Histone H3K27 acetylation is dispensable for enhancer activity in mouse embryonic stem cells

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Abstract

H3K27ac is well recognized as a marker for active enhancers and a great indicator of enhancer activity. However, its functional impact on transcription has not been characterized. By substituting lysine 27 in histone variant H3.3 with arginine in mouse embryonic stem cells, we diminish the vast majority of H3K27ac at enhancers. However, the transcriptome is largely undisturbed in these mutant cells, likely because the other enhancer features remain largely unchanged, including chromatin accessibility, H3K4me1, and histone acetylation at other lysine residues. Our results clearly reveal that H3K27ac alone is not capable of functionally determining enhancer activity.

Keywords: H3K27 acetylation, Enhancer, H3.3, Gene transcription

Background

Enhancers are critical regulatory elements that control spatial and temporal gene expression [1], and they are demarcated by distinct chromatin modifications [2]. In mammals, putative enhancers can be predicted by their local chromatin signatures, including H3K4me1 and H3K27ac [3–5]. Among these enhancer marks, H3K27ac is of particular interest because it distinguishes active enhancers from poised ones [6]. To date, a large body of literature has well established a role for H3K27ac in indicating enhancer activity. On the other hand, whether H3K27ac functionally determines enhancer activity is less clear because it is difficult to selectively target H3K27ac at enhancers. Nevertheless, it has been reported that H3K27ac plays an active role in cell identity control [7].

Enrichment of histone variant H3.3 and high histone turnover are other enhancer features [8–14]. Although mammalian H3.3 differs from canonical H3.1 by only five amino acids, H3.3 is deposited into specific chromatin regions [8, 9, 15, 16] by distinct histone chaperones [9, 17, 18] and is enriched for active histone modifications such as H3K4me3 and H3K27ac [19–21]. H3.3 is not essential for viability in Drosophila [22, 23]. However, complete loss of H3.3 in mice is embryonic lethal at an early stage and even cellular lethal as a result of mitotic defects [24]. Replacing lysine 27 with a nonmodifiable residue in one of the two genes encoding H3.3 in Drosophila causes male lethality and infertility and reduced global H3K27ac levels [25]. A similar reduction in H3K27ac levels was also observed in mouse embryonic stem cells (ESCs) depleted of H3.3 [26]. Therefore, we reasoned that targeting enhancer H3K27ac might be achievable by mutating both H3.3-encoding genes (H3f3a and H3f3b) and producing a homogenous population of H3.3 lysine 27-to-arginine (K27R) mutant histones in mouse ESCs.

Results and discussion

We utilized the CRISPR-Cas9 gene editing system to sequentially introduce mutations in both endogenous alleles of H3f3a and H3f3b, and the engineered mouse ESC clones were confirmed by Sanger sequencing (Additional file 2: Figure S1). We chose two H3.3K27R clones (Mut18 and Mut43) with similar H3.3 protein levels to those in wild-type (WT) ESCs for further investigations (Additional file 2: Figure S2a). The colony morphology and expression levels of key pluripotent genes were unchanged in H3.3K27R mutant cells compared with WT cells (Additional file 2: Figure S2b, S2c). We first measured the global H3K27ac level by Western blot analysis and observed a moderately reduced bulk H3K27ac level in mutant cells.
Fig. 1 (See legend on next page.)
declined at bivalent regions (Additional file 2: Figure S5a), which could be attributed to the incorporation of H3.3. On the other hand, the genome-wide distribution of H3K27me3 was mostly unchanged in response to the H3.3K27R mutation (Additional file 2: Figure S5b). We also specifically looked at the enhancers of important pluripotent genes, such as Pou5f1 (encoding Oct4) and Nanog, and observed a substantial reduction in H3K27ac (Fig. 1f).

To evaluate the functional impact of the H3.3K27R mutation on transcription, we performed mRNA-seq in WT and the two mutant lines in biological duplicates. Despite the global reduction in H3K27ac in H3.3K27R mutant ESCs, the transcriptome change was minimal; in fact, the $r$ value was 0.98 between the WT and mutant cells (Fig. 2a).

