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Dynamic chromatin accessibility and transcriptome changes following PDGF-BB treatment of bone-marrow derived mesenchymal stem cells

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Abstract

Background Mesenchymal stem cells (MSCs) are multipotent stem cells that are under investigation for use in clinical trials because they are capable of self-renewal and differentiating into different cell types under defined conditions. Nonetheless, the therapeutic effects of MSCs have been constrained by low engraftment rates, cell fusion, and cell survival. Various strategies have been explored to improve the therapeutic efficacy of MSCs, with platelet-derived growth factor (PDGF)-BB emerging as a promising candidate. To enhance our comprehension of the impact of PDGF-BB on the gene expression profile and chromosomal accessibility of MSCs, RNA-sequencing and analysis of chromatin accessibility profiles were conducted on three human primary MSCs in culture, both with and without stimulation by PDGF-BB.

Results Integrative analysis of gene expression and chromatin accessibility demonstrated that PDGF-BB treatment modified the chromatin accessibility landscape, marking regions for activation or repression through the AP-1 family transcription factors TEAD, CEBP, and RUNX2. These changes in AP-1 transcription factor expression, in turn, led to cell proliferation and differentiation potential towards osteoblasts, adipocytes, or chondrocytes. The degree of MSC differentiation varies among cells isolated from different donors. The presence of an enrichment of exosome-related genes is also noted among all the differentially expressed genes.

Conclusions In conclusion, the observed changes in AP-1 transcription factor expression not only induced cellular proliferation and differentiation, but also revealed variations in the degree of MSC differentiation based on donor-specific differences. Moreover, the enrichment of exosome-related genes among differentially expressed genes suggests a potential significant role for PDGF-BB in facilitating intercellular communication.

Keywords Mesenchymal stem cells, Platelet-derived growth factor (PDGF)-BB, Chromatin accessibility

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Background

Mesenchymal stem cells (MSCs) are self-renewing, multipotent stem cells originating in the bone marrow stroma and other tissues. MSCs have unique differentiation potential toward various mesenchymal lineages including osteoblast, adipocyte, and chondrocyte. MSCs have been employed in cardiac repair [1, 2], in enhancing bone marrow engraftment, reducing graft-versus-host disease, in generating connective tissue [3, 4], in endocrine and nervous system diseases, and in the repair of damaged musculoskeletal tissues [5]. Adult bone marrow-derived MSCs are easily cultivated and expanded, maintaining multipotency even after extended culture periods. This feature facilitates the use of cultured MSCs in both autologous and allogeneic transplants [3].

Nonetheless, the therapeutic effects of MSCs in numerous clinical trials have been constrained by low engraftment rates, cell fusion, and cell survival. Various strategies have been assessed to boost the therapeutic efficacy of MSCs. Platelet-derived growth factor (PDGF)-BB, a potent mitogen for MSC proliferation [6], has emerged as a promising candidate. PDGF is a dimeric glycoprotein consisting of either two A chains (-AA), two B chains (-BB), or an A/B heterodimer (-AB). Two types of PDGF receptor (PDGFR) have been identified: PDGFRα and PDGFRβ, where both subtypes are capable of binding PDGF [1]. Upon activation by PDGF, the receptor subunits dimerize and subsequently activate numerous signal transduction pathways, including JNK/c-Jun, PKC, ERK, JAK/STATs, and PI3K/Akt, which play profound roles in regulating cell growth, proliferation, and differentiation [1, 3, 6–9].

Previous research has shown that PDGF-BB can enhance stem cell-based bone regeneration [10, 11], stimulate angiogenesis [11, 12], promote proliferation [6, 13], and improve MSC-mediated cardioprotection [1]. To gain a deeper understanding of how this growth factor influences the function of MSCs, we aimed to characterize the response to PDGF-BB stimulation at system-wide levels, encompassing both the transcriptome and the chromatin accessibility landscapes. Our findings provide valuable insights into the complex regulatory mechanism through which PDGF-BB influences the function of MSCs. Such findings promote potential advancements in therapeutic applications and encourage further research in the field.

Results

ATAC-seq and RNA-seq libraries were prepared from three human bone marrow-derived MSCs (BM-MSCs) treated with or without 10 ng/ml PDGF-BB for 48 h. Changes in chromatin accessibility in response to PDGF-BB were analyzed using assay for transposase accessible chromatin with sequencing (ATAC-seq) and integrated with RNA-seq data. An average of 30 million high quality RNA-seq reads were mapped to the human reference genome, assembly hg38. Open chromatin regions from each sample were combined into a merged region set, resulting in a total of 248,171 regions. Our findings showed that these regions were located within 3 million base pairs around the transcription start site (TSS) of genes.

To visualize differences between the PDGF-BB treated and non-treated MSC samples, we performed principal component analysis (PCA) using normalized counts of the open chromatin regions (Figure S1A) and of the RNA-seq reads (Figure S1B). PCA results of both ATAC-seq and RNA-seq presented the same trend for each MSC sample. In both plots, PDGF-BB-treated and control cells from the same donor clustered together, suggesting that MSC cell origin was strongly correlated with donor-specific chromatin and gene expression patterns.

PDGF-BB treatment changes chromatin accessibility in BM-MSC

Differential accessibility analysis was performed using edgeR [14, 15]. We identified a total of 37,548 differential accessibility regions (DAR) at FDR-adjusted p value < 0.05 following PDGF-BB treatment of MSCs (Fig. 1A). Among these regions, 19,973 regions display increased accessibility, while 17,575 regions display decreased accessibility. Examples of increased and decreased chromatin accessibility are shown in Fig. 1B and C.

Approximately 17% of all chromatin-accessible regions were in promoter regions of genes (defined as the region between 5 kb upstream and 1 kb downstream of the transcription start site), ~40% of all open regions were in gene body regions, and the remaining 44% were in intergenic regions. Among all the regions with significant changes in chromatin accessibility following PDGF-BB treatment, there was a reduction in the percentage of open regions associated with promoters. Conversely, the percentage of open chromatin regions increased in gene body and intergenic regions (Fig. 1D). This finding suggests that changes in chromatin accessibility occur in both promoter and non-promoter regions, with a higher frequency observed in the non-promoter region.

