Experimental Hematology

Experimental Hematology 2018;62:1–6

REVIEW

MicroRNA-155 expression and function in AML: An evolving paradigm

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(Received 22 February 2018; revised 22 March 2018; accepted 23 March 2018)

Acute myeloid leukemia (AML) arises when immature myeloid blast cells acquire multiple, recurrent genetic and epigenetic changes that result in dysregulated proliferation. Acute leukemia is the most common form of pediatric cancer, with AML accounting for ~20% of all leukemias in children. The genomic aberrations that drive AML inhibit myeloid differentiation and activate signal transduction pathways that drive proliferation. MicroRNAs, a class of small (~22 nucleotide) noncoding RNAs that posttranscriptionally suppress the expression of specifically targeted transcripts, are also frequently dysregulated in AML, which may prove useful for the purposes of disease classification, prognosis, and future therapeutic approaches. MicroRNA expression profiles are associated with patient prognosis and responses to standard chemotherapy, including predicting therapy resistance in AML. miR-155 is the primary focus of this review because it has been repeatedly associated with poorer survival across multiple cohorts of adult and pediatric AML. We discuss some novel features of miR-155 expression in AML, in particular how the levels of expression can critically influence function. Understanding the role of microRNAs in AML and the ways in which microRNA expression influences AML biology is one means to develop novel and more targeted therapies.

Acute myeloid leukemia (AML) arises when immature myeloid blast cells acquire multiple, recurrent genetic and epigenetic changes that result in dysregulated proliferation. AML represents ~1–2% of all cancers and occurs more frequently over the age of 60 (incidence of 20 per 100,000) [1]. Acute leukemia is the most common form of pediatric cancer, with AML accounting for ~20% of all leukemias in children.

Broadly, the genomic aberrations that drive AML inhibit myeloid differentiation and activate signal transduction pathways that drive proliferation [2]. A range of cytogenetic and molecular aberrations involving hematopoietic transcription factors (e.g., CBFβ-MYH11) and molecular abnormalities, including mutations in key signaling molecules (e.g., Flt3-internal tandem duplication; FLT3-ITD), epigenetic modifiers, and spliceosome machinery, contribute to the heterogeneity of the disease with prognostic and therapeutic implications. This has been reviewed extensively elsewhere and several significant studies have been published recently that describe the genomic landscape of AML comprehensively [3–6]. MicroRNAs, a class of small (~22 nucleotide) noncoding RNAs that posttranscriptionally suppress the expression of specifically targeted transcripts, are also frequently dysregulated in AML, which may prove useful for the purposes of disease classification, prognosis, and future therapeutic approaches.

Profiling of microRNA expression across both adult and pediatric AML cohorts has shown that distinct microRNA signatures are associated with a range of different cytogenetic AML subtypes and, to a lesser extent, molecular subtypes of AML [7–12]. MicroRNA expression profiles are also associated with patient prognosis and responses to standard chemotherapy, notably predicting therapy resistance in AML (reviewed in Zebisch et al [13].). High expression of miR-199 and miR-191a, for example, correlates with poorer patient outcomes, including both shorter event-free survival and overall survival, in adult AML [11]. Most notably, miR-155, a microRNA that will be the primary focus of this review, has been repeatedly associated with poorer survival across multiple

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0301-472X/
https://doi.org/10.1016/j.exphem.2018.03.007

cohorts of adult and pediatric AML irrespective of underlying cytogenetic or molecular aberrations [6,14–16].

A much smaller subset of microRNAs, when overexpressed, are sufficient to drive AML development in murine models (e.g., miR-125b [17,18]) and have demonstrated roles in the pathogenesis of AML. Whether as a primary driver of AML or as a factor contributing to the function of other AML driver lesions, microRNAs are likely to regulate gene expression in ways that inhibit differentiation of AML cells, promote their proliferation, and block apoptosis pathways [19,20].

Understanding the role of microRNAs in AML thus provides new information to improve patient risk stratification and is potentially a means to develop novel and more targeted therapies. However, with advances in our understanding of microRNA biology in AML, a more multidimensional paradigm of how microRNA expression and function are regulated in AML emerges and must be considered.

