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Leukemogenesis: More Than Mutant Genes

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Abstract

Acute leukemias are characterized by recurring chromosomal aberrations and gene mutations which are critical to disease pathogenesis. It is now evident that epigenetic modifications including DNA methylation and histone modifications contribute significantly to the leukemogenic phenotype. An additional layer of epigenetic complexity is the pathogenetic role of microRNAs in leukemias, and their key role in the transcriptional regulation of tumor suppressor genes and oncogenes. The genetic heterogeneity of acute leukemias poses therapeutic challenges, but pharmacologic agents that target components of the epigenetic machinery hold promise as a part of the therapeutic arsenal for this group of diseases.

INTRODUCTION

Understanding the mechanisms of transformation related to chromosome translocations has had a profound impact on our understanding of carcinogenesis in general and of leukemogenesis and lymphomagenesis in particular. Identifying the genes at translocation breakpoints was the first step[1, 2]. Appropriately the first genes identified were oncogenes, *MYC* and *ABL* and this identification did two things: first for those who questioned the role of chromosome translocations in malignancy, it resolved any doubts because one of the partner genes in the 8;14 translocation (Burkitt lymphoma)[3, 4] and the 9;22 translocation (chronic myeloid leukemia)[5] was a bonafide oncogene[6, 7]; for those who had doubts about the relevance of oncogenes to human cancer (they were, after all, generally cloned from experimental mouse tumors), the discoveries showed their importance in human disease.

As translocation breakpoints in acute leukemia (see Table 1), especially acute myeloid leukemia (AML) were cloned, the picture became more murky. The 8;21 translocation (generally in acute myeloblastic leukemia)[8] and the 15;17 translocation[9] in a rare subtype of AML, acute promyelocytic leukemia (APL), each involved a gene critical for myeloid cell self-renewal, proliferation and/or differentiation but the partner gene was generally not active in myeloid cells. For the t(8;21) the active gene was *AML1* (also known as *RUNX1*), the DNA binding portion or alpha subunit of core binding factor (CBF), and the inactive gene was *ETO* (also known as *RUNX1T1*), a homolog of Drosophila *nervy* which is active in neurons[10]. For the t(15;17), the active gene was *RARA*[11, 12], retinoic acid receptor alpha, which was centrally involved in cell differentiation; its partner was a newly identified gene called *PML* for promyelocytic leukemia[12]. The fusion proteins *AML1-ETO* and *PML-RARA* were found to repress the transcription of wild type *AML1* and *RARA* target genes respectively by recruiting co-repressor complexes containing histone deacetylases (HDACs) (see Fig. 1)[13–17]. This was a revelation and immediately pointed to a potential therapeutic strategy, namely

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transcriptional derepression utilizing pharmacologic inhibitors of these co-repressor complex components.

In reality, the prototype for the successful therapeutic targeting of transcriptional repression by leukemic fusion proteins has been the use of all-trans retinoic acid (ATRA) for leukemias harboring the *PML-RARA* fusion[18, 19], whereas HDAC inhibitors (HDIs) have been less successful in treating other acute leukemias. We now know that histone alterations are not so simple, in fact, the "histone code" as far as we understand it is remarkably complex with a pattern that includes acetylation and methylation (mono, di and tri) that appears to proceed in a very ordered, complex and incompletely understood fashion[20]. In addition, there is a complex interplay of histone modifications and methylation that determines the state of chromatin structure and is critical to the regulation of gene transcription[21]. Clearly, in order to develop effective treatment that reverses transcriptional deregulation in leukemia, we must understand more about the precise details of these "marks" that are part of the epigenetic code.

To complicate matters, microRNAs (miRNAs, miRs) have been discovered[22]. These small (~22 nucleotide) non-protein coding RNAs pair to target mRNAs, usually at the 3' untranslated regions (3' UTRs), leading to degradation of mRNA or interfering with its translation into protein[22–24]. miRNAs appear to have powerful regulatory effects on many genes in various cancers[24–27], including leukemia[28–31]. Although our understanding of the regulation of miRNA expression is in its infancy, it is clear that mechanisms such as deletion, amplification, methylation (or other forms of epigenetic repression) are likely to play a role.

Besides the transcriptional and epigenetic deregulation conferred by oncogenic fusion proteins resulting from chromosomal translocations in acute leukemia, mutations involving specific genes that mediate critical signaling pathways, and mutations in key transcription factors also play a crucial role in leukemogenesis. For example *FLT3*, *KIT*, *NRAS*, *KRAS*, *CEBPA* and *NPM1* mutations have been described in AML, and *PAX5*, *TCF3*, *EBF1*, *LEF1* and *IKZF1* (also known as *IKAROS*) mutations in ALL. The role of these gene mutations in leukemogenesis is outside the scope of this review, and has been addressed recently in several other reports[32–34]. This review aims to describe the current understanding of epigenetic changes (including miRNA regulation), in acute leukemias, with a particular focus on acute leukemias characterized by balanced chromosomal aberrations.

EPIGENETIC CHANGES IN LEUKEMOGENESIS

The term epigenetics is generally used to refer to mitotically and meiotically heritable changes in gene expression that occur without alteration of the DNA coding sequence[35]. Epigenetic changes that underlie leukemogenesis have been described as falling into one of two major categories; changes in the DNA methylation (Box 1) state, or alterations in the histone modification (Box 2) pattern. Recent insights in this area suggest that these two major pathways of epigenetic modification act in concert to regulate gene transcription[21]. Compared with normal cells, cancer cells exhibit global DNA hypomethylation accompanied by aberrant methylation of CpG islands within gene promoters or coding regions[36]. In the context of leukemogenesis, aberrant CpG island methylation in promoter regions, for example of tumor suppressor genes such as cyclin-dependent kinase inhibitor 2B (*CDKN2B*), which encodes $p15^{INK4b}$ and *CDKN2A*, which encodes $p16^{INK4a}$ and $p14^{ARF}$, is a well described phenomenon, and is associated with transcriptional silencing, which also involves recruitment of methyl-binding proteins and HDACs to regions around the transcriptional initiation sites[37, 38].

In addition, the recurring chromosomal translocations in AML result in the generation of chimeric fusion genes which in many cases have been identified as transcriptional regulators

(see Table 1). A number of these fusion proteins result in leukemogenesis, at least in part by causing transcriptional deregulation via mechanisms linked to chromatin alterations (Fig. 1).