RNA-seq analysis indicates that PDGF-BB treatment of bone marrow-derived MSCs promote expression of genes involved in cell cycle and cell proliferation

Differential expression analysis detected 2,478 significantly upregulated and 2,482 significantly downregulated genes in the PDGF-BB treated MSCs compared to control (Fig. 2A, Table S1-S2) at FDR-adjusted p value < 0.05. Functional analysis of differential expression genes using hallmark gene sets further revealed that up-regulated genes were enriched in pathways that included cell cycle,

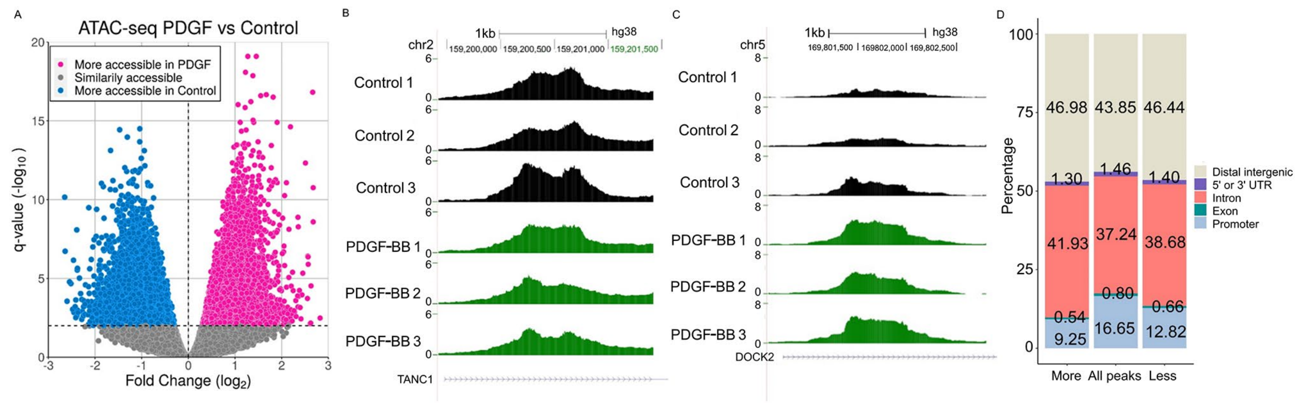

Fig. 1 PDGF-BB changes chromatin accessibility in MSC. (**A**) Volcano plot of ATAC-seq data from MSCs treated with and without PDGF-BB after 48 h. The pink dots correspond to increased accessibility upon PDGF-BB treatment. The blue dots correspond to decreased accessibility upon PDGF-BB treatment. (**B**) Examples of less accessible ATAC-seq open chromatin regions in PDGF-BB treated samples (green) compared with Control MSC samples (black). The y-axis represents normalized reads per million. (**C**) Examples of more accessible ATAC-seq open chromatin regions in PDGF-BB treated samples (green) compared with control MSC samples (black). The y-axis represents normalized reads per million. (**D**) Bar plots showing changes in the distribution of differential accessible regions in the genome. The numbers displayed in the bar plots represent the percentage of a genomic feature among all genomic features in all peaks, including more accessible regions and less accessible regions

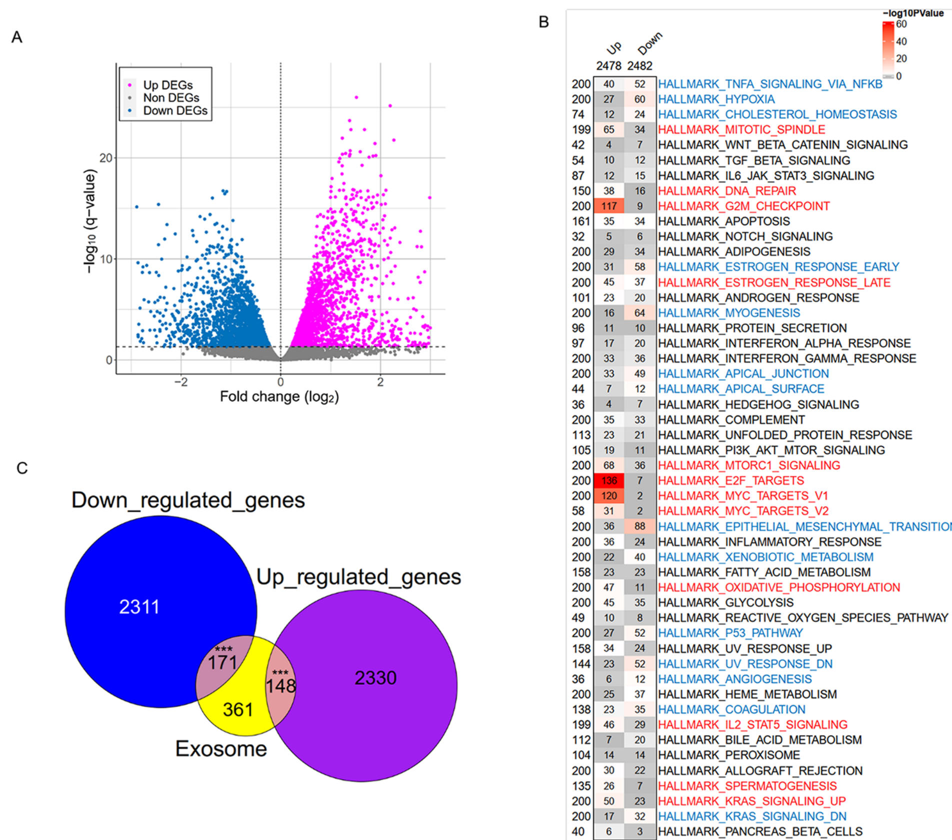

Fig. 2 Differential analysis of RNA-seq data from PDGF-BB treated and control samples. (**A**) Volcano plot of RNA-seq from MSCs treated with and without PDGF-BB for 48 h. The pink dots and blue dots correspond to up-regulated genes and down-regulated genes respectively upon PDGF-BB treatment. (**B**) Functional analysis of up-regulated and down-regulated genes after PDGF-BB treatment. The numbers in the first column indicate the size of each hallmark gene set. The second and third columns present the count of differentially expressed genes that are up-regulated and down-regulated, respectively. These genes overlap with the hallmark gene sets, and the total number of genes is displayed at the top. Hallmark gene sets that are significantly overrepresented in up-regulated genes are marked in red ($p < 0.01$), and those in down-regulated genes are indicated in blue. (**C**) Overlap of differentially expressed genes with exosome related genes. Significant overlaps ($p < 10^{-3}$) were marked asterisk

