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GENE REGULATION IN HEMATOPOIESIS: THE ESSENTIAL ROLE OF ENHANCERS IN LINKING GENOTYPE TO PHENOTYPE

[1*]Rab Nawaz Sathio, [2]Jawed Iqbal Bajeer, [3]Aamir Hussain

[1]Postgraduate Resident, Dept. of Pathology, Liaquat University of Medical & Health Sciences, Jamshoro
[2]Senior Lecturer, Dept. of Physiology, Bilawal Medical College @ Liaquat University of Medical & Health Sciences, Jamshoro
[3]Assoc. Prof. Dept. of Physiology, Bilawal Medical College @ Liaquat University of Medical & Health Sciences, Jamshoro

***Corresponding Author:** Rab Nawaz Sathio (rns.well@gmail.com)

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ABSTRACT

Blood consists of a diverse range of specialized cells, all emerging from a common progenitor, the hematopoietic stem cell (HSC), and possessing the same genetic makeup. The variety in cell types is the result of different transcriptional programs that are established during the process of hematopoiesis. In this process, gene expression is controlled by various elements, with enhancers being crucial. These segments of DNA attach to transcription factors, enhancing the transcription of the genes they influence. Hence, hematopoiesis entails activation of particular enhancer groups among HSCs and subsequent lineages, which are essential in dictating the gene groups responsible for cell shape and functionality. Interruptions in this precise process can lead to drastic effects, notably in hematologic malignancies, where the normal function of enhancer – driven transcriptional regulation is disrupted, and causing inappropriate expression of oncogenes that lead to cellular transformation. This review seeks to shed light on the fundamental aspects of enhancers with their critical part in the mechanism of transcription, focusing on their impact in both health and pathological hematopoiesis. We will discuss specific examples of enhancers that control major hematopoietic regulators and examine the key causes of enhancer deregulation in diseases such as leukemia and lymphoma.

Keywords: Hematopoiesis, Genes, Enhancers, Genotype, Phenotype.

Introduction

The hematopoietic system in humans is an intricate network of various cell types, each unique in shape and function, serving roles from immunological defense to distributing nutrients and aiding in blood clotting. All these cells, despite their varied functions, carry an identical genetic blueprint: a set of 46 chromosomes encompassing approximately 20,000 protein – coding genes. This similarity in genetic material poses an intriguing questions that how these cells with same genetic makeup diversity into multitude of cell types found in blood and contribute to the complex structure of human body, the solution lies in gene expression regulation[1,2]. Roughly half of the genes in humans are expressed universally, but there are specific genes that are crucial in defining the identity of a cell and are activated only in certain tissue types. These patterns of gene expression shift during the hematopoietic process, as the cells increasingly differentiate and compel to specific lineages[3]. Understanding the dynamics of active versus inactive genes, which uphold distinct transcriptional programs, requires looking past the gene – coding regions to a more obscure genome part.

The conception of gene regulation has its roots in the pioneering studies of Jacob and Monod on bacterial lactose metabolism. In their landmark research, they proposed that molecules known as repressors could bind to regulatory elements (operators) on DNA, thus controlling protein production through short – lived RNA intermediates[4]. This foundational concept, despite significant progress in the field, still underscore the two main mechanisms of transcriptional regulation. On one hand, transcription factors (TFs), which are DNA – binding molecules, function in *trans* to regulate gene expression through genome. Alternatively, the areas of non-coding DNA to which TFs bind are referred to as *cis* – regulator elements (CREs), which are specific to nearby genes. Within these CREs, enhancers stand out as crucial elements for establishing identity of the cell[4].

This review focuses on the enhancers and their role in the transcriptional regulation during the process of hematopoiesis, especially their involvement in malignant transformations.

Transcriptional Regulation Enhancers

Principles and Mechanisms of Transcriptional Regulation

The process of gene expression begins with transcription, where enzymes from the RNA polymerase family translate a DNA sequence into a corresponding RNA sequence. RNA polymerase II is chiefly responsible for transcribing all protein – coding genes as well as majority of noncoding genes, although RNA polymerase I and III handle the transcription of ribosomal RNA and particular small noncoding RNAs[5]. The transcription unfolds in three distinct phases: initiation, elongation, and termination, starting at a gene's 5'transcriptional start site (TSS) and moving towards the 3' end. Post – transcription, the mRNA produced from protein – coding genes undergoes translation in

ribosomes, where proteins are synthesized based on the codon sequences in the mRNA[6].

Control over when and where genes are expressed hinges on *cis* – regulatory elements (CREs), including promoters, enhancers, insulators, and silencers. The promoter, initially pinpointed by Monod et al, serves as a signal for the onset of transcription at a start of gene, steering RNA polymerase II into action[4]. At the heart of this process is the core promoter, a pivotal 50 – base pair stretch near the TSS, housing pre – initiation complex made up of RNA polymerase II and general transcription factors? Transcriptional factors, which latch onto specific DNA motifs, play a key role in adjusting the pace of initiation by drawing in components of the transcription apparatus. These factors operate via two key domains: DNA – binding domains that identify transcription factor – binding sites, and effector domains that interrelate by various proteins such as RNA polymerase II and transcriptional cofactors that either activate or repress transcription. The binding sites for transcription factors at CREs are densely packed and precisely organized to ensure effective team work among transcription factors, utilizing several cooperation methods such as direct protein interactions, DNA – mediated interactions, and other indirect strategies for nuanced transcriptional regulation[7,8].