Core binding factor leukemias

The fusion proteins resulting from the chromosomal translocations t(8;21)(q22;q22) (AML1-ETO) and inv(16)(p13q22) (CBFB-MYH11) (Table 1) have been identified as transcriptional repressors[15, 39, 40]. These fusion proteins are characterized by disruption of CBF, a heterodimeric transcription factor which is important in hematopoietic differentiation. CBF consists of an alpha unit, AML1 (the DNA binding component), and a beta unit, CBFB, which stabilizes AML1. In murine models, homozygous loss of *Aml1* or *Cbfb* is characterized by lack of definitive hematopoiesis and embryonic lethality[41, 42]. Wild-type AML1 acts as a transcriptional activator. In contrast, the fusion proteins repress transcription of AML1 target genes by recruiting HDACs directly or by co-operating with corepressors including NCOR1, SMRT and SIN3A[15–17, 43]. Recently, AML1-ETO has also been shown to recruit DNA methyltransferase-1 (DNMT1)[44]; this finding implies that transcriptional silencing of AML1 target genes occurs at least in part, by an interplay between histone deacetylation and promoter DNA methylation. AML1-ETO has also been shown to directly repress transcription of tumor suppressor genes such as *p14ARF* and *NF1* through the AML1 DNA binding domain[45, 46].

Acute Promyelocytic Leukemia

All patients with APL harbor the t(15;17) or one of its variants, that results in a fusion protein comprised of all but the first 30 amino acids of RARA[47], fused to a variable partner at the N-terminus[12, 48–52]. Wild-type RARA functions as a transcriptional activator, whereas the fusion protein acts as a transcriptional repressor via recruitment of the HDAC, NCOR1 and SMRT complex, DNMT1 and DNMT3A, repressive histone methyltransferases, and polycomb group proteins[53, 54]. In cells with t(15;17) (*PML-RARA*), treatment with pharmacologic doses of ATRA[18, 19] relieves this repression by allowing release of the N-CoR complex and recruitment of a co-activator complex which contains proteins with histone acetyltransferase (HAT) activity[55–57]. This results in activation of RARA target genes as well as transcription factors critical for normal hematopoiesis such as *SPI1* (also known as *PU.1*) and *C/EBP-beta* (*CEBPB*), with subsequent differentiation of leukemic cells[55–57]. Patients with APL have a high complete response rate with ATRA used in conjunction with chemotherapy, and APL has served as a paradigm for the successful therapeutic targeting of epigenetic changes in acute leukemia. Although the effects of ATRA on cellular differentiation are critical to its success in APL, ATRA has also been shown to result in degradation of PML-RARA[58], leading to growth arrest and a decline in leukemia-initiating cells or leukemic stem cells. Recently, the use of arsenic trioxide has also been found to result in the degradation of the PML-RARA fusion and apoptosis in APL cells, to have significant clinical efficacy in the therapy of APL, to complement the use of ATRA in the treatment of patients with APL, and to potentially obviate the need for chemotherapy in some patients with this disease[55, 57, 59]. Gene expression and proteomic profiling experiments following treatment with both of these compounds reveal effects on multiple genes including a pattern of upregulation of genes associated with myeloid differentiation and downregulation of genes enhancing cellular proliferation[55, 57, 60]. The synergistic effect observed with the combination of ATRA and arsenic in producing durable remissions in APL has also been linked to eradication of leukemic stem cells[58].

Leukemias that disrupt histone acetyltransferases

Besides the recruitment of HDACs, DNMTs and co-repressor complexes, the scope of epigenetic deregulation by chromosomal translocations in acute leukemias also includes a disruption of the actual enzymes that are involved in chromatin modification[61]. For example, HATs such as CBP and the closely related p300, as well as the monocytic leukemia zinc finger

(MOZ) and the related MORF (monocytic leukemia zinc finger protein related factor) are rearranged in chromosomal translocations in leukemia. MOZ and MORF belong to the MYST family of HATs. The t(8;16) and the t(10;16) each result in the fusion of two proteins with HAT activity, MOZ-CBP and MORF-CBP respectively[62, 63]. The MOZ-CBP fusion inhibits AML1 mediated transcription resulting in a differentiation block and the HAT domain of CBP was found to be indispensable in this regard[64]. In addition, the HAT domain of MOZ also has a critical role in hematopoiesis, with abrogation of the HAT activity in ES cell lines and murine cell lines leading to a significant reduction in the proliferation potential of hematopoietic precursors[65]. Therefore it is plausible that deregulation of CBP and MOZ-mediated acetylation by chromosomal rearrangements could lead to a disruption in the balance between proliferation and differentiation during hematopoiesis, and thus contribute to the leukemogenic phenotype.

The inv(8) fuses the HAT domain of MOZ to the transcription factor TIF2[66]. The MOZ HAT domain consists of a nucleosomal binding motif and an acetyltransferase catalytic domain (Acetyl-CoA binding domain). Murine models of the inv(8) fusion suggest that the nucleosomal binding domain of MOZ and the CBP interaction domain of TIF2 are essential for leukemogenesis, whereas the N-terminal PHD and Acetyl-CoA binding domain of MOZ are dispensable[67]. This implies that the MOZTIF2 fusion results in transcriptional deregulation via aberrant recruitment of CBP to nucleosomal regions targeted by MOZ; and the HAT activity of CBP may contribute at least in part to leukemogenesis.

MLL-associated Leukemias

The t(11;16) which fuses MLL (myeloid lymphoid leukemia or mixed lineage leukemia) and CBP is another example of a chromosomal translocation involving a protein with HAT activity[68]. *MLL* is located on chromosome band 11q23, has homology to the *Drosophila melanogaster trithorax* gene, especially in the SET domain, and is involved in both myeloid and lymphoid leukemias, as well as biphenotypic or mixed lineage leukemias, hence its name[69, 70]. *MLL* is involved in chromosomal translocations with over 60 different partner genes in acute leukemias, and the mechanism of leukemogenesis by MLL fusion proteins remains perplexing given the disparate nature of the multiple known partner genes which have nuclear or cytoplasmic functions[71, 72]. However, because CBP had long been recognized to be a HAT, the MLL-CBP fusion protein provided an initial insight into potential mechanisms of leukemogenesis induced by MLL fusions, and suggested that transcriptional deregulation via mechanisms linked to histone modifications and altered chromatin structure was important in disease pathogenesis[68].

