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Systemic analysis of osteoblast-specific DNA methylation marks reveals novel epigenetic basis of osteoblast differentiation

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ABSTRACT

DNA methylation is an important epigenetic modification that contributes to the lineage commitment and specific functions of different cell types. In this study, we compared ENCODE-generated genome-wide DNA methylation profiles of human osteoblast with 21 other types of human cells in order to identify osteoblast-specific methylation events. For most of the cell strains, data from two isogenic replicates were included, resulting in a total of 51 DNA methylation datasets. We identified 852 significant osteoblast-specific differentially methylated CpGs (DMCs) and 295 significant differentially methylated regions (DMRs). Significant DMCs/DMRs were not enriched in CpG islands (CGIs) and promoters, but more strongly enriched in CGI shores/shelves and in gene body and intergenic regions. The genes associated with significant DMRs were highly enriched in biological processes related to transcriptional regulation and critical for regulating bone metabolism and skeletal development under physiologic and pathologic conditions. By integrating the DMR data with the extensive gene expression and chromatin epigenomics data, we observed complex, context-dependent relationships between DNA methylation, chromatin states, and gene expression, suggesting diverse DNA methylation-mediated regulatory mechanisms. Our results also highlighted a number of novel osteoblast-relevant genes. For example, the integrated evidences from DMR analysis, histone modification and RNA-seq data strongly support that there is a novel isoform of *neurexin-2* (*NRXN2*) gene specifically expressed in osteoblast. *NRXN2* was known to function as a cell adhesion molecule in the vertebrate nervous system, but its functional role in bone is completely unknown and thus worth further investigation. In summary, we reported a comprehensive analysis of osteoblast-specific DNA methylation profiles and revealed novel insights into the epigenetic basis of osteoblast differentiation and activity.

1. Introduction

DNA methylation of cytosine is a crucial epigenetic mechanism for transcriptional regulation and has profound impacts on embryonic development, genomic imprinting, X-chromosome inactivation, and the pathogenesis of various human disorders (Weber et al., 2007). Though the regulatory function of DNA methylation is generally thought to be associated with transcriptional repression when occurring in gene promoter regions and with transcriptional activation when occurring in gene bodies (Jones, 2012; Ball et al., 2009; Rauch et al., 2009), recent studies revealed a far more complicated relationship between DNA methylation and gene expression. Both negative and positive correlations between methylation and expression have been observed across all gene structural regions, and DNA methylation can also regulate alternative splicing through effects on RNA Pol II elongation (Jones, 2012; Shukla et al., 2011; Chandra et al., 2014; Ehrlich and Lacey, 2013; Liu et al., 2013; Deaton et al., 2011; Lee et al., 2014; Varley et al., 2013), indicating that DNA methylation can have diverse, chromatin context- and cell type-dependent regulatory functions on transcription.

With recent advance in high-throughput technology for DNA methylation analysis (Sun et al., 2015), a number of studies have demonstrated that DNA methylation profiles vary in diverse human tissues and cell types (Jones, 2012; Lokk et al., 2014; Yang et al., 2015), which contribute to the regulation of cell type-specific gene expression and determine the differentiation and specific function of different cell types (Futscher et al., 2002; de la Rica et al., 2013; Tsumagari et al., 2013). For example, Ziller et al. (2013) found that 21.8% of autosomal CpGs showed dynamic DNA methylation changes in a range of human cell and tissue types and these dynamic CpGs co-localized with gene regulatory elements,

