The Regulatory Roles of Long Noncoding RNAs in Acute Myeloid Leukemia

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In this post-genomic era, long noncoding RNAs (lncRNAs) are rapidly gaining recognition for their crucial roles across diverse biological processes and contexts. The human blood system is no exception, where dozens of lncRNAs have been established as regulators of normal and/or malignant hematopoiesis, and where ongoing works continue to uncover novel lncRNA functions. Our review focuses on lncRNAs that are involved in the pathogenesis of acute myeloid leukemia (AML) and the mechanisms through which they control gene expression in this disease context. We also comment on genome-wide sequencing or profiling studies that have implicated large sets of lncRNAs in AML pathophysiology.

Keywords: acute myeloid leukemia, long noncoding RNA, regulatory RNA, antisense transcripts, competing endogenous RNA, chromatin looping, lncRNA profiles

BACKGROUND: ON IncRNAs AND AML

Hematopoietic stem cell (HSC) homeostasis and lifelong blood formation rely on a complex interplay between many different pathways. These include growth factors, signaling cascades, and transcription factors, all of which interact to control the fine balance between self-renewal, quiescence, proliferation, and differentiation (1). Deregulation of this critical interplay can result in malignancy. One example is acute myeloid leukemia (AML), a heterogeneous hematologic disease characterized by the uncontrolled proliferation of undifferentiated myeloid precursors (blasts) (2). This phenotype can be driven by genetic abnormalities that enhance self-renewal and block differentiation, such as chromosomal translocations [e.g., t(15;17)[PML-RARA], t(9;22)[BCR-ABL], inv(16)[CBFB-MYH11], t(8;21)[RUNX1-ETO], MLL rearrangements] and gene mutations (e.g., DNMT3A, NPM1, CEBPA, IDH1/2, TET2, FLT3-ITD), the majority of which are well-defined (3–6). However, our current understanding of the underlying genetic basis of AML hinges on decades of protein-centered research. The contribution of noncoding regions to the initiation, maintenance, and evolution of AML remains to be fully revealed.

It is well-known that only about 2% of the human genome encodes protein, while 70–90% is transcribed, giving rise to an enormous number and variety of noncoding transcripts (7, 8). Among these, long noncoding RNAs (lncRNAs) comprise a substantial proportion (9) that are drawing significant attention as novel regulatory RNAs with roles in diverse physiological processes. LncRNAs are operationally defined as transcriptional products over 200 nucleotides (nt) long that ostensibly lack open reading frames (10, 11). Based on their location relative to coding loci along the genome, they can be further subdivided into four groups: (1) sense, (2) antisense, (3) intronic, or (4) intergenic (12, 13). Some mechanisms
that have been described for lncRNAs, so far, include cooperating with gene regulatory circuits in the nucleus (e.g., transcription factor recruitment or chromatin looping), blocking translation, sequestering microRNAs (miRNAs), getting processed into small RNAs, and encoding micropeptides (14–18). Regulating chromatin configuration appears to represent a recurring mechanistic theme among newly characterized lncRNAs, but in general, lncRNA functions do not correspond with their current classification, and we cannot yet conclusively point to unifying pathways. Nonetheless, the picture is still incomplete, and when lncRNAs are better understood as a class, an improved, functionally defined classification system may emerge.

Given the recent rapid expansion of the lncRNA research field and its exposition of lncRNA involvement across a range of key biological processes, it is no surprise that lncRNAs also regulate the blood system. We now know a number of individual lncRNAs that influence lineage decisions along the hematopoietic hierarchy and whose deregulation can result in hematologic malignancy (19–29). These examples are discussed in comprehensive review articles that synthesize our present understanding of lncRNA in normal and malignant hematopoiesis (30–33). While these articles take a broad view on the intersection of hematology and lncRNA biology, this research area has grown to the point where targeted commentaries for different leukemia subtypes may prove beneficial—not least because of the context specificity of lncRNA expression, including between cancers (34, 35). Here, we provide a focused, updated review of lncRNAs that are specifically relevant to AML.