Subsequently, MLL was demonstrated to possess histone (H3K4) methyltransferase activity (and thus transcriptional activation properties) via its C-terminal SET domain[73, 74]. MLL and the tumor suppressor protein menin (encoded by multiple endocrine neoplasia type 1 (*MEN1*)), which binds MLL at its N-terminus, have been shown to associate with the homeobox A9 (*HOXA9*) promoter[75, 76], and recently, the chromatin associated protein, PSIP1 (also known as LEDGF), has been demonstrated to be a crucial cofactor for this interaction[77]. MLL H3K4 methyltransferase activity is associated with activation of MLL target genes including *Hoxa9*[74], which is important in the survival of *MLL*-rearranged leukemias[78]. The absence of menin and/or PSIP1 results in a failure of MLL and MLL fusions to regulate *Hoxa9* transcription, illustrating the importance of MLL/MENIN interaction in MLL fusion protein induced leukemogenesis[71, 77].

Despite the fact that the SET domain is associated with H3K4 methyltransferase activity and *Hoxa9* activation, it is consistently lost in the MLL fusions, except for the partial tandem duplication of MLL (MLL-PTD), where the SET domain and thereby the H3K4 methyltransferase activity is maintained[79]. However, MLL fusion-mediated leukemogenesis is not as simple as perturbed MLL-dependent H3K4 methylation[74]. Indeed, for several of the

MLL fusion proteins, the loss of the SET domain and H3K4 methyltransferase activity may be potentially compensated for by the acquisition of an alternative unique histone methyltransferase activity conferred by the partner proteins[71, 80]. For example, recent studies have shown that MLL fusion partners such as AF10, AF9, AF4 and ENL associate with the H3K79 histone methyltransferase DOT1L[81–83]. H3K79 methylation is also associated with transcriptional activation, and the acquisition of H3K79 methyltransferase activity has been demonstrated to be important for transformation by a subset of MLL fusion proteins[81, 84]. In a recently developed murine model of *MLL-AF4* leukemia, genome wide assessment of H3K79 methylation was performed using a ChIP-chip technique, and approximately 1000 promoters were found to be associated with increased H3K79 methylation compared with normal B cells[85]. Interestingly, siRNA mediated suppression of the H3K79 histone methyltransferase DOT1L decreased the expression of genes critical for MLL-fusion mediated leukemogenesis[85], suggesting that the modification of H3K79 methylation may be a potential therapeutic strategy in leukemias involving MLL-fusions.

MICRORNAS IN LEUKEMOGENESIS

Besides the two classic epigenetic modifications (i.e., DNA methylation and histone modifications), a third epigenetic mechanism has recently gained attention, namely miRNA regulation. MiRNAs are critical regulators of many physiological processes such as development, cell apoptosis, differentiation, and proliferation. Emerging evidence shows that altered miRNA expression is associated with various types of cancers[24–26]. In addition, miRNAs function in complex regulatory networks to regulate hematopoietic differentiation (see Supplementary Box 1 and Supplementary Table 1) and contribute to leukemogenesis (see Fig. 2, Table 2, and Supplementary Table 1), (see reviews[28–31]).

MiRNAs as oncogenes in acute leukemia

Several miRNAs have been shown to function as oncogenes in acute leukemia. Those that are the most well-studied are discussed in this section and included in Table 2.

The *mir-17-92* polycistron located at 13q31, which contains seven individual miRNAs including miR-17-5p, miR-17-3p, miR-18a, miR-19a, miR-20a, miR-19b-1, and miR-92-1, functions as an oncogene in various cancers including lymphoma, lung, colon, pancreas, prostate tumors, medulloblastoma, multiple myeloma[86–88]. Recently, Li *et al*.[89, 90] showed that miRNAs in the *mir-17-92* cluster are particularly overexpressed in acute leukemia cells bearing *MLL* rearrangements, which is at least partly owing to the genomic DNA amplification of the locus[91]. Retroviral transduction of the *mir-17-92* cluster significantly increased proliferation and colony-forming/replating capacity of mouse normal bone marrow progenitor cells alone and particularly, in cooperation with *MLL-ELL* fusion[91]. These data suggest that the *mir-17-92* cluster may play an important role in the development of *MLL*-associated leukemia. In normal hematopoiesis, *mir-17-92* plays an essential role in monocytopoiesis[92] and megakaryocytopoiesis[93], and in B cell development[94–96]. *mir-17-92* is down-regulated during monocytopoiesis and megakaryocytopoiesis and its forced expression represses monocytopoiesis (through targeting *AML1*)[92] and megakaryocytopoiesis[93]. *mir-17-92* inhibits B cell development at the pro-B to pre-B transition probably through targeting *PTEN* and *BIM*[94–96]. Thus, aberrant overexpresion of *mir-17-92* in leukemia would inhibit normal hematopoiesis and thereby contribute to leukemogenesis. In addition to the targets described above, E2F family transcription factors have also been suggested as both functional targets and regulators of *mir-17-92*[97–99]. Moreover, Li *et al*.[90] reported that 19 predicted targets of *mir-17-92* including *RASSF2* and *RB1*, were significantly down-regulated in *MLL*-rearranged leukemia and exhibit a significant inverse correlation with expression of the miRNAs. By use of a luciferase reporter assay, the direct regulation of *RASSF2* and *APP* have been

confirmed[90]. Thus, a group of target genes of *mir-17-92* have been identified and it is essential to determine which ones are critical in leukemogenesis.

miR-155 plays an important role in megakaryopoiesis/erythropoiesis[93, 100, 101] and lymphopoiesis[102]. Transgenic mice with forced expression of miR-155 initially exhibit a preleukemic pre-B cell proliferation evident in spleen and bone marrow, followed by a frank B cell malignancy, indicating that miR-155 can induce polyclonal expansion, favoring the occurrence of secondary genetic changes for full transformation[103]. In AML, miR-155 is specifically overexpressed in leukemia with internal tandem duplication of the receptor tyrosine kinase *FLT3* (*FLT3*-ITD)[104, 105], but the up-regulation of miR-155 is independent from FLT3 signaling[106]. miR-155 was reported to be overexpressed in a subset of AML (particularly, FAB-AML-M4 and M5), and sustained expression of miR-155 in hematopoietic stem cells caused a myeloproliferative disorder[107].

