Emerging role of long non-coding RNAs in normal and malignant hematopoiesis

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Abstract

Long noncoding RNAs (lncRNAs) have recently been discovered and are increasingly recognized as vital components of modern molecular biology. Accumulating evidence shows that lncRNAs have emerged as important mediators in diverse biological processes such as cell differentiation, pluripotency, and tumorigenesis, while the function of lncRNAs in the field of normal and malignant hematopoiesis remains to be further elucidated. Here, we widely reviewed recent advances and summarize the characteristics and basic mechanisms of lncRNAs and keep abreast of developments of lncRNAs within the field of normal and malignant hematopoiesis. Based on gene regulatory networks at different levels of lncRNAs participation, lncRNAs have been shown to regulate gene expression from epigenetics, transcription and post transcription. The expression of lncRNAs is highly cell-specific and critical for the development and activation of hematopoiesis. Moreover, we also summarized the role of lncRNAs involved in hematological malignancies in recent years. LncRNAs have been found to play an emerging role in normal and malignant hematopoiesis, which may provide novel ideas for the diagnosis and therapeutic targets of hematological diseases in the foreseeable future.

Keywords: Long non-coding RNA; Hematopoiesis; Hematological malignancies

Introduction

With the development of the genome-wide transcriptome studies, pervasive researches are currently focusing on non-protein-coding regulatory RNAs (ncRNAs), which were regarded as junk or noises of transcripts previously. The concept that non-coding RNAs do play crucial roles in regulating gene expression at the levels of transcription, RNA processing, and translation has been recognized for several years. According to the transcript size, ncRNAs are generally classified into two major groups: short ncRNAs (<200 nucleotides) or long ncRNAs (>200 nucleotides). MicroRNAs (miRNAs), a typical representative of short ncRNAs, have been best studied and known to induce mRNA degradation or block mRNA translation via the RNA interference pathway and recognized as powerful regulators of numerous genes and pathways in the pathogenesis of hematological diseases. In contrast, abundant long non-coding RNAs (lncRNAs) have recently been discovered and are increasingly recognized as vital components of modern molecular biology. Accumulating evidence shows that lncRNAs can modulate diverse biological processes such as cell proliferation, differentiation, pluripotency, apoptosis, and tumorigenesis. The exploration of the characteristics and mechanisms of them is at a relatively initial stage yet, and especially the function of lncRNAs in the field of hematopoiesis remains to be further elucidated. In this review, we will succinctly summarize the characteristics and mechanisms of lncRNAs and focus on the latest progress of lncRNAs in normal and malignant hematopoiesis.

Characteristics and Functions of Long Non-coding RNAs

Long non-coding RNAs are defined as a heterogeneous class of ncRNAs longer than 200 nucleotides which feature distinguish them from small regulatory RNAs such as miRNAs, small nucleolar RNAs (snoRNAs), piwi-interacting RNAs (piRNAs), short interfering RNAs (siRNAs), and other short RNAs. LncRNAs could be localized to the nucleus or cytoplasm and are most abundant in the nucleus, which is different from mRNAs that are mostly transported to the cytoplasm. According to the NONCODE database (current version v5.0, http://www.noncode.org), which is an integrated knowledge database dedicated to non-coding RNAs and currently recruits the lncRNA information of 17 species, there are 172,216 and 131,697 lncRNA transcripts of human and mouse at present, respectively.
Due to the high heterogeneity of the sequence, structure and biological function of lncRNAs, there are various classification methods so far. Generally, in light of their proximity to protein-coding mRNAs, lncRNAs could be classified into the following categories: (1) the long intergenic ncRNA (lincRNA), which comes from the region between two genes; (2) intronic lncRNA, which originates from the intron region of secondary transcript (sometimes mRNA precursor sequence); (3) sense lncRNA, which overlaps with one or more exons of another protein-coding gene of the synonymous chain; (4) antisense lncRNA, which overlaps with one or more exons of another protein-coding gene in the opposite strand; and (5) bidirectional lncRNAs, whose transcription start site is very close to the protein gene encoding on the antisense strand, but the direction of transcription is the opposite. Based on the features and special functions of lncRNAs, it also includes lncRNA-activating (lncRNA-a), transcribed pseudogene lncRNAs, telomere-associated ncRNAs (TERRAs), transcribed ultrasonoregions (T-UCRs), enhancer RNAs (eRNAs), circular RNAs, etc.