**lncRNAs IMPLICATED IN AML PATHOPHYSIOLOGY**

In recent years, several large-scale expression studies have implicated lncRNAs in the pathogenesis of AML by demonstrating the association of distinct lncRNA profiles with genetically defined subtypes of AML (27, 36–40). In the first study of this kind, Garzon et al. profiled lncRNA expression in patients over 60 years old with untreated cytogenetically normal (CN) AML. They discovered distinctive lncRNA signatures associated with major molecular subtypes of AML including FLT3-ITD, NPM1, CEBPA, IDH2, and RUNX1 mutations. Whole-transcriptome sequencing in a cohort of younger adults (<60 years old) similarly revealed lncRNA expression profiles associated with FLT3-ITD, NPM1, and CEBPA mutations (38). Interestingly, lncRNAs located antisense to HOX genes featured among those that were upregulated in NPM1-mutated cases, particularly in the cohort of older adults. In both studies, the authors derived a prognostic score based on a small subset of survival-associated lncRNAs and demonstrated its robust performance as an independent predictor of clinical outcome. Papaioannou et al. additionally correlated their prognostic lncRNAs with messenger RNA (mRNA) and miRNA expression, thereby linking the lncRNAs to cancer-related pathways like leukocyte activation, inflammation, and apoptosis through guilt by association. Last, a smaller study of CN-AML patients uncovered an lncRNA signature that depended on the mutational status of NPM1 (37). The authors identified and validated a minimal set of 12 lncRNAs that could discriminate between NPM1-mutated and NPM1-wild type cases and initiated the preliminary characterization of one lncRNA, XLOC_109948, which they propose is involved in drug sensitivity.

Besides recurrent mutations, distinct lncRNA profiles have also been associated with certain cytogenetic subgroups of AML (27, 39). For example, using unconventional library preparation and assembly approaches, Zhang et al. uncovered a set of four noncoding transcripts that are specifically and highly expressed in patients harboring the PML-RARA translocation. These included a novel, previously unannotated lncRNA, and MEG3, an established maternally expressed lncRNA (39). Our own group has also reported subtype-specific fingerprint lncRNAs for six major subgroups of pediatric AML [inv(16), t(8;21), t(10;11), t(9;11), acute megakaryoblastic leukemia (AMKL), and Down syndrome myeloid leukemia (ML-DS)] (27). We further compared these expression profiles with those of healthy blood populations and found HSC signatures that are upregulated in AML blasts. These lists and others, spanning across the human hematopoietic lineages and pediatric AML subtypes, are publicly available as an online resource (www.lncscape.de), which aims to provide an outline of the lncRNA landscape behind normal and malignant hematopoiesis.

The molecular and cytogenetic subtype-specific expression patterns of lncRNAs in AML samples suggest their crucial contribution to the pathophysiology of this disease. In addition, this phenomenon poises lncRNAs as attractive targets for new therapeutic approaches where the specific targeting of oncogenic proteins has been unsuccessful. However, regarding the role of subgroup-associated lncRNA profiles in leukemogenesis, it remains ambiguous whether they are significant in their own right or whether they represent passenger events driven by genetic aberrations affecting coding genes. Shedding light on this question to some extent is Mer et al.’s exploration of an lncRNA-centered stratification system for AML patients (40). Based solely on lncRNA expression, the authors distinguished four molecular subtypes that differ in prognoses and active pathways and that behave independently of the European Leukemia Net (ELN) risk classification groups. However, although these lncRNA-based subgroups lacked high concordance with conventional clinical or genetic factors, there was, nonetheless, some association with traditional molecular determinants such as mutations in CEBPA, NPM1, TP53, and FLT3-ITD (40). Another recent study of CN-AML cases with leukemia stem cell (LSC)-associated core-enriched gene expression signatures (CE-GES) discovered a set of 111 lncRNAs that strongly correlate with the LSC signature (41). One of the most

**Abbreviations:** ADR, Adriamycin; AML, acute myeloid leukemia; AMKL, acute megakaryoblastic leukemia; APL, acute promyelocytic leukemia; Ara-C, Cytarabine; ATRA, all-trans retinoic acid; ceRNA, competing endogenous RNA; CML, chronic myeloid leukemia; CN, cytogenetically normal; CRISPR, clustered regularly interspaced short palindromic repeats; DNMT, DNA methyltransferase; FLT3-ITD, FLT3 internal tandem duplications; HSC, hematopoietic stem cell; lncRNA, long noncoding RNA; LSC, leukemic stem cell; miRNA, microRNA; ML-DS, myeloid leukemia of Down syndrome; PRC, polycomb repressive complex.
upregulated LSC-associated lncRNAs, DANC~R, was confirmed in functionally validated LSC populations, and its knockdown was shown to reduce self-renewal capacity. The authors further demonstrated that targeting Dancer in vivo in a murine model of AML prolonged the survival of mice after secondary transplantation (41).

All of the above studies implicate lncRNAs in the pathogenesis of AML, and De Clara et al. and Bill et al. additionally indicated their functional relevance and therapeutic potential. Other publications featuring lncRNA-based prognostic scoring systems are also beginning to emerge (42–45). Even so, extensive functional work is required to delineate the interactions between lncRNAs and known driver events and to better understand how each factor can influence the development and progression of AML. With lncRNA research continuing to grow at such a rapid rate, it will soon become crucial that we integrate this body of knowledge into our disease models and treatment practices toward ultimately improving clinical outcomes for patients.