While core promoters are capable of initiating transcription on their own, they typically have minimal basal activity, often necessitating a boost from enhancers to achieve desired expression levels. Enhancers facilitate RNA polymerase II recruitment by linking up through promoters far away in the genome[9]. Additionally, other distant CREs play roles in gene regulation, such as silencers, which reduce transcription by drawing in repressive transcription factors, and insulators. The insulators bind to certain proteins like the CCCTC – binding factor or cohesin, creating looped domains that prevent cross – domain interactions while promoting interactions in same loop. The interplay among CREs and associated genes generally occurs in topologically associated domains (TADs), which are insulated areas that help orchestrate gene regulation[10].

Enhancers

Enhancers are specific DNA regions, often spanning a few hundred base pairs that contain sites for binding transcription factors (TFBS) and enhance transcription from associated promoter regions[11]. Initially identified in 1980s, the first remarkable enhanced was a 72 – base pair segment from SV40 virus, which notable boosted reporter gene transcription by approximately 200 – times, regardless of its proximity or direction relative to the gene[12]. The discovery of the first human enhancer soon followed in an intron of the immunoglobulin heavy chain (IGH) gene, distinguished by its activity exclusively in B cells[13]. These initial discoveries highlighted several critical attributes of enhances: their ability to elevate transcription in target genes, operate regardless of their orientation or distance to the gene, and frequently display tissue – specific activity. Moreover, enhancers retain their operational capacity across different genomic environments, a fact established in various reporter assays with inferences for disease

researches. Later investigations have steadily affirmed such attributes, emphasizing vital role of enhancers in controlling gene expression specific to particular tissues[14].

Current estimates of enhancers in the human genome vary greatly, ranging from about 40,000 to more than a million, depending on the analytical methods and tissue types studied. This figure significantly surpasses the number of identified promoters, although only a subset of these enhancers is active in any particular cell lineage. The predominance of transcription factor binding at enhancers underscores their essential function in managing tissue – specific gene expression and determining cellular identify[15]. Enhancers located in conserved genomic regions are especially crucial in processes like development and disease, with their activity closely linked to gene expression on a genome – wide scale. In systems like hematopoiesis, patterns of chromatin accessibility provide a more accurate reflection of cell types than gene expression alone[5]. Recent advances in single – cell analysis have shown a direct association between accessibility of CRE and expression of gene, with activation of enhancers often preceding gene transcription in differentiated cells. This has been corroborated in studies using Venus – YFP reporters in embryonic stem cells and bacterial methylation labeling in the differentiation of enterocytes[16–18].

CEBPA, a key regulator in myeloid cells, serves as a prime tissue – specific regulation example, with distinct enhancers being active among tissue expressing the gene as well as inactive in those where CEBPA is not expressed[19]. Enhancers can be broadly classified into two types: ubiquitous enhancers, active across a range of tissues, and progressive or tissue – specific enhances, confined to particular types of cells. The specificity of tissue is determined by the recruitment of particular transcription factors and cofactors, which depends on the transcription in the cell and convenience of their attachment enhancers sites[15]. For example, sites of binding for ETS, C/EBP, and NK-κB are available in enhancers specific to monocytes, while neuron enhancers are augmented with SOX and RFX proteins[20]. The binding of transcription factors enhances the specificity of developmental enhancers both in terms of tissue and genome, preventing unwanted transcriptional activity and allowing precise control over transcription patterns during cell differentiation. In hematopoietic stem and progenitor cells (HSPCs), a group of seven transcription factors – TAL1, LMO2, LYL1, ERG, GATA2, FLI1, and RUNX2 – often come together at CREs to collaboratively regulate gene expression[21].

While lineage – specific transcription factors (LDTFs) determine the set of tissue – specific enhancers among cell, all enhancers are not immediately functional. Few, termed inducible enhancers, are activated only upon the attachment of extra transcription factors in reaction to external or internal cues[22]. Such feature is particularly prevalent in flexible cells. For instance, macrophages exposed to ligands of TLR4 trigger pre – existing enhancers governing genes related to inflammatory responses[23]. These enhancers, primed by a combination of LDTFs, become fully operational following

stimulation by transcription factors. In a similar manner, CD4+ T cell adopt a regulatory phenotype after TCR stimulation, mainly through the activation of pre – set enhancers of FOXP3[24]. A subset of enhancers, known as latent enhancers, are newly formed in response to external stimuli, representing a small proportion of all enhancers activated while processes like differentiation in macrophages or in T cell regulation. The functionality of inducible enhancers is highly reliant on the presence of cohesin[25].

Activation of Target Promoters by Enhancers

Enhancers play a crucial role in refining gene expression by transferring regulatory signals to promoters through transcription factors (TFs) and transcriptional cofactors. These signals modulate transcription at various stages[26]. For instance, during initiation, certain proteins aid in assembling and stabilizing the Pre – initiation complex (PIC) and in recruiting RNA polymerase II (RNA pol II), as seen with the mediator complex and p300/CBP proteins. On the other hand, few essential promoters inherently attract increased RNA pol II levels and are primarily constrained by elongation. In such scenarios, their corresponding enhancers usually exhibit elevated protein levels that facilitate pause – release. Importantly, while the size of transcriptional bursts is fixed characteristic of essential promoter, the occurrence of such bursts can be enhances through developmental enhancers[27].

On intriguing aspect of enhancer biology in their capacity to activate genes located far away. Most enhancers are found with 200kb of their targeting promoters, having median space of about 120 kb, or 24 kb when considering only experimentally validated enhancers[28]. However, there are exceptions, such as the limb – specific enhancer of the SHH gene situated I Mb far and the hematopoietic enhancer of the MYC gene situated 1.7 Mb downstream. The facilitation of enhancer – mediated promoted activation is achieved through chromatin looping, a concept initially proposed in the 1980s and later substantiated by studies on the globin genes[29]. Further evidence included the opinion that artificial twisting between enhancer and promoter could trigger expression of gene. Live imaging analysis of Drosophila embryos also verified that close physical closeness among an enhancer and a promoter is linked to activation of transcription. While enhancer – promoter (E-P) connections are essential for expression of gene, they are not always adequate, as they can pre – exist prior activation. Therefore, additional elements like the presence of suitable TFs or biochemical compatibility between enhancers and promoters play a role. These pre – existing contacts may expedite transcription activation in response to exterior stimuli or during differentiation[30].

