REVIEW ARTICLE

Epigenetic regulation of megakaryopoiesis and platelet formation

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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 affecting the inner workings of the cell at different stages of growth, proliferation, differentiation, and apoptosis. These modifications also play important roles in megakaryocyte development and platelet biogenesis. However, the specific mechanisms underlying epigenetic processes and 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 patients' prognoses.

Introduction

Megakaryocytes and platelets play crucial roles in the growth, development, and physiological processes of mammals. As important members of the bone marrow hematopoietic microenvironment, megakaryocytes produce a large number of platelets that are released into the peripheral blood, where they play critical roles in hemostasis, coagulation, and immune regulation.[1] The traditional view suggests that megakaryocyte development is a cascade process involving the gradual differentiation of hematopoietic stem cells (HSC), multipotent progenitor cells, common myeloid progenitor cells, megakaryocyte-erythroid progenitor cells (MEP), and megakaryocyte progenitors into mature megakaryocytes.[2] Recent studies have confirmed the existence of a direct developmental pathway from hematopoietic stem/progenitor cells (HSPC) to megakaryocytes.[3] Moreover, megakaryocyte commitment appears to be the most direct pathway for HSC following disruption of their homeostasis.[4] Platelet generation requires the complete maturation of megakaryocytes, including volume increase, polyploid nucleus development (DNA content), organelle biosynthesis, and maturation of the demarcation membrane system. During the terminal phase of megakaryocyte maturation, proplatelets 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 platelets.[5] Among the processes involving key transcription factors 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 proto-oncogene friend leukemia integration 1 (FLI1) and GATA1 together activate specific genes responsible for regulating late-stage megakaryocyte progenitors. Additionally, runt-related transcription factor 1 (RUNX1) facilitates progenitor proliferation and regulates later stages of megakaryocyte maturation. Other transcription factors, 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 megakaryocyte development.[6]

Megakaryocyte development involves significant changes in morphology and function via programmed gene expression. Single-cell sequencing suggests that megakaryocytes are divisible into at least three functional subgroups related to platelet formation, hematopoietic microenvironment maintenance, and immunity.[1,7] Hence, specific transcriptional regulatory patterns may contribute to megakaryocyte 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 megakaryocyte development and platelet generation, such as myeloproliferative neoplasm,[9] multiple myeloma,[10] and rheumatoid arthritis.[11] Therefore, determining the epigenetic mechanisms underlying megakaryopoiesis and platelet 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), which regulate megakaryocyte development and platelet generation. Accordingly, this review is expected to provide an in-depth understanding of and comprehensive insights into megakaryocyte- and platelet-related disorders linked to hematopoietic system development.

DNA methylation in megakaryopoiesis and platelet formation

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 is initiated with the help of ten-eleven translocation 1 (TET1) protein which promotes the conversion of 5-methylcytosine 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 transcription factors 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.

Role of DNA methylation in megakaryopoiesis and platelet 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 multipotent progenitor cells into common myeloid progenitor cells and other corresponding lineages.[16]

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

Megakaryocyte 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 megakaryocytes from donor bone marrow according to ploidy and found regions, not the whole genome, which gradually underwent different degrees of methylation during polyploidization and megakaryocyte 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 transcription factors, including those of GATA1, a recombinant mothers against decapentaplegic homolog 1 (SMAD1), and T-cell acute

