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Epigenetic regulation of megakaryopoiesis and platelet formation

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Authors' contributions

BX and XY searched the references and wrote the article, and SC and JW designed the content of the article and the topic for discussion. ZW worked on literature search and language. All authors read and approved the final manuscript.

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Abstract:

Platelets, produced by megakaryocytes, play unique roles in physiological processes, such as hemostasis, coagulation, and immune regulation, while also contributing to various clinical diseases. During megakaryocyte differentiation, the morphology and function of cells undergo significant changes due to the programmed expression of a series of genes. Epigenetic changes modify gene expression without altering the DNA base sequence, effectively impacting the inner workings of the cell at different stages of growth, proliferation, differentiation, and apoptosis. These modifications also play an important role in megakaryocyte development and platelet biogenesis. However, the specific mechanisms underlying epigenetic processes or the vast epigenetic regulatory network formed by their interactions remain unclear. In this review, we systematically summarize the key roles played by epigenetics in megakaryocyte development and platelet formation, including DNA methylation, histone modification, and non-coding RNA regulation. We expect our review to provide a deeper understanding of the biological processes underlying megakaryocyte development and platelet formation and to inform the development of new clinical interventions aimed at addressing platelet-related diseases and improving patient prognoses.

Keywords: Epigenetics; Megakaryocyte; Platelet; DNA methylation; Histone modification; Non-coding RNA

Introduction

Megakaryocytes (MK) and platelets (PLT) play a crucial role in the growth, development, and physiological processes of mammals. As important members of the bone marrow hematopoietic microenvironment, MK produce a large number of PLT that are released into the peripheral blood, where they play critical roles in hemostasis, coagulation, and immune regulation.[1] The traditional view suggests that MK development is a cascade process involving the gradual differentiation of hematopoietic stem cells (HSC), multipotent progenitor cells (MPP), common myeloid progenitor cells (CMP), megakaryocyte-erythroid progenitor cells (MEP), and megakaryocyte progenitor (MKP) into mature MK.[2] Recent studies have confirmed the existence of a direct development pathway from hematopoietic stem/progenitor cells (HSPC) to MK.[3] Moreover, MK commitment appears to be the most direct pathway for hematopoietic stem cells (HSC) following disruption of their homeostasis.[4] PLT generation requires the complete maturation of MK, including volume increase, polyploid nucleus development (DNA content), organelle biosynthesis, and maturation of the demarcation membrane system (DMS). During the terminal phase of MK maturation, proplatelets (PPT) extend through the gaps between vascular endothelial cells and enter the blood, where they are released by hemodynamic forces, followed by their remodeling and fragmentation into individual PLT.[5] Among the processes involving key transcription factors (TF) that participate in these physiological processes, the interaction between GATA-binding factor 1 (GATA1) and friend of GATA1 (FOG1/ZFPM1) is essential for erythro-megakaryocytic differentiation, while the proto-oncogene friend leukemia integration 1 (FLI1) and GATA1 together activate specific genes responsible for regulating late-stage MKP. Additionally, runt-related transcription factor 1 (RUNX1) facilitates progenitor proliferation and regulates later stages of MK maturation. Other TF, including the MDS1

and EVI1 complex site protein (MECOM), nuclear factor red lineage 2 (NFE2), and ETS translocation variant 6 (ETV6), are also involved in MK development.[6]

MK development involves significant changes in morphology and function via programmed gene expression. Single-cell sequencing suggests that MK are divisible into at least three functional subgroups related to PLT formation, hematopoietic microenvironment maintenance, and immunity.[1,7] Hence, specific transcriptional regulatory patterns may contribute to MK commitment and its heterogeneous characteristics. As important regulatory mechanisms underlying gene expression, epigenetic processes occur at different stages of growth, proliferation, differentiation, and apoptosis without altering the DNA sequence (Figure 1).[8] Accompanied by the growth and development of the body, cells are continuously exposed to a dynamic environment; thus, the adaptable changes in gene activity or function brought about by epigenetics exhibit a high degree of plasticity and are often heritably preserved in offspring or daughter cells during mitosis or meiosis. These characteristics allow epigenetics to evolve with cell fate and ultimately help maintain specific cell states. Epigenetic dysfunction and mutations of related genes are partially associated with the pathogenesis and progression of disease, the symptoms of which manifest as abnormal MK development and PLT generation, such as myeloproliferative neoplasm (MPN),[9] multiple myeloma (MM),[10] and rheumatoid arthritis (RA).[11] Therefore, determining the epigenetic mechanisms underlying megakaryopoiesis and PLT formation is important.

This review systematically summarizes the important roles played by various epigenetic mechanisms, including DNA methylation, histone modification, and regulation via non-coding RNA (ncRNA), that regulate MK development and PLT generation. Accordingly, this review is expected to provide an in-depth understanding and comprehensive insights into MK- and PLT-related

disorders linked to hematopoietic system development.

1. Role of DNA methylation in megakaryopoiesis and PLT formation

1.1. Mechanisms of DNA methylation

In the mammalian genome, DNA methylation is a common gene silencing mechanism that is completed by several key enzymes. The DNA methyltransferases (DNMT) DNMT3a and DNMT3b are *de novo* methyltransferases that establish new methylation patterns in unmodified DNA, while DNMT1 copies the DNA methylation pattern from the template DNA strand to the newly synthesized daughter strand.[12] Demethylation occurs via the dilution effect of DNA replication and reduced DNMT1 activity, or it is initiated with the help of ten-eleven translocation 1 (TET1) protein which promotes the conversion of 5-methylcytosine (5-mC) to cytosine through active or passive demethylation processes.[13] DNA methylation is balanced by DNMT and TET,[14] enabling dynamic regulation of specific gene expression by recruiting TF that bind to DNA. DNA methylation patterns are cell-specific and preserve epigenetic “memory” during cell development to some extent. The internal and external environments dynamically modify these patterns during the subsequent life events of the cell.

1.2. Role of DNA methylation in megakaryopoiesis and PLT formation

DNA methylation regulates hematopoietic lineage commitment and contributes to the terminal differentiation and functional establishment of different cell types.[15] Studies investigating DNA methylation kinetics during hematopoietic development have shown that the preliminary stages of the hematopoietic lineage may involve different levels of total DNA methylation. Moreover, changes have been detected in the DNA methylation dynamics of lineage-determining genes during the

differentiation of MPP into CMP and other corresponding lineages.[16]

De novo methylation is the key step in MK formation. Dynamic changes in DNA methylation have been linked to the MK differentiation process, affecting several aspects, including DNA affinity, enhancer activity, TF expression, and cytokinesis.[15,17] Heuston et al. compared the genomic DNA methylation profiles of LSK (Lin^- $Sca\text{-}1^+c\text{-}Kit^+$ cells), CMP, CFU-MK, and immature MK populations and found that DNA methylation levels between MPP and MK lineages differed dramatically.[18] Indeed, increased levels of *de novo* methylation followed by a decrease in immature MK have been observed in CFU-MK cells.[15] Enhanced *de novo* DNA methylation in MK suggests that although MK possess a transcript profile similar to that of MPP, they are not subject to a "default" developmental program, indicating that DNA methylation occurs during MK differentiation.

MK maturation involves endomitosis and the exponential growth of cell ploidy, a process accompanied by progressive changes in DNA methylation patterns (Figure 2A). Farlik et al. performed DNA methylation sequencing of MKs from donor bone marrow according to ploidy and found regions, not the whole genome, that gradually underwent different degrees of methylation during polyploidization and MK maturation.[16] For example, DNA methylation levels at DNase I-hypersensitive and NFE2 binding sites progressively increase during cell development.[19] In contrast, DNA methylation is reduced at the promoter sites of hematopoietic TF, including those of GATA1, a recombinant mothers against decapentaplegic homolog 1 (SMAD1), and T-cell acute lymphoblastic leukemia protein 1 (TAL1).[20] Accompanying MK maturation, several MK-specified genes similarly undergo variational DNA methylation. For instance, Kanaji et al. demonstrated that dynamic changes in CpG methylation status in the 5′ proximal regulatory region, induced by thrombopoietin (TPO), may regulate the expression of megakaryocyte-specific genes, such as glycoprotein VI (*GPVI*).[21]

Single nucleotide polymorphisms (SNP) cause significant changes in epigenetics regulation. GATA1, which typically binds to the T/AGATA site, can only bind CGATA without CpG dinucleotide methylation. The c-kit proto-oncogene protein (Kit) serves as a target gene for GATA1. That is, mutation of the CGATA element on the Kit gene to TGATA prevents its methylation, facilitating GATA1 binding, and repressing Kit expression levels, ultimately reducing HSPC self-renewal ability and enhancing MEP accumulation (Figure 2B).[22] Additionally, platelet endothelial aggregation receptor 1 (PEAR1) is a transmembrane receptor involved in MK production and PLT activation.[23] The rs12041331 site on the PEAR1 enhancer is the first functional CpG island SNP (CpG-SNP) associated with PLT function, where multiple methylation-sensitive TF binding sites exist upstream. When the site represents the G allele, the nearby CpG site is fully methylated, and PEAR1 expression is increased. However, mutations of the G to A allele may otherwise lead to the loss of DNA methylation sites and the silencing of PEAR1 expression.[17]