According to recent advances, the characteristics of lncRNAs can be summarized as follows: Firstly, no different than mRNA, most lncRNAs whose promoter can also bind transcription factors are also polyadenylated, spliced, and modified with 5’-cap and poly-A tail, and also transcribed by RNA polymerase II (RNAP II). They have dynamic expression and different splicing modes during transcription.

Secondly, lncRNAs are relatively conservative in function although they contain fewer longer exons and lower evolutionary sequence conservation compared with mRNAs.

Thirdly, most lncRNAs are expressed at relatively lower levels but have more obvious temporal and spatial expression specificity compared with mRNAs in the process of tissue differentiation and development.

Up to now, the precise mechanism of lncRNA is not entirely clear. Nevertheless, based on gene regulatory networks at different levels of lncRNA participation, lncRNAs have been shown to regulate gene expression in three ways: epigenetics, transcription, and post-transcription.

LncRNAs in Erythropoiesis

Erythropoiesis is a developmental process that is critically controlled by multiple regulators to ensure the proper generation of mature red blood cells and the transportation of oxygen to tissues. Inspired by the “chromatin-state maps” approach pioneered by Gutmann et al., less than a decade ago, the Biomedical Research group of Cambridge identified the first erythroid-specific lncRNA lncRNA erythroid prosurvival (lincRNA-EPS), which could facilitate erythropoiesis by repressing the expression of Pycard, a proapoptotic gene, without altering erythroid differentiation. Subsequent studies also discovered multiple lncRNAs that are dynamically expressed during erythropoiesis and are targeted by key erythroid transcription factors such as GATA1, TAL1, or KLF1. AlncRNA-EC7 was one of the identified lncRNAs. Reduction of AlncRNA-EC7 expression in erythroblasts induced BAND3 (a major anion exchange protein present on erythrocyte membranes) gene expression via chromatin interactions between the AlncRNA-EC7 locus and the neighboring region of the BAND3 promoter leading to impaired erythrocyte maturation. Recently, a research group also released their findings that lncRNA Fas-antisense 1 (Fas-AS1 or Saf) was induced during differentiation through the activity of essential erythroid transcription factors GATA1 and KLF1. They further discovered that Saf was also negatively regulated by NF-xB and that over-expression of Saf in erythroblasts derived from CD34+ hematopoietic stem/progenitor cells of healthy donors could reduce surface levels of Fas and consequently conferred protection against Fas-mediated cell death signals. Although current studies of lncRNAs in erythropoiesis are largely done in the murine models, these advances expand the repertoire of lncRNA functions and provide a novel genetic pathway that can be exploited with effective targets for the treatment of various anemia-related diseases in the future.

LncRNAs in Myeloid Hematopoiesis

Almost a decade ago, Zhang et al. unveiled the first myeloid lineage-specific lncRNA HOXAIR (HOX antisense intergenic RNA myeloid 1) which is transcribed between the human HOXA1 and HOXA2 genes. In-depth research revealed that HOXAIR1 contributed to three-dimensional conformational changes of chromosomes, regulating mRNA stability and abundance, or the role of competitive endogenous RNA (ceRNA).
which were required for the temporal collinear activation of HOXA genes. Functional studies showed that knockdown of HOTAIRM1 could quantitatively impair all-trans retinoic acid (ATRA)-induced myeloid differentiation and selectively attenuated differentiation-related transcripts such as CD11b, CD18, HOXAI, and HOXA4. Subsequent studies showed that the master transcription factor PU.1 during myeloid differentiation could directly activate the expression of HOTAIRM1 through binding to the regulatory region of HOTAIRM1. A recent study revealed that the high expression of HOTAIRM1 could enhance ATRA-induced PML-RARA degradation by affecting autophagic flux. Conclusively, these advances indicate that HOTAIRM1 may be a novel potential therapeutic target for acute promyelocytic leukemia (APL).

The IncRNA EGO (eosinophil granule ontogeny) is a novel, nested, non-coding RNA, expressed during eosinophil development from CD34+ human HSCs and mature eosinophils. RNA silencing assays investigated that EGO regulated granule protein MBP (major basic protein) and EDN (eosinophil derived neurotoxin) transcript expression in developing CD34 hematopoietic progenitors.