**MECHANISMS OF lncRNA-MEDIATED GENE REGULATION IN AML**

Starting only a few years ago, the field of lncRNA research underwent—and is continuing to undergo—an exponential expansion. Dozens of articles are published per month describing lncRNA functions in a wide variety of healthy and diseased cell contexts. While this is very exciting for those of us who dream of someday being able to look up lncRNA functions as easily as we currently do for proteins, the field is still young, and in-depth mechanistic investigation is missing for many newly “characterized” lncRNAs. Further complicating the situation, some lncRNAs may produce different outcomes on gene regulation in different cell contexts or act via multiple pathways in a single context. Given the current, incomplete state of the literature—where, in many cases, one lncRNA is reported in a range of cell types along with an equal range of dissimilar mechanisms—it is difficult to distinguish between these two possibilities. By extension, it is also challenging to group lncRNAs based on their mechanisms. Nonetheless, we compiled a catalog of lncRNAs that have specifically been shown to play regulatory roles in AML cells (Table 1). While the list is not exhaustive, it summarizes the best examples of lncRNAs in AML pathogenesis and their mechanisms of action, which are discussed in detail in the rest of this review.

**COMPETING ENDOGENOUS RNA~S**

The deregulation of HOX genes has long been accepted as an important mechanism of leukemogenesis, often in connection with chromosomal translocations involving MLL (77). It is therefore unsurprising that lncRNAs transcribed from the HOX loci have also been discovered to exert regulatory roles in AML. For example, HOTAIRM1 is an intergenic lncRNA (lincRNA) located between HOXA1 and HOXA2 that is transcribed antisense to the HOXA cluster (46), and it is one of the best-studied lncRNAs in the blood system. Under ordinary circumstances, HOTAIRM1 expression is restricted to the myeloid lineage (46). In the NB-4 human acute promyelocytic leukemia (APL) cell line, it is strongly upregulated during all-trans retinoic acid (ATRA)-driven granulocytic differentiation, where it selectively modulates the induction of HOXA1/HOXA4 and of the myeloid maturation markers CD11b, CD18, and CD11c, meanwhile repressing CD49d (46, 47). HOTAIRM1 was shown to promote ATRA-driven cell cycle arrest, suggesting that HOTAIRM1-mediated gene expression changes may regulate a switch from a proliferative phase toward granulocytic maturation (47). An alternate, detailed mechanism put forward by Chen et al. contends that HOTAIRM1 regulates autophagy and thus the degradation of the PML-RARA oncoprotein, which characterizes APL and drives leukemogenesis in this subtype (48). HOTAIRM1 appears to accomplish this by acting as a competing endogenous RNA (ceRNA) and thereby sequestering miR-20a, miR-106a, and miR-125b away from their target mRNAs in the autophagy pathway—ULK1, E2F1, and DRAM2 (48) (Figure 1A).

While these findings provide an elegant trans mechanism for HOTAIRM1, another study from around the same time frame proposed a cis-regulatory mode of action within the HOXA cluster. Wang et al. reported HOTAIRM1’s involvement in controlling the three-dimensional chromatin organization behind HOXA gene activation in NT2-D1 carcinoma cells (49). Interestingly, modulating HOTAIRM1 exerted opposite effects on HOXA4/5 expression in NB-4 and NT2-D1 cells and produced chromatin conformation changes in NT2-D1 that were absent in NB-4 cells. These findings provide experimental evidence of the often-cited tissue-specific manner of lncRNA function (7–9) and offer a reminder to exercise caution when generalizing their mechanisms across cell types and contexts. Notably, both studies identified multiple HOTAIRM1 isoforms, which may explain the differences in HOTAIRM1-regulated HOXA gene expression between the two cell lines (48, 49). The primary HOTAIRM1 transcript detected in ATRA-treated NB-4 cells was ∼500 nt long (48, 49), whereas ATRA-induced NT2-D1 cells produced an unspliced transcript of over 4 kb as well as an ∼1.1-kb spliced form (49).

Other ceRNAs arising from the HOX loci include HOXAS2 (50, 51) and HOTAIR (52), which were reported to target miR-510-3p and miR-193a, respectively. In contrast to the antileukemic role of HOTAIRM1, HOXAAS2, and HOTAIR purportedly promote AML cell survival and proliferation by freeing oncogenic pathways from the control of tumor suppressor miRNAs. HOTAIR was additionally reported to induce EZH2-dependent epigenetic silencing of the tumor suppressor gene p15 (53), implying that some lncRNAs may function through multiple pathways to coordinate multilevel gene regulation. This finding is consistent with studies in other cell contexts describing how HOTAIR recruits polycomb repressive complex 2 (PRC2) and the LSD1/coREST/REST complex to regulate chromatin dynamics and epigenetic silencing [see Ref. (78) for a review]. On the other hand, a decisive study in human embryonic kidney 293 (HEK293) cells found that artificially tethering HOTAIR to chromatin led to local conformational changes and transcriptional repression independent of PRC2 (79). The matter of whether PRC2–lncRNA