cell division, DNA repair, mTOR signaling, IL2-STAT5 signaling, and KRAS signaling. Genes exhibiting down-regulation were found to be enriched in pathways including NFKB signaling, hypoxia, early estrogen response, epithelial mesenchymal transition, P53 signaling, and angiogenesis. (Fig. 2B). Significant enrichment of both up-regulated and down-regulated genes was noted in relation to exosome-related genes (Fig. 2C), indicating the important roles of PDGF-BB signaling in cell to cell communication.

Although numerous genes within the hallmark angiogenesis gene set show down-regulation, several angiogenic growth factors, cytokines, and chemokines exhibit up-regulation. This includes vascular endothelial growth factor C (VEGF-C), hepatocyte growth factor (HGF), chemokines like KC (keratinocyte chemoattractant), FGF-1, IL-8, MMP1, and insulin-like growth factor-binding proteins (IGFBP)-1.

Motif enrichment analysis reveals that changes in chromatin accessibility after PDGF-BB treatment are associated with the bindings of AP-1 family transcription factors, TEAD, CEBPA, and RUNX2

To gain a better understanding of what regulatory pathways are altered in MSCs after PDGF-BB treatment, we investigated the enrichment of motifs related to transcription factors (TFs) within the differentially accessible chromatin regions. Using all genomic regions as the background, we performed enrichment analyses for all accessible regions using HOMER [16]. Our findings revealed a comparable set of enriched motifs, consistent with those illustrated in Ho et al. [17].

The TF motif enrichment in the more accessible regions in the PDGF-BB treated samples (Fig. 3A) indicated that most of the enriched transcription factors belong to the AP-1 family. Many of these transcription factors are known to regulate the expressions of the genes which are associated with cell proliferation, differentiation, cell apoptosis, and response to various stimuli such

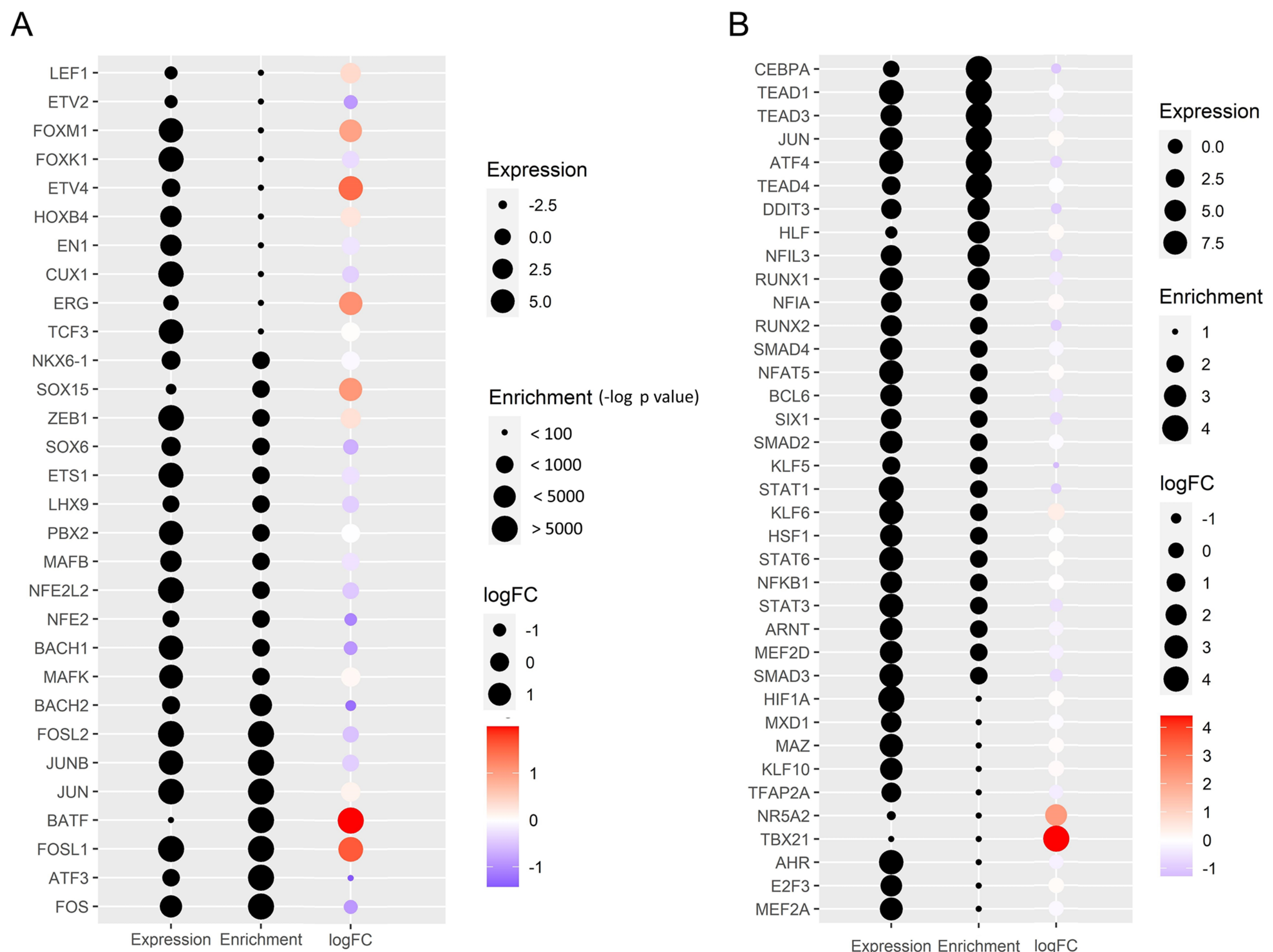

Fig. 3 Top enriched transcription factor (TF) motifs in differential accessibility regions between PDGF-BB treated and control samples. The gene expression level of each TF, the significance of enrichment, and the log fold-change for more open chromatin regions (**A**) and more closed regions (**B**) are shown

as cytokines, growth factors, and stress signals. Notably, transcription factors ATF3, FOS, FOSL1, FOSL2, JUN, and JUNB were significantly enriched, with *p* values $< 10^{-1000}$. Most of these transcription factors exhibited high expression levels, and some also demonstrated differential expressions. When combined with motif enrichment analysis of differentially accessible regions in PDGF-BB treated samples, this observation underscores a significant regulatory impact exerted by these transcription factors.

