Received: 7 June 2019 | Accepted: 9 December 2019

DOI: 10.1002/jcb.29619

Journal of Cellular Biochemistry WILEY

RESEARCH ARTICLE

A switch in bidirectional histone mark leads to differential modulation of lincRNAs involved in neuronal and hematopoietic cell differentiation from their progenitors

Md. Wahid Murad | Md. Abdullah-Al-Kamran Khan | Md. Sajedul Islam | Abul Bashar Mir Md. Khademul Islam

Department of Genetic Engineering and Biotechnology, University of Dhaka, Dhaka, Bangladesh

Correspondence
Abul Bashar Mir Md. Khademul Islam, Department of Genetic Engineering and Biotechnology, University of Dhaka, Dhaka–1000, Bangladesh.
Email: khademul@du.ac.bd

Funding information
Biotechnology Research Centre (BRC), University of Dhaka, Bangladesh; University Grants Commission (UGC), Bangladesh

Abstract

Long intergenic noncoding RNAs (lincRNAs) are more than 200 bases long, transcribed from intergenic genomic regions and do not undergo translation. They have regulatory roles in differentiation and development. However, how their transcription is activated and how their expression is differentially modulated in differentiation is quite unclear. In this study, we explored and analyzed data at the transcriptomic and epigenetic level to address these questions. Here, we identified novel lincRNAs that are differentially expressed in neuronal and hematopoietic differentiation and showed that such differential modulations are achieved under epigenetic regulations. lincRNAs that are upregulated in mature cells than in progenitor are activated from a bivalent poised state where activating H3K4me3/H3K9ac/H3K27ac and suppressive H3K9me3/H3K27me3 marks are colocalized. And, lincRNAs that are downregulated in mature cells after differentiation are suppressed by the addition of H3K9me3/H3K27me3 marks. Moreover, here we show a tissue-specific expression pattern of lincRNAs in various cell lines and normal tissues. The study reveals bidirectional histone marks as an epigenetic means of directing the differential expression of lincRNAs which are found to be involved in the process of cellular differentiation.

KEYWORDS
differentiation, epigenetics, histone-modification, lincRNA, RNA-Seq

1 | INTRODUCTION

Once the non-genic part of the human genome was thought to be "Junk DNA". This noncoding part of the genome can be attributed to a variety of regulatory functions. Almost 80% of the genome has been assigned to have biochemical functions, particularly, regions outside the well-studied coding regions.[1] An incredibly significant amount of

Abbreviations: CD, cluster of differentiation; ChIP, chromatin immunoprecipitation; ENCODE, encyclopedia of DNA elements; FPKM, fragments per kilobase of transcript per million mapped reads; H4K20me1, histone 4 lysine 20 monomethylation; H3K27ac, histone 3 lysine 27 acetylation; H3K27me3, histone 3 lysine 27 trimethylation; H3K4me1, histone 3 lysine 4 monomethylation; H3K4me3, histone 3 lysine 4 trimethylation; H3K9ac, histone 3 lysine 9 acetylation; H3K9me3, histone 3 lysine 9 trimethylation; HPC, hematopoietic progenitor cell; lincRNA, long intergenic noncoding RNA; lncRNA, long noncoding RNA; NPC, neural progenitor cell; RPKM, reads per kilobase of transcript per million mapped reads; TSS, transcription start site.

functional noncoding RNAs are discovered to be transcribed from the intergenic regions in the genome.[2] Intergenic noncoding transcripts sized larger than 200 nucleotides are termed "long intergenic noncoding RNAs (lincRNAs)".[3] Lengths of the lincRNAs can be of several hundred bases or even larger up to tens of thousands bases.[4] They are more evolutionarily diverse than messenger RNAs (mRNAs). They are more tissue specific than coding genes and are not as efficiently spliced as mRNAs. They show similar stability as mRNAs.[5,6] LincRNAs are transcriptionally activated and processed similarly to mRNAs. Upon their transcription by RNA polymerase II, then they are 5′ capped and 3′ polyadenylated. Similar to mRNAs, actively transcribed lincRNAs' promoter has abundant histone 3 lysine 4 trimethylation (H3K4me3) and its transcribed region has trimethylation of H3K36, which is a mark of transcription by RNA polymerase II. Discovering novel lincRNAs are possible by identifying this distinctive K4-K36 domain.[5] Human transcriptome consists of about 91 013 expressed genes. Over 68% (58 648) of them are long noncoding RNAs (lncRNAs) and 72% lncRNAs are intergenic. So, out of 42 000 human lincRNAs, only about 9000 are annotated. Seventy nine percent (79%) of the lincRNAs are still unannotated.[7] LincRNAs are known to perform diverse functions, for example, regulation of gene expression, regulation of epigenetic modification, signaling in developmental cues, signaling in DNA damage response, regulation of pluripotency, signaling transcriptional activity with spatial and temporal specificity, guiding localization of ribonucleoprotein complexes, regulation of differentiation and so on.[8-10] However, specific functional role and mode of action of only about 1% of these lincRNAs are known. Functions of most of them are yet to be characterized.[4]

Differential modulation of lincRNAs have been observed in various cellular differentiation processes and specific roles of some lincRNAs are discovered in developmental processes.[11] LincRNAs can function to maintain stem cell renewal in a repression of repressor manner by acting as miRNA sponge. For example, lincRNA Regulator of Reprogramming (RoR) prevents repression of some core transcription factors like—Oct4, Sox2, Nanog, by inhibiting miRNA miR-145, thereby, keeping self-renewal genes active.[12] Metastasis associated lung adenocarcinoma transcript 1 (MALAT1) plays significant roles in myogenic differentiations,[13] nuclear paraspeckle assembly transcript 1 (NEAT1) was found to be crucial in myeloid differentiation in acute leukemia,[14] antisense RNA of sperm acrosome membrane-associated 6 (SPACA6-AS) plays role in embryonic differentiations.[15] Differentially modulated lincRNAs are found to be associated with the divergence of T cell and B cell lineages,[16] in neurogenesis,[17] osteogenic differentiation,[18] chondrogenic differentiation,[19] and muscle differentiation.[20] They are also involved in embryonic development,[21] brain development,[22] and cardiac development.[23] These diverse regulatory lincRNAs show cell-specific manner of expression, where epigenetic regulations mark their cell specificity. Differences in the density of epigenetic footprints, like histone 3 lysine 4 monomethylation (H3K4me1) and H3K27me3, near the transcription start site (TSS) of the lincRNAs, are the major role players here.[24] However, epigenetic regulations behind the differential modulation of lincRNAs in the course of differentiation to mature cells from their progenitors are yet to be elucidated.

In this study, first, the expression pattern of lincRNAs in different cell lines and normal tissues was studied using RNA-Seq data to get a global overview of their expression. Then, lincRNAs that are differentially expressed in neuronal and hematopoietic differentiation were identified. Finally, the roles of epigenetic means in this differential modulation were sought by analyzing chromatin immunoprecipitation (ChIP)-Seq data of histone epigenetic marks. We hypothesized that differentially modulated lincRNAs that act in diverse epigenetic regulations in neuronal and hematopoietic differentiations are, themselves, epigenetically regulated by bivalent histone marks.