Regulation of MK development may be achieved by altering methylase activity. The Notch1 acceptor intracellular domain (N1IC) and E26 avian erythroblast virus transcription factor-1 (Ets1) downregulate cellular DNMT3B levels, affecting the methylation state of the transient acceptor potential ankyrin 1 (TRPA1) promoter and increasing MK differentiation ability (Figure 2C).[24] Saran et al. studied the role played by MK-derived microvesicles in the differentiation and maturation of MK and found that microbubbles could promote the production of MK by downregulating the expression of DNMT1, DNMT3a, and DNMT3b and inhibiting Notch homolog 1 (NOTCH1) promoter methylation.[25]

2. Role of histone modifications in megakaryopoiesis and PLT formation

2.1 Mechanisms underlying histone modification

Histone modifications have critical roles in myriad biological processes, with over 60 histone markers discovered to date.[26] Several catalytic enzymes orchestrate the balance of these epigenetic markers. While histone acetyltransferase (HAT) and methyltransferases (HMT) transfer modification groups to target amino sites, histone deacetylase (HDAC) and demethylases (HDM) act as "erasers" by removing the corresponding group from histones or as a transcriptional co-inhibitor to alter chromatin accessibility and reduce gene transcription. Generally, histone epigenetic markers can be divided into two groups according to their effects: activation markers associated with euchromatin regions, particularly transcriptional enhancers and promoter regions (e.g., H3R2me2, H3K4me2, H3K4me3, H3K9ac, H3K14ac, K3K27ac, and H4K16ac), and repressive markers involved in the formation of specific chromatin structures and associated with transcriptional silencing regions of the genome (e.g., H3K9me3, H3K27me1, H3K27me3, H4K20me3, etc.).[27]

2.2 Histone acetylation and deacetylation in megakaryopoiesis and PLT formation

Histone acetylation affects the expression of lineage-specific genes and TF related to MK. Fli-1 and GATA-1 are essential for MK development, with binding sites on the promoters of genes encoding GPIIb, GPIX, and myeloproliferative leukemia protein (MPL). H3 acetylation of the MPL and GPIX promoters occupied by Fli-1 is directly related to gene expression.[28] The maintenance of histone acetylation at the GATA1 site requires an enhancer—HS-3.5 (3.5 kb upstream of the GATA1 hematopoietic transcription start site)—for GATA1 expression, playing a key role at the MKP level.[29] During MK differentiation and maturation, increased H3 acetylation in the promoter region of *EVI1* is regulated by RUNX1, leading to transcription activation.[30] Meanwhile, RUNX1 inhibits gene transcription by interacting with transcriptional co-inhibitors, including the histone deacetylase

complex subunit Sin 3a (mSin3A), transducin-like enhancer of split 1 (TLE), and HDAC, suggesting that it may have a dual regulatory role in acetylation and deacetylation during MK development.[30] The interaction between the KIX domain of CREB-binding protein (CBP)/p300 and c-Myb can synergistically regulate the expression of c-Myb-dependent genes, impacting MK development and increasing PLT counts.[31] Moreover, certain metabolites affect histone acetylation during MK activity, providing clinical treatment targets to improve patient PLT levels via diet supplementation. Dürholz et al. found that the metabolic products of intestinal bacteria, such as the tri-carbon short-chain fatty acid propionate, significantly increased histone H3 acetylation (H3K27) and propionylation (H3K23), thereby upregulating the expression of neurooncological viral antigen 2 (NOVA2), NOTCH3, and solid carrier family 44 member 2 (SLC44A2). This described a new nutritional axis that affects the formation and function of PLT.[11] Moreover, Xie et al. found that H3 acetylation may be promoted by ketone-derived β-hydroxybutyrate (β-OHB) through the inhibition of endogenous HDAC1/2 and increasing the acetyl-CoA supply, effectively inducing GATA1 and NFE2 expression.[32]

HDAC—a scavenger of histone acetylation modifications—is involved in the dynamic regulation of acetylation, which affects MK development and PLT formation. HDAC1 and HDAC2 are located in the nucleosome remodeling and histone deacetylase (NuRD) complex—a large multi-subunit complex comprising chromatin remodeling ATPase and histone deacetylase,[33] which interacts with FOG-1 to activate the “GATA switch” during the final differentiation of MK. FOG-1 recruits NuRD via the interaction between its conserved N-terminal motif and the MTA2 subunit of NuRD, inhibiting the incorrect expression of MK-specific genes that regulate lineage commitment during MK proliferation and maturation.[34,35] In addition to affecting histone modification, Messaoudi et al. found that HDAC6 inhibition causes excessive cortactin acetylation, leading to actin assembly

dysregulation and terminal differentiation of MK and, thus, PLT formation[36]. HDAC11 also regulates genes related to mitosis or G2/M transformation and promotes the clonal growth of myeloproliferative neoplasm samples via the JAK/STAT pathway, leading to abnormal megakaryopoiesis and the exacerbation of thrombocytopenia[37].[37]

2.3 Role of histone methylation and demethylation in megakaryopoiesis and PLT formation

Histone methylation or demethylation may regulate gene expression, selective splicing, and the accessibility of TFs or regulatory complexes, among other processes, ultimately influencing MK development. MPL histone methylation and H3K4me3 and H4 acetylation are regulated by Ott1—a spliceosomal component associated with HDAC3—and the histone methyltransferase SET domain containing 1b (Setd1b), thereby regulating the selective splicing of MPL and modifying the effect of the TPO-MPL axis.[38] In addition, MK are amplified in the bone marrow of *Setd2*-deficient mice. Knocking out SETD2—a H3K36 trimethyltransferase—removes the inhibition of the nuclear receptor binding SET domain protein 1/2/3 (NSD1/2/3) transcriptional complex, which subsequently recruits the super extension complex and regulates the extension of RNA polymerase II, resulting in increased expression of target genes, including *Gata1*, *Gata3*, V-Myc avian myelocytomatosis viral oncogene homolog (*Myc*), and erythroid-specific Kruppel like factor 1 (*Klf1*), among others, ultimately leading to the commitment of HSC to MK.[39] During most of the late MK transcriptional program, TF such as NFE2,[40] FLI1, and RUNX1[41] regulate terminal differentiation in maturing MK by acting on H3K4me2-enriched common binding sites on enhancer sequences.[42]

Several demethylases and related factors participate in MK development and PLT formation. For example, JARID1A is a H3K4 demethylase. In response to MK differentiation signals, the replication origin binding protein (RepID) dissociates from chromatin, preventing JARID1A and its

interaction partner, Cullin 4-RING E3 ubiquitin ligase complex (CRL4A), from loading onto the promoter of disabled homolog 2 (*DAB2*), effectively reducing H3K9me3 and increasing H3K4me3. Such histone modifications mediate the restoration of the *DAB2* promoter region from a heterochromatin to an euchromatin state, relieving DAB2-based transcriptional inhibition, playing a crucial role in MK differentiation.[43] Plant homeodomain finger 2 (PHF2)—a member of the transcription factor Jumonji (JmjC) family of histone demethylases—binds to the p53 promoter and regulates the expression of p53 and its target genes, i.e., cyclin-dependent kinase inhibitor 1A (*CDKN1A*) and tumor protein p53 inducible protein 3 (*TP53I3*), by demethylating the repressive marker H3K9me2, which in turn affects MK differentiation.[44] During megakaryopoiesis, upregulation of the H3K27 demethylase Jumonji domain containing 3 (JMJD3) affects integrin alpha 2 B (ITGA2B) expression, regulating the commitment of HSPC to MK.[45] Meanwhile, zinc finger protein (ZNF300) alters H3K9ac and H3K9me3, enhancing MK differentiation induced by 12-O-tetradecanoylphorbol-13 acetate (PMA).[46] Moreover, to modify the chromatin structure, GFI1 and GFI1B recruit HDAC and lysine-specific demethylase (LSD1) to the promoter to regulate gene expression and enhance MK development.[47] In addition, under the dual regulatory effect of the co-inhibitory factor REST corepressor (CoREST), LSD1 can mediate the demethylation of H3K4 or H3K9 residues, regulate Gfi-1/1b expression, and form the Gfi-1b/CoREST/LSD1 ternary complex, impacting the expression of key genes in lineage development and ultimately MK differentiation and PLT formation.[48] LSD1 inhibitors can interfere with the regulation of LSD1 methylation and impact the LSD1–GFI1B axis function, causing MKP accumulation and decreased PLT.[49] At the chromosome level, histone modification extensively affects the openness of genes, forming a gene regulatory network. In Figure 3, we integrated these mechanisms to map a histone modification

network in MK development.

3. Role of ncRNAs in megakaryopoiesis and PLT formation

3.1 Mechanism of ncRNAs

ncRNAs, including microRNA (miRNA), long non-coding RNA (lncRNA), circular RNA (circRNA), small cytoplasmic RNA (scRNA), and small nucleolar RNA (snoRNA), are functional RNA molecules that cannot be translated into proteins but rather exert regulatory effects. Of these, miRNAs have been studied the most extensively. By binding to the 3′ -untranslated region (3′ -UTR), miRNA promote the degradation of target mRNA and inhibit translation.[50] In addition to miRNAs, the role of lncRNA in MK development has attracted considerable research attention.[51] lncRNA are classified according to their length, function, subcellular localization or origin, biogenesis pathway, and association with specific biological processes, etc.[52] Although the sequences, secondary structures, and regulatory mechanisms of most lncRNA remain unknown, their participation in cell development via the effects they exert on the miRNA regulation of protein translation by acting as competitive RNA,[53] remolding the chromatin structure,[54] and interacting with specific TF[55] has been revealed. In this section, we focus on the effects of miRNA and lncRNA on MK development and differentiation.