We then divided all enhancers into active and poised groups according to the H3K27ac signal in WT cells. Strikingly, H3K27ac was almost completely depleted at active enhancers in H3.3K27R mutant ESCs (Fig. 1d). These results suggest that the vast majority of H3K27ac at active enhancers is on H3.3 and not on H3.1 or H3.2. This is consistent with high nucleosome turnover [13, 14] and high H3.3 occupancy ([27], Additional file 2: Figure S4) at enhancers. In contrast, H3K27ac signals around gene transcription start sites (TSSs) were moderately decreased in the mutants (Fig. 1e). H3.3 has been reported to promote the establishment of H3K27me3 at the bivalent promoters of developmentally regulated genes [28]. We mapped the distribution of H3K27me3 by ChIP-seq in WT and H3.3K27R mutant ESC lines and observed that H3K27me3 signals were moderately declined at bivalent regions (Additional file 2: Figure S5a), which could be attributed to the incorporation of the nonmodifiable H3.3K27R mutant histone. On the
Fig. 2 (See legend on next page.)
H3K27ac is catalyzed by cyclic AMP response charges on histone tails, attenuate the interaction between Figure S10). Lysine acetylation events neutralize positive ESCs (Fig. 2f, g), suggesting that enhancer identities in (ATAC-seq) revealed that the open state of enhancers and accessible chromatin with high-throughput sequencing accessibility as determined by the amount of transposase-mutant ESCs (Additional file 2: Figure S9). Multiple lysine mouse ESCs are well maintained. Similarly, H3K4me3 was mildly affected at enhancers and TSSs (Additional file 2: Figure S8). Chromatin accessibility as determined by the amount of transposase-accessible chromatin with high-throughput sequencing (ATAC-seq) revealed that the open state of enhancers and TSSs remained largely unchanged in H3.3K27R mutant ESCs (Fig. 2f, g), suggesting that enhancer identities in mouse ESCs are well maintained. Similarly, H3K4me3 was well maintained at H3K27ac-occupied TSSs in H3.3K27R mutant ESCs (Additional file 2: Figure S9). Multiple lysine residues can be acetylated, and these acetylation events, such as H3K9ac, H3K18ac, H3K23ac, H3K122ac, and histone H4 acetylation, tend to be coenriched. ChIP-seq profiling of H3K9ac, H3K18ac, H3K122ac, H4K5ac, and H4K8ac in WT and H3.3K27R mutant ESCs showed that these acetylation events were generally unchanged or only mildly affected at enhancers and TSSs (Additional file 2: Figure S10). Lysine acetylation events neutralize positive charges on histone tails, attenuate the interaction between histone tails and DNA, and lead to chromatin opening [31]. H3K27ac is catalyzed by cyclic AMP response element-binding protein (CBP) and its paralogous protein P300, which also catalyze acetylation at many other histone lysine residues [32–34]. Therefore, a synergistic action by multiple acetylation events at these different residues is much more crucial for transcription than acetylation at a solo site, such as H3K27. Consistently, RNA Pol II occupancy was unaltered around the TSSs (Fig. 2h, i), reflecting globally undisturbed transcription initiation.

Among multiple histone lysine acetylation sites, H3K27ac is specifically utilized as a marker for active enhancers. Using chromatin accessibility as a proxy for promoter and enhancer activity, we analyzed the correlations between each individual acetylation ChIP-seq and ATAC-seq signals at promoters and enhancers. Pearson correlation revealed that indeed H3K27ac is the best marker associated with active enhancer, but acetylation events at other lysine residues also display positive correlations with enhancer accessibility (Additional file 2: Figure S11).

**Conclusions**

Our results clearly indicate that the depletion of H3K27ac does not affect enhancer activity in mouse ESCs, whether it may have a stronger impact in other cells types such as somatic cells and cancer cells can be addressed using the same approach in future.

H3K27ac is and remains to be the best marker for enhancer indication. However, the depletion of H3K27ac at enhancer regions does not affect chromatin accessibility, gene transcription, and self-renewal of mouse ESCs. Therefore, maintenance of enhancer activity does not solely depend on H3K27ac; instead, H3K27ac has to work in concert with acetylation events on other histone lysine residues.

**Methods**

**Cell culture and editing**

J1 mouse ES cells [35] were cultured in standard ES medium in the presence of feeder cells. Prior to harvesting, ESCs were cultured under the feeder-free condition for two passages to remove feeder cells. Gene editing was performed with CRISPR-Cas9 gene editing technology. Each gene was targeted by two sgRNAs: H3f3a: 5′-GCTTAATTAGCGCTCGACAC-3′, 5′-GCTTAATTAGCGCTCGACAC-3′; H3f3b: 5′-TGG TGCCAGACGCTGCCGA-3′, 5′-TGG TGCCAGACGCTGCCGA-3′. Detailed strategies are presented in Additional file 1.