2 | MATERIALS AND METHODS

2.1 | RNA-Seq transcriptome profiling data and ChIP-Seq data analysis

Normalized RNA-Seq transcriptome profiling data in fragments per kilobase of transcript per million mapped reads (FPKM) was obtained for 30 different cell lines, each with a replicate, through ENCODE experiment matrix (https://genome.ucsc.edu/ENCODE/dataMatrix/encodeDataMatrixHuman.html).[25,26] All the data were processed to form one continuous data matrix. Similarly, normalized RNA-Seq transcriptome profiling data in reads per kilobase of transcript per million mapped reads (RPKM) were obtained for 39 different types of normal human tissues from ENCODE data portal (https://www.encodeproject.org) and was combined in a continuous data matrix. In these matrices, the genes were filtered using list of lincRNAs from Ensembl biomart version 84 (GRCh38.p5).

For neuronal differentiation, RNA-Seq transcription profiling data and ChIP-Seq histone modification data of H3K4me1, H3K4me3, H3K9ac, H3K9me3, H4K20me1, H3K27ac, and H3K27me3 were obtained from ENCODE data portal for *Homo sapiens* neural progenitor cell (NPC) derived from H9 (Table S1) and *Homo sapiens* bipolar spindle neuron derived from induced pluripotent stem cell (Table S1). For hematopoietic differentiation, RNA-Seq transcription profiling data and ChIP-Seq histone

modification data were obtained from ENCODE data portal (Table S1) for cluster of differentiation 34+ (CD34+) mobilized hematopoietic progenitor cells (HPCs), CD20+ B cells and CD14+ monocytes.

2.2 | Expression landscape study

The expression data matrix of cell lines was processed using Genesis (version 1.7.7).[27] At first, the lincRNA genes were sorted by descending average FPKM values and top 200 highly expressed lincRNA genes were selected. Then, the cell lines were clustered using Manhattan distance and complete linkage. Similarly, the expression data matrix of normal tissues was processed in Genesis (version 1.7.7). Upon sorting the lincRNA genes by descending average RPKM values and selecting top 500 highly expressed lincRNA genes, the tissues were clustered by Pearson correlation distance and complete linkage.

2.3 | Differential expression analysis of lincRNAs

FPKM values of lincRNAs of both the progenitor and differentiated cells were taken in a continuous data matrix for each differentiation pair. The mean FPKM value of each gene in all samples was then subtracted from each FPKM value. The lincRNAs in the mean centered matrix were clustered in Cluster 3.0[28] and the differentially expressed lincRNAs were selected manually by an approximate cutoff difference 3.0 from mean for neuronal cells and 1.0 for hematopoietic cells using Gitools 1.8.4.[29]

2.4 | Histone density profile analysis from ChIP-Seq data

ChIP-Seq data of both progenitor and differentiated cells were analyzed to determine the average density of histone modification marks by read depths for each differentially modulated set of lincRNAs on 5000 base pair (bp) at both sides of the TSS by using in-house R scripts.

2.5 | Functional annotation

For determining what sort of molecular interactions and functions the differentially modulated lincRNAs are involved in, previously known functions of some of the differentially expressed lincRNAs were retrieved from the LncRNA and Disease Database (LncRNADisease).[30]

3 | RESULTS

3.1 | lincRNAs show tissue-specific expression pattern

To understand which lincRNAs are expressed globally in various tissue types and which are more restricted to individual tissue types, normalized gene expression (FPKM/RPKM) data on normal cells as well as cell lines from ENCODE data portal were plotted in a heatmap. The landscape of expression of lincRNAs generated for 30 different cell lines showed that few lincRNAs are constitutively expressed in all cell lines (Figure 1A). MALAT1 and NEAT1 are two of the most expressed lincRNAs found in all the cell lines with an average FPKM of 178.65 and 25.36, respectively (Table S2). Whereas most of the lincRNAs showed similar expression patterns in cell lines from similar organs or tissues. Like, lincRNA LINC00704 showed comparatively higher expression in cell lines that are derived from blood vessel. This strongly suggests the tissue-specific expression pattern of lincRNAs. Similarly, as for the cell lines, a landscape of lincRNA expression was prepared to get a global expression pattern of lincRNAs in 39 normal tissues or organs. Here also, very few lincRNAs are found to express commonly in all types of normal tissues (Figure 1B). SPACA6P-AS is the most expressed lincRNA found in all the normal tissues with an average RPKM of 315.02 (Table S3). MALAT1 and NEAT1 are also expressed in all the normal tissues with an average RPKM of 8.73 and 2.71, respectively. Yet, most of the lincRNAs showed tissue-specific high expression. For example, lincRNA CTC-523E23.6 showed the expression in only testis but not in other organs and lincRNA RP11-545M17.1 is more expressed in pancreas compared with other organs. These two heatmaps strongly suggest a tissue-specific manner of lincRNA expression.

3.2 | LincRNAs are differentially expressed in differentiated neurons

LincRNAs are implicated in the processes of cellular differentiation. In neuronal differentiation, eighty-one (81) lincRNAs have been identified that showed differential expression (DE) in differentiated bipolar spindle neurons compared with NPC. Among them, 47 lincRNAs are overexpressed (Figure 2A) and 34 lincRNAs are underexpressed in neurons than in NPCs (Figure 2B).