The TF motif enrichment in the less accessible regions in PDGF-BB treated samples (Fig. 3B) showed that the AP-1 family members JUN and ATF4 were enriched. It suggests that the transcriptional program in PDGF-BB treated samples may be orchestrated primarily through the AP-1 family of transcription factors, which possibly mediate this regulation by changing the chromatin accessibility landscape. In addition, transcription factors critical for regulating osteogenesis and adipogenesis such as TEAD, CEBPA, and RUNX2 [18, 19] were enriched in the closed regions.

Integrative transcriptome and chromatin accessibility analysis discloses pathways and processes involved in PDGF-BB treatment effects

To investigate the effects of changes in chromatin accessibility on gene expression after PDGF-BB treatment of MSCs, we integrated the RNA-seq and ATAC-seq of the same samples. We observed that genes possessing increased accessibility in their promoter or enhancer regions exhibited more significant expression changes when comparing PDGF-BB treated samples with controls, and conversely, genes with less accessible promoter or enhancer regions showed smaller expression changes (Fig. 4A). Overlaps of the significant differentially expressed genes and the genes with differentially accessible promoters or enhancers (Fig. 4B) are statistically significant. The substantial alteration in chromatin accessibility was associated with a corresponding significant change in gene expression (Fig. 4C), which demonstrates the consistency of chromatin accessibility and the gene expression. As a result, we identified 760 genes that were consistently up or down regulated both at the chromatin accessibility and transcriptional levels, which represented a shortlist of PDGF-BB-related candidate genes.

To elucidate the biological pathways dysregulated with treatment of PDGF-BB, we examined the enrichment of hallmark gene sets of the consistently PDGF-BB up or down-regulated candidate genes using hypergeometric test (Fig. 4D). Genes with both up-regulated expression and increased accessibility in PDGF-BB treated samples are enriched in PI3K-AKT signaling, IL2-STAT5 signaling, KRAS signaling, and E2F targets. In contrast, genes with both down-regulated expression and decreased accessibility in PDGF-BB treated samples are enriched for estrogen response, epithelial-mesenchymal transition, and coagulation. This may indicate that PDGF-BB stimulates PI3K-AKT signaling, IL2-STAT5 signaling, KRAS signaling, and E2F targets to regulate gene expression through chromatin change.

In a previous study of PDGF-BB effects on MSCs, it was reported that distinct pathways with opposing actions were activated by PDGF-BB [20]. The PI3K/Akt signaling was identified as the main contributor to MSC proliferation in response to PDGFRα activation. Additionally, activation of Erk by PDGFR signaling strongly inhibited the adipocytic differentiation of MSCs by blocking PPAR and CEBP expression [21]. The induced Akt and Erk pathways regulate opposing fate decisions of proliferation and differentiation [20]. In our study, the PI3K/Akt signaling was not enriched based on gene expression changes alone (Fig. 2B). However, when considering chromatin accessibility changes, it became apparent that this pathway is enriched in more accessible, up-regulated genes. This indicates that changes in chromatin accessibility were a consequence of the regulation in the PI3K/Akt signaling pathway.

PDGF-BB treatment changes gene expression and chromatin accessibility patterns of key genes in MSCs and in osteogenic, adipogenic, and chondrogenic differentiated cells

To further elucidate differential potential of MSCs after PDGF-BB treatment, we investigate the gene expression and chromatin accessibility changes of key marker genes of MSCs, osteogenic, adipogenic, and chondrogenic cells. Some marker genes were observed up-regulated, while some were not changed or even down-regulated. These suggest the diverse effects of PDGF-BB treatment on MSCs for the selected marker genes. The majority of examined adipogenic marker genes demonstrated elevated gene expression with increased accessibility on gene promoters. In contrast, most examined osteogenic marker genes show decreased expression under our experimental conditions.

We examined the transcriptional and chromatin accessibility changes associated with the differentiation of PDGF-BB-treated MSCs towards adipocytes compared to osteoblasts (Fig. 5A). Genes selectively associated with osteogenesis, adipogenesis, and those exhibiting characteristics of both osteogenic and adipogenic differentiation were derived from Rauch's research [22]. In their study, it was found that undifferentiated MSCs express osteoblast-selective genes at higher levels than adipocyte-selective genes, as demonstrated by bulk RNA-seq and single-cell RNA- seq. After differentiation, the expression of osteoblast genes was higher compared to the expression of adipogenic genes. Overall, these data