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particularly enhancers and transcription-factor-binding sites, allowing identification of key lineage-specific regulators. In addition, Rica et al. (2013) identified hyper-/hypo-methylation changes in several thousand genes during in vitro monocyte-to-osteoclast differentiation, including all relevant osteoclast differentiation and function categories. DNA methylation has also been implicated in the regulation of differentiation and function of osteoblasts, the bone-forming cell with main function of mineralizing the bone matrix (Eslaminejad et al., 2013). For example, the promoter of *osteocalcin* gene, a gene solely expressed by osteoblasts, is highly methylated in cells not expressing osteocalcin, including the mesenchymal stem cells (MSCs) (Villagra et al., 2002). Interestingly, during in vitro MSC-to-osteoblast differentiation, as the osteocalcin gene becomes increasingly expressed, CpG methylation of the osteocalcin promoter is significantly reduced (Villagra et al., 2002). Similarly, reduced DNA methylation along with transcriptional upregulation were also observed for two additional osteogenic genes, namely, *alpha 1 type I collagen* (*COL1A1*) and *osteopontin* (Arnsdorf et al., 2010). In addition to hypomethylation mediated gene activation, hypermethylation induced silencing of specific genes were also crucial in osteoblast differentiation. For instance, Hsiao et al. (2010) found that *Trip10* (thyroid hormone receptor interactor 10), an adaptor protein involved in diverse cellular functions, shows significant alterations in promoter methylation and mRNA levels during lineage-specific induction of human bone marrow-derived MSCs. Remarkably, targeted induction of *Trip10* promoter methylation resulted in reduced *Trip10* expression and accelerated MSC differentiation towards osteogenic lineage at the expense of MSC-to-adipocyte differentiation. Taken together, these results strongly supported that DNA methylation plays a significant role in mediating cell-specific gene transcription and interfering with cell fate determination, including osteoblast differentiation.

In this study, we compared the genome-wide DNA methylation profiles between human osteoblasts and a wide range of other types of human cells in order to identify and characterize osteoblast-specific methylation patterns on a global scale. The purpose is to identify those genes and regulatory mechanisms underlying specific functions of osteoblasts. Our results revealed many osteoblastic hyper-/hypo-methylated CpGs across the genome. By integrating the DNA methylation patterns with transcriptomic and other epigenomic profiles, we further showed that these osteoblastic-specific methylation events were enriched in regulatory regions beyond the promoter areas and may influence gene expression and the use of alternative promoters in a cell-type specific manner. Collectively, these data may provide novel insight into the regulatory role of DNA methylation in osteoblast differentiation and functioning.

2. Results and discussion

2.1. Identification and characterization of osteoblast-specific DMCs/DMRs

We compared ENCODE-generated DNA methylation profiles of osteoblasts with those of 20 different types of non-transformed human cell strains plus Epstein-Barr virus-transformed lymphoblastoid cell lines (LCLs) (Supplementary Table 1). For most of the cell strains, DNA methylation data generated by reduced representation bisulfite sequencing (RRBS) from two isogenic replicates were included, resulting in a total of 51 DNA methylation datasets. The number of CpGs assessed per sample ranged from 960,300 to 1,489,630, including ~31.6–43.7% of CpGs with sequence coverage ≥10× (Supplementary Table 1). We compared a total of 182,518 CpGs with coverage ≥10× across all 51 samples and identified 852 significant differentially methylated CpGs (DMCs) with stringent criteria ($q < 0.01$, difference in methylation ≥50%), which were distributed across the entire genome (Supplementary Fig. 1). Hierarchical clustering analysis using the significant DMCs correctly grouped cells from similar tissues and of similar biological functions (Supplementary Fig. 2). Interestingly, we observed high similarity of the DNA methylation patterns between osteoblast and skeletal muscle myoblast. This is not completely unexpected, because both osteoblast and myoblast are mesodermal descendent of the bone-marrow mesenchymal stem cells (BMSCs) (Gimble et al., 2008). Moreover, it has been shown that myoblastic cells can be differentiated into osteoblastic cells (Tanaka et al., 2012), and the muscle-derived MSCs were more effective in differentiation into osteoblastic cells than BMSCs (Glass et al., 2011). In fact, a high similarity of chromatin states between osteoblast and skeletal muscle myoblast has also been observed in the NIH Roadmap Epigenomics project (C. Roadmap Epigenomics et al., 2015).