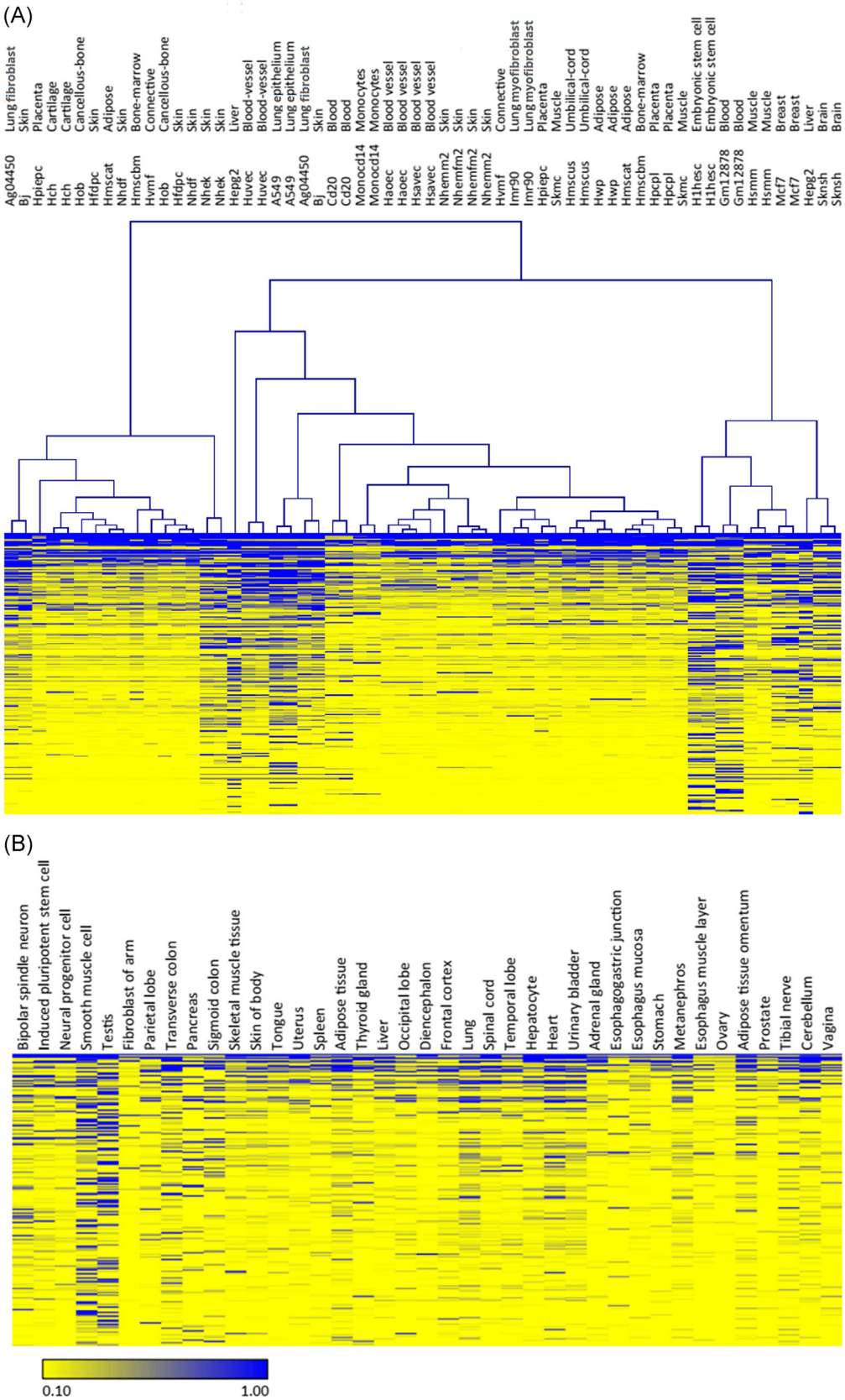

FIGURE 1 Landscape of lincRNA expression. A, lincRNA expression in 30 different cell lines, each with a replicate is shown. Only the average FPKM values of the top 200 highly expressing lincRNAs are shown. B, lincRNA expression in 39 different normal tissues or organs is shown. The average RPKM values of the top 500 highly expressing lincRNAs are shown. Color towards blue indicates a high level of expression, whereas color towards yellow indicates a low level of expression in an FPKM/RPKM scale. FPKM, fragments per kilobase of transcript per million mapped reads; lincRNA, long intergenic noncoding RNA; RPKM, reads per kilobase of transcript per million mapped reads

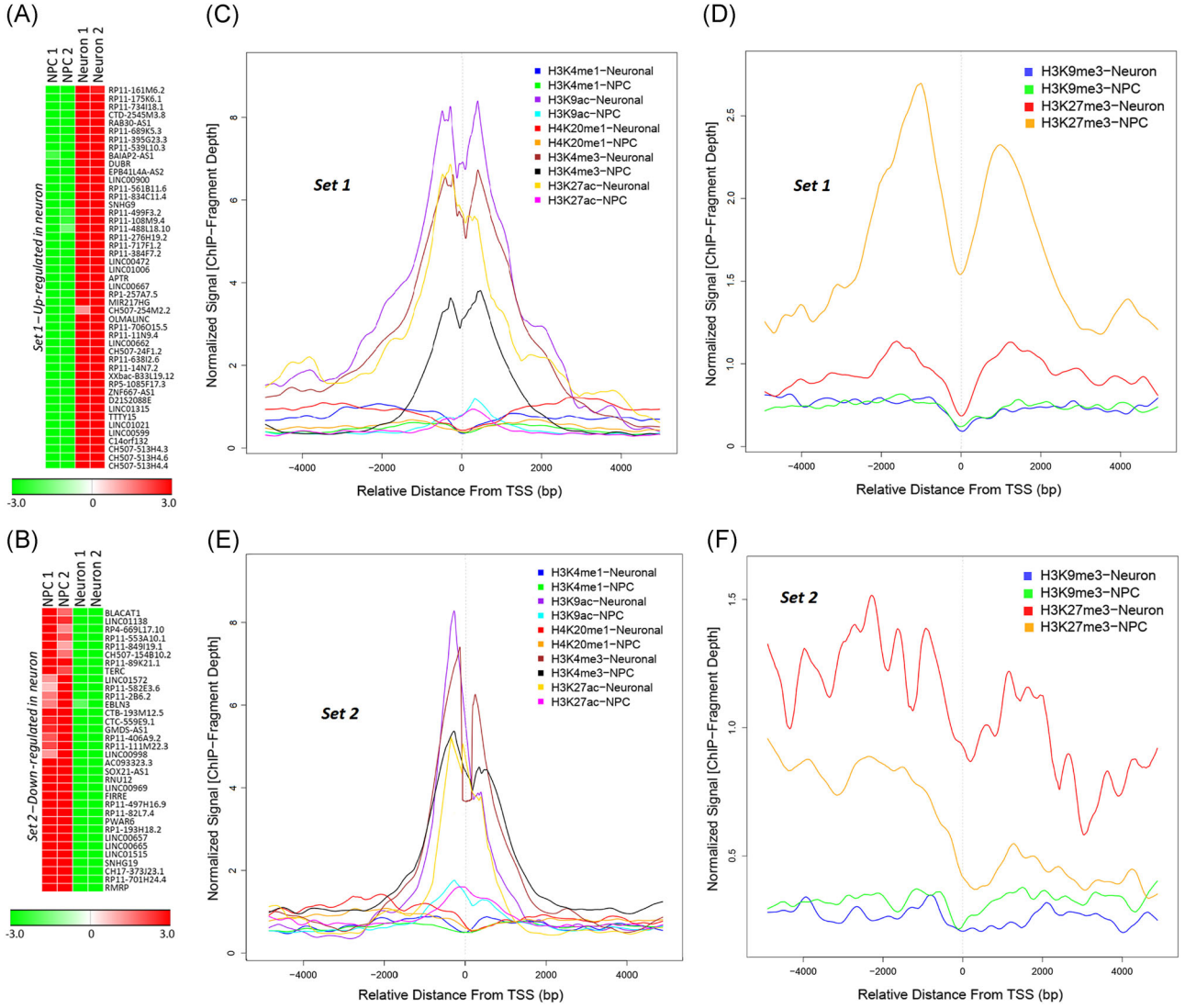

FIGURE 2 Differentially expressed lincRNAs in neuronal differentiation and their corresponding histone profiles. A, Heatmap showing 47 lincRNAs that are upregulated in neurons compared with NPCs (B) and 34 lincRNAs that are downregulated in neurons than in NPCs in a color coded scale. The heatmaps of lincRNAs show mean centered FPKM expression. Color towards green indicates low-expression and color towards red indicates higher-expression of lincRNAs. Average density profile of (C) activating histone modification marks, and (D) suppressive histone modification marks are shown on 5000 bp region at both sides of the TSS of lincRNAs that are upregulated in neurons than in NPCs. Again, average density profile of (E) activating histone modification marks, and (F) suppressive histone modification marks are shown on 5000 bp region at both sides of TSS of lincRNAs that are downregulated in neurons than in NPCs. (Curves are smoothened using loess regression with 5% smoothing span). bp, base pair; FPKM, fragments per kilobase of transcript per million mapped reads; lincRNA, long intergenic noncoding RNA; NPC, neural progenitor cell; TSS, transcription start site

3.3 | Differential expression of lincRNAs in neuronal differentiation is associated with changes in histone marks

As we find some lincRNAs are differentially expressed in neuronal differentiation, we sought if histone plays role in the regulation of the DE. We have generated histone mark profiles for regions around the TSS of the DE lincRNAs in neurons and NPCs. Average histone modification mark density profiles showed that, for the lincRNAs that are upregulated in neurons; the average density of activation marks, H3K9ac, H3K27ac, and H3K4me3, has increased and the average density of suppression mark, H3K27me3, has decresed in neuron than in progenitor (Figure 2C,D). Whereas, for downregulated lincRNAs in neurons, average density of H3K27me3 has increased in neurons than in NPCs when at the same time activation marks were also present in the downregulated lincRNAs (Figure 2E,F). The presence of higher suppressive marks in progenitor in the upregulated lincRNAs suggests that they are activated from a bivalently marked epigenetically poised state by environmental cues. Again, higher suppressive marks in downregulated lincRNAs