3.2 miRNA mediate megakaryopoiesis and PLT formation

miRNA play an important role in megakaryopoiesis during different developmental phases, with some potentially interacting with regulatory factors to modulate MK development, forming a complex regulatory network (Table 1).[56] As successors of MK, PLT receive a diverse and complex miRNA lineage, indicating that processes associated with the epigenetic regulation of MK are

significant in elucidating the physiological effects and functions of PLT.[57]

miRNA promote MK development by affecting TF and gene expression. For example, miR-34a downregulates c-Src tyrosine kinase (c-SRC) and phosphorylates (Tyr416) c-SRC proteins, inhibiting the RAF/MEK/ERK signaling pathway and reducing proliferation.[58] miR-105 reduces c-Myb protein levels to enhance megakaryopoiesis in primitive and definitive hematopoiesis,[59] while c-Myb transactivates miR-4863p expression via the c-Myb/miR-486-3p/MAF axis, downregulating MAF and inhibiting MK production.[60] In $CD34^+$ cells, high miR-146b (miR-146b-5p) expression directly affects PLT-derived growth factor receptor alpha (PDGFRA), inhibits the PDGFRA/JNK/JUN/HEY1 pathway, and increases the expression of GATA1, which positively regulates the transcription of miR-146b, ultimately promoting the differentiation of MK via the miR-146b/PDGFRA/GATA1 feedforward pathway.[61] Recently, Gutti et al. discovered developmental differences in the expression of several miRNA between neonatal and adult MK.[62] Among those upregulated in neonatal MK, miR-99a represses the tumor suppressor protein CTDSPL, enhances RB phosphorylation, and releases E2F1, which induces the G1/S transition by increasing the expression of several cyclins.[63] Meanwhile, miR-125b downregulates the p53 pathway (p53, BAK1, and CDK6) in CB-MK, contributing to their rapid proliferation and increasing the abundance of MK.[64] Additionally, miR-125b and miR-99 may contribute to vincristine resistance in childhood acute megakaryoblastic leukemia (AMeL) and may, thus, represent potential therapeutic strategies.[65] miR-9, which targets RUNX1 and increases the rate of cell proliferation, was found to be more enriched in CB- compared to PB-derived mature MK, providing a potential target for neonatal thrombocytopenia and other PLT disorders.[66]

In contrast, some miRNAs inhibit MK development. For example, let-7b, which is

downregulated in neonate compared with adult MK, inhibits the WNT signaling pathway by regulating FZD4 (frizzled family receptor 4) and inducing mitochondrial biogenesis, thereby regulating proliferation and differentiation during MK development.[62] Meanwhile, miR-28a, miR-708, and miR-151 regulate MK differentiation by inhibiting MPL expression and interfering with the TPO–MPL signaling pathway.[67] Moreover, miR-125b—expressed in human MEP—is downregulated during MK differentiation; its overexpression may lead to excessive MKP and MEP proliferation and self-renewal by inhibiting their targets, i.e., DICER1 and tumor suppressor 18 (ST18).[68] Additionally, miR-27a exerts a regulatory role in MK differentiation by downregulating RUNX1 expression via a feedback loop. Similarly, miR-9, miR-18a, miR-30c, and miR-199a reduce RUNX1 levels during MK production.[69] Moreover, the transplantation of HSC overexpressing miR-155 into irradiated mice effectively decreases MK abundance in the bone marrow.[70] Meanwhile, miR-155 may induce the differentiation of MEP into MKP by reducing ETS1 and MEIS1 expression.[71] During MK maturation and PLT generation, GPIbα promotes endomitosis and MK maturation, while miR-10a and miR-10b target the 3′-UTR of *GPIBA* mRNA and decrease GPIbα expression, ultimately restricting the normal progression of late megakaryopoiesis and PLT production.[72] Additionally, miR-146a affects MK generation by regulating the expression of TNF receptor-associated factor 6 (TRAF6) reducing chemokine C-X-C motif receptor 4 (CXCR4), reducing the proliferation and maturation rate of MK cells and inhibiting the formation of MK-specific colonies.[73,74]

3.3 lncRNA mediate megakaryopoiesis and PLT formation

Recent years have witnessed lncRNA as sophisticated regulators during hematopoiesis,[75] suggesting that they may also affect megakaryopoiesis and PLT formation (Table 1). HOX antisense

intergenic RNA myeloid 1 (HOTAIRM1) impacts the p53-mediated regulation of cyclin D1 by sponging miR-125b to promote MK maturation. Meanwhile, HOTAIRM1 suppression may lead to abnormal MK differentiation and maturation.[76] Just proximal to XIST (JPX) is likely involved in releasing TGF-β receptor expression by sponging oncogenic miRNA (miR-9-5p, miR-17-5p, and miR-106-5p) during MK differentiation, augmenting the activity of ERK1/ERK2 and PI3K/AKT pathways, which in turn enhances the polyploidization and terminal maturation of MK.[77] Differentiation-antagonizing non-coding RNA (DANCR)—a lncRNA highly expressed at the MEP stage—promotes erythroid differentiation by compromising MK differentiation and coordinating with chromatin accessibility and TF, such as RUNX1.[78] Knocking down nuclear paraspeckle assembly transcript 1 (NEAT1), which is abundant in PLT, may lead to decreased cell differentiation, PLT-like particle activity, and IL-8 levels. Meanwhile, weakening the interaction between NEAT1 and splicing production factor proline/glutamine-rich (SFPQ) via NEAT1 knockdown inhibits IL-8 expression, causing PLT hyperactivation and promoting thrombosis, as well as MK proliferation.[79] Antisense-RNA-binding motif protein 15 (AS-RBM15)—a human antisense lncRNA transcribed head-to-head with RBM15—augments CAP-dependent RBM15 protein translation and promotes MK terminal differentiation.[80]

4. Epigenetics modulation of megakaryopoiesis and PLT formation in clinical application

Nowadays, improving the prognosis of clinical MK- and PLT-related diseases by regulating epigenetics has now become popular. Targeting DNA methylation has become a strategy to interfere with disease development and progression. Low doses of demethylating agent desitabine (DAC) significantly increase the number of mature polyploid MK and the number of released PLT in Myelodysplastic syndrome (MDS) patients accompanied by abnormal DNA methylation and a

significant decrease in PLT count.[81] Meanwhile, it has also exhibited potential in treating other thrombocytopenic diseases, such as immune thrombocytopenic purpura.[82]

Meanwhile, numerous studies have focused on regulating histone modifications to improve clinical treatment outcomes. For example, Liu et al. found that inhibitors of the frequently stained histone lysine methyltransferase (EHMT) can significantly promote the generation of megakaryocytes in umbilical cord blood and thus increase the number of PLTs. This has the capacity to be exploited to achieve large-scale *in vitro* PLT preparation to meet clinical resource needs.[83] Use of certain HDAC inhibitors, such as abexinostat,[84] panobinostat,[85] and belinostat,[86] as well as HDM inhibitors like INCB059872, may be followed by drug-induced thrombocytopenia resulting from dysregulated megakaryopoiesis. Therefore, further exploration of the specific mechanisms by which histone modification affects PLT generation will provide insights regarding the rational use of inhibitor drugs in clinical practice. In a clinical trial evaluating the combinatorial effect of the HDAC6 inhibitor, Ricolinostat, and chemotherapy for treating MM, thrombocytopenia was found to potentially be associated with the Ricolinostat dose but not chemotherapy. This highlights the regulatory effect of HDAC6 on PLT generation and is therapeutic capacity to prevent PLT-related side effects.[87] Similarly, Ito et al. found that secreted nardilysin (NRDC), which has been shown to affect PLT yield, can coordinate with HDAC6 to regulate microtubule conformation and remodeling through deacetylation/acetylation, further facilitating PLT shedding.[88]

To date, diverse ncRNA, including miRNA and lncRNA, have been characterized as well as their modes of action in different cell types. These RNA are anticipated to serve as key targets for treating various diseases as they regulate a broad array of biological processes. Indeed, several ncRNA-based therapeutics have been approved.[89] However, due to the dynamic changes within the

physiological environment, the complex intracellular molecular cocreation within cell development, and the single-target specificity required, reports on ncRNA-targeted therapy for abnormal megakaryopoiesis and PLT release are lacking.

5. Conclusions and future directions

Epigenetics plays an important role in stabilizing cell type and abundance in the hematopoietic system and responding to stressful stimuli.[8] Commitment to MK occurs early in the hematopoietic process, with the MK heterogeneity likely determined at this stage. Polymorphisms in the transcriptomic and epigenetic programs of cell populations form the molecular basis for population heterogeneity. The epigenetic mechanisms described in this review primarily regulate gene expression, transcription, and translation, thus, promoting or repressing the physiological development of MK and PLT production. These findings further elucidate the complex molecular mechanisms underlying the growth and development of MK, providing new insights into PLT formation.