Of the total 852 DMCs, 685 (80.40%) were hypermethylated and 167 (19.60%) were hypomethylated, in osteoblasts vs. other cell types. While the majority of the DMCs was mapped to CpG islands (CGIs), DMCs were more strongly enriched in non-CGI regions, including CGI shore ($p = 5.73 \times 10^{-7}$, Fisher's exact test), CGI shelf ($p = 7.54 \times 10^{-11}$) and open sea ($p = 4.54 \times 10^{-11}$), when taking into account of the number of CpGs tested in each CpG annotation class (Fig. 1A). We observed a marked difference in the distributions with respect to CGIs between hyper- and hypo-methylated DMCs (Fig. 1A, $p = 1.34 \times 10^{-64}$), with the over majority (78%) of hypermethylated DMCs associated with CGIs, in contrast to hypomethylated DMCs, which were mainly mapped to open sea (~56%) and relatively infrequent in CGIs (~13%). Interestingly, the enrichment of cell lineage-/tissue-specific DNA methylation events in non-CGI regions but depletion in CGIs have also been observed by others (Lokk et al., 2014; Yang et al., 2015; Byun et al., 2009; Slieker et al., 2013), highlighting the importance of exploring the functional significance of non-CGI methylation.

We considered the location of DMCs across different parts of individual genes. We observed a significant depletion of DMCs in 5′-untranslated regions (UTRs) ($p = 4.2 \times 510^{-34}$) and promoters ($p = 7.87 \times 10^{-21}$) but a significant enrichment of DMCs in exons ($p = 1.64 \times 10^{-07}$), 3′UTR ($p = 3.89 \times 10^{-09}$) as well as intergenic regions (p

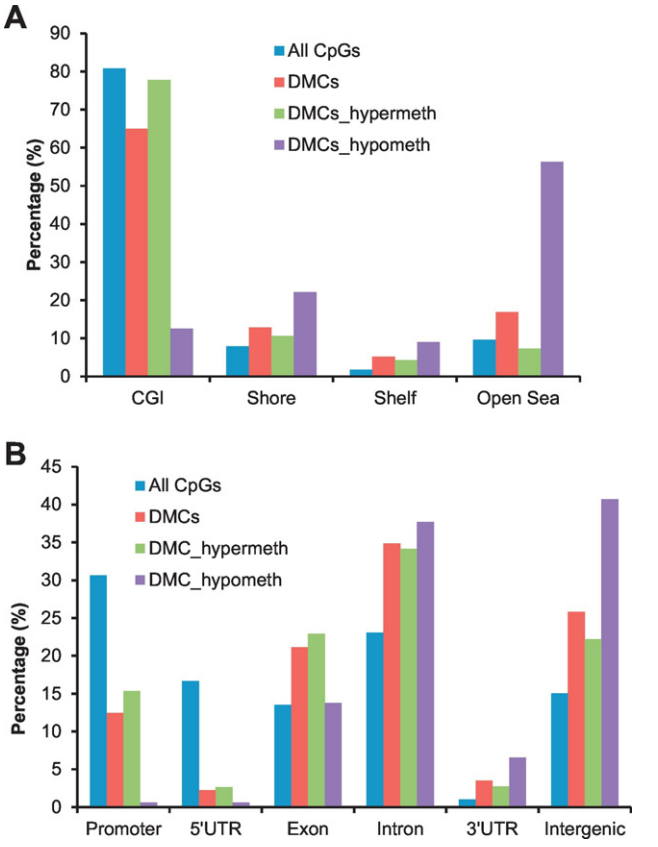

Fig. 1. Distribution of all 182,518 tested CpGs and 852 significant osteoblast-specific DMCs, including 685 hypermethylated and 167 hypomethylated DMCs, across (A) different regions related to CGIs and (B) different genic regions.