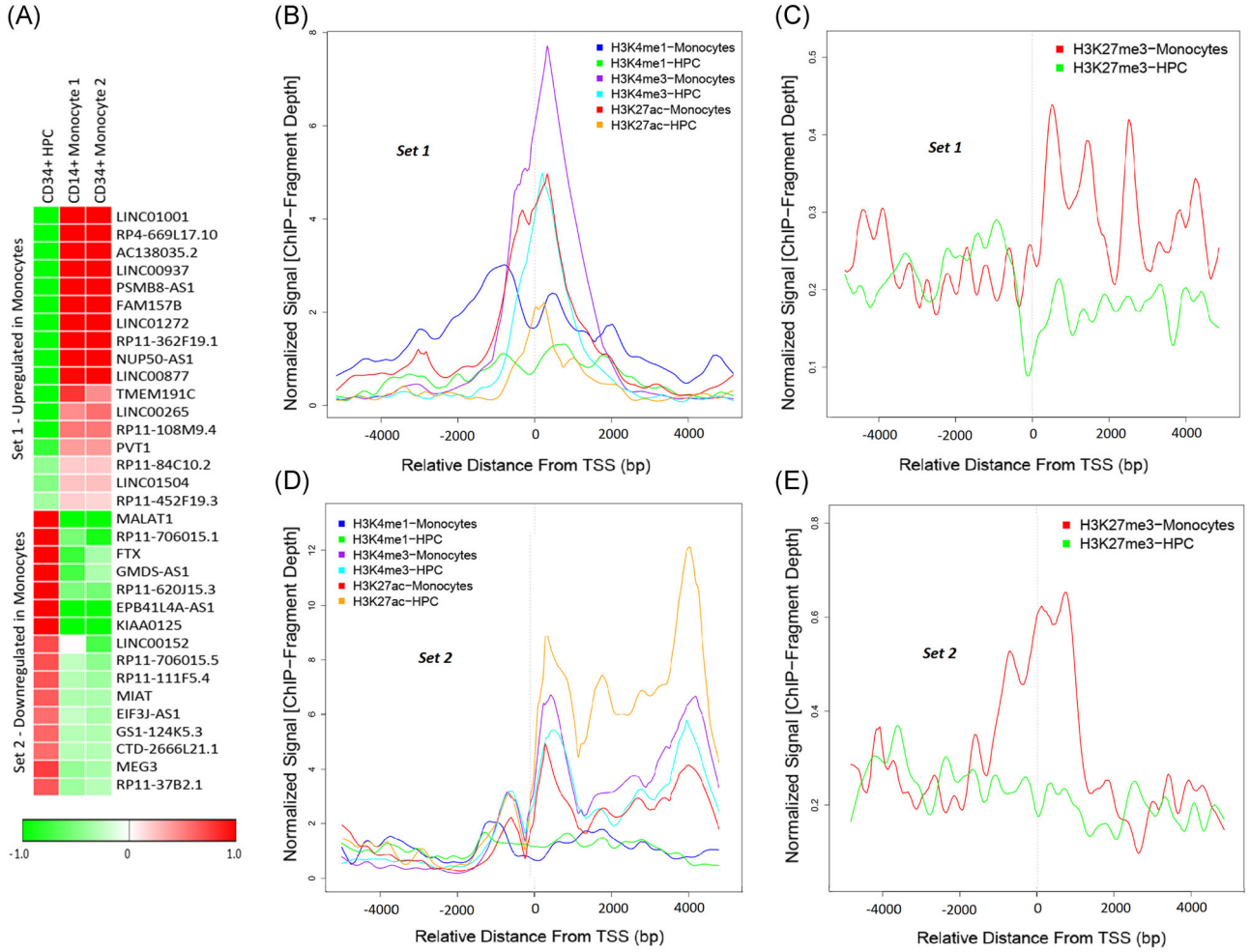

FIGURE 3 Differentially expressed lincRNAs in CD14+ monocyte differentiation and their corresponding histone profiles. A, Heatmap showing 33 lincRNAs that are differentially expressed in CD14+ monocytes than in CD34+ HPC in a color coded scale. Set 1 shows 17 upregulated lincRNAs and set 2 shows 16 downregulated lincRNAs in monocytes. The heatmap of lincRNAs shows mean centered FPKM expression. Color towards green indicates lower expression and color towards red indicates higher expression of lincRNAs. Average density profile of (B) activating histone modification marks, and (C) suppressive histone modification marks are shown on 5000 bp region at both sides of TSS of lincRNAs that are upregulated in CD14+ monocytes than in HPC. Again, average density profile of (D) activating histone modification marks, (E) suppressive histone modification marks are shown on 5000 bp region at both sides of TSS of lincRNAs that are downregulated in CD14+ monocytes than in HPC. (Curves are smoothened using loess regression with 5% smoothing span). bp, base pair; FPKM, fragments per kilobase of transcript per million mapped reads; HPC, hematopoietic progenitor cell; lincRNA, long intergenic noncoding RNA; TSS, transcription start site

after differentiation suggests that similar extrinsic or intrinsic environmental signals may have epigenetically silenced these genes. We observed no remarkable change in the average density of H3K4me1, H4K20me1, and H3K9me3 marks in neuron than in neural progenitor.

3.4 | LincRNAs are differentially modulated in hematopoietic differentiation

Now, cells of hematopoietic lineage were studied to check if their differentiation accompanied any epigenetic controls similar to neuronal differentiation. For this purpose, differentiation of CD14+ monocytes and CD20+ B cells from CD34+ mobilized HPC were individually studied. For the CD14+ monocytes, 33 differentially expressed lincRNAs have been identified (Figure 3A). Comparing CD14+ monocytes to CD34+ HPCs, among the 33 identified DE lincRNAs, 17 are upregulated and 16 are downregulated. For the CD20+ B cells, 44 differentially expressed lincRNAs have been identified (Figure 4A). Among them, 25 are upregulated and 19 are downregulated in CD20+ cells compared with the CD34+ HPCs. Among the differentially expressed lincRNAs in these two hematopoietic