However, the mechanisms underlying epigenetic modification far exceed those described here. In addition to common methylation and acetylation, histone modifications include butyrylation and crotonylation,[26] among others. Moreover, adenosine-to-inosine site-selective editing ("A-to-I editing") mediated by the adenosine deaminase acting on RNA plays an important role in HSC maintenance, as well as the regulation of interferon signaling, the terminal stage of B lymphocyte development and their maintenance in peripheral blood, and erythroid lineage development.[90] Regarding DNA modification, in addition to 5-mC modification, N6-methyladenine (6-mA) modification in bacteria has recently been identified in some eukaryotes.[91] In RNA, the N6-methyl-

adenosine (m^6A) modification is essential to myriad aspects of hematopoietic lineage development and function, including HSC self-renewal, expansion of HSC and pluripotent progenitor cells, HSPC formation, and promotion of erythrocyte differentiation.[92] In addition to miRNA and lncRNA, other types of ncRNA have unique roles in regulating MK and PLT production, warranting further exploration. Moreover, although these epigenetic modifications play critical roles in the hematopoietic system, their functions in megakaryopoiesis and thrombopoiesis have not yet been reported. Additionally, several epigenetic mechanisms may coexist, forming a regulatory network. For instance, in addition to allele-specific DNA methylation, PEAR-1 can achieve higher expression. The intron region carrying its CpG SNP exhibits enrichment in histone-modified H3K4me1 and H3K27ac, which may prove beneficial for PEAR-1 expression.[17]

As the interest in MK development and PLT formation continues to expand, researchers in the field have come to focus on three main aspects. The first pertains to abnormalities in the hematopoietic system, which are not only limited to the diseases of MK development, such as AmeL,[93] but also to others, such as MPN and MM.[9,10,94] PLT formation abnormalities have also been evaluated, including certain epigenetic mutations. Second, to alleviate the shortage of blood sources in transfusion medicine, research has centered on *in vitro* PLT formation, revealing that epigenetic regulation shows potential as a process that can be utilized to improve *in vitro* PLT formation.[95] For instance, Ito et al. reported on the important physical regulatory effect of turbulence on PLT formation and developed a turbulent flow-based bioreactor, VerMES, providing an innovative strategy for *ex vivo* PLT manufacturing.[88] Third, studies on MK based on single-cell sequencing have revealed that just as the roles played by MK and PLT in the hematopoietic system are important, so too are the roles they play as immune regulatory cells in infectious[96] and autoimmune diseases[97]. The

formation of an immune-MK subpopulation and the engineered transformation of PLT are inextricably linked to gene expression and regulation. Thus, research on the roles of epigenetics in these areas may be of considerable benefit.

Abbreviations：

acetyl-CoA, acetyl coenzyme A; AmeL, acute megakaryoblastic leukemia; AS-RBM15, antisense-RNA binding motif protein 15; A-to-I editing, adenosine to inosine site selective editing; CBP, CREB binding protein; CDKN1A, cyclin-dependent kinase inhibitor 1A; CFU-MK, megakaryocyte colony forming unit; circRNA, circular RNA; c-Kit, c-kit proto-oncogene protein; CMP, common myeloid progenitor; c-Myb, myeloblastosis viral oncogene homolog/MYB proto-oncogene; CoREST, co-inhibitory factor REST corepressor; CpG-SNP, CpG island-single nucleotide polymorphisms; CRL4A, cullin 4-RING E3 ubiquitin ligase complex; c-SRC, c-src tyrosine kinase; CXCR4, chemokine C-X-C-motif receptor 4; DAB2, disabled homolog 2; DAC, demethylating agent desitabine; DANCR, differentiation antagonizing non-coding RNA; DICER1, endoribonuclease dicer; DMS, demarcation membrane system; DNMT, DNA methyltransferase; ERK, extracellular regulated protein kinases; ETS1, E26 avian erythroblastosis virus transcription factor-1; EVI1, ecotropic viral integration site 1; FLI1, proto-oncogene friend leukemia integration 1; FOG1, friend of GATA1; FZD4, frizzled family receptor 4; GFI1, growth factor independence 1; GPVI, glycoprotein VI; H3K27me3, H3K27 trimethylation; HAT, histone acetyltransferase; HDAC, histone deacetylase; HDM, histone demethylase; HEY1, Hes related family bHLH transcription factor with YRPW motif 1; HMT, histone methyltransferase; HOTAIRM1, HOX antisense intergenic RNA myeloid 1; HSC, hematopoietic stem cell; HSPC, hematopoietic stem/progenitor cell; IL, interleukin;

ITGA2B, monoclonal antibody to integrin alpha 2B; JMJD3, Jumonji domain containing 3; JPX, just proximal to XIST; KLF1, Kruppel-like factor 1; LSD1, lysine specific demethylase 1; LSK, Lin-Sca-1+c-Kit+ cell; m6A, N6-methyl-adenosine; MAF, v-maf musculoaponeurotic fibrosarcoma oncogene homolog; MECOM, MDS1 and EVI1 complex site protein; MEIS1, Homeobox Protein Meis1; MEK, mitogen-activated protein kinase; MEP, megakaryocyte–erythroid progenitor cell; miRNA/miR, microRNA; MK, megakaryocyte; MKP, megakaryocyte progenitor; MM, multiple myeloma; MPL, MPL proto-oncogene/thrombopoietin receptor; MPN, myeloproliferative neoplasms; MPP, multipotent progenitor; mSin3A, histone deacetylase complex subunit Sin 3a; Myc, V-Myc avian Myelocytomatosis Viral Oncogene Homolog; N1IC, Notch1 receptor intracellular domain; ncRNA, non-coding RNA; NEAT1, nuclear paraspeckle assembly transcript 1; NFE2, nuclear factor erythroid 2; NOTCH, Notch homolog; NOVA2, neuro-oncological ventral antigen 2; NSD, nuclear receptor binding SET domain protein; NuRD, nucleosome remodeling complex; NRDC, nardilysin; p300, E1A binding protein p300; PDGFRA, platelet-derived growth factor receptor alpha; PEAR1, platelet endothelial aggregation receptor 1; PHF2, plant homeodomain finger 2; PLT, platelet; PMA, 12-O-tetradecanoylphorbol-13-acetate; PPT, proplatelet; RA, rheumatoid arthritis; RAF, Raf-1 proto-oncogene, serine/threonine kinase; RepID, replication origin binding protein; RUNX1, runt-related transcription factor 1; scRNA, small cytoplasmic RNA; SETD, SET domain containing; STAT, signal transducer and activator of transcription; SFPQ, splicing production factor proline/glutamine-rich; SLC44A2, solute carrier family 44 member 2; SMAD1, recombinant mothers against decapentaplegic homolog 1; snoRNA, small nucleolar RNA; ST18, suppression of tumorigenicity 18; TAL1, T-cell acute lymphocytic leukemia protein 1; TET, ten-eleven translocation protein; TF, transcription factor; TLE, transducin-like enhancer of split 1; TP53I3, tumor protein p53 inducible

protein 3; TPO, thrombopoietin; TRAF6, TNF receptor associated factor 6; TRPA1, transient receptor potential ankyrin 1; ZNF300, zinc finger protein; β-OHB, β-hydroxybutyrate salts; 3′-UTR, 3′-untranslated region; 5-mC, 5-methylcytosine.; 6-mA, N6-methyladenine.

REFERENCES

1. Pariser DN, Hilt ZT, Ture SK, et al. Lung megakaryocytes are immune modulatory cells. J Clin Invest. 2021;131(1):e137377.

2. Woolthuis CM, Park CY. Hematopoietic stem/progenitor cell commitment to the megakaryocyte lineage. Blood. 2016;127(10):1242-1248.

3. Haas S, Hansson J, Klimmeck D, et al. Inflammation-Induced Emergency Megakaryopoiesis Driven by Hematopoietic Stem Cell-like Megakaryocyte Progenitors. Cell Stem Cell. 2015;17(4):422-434.

4. Sakurai M, Ishitsuka K, Ito R, et al. Chemically defined cytokine-free expansion of human haematopoietic stem cells. Nature. 2023;615(7950):127-133.

5. Alicia Bornert, Julie Boscher, Fabien Pertuy, et al. Cytoskeletal-based mechanisms differently regulate in vivo and in vitro proplatelet formation. Haematologica. 2020;106(5):1368-1380.

6. Noh J-Y. Megakaryopoiesis and Platelet Biology: Roles of Transcription Factors and Emerging Clinical Implications. Int J Mol Sci. 2021;22(17):9615.

7. Wang H, He J, Xu C, et al. Decoding Human Megakaryocyte Development. Cell Stem Cell. 2021;28(3):535-549.

8. Raghuwanshi S, Dahariya S, Kandi R, et al. Epigenetic Mechanisms: Role in Hematopoietic Stem Cell Lineage Commitment and Differentiation. Curr Drug Targets. 2018;19(14):1683-1695.

9. Shimizu T, Kubovcakova L, Nienhold R, et al. Loss of Ezh2 synergizes with JAK2 -V617F in initiating myeloproliferative neoplasms and promoting myelofibrosis. J Exp Med. 2016;213(8):1479-1496.