Complexity of microRNA-155 expression and function in AML

MicroRNA-155 was first identified as the product of the B-cell integration cluster (*bic*) gene (21q21.3; *MIR155HG*), a gene frequently upregulated in B-cell lymphoma [21,22]. In the hematopoietic system, a diverse range of functions for miR-155 have been described, ranging from the regulation of immune responses to inflammation to myeloid lineage specification. Mice deficient for *MIR155HG* have significant defects in B-cell germinal center formation, B-cell antibody class switching, T-cell cytokine response, and dendritic cell antigen presentation [23–25]. Although no impairments in the development of normal myeloid and lymphoid subsets were observed in miR-155-knockout animals, enforced expression of miR-155 in hematopoietic stem cells (HSCs) can block myeloid, erythroid, and possibly even megakaryocytic differentiation in vitro [26]. Enforced expression of miR-155 in HSCs also results in a myeloproliferative phenotype in vivo, suggesting that miR-155 may have a role in normal myeloid cell development [27]. In the context of inflammation, treatment of macrophages or B cells with lipopolysaccharide (LPS) in vitro and in vivo or through activation of toll-like receptor (TLR) signaling also induces miR-155 expression [27–29].

In cancer, miR-155 is commonly overexpressed and is best established as an oncogenic driver of B-cell lymphoma. Elevated miR-155 expression has been detected in many subtypes of B-cell lymphoma [21,30] and transgenic expression of miR-155 in mouse models is sufficient to drive the development of B-cell lymphoma in vivo [31]. In AML, numerous studies have demonstrated that miR-155 expression is elevated in adult and pediatric AML, particularly in the Flt3-ITD bearing cytogenetically normal AML [8,10,11,32,33]. However, until recently, the functional role of miR-155 in the pathogenesis of AML remained somewhat puzzling. Although enforced expression of miR-155 in vivo resulted in a myeloproliferative phenotype and blocked myeloid differentiation in some settings, a contrasting apparent tumor suppressor functions of miR-155 in AML cell lines has also been reported [34,35].

Our recent discovery of a dose-dependent role for miR-155 may resolve this dichotomy of miR-155 function in AML [15] (Fig. 1). We showed that the effects of miR-155 in AML are critically dependent on the levels to which miR-155 is expressed. Enforced expression of miR-155 to high levels (>50-fold above controls) demonstrated antitumor activity in three models of AML (MLL-AF9, MLL-ENL, and HoxA9/Meis1), reducing the clonogenic potential and proliferation of AML cell lines in vitro. Conversely, the oncogenic functions of miR-155 in AML were mediated within a defined range of *intermediate* expression (~10- to 50-fold above controls), were associated with increased tumor burden in our AML model in vivo, and were associated with poorer prognosis in pediatric AML patients.

Most notably, our data showed that these contrasting effects on phenotype in AML were a consequence of alternate target selection and distinct transcriptional changes induced by miR-155 at the "high" and "intermediate" levels of expression, with little overlap in the differentially expressed genes between the two conditions [15]. Intermediate miR-155 expression was characterized by the potentially direct repression of genes associated with myeloid differentiation and/or loss of function in AML [36–42], such as *Spi1*, *Cebpb*, and *Pml*, among other genes and gene pathways. We also observed significant downregulation of another gene, *Tle2*, at intermediate miR-155 expression levels, which had not been previously implicated in the oncogenic functions of miR-155. *Tle2* is a member of the Groucho/Tle family of transcriptional corepressors that can compete with β-catenin binding to the Tcf/Lef transcriptional complex and subsequent activation of genes downstream of the Wnt signaling pathway [43]. Recent studies have highlighted the importance of the Wnt/β-catenin pathway in AML [44,45] and suggested that *Tle2* downregulation by miR-155 potentially functions to augment Wnt/β – catenin signaling. The high miR-155 expression level, in contrast, was associated with downregulation of genes normally activated in AML and/or required for AML maintenance [46–49], such as *Myb* and *Kit*. This was consistent with its potential tumor-suppressor-like effects in AML.