| LncRNA       | Putative role    | Function in AML cells                                | Mechanism of action                                                                 | References |
|--------------|------------------|------------------------------------------------------|-------------------------------------------------------------------------------------|------------|
| HOTAIRM1     | Tumor suppressor | Regulates myeloid maturation, cell cycle, and autophagy | Activates proximal HOX4 and CD11b/CD18/CD11c expression and represses CD49d; sponges miR-20a/miR-106b/miR-125b | (46–49)    |
| HOX4-AS2     | Oncogenic        | Mediates resistance to apoptosis and Adriaycin       | Sponges miR-520c-3p, thereby increasing S100A4                                        | (50, 51)   |
| HOTAIR       | Oncogenic        | Promotes leukemic phenotypes by modulating c-KIT and p15 | Sponges miR-193a away from c-KIT and epigenetically silences p15, perhaps via PRC2 or LSD1? | (52, 53)   |
| CCAT1        | Oncogenic        | Inhibits myeloid maturation and promotes proliferation | Sponges miR-155 away from c-MYC                                                      | (54)       |
| UCA1         | Oncogenic        | Promotes chemoresistance, glycolysis, and proliferation; activated by CEBPα-p30 | Sponges miR-125a away from HK2, and miR-126 from RAC1; translationally represses p27? | (55–57)    |
| PU.1-AS      | Oncogenic*       | Negatively regulates PU.1 expression                 | Interferes with PU.1 translation by binding eIF4A                                       | (58)       |
| IRAIN        | Tumor suppressor* | Regulates expression of IGF1R in cis                 | Mediates enhancer looping to IGF1R promoter                                           | (59)       |
| PVT1         | Oncogenic        | Regulates expression of MYC in cis; promotes proliferation and survival | Alternate TSS are enhancers that loop to MYC<sup>C</sup>; promoter acts as a DNA boundary element | (60–62)    |
| RUNXOR       | Oncogenic<sup>5</sup> | Upregulated in AML blasts and after Ara-C treatment; likely regulates RUNX1 | Interacts with chromatin to form intra/interchromosomal loops, and with RUNX1 and EZH2 | (63)       |
| GAS6-AS2     | Oncogenic        | Mediates resistance to cytotoxic (Ara-C)             | Positively regulates GAS1 and 4XL expression in the latter case via promoter methylation by DNMT<sup>1</sup> | (64)       |
| TUG1         | Oncogenic        | Confers Adriaycin resistance                         | Epigenetically silences miR-34a via EZH2-dependent deposition of H3K27me3 at the promoter | (65)       |
| MIR100HG/MONC | Oncogenic        | Required for maintenance and self-renewal of AMLK    | Enhances erythroid progenitors                                                        | (66)       |
| LINC-223     | Tumor suppressor | Promotes monocytic differentiation                   | Acts as a decoy against oncogenic miR-125 family members                              | (67)       |
| ANRIL        | Oncogenic        | Maintains AML cell survival/proliferation and regulates glucose metabolism | Activates ADIPOR1/AMPK/SIRT1 expression                                                | (68)       |
| NEAT1        | Tumor suppressor | Decreases proliferation and increases apoptosis      | May act as a ceRNA on the miR-23a-3p/SMC1A axis                                        | (69)       |
| H19          | Oncogenic        | Behaves like an oncogenic lncRNA; may be involved in telomerase activity | May act as a ceRNA on miR-19/ID2; mediates hTERT2/hTR interaction                      | (70–72)    |
| CASC15       | Oncogenic<sup>6</sup> | Promotes myeloid over B-cell development             | May positively regulate SOX4 transcription via YY1?                                     | (73)       |
| CCDC26       | Tumor suppressor* | May regulate cellular response to starvation!       | May negatively regulate c-KIT!                                                       | (74)       |
| MEG3         | Tumor suppressor | Acts as a tumor suppressor, is regulated by WT1/TET2  | Activates p53, downregulates DNMT3A through MDM2/RB1                                  | (75)       |
| WT1-AS       | Tumor suppressor* | Mediates WT1 induction under hypoxic conditions      | Regulates WT1 expression in cis                                                       | (76)       |

The order corresponds to their sequence of appearance in the manuscript. The lncRNAs are grouped according to their mechanisms of action, with different groups separated by double lines in the table.

<sup>1</sup> Mechanism described in other tumor contexts; remains to be confirmed in AML.

<sup>2</sup> Mechanism described in K562 cells (chronic myeloid leukemia, CML); implicated in AML.

<sup>3</sup> Speculation based on the regulation of coding genes; cellular phenotype not yet characterized.

<sup>4</sup> Experiment conducted in the murine system; remains to be validated in human cells.