**ChIP-seq**

ChIP experiments were performed as described previously [36] with minor modifications. In brief, cells were
cross-linked with 1% formaldehyde for 9 min at room temperature. For RNA Pol II ChIP, cells were pre-cross-linked with 0.2 mM disuccinimidyl glutarate (DSG; ProteoChem, c1104) for 30 min at room temperature. Chromatin shearing was assisted with additional 0.1% sodium deoxycholate (0.5% for dual cross-linked samples). Adequate antibodies as indicated in the manufacturers’ instructions were used for precipitations. Library construction for deep sequencing was performed with KAPA Hyper Prep Kit (KAPA, KK8504) according to the manufacturers’ instructions.

**Antibodies**

Antibodies against histone H3 (Abcam, ab1791), H3.3 (Millipore, 09-838), H3K4me1 (Active Motif, 39297), H3K4me3 (Millipore, 07-473), H3K9ac (Abcam, ab4441), H3K18ac (Active Motif, 39755), H3K27ac (Active Motif, 39133), H3K27me3 (Cell Signaling, 9733S), H3K22ac (Abcam, ab33309), histone H4 (ABclonal, A1131), H4K5ac (Active Motif, 39699), H4K8ac (Active Motif, 61103), and RNA Pol II (Active Motif, 39097) were obtained commercially.

**Sequencing and bioinformatic analysis**

ChIP-seq and ATAC-seq libraries were sequenced (PE150) with Illumina NovaSeq 6000 (see Additional file 3: Table S1). mRNA-seq libraries were sequenced with the MGIDEQ-2000 (PE150) (see Additional file 3: Table S3). Mouse genome sequences (mm10) and *Drosophila* genome sequences (dm6) were concatenated to be used as the reference genome. H3.3 ChIP-seq data were downloaded from GEO database under the accession number GSE117035 [27]. Please see Additional file 1 for the detailed data analysis strategies.

**Supplementary information**

Supplementary information accompanies this paper at https://doi.org/10.1186/s13059-020-01957-w.

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**Additional file 1.** Methods and supplementary references. (PDF 121 kb)

**Additional file 2.** *Figure S1.* Strategy for CRISPR-Cas9-mediated gene editing at H3f3a and H3f3b loci. *Figure S2.* H3.3K27R mutation does not affect the H3.3 protein level and pluripotent state of ESCs. *Figure S3.* Genome-wide decrease in H3K27ac levels in individual H3.3K27R mutant mouse ESC lines. *Figure S4.* Averaged profiles of H3.3 at active enhancers and H3K27-occupied promoters in mouse ESCs. *Figure S5.* H3.3K27R mutation does not affect the genome-wide distribution of H3K27me3 in mouse ESCs. *Figure S6.* Heatmap clustering of differentially expressed genes in WT and H3.3K27R mutant mouse ESC lines. *Figure S7.* H3.3K27R mutation does not affect the transcription of genes associated with super enhancers in mouse ESCs. *Figure S8.* H3K4me1 distribution at enhancers and H3K27ac-occupied promoters in WT and H3.3K27R mutant ESC lines. *Figure S9.* H3K4me3 distribution at H3K27ac-occupied promoters in WT and H3.3K27R mutant ESC lines. *Figure S10.* Averaged profiles of H3K9ac, H3K18ac, H3K22ac, H4K5ac, and H4K8ac at enhancers and H3K27ac-occupied promoters in WT and H3.3K27R mutant ESC lines. *Figure S11.* Barplots show Pearson correlation coefficients between ChIP-seq signal of histone acetylation and chromatin accessibility (ATAC-seq signal). (PDF 2893 kb)

**Additional file 3.** *Table S1.* Sequencing depth and mapping efficiency of ChIP-seq and ATAC-seq experiments. *Table S2.* Peak numbers called in ATAC-seq and ChIP-seq experiments. *Table S3.* Stats for mRNA-seq experiments. *Table S4.* Sequences of DNA oligos used in this study. (PDF 459 kb)

**Additional file 4.** Review history.

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**Peer review information**

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**Review history**

The review history is available as Additional file 4.

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**Availability of data and materials**

The methods in detail and datasets supporting the conclusions of this report are included within the article and its additional files. All deep sequencing data reported in this paper have been submitted to the NCBI Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo) under the accession number GSE141525 [37]. All biological materials and bioinformatics analysis scripts used in this study are available from the corresponding author on reasonable request.

**Authors’ contributions**

BZ and JX conceived, designed, and supervised the project. TZ performed the majority of the experiments. ZZ performed the bioinformatics analyses. QD assisted in gene editing. TZ, JX, ZZ, and BZ wrote the paper. All authors contributed to, read, and approved the final manuscript.

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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