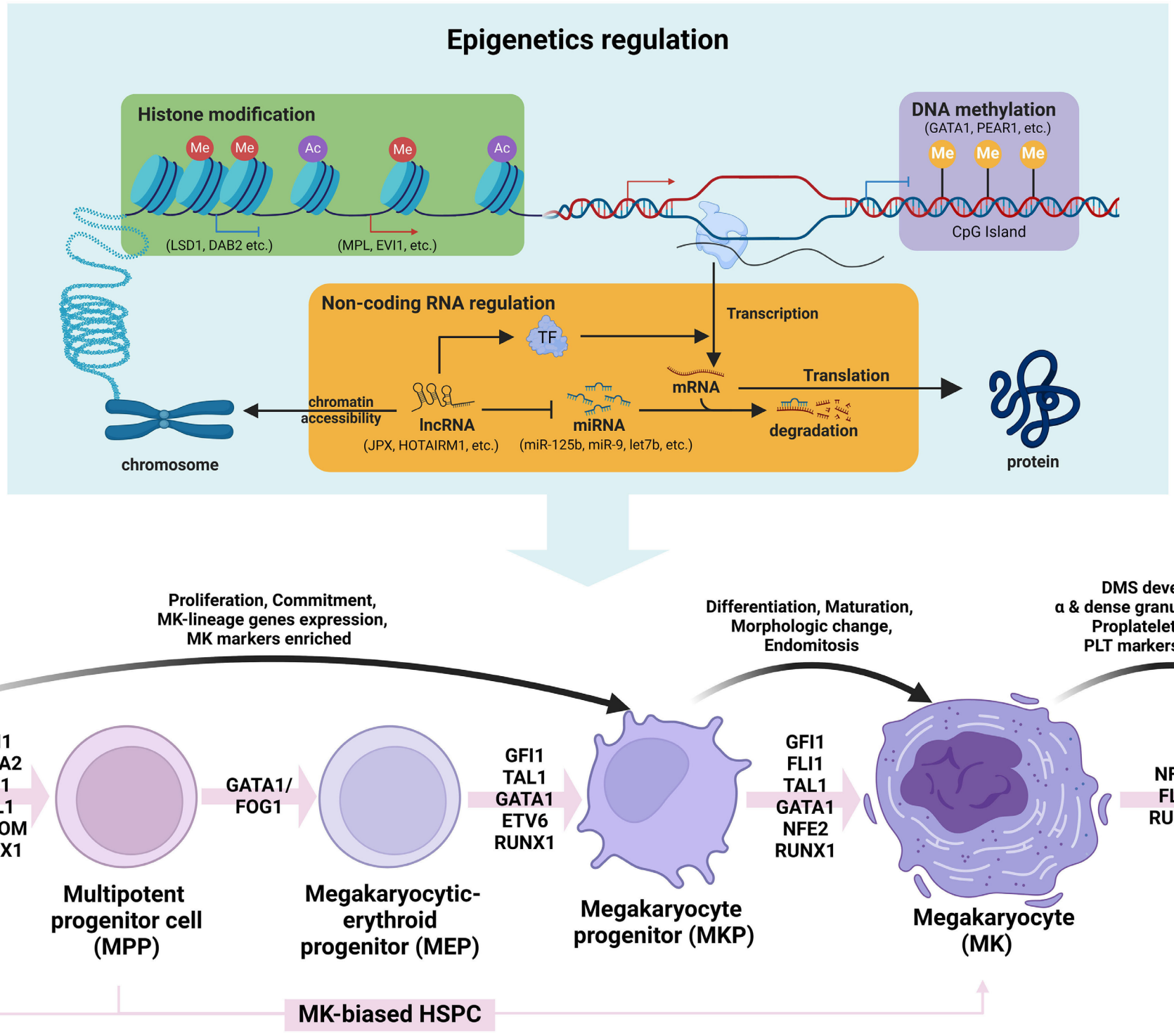

Figure 1. Epigenetic regulation involved in the pathway of differentiation starting from a hematopoietic stem cell through terminal differentiation to megakaryocytes and platelet formation and related transcription factors. TF: transcription factor; HSPC: hematopoietic stem/progenitor cell; DMS: demarcation membrane system.