**Lymphoid Differentiation-related IncRNAs**

CD4+ T cells play central roles in mediating adaptive immunity against various pathogens. With T-cell receptor activation by specific cytokines, naive CD4+ T cells may differentiate into one of several lineages of T helper (Th) cells, including Th1, Th2, Th17, and inducible regulatory T cell (iTreg), as defined by their pattern of cytokine production and function. The diversity of CD4+ T-cell subsets enables the adaptive immune system to adapt to many challenges during the expression of genes encoding cytokines and transcription factors. NeST (Nettoie Salmonella pas Theiler’s; cleanup Salmonella not Theiler’s), formally known as Tmevpg1 or IFNG-AS1, is a long non-coding RNA specifically expressed in Th1 cells by a T-bet (a Th1-specific key transcription factor) dependent mechanism. NeST RNA was found to bind WDR5, a component of the histone H3 lysine 4 methyltransferase complex and to alter histone 3 methylation at the interferon-gamma locus, ultimately leading to the regulation of the expression of IFN-γ. Another Th1-specific IncRNA is linc-MAF-4 whose expression is negatively correlated with MAF, a Th2-associated transcription factor. Studies suggest that linc-MAF-4 could
Table 1: Long non-coding RNAs involved in the normal hematopoiesis.

| Hematopoiesis type | LncRNAs | Function | References |
|--------------------|---------|----------|------------|
| Erythropoiesis | LncRNA-EPS | Promotes erythropoiesis by inhibiting the expression of PyCARD | [36,37] |
| | LincRNA-EC7 | Down-regulation induces gene BAND3 expression and leads to impaired erythrocyte maturation | [38] |
| | Fas-AS1 (or Saf) | Induced during differentiation through the activity of essential erythroid transcription factors GATA-1 and KLF1 | [39,40] |
| Leukemogenesis | HOTAIRMI | Knockdown results in quantitatively impaired ATRA-induced myeloid differentiation and selectively attenuated differentiation-related transcripts | [41,42] |
| | EGO | Regulates granule protein MBP and EDN transcript expression in developing CD34 hematopoietic progenitors | [43] |
| Th1 CD4+ T | Nest (Tmevpg1 or IFNG-AS1) | Binds to WDR5 and alters histone 3 methylation at the IFN-γ locus in Th1 cells by a T-bet dependent mechanism | [44,45] |
| | Linc-MAF-4 | Regulates MAF transcription by recruitment of chromatin modifiers and down-regulation could skew T-cell differentiation toward Th2 | [46] |
| Th2 CD4+ T | Linc-R-Ccr2-5'AS | Co-regulated by the same regulatory elements and might have shared functions with GATA3 in Th2-cell response | [47,48] |
| | GATA3-AS1 | Transcribed from the RAD50 locus and significantly required for expression of genes encoding Th2 cytokines | [49] |
| Th17 CD4+ T | Rmrp | Regulates the function of RORγt transcriptional complexes at a subset of critical genes in the Th17 effector program | [50] |
| Treg | Flicr | Dampens the Treg signature and may lower Treg stability, allowing stronger antiviral responses by destabilizing Foxp3 | [51] |
| | Lnc-EGFR | Positively correlates with expression of EGRF/Foxp3 and augments immunosuppression by promoting Treg cell differentiation | [52] |
| CD8+ T | LncRNA-CD244 | Mediates IFN-γ and TNF-α expression and improves protective immunity of CD8+ T cells by interacting with EZH2 | [53] |
| B cell | lncRNA-CSR | Regulate the differentiation and antibody response of B cell | [54] |
| | CRNDE | Its expression in primarily pre-B1, pre-B2, and centroblasts is consistent with its role as a metabolic regulator | [55] |

LncRNA-EPS: LncRNA erythroid prosurvival; Fas-AS1 (or Saf): Fas-antisense 1; HOTAIRMI: HOX antisense intergenic RNA myeloid 1; EGO: Eosinophil granule ontology; Nest (Tmevpg1 or IFNG-AS1): Nettou Salmonella palas Theiler’s; cleanup Salmonella not Theiler’s; GATA3-AS1: GATA3-Antisense1; TH2-LCR: TH2-locus control region; Flicr: Foxp3 long intergenic non-coding RNA; Lnc-EGFR: Lnc-epidermal growth factor receptor; LncRNA-CSR: LncRNA-class switch DNA recombination; CRNDE: Colorectal neoplasia differentially expressed; PyCARD: Human apoptosis-associated speck-like protein containing a CARD; KLF1: Kruppel-like factor 1; ATRA: All-trans retinoic acid; MBP: Major basic protein; EDN: Eosinophil derived neurotoxin; RAD50: Recombinant DNA repair protein; RORγt: Retinoid-related orphan receptor gamma-t; EZH2: Enhancer of zeste homolog 2.