10. Kuang C, Xia M, An G, et al. Excessive serine from the bone marrow microenvironment impairs megakaryopoiesis and thrombopoiesis in Multiple Myeloma. Nat Commun. 2023;14(1):2093.

11. Dürholz K, Schmid E, Frech M, et al. Microbiota-Derived Propionate Modulates Megakaryopoiesis and Platelet Function. Front Immunol. 2022;13:908174.

12. Moore LD, Le T, Fan G. DNA Methylation and Its Basic Function. Neuropsychopharmacology. 2013;38(1):23-38.

13. Liu W, Wu G, Xiong F, Chen Y. Advances in the DNA methylation hydroxylase TET1. Biomark Res. 2021;9(1):76.

14. Peixoto P, Cartron P-F, Serandour AA, Hervouet E. From 1957 to Nowadays: A Brief History of Epigenetics. Int J Mol Sci. 2020;21(20):7571.

15. Farlik M, Halbritter F, Müller F, et al. DNA Methylation Dynamics of Human Hematopoietic Stem Cell Differentiation. Cell Stem Cell. 2016;19(6):808-822.

16. Ji H, Ehrlich LIR, Seita J, et al. Comprehensive methylome map of lineage commitment from haematopoietic progenitors. Nature. 2010;467(7313):338-342.

17. Izzi B, Pistoni M, Cludts K, et al. Allele-specific DNA methylation reinforces PEAR1 enhancer activity. Blood. 2016;128(7):1003-1012.

18. Heuston EF, Keller CA, Lichtenberg J, et al. Establishment of regulatory elements during erythro-megakaryopoiesis identifies hematopoietic lineage-commitment points. Epigenetics Chromatin. 2018;11(1):22.

19. Lecine P, Villeval JL, Vyas P, Swencki B, Xu Y, Shivdasani RA. Mice lacking transcription factor NF-E2 provide in vivo validation of the proplatelet model of thrombocytopoiesis and show a platelet production defect that is intrinsic to megakaryocytes. Blood. 1998;92(5):1608-1616.

20. Tijssen MR, Cvejic A, Joshi A, et al. Genome-wide Analysis of Simultaneous GATA1/2, RUNX1, FLI1, and SCL Binding in Megakaryocytes Identifies Hematopoietic Regulators. Dev Cell. 2011;20(5):597-609.

21. Kanaji S, Kanaji T, Jacquelin B, et al. Thrombopoietin initiates demethylation-based transcription of GP6 during megakaryocyte differentiation. Blood. 2005;105(10):3888-3892.

22. Yang L, Chen Z, Stout ES, et al. Methylation of a CGATA element inhibits binding and regulation by GATA-1. Nat Commun. 2020;11(1):2560.

23. Izzi B, Noro F, Cludts K, Freson K, Hoylaerts MF. Cell-Specific PEAR1 Methylation Studies Reveal a Locus that Coordinates Expression of Multiple Genes. Int J Mol Sci. 2018;19(4):1069.

24. Chen J-L, Ping Y-H, Tseng M-J, et al. Notch1-promoted TRPA1 expression in erythroleukemic cells suppresses erythroid but enhances megakaryocyte differentiation. Sci Rep. 2017;7:42883.

25. Chattapadhyaya S, Haldar S, Banerjee S. Microvesicles promote megakaryopoiesis by regulating DNA methyltransferase and methylation of Notch1 promoter. J Cell Physiol. 2020;235(3):2619-2630.

26. Tan M, Luo H, Lee S, et al. Identification of 67 Histone Marks and Histone Lysine Crotonylation as a New Type of Histone Modification. Cell. 2011;146(6):1016-1028.

27. Butler JS, Dent SYR. The role of chromatin modifiers in normal and malignant hematopoiesis. Blood. 2013;121(16):3076-3084.

28. Jackers P, Szalai G, Moussa O, Watson DK. Ets-dependent Regulation of Target Gene

Expression during Megakaryopoiesis. J Biol Chem. 2004;279(50):52183-52190.

29. Guyot B, Murai K, Fujiwara Y, et al. Characterization of a megakaryocyte-specific enhancer of the key hemopoietic transcription factor GATA1. J Biol Chem. 2006;281(19):13733-13742.

30. Maicas M, Vázquez I, Vicente C, et al. Functional characterization of the promoter region of the human EVI1 gene in acute myeloid leukemia: RUNX1 and ELK1 directly regulate its transcription. Oncogene. 2013;32(16):2069-2078.

31. Kasper LH, Fukuyama T, Lerach S, et al. Genetic Interaction between Mutations in c-Myb and the KIX Domains of CBP and p300 Affects Multiple Blood Cell Lineages and Influences Both Gene Activation and Repression. PLoS One. 2013;8(12):e82684.

32. Xie S, Jiang C, Wu M, et al. Dietary ketone body–escalated histone acetylation in megakaryocytes alleviates chemotherapy-induced thrombocytopenia. Sci Transl Med. 2022;14(673):eabn9061.

33. Wilting RH, Yanover E, Heideman MR, et al. Overlapping functions of Hdac1 and Hdac2 in cell cycle regulation and haematopoiesis. EMBO J. 2010;29(15):2586-2597.

34. Miccio A, Wang Y, Hong W, et al. NuRD mediates activating and repressive functions of GATA-1 and FOG-1 during blood development. EMBO J. 2010;29(2):442-456.

35. Gao Z, Huang Z, Olivey HE, Gurbuxani S, Crispino JD, Svensson EC. FOG-1-mediated recruitment of NuRD is required for cell lineage re-enforcement during haematopoiesis. EMBO J. 2010;29(2):457-468.

36. Messaoudi K, Ali A, Ishaq R, et al. Critical role of the HDAC6-cortactin axis in human megakaryocyte maturation leading to a proplatelet-formation defect. Nat Commun. 2017;8(1):1786.

37. Yue L, Sharma V, Horvat NP, et al. HDAC11 deficiency disrupts oncogene-induced hematopoiesis in myeloproliferative neoplasms. Blood. 2020;135(3):191-207.

38. Xiao N, Laha S, Das SP, Morlock K, Jesneck JL, Raffel GD. Ott1 (Rbm15) regulates thrombopoietin response in hematopoietic stem cells through alternative splicing of c-Mpl. Blood. 2015;125(6):941-948.

39. Zhou Y, Yan X, Feng X, et al. Setd2 regulates quiescence and differentiation of adult hematopoietic stem cells by restricting RNA polymerase II elongation. Haematologica. 2018;103(7):1110-1123.

40. Vukadin L, Kim J-H, Park EY, et al. SON inhibits megakaryocytic differentiation via repressing RUNX1 and the megakaryocytic gene expression program in acute megakaryoblastic leukemia. Cancer Gene Ther. 2021;28(9):1000-1015.

41. Majumder A, Dharan AT, Baral I, et al. Histone chaperone HIRA dictate proliferation vs

differentiation of chronic myeloid leukemia cells. FASEB Bioadv. 2019;1(9):525-537.

42. Zang C, Luyten A, Chen J, Liu XS, Shivdasani RA. NF-E2, FLI1 and RUNX1 collaborate at areas of dynamic chromatin to activate transcription in mature mouse megakaryocytes. Sci Rep. 2016;6:30255.

43. Jo J-H, Park J-U, Kim Y-M, et al. RepID represses megakaryocytic differentiation by recruiting CRL4A-JARID1A at DAB2 promoter. Cell Commun Signal. 2023;21(1):219.

44. Yang J, Ma J, Xiong Y, et al. Epigenetic regulation of megakaryocytic and erythroid differentiation by PHF2 histone demethylase. J Cell Physiol. 2018;233(9):6841-6852.

45. Dumon S, Walton DS, Volpe G, et al. Itga2b regulation at the onset of definitive hematopoiesis and commitment to differentiation. PloS One. 2012;7(8):e43300.

46. Yan F-J, Fan J, Huang Z, Zhang J-J. ZNF300 tight self-regulation and functioning through DNA methylation and histone acetylation. Cell Biosci. 2017;7:33.

47. Beauchemin H, Möröy T. Multifaceted Actions of GFI1 and GFI1B in Hematopoietic Stem Cell Self-Renewal and Lineage Commitment. Front Genet. 2020;11:591099.

48. Saleque S, Kim J, Rooke HM, Orkin SH. Epigenetic Regulation of Hematopoietic Differentiation by Gfi-1 and Gfi-1b Is Mediated by the Cofactors CoREST and LSD1. Mol Cell. 2007;27(4):562-572.

49. Johnston G, Ramsey HE, Liu Q, et al. Nascent transcript and single-cell RNA-seq analysis defines the mechanism of action of the LSD1 inhibitor INCB059872 in myeloid leukemia. Gene. 2020;752:144758.

50. Li H, Zhao H, Wang D, Yang R. microRNA regulation in megakaryocytopoiesis. Br J Haematol. 2011;155(3):298-307.

51. Guo X, Gao L, Wang Y, Chiu DKY, Wang T, Deng Y. Advances in long noncoding RNAs: identification, structure prediction and function annotation. Brief Funct Genomics. 2016;15(1):38-46.

52. Jarroux J, Morillon A, Pinskaya M. History, Discovery, and Classification of lncRNAs. Adv Exp Med Biol. 2017;1008:1-46.