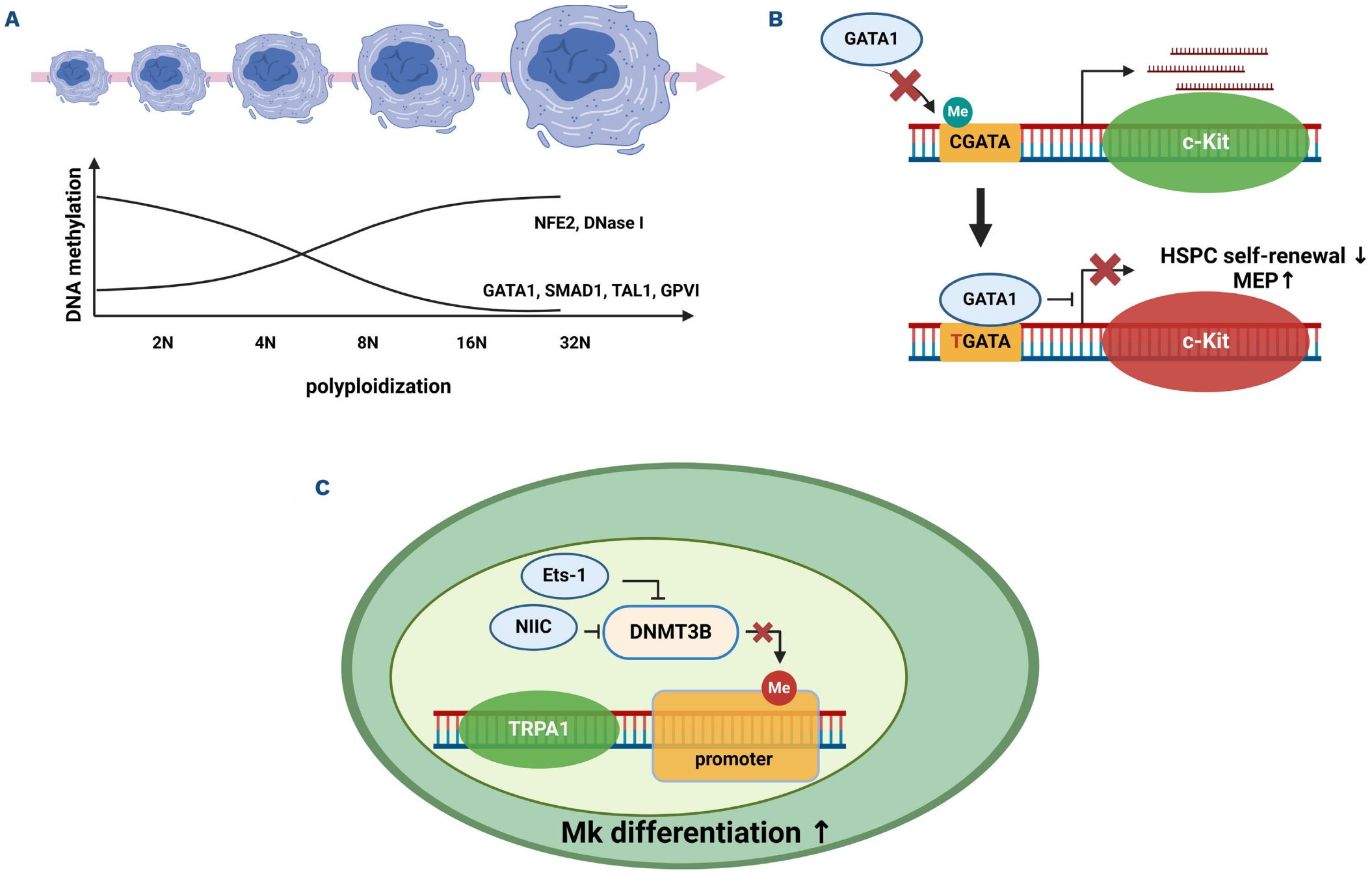

Figure 2. The mechanisms underlying DNA methylation during the regulation of megakaryopoiesis and platelet formation. (A) During the polyploidization of megakaryocytes, several critical genes related to megakaryocyte development are differentially methylated. (B) A single base alteration may induce significant changes in gene expression and lineage commitment. (C) Megakaryocyte differentiation can be affected by the activity of DNA methylase. HSPC: hematopoietic stem/progenitor cell; MEP: megakaryocyte–erythroid progenitor cell; Mk: megakaryocyte.

lymphoblastic leukemia protein 1 (TAL1).[20] Accompanying megakaryocyte maturation, several megakaryocyte-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 (*GP6*).[21]

Single nucleotide polymorphisms cause significant changes in epigenetic 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 megakaryocyte production and platelet activation.[23] The rs12041331 site on the PEAR1 enhancer is the first functional CpG island single nucleotide polymorphism associated with platelet function, where multiple methylation-sensitive transcription factor 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 megakaryocyte 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 megakaryocyte differentiation ability (Figure 2C).[24] Saran *et al.* studied the role played by megakaryocyte-derived microvesicles in the differentiation and maturation of megakaryocytes and found that microbubbles could promote the production of megakaryocytes by downregulating the expression of DNMT1, DNMT3a, and DNMT3b and inhibiting Notch homolog 1 (NOTCH1) promoter methylation.[25]

Histone modifications in megakaryopoiesis and platelet formation

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 and methyltransferases transfer modification groups to target amino sites, histone deacetylase (HDAC) and demethylases act as “erasers” by removing the corresponding group from histones or as transcriptional co-inhibitors 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, and H4K20me3).[27]