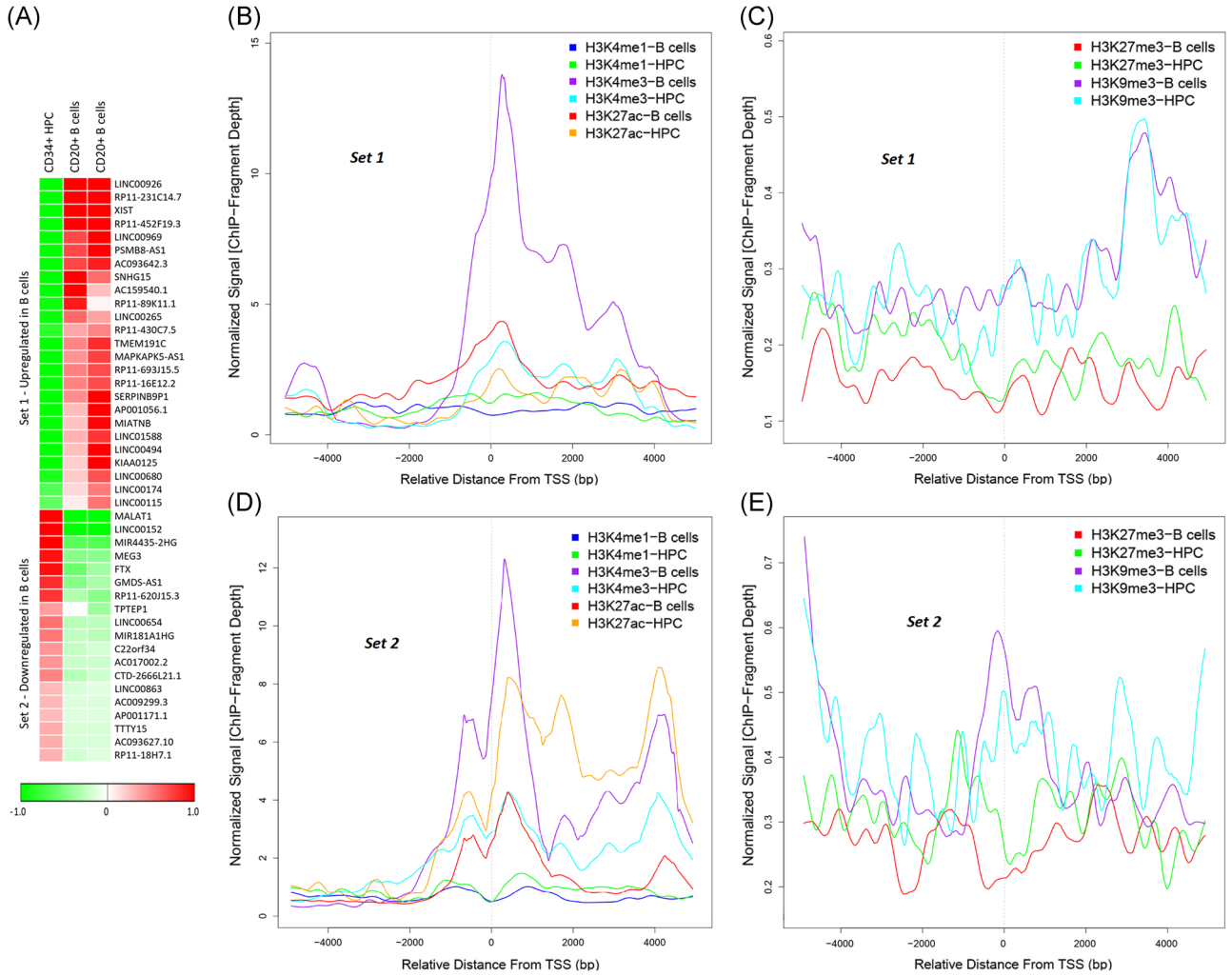

FIGURE 4 Differentially expressed lincRNAs in CD20+ B cell differentiation and their corresponding histone profiles. A, Heatmap showing 44 lincRNAs that are differentially expressed in CD20+ B cells than in CD34+ HPC in a color coded scale. Set 1 shows 25 upregulated lincRNAs and set 2 shows 19 downregulated lincRNAs in B cells. The heatmap of lincRNAs shows mean centered FPKM expression. Color towards green indicates low expression and color towards red indicates higher expression of lincRNAs. Average density profile of (B) activating histone modification marks, (C) suppressive histone modification marks are shown on 5000 bp region at both sides of TSS of lincRNAs that are upregulated in CD20+ B cells than in HPC. Again, average density profile of (D) activating histone modification marks, and (E) suppressive histone modification marks are shown on 5000 bp region at both sides of TSS of lincRNAs that are downregulated in CD20+ B cells than in HPC. (Curves are smoothened using loess regression with 5% smoothing span). bp, base pair; FPKM, fragments per kilobase of transcript per million mapped reads; HPC, hematopoietic progenitor cell; lincRNA, long intergenic noncoding RNA; TSS, transcription start site

differentiations, CD14+ and CD20+ cells have four common upregulated (Figure 5A) and seven common downregulated (Figure 5B) lincRNAs. A comparative heatmap of expression for the differentially expressed lincRNAs among CD34+, CD14+, and CD20+ cells is shown in Figure 5C. These findings show that similarly to neuronal differentiation, lincRNAs are also differentially modulated in hematopoietic differentiation indicating a general role of lincRNAs in cellular differentiation.

3.5 | Differential modulation of lincRNAs in hematopoietic differentiation are also epigenetically regulated

Average histone mark density profiles were produced for the differentially expressed lincRNAs in hematopoietic differentiation to check if they show similar epigenetic regulation by histones in their differential modulation like in neuronal differentiation. Density profiles were calculated around the TSS of DE lincRNAs. H3K4me3 patterns were different in