miR-196a and b are significantly upregulated in AMLs bearing *NPM1* mutations[104] and in *MLL*-associated pediatric ALL[108], as well as in *MLL*-associated AML[89]. During mouse embryonic stem (ES) cell differentiation, Popovic et al.[109] showed that *MLL* normally regulates expression of miR-196b in a pattern similar to that of the surrounding *Hox* genes, *Hoxa9* and *Hoxa10*. Within the hematopoietic lineage, the expression level of miR-196b reached a peak in short-term repopulating HSCs and then decreased as cells became more differentiated. Leukemogenic MLL fusion proteins caused overexpression of miR-196b, while treatment of *MLL-AF9* transformed bone marrow cells with miR-196-specific antagomir oligos abrogated their replating potential in methylcellulose. Forced expression of miR-196b in bone marrow progenitor cells led to increased proliferative capacity and survival, as well as a partial block in differentiation[109]. Consistently, miR-196 (and miR-21) is significantly down-regulated by the transcriptional repressor GFI1 during the transition from CMPs (common myeloid progenitors) to GMPs (granulocyte-macrophage progenitors), and forced expression of miR-196b (particularly, when co-expressed with miR-21) significantly blocks granulopoiesis[110]. Thus, miR-196 contributes to leukemogenesis likely through enhancing proliferation while blocking differentiation of hematopoietic progenitor cells.

MiRNAs as tumor suppressor genes in acute leukemia

miRNAs have also been shown to function as tumor suppressors in acute leukemia (Table 2), although data in this area are more limited than for those miRNAs that act as oncogenes. The let-7 family is a well-known tumor suppressor gene family, and functions as a negative regulator of a set of oncogenes including *RAS* (*NRAS* and *KRAS*) and *HMGA2*[111, 112]. In acute leukemia, let-7b and c were downregulated in CBF leukemia cases[104]. Upon treatment of APL primary leukemia samples and cell lines with ATRA, let-7a-3, let-7c, and let-7d were upregulated, whereas their target *RAS* was downregulated[113]. The tumor suppressor property of miR-15a/16-1 was first highlighted by the findings that they were deleted or downregulated in 68% of chronic lymphocytic leukemias (CLLs) and targeted *BCL2*, an anti-apoptotic gene[114, 115]. In AML, miR-15a/b and miR-16-1 are upregulated while their target *BCL2* is down-regulated in APL cells after treatment with ATRA[113]. In addition, a MYB-miR-15a autoregulatory feedback loop was reported in which miR-15a targeted MYB and blocked the cells in the G1 phase of cell cycle, while MYB bound the promoter region of miR-15a and was required for miR-15a expression; moreover, MYB and miR-15a expression were inversely correlated in cells undergoing erythroid differentiation[116].

MiRNA expression profiling in acute leukemia

In a large-scale, genome-wide miRNA profiling study in AMLs, Li *et al*.[89] observed distinct miRNA expression patterns for t(15;17), translocations involving *MLL*, and CBF AMLs including both t(8;21) and inv(16) leukemias. Expression signatures of a minimum of two (i.e.,

miR-126/126*), three (i.e., miR-224, miR-376c/ miR-368, and miR-382), and seven (miR-17-5p and miR-20a, plus the aforementioned five) miRNAs could accurately discriminate CBF, t(15;17), and *MLL*-rearranged AMLs, respectively, from each other[89]. Similarly, Jongen-Lavrencic *et al*.[104] showed that miRNA signatures correlated with cytogenetic and molecular subtypes of AML (i.e., AMLs with t(8;21), t(15;17), inv(16), *NPM1*, and *CEBPA* mutations). For example, all 6 AML cases with t(15;17) aggregated in a cluster; a significant upregulation of miR-10a/b and miR-196a/b was identified in AMLs bearing *NPM1* mutations, and of miR-155 in AMLs carrying *FLT3*-ITD. Notably, they also observed a significant upregulation of miR-126 in CBF leukemia and of miR-224 and miR-382 in t(15;17) AML[104]. The specific miRNA signature of t(15;17) (APL) cases and the up-regulation of miR-10a/b in AMLs bearing *NPM1* mutations and of miR-155 in AMLs carrying *FLT3*-ITD were also reported by others[105, 106, 117]. Marcucci *et al*. reported that miR-181a, a*, b, c and d, miR-128, miR-192, miR-219-1-3p, miR-224, miR-335, and miR-340 were up-regulated whereas miR-34a and miR194 were down-regulated in cytogenetically normal AMLs with *CEBPA* mutations[118].

In addition, the expression signature of some miRNAs was associated with outcome and survival of leukemia patients. Garzon *et al*.[105] showed that patients with high expression of miR-191 and miR-199a had significantly worse overall and event-free survival than AML patients with low expression. In cytogenetically normal AMLs, Marcucci *et al*.[119] found that expression signature of miR-181a (2 probes) and miR-181b (3 probes) was associated with good outcome whereas that of miR-124, miR-128, miR-194, miR-219-5p, miR-220a, and miR-320 (2 probes) was associated with poor outcome.

In a study of 17 ALL and 52 AML cases, Mi *et al*.[120] identified 27 miRNAs that were differentially expressed between ALL and AML. Among them, miR-128a and b were significantly overexpressed, whereas let-7b and miR-223 were significantly down-regulated in ALL compared to AML. Using the expression signatures of a minimum of two of these four miRNAs could distinguish ALL and AML with >95% accuracy, indicating that expression signatures of as few as two miRNAs could accurately discriminate ALL from AML[120]. Notably, significant overexpression of miR-128 in ALL was also reported elsewhere[108, 121].

Thus, miRNA signatures correlate with cytogenetic and molecular subtypes of acute leukemia, as well as outcome of leukemia patients. Further large-scale miRNA expression profiling assays conducted by different groups is critical to identify the miRNAs that can serve as reliable biomarkers for diagnosis and prognosis and/or as therapeutic targets of leukemia.