Histone acetylation and deacetylation in megakaryopoiesis and platelet formation

Histone acetylation affects the expression of lineage-specific genes and transcription factors related to megakaryocytes. Fli-1 and GATA-1 are essential for megakaryocyte 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 megakaryocyte progenitor level.[29] During megakaryocyte 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 megakaryocyte development.[30] The interaction between the KIX domain of CREB-binding protein (CBP)/p300 and myeloblastosis viral oncogene homolog (c-Myb) can synergistically regulate the expression of c-Myb-dependent genes, affecting megakaryocyte development and increasing platelet counts.[31] Moreover, certain metabolites affect histone acetylation during megakaryocyte activity, providing clinical treatment targets to improve patients’ platelet levels via dietary 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 platelets.[11] Moreover, Xie *et al.* found that H3 acetylation may be promoted by ketone-derived β-hydroxybutyrate through the inhibition of endogenous HDAC1/2 and increasing the supply of acetyl coenzyme A, effectively inducing GATA1 and NFE2 expression.[32]

HDAC – a scavenger of histone acetylation modifications – is involved in the dynamic regulation of acetylation, which affects megakaryocyte development and platelet 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 megakaryocytes. FOG-1 recruits NuRD via the interaction between its conserved N-terminal motif and the MTA2 subunit of NuRD, inhibiting the incorrect expression of megakaryocyte-specific genes that regulate lineage commitment during megakaryocyte proliferation and maturation.[34,35] In addition to affecting histone modification, Messaoudi *et al*. found that HDAC6 inhibition causes excessive cortactin acetylation, leading to dysregulation of actin assembly and terminal differentiation of megakaryocytes and, thus, platelet 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]

Role of histone methylation and demethylation in megakaryopoiesis and platelet formation

Histone methylation or demethylation may regulate gene expression, selective splicing, and the accessibility of transcription factors or regulatory complexes, among other processes, ultimately influencing megakaryocyte 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, megakaryocytes 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 megakaryocytes.[39] During most of the late megakaryocyte transcriptional program, transcription factors such as NFE2,[40] FLI1, and RUNX1[41] regulate terminal differentiation in maturing megakaryocytes by acting on H3K4me2-enriched common binding sites on enhancer sequences.[42]

Several demethylases and related factors participate in megakaryocyte development and platelet formation. For example, JARID1A is a H3K4 demethylase. In response to megakaryocyte 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 a euchromatin state, relieving DAB2-based transcriptional inhibition, playing a crucial role in megakaryocyte 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 megakaryocyte 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 megakaryocytes.[45] Meanwhile, zinc finger protein (ZNF300) alters H3K9ac and H3K9me3, enhancing megakaryocyte differentiation induced by 12-O-tetradecanoylphorbol-13 acetate.[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 megakaryocyte 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, affecting the expression of key genes in lineage development and, ultimately, megakaryocyte differentiation and platelet formation.[48] LSD1 inhibitors can interfere with the regulation of LSD1 methylation and have an impact on the function of the LSD1–GFI1B axis, causing megakaryocyte progenitor accumulation and decreased platelets.[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 megakaryocyte development.

Non-coding RNA in megakaryopoiesis and platelet formation

Mechanisms of non-coding RNA

ncRNA, 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, miRNA have been studied the most extensively. By binding to the 3′-untranslated region, miRNA promote the degradation of target mRNA and inhibit translation.[50] In addition to miRNA, the role of lncRNA in megakaryocyte development has attract-