Interactions are promiscuous or functionally specific in nature remains highly debated, with abundant evidence for both sides of the argument merit a separate review [or three: Refs. (80–82)].

To date, there exist two other examples of lncRNAs that appear to regulate gene expression in AML by titrating miRNAs away from their endogenous mRNA targets. First, CCAT1 was shown to inhibit myeloid maturation and promote proliferation by reducing miR-155 availability and consequently raising c-MYC levels in AML cells (54). Second, UCA1 was reported to promote chemoresistance via the miR-125b/HK2 axis (57) and sustain proliferation and survival through miR-126/RAC1 (56). Of note, UCA1 was first described in AML as a regulatory target gene of CEBPα-p30 (55)—the 30-kDa isoform that results from mutations in CEBPA. Hughes et al. showed that both wild-type CEBPα and the p30 protein bind the UCA1 promoter but produce opposite effects on UCA1 expression. Using K562 cells, they outlined UCA1’s oncogenic role in suppressing translation of the cell cycle regulator p27<sup>kip1</sup> through competing for hnRNP
FIGURE 1 | Some examples of IncRNA mechanisms that unfold in the cytoplasmic compartment. Dashed lines specify pathways that are reduced upon IncRNA expression, whereas solid lines indicate outcomes of IncRNA expression. The examples are ordered in a counterclockwise manner. (A) HOTAIRM1 acts as a decoy for miR-20a, miR-106b, and miR-125b in NB-4 cells, titrating them away from mRNAs that encode regulators of autophagy, and thereby enhancing the degradation of PML-RARA oncoprotein through this pathway. HOTAIRM1 also modulates the expression of HOXA genes and of cell surface lineage markers CD11c and CD49d through an undetermined mechanism. (B) In contrast to wild-type CEBPα, truncated CEBPα-p30 transactivates the UCA1 promoter in AML cells with CEBPα mutations. The UCA1 transcript functions as a sponge on miR-125a and miR-126 and additionally competes with the p27kip1 mRNA for stabilizing interactions with hnRNP I. (C) PU.1-AS lncRNAs are polysome associated and interfere with PU.1 protein expression by competitively binding the translation initiating factor eIF4A. I—a mechanism that was originally proposed in breast cancer (83) (Figure 1B). Another notable study defines UCA1 as an RNA scaffold that is vital for normal erythrocyte development and heme biosynthesis (84).

OTHER CYTOPLASMIC TRANS-REGULATORY MECHANISMS

As illustrated by the example of UCA1, there is evidence of IncRNAs acting at the level of translation to regulate gene expression. In AML, the remainder of this group is represented by PU.1-AS, which acts on the mRNA of PU.1, its antisense coding gene, to reduce its translation (58). The PU.1-AS transcripts originate from an intronic promoter in PU.1, which encodes a vital transcription factor for normal hematopoiesis and whose downregulation can result in leukemogenesis [for a review, see (85)]. PU.1-AS was discovered to negatively regulate PU.1 expression, through a mechanism whereby PU.1-AS antagonizes PU.1 translation by selectively binding to the initiation factor eIF4A (58) (Figure 1C). PU.1-AS also appeared to interfere with translation elongation through an unknown mechanism. The implications of these findings on the pathophysiology of AML cells remain to be elucidated as well.

TRANSCRIPTIONAL/EPIGENETIC REGULATION IN THE NUCLEUS

In cis

Besides acting on miRNAs or mRNAs in the cytoplasm, IncRNAs can also control gene expression in the nucleus
at the level of transcription, epigenetics, or chromatin state. They can accomplish this in cis by recruiting transcription factors and epigenetic modifiers to their site of transcription or by assisting the formation of enhancer–promoter loops or other chromatin conformations. **IRAIN** is one such lncRNA. It is transcribed antisense to the gene encoding IGF1R—a key component of the PI3K/AKT signaling pathway that is constitutively active in AML—and expressed exclusively from the paternal allele in hematopoietic and leukemia cells (59). The transcript itself shows evidence of interacting with neighboring chromatin regions including the **IGF1R** promoter and an intronic enhancer (59) (Figure 2A). Indeed, Sun et al. showed that the **IRAIN** RNA is needed for an enhancer–promoter contact between these two elements. However, the impact of **IRAIN** on AML pathogenesis and whether IRAIN-dependent enhancer–promoter loop formation influences the expression of IGF1R or other genes remain to be seen. Initial hints from patient data suggest an inverse relationship between **IRAIN** and AML aggressiveness (59).