MiRNAs as targets and effectors of the epigenetic machinery

It is becoming clear now that not only do miRNAs themselves act in an epigenetic way by post-transcriptional regulation of expression of target genes, but they can also be targets of the epigenetic machinery, as well as effectors of DNA methylation and histone modifications. These functions may all have crucial roles in leukemogenesis.

As examples of miRNAs as targets of the epigenetic machinery, their aberrant expression in acute leukemia is directly associated with DNA methylation. For example, the elevated expression of miR-126/126* in CBF AMLs and of miR-128 in ALL was associated with promoter demethylation[89, 120]. In addition, the fusion oncoproteins that arise from chromosomal translocations have been associated with epigenetic silencing of miRNAs. For example, expression of miR-223 is down-regulated by the AML1-ETO fusion resulting from t(8;21) in AML[122] owing to a heterochromatic silencing of the miR-223 genomic region triggered directly by AML1-ETO. Increasing miR-223 expression through demethylation restores differentiation of leukemic blasts[122]. Similarly, transcriptional repression of miR-210 and miR23/24 by PML-RARA was reported in APL with the t(15;17)[123].

Histone modifications may also play a role in regulation of miRNA expression in acute leukemia. Roman-Gomez et al.[124] observed high levels of dimethylation of H3 lysine 9 (K9H3me2) and/or low levels of trimethylation of H3 lysine 4 (K4H3me3) (these are patterns of histone modifications underlying a closed chromatin structure associated with repressive gene expression) in CpG islands around 13 miRNAs. Their analysis of 353 ALL primary patients showed that 65% of the ALL samples had at least one miRNA methylated. Notably, patients with miRNA methylation had a significantly poorer disease-free survival (DFS; 24% vs. 78%) and overall survival (OS; 28% vs. 71%) than patients with unmethylated miRNAs. Multivariate analysis demonstrated that methylation profile was an independent prognostic factor for predicting DFS and OS. Their results suggest that aberrant miRNA methylation is a common phenomenon in ALL and miRNA methylation profile might be important in predicting the clinical outcome of ALL patients[124].

On the other hand, miRNAs may also function as effectors of the epigenetic machinery. Two recent studies[125, 126] show that miR-290, a mouse ES cell-specific miRNA, controls DNA methylation and telomere recombination *via* retinoblastoma-like 2 (Rbl2)-dependent regulation of DNMTs in mouse ES cells. In leukemia, forced expression of miR-29b in AML cells induced global DNA hypomethylation and re-expression of tumor suppressor genes including $p15^{INK4b}$ and ESR1 by targeting DNMT3A and DNMT3B directly and DNMT1 indirectly[127].

EPIGENETIC THERAPY IN ACUTE LEUKEMIA

Unlike gene deletions which lead to irreversible loss of function, transcriptional repression by epigenetic mechanisms such as histone deacetylation and promoter DNA methylation can be reversed by pharmacologic inhibitors of such processes. One of the best known and most successful examples of “targeted therapy” that can induce epigenetic changes, is the use of ATRA in the therapy of APL, which has been described above. Because epigenetic mechanisms are critical to the pathogenesis of acute leukemias as a whole, there has been a significant interest in the clinical and translational investigation of agents that target the epigenome in these diseases.

Histone deacetylase inhibitors (HDIs)

HDIs have been associated with effects on a variety of genes including those involved with cell cycle regulation, apoptosis and angiogenesis. HDIs have been demonstrated to exert anti-tumor effects *in vitro* and *in vivo*, and are now in clinical trials in acute leukemias, as well as in related neoplastic disorders of the bone marrow such as myelodysplastic syndromes (MDS) and myeloproliferative neoplasms (MPN). In preclinical studies, HDIs have been shown to induce differentiation of APL cell lines including those resistant to standard differentiating therapy with ATRA[128–130]. They abolish tumors with t(15;17) in nude mice, and induce remissions in transgenic mice models of ATRA resistant APL[130, 131]. HDIs have also been demonstrated to induce differentiation and apoptosis of leukemia cell lines and primary leukemia blasts with the t(8;21)[39, 132–134]. In the clinical setting, therapy with the HDI sodium phenylbutyrate has been shown to restore ATRA responsiveness in a patient with APL who had experienced multiple relapses and was clinically resistant to therapy with ATRA alone[135]. Treatment with phenylbutyrate induced a complete clinical and cytogenetic remission and a time-dependent histone acetylation in peripheral blood and bone marrow mononuclear cells[135]. Butyrates however are short chain fatty acids and generally not very potent in inhibiting HDACs[136]. More potent HDIs including hydroxamic acids, cyclic tetrapeptides and benzamides have been developed and are under clinical investigation[137]. To date however, the only tumor where significant efficacy has been demonstrated in the clinic is advanced primary cutaneous T cell lymphoma, where the HDI SAHA (vorinostat) was recently FDA approved[138].

The majority of clinical trials utilizing HDIs as single agents in patients with advanced AML or myelodysplastic syndromes have demonstrated limited clinical activity[139–147] (Table 3). Recently published pre-clinical studies have also focused on distinct cytogenetic subsets of AML such as CBF AML, a cytogenetic subset that would be hypothesized to be particularly amenable to therapy with this class of drugs, based on transcriptional repression *via* HDAC recruitment by the oncogenic fusion proteins[132, 133]. In a recent clinical trial, transient antileukemic activity was demonstrated in patients with advanced CBF leukemia treated with the HDI Romidepsin, and this was associated with upregulation of AML1-ETO target genes[146]. Given the relatively limited single agent activity of HDIs thus far in acute leukemias, ongoing trials are investigating the combination of HDIs with other agents, including DNMT inhibitors (Table 3).