**Regulate MAF transcription by recruiting chromatin modifiers and that down-regulation of linc-MAF-4 could skew T-cell differentiation toward the Th2 phenotype.**

Hu et al. [47,48] identified 1524 lincRNA clusters from early T-cell progenitors to terminally differentiated T-helper subsets, among which *linc-R-Ccr2-5'AS*, regulated by GATA-3 (a zinc-finger transcription factor, highly expressed in Th2 cells and critical to Th2 differentiation by regulating Th2 gene expression), was considered to be an essential part of the regulation in Th2-specific gene expression and important for Th2 cell migration. In the same year, another research group also reported a *GATA3*-associated lncRNA *GATA3-AS1* which was specifically expressed in primary Th2 cells. Their results indicate that the expression of *GATA3-AS1* and GATA3 might be co-regulated by the same regulatory elements and might have shared functions in Th2 cell responses. [49] By whole-genome sequencing (RNA-seq), Spurlock et al. [50] identified a cluster of antisense lncRNAs *TH2-LCR*, which was transcribed from the RAD50 locus that is co-expressed with *IL4*, *IL5*, and *IL13* genes under Th2 polarizing conditions. Their analyses demonstrated that *TH2-LCR* is significantly required for the expression of genes encoding Th2 cytokines.

The differentiation of Th17 cells requires the nuclear hormone receptor RORγt which focuses on the activity of a cytokine-regulated transcriptional network including genes encoding the signature Th17 cytokines (*IL-17A, IL-17F, IL-22*). Huang and colleagues identified the lncRNA *Rmrp*, RNA component of mitochondria RNA-processing endoribonuclease (*RNase MRP*), as a key *DDX5* (DEAD-box protein 5, an RNA helicase possessing an important role in gene expression)-associated RNA, which could regulate the function of RORγt transcriptional complexes at a subset of critical genes implicated specifically in the Th17 effector program. [51,52] Regulatory T cells (Tregs) characterized by the transcription factor *Foxp3* are a fundamental component in maintaining immune homeostasis by negatively regulating several immunocyte lineages, especially during autoimmune, tumor, and lymphoproliferative pathologies. [53] A
recent study identified a lncRNA Flicr whose expression profile and genomic localization displayed Treg specificity, partially overlapping Foxp3. Further assays revealed that Flicr dampens the Treg signature and may lower Treg stability, allowing stronger antiviral responses by destabilizing Foxp3. Another novel Treg-related lncRNA is lnc-EGFR (lnc-epidermal growth factor receptor), whose up-regulation positively correlates with the expression of EGFR/Foxp3. Mechanism research shows that lnc-EGFR is a potential enhancer of EGFR and its downstream AP-1/NF-AT1 axis, and could augment immunosuppression by promoting Treg cell differentiation which may offer a potential therapeutic target for certain carcinomas.

Wang et al. revealed that the expression of CD244, a T-cell-inhibitory molecule in CD8+ T-cell immune responses during tuberculosis (TB) infection, correlated with high levels of a lncRNA IncRNA-CD244. Functional assays demonstrated that IncRNA-CD244 could mediate IFN-γ and TNF-α expression and improve the protective immunity of CD8+ T cells by interacting with EZH2 (enhancer of zeste homolog 2, a chromatin-modification enzyme).

B cells develop from the common lymphoid progenitor cells in the bone marrow and the initial antigen-independent phase is characterized by immunoglobulin gene rearrangements. As the central drivers of immune humoral response, B cells development and function are influenced by a series of gene regulation. Using RNA-seq and de novo transcript assembly, researchers have identified several lncRNAs involved in the development, activation, proliferation, and differentiation of B cells, such as IncRNA-CSR and CRNDE.

**Long Non-coding RNAs in Malignant Hematopoiesis**

In addition to the normal hematopoietic process regulated by a variety of lncRNAs, abnormal interference of lncRNA regulation also inevitably leads to hematopoietic dysfunction, mainly in the occurrence of leukemia, lymphoma, and myeloma. At present, several lncRNAs related to hematological malignancies have been identified and summarized in the following sections [Table 2 and Figure 3].

**AML-related lncRNAs**

The expression of nuclear paraspeckle assembly transcript 1 (NEAT1), a novel lncRNA localized specifically to nuclear paraspeckles, has a vital regulatory role in many human malignancies and was indicated to be repressed by PML-RARα in de novo APL samples. Furthermore, significant NEAT1 up-regulation was observed during (ATRA)-induced NB4 cell differentiation.