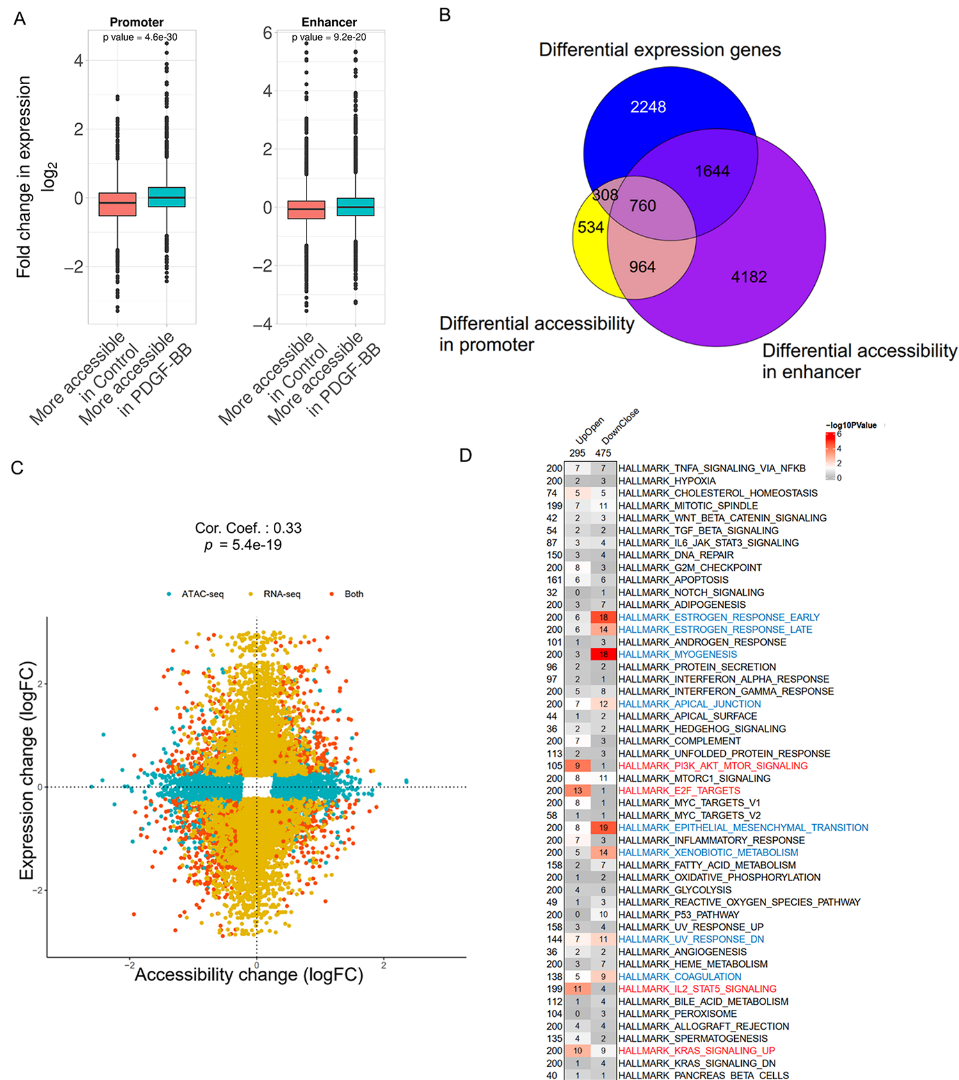

Fig. 4 Integration of ATAC-seq and RNA-seq. (**A**) Gene expression changes in differential accessibility regions. (**B**) Overlap of genes with differential expression and genes with differential accessibility. (**C**) Correlation of changes between ATAC-seq and RNA-seq. (**D**) Functional analysis of genes associated with both up-regulated expression and more accessible regions in PDGF-BB treatment samples and associated with both down-regulated expression and less accessible regions in PDGF-BB treated samples. The numbers outside of the table are the size of each set of hallmark genes (on the left of the table) and up/down regulated genes (DEGs, on the top of the table). The numbers inside the table are the number of genes overlapping between the hallmark gene sets and DEGs. Significantly ($p < 0.01$) overrepresented hallmark gene sets in genes associated with more open regions are colored red, while significantly overrepresented hallmark gene sets in genes associated with more closed regions are colored blue

demonstrate that osteogenesis involves the induction of numerous genes already active in MSCs, while adipogenesis involves the downregulation of several MSC genes along with the significant upregulation of a substantial group of genes not active in MSCs. The study's analysis, presented in Fig. 5A, compares the ratio of changes in chromatin accessibility and transcriptional expression among selective gene groups. It reveals that the percentage change of osteogenic selective genes is more significant than that of adipogenic selective genes. This trend is observed not only in osteogenic selective genes but also in both osteogenic and adipogenic selective genes, as well as all genes collectively. These findings suggest that osteogenic selective genes experience more comprehensive changes overall in response to PDGF-BB treatment compared to adipogenic selective genes, encompassing alterations in all genes examined.

The transcription factor (TF) – chromatin regulatory network following PDGF-BB treatment reveals key factors involved transcriptional and chromatin accessibility changes

The regulatory network was inferred using PECA [23] from both RNA-seq and ATAC-seq data (Fig. 6A). Among the major TFs in the network, their target genes are enriched for extracellular matrix, ossification, blood