DNA methyltransferase (DNMT) inhibitors

Although DNMT inhibitors have been in existence for several decades, they were originally utilized at very high doses which resulted in significant cytotoxicity, and an unacceptable toxicity profile[148]. At lower doses however, the DNA demethylating and differentiating effects of these drugs predominate, and they are active in a broad range of myeloid neoplasms including MDS, MPN, and AML[149]. The DNMT inhibitors in clinical and/or preclinical development fall into 2 broad categories, namely nucleoside analogues and non-nucleoside demethylating agents (reviewed in[137, 149]). The prototypic nucleoside analogue DNMT inhibitors 5-azacytidine (5-Aza, azacitidine) and 5-aza-2'deoxycytidine (decitabine), both get incorporated into DNA (5-Aza also gets incorporated into RNA) and form a covalent complex with the DNMT enzyme resulting in trapping and degradation of the enzyme and progressive loss of DNMT activity within cells. Both compounds have recently been approved by the FDA for the treatment of MDS[150–152]. Objective response rates (complete and partial responses) in these trials have been in the 20 to 30% range, but an additional 20 to 30% of patients derive clinical benefit in terms of improvement in blood counts or transfusion requirements (so-called hematologic improvement), despite evidence of persistence of significant disease in the bone marrow[150, 152, 153]. Several of the trials conducted in MDS have included patients who have a myeloblast count of 20 to 30% in the bone marrow (and are therefore considered to have AML), and in these patients the overall response rates including hematologic improvement have been in the 35% to 48% range[154]. There are now several early phase trials utilizing DNMT inhibitors, either as single agents or in combination with other agents, that confirm the clinical activity of this group of drugs in AML[155–162], including elderly patients with AML who are unable to tolerate standard cytotoxic chemotherapy[155–157].

It is important to note that these drugs may require several cycles of administration for activity to be demonstrated, and this may be due at least in part to the fact that repetitive administration is necessary for progressive demethylation and epigenetic modulation of critical genes such as cell cycle regulatory and proapoptotic genes. In addition, the effects of these drugs on methylation are not permanent, and therefore chronic exposure is required to maintain the effects. The clinical activity of these agents is thought to be mediated via reversal of epigenetic silencing and there are a few published clinical trials that support this hypothesis[158, 163]. However, in general this has been challenging to prove conclusively in the clinical setting[156, 160, 164] (Table 3), and it is likely that alternative mechanisms of action such as induction of DNA damage[159, 165–167] may contribute to the pleiotropic effects of these drugs.

Efforts are ongoing to develop DNMT inhibitors that have greater selectivity for cancer cells and that are suitable for chronic oral administration in the clinical setting. In addition, given the interrelationship between DNA methylation and histone modifications in the regulation of gene expression[168], it is not surprising that HDIs and DNMT inhibitors are being combined in clinical trials in AML and MDS (Table 3); in an effort to optimize the antitumor activities of

these agents and recapitulate the synergistic interaction that has been demonstrated in the preclinical setting. Several of these published trials have been conducted using relatively low potency HDIs[155–158] and have shown the feasibility of this approach, but have not demonstrated a clear contribution of the HDIs to the clinical or biologic activity of the combination[155]. The results from ongoing randomized trials utilizing newer generation, more potent HDIs will be necessary to validate the hypothesized synergy between these two classes of epigenetic modulators in patients with AML and MDS.

MiRNAs as Potential Therapeutic Targets and Tools

Because they can function as oncogenes or tumor suppressor genes in leukemogenesis, miRNAs also have the potential to serve as therapeutic targets or tools. miRNA-based cancer gene therapy offers the theoretical appeal of targeting multiple gene networks that are controlled by a single, aberrantly expressed miRNA[169]. Reconstitution of tumor-suppressive miRNA, or sequence-specific knockdown of oncogenic miRNAs by “antagomir oliogos”, has produced favorable antitumor outcomes in experimental models[169]. In addition, the efficacy of some existing clinical therapeutic approaches may be mediated via modulation of miRNA expression. For example, tumor suppressor miRNA upregulation has been demonstrated with the use of ATRA in APL cell lines and primary leukemia samples[113, 123] and the DNMT inhibitor 5-azacytidine has been associated with reversal of epigenetic silencing of a miRNA linked to the differentiation block in AML1-ETO AML blasts[122].

However, there are still many issues to be resolved prior to consideration of conducting miRNA-based clinical therapy including dosage, efficacy, functionality, delivery, non-specific toxicity, and immune activation[24, 169]. In addition, because of the redundancy of some miRNA families or functional redundancy of a set of miRNAs that are not in a family, targeting a single member might not be sufficient in terms of gene therapy. In such cases, targeting several miRNAs simultaneously would be critical. Furthermore, some miRNAs may play a different role (as oncogene or tumor suppressor) depending on the cellular context. For example, the *mir-17-92* cluster is a well-known oncogene in various types of cancers[86, 87, 89, 170, 171] including leukemia[89, 104, 108, 109], but may it function as a tumor suppressor gene in breast cancer[172, 173]. Therefore, before considering a potential clinical application, it is important to understand the expression pattern and potential role of the candidate miRNA(s) in other tissues to avoid causing undesirable side effects.

CONCLUSIONS AND PERSPECTIVES

The complexity and biologic heterogeneity of acute leukemias pose significant challenges to making therapeutic advances. It is clear however that an in-depth understanding of the biology is essential to making meaningful progress. The oncogenic fusion proteins in acute leukemia associate in macromolecular complexes, lack intrinsic enzymatic activity and are therefore not easily “druggable” in contrast for example, to the clinical development of ABL kinase inhibitors in CML. Epigenetic and transcriptional therapeutic strategies that focus on disruption of the association of oncoproteins with substrate DNA, or interference with key molecules that oncoproteins may associate with to promote leukemia (such as disruption of the MLL/MENIN interaction) hold promise for the future. In addition, besides the ongoing clinical studies of HDIs and DNMT inhibitors, other components of the epigenetic regulatory machinery such as histone methyltransferases, histone demethylases, HATs, and sirtuins are potential targets for future anti-cancer and antileukemia therapy[174]. The importance of miRNAs in enhancing our basic perceptions about the pathobiology of leukemias as well as their therapeutic potential and some of the potential pitfalls that could be encountered in clinical translation have been mentioned above. Given the importance of co-operating gene mutations in signaling pathways in the generation of the acute leukemia phenotype, it is likely that the most effective treatment

strategies in the future will involve a combination of rationally designed transcriptional treatment approaches with those that inhibit relevant activated signal transduction molecules.