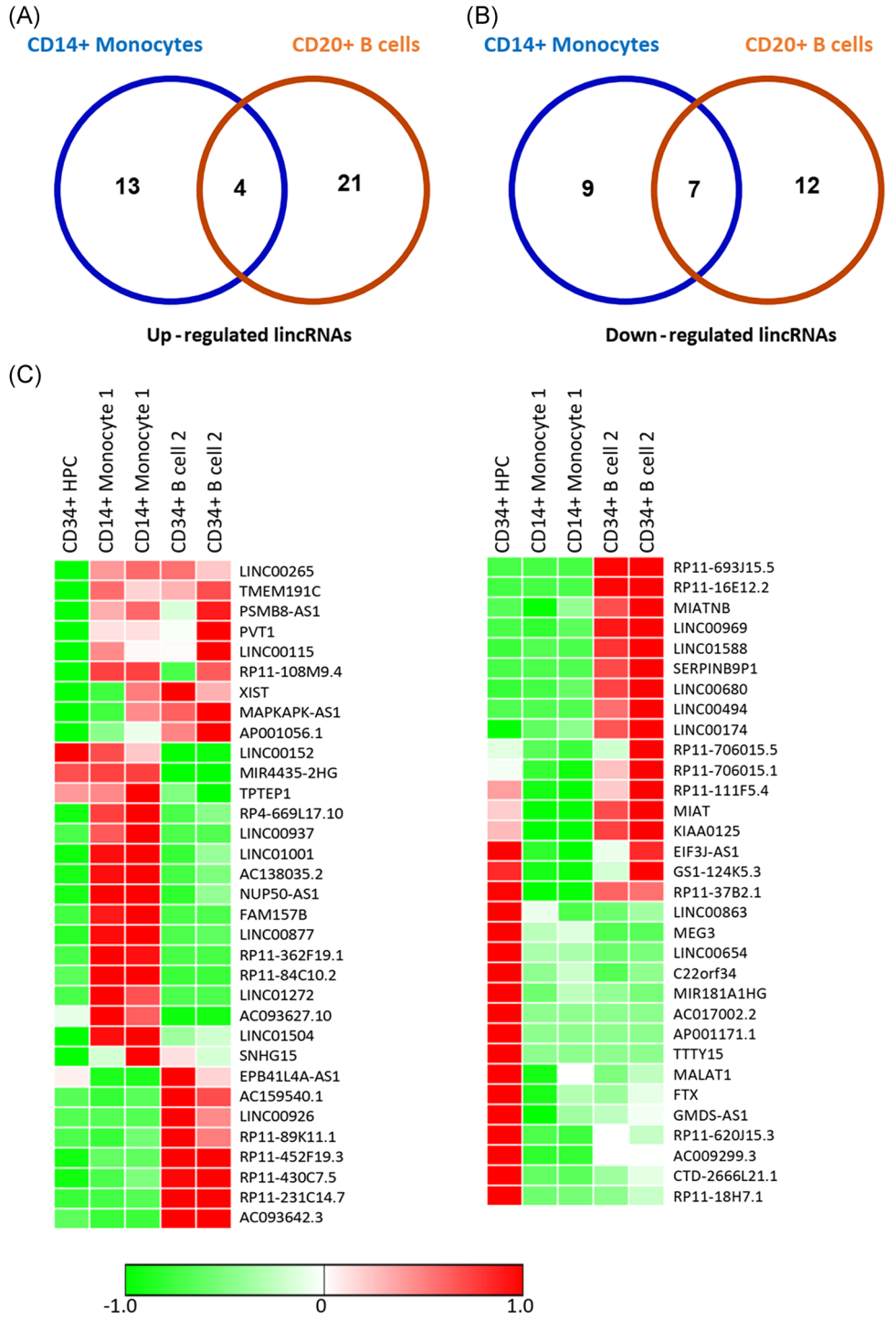

FIGURE 5 Comparison of differential expression of lincRNAs in CD14+ monocyte and CD20+ B cell differentiation. A, Venn diagram showing CD14+ and CD20+ cells have four common upregulated lincRNAs, and (B) seven common downregulated lincRNAs compared with CD34+ HPCs. C, Comparative heatmap of differentially expressed lincRNAs in CD34+ HPCs, CD14+ monocytes, and CD20+ B cells in a color coded scale. Expression of lincRNAs is shown in mean centered FPKM values. Color towards green indicates low expression and color towards red indicates higher expression of lincRNAs. FPKM, fragments per kilobase of transcript per million mapped reads; HPC, hematopoietic progenitor cell; lincRNA, long intergenic noncoding RNA

upregulated and downregulated lincRNAs in both the CD14+ (Figures 3B,D) and CD20+ cells (Figures 4B,D). Activating H3K4me1, H3K4me3, and H3K27ac were relatively higher in CD14+ monocytes and lower in the CD34+ HPCs for the upregulated set of lincRNAs in monocytes (Figure 3B); while the suppression marks H3K27me3 was in higher level in CD14+ monocytes for the downregulated lincRNAs in monocytes (Figure 3E). Also, activation histone

TABLE 1 Functions of differentially expressed lincRNAs in hematopoietic differentiation

LincRNA	Interaction partner	Level of interactions	Type of interactions	Reference
MALAT1	CREB	DNA-TF	Regulatory	Koshimizu et al[31]
MALAT1	IBP160, SRm160, RNPS1	RNA-protein	Regulatory	Miyagawa et al[32]
MALAT1	PSF, p54nrb	RNA-protein	Binding	Nakagawa et al[33]
MALAT1	Pc2	DNA-protein	Regulatory	Yang et al[34]
MALAT1	SR	RNA-protein	Binding	Tripathi et al[35]
MALAT1	SR protein family	RNA-protein	Regulatory	Anko and Neugebauer[36]
MALAT1	SRSF1	RNA-protein	Regulatory	Anko and Neugebauer[36]
MEG3	cAMP	DNA-TF	Regulatory	Zhao et al[37]
MEG3	GDF15	RNA-DNA	Regulatory	Zhou et al[38]
MEG3	p53	RNA-DNA	Regulatory	Clark and Mattick[39]
MEG3	p53	RNA-protein	Binding	Zhou et al[38]
MEG3	p53	RNA-protein	Regulatory	Lipovich et al[40]
MEG3	p53	RNA-RNA	Co-expression	Zhang et al[41]
MEG3	Tp53	RNA-DNA	Regulatory	Liao et al[42]
MIAT	IRES-GFP	DNA-DNA	Regulatory	Rapicavoli et al[43]
PVT1	c-Myc	DNA-TF	Regulatory	Carramusa et al[44]
PVT1	c-Myc	RNA-DNA	Regulatory	Carramusa et al[44]
PVT1	P53	DNA-TF	Regulatory	Barsotti et al[45]
XIST	PRC2	RNA-protein	Binding	Khalil et al[46]
XIST	TAP/NXF1	RNA-protein	Binding	Cohen and Panning[47]

Note: Description of the interactions is provided in (Table S4).
Abbreviation: lincRNA, long intergenic noncoding RNA.

marks H3K4me3 and H3K27ac were found more represented in the B-cells compared with the CD34+ cells around the promoters of upregulated lincRNAs in B-cells (Figure 4B). Also, suppression histone mark H3K9me3 was more prominent in the B-cells for the downregulated lincRNAs in B-cells (Figure 4E). We found no change in the average density of suppression marks at TSS of the upregulated lincRNAs in both monocytes and B-cells compared with CD34+ progenitors (Figures 3C and 4C). Activation/suppression marks showed similar pattern earlier in neuronal differentiation suggesting a similar differential modulation of lincRNAs through the epigenetic regulation by histone marks during hematopoietic differentiation.

3.6 | Differentially expressed lincRNAs play important regulatory functions in hematopoietic differentiation

To understand the type of molecular interactions and functions the DE lincRNAs are involved in, interaction data of DE lincRNAs in hematopoietic differentiation have been retrieved from the LncRNADisease database (Table 1). It is found that the DE lincRNAs render regulatory functions via RNA-protein or RNA-DNA interactions in important cellular aspects, like—growth differentiation factor stimulation, RNA interference-mediated suppression, and nuclear localization etc.

4 | DISCUSSION

Long intergenic noncoding RNAs (lincRNAs) are the most abundant type of genes in the human genome.[7] These >200 bp long, non-protein-coding transcripts are associated with many regulatory functions[8-10] even though their regulatory mechanisms are still elusive. This phenomenon has led to our analysis of the landscapes of expression of lincRNAs across cell lines and normal tissues, and possible epigenetic mechanisms by which they are regulated. Their pattern of expression indicates a tissue-specific expression (Figure 1) because similar types of tissues are clustered together based on lincRNA expression profile. This finding supports the earlier report that lincRNAs are expressed in a tissue-specific manner.[5,6] Tissue-specific differential expression could assist in functional annotation of unannotated lincRNAs that show similar expression to known lincRNAs or other known genes in the same tissue.[48] And, the genes that the lincRNAs regulate or are regulated by could be detected

based on what unique genes are expressed in a specific tissue.[49]

To determine the implication of lincRNAs in cellular differentiation, differentially expressed lincRNAs were identified for neuronal and hematopoietic cells and the differentially expressing modules of lincRNAs were further studied to determine any role of epigenetic regulations, such as histone modifications that may be associated with their differential modulation (Figures 2-4). We observed interesting differences in the densities of histone marks associated with transcriptional activation, H3K4me3, H3K9ac, and H3K27ac, and marks that are repressive, H3K9me3 and H3K27me3, between progenitor cells and differentiated mature cells. In neuronal differentiation, although higher levels of activating H3K4me3/H3K9ac/H3K27ac marks and lower level of suppressive H3K27me3 mark was observed for upregulated lincRNA genes in neuron than in NPC, both activating and suppressive marks were present in the progenitor. The presence of both activating and suppressive marks in progenitor strongly suggests a bivalent poised state before differentiation, that is, a probable integration of intrinsic and extrinsic environmental and systematic signals drives them to differentiation cues from an epigenetically poised state. On the other hand, relatively more suppressive marks colocalized with activating marks in the downregulated lincRNAs in neurons than in NPC. Such histone tag pattern indicates that again a probable integration of similar signals drives them to epigenetically poised state from a highly activated state by addition of suppressive marks. In hematopoietic differentiation, H3K4me3/H3K27ac data showed relatively higher densities in both differentiated CD14+ and CD20+ cells than CD34+ progenitors for upregulated genes similar to neuron. Again, similarly to neuronal differentiation, for downregulated genes, we observed an increase in the density of H3K9me3/H3K27me3 marks in differentiated CD14+ and CD20+ cells than CD34+ progenitors. It means similar epigenetic factors may be involved in hematopoietic differentiation like neuronal differentiation. These discoveries elucidate that the regulatory lincRNAs are themselves regulated by epigenetic means and support the hypothesis of the study. Such epigenetic regulations are already established for the protein coding genes. In self-renewing, pluripotent cells, lineage control genes are known to be silenced by bivalent chromatin modification (H3K27me3) while their subsequent activation during differentiation by "poising" (H3K4me3).[50] Such bivalently poised regions are known to be co-occupied by H3K4me3 and H3K27me3 marks.[51] However, it was not clear earlier that lincRNAs are also subjected to similar epigenetic regulations. These insights may direct towards a better understanding of the complex network of interactions in the process of differentiation and establishment of novel treatment of neurological and hematopoietic diseases through the controlled proliferation of renewable progenitors.