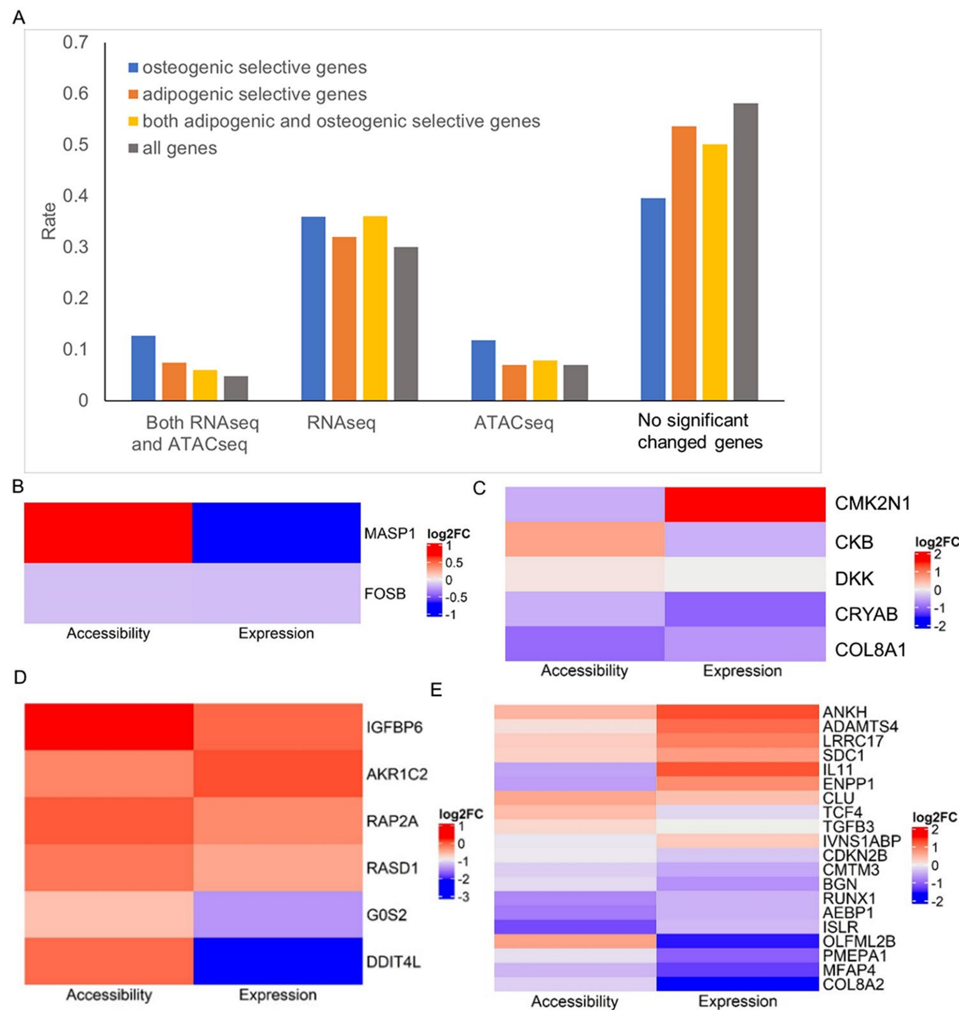

Fig. 5 Chromatin accessibility change and gene expression change of osteogenic and adipogenic selective genes (**A**), key marker genes of MSC (**B**), Osteogenic (**C**), Adipogenic (**D**), and Chondrogenic (**E**) cells

vessel development, the WNT signaling pathway, the BMP signaling pathway, ECM-receptor interaction, the PI3K-Akt signaling pathway, focal adhesion and paracrine signaling- important processes or functions within MSCs (Fig. 6B).

Several transcription factors (TFs), including CEBPB, CEBPD, ETS1, FOXC1, FOXO3, JUN, MAFF, MAFG, MAFK, TCF7, TEAD1, and TEAD3, have been identified. Their predicted target genes are significantly associated with the extracellular matrix. This finding underscores the intricate processes and functions that are involved in the regulation of the extracellular matrix, highlighting the diverse roles these TFs play in their modulation and maintenance.

Similar to earlier findings from motif enrichment analysis, TEAD and CEBP transcription factors are predominantly featured within the control-specific network. The downregulation of these factors has a consequential impact on their target genes, subsequently influencing numerous biological processes, including ECM-receptor interactions and the PI3K-Akt signaling pathway.

The target genes of TCF7 are implicated in a wide array of biological functions, including protein digestion and absorption, ECM-receptor interactions, response to BMP, cellular response to growth factor stimulus, PI3K-Akt signaling pathway, and the transforming growth factor beta (TGF-β) receptor signaling pathway. This highlights the multifaceted role of TCF7 in regulating critical pathways that contribute to cellular processes and responses.

Discussion

After PDGF-BB treatment, motif enrichment analyses of up-regulated genes with more open chromatin status and down-regulated genes with a more open chromatin status reveal similar top-enriched motifs. Additionally, similar top enriched motifs are also observed in all more open chromatin regions, regardless of gene expression change status. This suggests that despite the overall consistency

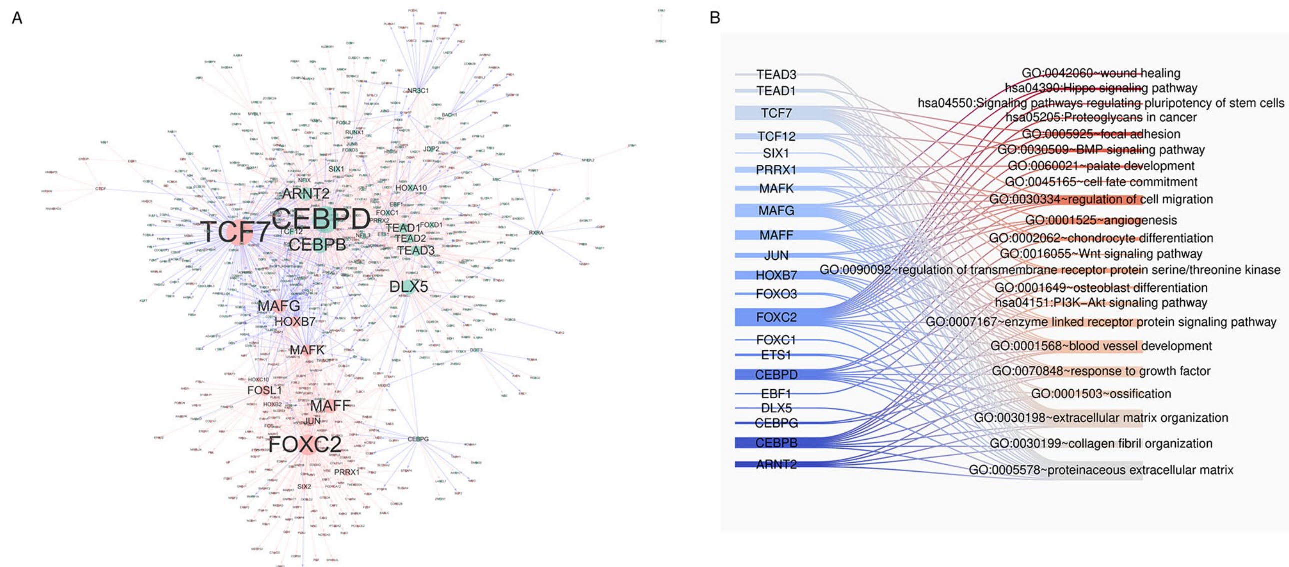

Fig. 6 Network derived from PECA analysis (**A**). Enriched pathway/GO for the targets of the major TF (**B**)

between gene expression and chromatin accessibility, gene expression is fine-tuned by transcription factors and their coactivators or corepressors. We observe the same enriched motifs in all more closed chromatin regions as in the more open regions with differential gene expression. We believe PDGF-BB alters the landscape of chromatin accessibility, making the cell competent for functions encoded in the more open regions.