Box 1: DNA methylation and associated enzymes

DNA methylation is mediated by DNA methyltransferases (DNMTs) which catalyze the conversion of cytosine residues which precede guanosine (CpG) to 5-methylcytosine, by the covalent addition of a methyl group at the 5-carbon position of the cytosine[175]. These CpG residues are underrepresented in the genome as a whole and occur at only 5 to 10 % of the predicted frequency. This underrepresentation is likely linked to the propensity of methylated cytosine to undergo spontaneous deamination to thymidine, leading to progressive depletion of CpG dinucleotides over time. CpG residues cluster particularly in promoter regions of genes, in so called CpG islands, are generally unmethylated in normal cells, and are associated with hyperacetylated histones and an open chromatin configuration, which facilitate accessibility to transcription factors and transcriptional activation. CpG island methylation in the promoter regions of genes is associated with transcriptional repression, gene silencing and a condensed chromatin state and is seen physiologically, for example, in the context of genes silenced on the inactive X chromosome and imprinted genes.

The known enzymatically active DNMTs include DNMT3A and DNMT3B which are de novo methylases and bind to both unmethylated and hemimethylated CpG sites, while DNMT1 is responsible for maintaining DNA methylation patterns and binds preferentially to hemimethylated DNA.

Box 2: Histone modifications and associated enzymes

Structural studies have revealed that the N-terminal tails of the histones protrude outward from the nucleosome and are subject to various post-translational modifications including acetylation, methylation, ubiqitination, phosphorylation, sumoylation and ADP-ribosylation (reviewed in [Ref. 176]). Histone acetylation is associated with transcriptionally active chromatin (euchromatin) and is catalyzed by histone acetyltransferases (HATs). A number of transcriptional co-activators including CREB binding protein (CBP) and p300, Gcn5/pCAF and SRC-1 have been shown to possess HAT activity. Conversely, transcriptional co-repressor complexes such as nuclear co-repressor 1 (NCOR1), NCOR2 (also known as SMRT) and SIN3A have been shown to contain subunits with histone deacetylase (HDAC) activity.

Histone methylation is catalyzed by histone methyltransferases and can occur on lysine and/or arginine residues. In contrast to histone acetylation which results in a transcriptionally active state, histone methylation can result in activation or repression of transcription depending on the residue that is affected. For example, histone H3 lysine 4 (H3K4) methylation is recognized as an active mark associated with actively transcribed genes, while H3K20 trimethylation or H3K9 methylation are inactive marks associated with transcriptional repression and heterochromatic states. Histone methyltransferases also tend to be more specific with regard to their histone substrates, in contrast to HATs for example, and contain a conserved SET domain. Some of the histone methyltransferases identified thus far in mammalians include SETD7, SMYd3 and MLL (catalyze H3K4 methylation); SUV39H1, euchromatic histone-lysine N-methyltransferase 2 (EHMT2), EHMT1, SETDB1 (catalyze H3K9 methylation); EZH2 (H3K27 methylation); and DOT1L (H3K79 methylation).

Histone demethylases have recently been identified and include amine oxidases such as LSD1 which can demethylate H3K4 or H3K9 depending on the associated protein, and JmjC family members which demethylate mono-, di-, or tri-methylated lysine.

AT-A-GLANCE SUMMARY

- Acute leukemias, arising from neoplastic transformation of uncommitted or partially committed hematopoietic stem cells, are characterized by recurring chromosomal aberrations and gene mutations which are critical to disease pathogenesis.
- The recurring chromosomal translocations in acute myeloid leukemia (AML) result in the generation of chimeric fusion proteins, which in many cases function as transcriptional regulators. These include AML1-ETO (generated by t(8;21)), CBFB-MYH11 (generated by inv(16) or t(16;16)), PML-RARA (generated by t (15;17)), MOZ-CBP (generated by t(8;16)), MORF-CBP (generated by t(10;16)), MOZ-TIF (generated by inv(8)), and MLL fused with various partners (generated by t(11q23)). They contribute to leukemogenesis, at least partially by causing transcriptional deregulation via epigenetic modifications.
- Epigenetic modifications including DNA methylation or demethylation and histone changes lead to activation or repression of gene expression. Aberrant epigenetic changes occur frequently in acute leukemias. Fusion genes resulting from chromosome translocations may be regulators or mediators of the epigenetic machinery.
- MicroRNA regulation may also contribute significantly to leukemogenesis. Some microRNAs function as oncogenes or tumor suppressor genes in acute leukemias. microRNA signatures correlate with cytogenetic and molecular subtypes of acute leukemias, and some microRNA signatures are associated with outcome or survival of acute leukemias.
- It is evident that not only do microRNAs themselves function in an epigenetic manner by post-transcriptional regulation of expression of target genes, but they can also be targets of the epigenetic machinery and effectors of DNA methylation and histone modifications. These functions may all be involved in leukemogenesis.
- Although the genetic heterogeneity of acute leukemias poses therapeutic challenges, drugs or small molecules that target components of the epigenetic machinery hold great promise in the treatment of leukemias. The use of all-trans retinoic acid (ATRA) in the therapy of acute promyelocytic leukemia (APL) is one of the best known and most successful examples of “targeted therapy” involved in epigenetic changes and progress has been made in the clinical trials of histone deacetylase inhibitors (HDIs) and DNA methyltransferase (DNMT) inhibitors; however, more effective treatment strategies are needed for therapeutic advances.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Janet D. Rowley, M.D. Dr. Rowley is the Blum Riese Distinguished Service Professor of Medicine, Molecular Genetics & Cell Biology and Human Genetics, The University of Chicago. Dr. Rowley's laboratory focuses on understanding genetic changes and their functional consequences in human acute myeloid leukemia. This is done by mapping and cloning new chromosome translocation breakpoints and by analyzing the genomic structure of breakpoints in common translocations. Dr. Rowley has used SAGE and microarray analysis to identify mRNAs/miRNAs that are aberrantly expressed in leukemia patients with common translocations. Using data from these analyses, she has identified pathways that are activated by different translocations, which may lead to improved diagnosis and therapy.