53. Paraskevopoulou MD, Hatzigeorgiou AG. Analyzing MiRNA-LncRNA Interactions. Methods Mol Biol. 2016;1402:271-286.

54. Han P, Chang C-P. Long non-coding RNA and chromatin remodeling. RNA Biol. 2015;12(10):1094-1098.

55. Dykes IM, Emanueli C. Transcriptional and Post-transcriptional Gene Regulation by Long Non-coding RNA. Genomics Proteomics Bioinformatics. 2017;15(3):177-186.

56. Raghuwanshi S, Dahariya S, Musvi SS, et al. MicroRNA function in megakaryocytes. Platelets. 2019;30(7):809-816.

57. Edelstein LC, McKenzie SE, Shaw C, Holinstat MA, Kunapuli SP, Bray PF. MicroRNAs in platelet production and activation. J Thromb Haemost. 2013;11 Suppl 1:340-350.

58. Yang Y, Ding L, Guo Z-K, et al. The epigenetically-regulated miR-34a targeting c-SRC suppresses RAF/MEK/ERK signaling pathway in K-562 cells. Leuk Res. 2017;55:91-96.

59. Kamat V, Paluru P, Myint M, French DL, Gadue P, Diamond SL. MicroRNA screen of human embryonic stem cell differentiation reveals miR-105 as an enhancer of megakaryopoiesis from adult CD34+ cells. Stem Cells. 2014;32(5):1337-1346.

60. Bianchi E, Bulgarelli J, Ruberti S, et al. MYB controls erythroid versus megakaryocyte lineage fate decision through the miR-486-3p-mediated downregulation of MAF. Cell Death Differ. 2015;22(12):1906-1921.

61. Zhai P-F, Wang F, Su R, et al. The regulatory roles of microRNA-146b-5p and its target platelet-derived growth factor receptor α (PDGFRA) in erythropoiesis and megakaryocytopoiesis. J Biol Chem. 2014;289(33):22600-22613.

62. Undi RB, Gutti U, Gutti RK. Role of let-7b/Fzd4 axis in mitochondrial biogenesis through wnt signaling: In neonatal and adult megakaryocytes. Int J Biochem Cell Biol. 2016;79:61-68.

63. Kandi R, Gutti U, Undi R, Sahu I, Gutti RK. Understanding thrombocytopenia: physiological role of microRNA in survival of neonatal megakaryocytes. J Thromb Thrombolysis. 2015;40(3):310-316.

64. Kandi R, Undi R, Gutti RK. MiR-125b regulates cell proliferation and survival in neonatal megakaryocytes. Ann Hematol. 2014;93(6):1065-1066.

65. Kandi R, Gutti U, Saladi RGV, Gutti RK. MiR-125b and miR-99a encoded on chromosome 21 co-regulate vincristine resistance in childhood acute megakaryoblastic leukemia. Hematol Oncol Stem Cell Ther. 2015;8(2):95-97.

66. Raghuwanshi S, Gutti U, Kandi R, Gutti RK. Micro RNA □9 promotes cell proliferation by regulating RUNX 1 expression in human megakaryocyte development. Cell Prolif. 2018;51(1):e12414.

67. Girardot M, Pecquet C, Boukour S, et al. miR-28 is a thrombopoietin receptor targeting microRNA detected in a fraction of myeloproliferative neoplasm patient platelets. Blood. 2010;116(3):437-445.

68. Klusmann J-H, Li Z, Böhmer K, et al. miR-125b-2 is a potential oncomiR on human chromosome 21 in megakaryoblastic leukemia. Genes Dev. 2010;24(5):478-490.

69. Ben-Ami O, Pencovich N, Lotem J, Levanon D, Groner Y. A regulatory interplay between

miR-27a and Runx1 during megakaryopoiesis. Proc Natl Acad Sci U S A. 2009;106(1):238-243.

70. O'Connell RM, Rao DS, Chaudhuri AA, et al. Sustained expression of microRNA-155 in hematopoietic stem cells causes a myeloproliferative disorder. J Exp Med. 2008;205(3):585-594.

71. Romania P, Lulli V, Pelosi E, Biffoni M, Peschle C, Marziali G. MicroRNA 155 modulates megakaryopoiesis at progenitor and precursor level by targeting Ets-1 and Meis1 transcription factors. Br J Haematol. 2008;143(4):570-580.

72. Zhang Z, Ran Y, Shaw TS, Peng Y. MicroRNAs 10a and 10b Regulate the Expression of Human Platelet Glycoprotein Ibα for Normal Megakaryopoiesis. Int J Mol Sci. 2016;17(11):1873.

73. Labbaye C, Spinello I, Quaranta MT, et al. A three-step pathway comprising PLZF/miR-146a/CXCR4 controls megakaryopoiesis. Nat Cell Biol. 2008;10(7):788-801.

74. Starczynowski DT, Kuchenbauer F, Argiropoulos B, et al. Identification of miR-145 and miR-146a as mediators of the 5q– syndrome phenotype. Nat Med. 2010;16(1):49-58.

75. Sangeeth A, Malleswarapu M, Mishra A, Gutti RK. Long Non-Coding RNAs as Cellular Metabolism and Haematopoiesis Regulators. J Pharmacol Exp Ther. 2023;384(1):79-91.

76. Dahariya S, Raghuwanshi S, Thamodaran V, Velayudhan SR, Gutti RK. Role of Long Non-Coding RNAs in Human-Induced Pluripotent Stem Cells Derived Megakaryocytes: A p53, HOX Antisense Intergenic RNA Myeloid 1, and miR-125b Interaction Study. J Pharmacol Exp Ther. 2023;384(1):92-101.

77. Dahariya S, Raghuwanshi S, Sangeeth A, Malleswarapu M, Kandi R, Gutti RK. Megakaryoblastic leukemia: a study on novel role of clinically significant long non-coding RNA signatures in megakaryocyte development during treatment with phorbol ester. Cancer Immunol Immunother. 2021;70(12):3477-3488.

78. Ren Y, Zhu J, Han Y, et al. Regulatory association of long noncoding RNAs and chromatin accessibility facilitates erythroid differentiation. Blood Adv. 2021;5(23):5396-5409.

79. Bian W, Chen W, Jiang X, et al. Downregulation of Long Non-coding RNA Nuclear Paraspeckle Assembly Transcript 1 Inhibits MEG-01 Differentiation and Platelet-Like Particles Activity. Front Genet. 2020;11:571467.

80. Tran N, Su H, Khodadadi-Jamayran A, et al. The AS-RBM15 lncRNA enhances RBM15 protein translation during megakaryocyte differentiation. EMBO Rep. 2016;17(6):887-900.

81. Traina F, Visconte V, Elson P, et al. Impact of molecular mutations on treatment response to DNMT inhibitors in myelodysplasia and related neoplasms. Leukemia. 2014;28(1):78-87.

82. Zhou H, Hou Y, Liu X, et al. Low-dose decitabine promotes megakaryocyte maturation and platelet production in healthy controls and immune thrombocytopenia. Thromb Haemost. 2015;113(5):1021-1034.

83. Liu Y, Zhao J, Wang Y, et al. Augmented Production of Platelets From Cord Blood With Euchromatic Histone Lysine Methyltransferase Inhibition. Stem Cells Transl Med. 2022;11(9):946-958.

84. Ali A, Bluteau O, Messaoudi K, et al. Thrombocytopenia induced by the histone deacetylase inhibitor abexinostat involves p53-dependent and -independent mechanisms. Cell Death Dis. 2013;4(7):e738.

85. Iancu-Rubin C, Gajzer D, Mosoyan G, Feller F, Mascarenhas J, Hoffman R. Panobinostat (LBH589)-induced acetylation of tubulin impairs megakaryocyte maturation and platelet formation. Exp Hematol. 2012;40(7):564-574.

86. Peer CJ, Hall OM, Sissung TM, et al. A population pharmacokinetic/toxicity model for the reduction of platelets during a 48-h continuous intravenous infusion of the histone deacetylase inhibitor belinostat. Cancer Chemother Pharmacol. 2018;82(3):565-570.

87. Vogl DT, Raje N, Jagannath S, et al. Ricolinostat, the First Selective Histone Deacetylase 6 Inhibitor, in Combination with Bortezomib and Dexamethasone for Relapsed or Refractory Multiple Myeloma. Clin Cancer Res. 2017;23(13):3307-3315.

88. Ito Y, Nakamura S, Sugimoto N, et al. Turbulence Activates Platelet Biogenesis to Enable Clinical Scale Ex Vivo Production. Cell. 2018;174(3):636-648.

89. Sangeeth A, Malleswarapu M, Mishra A, Gutti RK. Long Non-coding RNA Therapeutics: Recent Advances and Challenges. Curr Drug Targets. 2022;23(16):1457-1464.

90. Liddicoat BJ, Hartner JC, Piskol R, et al. Adenosine-to-inosine RNA editing by ADAR1 is essential for normal murine erythropoiesis. Exp Hematol. 2016;44(10):947-963.

91. Chen L-Q, Zhao W-S, Luo G-Z. Mapping and editing of nucleic acid modifications. Comput Struct Biotechnol J. 2020;18:661-667.

92. Kuppers DA, Arora S, Lim Y, et al. N6-methyladenosine mRNA marking promotes selective translation of regulons required for human erythropoiesis. Nat Commun. 2019;10(1):4596.