How miR-155 can mediate such contrasting effects on the transcriptome when expressed to different levels is not well understood. MicroRNAs recognize and bind their mRNA targets via extensive or partial Watson–Crick pairing of the microRNA seed sequence (nucleotides 2–8 of the microRNA; Fig. 1) with a complementary sequence typically at the 3′ untranslated region of its target mRNA (see pairing of *Spi1* and murine miR-155 in Fig. 1) [50]. However, factors such as cellular context and the strength of interactions between a microRNA and its target ultimately influence outcome of microRNA expression in any given cell. MicroRNAs are known to have different, even opposite, functional effects in different contexts. For example, the miR-17~92 cluster acts as an oncomiR in hepatocellular and colorectal carcinoma

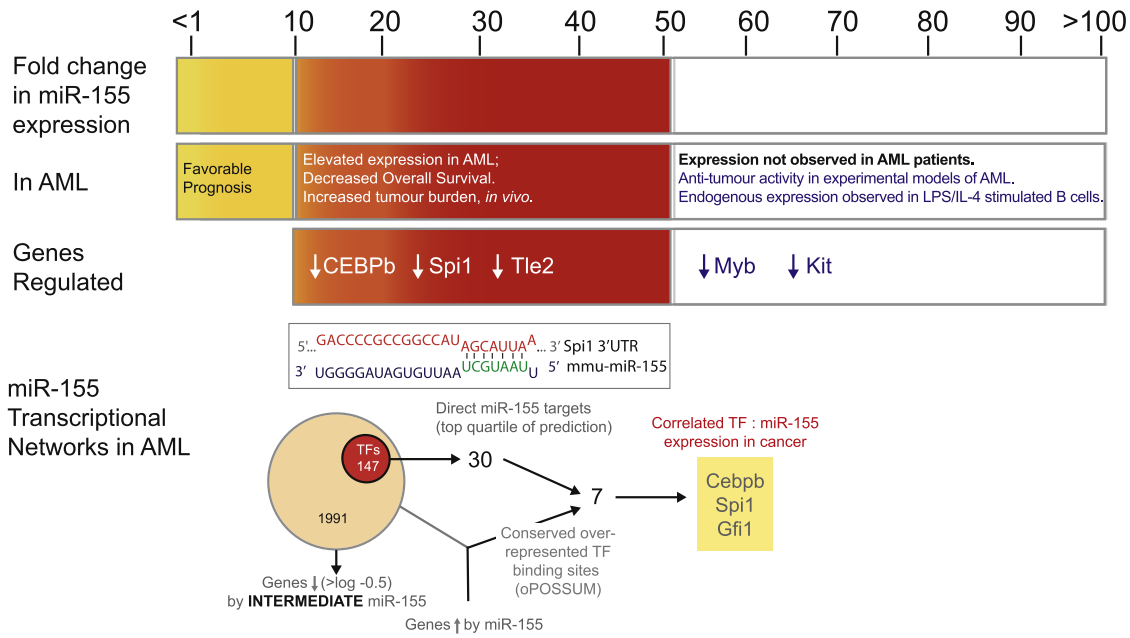

Figure 1. miR-155 function in AML is strongly influenced by the levels to which the microRNA is expressed. The oncogenic functions of miR-155 in AML are mediated within a defined range of intermediate miR-155 expression (between ~10- and 50-fold expression relative to nonleukemic controls) that reflects the true elevated miR-155 expression in patients that is associated with poorer overall survival and increased tumor burden in murine model of AML. Patients with <10-fold miR-155 expression have more favorable outcomes. In contrast, high levels of miR-155 (>50-fold expression) are not observed in pediatric AML patients and in vitro correlates with tumor suppressor-like activity in experimental models of AML. Instead, the high levels of miR-155 correlate with endogenous expression of miR-155 observed upon activation of B cells with an inflammatory stimulus of LPS and IL-4. These contrasting effects of different miR-155 expression levels in AML are explained by the differences in genes regulated at intermediate (*Cebpb*, *Spi1*, and *Tle2*) and high (*Myb* and *Kit*) miR-155 expression levels. These include direct miR-155 targets with complementary sites to the miR-155 seed sequence (green), such as *Spi1* depicted here, as well as secondary targets of miR-155. This may be of particular consequence when differentially regulated genes are themselves transcription factors that in turn control a host of downstream targets, as illustrated here. (The *Spi1* 3′ untranslated region and mmu-miR-155 sequences were obtained from Target Scan http://www.targetscan.org/mmu_71/).