As per loop extrusion hypothesis, chromatin loops are created by looping action of Structural Maintenance of Chromosomes (SMC) proteins such as cohesin or condensin. This process continues until it encounters a CTCF protein aligned correctly. This mechanism is crucial both

for E-P loop formation and TAD boundary establishment. While CTCF is commonly present at TAD boundaries, it appears less frequently at E-P loop sites. Instead, these cell – specific interactions sites often contain a DNA – binding zinc factor known as YY1. Depleting YY1 alters expression of gene and disrupts E-P loops, that are reestablished once levels of YY1 are replenished[31]. The mediator complex is too involved in short – range connections alongside cohesin, however it appears to function more as a facilitator of information transfer from TFs to RNA pol II rather than as a structural component, aiding in the formation of the pre – initiation complex. Moreover, cohesin at the non-CTCF places might be alleviated by additional TFs[32].

Consistent with this model, eliminating either cohesin or CTCF caused the depletion of all loops mediated by CTCF. Though, the impact of these alterations on gene regulation is relatively modest, indicating that more complex spatial organization layers exist outside loops mediated by cohesin[33]. For instance, LDB1, an adaptor protein forming loops when recruited through TFs, without directly binding to DNA, plays a role in this complex organization[34]. Research by Hsieh et al. suggests that E-P contacts persist even after the acute depletion of these architectural proteins, proposing a model where such proteins are essential for loop formation but not necessarily for their maintenance[35].

Role of Enhancers in Hematopoiesis

The process of hematopoiesis is intricately managed, ensuring a consistent production of diverse blood cell types. It initiates from hematopoietic stem cells (HSCs), which evolve into specialized precursor cells and eventually mature into fully functioning blood cells. During this progression, there are substantial epigenetic shifts including chromatin restructuring, DNA methylation patterns changing, histone tail alterations, and variations in chromatin connectivity. These changes are orchestrated by several essential transcription factors (TFs), pivotal in defining cell destiny and preserving cellular identity, with their expression being tightly controlled[36,37].

In this mechanism, enhancers play a crucial role, particularly in cell – specific gene expression. This is evidenced by the discovery that single – nucleotide polymorphism (SNPs) influencing blood characteristics and diseases frequently occur in areas presumed to be enhancers. These SNPs represent a considerable fraction, roughly 19% to 46% of the genetic variability linked to these blood traits. Interestingly, SNPs associated with specific blood properties tend to be enriched in enhancers regions that are active in corresponding cell types[38]. For examples, SNPs affecting coagulation traits are predominantly found in enhancer areas active in megakaryocytes. Such genetic variants typically modify the binding sites of hematopoietic TFs and are linked to alterations among chromatin openness[39]. Experimental disruptions in certain hematopoietic enhancers in animal models have led to significant blood – related issues. These include neutropenia and a reduction in HSCs in mice without a CEBPA enhancer, unsuccessful HSC generation in mice with an eliminated

GATA2 enhancer, and the induction of fetal hemoglobin production by altering the BCL11a enhancer[40–42].

Various theories have been proposed to elucidate the development of lineage – related transcriptional processes in hematopoiesis. One model suggests that as cells evolve towards specialization, there is a new formation and activation of regulatory regions[43]. On the other hand, the multilineage priming models argues that early progenitors hold potential for various differentiation paths, which become progressively limited. This blank state model is based on single – cell RT-PCR findings in HSPCs that showed simultaneous expression of markers specific to lineages at small levels prior commitment to particular lineage. In this model, myelo – erythroid genes are exclusive to common myeloid progenitors (CMPs), while B and T lymphoid lineage genes appear only among common lymphoid progenitors (CLPs)[43].

With introduction of next – generation sequencing (NGS), it became feasible to differentiate such models. Single – cell ATAC – seq revealed that in HSCs, chromatin is initially more reachable but become more condensed as cells differentiate. However, this is mainly observed in promoters, while new open chromatin areas at enhancers are often formed during differentiation[44]. Histone profiling, including markers like H3K4me1, H3Kme2, H3K4me3, and H3K27ac, indicated that lineage commitment in hematopoiesis is associated with extensive chromatin landscape changes. About 90% of enhancers alter state, with 60% being active in HSCs and specific lineages, supporting multilineage priming. However, the remaining are formed anew during differentiation[45]. The establishment of new enhancers primarily occurs at critical points, such as in CMPs and granulocyte – monocyte progenitors (GMPs) during myelopoiesis. Additionally, histone mark acquisition occurs subsequently, characteristically opening with H3K4me1/2 at poised enhancers in initial progenitors and progressing to H3K27ac with the onset of transcription[38].

The current consensus is that hematopoiesis adheres to a hybrid model combining restricted multilineage priming a new enhancer activation. However, even newly developed enhancers undergo priming prior their full activation in future hematopoiesis stages, as indicated by presence of H3K4me1 in enhancers of multipotent progenitors[46]. This is further supported by the observation that H3K4me1 – based clustering aligns progenitors with their mature cell counterparts, unlike RNA – seq data[47]. Additionally, a research integrating RNA-seq and ATAC-seq across main types of blood cells revealed that availability of chromatin in HSCs/MPPs precedes transcriptional alterations in future phases[48].