93. Malinge S, Chlon T, Doré LC, et al. Development of acute megakaryoblastic leukemia in Down syndrome is associated with sequential epigenetic changes. Blood. 2013;122(14):e33-e43.

94. Malherbe JAJ, Fuller KA, Arshad A, et al. Megakaryocytic hyperplasia in myeloproliferative neoplasms is driven by disordered proliferative, apoptotic and epigenetic mechanisms. J Clin Pathol. 2016;69(2):155-163.

95. Qin J, Zhang J, Jiang J, et al. Direct chemical reprogramming of human cord blood erythroblasts to induced megakaryocytes that produce platelets. Cell Stem Cell. 2022;29(8):1229-1245.

96. Valet C, Magnen M, Qiu L, et al. Sepsis promotes splenic production of a protective platelet pool with high CD40 ligand expression. J Clin Invest. 2022;132(7):e153920.

97. Wang Y, Xie X, Zhang C, et al. Rheumatoid arthritis, systemic lupus erythematosus and primary Sjögren's syndrome shared megakaryocyte expansion in peripheral blood. Ann Rheum Dis. 2022;81(3):379-385.

Table 1 legend

Several non-coding RNA and their influences on megakaryopoiesis and PLT formation.

ncRNA		Target	Effect	Reference
miRNA	miR-34a	c-SRC	Inhibit MK proliferation	58
	miR-105	c-Myb	Promote megakaryopoiesis	59
	miR-486-3p	MAF	Inhibit megakaryopoiesis	60
	miR-146b-5p	PDGFRA	Promote MK differentiation	61
	let-7b	FZD4	Inhibit proliferation	62
	miR-99a	CTDSPL	Promote MK proliferation	63
		p53	Promote MK proliferation	64
	miR-125b	DICER1 ST18	Increase proliferation of MKP and MEP, inhibit megakaryopoiesis	68
	miR-9 miR-27a miR-18a miR-30c miR-199a miR-28a miR-151 miR-708	RUNX1	Promote MK proliferation, inhibit MK differentiation	66, 69
		MPL	Inhibit MK differentiation	67
	miR-155	ETS1 MEIS1	Inhibit MK proliferation and differentiation	71
	miR-10a miR-10b	GPIbα	Inhibit MK maturation and PLT production	72
	miR-146a	TRAF6	Inhibit megakaryopoiesis	73
		CXCR4	Inhibit MK proliferation and maturation	74
lncRNA	HOTAIRM1	miR-125b	Promote MK differentiation	76
	JPX	miR-9-5p miR-17-5p miR-106-5p	Enhance MK polyploidization and terminal maturation	77
	DANCR	TFs (RUNX1 etc.) chromatin (accessibility)	Inhibit MK differentiation	78
	NEAT1	SFPQ	Promote MK proliferation and differentiation	79
	AS-RBM15	RBM15	Promote MK differentiation	80