[51,52], yet behaves as a tumor suppressor in cervical cancer [53]. This is the case with a surprisingly high number of microRNAs, with the most obvious explanation being the differential expression of target genes between different cells. We find a similar situation with miR-155, although, in contrast to the previous example, the differential effect we report is a function of the level of miR-155 expression rather than different cellular contexts [15]. Therefore, variation in the repertoire of expressed target genes cannot be the underlying mechanism. A similar observation was also made with regard to the let7a~7f and miR-17~92 clusters, with a shift in targets in response to varying microRNA levels changing the biological effect of the microRNA itself [54]. The main determinant for an individual microRNA to inhibit a single given transcript is the strength of the interaction interface between the RNA molecules, which is dependent on factors including the stoichiometry of the participant RNAs and levels of other binding partners within the transcriptomic milieu [55]. One would anticipate the lower a microRNA is expressed, the less likely it is to exert a functionally significant degree of suppression of a highly expressed mRNA. As microRNA levels increase, one anticipates that a threshold of functional significance will be reached for progressively more highly expressed mRNAs [50,56]. This is the exact observation that we made with miR-155, with intermediate miR-155 expression tending to target more lowly expressed genes [15].

Another intriguing observation from the analysis of the differentially expressed genes in high and intermediate miR-155 expression in our AML models was the significant differential regulation of key transcriptional networks. MicroRNAs commonly target transcription factors that themselves regulate entire networks of gene expression through direct regulation of transcription. From the early days of microRNA network biology, an enrichment of transcription factor targets was noted and now a great many examples exist of, not only microRNAs targeting transcription factors, but the transcription factors themselves also reciprocally targeting the microRNA and thus forming coregulatory loops [57]. Such a network motif occurs significantly more commonly than would be expected by chance [58]. Such is the importance of microRNA: transcription factor interactions: that most changes in gene expression in response to microRNA perturbation actually occur at the transcriptional and not the posttranscriptional level, consistent with the special significance of transcription factor targeting [59]. We speculate this may at least partially underlie the differential effects of

intermediate and high miR-155 expression. For example, we identified 30 transcription factors that are putative direct targets suppressed by intermediate miR-155 expression (unpublished observation). Binding sites for seven of these were found to be enriched within the set of genes that were differentially expressed between intermediate and high miR-155 expression (Fig. 1). The expression of three of these genes, *Cebpb*, *Gfi1*, and *Spi1*, was negatively correlated with that of miR-155 across AML and other cancer expression datasets, consistent with roles for these transcription factors in mediating the differential outcomes of intermediate and high miR-155 expression. Interestingly, *Cebpb* (and/or *Cebpa*) [42,60,61], *Gfi1* [62,63], and *Spi1/PU.1* [36–39] have each been extensively implicated in both hematopoiesis and leukemia.

miR-155 expression: a balance between inflammation and cancer

A key observation from our study was that the high miR-155 expression levels (>50-fold above controls) associated with the repression of AML cell growth in vitro and was not observed in any of our pediatric AML patients, closely correlated instead with the endogenous expression levels of miR-155 achieved in activated B cells exposed to an inflammatory stimulus of LPS and interleukin-4 (IL-4) [15]. Certainly without this evidence, it is plausible to think that the antitumor activity of high miR-155 expression in our experimental models might be an artifact of enforcing miR-155 to supraphysiological levels. Our finding, however, that in an endogenous context, miR-155 expression can also increase to levels >50-fold (~60–90 fold) in response to LPS and IL-4 stimulation compared with naive B cells, countered this argument. Consistently, high miR-155 expression levels in our AML models also upregulated the expression of many genes associated with interferon signaling and inflammation. This suggests that high miR-155 expression enforced in our models has a physiological correlate corresponding to miR-155's function in inflammation and/or immune responses. In addition, these data highlight that, in normal blood cells, a fine balance of miR-155 expression needs to be maintained to mediate its inflammatory/immune functions while avoiding malignant consequences. Recent findings by Wallace et al. [64] further emphasize this distinction between miR-155 function in inflammation versus cancer, showing that, in cells with Flt3-ITD mutations, miR-155 promotes the expansion of myeloid cells by repressing interferon signaling. This highlights the importance of understanding how miR-155 expression is regulated in a physiological setting and the mechanisms that perturb this regulation to drive cancer development.