The dynamic activity of enhancers in hematopoiesis is associated with their occupation by transcription factors (TFs), particularly master regulators that define fate of cell. These TFs usually act as pioneer factors, remodeling chromatin to allow other TFs to bind. Expression of these master regulators can direct differentiation into specific lineages or even reprogram committed cells into different lineages. A group of these master regulators forms a TFs

heptad that simultaneously attach CREs linked to genes essential in development of hematopoiesis, including those programming the TFs. While these seven TFs often co – localize in HSPCs, specific combinations are unique to certain progenitors, influencing fat decisions and regulating expression of genes specific to lineage[49]. Interestingly, availability of chromatin at just nine enhancers attached by the heptad can calculate identity of cell in initial hematopoiesis phases. Furthermore, heptad attachment may occur before E-P loops formation in more mature cells, suggesting that it is a stepwise progression[50].

Hematopoietic Master Regulators Control by Enhancers

The creation of lineage – specific processes includes changes in a vast number of enhancers, yet the ones governing master regulators stand out for their critical role in activating and maintaining other enhancers unique to each cell type.

PU.1

SPI1 gene, encoding the PU.1 protein, is integral to development of both lymphoid and myeloid cells. It is crucial for producing granulocyte – monocyte progenitors and common lymphoid progenitors, but not for the generation of erythrocytes or megakaryocytes[51]. B-cell differentiation is linked to lower PU.1 levels, while higher concentration drive myelopoiesis, often to the exclusion of other lineages. In the realm of myeloid lineage, an increase in PU.1 predominantly encourages macrophage commitment over granulocyte development, and a reduction in PU.1 is associated with an increase in neutrophil count. Present in initial T-cell precursors until DN2 stage, PU.1 must decrease for complete maturation of T-cell. The crucial role of PU.1 in such mechanisms is due to its ability as a developer factor to initiate nucleosome remodeling in enhancers specific to macrophages, leading to the recruitment of H3K27ac and H3K4me1[52].

Expression of PU.1 is stringently regulated through multiple distal enhancer. A prominent enhancer, situated roughly – 15kb upregulation of the SPI1 gene (or -14kb in rats), is essential for maintaining optimal levels of PU1. Removal of this enhancer, known as the upstream regulatory element (URE), results in a significant drop of about 80% in PU1. Levels, impairing hematopoietic stem cell functionality and hindering the completion of myeloid differentiation, which could lead to leukemia. The influence of the -15kb enhancer across the blood cell types designates it as a broad hematopoietic enhancer. The specific transcriptional regulation in every type of cell managed by interactions to additional upregulating *cis* – regulatory elements. Among these is a -12kb enhancer which is active in myeloid lineage but not in B cells. The stimulation of the -12kb enhancer is controlled indirectly by interaction of C/EBPα with URE, alongside PU.1, forming a self – regulatory loop. In B cells, the recruitment of lymphoid transcription factors to URE is considered adequate to initiate PU.1 transcription, bypassing the need for the -12kb enhancer and leading to

the reduced level of PU.1 necessary for B-cell lineage differentiation[53].

C/EBPα

The C/EBP alpha (C/EBPα), a pivotal regulator programmed by CEBPA gene, is involved in the development of myeloid cells, including the formation of granulocyte-monocyte progenitors and the processes of ganulopoiesis and monopoiesis. Remarkably, high levels of C/EBPα can initiate a myeloid differentiation pathway among lymphoid progenitor cells and even re-program the B cells to the macrophages. Beginning its expression in early myeloid progenitor cells, C/EBPα orchestrates the progression of granulocyte-monocyte progenitors, simultaneously suppressing the development of erythroid cells. It acts as a regulatory support at the granulocyte-monocyte progenitor stage, with elevated levels triggering granulocyte differentiation through the activation of genes such as GFI1 and CEBPE, and lower levels favoring monopoiesis. This process is characterized by C/EBPα interaction with both established and newly emerging enhancers during differentiation, including those linked to PU.1, indicating its potential as innovator factor[54].

CEBPA gene expression in myeloid lineage cells is significantly influenced through a +42kb downstream enhancer (+37kb in mouse models), actively specifically in blood – related tissues. While several enhancers in proximity to CEBPA show H3K27ac markings, only the +42kb and +9kb enhancers are active in hematopoietic stem and progenitor cells. The +42kb enhancer, binding preferentially with hematopoietic transcription factors, appears to be pivotal in kick – starting CEBPA expression, facilitating evolution from common myeloid progenitors into granulocyte-monocyte progenitors[55]. Disruption of this enhancer among mice impedes the progression from common myeloid progenitors into granulocyte-monocyte progenitors, causing stark absence of granulocytes. Activation of this enhancer is influenced in part by the binding of RUNX1, and its absence results in diminished CEBPA expression and compromised granulopoiesis. The role of additional regulatory components, predominantly the +9kb enhancer, for modulating expression of CEBPA, remains a subject for further study[54,55].

GATA2

Belonging to GATA family of zinc – finger transcription factors, GATA2 is distinguished by its binding capability to (A/T) GATA (A/G) sequence, commonly identified as WGATAR. The GATA protein family participates in a specialized switching process, where various GATA proteins sequentially replace one another during key stages of cell differentiation[56]. An example of this is in erythropoiesis, where GATA1 supersedes GATA2 at its own promoter site, resulting in the reduction of GATA2 transcription. GATA2 plays an important part in growth and maintenance of hematopoietic stem cells (HSCs), their development during the embryonic stage, and in the activities of granulocyte-monocyte progenitors. It is prominently expressed in HSCs, initial stages of myeloid progenitors, as well as in erythroid cells[57].