MSCs are a multipotent cell type that can differentiate into several distinct lineages. Two key transcription factors, RUNX2 and PPARγ, have been reported by various studies to drive MSCs to differentiate into either osteoblasts or adipocytes, respectively [22, 24, 25]. The balance between MSC osteoblast and adipocyte differentiation is disrupted in various human diseases. For example, decreased bone formation accompanied by an increase in bone marrow adipogenesis occurs with aging and immobility or following corticosteroid use, whereas increased bone formation is observed in patients with progressive osseous hyperplasia who form heterotopic bone within their adipose tissue [25].

The 14-3-3-binding protein, a transcriptional coactivator with PDZ-binding motif (TAZ), functions as a molecular rheostat to modulate MSC differentiation. The ectopic expression of TAZ in C3H10T1/2 MSCs fosters commitment to the osteoblast lineage by activating of RUNX2-dependent genes, while concurrently suppressing adipocyte differentiation through the repression of PPARγ transcriptional activity [25]. In the current study, TAZ expression exhibited a significant increase, in tandem with more accessible chromatin, which could enhance RUNX2-mediated osteoblast differentiation and suppress PPARγ-stimulated adipocyte differentiation. Indeed, MSC differentiation is a supplicated interaction among various transcription factors guide the transformation of these cells. In adipogenesis, CEBPα and PPARγ collaborate to drive MSC differentiation into adipocytes [26]. Reduced MAF levels can boost CEBPα activity and enhance adipogenesis. Disruption in CEBP expression, like downregulation, severely interrupts MSC differentiation to adipocyte, highlighting CEBP's crucial role [27].

FOXP1 has been reported to increase MSCs osteogenic differentiation. The reduction of FOXP1 expression in aging MSCs leads to an enhancement of adipogenesis at the expense of osteogenesis. The molecular explanation for these findings is that FOXP1 inhibits adipocyte differentiation by interacting with CEBPB and promotes osteogenic differentiation through repression of NOTCH signaling pathway [28]. In this study, the chromatin of FOXP1 was significantly open, while the gene expression was not significantly increased. The opening of chromatin at FOXP1 loci makes it competent for osteogenic differentiation.

In addition, we observed upregulation of two other genes, HOXB7 and FOXC2. FOXC2's target genes are significantly enriched for mesenchymal cell development and extracellular structure organization. Furthermore, FOXC2 is involved in the regulation of osteogenesis and adipogenesis [29, 30]. HOXB7 acts as an activator of FOXC2. The increased expression of HOXB7 resulted in enhanced cell proliferation, reduced cellular senescence, and promoted osteogenic differentiation [28].

Furthermore, alongside these observations, we noted two additional hub genes, TCF7 and TEAD1, which exhibit specificity in targeting distinct sets of ECM-receptor interaction genes. Moreover, within the PI3K-Art signaling pathway, their target genes predominantly differ. Notably, TCF7 predominantly exerts repressive

effects on target genes, while TEAD1 acts as an activator. Consequently, the upregulation of TCF7 expression and concurrent decreased gene expression of TEAD1 in PDGF-BB samples result in a reduction of target gene expression within both pathways. This observation is consistent with the RNA-seq analysis findings pertaining to downregulated genes. Given the context dependent activation and repression function of TCF7 [31] and TEAD1 [32], it remains to be investigated whether such alterations in TCF7 and TEAD1 expression would influence MSC differentiation fate, thus warranting further exploration in future studies.

In our study, mesenchymal stem cells (MSCs) were exposed to PDGF-BB for a duration of 48 h. However, it is important to acknowledge that the dynamics of cellular responses and regulatory mechanisms can vary significantly depending on the duration of exposure to stimuli. Therefore, it remains to be elucidated whether longer treatment durations, such as a week or throughout the entire differentiation process, would result in alterations in the regulatory network compared to the observed effects after 48 h. Extended exposure to PDGF-BB may induce additional signaling cascades or trigger feedback mechanisms that could modulate the regulatory landscape governing MSC behavior and differentiation. Further investigation into the effects of prolonged PDGF-BB exposure durations is warranted to comprehensively understand the temporal dynamics of MSC responses and the regulatory network involved in this process.

Conclusions

Our findings, consistent with existing literature, indicate that PDGF-BB may induce osteogenic effects on mesenchymal stem cells (MSCs). However, the specific outcomes are contingent upon various factors such as the origin of the cells, experimental conditions, and cellular context. Previous studies have shown that PDGF-BB promotes osteogenic differentiation while inhibiting adipogenic direction in MSCs [3, 10, 33]. In contrast, bone marrow-derived MSCs exhibited no significant difference in mineralization and downregulated osteogenic genes following PDGF-BB treatment, whereas adipose-derived stromal/stem cells displayed increased calcium production per cell and upregulated osteogenic genes under identical PDGF-BB conditions [4, 13]. These findings underscore the intricate interplay between PDGF-BB and MSCs, emphasizing the need for further research to elucidate the underlying mechanisms and optimize therapeutic approaches for bone regeneration.

Our results demonstrate that the expression of genes associated with cell cycle progression and angiogenesis markers was upregulated following PDGF-BB treatment. This finding aligns with previous studies conducted by Qiu et al. (2013) [6] and Lopatina et al. (2014) [12], as well as the findings reported by Rezaie et al. (2019), Xu et al. (2017), and Xie et al. (2014) [11, 34, 35]. These studies collectively support the notion that PDGF-BB treatment induces upregulation of genes related to cell cycle regulation and angiogenesis.

This study has generated lists of differential genes and chromatin regions in response to PDGF-BB treatment in MSCs, which can serve as valuable resources for identifying PDGF-BB response genes and chromatin elements. PDGF-BB enhances proliferation through multiple ways in different cells [36–40]. In this study, PDGF-BB treatment is shown to modulate the chromatin accessibility landscape through the activity of AP-1 family transcription factors, TEAD, CEBPA, and RUNX2. Specifically, the AP-1 family of transcription factors predominantly leads to the opening of target chromatin regions, while reduced expression of CEBPA, TEAD, and RUNX2 correlates with decreased accessibility of target chromatin regions. The ultimate fate of proliferation or differentiation, whether towards osteogenesis or adipogenesis, is governed by the interplay between transcription factors and co-factors, regulated by processes such as phosphorylation and other modifications.