This study has showed the tissue-specific expression of lincRNAs. Furthermore, differentially expressed lincRNAs probably playing role in differentiation, have been identified in neuronal and hematopoietic differentiations from their respective progenitors and roles of histone modifications in this differential modulation of lincRNAs have been brought in front. We have detected that the lincRNAs which are to be involved in epigenetic regulation of differentiation are themselves epigenetically regulated by histone modifications. Our findings would be helpful for further in vivo or in vitro experiments for the better understanding of the functional roles and mechanisms exerted by the lincRNAs.

ACKNOWLEDGMENTS

ABMMKI acknowledges the support from Biotechnology Research Centre (BRC), University of Dhaka, Bangladesh; University Grants Commission (UGC), Bangladesh and The World Academy of Sciences (TWAS). The project was funded by the grant from Biotechnology Research Centre (BRC), University of Dhaka, Bangladesh and University Grants Commission (UGC), Bangladesh. MWM and MSI received NST fellowships from the Ministry of Science and Technology, Bangladesh.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTION

ABMMKI conceived the project. MWM and MAAKK collected the data. MWM and MAAKK performed the analyses and interpreted the results. MWM, MAAKK, MSI, and ABMMKI wrote the manuscript. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

We analysed publicly available data. All data are available at public repositories.

ORCID

Abul Bashar Mir Md. Khademul Islam http://orcid.org/0000-0002-7274-0855

REFERENCES

1. TheEncodeProjectConsortium. An integrated encyclopedia of DNA elements in the human genome. *Nature*. 2012;489(7414): 57-74. https://doi.org/10.1038/nature11247
2. Mattick JS. The genetic signatures of noncoding RNAs. *PLoS Genet*. 2009;5(4):e1000459. https://doi.org/10.1371/journal.pgen.1000459
3. Hangauer MJ, Vaughn IW, McManus MT. Pervasive transcription of the human genome produces thousands of previously unidentified long intergenic noncoding RNAs. *PLoS Genet*. 2013; 9(6):e1003569. https://doi.org/10.1371/journal.pgen.1003569
4. Ding X, Zhu L, Ji T, et al. Long intergenic non-coding RNAs (LincRNAs) identified by RNA-seq in breast cancer. *PLoS One*. 2014;9(8):e103270. https://doi.org/10.1371/journal.pone.0103270
5. Ma L, Bajic VB, Zhang Z. On the classification of long non-coding RNAs. *RNA Biol*. 2013;10(6):924-933. https://doi.org/10.4161/rna.24604
6. Melé M, Mattioli K, Mallard W, Shechner DM, Gerhardinger C, Rinn JL. Chromatin environment, transcriptional regulation, and splicing distinguish lincRNAs and mRNAs. *Genome Res*. 2017;27(1):27-37.
7. Iyer MK, Niknafs YS, Malik R, et al. The landscape of long noncoding RNAs in the human transcriptome. *Nat Genet*. 2015; 47(3):199-208. https://doi.org/10.1038/ng.3192
8. Guttman M, Donaghey J, Carey BW, et al. lincRNAs act in the circuitry controlling pluripotency and differentiation. *Nature*. 2011;477(7364):295-300.
9. Ranzani V, Rossetti G, Panzeri I, et al. The long intergenic noncoding RNA landscape of human lymphocytes highlights the regulation of T cell differentiation by linc-MAF-4. *Nat Immunol*. 2015;16(3):318-325. https://doi.org/10.1038/ni.3093
10. Wang KC, Chang HY. Molecular mechanisms of long noncoding RNAs. *Mol Cell*. 2011;43(6):904-914. https://doi.org/10.1016/j.molcel.2011.08.018
11. Fatica A, Bozzoni I. Long non-coding RNAs: new players in cell differentiation and development. *Nat Rev Genet*. 2014;15(1):7-21. https://doi.org/10.1038/nrg3606
12. Wang Y, Xu Z, Jiang J, et al. Endogenous miRNA sponge lincRNA-RoR regulates Oct4, Nanog, and Sox2 in human embryonic stem cell self-renewal. *Dev Cell*. 2013;25(1):69-80. https://doi.org/10.1016/j.devcel.2013.03.002
13. Chen X, He L, Zhao Y, et al. Malat1 regulates myogenic differentiation and muscle regeneration through modulating MyoD transcriptional activity. *Cell Discov*. 2017;3(1):17002. https://doi.org/10.1038/celldisc.2017.2
14. Zeng C, Xu Y, Xu L, et al. Inhibition of long non-coding RNA NEAT1 impairs myeloid differentiation in acute promyelocytic leukemia cells. *BMC Cancer*. 2014;14(1):693. https://doi.org/10.1186/1471-2407-14-693
15. Tani H, Matsutani T, Aoki H, et al. Identification of RNA biomarkers for chemical safety screening in neural cells derived from mouse embryonic stem cells using RNA deep sequencing analysis. *Biochem Biophys Res Commun*. 2019;512(4):641-646. https://doi.org/10.1016/j.bbrc.2018.11.141
16. Casero D, Sandoval S, Seet CS, et al. Long non-coding RNA profiling of human lymphoid progenitor cells reveals transcriptional divergence of B cell and T cell lineages. *Nat Immunol*. 2015;16(12):1282-1291. https://doi.org/10.1038/ni.3299
17. Lin M, Pedrosa E, Shah A, et al. RNA-seq of human neurons derived from iPS cells reveals candidate long non-coding RNAs involved in neurogenesis and neuropsychiatric disorders. *PLoS One*. 2011;6(9):e23356. https://doi.org/10.1371/journal.pone.0023356
18. Wang L, Wang Y, Li Z, Li Z, Yu B. Differential expression of long noncoding ribonucleic acids during osteogenic differentiation of human bone marrow mesenchymal stem cells. *Int Orthop*. 2015; 39(5):1013-1019. https://doi.org/10.1007/s00264-015-2683-0
19. Wang L, Li Z, Li Z, Yu B, Wang Y. Long noncoding RNAs expression signatures in chondrogenic differentiation of human bone marrow mesenchymal stem cells. *Biochem Biophys Res Commun*. 2015;456(1):459-464. https://doi.org/10.1016/j.bbrc.2014.11.106
20. Neguembor MV, Jothi M, Gabellini D. Long noncoding RNAs, emerging players in muscle differentiation and disease. *Skelet Muscle*. 2014;4(1):8. https://doi.org/10.1186/2044-5040-4-8
21. Ulitsky I, Shkumatava A, Jan, Calvin H, Sive H, Bartel David P. Conserved function of lincRNAs in vertebrate embryonic development despite rapid sequence evolution. *Cell*. 2011; 147(7):1537-1550. https://doi.org/10.1016/j.cell.2011.11.055
22. Sauvageau M, Goff LA, Lodato S, et al. Multiple knockout mouse models reveal lincRNAs are required for life and brain development. *Elife*. 2013;2:e01749. https://doi.org/10.7554/eLife.01749
23. Scheuermann JC, Boyer LA. Getting to the heart of the matter: long non-coding RNAs in cardiac development and disease. *EMBO J*. 2013;32(13):1805-1816.
24. Amin V, Harris RA, Onuchic V, et al. Epigenomic footprints across 111 reference epigenomes reveal tissue-specific epigenetic regulation of lincRNAs. *Nat Commun*. 2015;6:6370. https://doi.org/10.1038/ncomms7370
25. Rosenbloom KR, Dreszer TR, Pheasant M, et al. ENCODE whole-genome data in the UCSC Genome Browser. *Nucleic Acids Res*. 2010;38(suppl 1):D620-D625.
26. Thomas DJ, Rosenbloom KR, Clawson H, et al. The ENCODE project at UC Santa Cruz. *Nucleic Acids Res*. 2007;35(suppl 1): D663-D667.
27. Sturn A, Quackenbush J, Trajanoski Z. Genesis: cluster analysis of microarray data. *Bioinformatics*. 2002;18(1):207-208.
28. de Hoon MJL, Imoto S, Nolan J, Miyano S. Open source clustering software. *Bioinformatics*. 2004;20(9):1453-1454.
29. Perez-Llamas C, Lopez-Bigas N. Gitools: analysis and visualisation of genomic data using interactive heat-maps. *PLoS One*. 2011;6(5):e19541. https://doi.org/10.1371/journal.pone.0019541
30. Chen G, Wang Z, Wang D, et al. LncRNADisease: a database for long-non-coding RNA-associated diseases. *Nucleic Acids Res*. 2013;41(D1):D983-D986.
31. Koshimizu TA, Fujiwara Y, Sakai N, Shibata K, Tsuchiya H. Oxytocin stimulates expression of a noncoding RNA tumor marker in a human neuroblastoma cell line. *Life Sci*. 2010; 86(11-12):455-460. https://doi.org/10.1016/j.lfs.2010.02.001
32. Miyagawa R, Tano K, Mizuno R, et al. Identification of cis- and trans-acting factors involved in the localization of MALAT-1 noncoding RNA to nuclear speckles. *RNA*. 2012;18(4):738-751. https://doi.org/10.1261/rna.028639.111
33. Nakagawa S, Naganuma T, Shioi G, Hirose T. Paraspeckles are subpopulation-specific nuclear bodies that are not essential in mice. *J Cell Biol*. 2011;193(1):31-39. https://doi.org/10.1083/jcb.201011110
34. Yang L, Lin C, Liu W, et al. ncRNA- and Pc2 methylation-dependent gene relocation between nuclear structures mediates