Another example of a cis-regulatory lncRNA is **PVT1**. The **PVT1** gene lies within the human chromosome 8q24 region, which is frequently disrupted in human cancers (i.e., by rearrangements or amplifications) and which harbors numerous MYC enhancer elements—including several within the **PVT1** gene locus (86). A landmark paper by Tseng et al. demonstrated **PVT1** dependence in breast cancer cells with **MYC** copy number increase (87). Consistent with the oncogenic role of **PVT1** in other cancers, its knockdown reduced proliferation and induced apoptosis in AML cell lines (60, 62, 88) and was accompanied by **MYC** downregulation in some (60, 88). A CRISPRi tiling screen covering 3 Mb of the locus identified alternative transcription start sites of two **PVT1** isoforms as enhancers that loop to the **MYC** promoter in K562 cells (61). Interestingly, in breast cancer cells, a tumor suppressor role was recently ascribed to the **PVT1** promoter, which is proposed to act as a DNA boundary element that silences **MYC** from its downstream enhancers (89).

The **RUNX1** genetic locus on human chromosome 21 also plays a critical role in normal and malignant hematopoiesis [for a review, see Ref. (90)]. It is frequently disrupted by chromosomal aberrations in AML, such as the t(8;21) translocation that occurs in 30–40% of AML cases. In addition, the locus produces a rather unconventional lncRNA named **RUNXOR**, which is transcribed as a 216-kb unspliced sense RNA from a promoter several kilobases upstream of **RUNX1** and which, thus, overlaps the **RUNX1** introns and exons (63). Wang et al. found **RUNXOR** to be upregulated in AML patient samples compared to healthy controls, and in K562 cells following cytarabine (Ara-C) treatment. In KG-1 cells, the **RUNXOR** transcript showed evidence of interacting via its 3’ end with the **RUNX1** promoters and enhancers (63). It also appeared to form an intrachromosomal contact with the most common **RUNX1** intronic translocation breakpoint, as well as interchromosomal contacts with distant loci including **EVI1** and **ETO**—recurrent **RUNX1** translocation partners in hematologic malignancies (Figure 2B). These latter findings suggest a revolutionary model where **RUNXOR** may physically mediate the chromosomal translocation process. Lastly, Wang et al. showed EZH2 and RUNX1 binding to the **RUNXOR** RNA, thereby implying its involvement in recruiting and directing these transcription factors (63).

**Bidirectional Promoters**

The last few years have seen a steep rise in the application of large-scale CRISPR/Cas9 screens toward functionalizing lncRNAs (64, 91–98). These have been carried out in various cell systems with unified outcomes in several respects: (1) lncRNAs are indeed important in a range of cellular contexts; (2) whether they are essential or not appears to be cell type specific, sometimes even between cell lines derived from the same cancer type; and (3) the screens tend to have low hit identification rates, which may
be due in part to the context-restricted manner of lncRNA expression and function.

A recent study by Bester et al. utilized a CRISPR activation (CRISPRa)-based approach to identify lncRNAs that influence cytarabine (Ara-C) resistance in AML cell lines (64). Their screen identified GAS6/GAS6-AS2 as an enriched coding-noncoding gene pair in Ara-C-resistant cells. GAS6/GAS6-AS2 are transcribed in opposite directions from a bidirectional promoter—a "head-to-head" configuration that qualifies GAS6-AS2 as a divergent lncRNA. Divergent lncRNAs have been linked with the genetic loci of essential developmental transcription factors and show evidence of regulating their expression in cis (99–101). Consistent with this model, GAS6-AS2 and GAS6 expression were strongly correlated across 760 cancer cell lines, and antisense oligonucleotide-based GAS6-AS2 knockdown also caused a significant downregulation of GAS6 (64). In addition, AXL, the receptor of the GAS6 ligand in the TYRO3–AXL–MERTK pro-survival signaling cascade, was reduced at the mRNA level, suggesting a coexisting trans mechanism for GAS6-AS2. Based on changes in AXL promoter methylation upon GAS6-AS2 knockdown and on RNA immunoprecipitation assays in K562 cells, the authors speculate that GAS6-AS2 coordinates the activity of DNA methyltransferase proteins at the AXL promoter, on top of regulating GAS6 expression (64) (Figure 2C).

In Trans

As exemplified by GAS6-AS2, lncRNA transcripts can diffuse away from their genomic origin to regulate distant loci by interfacing with nuclear regulatory complexes or by acting as a scaffold for the assembly of transcriptional machinery. TUG1, unlike GAS6-AS2, seems to function exclusively in trans, at least so far [refer to reviews (102, 103)]. Its expression is elevated in AML patients and cell lines (65, 104) and may be linked to aurora kinase (AURKA) protein levels, leading to protection against apoptosis (104). Recent work by Li et al. moreover provides compelling evidence of TUG1's role in promoting chemoresistance (65). Not only was TUG1 upregulated in AML patients compared to healthy controls, but it was also particularly high in Adriamycin (ADR)-resistant leukemia cells relative to ADR-sensitive samples (65). In a series of complementary overexpression and knockdown experiments in HL-60 (sensitive) and HL-60/ADR (resistant) cells, Li et al. robustly demonstrated that TUG1 confers ADR resistance through EZH2-mediated epigenetic silencing of miR-34a (65) (Figure 2D).