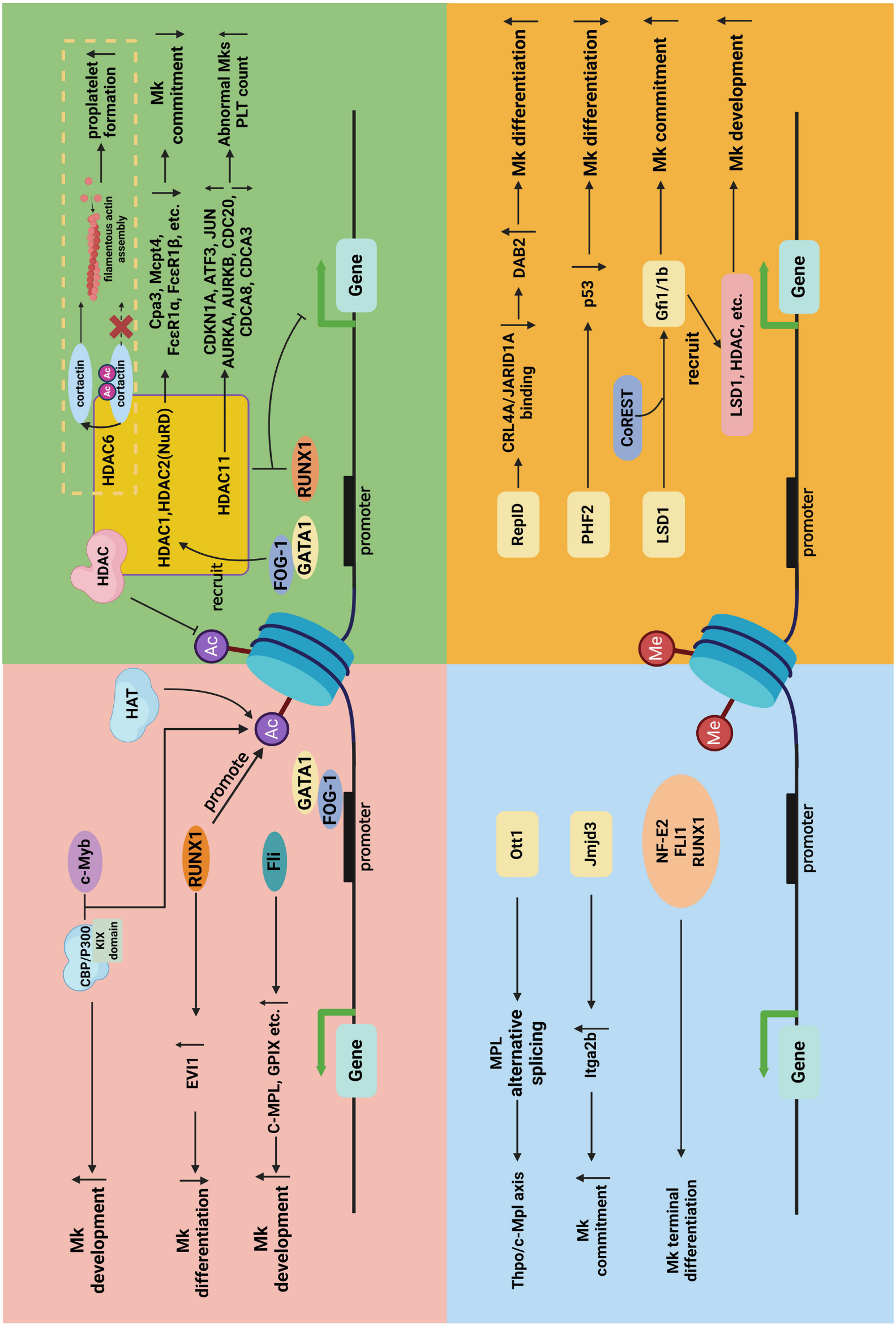

Figure 3. Histone modification during megakaryopoiesis and platelet formation. The expression of several critical genes is regulated by acetylation (top, left), deacetylation (top, right), methylation (bottom, left) and demethylation (bottom, right) of histones with the mediation of several transcription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

ed 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 transcription factors[55] have been revealed. In this section, we focus on the effects of miRNA and lncRNA on megakaryocyte development and differentiation.

MicroRNA mediate megakaryopoiesis and platelet formation

miRNA (miR) play an important role in megakaryopoiesis during different developmental phases, with some potentially interacting with regulatory factors to modulate megakaryocyte development, forming a complex regulatory network (Table 1).[56] As successors of megakaryocytes, platelets receive a diverse and complex miRNA lineage, indicating that processes associated with the epigenetic regulation of megakaryocytes are significant in elucidating the physiological effects and functions of platelets.[57]

miRNA promote megakaryocyte development by affecting transcription factors 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 megakaryocyte production.[60] In $CD34^+$ cells, high miR-146b (miR-146b-5p) expression directly affects platelet-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 megakaryocytes 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 megakaryocytes.[62] Among those upregulated in neonatal megakaryocytes, miR-99a represses the tumor suppressor protein CTDSPL, enhances RB phosphorylation, and releases E2F1, which induces G1/S transition by increasing the expression of several cyclins.[63] Meanwhile, miR-125b downregulates the p53 pathway (p53, BAK1, and CDK6) in cord blood megakaryocytes, contributing to their rapid proliferation and increasing the abundance of megakaryocytes.[64] Additionally, miR-125b and miR-99 may contribute to vincristine resistance in childhood acute megakaryoblastic leukemia 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 mature megakaryocytes derived from cord blood than those derived from peripheral blood, providing a potential target for neonatal thrombocytopenia and other platelet disorders.[66]