The critical function of GATA2 in hematopoietic processes demands meticulous control over its expression. This control is mediated by several

enhancers, which also function at sites for GATA switch. These include a +9.9 kb enhancer within an intron (+9.5 kb in mice), various enhancers located proximally, and a distant enhancer at -110 kb (-77 kb in mice). Although, proximal enhancers are not vital for expression of GATA2 or hematopoiesis, the absence of +9.5 kb or -77 kb enhancers drastically lowers the levels of GATA2, adversely affecting normal hematopoietic function. The -77 kb enhancer primarily affects GATA2 expression during the commitment to the myeloid lineage, whereas the +9.5 kb enhancer is crucial for the development of HSCs. In the humans, mutations that inactivate the +9.9 kb enhancer can lead to GATA2 deficiency. This condition is marked by a range of cytopenias, increased susceptibility to infections, and a heightened risk of increasing acute myeloid leukemia (AML) and familial myelodysplastic syndromes (MDS)[58].

PAX5

PAX5 plays an essential role as a transcription factor for ensuring the definitive commitment to the B-cell lineage, activating genes that are specific to B-cells and inhibiting those of alternate lineages. This action builds upon the initial direction provided to CLP cells by transcription factors such as TCF3 (E2A) and EBF1. While the formation of pro-B cells can occur in the absence of PAX5, these cells are incapable of progressing to later stages, often expressing genes characteristics of different lineages[59]. PAX5 is exclusively present in cells within the B-cell lineage, extending from pro-B cells into fully mature B-lymphocytes, and it ongoing presence is crucial for maintaining their distinct features. Studies have demonstrated that eliminating PAX5 in mice leads to regression of these cells back to a more primitive progenitor state. PAX5 is known for directly interacting with enhancer regions, recruiting factors that remodel chromatin and modify histones to either activate or repress the target genes[60].

PAX5 expression itself is governed by a particular enhancer situated in its fifth intron, which is activated by factors including PU.1, IRF4, IRF8, and NF-$_{\kappa}$B. Within embryonic stem cells (ESCs), this area is initially repressed through DNA methylation, then undergoes demethylation as hematopoiesis commences, becoming active in a certain group of CLPs, presumably those destined to develop into B-cells. This activation persists across all stages of B-cell development, reducing only when the cells differentiate into plasma cells. On the other hands, its promoter in ESCs is initially inactivated by H3K27me3, but is later remodeled during lymphopoiesis due to the binding of EBF1[61].

The foregoing scenarios offer an indication into elaborate systems that regulate expression of gene in both spatial and temporal dimensions. They highlight the challenges associated with the study of enhancers and reflect previously mentioned traits, including ability of enhancers to operate over considerable distances and existence of various enhancers that collectively moderate transcriptional outcomes.

Conclusion

In recent years, a significant strides have been made in understanding how genes are regulated, thanks to breakthroughs in technologies like genome – wide sequencing and gene editing. This progress has highlighted the importance of enhancers in controlling gene expression specific to tissue types, vital for both maintaining identify of cells and their response to external factors. These enhances play a pivotal role in the differentiation of hematopoietic cells, either triggering or suppressing gene activity as cells mature. Disruptions in this process can lead to abnormal gene expression patterns, which in turn may contribute to the development of cancers. Such disruptions can arise from different processes, including the misappropriation of enhancers or the formation of new enhancers, influencing the expression of cancer – related genes or the suppression of genes that prevent tumors.

References

1. Kumar A, D'Souza SS, Thakur AS. Understanding the Journey of Human Hematopoietic Stem Cell Development. Stem Cells Int [Internet]. 2019 [cited 2024 Jan 23];2019:2141475. Available from: /pmc/articles/PMC6526542/
2. Zhang Y, Gao S, Xia J, Liu F. Hematopoietic Hierarchy – An Updated Roadmap. Trends Cell Biol. 2018 Dec 1;28(12):976–86.
3. Pollen AA, Kilik U, Lowe CB, Camp JG. Human-specific genetics: new tools to explore the molecular and cellular basis of human evolution. Nat Rev Genet. 2023;24(1):687–711.
4. Lewis M. A tale of two repressors – a historical perspective. J Mol Biol [Internet]. 2011 May 5 [cited 2024 Jan 23];409(1):14–27. Available from: /pmc/articles/PMC3104267/
5. Guo J. Transcription: the epicenter of gene expression. J Zhejiang Univ Sci B [Internet]. 2014 [cited 2024 Jan 23];15(5):409–411. Available from: /pmc/articles/PMC4076597/
6. Xu B, Liu L, Song G. Functions and Regulation of Translation Elongation Factors. Front Mol Biosci [Internet]. 2021 Jan 19 [cited 2024 Jan 23];8:816398. Available from: /pmc/articles/PMC8807479/
7. Sahu B, Hartonen T, Pihlajamaa P, Wei B, Dave K, Zhu F, et al. Sequence determinants of human gene regulatory elements. Nat Genet [Internet]. 2022 Feb 21 [cited 2024 Jan 23];54(3):283–94. Available from: https://www.nature.com/articles/s41588-021-01009-4
8. Riethoven JJM. Regulatory regions in DNA: promoters, enhancers, silencers, and insulators. Methods Mol Biol [Internet]. 2010 [cited 2024 Jan 23];674:33–42. Available from: https://pubmed.ncbi.nlm.nih.gov/20827584/
9. Haberle V, Stark A. Eukaryotic core promoters and the functional basis of transcription initiation. Nat Rev Mol Cell Biol [Internet]. 2018 Jun 26 [cited 2024 Jan 23];19(10):621–37. Available from: https://www.nature.com/articles/s41580-018-0028-8
10. Zhang Y, See YX, Tergaonkar V, Fullwood MJ. Long-Distance Repression by Human Silencers: Chromatin Interactions and Phase Separation in Silencers. Cells [Internet]. 2022 May 1 [cited 2024 Jan 23];11(9):1560. Available from: /pmc/articles/PMC9101175/
11. Spitz F, Furlong EEM. Transcription factors: from enhancer binding to developmental control. Nat Rev Genet [Internet]. 2012 Aug 7 [cited 2024 Jan