Another group performed transcriptome-wide lncRNA expression profiling of acute myeloid leukemia (AML) and identified that lncRNAs up-regulated in AML are associated with a lower degree of DNA methylation and
Table 2: Long non-coding RNAs involved in the malignant hematopoiesis.

| Hematologic disease | LncRNAs | Function | References |
|---------------------|---------|----------|------------|
| APL                 | NEAT1   | Repressed by PML-RARα in de novo APL | [70,71] |
| AML                 | RUNX0R  | Interactions with the RUNX1 promoter and enhancer and up-regulated in AML | [72] |
|                     | IRAIN   | Interactions with the IGFR1 promoter and enhancer DNA sequences and down-regulated in AML | [73] |
|                     | LOC285758 | Regulates proliferation of AML cell lines by enhancing the expression of HDAC2 | [74,75] |
|                     | CCDC26  | Controls growth of myeloid leukemia cells through regulating the expression of KIT in patients with relapsed AML | [76] |
| CML                 | PLIN2   | Positive correlates with CEBPA in CML via GSK3 and Wnt/β-catenin signaling | [77] |
|                     | LncRNA-BGL3 | Functions as a ceRNA for binding a subset of microRNAs to cross-regulate PTEN expression | [78,79] |
|                     | HOTAIR  | Plays a crucial role in the development of MDR by imatinib by a PI3K/Akt-dependent way | [80] |
|                     | HULC    | Positively correlates with imatinib-induced apoptosis of CML cells and inhibits c-Myc expression and PI3K/Akt pathway activity | [81] |
|                     | UCA1    | Important modulator of multidrug resistance protein-1 for IM resistance in CML | [82] |
| ALL                 | LUNAR1  | Correlates with its coding neighbor gene IGFR1 and up-regulated in primary T-ALL samples with a Notch mutation | [83,84] |
|                     | BALR-2  | Knockdown leads to apoptosis of B-ALL cell lines and up-regulation correlates with poor patient response to prednisone | [85] |
| CLL                 | CRNDE   | Hypermethylation correlates with a poor outcome in patients with CLL | [86] |
|                     | AC012065.7 | Hypomethylation correlates with a poor outcome in patients with CLL | [86] |
|                     | MIAT    | Positively correlates with aggressive CLL carrying either 17p-, 11q-, or 13p-deletions | [87] |
| B-cell lymphoma     | FAS-AS1 | Regulates Fas-mediated apoptosis by inhibiting EZH2 in NHL | [88,89] |
| Burkitt lymphoma    | MINCR   | Knockdown associated with a reduction in MYC binding to the promoters of certain cell cycle genes in MYC-positive Burkitt lymphomas | [90] |
| DLBCL               | PANDA   | Down-regulated in patients with DLBCL and functions as a tumor suppressor gene through silencing MAPK/ERK signaling pathway | [91,92] |
| HL                  | LINC00116 and LINC00461 | Over-expressed in HL | [93] |
| MM                  | FLJ42351 | A remarkable RS cell-specific expression in HL | [93] |
|                     | MEG3    | Plays an essential role in osteogenic differentiation in bone marrow MSCs partly by activating transcription of BMP4 in MM | [94] |
| MDS                 | MALAT1  | Initiates the activation of Sp1 on LTB P3 promoter in MSCs from MM | [95] |
|                     | Linc-RPIA | Binds miR-429 and may be involved in the regulation of tumor-related genes, including FOXO1 and TP53 | [96] |
| AA                  | TDRG1   | Regulated by FGFI and enhance the proliferation of BMSCs | [97] |

APL: Acute promyelocytic leukemia; AML: Acute myeloid leukemia; CML: Chronic myeloid leukemia; T-ALL: T-cell acute lymphocytic leukemia; B-ALL: B-cell acute lymphocytic leukemia; CLL: Chronic leukemia; DLBCL: Diffuse large B-cell lymphoma; NHL: Non-Hodgkin lymphoma; HL: Hodgkin lymphoma; MM: Multiple myeloma; MDS: Myelodysplastic syndrome; AA: Aplastic anemia; ceRNA: Competitive endogenous RNA; NEAT1: Nuclear paraspeckle assembly transcript 1; HDAC2: Histone deacetylase 2; IGFR1: Insulin-like growth factor 1 receptor; PI3K: Phosphatidylinositol 3-kinase; CDK: Cyclin-dependent kinase; PTEN: Gene of phosphate and tension homology deleted on chromosome 10; MDR: Multidrug resistance; IM: Imatinib; EZH2: Enhancer of zeste homolog 2; MAPK/ERK: Mitogen-activated protein kinase/extracellular regulated protein kinases; RS: Reed-Sternberg; MSC: Mesenchymal stem cell; BMP4: Bone morphogenetic protein 4; LTBP3: Latent-transforming growth factor beta-binding protein 3; IPSS: International prognostic scoring system; FGFI: Fibroblast growth factor 1.