In contrast, some miRNA inhibit megakaryocyte development. For example, let-7b, which is downregulated in neonatal compared with adult megakaryocytes, inhibits the WNT signaling pathway by regulating frizzled family receptor 4 (FZD4) and inducing mitochondrial biogenesis, thereby regulating proliferation and differentiation during megakaryocyte development.[62] Meanwhile, miR-28a, miR-708, and miR-151 regulate megakaryocyte differentiation by inhibiting MPL expression and interfering with the TPO-MPL signaling pathway.[67] Moreover, miR-125b – expressed in human MEP – is downregulated during megakaryocyte differentiation; its overexpression may lead to excessive megakaryocyte progenitor 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 megakaryocyte differentiation by downregulating RUNX1 expression via a feedback loop. Similarly, miR-9, miR-18a, miR-30c, and miR-199a reduce RUNX1 levels during megakaryocyte production.[69] Moreover, the transplantation of HSC overexpressing miR-155 into irradiated mice effectively decreased megakaryocyte abundance in the bone marrow.[70] Meanwhile, miR-155 may induce the differentiation of MEP into megakaryocyte progenitors by reducing ETS1 and MEIS1 expression.[71] During megakaryocyte maturation and platelet generation, GPIbα promotes endomitosis and megakaryocyte maturation, while miR-10a and miR-10b target the 3′-untranslated region of *GPIBA* mRNA and decrease GPIbα expression, ultimately restricting the normal progression of late megakaryopoiesis and platelet production.[72] Additionally, miR-146a affects megakaryocyte 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 megakaryocytes and inhibiting the formation of megakaryocyte-specific colonies.[73,74]

Long non-coding RNA mediate megakaryopoiesis and platelet formation

Recent years have witnessed lncRNA as sophisticated regulators during hematopoiesis,[75] suggesting that they may also affect megakaryopoiesis and platelet formation (Table 1). HOX antisense intergenic RNA myeloid 1 (HOTAIRM1) impacts the p53-mediated regulation of cyclin D1 by sponging miR-125b to promote megakaryocyte maturation. Meanwhile, HOTAIRM1 suppression may lead to abnormal megakaryocyte differentiation and maturation.[76] Just proximal to XIST (JPX) is likely involved in releasing transforming growth factor-β receptor expression by sponging oncogenic miRNA (miR-9-5p, miR-17-5p, and miR-106-5p) during megakaryocyte differentiation, augmenting the activity of ERK1/ERK2 and PI3K/AKT pathways, which in turn enhances the poly-

ploidization and terminal maturation of megakaryocytes.[77] Differentiation-antagonizing non-coding RNA (DANCR) – a lncRNA highly expressed at the MEP stage – promotes erythroid differentiation by compromising megakaryocyte differentiation and coordinating with chromatin accessibility and transcription factors, such as RUNX1.[78] Knocking down nuclear paraspeckle assembly transcript 1 (NEAT1), which is abundant in platelets, may lead to decreased cell differentiation, platelet-like particle activity, and interleukin-8 levels. Meanwhile, weakening the interaction between NEAT1 and splicing production factor proline/glutamine-rich (SFPQ) via NEAT1 knockdown inhibits interleukin-8 expression, causing platelet hyperactivation and promoting thrombosis, as well as megakaryocyte 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 megakaryocyte terminal differentiation.[80]

Clinical application of epigenetic modulation of megakaryopoiesis and platelet formation

Improving the prognosis of clinical megakaryocyte- and

Table 1. Several non-coding RNA and their influences on megakaryopoiesis and platelet formation.