23];13(9):613–26. Available from: https://www.nature.com/articles/nrg3207
12. Wang X, Cairns MJ, Yan J. Super-enhancers in transcriptional regulation and genome organization. Nucleic Acids Res. 2019;47(22):11481–11496.
13. Rodriguez OL, Safonova Y, Silver CA, Shields K, Gibson WS, Kos JT, et al. Genetic variation in the immunoglobulin heavy chain locus shapes the human antibody repertoire. Nat Commun [Internet]. 2023 Jul 21 [cited 2024 Jan 23];14(1):1–18. Available from: https://www.nature.com/articles/s41467-023-40070-x
14. Panigrahi A, O'Malley BW. Mechanisms of enhancer action: the known and the unknown. Genome Biol [Internet]. 2021 Apr 15 [cited 2024 Jan 23];22(1):1–30. Available from: https://genomebiology.biomedcentral.com/articles/10.1186/s13059-021-02322-1
15. Pennacchio LA, Bickmore W, Dean A, Nobrega MA, Bejerano G. Enhancers: five essential questions. Nat Rev Genet [Internet]. 2013 Apr [cited 2024 Jan 23];14(4):288–95. Available from: /pmc/articles/PMC4445073/
16. Shu M, Hong D, Lin H, Zhang J, Luo Z, Du Y, et al. Single-cell chromatin accessibility identifies enhancer networks driving gene expression during spinal cord development in mouse. Dev Cell [Internet]. 2022 Dec 19 [cited 2024 Jan 23];57(24):2761–75. Available from: https://pubmed.ncbi.nlm.nih.gov/36495874/
17. Zu S, Li YE, Wang K, Armand EJ, Mamde S, Amaral ML, et al. Single-cell analysis of chromatin accessibility in the adult mouse brain. Nature [Internet]. 2023 Dec 13 [cited 2024 Jan 23];624(7991):378–89. Available from: https://www.nature.com/articles/s41586-023-06824-9
18. Nair VD, Vasoya M, Nair V, Smith GR, Pincas H, Ge Y, et al. Differential analysis of chromatin accessibility and gene expression profiles identifies cis-regulatory elements in rat adipose and muscle. Genomics. 2021 Nov 1;113(6):3827–41.
19. Repele A, Krueger S, Bhattacharyya T, Tuineau MY, Manu. The regulatory control of Cebpa enhancers and silencers in the myeloid and red-blood cell lineages. PLoS One. 2019;14(6):e0217580.
20. Wang L, Feng J, Deng Y, Yang Q, Wei Q, Ye D, et al. CCAAT/Enhancer-Binding Proteins in Fibrosis: Complex Roles Beyond Conventional Understanding. Research [Internet]. 2022 Jan [cited 2024 Jan 23];2022:9891689. Available from: https://pubmed.ncbi.nlm.nih.gov/36299447/
21. Bujko K, Kucia M, Ratajczak J, Ratajczak MZ. Hematopoietic Stem and Progenitor Cells (HSPCs). Adv Exp Med Biol [Internet]. 2019 [cited 2024 Jan 23];1201:49–77. Available from: https://pubmed.ncbi.nlm.nih.gov/31898781/
22. Park YK, Lee JE, Yan Z, McKernan K, O'Haren T, Wang W, et al. Interplay of BAF and MLL4 promotes cell type-specific enhancer activation. Nat Commun [Internet]. 2021 Mar 12 [cited 2024 Jan 23];12(1):1–16. Available from: https://www.nature.com/articles/s41467-021-21893-y
23. Swanson L, Katkar GD, Tam J, Pranadinata RF, Chareddy Y, Coates J, et al. TLR4 signaling and macrophage inflammatory responses are dampened by GIV/Girdin. Proc Natl Acad Sci U S A [Internet]. 2020 Oct 27 [cited 2024 Jan 23];117(43):26895–906. Available from: https://pubmed.ncbi.nlm.nih.gov/33055214/
24. Li Y, Xu M, Li Y, Zhang Z, Gu W, Halimu G, et al. Induction of CD4+ regulatory T cells by stimulation with Staphylococcal Enterotoxin C2 through different signaling pathways. Biomed Pharmacother [Internet]. 2021 Nov 1 [cited 2024 Jan 23];143:112204. Available from:

https://pubmed.ncbi.nlm.nih.gov/34560552/
25. Ostuni R, Piccolo V, Barozzi I, Polletti S, Termanini A, Bonifacio S, et al. Latent enhancers activated by stimulation in differentiated cells. Cell [Internet]. 2013 Jan 7 [cited 2024 Jan 23];152(2):157–71. Available from: https://pubmed.ncbi.nlm.nih.gov/23332752/
26. Sartorelli V, Lauberth SM. Enhancer RNAs are an important regulatory layer of the epigenome. Nat Struct Mol Biol [Internet]. 2020 Jun 8 [cited 2024 Jan 23];27(6):521–8. Available from: https://www.nature.com/articles/s41594-020-0446-0
27. Chen X, Qi Y, Wu Z, Wang X, Li J, Zhao D, et al. Structural insights into preinitiation complex assembly on core promoters. Science (80-) [Internet]. 2021 Apr 30 [cited 2024 Jan 23];372(6541):8490. Available from: https://pubmed.ncbi.nlm.nih.gov/33795473/
28. Fulco CP, Nasser J, Jones TR, Munson G, Bergman DT, Subramanian V, et al. Activity-by-contact model of enhancer–promoter regulation from thousands of CRISPR perturbations. Nat Genet [Internet]. 2019 Nov 29 [cited 2024 Jan 23];51(12):1664–9. Available from: https://www.nature.com/articles/s41588-019-0538-0
29. Lettice LA, Heaney SJH, Purdie LA, Li L, de Beer P, Oostra BA, et al. A long-range Shh enhancer regulates expression in the developing limb and fin and is associated with preaxial polydactyly. Hum Mol Genet [Internet]. 2003 Jul 15 [cited 2024 Jan 23];12(14):1725–35. Available from: https://pubmed.ncbi.nlm.nih.gov/12837695/
30. Chen H, Levo M, Barinov L, Fujioka M, Jaynes JB, Gregor T. Dynamic interplay between enhancer–promoter topology and gene activity. Nat Gen [Internet]. 2018 Jul 23 [cited 2024 Jan 23];50(9):1296–303. Available from: https://www.nature.com/articles/s41588-018-0175-z
31. Davidson IF, Peters JM. Genome folding through loop extrusion by SMC complexes. Nat Rev Mol Cell Biol [Internet]. 2021 Mar 25 [cited 2024 Jan 23];22(7):445–64. Available from: https://www.nature.com/articles/s41580-021-00349-7
32. Allen BL, Taatjes DJ. The Mediator complex: a central integrator of transcription. Nat Rev Mol Cell Biol [Internet]. 2015 Feb 18 [cited 2024 Jan 23];16(3):155–66. Available from: https://www.nature.com/articles/nrm3951
33. Liu Y, Dekker J. CTCF–CTCF loops and intra-TAD interactions show differential dependence on cohesin ring integrity. Nat Cell Biol [Internet]. 2022 Oct 6 [cited 2024 Jan 23];24(10):1516–27. Available from: https://www.nature.com/articles/s41556-022-00992-y
34. Liu G, Dean A. Enhancer long-range contacts: The multi-adaptor protein LDB1 is the tie that binds. Biochim Biophys Acta Gene Regul Mech [Internet]. 2019 Jun 1 [cited 2024 Jan 23];1862(6):625–33. Available from: https://pubmed.ncbi.nlm.nih.gov/31022553/
35. Hsieh T-HS, Cattoglio C, Slobodyanyuk E, Hansen AS, Darzacq X, Tjian R. Enhancer-promoter interactions and transcription are largely maintained upon acute loss of CTCF, cohesin, WAPL or YY1. Nat Genet. 2022;54(12):1919–32.
36. Lee JY, Hong SH. Hematopoietic Stem Cells and Their Roles in Tissue Regeneration. Int J Stem Cells [Internet]. 2020 Mar 1 [cited 2024 Jan 23];13(1):12. Available from: /pmc/articles/PMC7119209/
37. Rodrigues CP, Shvedunova M, Akhtar A. Epigenetic Regulators as the Gatekeepers of Hematopoiesis. Trends Genet. 2021 Feb 1;37(2):125–42.
38. Maurya SS. Role of Enhancers in

Development and Diseases. Epigenomes. 2021;5(4):21.
39. van Arensbergen J, Pagie L, FitzPatrick VD, de Haas M, Baltissen MP, Comoglio F, et al. High-throughput identification of human SNPs affecting regulatory element activity. Nat Genet [Internet]. 2019 Jun 28 [cited 2024 Jan 23];51(7):1160–9. Available from: https://www.nature.com/articles/s41588-019-0455-2
40. Avellino R, Mulet-Lazaro R, Havermans M, Hoogenboezem R, Smeenk L, Salomonis N, et al. Induced cell-autonomous neutropenia systemically perturbs hematopoiesis in Cebpa enhancer-null mice. Blood Adv [Internet]. 2022 Mar 8 [cited 2024 Jan 23];6(5):1406–19. Available from: https://pubmed.ncbi.nlm.nih.gov/34814180/
41. You X, Chang YI, Kong G, Ranheim EA, Zhou Y, Johnson KD, et al. Gata2 −77 enhancer regulates adult hematopoietic stem cell survival. Leukemia [Internet]. 2020 Jun 27 [cited 2024 Jan 23];35(3):901–5. Available from: https://www.nature.com/articles/s41375-020-0942-6
42. Basak A, Sankaran VG. Regulation of the fetal hemoglobin silencing factor BCL11A. Ann N Y Acad Sci [Internet]. 2016 Mar 1 [cited 2024 Jan 23];1368(1):25. Available from: /pmc/articles/PMC4870126/
43. Arendt D, Musser JM, Baker CVH, Bergman A, Cepko C, Erwin DH, et al. The origin and evolution of cell types. Nat Rev Genet [Internet]. 2016 Nov 7 [cited 2024 Jan 23];17(12):744–57. Available from: https://www.nature.com/articles/nrg.2016.127
44. Jiang P, Zhang Z, Hu Y, Liang Z, Han Y, Li X, et al. Single-cell ATAC-seq maps the comprehensive and dynamic chromatin accessibility landscape of CAR-T cell dysfunction. Leukemia [Internet]. 2022 Nov 1 [cited 2024 Jan 23];36(11):2656–68. Available from: https://pubmed.ncbi.nlm.nih.gov/35962059/
45. Barsoum M, Sayadi-Boroujeni R, Stenzel AT, Bussmann P, Lüscher-Firzlaff J, Lüscher B. Sequential deregulation of histone marks, chromatin accessibility and gene expression in response to PROTAC-induced degradation of ASH2L. Sci Rep. 2023;13:22565.
46. Choukrallah MA, Song S, Rolink AG, Burger L, Matthias P. Enhancer repertoires are reshaped independently of early priming and heterochromatin dynamics during B cell differentiation. Nat Commun [Internet]. 2015 Oct 19 [cited 2024 Jan 23];6(1):1–11. Available from: https://www.nature.com/articles/ncomms9324
47. Local A, Huang H, Albuquerque CP, Singh N, Lee AY, Wang W, et al. Identification of H3K4me1-associated proteins at mammalian enhancers. Nat Genet [Internet]. 2017 Dec 18 [cited 2024 Jan 23];50(1):73–82. Available from: https://www.nature.com/articles/s41588-017-0015-6
48. Kim SS, Truong B, Jagadeesh K, Dey KK, Shen AZ, Raychaudhuri S, et al. Leveraging single-cell ATAC-seq and RNA-seq to identify disease-critical fetal and adult brain cell types. Nat Commun [Internet]. 2024 Jan 17 [cited 2024 Jan 23];15(1):1–11. Available from: https://www.nature.com/articles/s41467-024-44742-0
49. Huang J, Liu X, Li D, Shao Z, Cao H, Zhang Y, et al. Dynamic Control of Enhancer Repertoires Drives Lineage and Stage-Specific Transcription during Hematopoiesis. Dev Cell. 2016;36(1):9–23.
50. Klemm SL, Shipony Z, Greenleaf WJ. Chromatin accessibility and the regulatory epigenome. Nat Rev Genet [Internet]. 2019 Jan 23 [cited 2024 Jan 23];20(4):207–20. Available from: https://www.nature.com/articles/s41576-018-0089-8