Accumulating evidence has shown that the insulin-like growth factor type 1 receptor (IGFR1) is one of the most important regulators in the progression and therapeutic resistance of AML. A novel intragenic lncRNA IRAIN was discovered to directly interact with the IGFR1 promoter and enhance chromatin DNA sequences. Moreover, IRAIN was down-regulated both in leukemia cell lines and high-risk patients with AML.
Distinctive lncRNA profiles were found associated with common recurrent mutations in AML, such as FLT3-ITD (internal tandem duplications in the FLT3 gene) or NPM1, CEBPA, IDH2, and RUNX1 genes.\textsuperscript{[101]} Utilizing a novel R3C (RNA-guided chromatin conformation capture) method,\textsuperscript{[102]} Wang et al\textsuperscript{[72]} described a RUNX1-intragenic lncRNA RUNXOR (RUNX1 overlapping RNA), which is transcribed by an upstream promoter and overlaps with RUNX1 (one of the most frequently mutated genes in AML\textsuperscript{[103]}). RUNXOR was up-regulated in both AML samples and Ara-C-treated cell lines. The in-depth study showed that RUNXOR directly interacted with the RUNX1 promoter and enhancer chromatin DNA sequences through its 3'‐terminal fragment.
Another lncRNA CCDC26, which is thought to transcriptionally regulate a set of genes and associated with pediatric AML,\textsuperscript{104,105} was also proved to controls growth of myeloid leukemia cells through regulation the expression of \textit{KIT}.\textsuperscript{76} A receptor tyrosine kinase that have been considered to up-regulated in leukemia stem cells from patients with AML who relapsed after chemotherapy.\textsuperscript{106}

\textbf{CML-related lncRNAs}

A comprehensive analysis of lncRNAs in human chronic myeloid leukemia (CML) cells was performed and a novel lncRNA \textit{lncRNA-BGL3} was observed to serve as a key regulator of \textit{Bcr-Abl}-mediated cellular transformation. Functional assays suggested that \textit{lncRNA-BGL3} was highly induced in response to the disruption of \textit{Bcr-Abl} expression or by inhibiting \textit{Bcr-Abl} kinase activity in cell lines and patients with CML. Notably, \textit{lncRNA-BGL3} may function as a ceRNA that binds a subset of microRNAs to cross-regulate \textit{PTEN} (phosphatase and tensin homolog, a critical tumor suppressor gene) expression.\textsuperscript{78,79}

Hughes et al\textsuperscript{87} identified more than 900 lncRNAs which are regulated by \textit{CEBPA} (CCAAT/enhancer-binding protein-\alpha), a critical regulator of myeloid differentiation, and many of them are induced during myeloid differentiation of AML cell lines including \textit{lncRNA PLIN2}. Another group recently further investigated the potential roles of \textit{CEBPA}-related lncRNA \textit{PLIN2} during CML. Results indicated that both \textit{CEBPA} and \textit{PLIN2} were up-regulated in the process of CML and there was a positive correlation between \textit{CEBPA} and \textit{PLIN2} in patients with CML. Moreover, they found that \textit{CEBPA}-mediated up-regulation of \textit{PLIN2} expression promotes the development of CML via GSK3 and Wnt/\beta-catenin signaling.\textsuperscript{107}

Recent studies have also reported some newly discovered lncRNAs that are associated with CML. For instance, lncRNA \textit{HOTAIR} may play a crucial role in the development of multidrug resistance (MDR) to imatinib in CML via a \textit{PI3K/Akt}-dependent mechanism.\textsuperscript{80} Another lncRNA \textit{HULC} was also revealed to be positively correlated with imatinib (IM)-induced apoptosis of CML cells and lead to the reduction of c-Myc expression and inhibition of \textit{PI3K/Akt} pathway activity.\textsuperscript{81} lncRNA \textit{UCAI} was identified as another important modulator of multidrug resistance protein-1 (MDR1) which is considered as the main reason for IM resistance in CML cells.\textsuperscript{82}