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

ncRNA: non-coding RNA; miRNA/miR: microRNA; c-SRC: c-src tyrosine kinase; MK: megakaryocyte; c-Myb: myeloblastosis viral oncogene homolog/MYB proto-oncogene; MAF: v-maf musculoaponeurotic fibrosarcoma oncogene homolog; PDGFRA: platelet-derived growth factor receptor alpha; FZD4: frizzled family receptor 4; CTDSPL: CTD (carboxy-terminal domain, RNA polymerase II, polypeptide A) small phosphatase-like; DICER1: endoribonuclease dicer; MKP: megakaryocyte progenitor cell; MEP: megakaryocyte-erythroid progenitor cell; ST18: suppression of tumorigenicity 18; RUNX1: runt-related transcription factor 1; MPL: myeloproliferative leukemia protein proto-oncogene/thrombopoietin receptor; ETS1: E26 avian erythroblastosis virus transcription factor-1; MEIS1: homeobox protein Meis1; GP1bα: glycoprotein 1b alpha; PLT: platelet; TRAF6: TNF receptor associated factor 6; CXCR4: chemokine C-X-C-motif receptor 4; lncRNA: long non-coding RNA; HOTAIRM1: HOX antisense intergenic RNA myeloid 1; JPX: just proximal to XIST; TF: transcription factors; DANCR: differentiation antagonizing non-coding RNA; NEAT1: nuclear paraspeckle assembly transcript 1; SFPQ: splicing production factor proline/glutamine-rich; AS-RBM15: antisense-RNA binding motif protein 15; RBM15: RNA binding motif protein 15.

platelet-related diseases by regulating epigenetics has become popular. Targeting DNA methylation has become a strategy to interfere with disease development and progression. Low doses of the demethylating agent decitabine significantly increase the number of mature polyploid megakaryocytes and the number of platelets released in myelodysplastic syndrome patients, accompanied by abnormal DNA methylation and a significant decrease in platelet count.[81] 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 can significantly promote the generation of megakaryocytes in umbilical cord blood and thus increase the number of platelets. This has the capacity to be exploited to achieve large-scale *in vitro* platelet preparation to meet clinical resource needs.[83] Use of certain HDAC inhibitors, such as abexinostat,[84] panobinostat,[85] and belinostat,[86] as well as histone demethylase inhibitors, such as INCB059872, may be followed by drug-induced thrombocytopenia resulting from dysregulated megakaryopoiesis. Therefore, further exploration of the specific mechanisms by which histone modification affects platelet 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 multiple myeloma, thrombocytopenia was found to be potentially associated with the ricolinostat dose but not chemotherapy. This highlights the regulatory effect of HDAC6 on platelet generation and its therapeutic capacity to prevent platelet-related side effects.[87] Similarly, Ito *et al*. found that secreted nardilysin, which has been shown to affect platelet yield, can coordinate with HDAC6 to regulate microtubule conformation and remodeling through deacetylation/acetylation, further facilitating platelet shedding.[88]

To date, diverse ncRNA, including miRNA and lncRNA, have been characterized together with 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 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 platelet release are lacking.

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 megakaryocytes occurs early in the hematopoietic process, with megakaryocyte 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 megakaryocyte and platelet production. These findings further elucidate the complex molecular mechanisms underlying the growth and development of megakaryocytes, providing new insights into platelet 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 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 modifications, in addition to modification of 5-methylcytosine, N6-methyladenine modification in bacteria has recently been identified in some eukaryotes.[91] In RNA, the N6-methyl-adenosine 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 megakaryocyte and platelet 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 island single nucleotide polymorphism exhibits enrichment in histone-modified H3K4me1 and H3K27ac, which may prove beneficial for PEAR-1 expression.[17]

As the interest in megakaryocyte development and platelet 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 megakaryocyte development, such as acute megakaryoblastic leukemia,[93] but also to others, such as myeloproliferative neoplasms and multiple myeloma.[9,10,94] Platelet formation abnormalities have also been evaluated, including certain epigenetic mutations. Second, to alleviate the shortage of blood resources in transfusion medicine, research has centered on

in vitro platelet formation, revealing that epigenetic regulation shows potential as a process that can be utilized to improve *in vitro* platelet formation.[95] For instance, Ito *et al*. reported on the important physical regulatory effect of turbulence on platelet formation and developed a turbulent flow-based bioreactor, VerMES, providing an innovative strategy for *ex vivo* platelet manufacturing.[88] Third, studies on megakaryocytes based on single-cell sequencing have revealed that just as the roles played by megakaryocytes and platelets in the hematopoietic system are important, so too are the roles they play as immune regulatory cells in infections[96] and autoimmune diseases.[97] The formation of an immune-megakaryocyte subpopulation and the engineered transformation of platelets are inextricably linked to gene expression and regulation. Thus, research on the roles of epigenetics in these areas may be of considerable benefit.

Disclosures

No conflicts of interest to disclose.

Contributions

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

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