51. Zhang G, Lu J, Zheng J, Mei S, Li H, Zhang X, et al. Spi1 regulates the microglial/macrophage inflammatory response via the PI3K/AKT/mTOR signaling pathway after intracerebral hemorrhage. Neural Regen Res [Internet]. 2024 Jan [cited 2024 Jan 23];19(1):161–170. Available from: /pmc/articles/PMC10479839/
52. Zhao X, Bartholdy B, Yamamoto Y, Evans EK, Alberich-Jordà M, Staber PB, et al. PU.1-c-Jun interaction is crucial for PU.1 function in myeloid development. Commun Biol [Internet]. 2022 Sep 14 [cited 2024 Jan 23];5(1):1–15. Available from: https://www.nature.com/articles/s42003-022-03888-7
53. Ebralidze AK, Guibal FC, Steidl U, Zhang P, Lee S, Bartholdy B, et al. PU.1 expression is modulated by the balance of functional sense and antisense RNAs regulated by a shared cis-regulatory element. Genes Dev [Internet]. 2008 Aug 8 [cited 2024 Jan 23];22(15):2085–2092. Available from: /pmc/articles/PMC2492744/
54. Pulikkan JA, Tenen DG, Behre G. C/EBPα deregulation as a paradigm for leukemogenesis. Leukemia [Internet]. 2017 Jul 19 [cited 2024 Jan 23];31(11):2279–85. Available from: https://www.nature.com/articles/leu2017229
55. Braun TP, Okhovat M, Coblentz C, Carratt SA, Foley A, Schonrock Z, et al. Myeloid lineage enhancers drive oncogene synergy in CEBPA/CSF3R mutant acute myeloid leukemia. Nat Commun [Internet]. 2019 Nov 29 [cited 2024 Jan 23];10(1):1–15. Available from: https://www.nature.com/articles/s41467-019-13364-2
56. Tanaka H, Takizawa Y, Takaku M, Kato D, Kumagawa Y, Grimm SA, et al. Interaction of the pioneer transcription factor GATA3 with nucleosomes. Nat Commun [Internet]. 2020 Aug 18 [cited 2024 Jan 25];11(1):1–10. Available from: https://www.nature.com/articles/s41467-020-17959-y
57. Ferreira R, Ohneda K, Yamamoto M, Philipsen S. GATA1 Function, a Paradigm for Transcription Factors in Hematopoiesis. Mol Cell Biol [Internet]. 2005 Feb 1 [cited 2024 Jan 25];25(4):1215–1227. Available from: /pmc/articles/PMC548021/
58. You X, Chang YI, Kong G, Ranheim EA, Zhou Y, Johnson KD, et al. Gata2 −77 enhancer regulates adult hematopoietic stem cell survival. Leukemia [Internet]. 2020 Jun 27 [cited 2024 Jan 25];35(3):901–5. Available from: https://www.nature.com/articles/s41375-020-0942-6
59. Cobaleda C, Schebesta A, Delogu A, Busslinger M. Pax5: the guardian of B cell identity and function. Nat Immunol [Internet]. 2007 Apr 17 [cited 2024 Jan 25];8(5):463–70. Available from: https://www.nature.com/articles/ni1454
60. McManus S, Ebert A, Salvagiotto G, Medvedovic J, Sun Q, Tamir I, et al. The transcription factor Pax5 regulates its target genes by recruiting chromatin-modifying proteins in committed B cells. EMBO J [Internet]. 2011 May 6 [cited 2024 Jan 25];30(12):2388–404. Available from: https://www.embopress.org/doi/10.1038/emboj.2011.140
61. Decker T, Pasca di Magliano M, McManus S, Sun Q, Bonifer C, Tagoh H, et al. Stepwise Activation of Enhancer and Promoter Regions of the B Cell Commitment Gene Pax5 in Early Lymphopoiesis. Immunity [Internet]. 2009 Apr 17 [cited 2024 Jan 25];30(4):508–20. Available from: http://www.cell.com/article/S1074761309001368/fulltext