We also observed enrichment of exosome-related genes among differentially expressed genes. PI3K/Akt signaling pathway genes such as COL1A1, COL1A2, COL4A1, COL4A2, COL4A3, COMP, CSF1, FN1, INSR, ITGA11, ITGAV, PDGFA, PDGFRB, THBS1, THBS2, VTN are down regulated. It remains to be elucidated how these genes influence cell-cell communication.

In conclusion, the function of mesenchymal stem cells (MSCs), whether it involves differentiation, proliferation, or paracrine signaling, is regulated by a diverse array of factors, including the source of cells, duration of stimulation, and culture conditions. Therefore, it is essential to investigate methodologies that enhance regulatory mechanisms to optimize MSC functionality as a source of cell therapy. These research endeavors are critical for advancing our understanding of MSC differentiation and its related pathways, holding significant promise for progress in regenerative medicine and tissue engineering.

Materials and methods

MSCs: PoieticsTM Normal Human Bone Marrow Derived Mesenchymal Stem Cells (hMSC) are isolated from normal (non-diabetic) adult human bone marrow withdrawn from bilateral punctures of the posterior iliac crests of normal volunteers. Three PoieticsTM hMSC cells, namely 36,015, 36,461, and 36,550, sourced from three distinct donors, were acquired from Lonza. These cells were designated as follows: 1 (batch 18TL 169252 from donor 36015), 2 (batch 18TL 241909 from donor 36461), and 3 (batch 18TL 262066 from donor 36550). The MSC cells were grown in a humidified chamber

maintained at 37 °C and 5% CO2, utilizing MSCBMTM Basal Media (PT-3238) and MSCGMTM SingleQuots Supplement Kit (PT-4105) obtained from Lonza. Media was changed every three days.

Following our previous study [1], PDGF-BB dose of 10 ng/ml was found the minimum concentration required to elicit maximum VEGF release from the cells. Additionally, 10 ng/ml PDGF-BB rapidly activates ERK and AKT, likely within 5 min. Therefore, MSCs were cultured in media supplemented with 10 ng/ml PDGF-BB (Invitrogen) for 48 h as the treated sample, while the control sample was cultivated without PDGF-BB. Therefore, we have 6 samples based on the donor cells and treatment conditions: Control 1, Control 2, Control 3, PDGF-BB 1, PDGF-BB 2, and PDGF-BB 3. Control and PDGF-BB treated MSCs were concurrently collected from distinct plates for ATACseq and RNAseq analyses separately.

ATAC-seq: Assays were performed in biologic duplicates with approximately 50,000 cells, employing modifications of the original method by [41], previously described [42]. Briefly, cells were harvested using trypsin-EDTA, washed with cold PBS, centrifuged at 300 x *g* for 5 min, and then re-suspended in cold PBS. The collected cells were lysed in cold lysis buffer (10 mM Tris-HCl, pH 7.4, 10 mM NaCl, 3 mM MgCl2 and 0.1% IGEPAL CA-630) and the nuclei were pelleted and resuspended in Tn5 enzyme and transposase buffer (Illumina Nextera® DNA library preparation kit, FC-121-1030). Libraries were amplified using the NEBNext High-Fidelity 2X PCR Master Mix and 1.25 µM of custom Nextera PCR primers 1 and 2 [41]. AMPure XP beads (Beckman Coulter) were utilized to purify the transposed DNA and the amplified PCR products. The resulting ATAC-seq libraries were sequenced in Illumina NovaSeq 6000 at CMG of Indiana University School of Medicine, generating paired-end 100 bp reads. Illumina adapter sequences and low-quality base calls were trimmed from the paired-end reads using Trim Galore v0.4.3. The resulting high-quality reads were aligned to the human reference genome hg38 using bowtie2 (version 2.3.2) with parameters "-X 2000 --no-mixed --no-discordant". Duplicate reads were discarded with Picard (https://broadinstitute.github.io/picard/). Reads mapped to mitochondrial DNA, along with low mapping quality reads (MAPQ < 10), were excluded from further analysis.

Differential chromatin accessibility analysis was conducted using MACS2 for region calling to identify open chromatin region under different conditions. A total of 248,171 chromatin accessibility regions were identified, and accessibility for promoter region and enhancer regions was analyzed separately. To classify the types of chromatin accessibility regions and their corresponding target genes, EnhancerAtlas [43] was employed, as it provides predictions for both. Subsequently, edgeR [14, 15] was utilized for differential chromatin accessibility analysis. Significantly differential accessibility regions under various conditions were identified by applying a filter for FDR < 0.05.

RNA-seq data: Total RNA from MSCs treated with or without PDGF-BB for 48 h in biologic duplicates was extracted using the miRNeasy kit (Qiagen), and mRNA was used for sequencing. edgeR was employed for differential gene expression analysis between the control and PDGF-BB treated groups.

Integration of RNA-seq and ATAC-seq data: To establish a relationship between chromatin accessibility and gene expression changes, regions of chromatin accessibility located within 5 kb upstream and 1 kb downstream of a gene were considered to be linked to that gene. This approach helps to identify how changes in the accessibility of these chromatin regions correlate with changes in gene expression. PECA [23] was utilized to infer gene regulatory network from both RNA-seq and ATAC-seq data.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12864-024-10861-7.

Supplementary Material 1

Supplementary Material 2

Author contributions

S.L.: bioinformatics data analysis and manuscript writing; X.C.: conducted molecular biology experiments and manuscript writing; J.R., X.Y., F.F., P.M., H.G., J.W., and Y.L.: manuscript writing; Y.W.: provided funding resources, concept and design, and manuscript writing.

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Data availability

The raw data supporting the conclusions of this article can be found in the Gene expression omnibus (GEO) with accession numbers GSE272114 for RNA-seq and GSE272136 for ATAC-seq. The original contributions presented in this study are included in the article or supplementary materials, further inquiries can be directed to the corresponding authors.

Declarations

Ethics approval and consent to participate

Since we used commercially available cells derived from human donors, an IRB document is not necessary. All experimental procedures have been approved under Indiana University Institutional Biosafety Committee (IBC) Protocol #1526.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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