SMALL RNA HOST GENES

A number of miRNA host genes have been described to carry out independent regulatory functions as lncRNAs, separate from their miRNA products. Our group demonstrated this for MONC and MIR100HG, each of which harbors a three-miRNA cluster that is transcribed as a single polycistron (tricistron): miR-99a∼125b-2 on chromosome 21 and miR-100∼125b-1 on chromosome 11, respectively (66). The tricistrons are homologs of each other and were shown to protect AML blasts from transforming growth factor-β1 (TGF-β1)-mediated cell cycle arrest and apoptosis by shifting the overall balance of cellular signaling toward the Wnt pathway (105). This finding has since been corroborated by Lu et al., who showed that MIR100HG-derived miR-100 and miR-125b promote cetuximab resistance through the coordinated repression of five negative regulators of Wnt/β-catenin (106). They additionally discovered a double-negative feedback loop between MIR100HG and GATA6, in which GATA6-mediated MIR100HG repression is relieved through miR-125b targeting of the GATA6 mRNA. Meanwhile, miR-125b appears to act as an oncomiR in megakaryoblastic leukemias (107). Returning to the lncRNA host genes, MIR100HG and MONC expressions are elevated in AML cell lines of the megakaryoblastic subtype (AMKL) (66) and are required for the self-renewal and maintenance of AMKL cells. When overexpressed in CD34+ cord blood cells, we saw that ectopic MONC expression interfered with hematopoietic lineage decisions and enhanced the growth of immature erythroid progenitors.

LINC-223, the precursor of miR-223, is a second example of a miRNA host transcript with independent lncRNA functions (67). Its expression is induced in HL-60 cells upon vitamin D3-driven monocytic differentiation, without a concomitant increase in miR-223, and similar effects were observed during monocytic differentiation of CD34+ cord blood progenitors (67). In order to interrogate LINC-223 specifically, without contribution from miR-223, the authors generated a LINC-223 derivative lacking the requisite region for DROSHA cleavage and miRNA processing. In HL-60 cells, ectopic expression of this construct impaired proliferation and cell cycle progression, meanwhile promoting monocytic differentiation (67). Mechanistically, the authors robustly demonstrated LINC-223's role as a ceRNA decoy of the oncogenic miR-125 family members (miR-125a, miR-125b-1, and miR-125b-2) and implicated this lncRNA in the regulation of the transcription factor IFN4, which is typically targeted by miR-125.

OTHER REGULATORY lncRNAs

In this section we describe lncRNAs that show experimental evidence of regulating gene expression, but that remain ambiguous as to their precise mechanisms of action. These lncRNAs lack defined interaction partners and/or functional rescue data, making it difficult to robustly discriminate cis from trans modes of action or determine direct influence on chromatin architecture, RNA stability, translation, or regulation on any other level. Given the depth of data needed to conclusively resolve lncRNA mechanisms, many newly characterized lncRNAs (or well-known lncRNAs that are investigated in a new cell context) predictably fall in this category.

ANRIL, an lncRNA transcribed from the INK4A–ARF–INK4B cluster on chromosome 9, is known for acting through PRC1/2 to regulate the expression of p15INK4B and p16INK4A in cis and for repressing distant genes in trans [for a review, refer to Ref. (108)]. Polymorphisms affecting ANRIL have been associated with risk for various diseases including cancer, albeit with a wide
range of reported impacts on the transcript itself, and ANRIL gain of function also gives rise to diverse cell type-dependent results despite universally increasing oncogenic phenotypes like proliferation and survival (108). This observation again highlights how lncRNAs may have context-dependent functions and discourages their generalization (108). With respect to AML, ANRIL was described to be essential for AML maintenance and to positively regulate glucose metabolism through ADIPOR1 and its targets, AMPK and SIRT1 (68).

The NEAT1 lncRNA is an essential structural component of paraspeckles and plays an indispensable role in their spatial organization, possibly through long-range interactions between NEAT1 transcripts (109). Dysregulation of NEAT1 has been reported in human cancers: interestingly, it is upregulated in various solid tumors, whereas it is downregulated in hematologic malignancies [for recent reviews, refer to Refs. (110, 111)]. Consistent with this, NEAT1 overexpression led to decreased proliferation and increased apoptosis in primary AML blasts and THP-1 cells (69). Zhao et al. suggested the mir-23a-3p/SMC1A axis as a downstream effector of NEAT1 and showed that its perturbation recapitulates the NEAT1 phenotype. Another earlier study in the APL cell line NB-4 found a subtle inverse correlation between NEAT1 and PML-RARA expression and showed the upregulation of NEAT1 upon ATRA-induced differentiation (112).