gene activation programs. *Cell*. 2011;147(4):773-788. https://doi.org/10.1016/j.cell.2011.08.054
35. Tripathi V, Ellis JD, Shen Z, et al. The nuclear-retained noncoding RNA MALAT1 regulates alternative splicing by modulating SR splicing factor phosphorylation. *Mol Cell*. 2010;39(6):925-938. https://doi.org/10.1016/j.molcel.2010.08.011
36. Anko ML, Neugebauer KM. Long noncoding RNAs add another layer to pre-mRNA splicing regulation. *Mol Cell*. 2010;39(6):833-834. https://doi.org/10.1016/j.molcel.2010.09.003
37. Zhao J, Zhang X, Zhou Y, Ansell PJ, Klibanski A. Cyclic AMP stimulates MEG3 gene expression in cells through a cAMP-response element (CRE) in the MEG3 proximal promoter region. *Int J Biochem Cell Biol*. 2006;38(10):1808-1820. https://doi.org/10.1016/j.biocel.2006.05.004
38. Zhou Y, Zhong Y, Wang Y, et al. Activation of p53 by MEG3 non-coding RNA. *J Biol Chem*. 2007;282(34):24731-24742. https://doi.org/10.1074/jbc.M702029200
39. Clark MB, Mattick JS. Long noncoding RNAs in cell biology. *Semin Cell Dev Biol*. 2011;22(4):366-376. https://doi.org/10.1016/j.semcdb.2011.01.001
40. Lipovich L, Johnson R, Lin CY. MacroRNA underdogs in a microRNA world: evolutionary, regulatory, and biomedical significance of mammalian long non-protein-coding RNA. *Biochim Biophys Acta*. 2010;1799(9):597-615. https://doi.org/10.1016/j.bbagrm.2010.10.001
41. Zhang X, Zhou Y, Klibanski A. Isolation and characterization of novel pituitary tumor related genes: a cDNA representational difference approach. *Mol Cell Endocrinol*. 2010;326(1-2):40-47. https://doi.org/10.1016/j.mce.2010.02.040
42. Liao Q, Liu C, Yuan X, et al. Large-scale prediction of long non-coding RNA functions in a coding-non-coding gene co-expression network. *Nucleic Acids Res*. 2011;39(9):3864-3878. https://doi.org/10.1093/nar/gkq1348
43. Rapicavoli NA, Poth EM, Blackshaw S. The long noncoding RNA RNCR2 directs mouse retinal cell specification. *BMC Dev Biol*. 2010;10:49. https://doi.org/10.1186/1471-213x-10-49
44. Carramusa L, Contino F, Ferro A, et al. The PVT-1 oncogene is a Myc protein target that is overexpressed in transformed cells. *J Cell Physiol*. 2007;213(2):511-518. https://doi.org/10.1002/jcp.21133
45. Barsotti AM, Beckerman R, Laptenko O, Huppi K, Caplen NJ, Prives C. p53-Dependent induction of PVT1 and miR-1204. *J Biol Chem*. 2012;287(4):2509-2519. https://doi.org/10.1074/jbc.M111.322875
46. Khalil AM, Guttman M, Huarte M, et al. Many human large intergenic noncoding RNAs associate with chromatin-modifying complexes and affect gene expression. *Proc Natl Acad Sci USA*. 2009;106(28):11667-11672. https://doi.org/10.1073/pnas.0904715106
47. Cohen HR, Panning B. XIST RNA exhibits nuclear retention and exhibits reduced association with the export factor TAP/NXF1. *Chromosoma*. 2007;116(4):373-383. https://doi.org/10.1007/s00412-007-0100-1
48. Shyamsundar R, Kim YH, Higgins JP, et al. A DNA microarray survey of gene expression in normal human tissues. *Genome Biol*. 2005;6(3):R22. https://doi.org/10.1186/gb-2005-6-3-r22
49. Zhang W, Morris QD, Chang R, et al. The functional landscape of mouse gene expression. *J Biol*. 2004;3(5):21. https://doi.org/10.1186/jbiol16
50. De Gobbi M, Garrick D, Lynch M, et al. Generation of bivalent chromatin domains during cell fate decisions. *Epigenetics Chromatin*. 2011;4(1):9. https://doi.org/10.1186/1756-8935-4-9
51. Sati S, Ghosh S, Jain V, Scaria V, Sengupta S. Genome-wide analysis reveals distinct patterns of epigenetic features in long non-coding RNA loci. *Nucleic Acids Res*. 2012;40(20):10018-10031. https://doi.org/10.1093/nar/gks776

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Murad MW, Khan MA-A-K, Islam MS, Islam ABMMK. A switch in bidirectional histone mark leads to differential modulation of lincRNAs involved in neuronal and hematopoietic cell differentiation from their progenitors. *J Cell Biochem*. 2020;121:3451–3462. https://doi.org/10.1002/jcb.29619