\textbf{T-lymphocytic leukemia-related lncRNAs}

\textit{LUNAR1} (LeUkemia-induced non-coding activator RNA) is a pivotal lncRNA involved in T-cell leukemia (T-ALL), an aggressive hematological neoplasm derived from malignant T-lymphocyte progenitors with aberrant \textit{NOTCH1} signaling.\textsuperscript{108} Evidence indicated that \textit{LUNAR1} is highly correlated with its coding neighbor gene \textit{IGF1R}, which has been previously suggested to play a role in T-ALL.\textsuperscript{83,84} Trimarchi et al\textsuperscript{84} have revealed that the expression of \textit{LUNAR1} was up-regulated in primary T-ALL samples especially in ones with a Notch mutation, while down-regulated upon Notch inhibition.

\textbf{B-lymphocytic leukemia-related lncRNAs}

Unbiased microarray profiling was performed on human B-ALL samples and indicated that the expression of a subset of lncRNAs, termed BALRs (B-ALL-associated long RNAs), corresponds with specific cytogenetic abnormalities in B-ALL. A functional assay suggested that knockdown of \textit{BALR-2} (a lncRNA among BALRs) led to apoptosis of B-ALL cell lines alone and up-regulated \textit{BALR-2} was correlated with poor patient response to prednisone and worse overall survival.\textsuperscript{85}

By employing methyl-CpG-binding domain protein-enriched genome-wide sequencing (MBD-Seq), Subhash et al\textsuperscript{86} identified 5800 hypermethylated and 12,570 hypomethylated chronic lymphocytic leukemia (CLL)-specific differentially methylated genes (cDMDGs), among which hypermethylated \textit{CRNDE} and hypomethylated \textit{AC012065.7} were two novel lncRNAs validated in CLL samples. Notably, survival analysis revealed that hypermethylation of \textit{CRNDE} and hypomethylation of \textit{AC012065.7} were correlated with poor outcome in patients with CLL. In addition, lncRNA \textit{MIAT} is another lncRNA whose expression level was considered to be positively correlated with the aggressive CLL forms carrying either 17p-deletion, 11q-deletion, or trisomy 12 over indolent form carrying 13p-deletion.\textsuperscript{87}

\textbf{Lymphoma and multiple myeloma-related lncRNAs}

Viré et al\textsuperscript{88} firstly reported a lncRNA corresponding to an antisense transcript of Fas (\textit{FAS-AS1}) which could effectively regulate Fas-mediated apoptosis in non-Hodgkin lymphoma (NHL). Results suggested that the \textit{FAS-AS1} expression was repressed because of its promotor being hyper-methylated by \textit{EZH2} (enhancer of zeste homologue 2), a histone-lysine N-methyltransferase enzyme that participates in histone methylation and leads to transcriptional repression, which is often mutated or over-expressed in lymphomas.\textsuperscript{88} Functional assays indicated that treatment with Bruton’s tyrosine kinase (\textit{BTK}) inhibitor or \textit{EZH2} knockdown significantly could decrease the levels of \textit{EZH2} and then enhancing Fas-mediated apoptosis, which may provide a novel therapeutic target in lymphomas.\textsuperscript{89}

Burkitt lymphoma is an aggressive hematological neoplasm with a poor prognosis and the deregulation of the oncogenic transcription factor \textit{MYC} is considered to be the major driving force in lymphoma development.\textsuperscript{109} Doose et al\textsuperscript{80} identified 13 lncRNAs differentially expressed in IG-MYC-positive Burkitt lymphoma, among which a lncRNA named \textit{MYC-induced lncRNA (MINCR)} showing a strong correlation with \textit{MYC} expression in MYC-positive lymphomas. \textit{MINCR} knockdown was associated with a reduction in \textit{MYC} binding to the promoters of selected cell cycle genes. These findings suggested novel therapeutic opportunities for the fight against not only malignant lymphomas but possibly, all cancers that rely on \textit{MYC} expression.