H19 was among the first-discovered lncRNAs and has since been shown to play essential roles during development and tumorigenesis [see reviews (113, 114)]. H19 is also quite established in the blood system, where it is one of the few lncRNAs that have been characterized in both normal and malignant hematopoiesis. The most recent data come from a single-cell resolution lncRNA landscape of murine HSC development, which identified H19 as a critical regulator of endothelial-to-HSC transition, independent of its derivative mir-675 (115). This study further demonstrated how H19 acts in trans to modulate promoter methylation of key hematopoietic transcription factors like Runx1 and Spi1. In human AML cell lines, H19 knockdown caused a decrease in proliferation and increase in apoptosis (70, 72), while elevated expression correlated with poor treatment response and overall survival in patients (72). Since its expression was observed to reciprocate that of miR-19, H19 was proposed to act as a ceRNA upon the mir-19/ID2 axis (70). It has also been linked to telomerase activity in the NB-4 cell line, where elevated H19 disrupted the interaction between TERT and TR (71). Additional studies are required to determine whether H19 regulates both circuits at once and to resolve the mechanistic details surrounding its mode of action.

Moving this discussion to less renowned lncRNAs, CASC15 was originally reported as a tumor suppressor in neuroblastoma (116), though it has since been found to promote oncogenic processes in melanoma (117) and other solid tumors. A study from Fernando et al. found elevated CASC15 levels in AML and B-cell acute lymphoid leukemia (B-ALL) patients with RUNXI rearrangements (respectively, t(8;21)[RUNX1-ETO] and t(12;21)[RUNX1-ETV6]) compared to other cytogenetic profiles (73). Enforced expression of Casc15 in normal murine bone marrow cells impaired their engraftment and led to a myeloid differentiation bias concurrent with defective B-cell development. The CASC15 gene lies next to that of the B-cell transcription factor SOX4, and the pair show coordinated expression across AML and B-ALL cell lines—once again, with elevated expression in instances with RUNX1 translocations (REH, Kasumi-1, and SKNO-1) (73). Using several strategies to deplete CASC15 in B-ALL cell lines, Fernando et al. demonstrated its regulatory action on SOX4 and mechanistic synergy with the YY1 transcription factor. It remains unclear whether this occurs in cis or in trans, and the mechanism has yet to be validated in AML cells.

Along with MYC and PVT1, the CCDC26 lncRNA gene lies in the human chromosome 8q21 region that is frequently amplified in cancer (86). Despite being expressed at roughly one copy per cell in myeloid leukemia cell lines, it is upregulated in these cells compared to other cancer types—including lymphoid malignancies—in which CCDC26 expression is not detectable (74). Knockdown clones derived from K562 cells showed increased proliferation rates in serum-depleted media, as well as reduced cell death compared to control cells. This oncogenic phenotype was attributed to the induction of KIT, which was among the most upregulated genes in CCDC26 knockdown cells (74).

MEG3 is a well-known tumor suppressor lncRNA, and its expression is lost—mostly through promoter methylation—in a growing list of human cancers (118). MEG3 was first shown to activate p53 transcription in carcinoma cell lines (119), but a more recent study also discovered a p53-independent pathway where MEG3 downregulates DNMT3A via MDM2/RB signaling to suppress leukemogenesis (75). The latter study further connected the transcriptional regulation of MEG3 to WT1/TET2 (75), two frequently inactivated genes in AML. Notably, the WT1 gene locus produces its own antisense lncRNA, WT1-AS, which is frequently disrupted by aberrant splicing in AML patients (120). WT1-AS and WT1 expression have been linked to hypoxia-induced, TET2-dependent demethylation of the CpG island in intron 1 of WT1 (76). WT1-AS additionally appears to be necessary for WT1 expression but failed to induce WT1 in trans (76). Altogether, these data afford a glimpse into how complex gene regulatory networks can be steered by lncRNAs.

CONCLUDING REMARKS

There is obviously substantial work that remains before we can claim an in-depth understanding of how lncRNAs contribute to AML biology or, indeed, to biology in general. Nevertheless, individual examples provide important insights into the regulatory roles of a few lncRNAs in the context of AML. From these, it is apparent that lncRNAs are crucial players in coordinating both novel as well as established oncogenic
gene expression pathways across this heterogeneous genetic disease, whether they act upon spatial chromatin organization, epigenetics, competitive miRNA binding, or other processes. We look forward to witnessing how this new layer of regulatory complexity mediated by lncRNAs will integrate into our current protein-centric view of human health and disease and to unraveling the exquisite gene networks that will surely emerge.

AUTHOR CONTRIBUTIONS

MN drafted the manuscript. DH and J-HK revised the content and approved the manuscript for publication.

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