In AML, miR-155 is known to be regulated by multiple mechanisms. Oncogenic proteins and fusions such as Flt3-ITD and MLL fusions, functioning through HoxA9 and Meis1, are known to upregulate miR-155 expression in AML [33,65–67]. In addition, we demonstrated in pediatric AML that the *MIR155HG* locus is also differentially methylated, potentially accounting for elevated expression of miR-155 in the disease [15]. In inflammation, upregulation of miR-155 expression has been largely attributed to transcriptional regulation of the *MIR155HG* locus by AP-1 and nuclear factor kappa beta (NF-κB) downstream of TLR signaling [68]. Interestingly, NF-κB is also implicated in the regulation of miR-155 expression downstream of Flt3-ITD in AML and inhibition of NF-κB activation with MLN4924 (pevonedistat) was shown to reduce miR-155 expression and to delay tumor progression in vivo [65]. However, how these mechanisms distinctly induce miR-155 expression to elevated levels in cancer and very high levels in inflammation has yet to be determined.

Clinical implications of miR-155 expression in AML

Evidence in support of an oncogenic role for miR-155 in AML is accumulating, with more recent findings demonstrating cooperation between enforced miR-155 expression and loss of C/EBPA in the development of AML [69]. What remains to be established, however, is the requirement of miR-155 expression in the maintenance and progression of AML. Schneider et al. reported that, despite the induction of miR-155 by MLL fusion genes, the deletion of miR-155 did not affect the onset or latency of MLL-AF9- or MLL-ENL-driven leukemias in vivo [67]. Nevertheless, the consistent and strong association of miR-155 expression with poor prognosis in adult and pediatric AML [14–16] suggests that miR-155 is likely to confer some advantage in tumor maintenance and progression. This is important when considering if and how miR-155 may be targeted therapeutically in AML.

Anti-miR-155 strategies in preclinical mouse models of B-cell lymphoma showed good success, causing regression of tumors in vivo [70]. However, if miR-155 is not required for AML maintenance as suggested, then inhibition of miR-155 may ultimately be an ineffective strategy and one might expect to select rapidly for tumor clones that tolerate low, or no, miR-155 expression. Because the induction of miR-155 to high levels in AML, well beyond the levels expressed in cancer, can have antitumor effects by targeting genes required for AML maintenance and progression, it is intriguing and tempting to speculate that, rather than trying to lower miR-155 expression, increasing miR-155 expression levels in AML may be more therapeutically effective. How this might be achieved requires a more nuanced understanding of the regulation of miR-155 expression in different cellular contexts. MicroRNA-based therapy offers significant promise that has seen multiple ongoing preclinical and early-phase human trials, although it should be pointed out that substantial hurdles remain regarding the safety of high/frequent dose regimes and targeted delivery strategies [71].

Concluding remarks

The discovery of microRNAs just over two decades ago has presented new insights into the molecular mechanisms that underpin AML development and progression, as well as novel

approaches to diagnosis, prognosis, and treatment of the disease. The analysis of the pathways regulated by microRNAs and how they overlap with commonly dysregulated pathways in AML leads to a better understanding of the core genes and gene networks that are central to the pathogenesis and maintenance of the disease. However, the complexity of microRNA biology, exemplified by the differential targeting of genes in response to different microRNA levels, poses significant new challenges both for the understanding of AML and the development of future therapeutic strategies.

Acknowledgments

NN and PGE are supported by the Victorian Government's Operational Infrastructure Support Program, a National Health and Medical Research Council project grant (APP1103244), and by the Children's Cancer Foundation (to PGE). CPB is supported by a Florey Fellowship from the Royal Adelaide Hospital Research Foundation and from the Australian Health and Medical Research Council (GNT1034633 and GNT1069128).

Conflict of interest

The authors declare no competing financial interests.

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