The long non-coding RNA \textit{PANDA} (P21-associated ncRNA DNA damage activated), which is induced in a
p53-dependent manner and interacts with the transcription factor NF-YA to limit expression of pro-apoptotic genes,⁹¹ was recently reported to be down-regulated in patients with diffuse large B-cell lymphoma (DLBCL) and functioned as a tumor suppressor gene through silencing MAPK/ERK signaling pathway.¹²

A differential expression profiling between Hodgkin lymphoma (HL) and normal germinal center (GC)-B cells was performed and selected two lncRNAs (LINC00116 and LINC00461) which was over-expression in HL, DLBCL and lymphoblastoid cell lines, and another lncRNA (FLJ42351) which has a remarkable Reed-Sernberg (RS) cell-specific expression in HL and part of Burkitt lymphoma cell lines.⁹³

Multiple myeloma (MM) is another large class of hematological malignancy characterized by the impaired osteogenic differentiation of mesenchymal stromal cells (MSCs). Zhuang et al.¹¹⁰ revealed that lncRNA maternally expressed gene 3 (MEG3, a tumor suppressor) played an essential role in osteogenic differentiation in bone marrow MSCs, partly by activating transcription of BMP4, a member of the transforming growth factor (TGF) family and participate in embryonic development, hematopoietic development, and mesenchymal development.⁹⁴,¹¹⁰ Functional assays indicated that MEG3 knockdown significantly reduced the expression of key osteogenic markers, including Runt-related transcription factor 2, osterix, and osteocalcin.¹¹⁰

Furthermore, another research group also reported an MM-related lncRNA MALAT1 (metastasis-associated lung adenocarcinoma transcript 1), which have been previously reported, and critical for the development and activation of numerous cancers.¹¹¹,¹¹² could initiate the activation of the key transcription factor Sp1 on Latent TGF-binding proteins (LTBP3, an important regulator for efficient secretion, folding, and activation of TGF-s and regulates the bioavailability of TGF- especially in the bone)¹¹³ promoter in MSCs from MM.⁹³

**Myelodysplastic syndromes and aplastic anemia-related lncRNAs**

Myelodysplastic syndromes (MDS) are a group of myeloid clonal diseases with a high risk of transformation to AML.¹¹⁴ Up to now, the molecular pathogenesis of MDS remains to be explored. Liu et al.⁹⁰ have developed a network-based lncRNA co-module function annotation method, which integrated correlations between lncRNA, protein-coding genes and non-coding miRNAs, and generated lncRNA expression profiles from the HSCs from patients with MDS and healthy donors. Linc-RPLA was identified to potentially bind miR-429, which act as tumor-suppressor, and may be involved in the regulation of tumor-related genes, including FOXO1 and TP53.⁹⁰ A recent study revealed HOXB-AS3, a lncRNA located at the human HOXB cluster, maybe a potential risk factor in myeloid neoplasm especially MDS.¹⁰⁷ They demonstrated that high expression of HOXB-AS3 could promote myeloid cell proliferation which was consistent with previous researches.¹⁰⁷,¹¹⁵ Furthermore, clinical correlation analysis also showed that HOXB-AS3 was an adverse prognostic marker in patients with low IPSS index, compared with higher risk ones.⁹⁷

Aplastic anemia (AA) is a group of hematopoietic failure syndrome caused by multiple factors.¹¹⁶ At present, the underlying molecular mechanisms of AA are largely unknown. In a research of bone marrow mesenchymal stem cells (BMSCs) differentiation from AA patients, Jiang et al.⁹⁸ found fibroblast growth factor 1 (FGF1) could regulate the expression of lncRNA TDRG1 through promoting acetylation in the TDRG1 promoter, thus enhancing the proliferation of BMSCs. This finding is a novel insight into the treatment of aplastic anemia patients. However, more research is needed to deepen the explanation of the relationship between lncRNA and AA.

**Conclusion and Future Perspectives**

To date, there is a large body of evidence to suggest that long non-coding RNAs are becoming fundamental regulators in diverse biological processes including cell proliferation, differentiation, pluripotency, apoptosis, and tumorigenesis and are increasingly recognized as vital components of modern molecular biology. With the publication of various studies in succession, the role of lncRNAs in hematopoiesis shows up prominently. Here we widely reviewed recent advances and summarize the characteristics and basic mechanisms of lncRNAs and keep abreast of developments of lncRNAs within the field of normal and malignant hematopoiesis. Based on gene regulatory networks at different levels of lncRNAs participation, lncRNAs have been shown to regulate gene expression from epigenetics, transcription, and post-transcription. The expression of lncRNAs is highly cell-specific and critical for the development and activation of hematopoiesis. Moreover, we also summarized the role of lncRNAs involved in hematological malignancies in recent years. With the advent of the post-genome era, there must be many additional hematopoiesis-related lncRNAs to be discovered and the underlying precise mechanisms also need to be further excavated. We believe that lncRNAs may provide novel ideas for the diagnosis and therapeutic targets of hematological diseases in the foreseeable future.

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**Conflicts of interest**

None.

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