + cells), penis and two cell lines, H1 and neurosphere culture cells. Some tissues like brain and breast had datasets corresponding to different developmental time points.

Multiple datasets corresponding to brain and breast were included in the analysis. This includes datasets corresponding to 85 days, 96 days, 122 days, 17 weeks and 20 weeks for brain, 26 years, 36 years, and 39 years for luminal epithelial cells, 18 years, 26 years and 36 years for myoepithelial cells, 18 years, 26 years, 36 years, and 37 years for breast and one sample which corresponded to an 18 year mammary epithelial cell. The datasets and summary of reads are shown in Table 1. The bisulfite sequencing dataset was downloaded for the H1 cell line from the NIH Human Epigenome Roadmap project (ID GSM429321). The protocol used specific antibodies to 5-methyl cytosine (Eurogentec, catalog: BI-MECY-0100), which have also been used in many other studies.

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