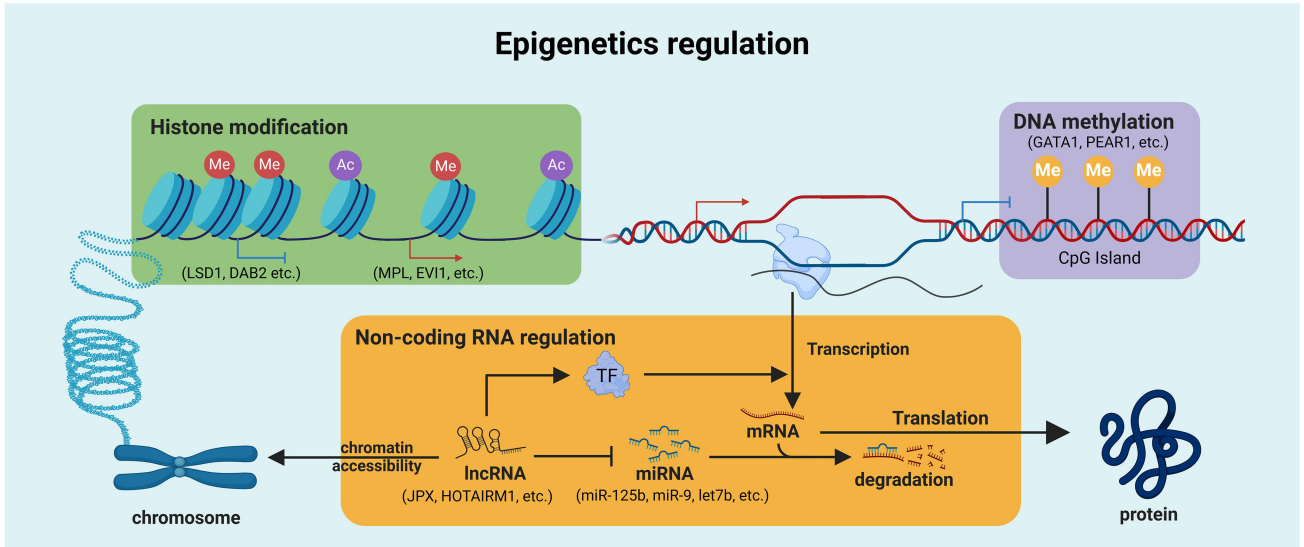
Table 1

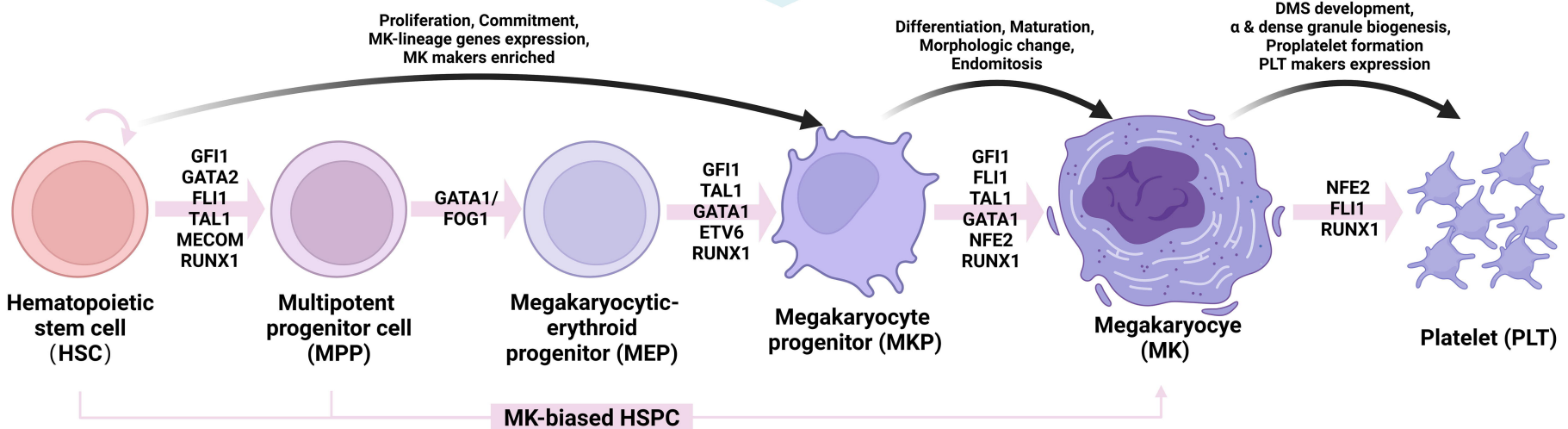
Figure legends

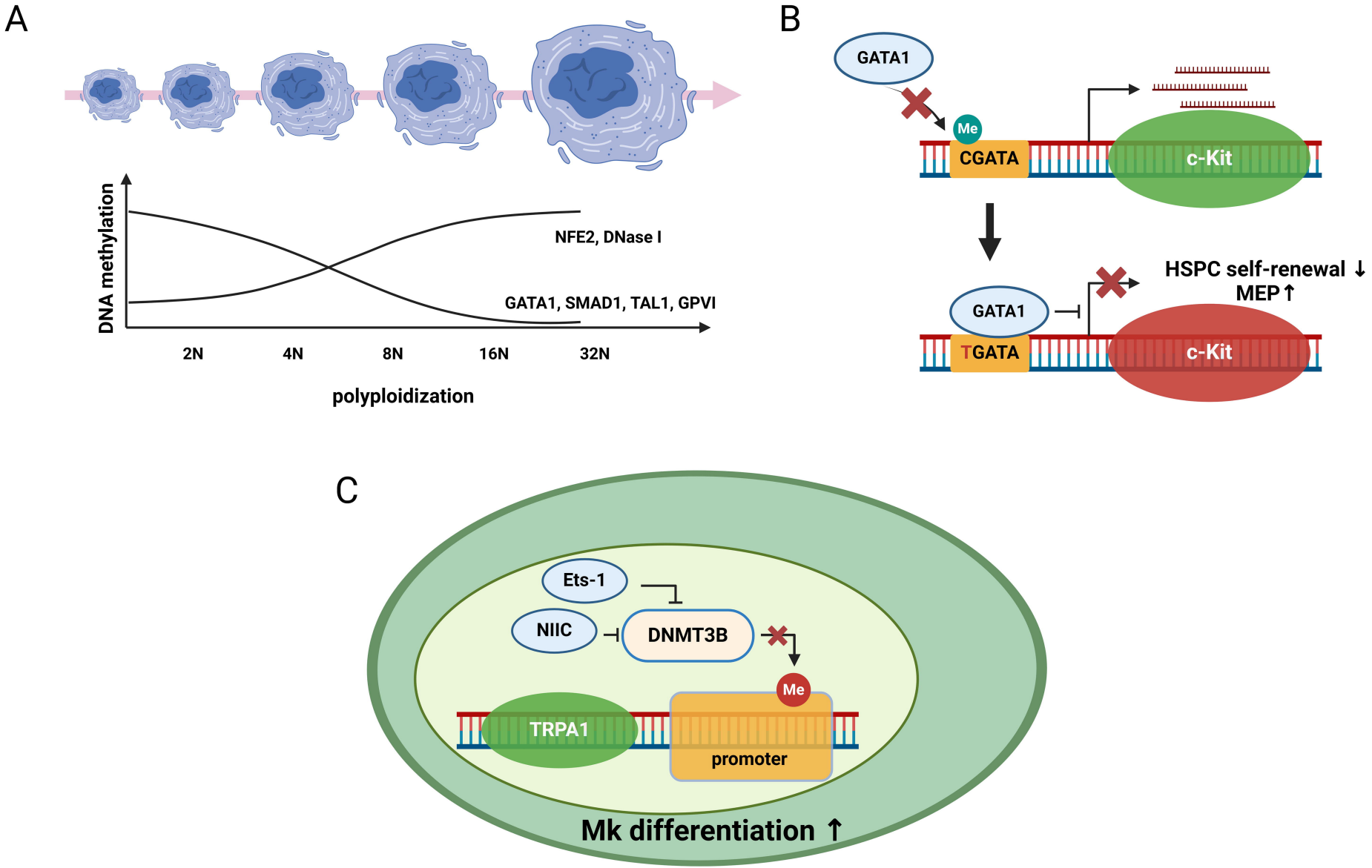
Figure 1| Epigenetic regulation involved in differentiation pathway starting from HSC through terminal differentiation to MK and PLT formation and related transcription factors.

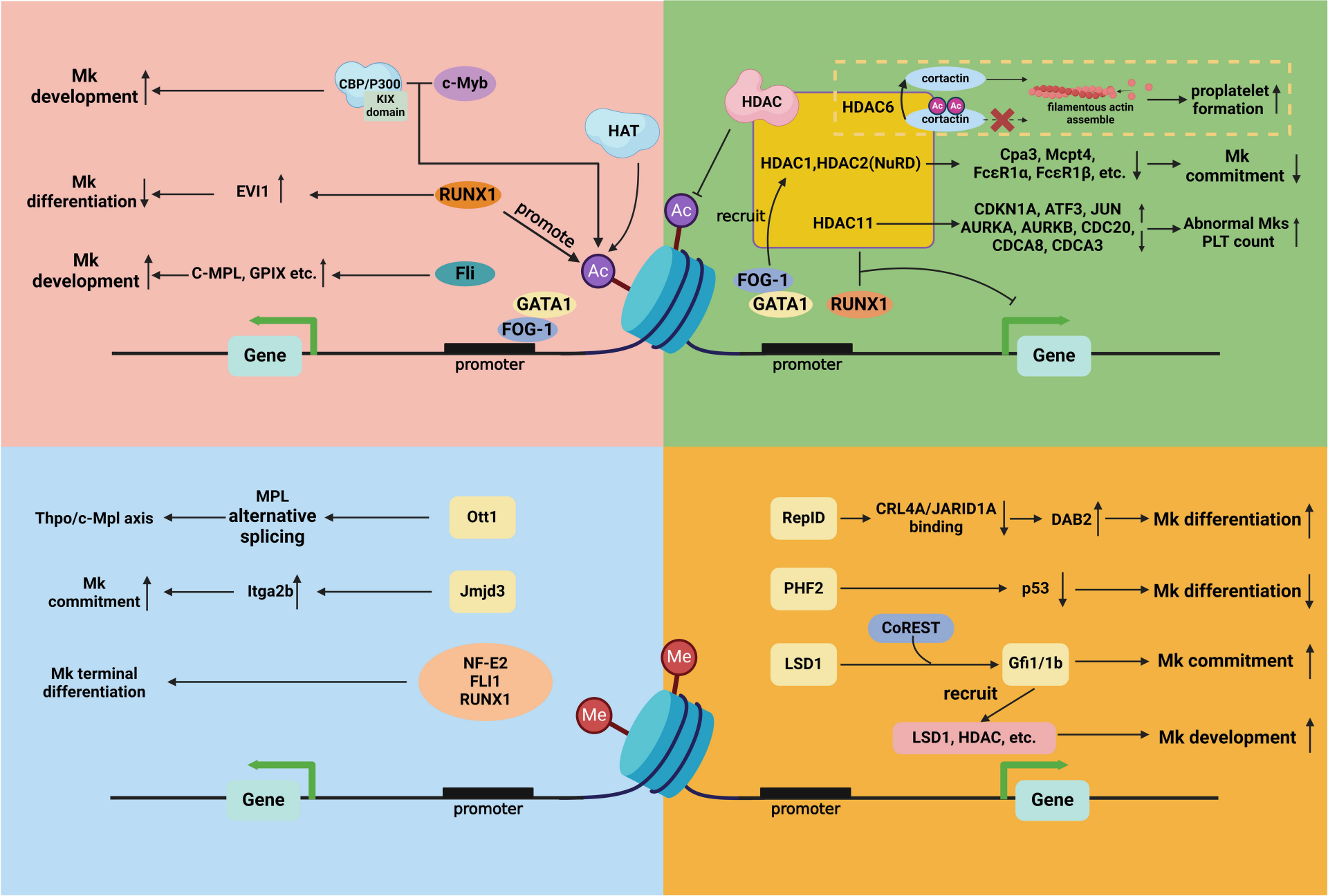
Figure 2| The mechanisms underlying DNA methylation during the regulation of megakaryopoiesis and PLT formation. A) During the polyploidization of MK, several critical genes related to MK development are differentially methylated. B) A single base alteration may induce significant changes on genes expression and lineage commitment. C) MK differentiation can be affected by the activity of DNA methylase.

Figure 3| Histone modification during megakaryopoiesis and PLT formation. The expression of several critical genes is regulated by acetylation (top, left), deacetylation (top, right), methylation (bottom, left) and demethylation (bottom, right) of histones with the mediation of several TFs and enzyme complexes.


Epigenetics regulation
Histone modification
Me
Me
Ac
Me
Ac
(LSD1, DAB2 etc.)
(MPL, EVI1, etc.)
DNA methylation
(GATA1, PEAR1, etc.)
Me
Me
Me
CpG Island
Non-coding RNA regulation
TF
Transcription
mRNA
Translation
chromatin accessibility
lncRNA
(JPX, HOTAIRM1, etc.)
miRNA
(miR-125b, miR-9, let7b, etc.)
degradation
chromosome
protein


Proliferation, Commitment,
MK-lineage genes expression,
MK makers enriched
Differentiation, Maturation,
Morphologic change,
Endomitosis
DMS development,
α & dense granule biogenesis,
Proplatelet formation
PLT makers expression
GFI1
GATA2
FLI1
TAL1
MECOM
RUNX1
GATA1/
FOG1
GFI1
TAL1
GATA1
ETV6
RUNX1
GFI1
FLI1
TAL1
GATA1
NFE2
RUNX1
NFE2
FLI1
RUNX1
Hematopoietic stem cell (HSC)
Multipotent progenitor cell (MPP)
Megakaryocytic-erythroid progenitor (MEP)
Megakaryocyte progenitor (MKP)
Megakaryocye (MK)
Platelet (PLT)
MK-biased HSPC


A
DNA methylation
NFE2, DNase I
GATA1, SMAD1, TAL1, GPVI
2N
4N
8N
16N
32N
polyploidization
B
GATA1
Me
CGATA
c-Kit
HSPC self-renewal ↓
MEP ↑
GATA1
TGATA
c-Kit
C
Ets-1
NIIC
DNMT3B
Me
TRPA1
promoter
Mk differentiation ↑


Mk development
CBP/P300
KIX domain
c-Myb
HAT
Mk differentiation
EVI1
RUNX1
promote
Mk development
C-MPL, GPIX etc.
Fli
Ac
GATA1
FOG-1
Gene
promoter
HDAC
HDAC6
cortactin
Ac
cortactin
filamentous actin assemble
proplatelet formation
HDAC1,HDAC2(NuRD)
Cpa3, Mcpt4, FcεR1α, FcεR1β, etc.
Mk commitment
recruit
HDAC11
CDKN1A, ATF3, JUN AURKA, AURKB, CDC20, CDCA8, CDCA3
Abnormal Mks PLT count
FOG-1
GATA1
RUNX1
promoter
Gene
Thpo/c-Mpl axis
MPL alternative splicing
Ott1
Mk commitment
Itga2b
Jmjd3
Mk terminal differentiation
NF-E2
FLI1
RUNX1
Me
Me
Gene
promoter
RepID
CRL4A/JARID1A binding
DAB2
Mk differentiation
PHF2
p53
Mk differentiation
CoREST
LSD1
Gfi1/1b
Mk commitment
recruit
LSD1, HDAC, etc.
Mk development
promoter
Gene