GLOSSARY

Histones	the chief protein components of chromatin, which plays an important role in DNA packaging, chromosome stabilization and gene expression. Histones form the core component of nucleosomes
Histone classes	There are six classes of histones (H1, H2A, H2B, H3, H4, and H5) organized into two super classes including core histones (i.e., H2A, H2B, H3 and H4) and linker histones (i.e., H1 and H5)
Histone code	the “rules” governing the pattern of covalent histone tail modifications. Histone tail modifications play an important role in the chromatin structure, and thereby play an important role in regulation of gene expression
Histone deacetylases	enzymes that regulate chromatin structure and function through the removal of the acetyl group from the lysine residues of core nucleosomal histones

Nucleosomes	the basic unit of chromatin and consist of approximately 146 base pairs of DNA wound around an octameric core of histone proteins: an H3-H4 tetramer and two H2A-H2B dimers
Normal hematopoiesis	a developmental process by which all types of blood cells of the body are continuously produced by rare puripotent self-renewing hematopoietic stem cells (HSCs). In normal adults, hematopoiesis occurs primarily in marrow and lymphatic tissues
Leukemic blasts	abnormal immature white blood cells that are malignant (neoplastic), and typically found in the bone marrow and peripheral blood of patients with acute leukemia
Acute leukemia	a type of malignancy that results in the relatively rapid growth of abnormal immature white blood cells (myeloid or lymphoid leukemic blasts) in the marrow and blood and inhibition of normal hematopoiesis
FAB classification of AML	The French-American-British (FAB) classification system divided AML into 8 subtypes, M0 through to M7, based on the type of cells from which the leukemia developed and how mature the cells are
Myelodysplastic syndrome (MDS)	a group of clonal hematopoietic stem cell disorders characterized by cytopenias (low blood counts) and ineffective hematopoiesis, dysplasia in one or more myeloid cell lines, and an increased risk of transformation to AML
Hematopoietic stem cells (HSCs)	multipotent stem cells that are composed of short-term repopulating (STR) and long-term repopulating (LTR) stem cells. STR HSC can sustain the hematopoietic system for only a short term, whereas LTR HSC can reconstitute hematopoiesis for life
Chromosome abnormalities	alterations in the number or the structure of one or more chromosomes. In leukemia or cancer, chromosome abnormalities usually occur when there is an error in cell division following mitosis; they can be either numerical or structural, or both
Chromosome translocation	a structural abnormality resulting from rearrangement of pieces generally between two non-homologous chromosomes
Antagomir oligos (AMOs)	a class of chemically engineered antisense oligonucleotides that are complementary to either the mature miRNAs or their precursors an are used to specifically inhibit the activity of endogenous miRNAs, probably through irreversibly binding the miRNAs. Antagomirs are now used as a method to constitutively inhibit the activity of specific miRNAs

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Reference 5 (Rowley J.D., Nature, 1973): showing that the Philadelphia chromosome, a specific chromosomal abnormality associated with chronic myelogenous leukemia (CML), was the result of a reciprocal translocation between chromosome 9 and 22.

References 13 & 14 (Grignani F., et al., Nature, 1998; Lin R.J., et al. Nature, 1998): demonstrating that the PML-RARA and PLZF-RARA fusion oncoproteins in acute promyelocytic leukemia result in transcriptional repression of retinoic acid target genes via recruitment of N-CoR-histone deacetylase complex. A molecular explanation for the clinical efficacy of ATRA in APL with the PML-RARA fusion was also provided.

Reference 15 (Wang J., et al., PNAS, 1998): highlighting the fact that the mechanism of transcriptional repression by the AML1-ETO fusion protein in AML is via recruitment of the N-CoR complex. This finding has clinical translational relevance, as it implies that effective inhibitors of such repressor complexes may provide therapeutic benefit in this subset of AML

Reference 18 (Huang M.E, et al., Blood, 1988): demonstrating the efficacy of ATRA in the treatment of APL patients. It was the first example of genotype effective treatment of translocation-associated AML.

Reference 62 (Borrow J., et al., Nat Genet., 1996): highlighting the involvement of a histone acetyltransferase in chromosomal translocations in AML, and providing evidence that disruption of chromatin modifying enzymes is associated with leukemogenesis.

Reference 68 (Sobulo O,M., et al., PNAS, 1997): highlighting the involvement of a HAT in an *MLL*-associated leukemia, and providing an early insight that alluded to transcriptional deregulation via histone/chromatin modifications as being important in *MLL*-mediated leukemogenesis.

Reference 73 (Nakamura T., et al., Mol Cell, 2002): showing that MLL exists within a very large multiprotein complex, that the SET domain has histone methyltransferase (H3K4) activity and that this multiprotein complex associates with the promoter of target genes such as *HOXA9*.

Reference 74 (Milne T.A., et al., Mol Cell, 2002): showing that although the SET domain of MLL is an H3K4 methyltransferase associated with Hox gene activation, a leukemogenic MLL fusion protein (MLL-AF9) that activates *HOX* expression had no effect on H3K4 methylation. This implies that the mechanism for *MLL*-fusion gene activation and leukemogenesis is not merely via perturbation of H3K4 methylation.

Reference 85 (Krivtsov A.V., et al., Cancer Cell, 2008): highlighting increased H3K79 methylation on a genome wide analysis, associated with a murine model of *MLL*-fusion leukemia (*MLL-AF4*), and providing some evidence for the clinical translational potential of inhibition of H3K79 as a therapeutic strategy in leukemias involving MLL fusions.

Reference 89 (Li Z., et al., PNAS, 2008): demonstrating that specific alterations in miRNA expression distinguish AMLs with common translocations and implies that the deregulation of specific miRNAs may play a role in the development of leukemia with these associated genetic rearrangements.

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Reference 104 (Jongen-Lavrencic M., et al., Blood, 2008): revealing distinctive microRNA signatures that correlate with cytogenetic and molecular subtypes of AML (ie, AMLs with t (8;21), t(15;17), inv(16), NPM1, and CEBPA mutations).

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Reference 109 (Popovic R., et al., Blood, 2009): suggesting a mechanism whereby increased expression of miR-196b by MLL fusion proteins significantly contributes to leukemia development.

Reference 119 (Marcucci G., et al., N Engl J Med, 2008): reporting that a microRNA signature was associated with the clinical outcome of adults under the age of 60 years who had cytogenetically normal AML and high-risk molecular features.

Reference 120 (Mi S., et al., PNAS, 2007): showing that expression signatures of as few as two miRNAs could accurately discriminate ALL from AML.

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Reference 127 (Garzon R., et al., Blood, 2009): showing that miR-29b targets DNMTs, thereby resulting in global DNA hypomethylation and reexpression of hypermethylated, silenced genes in AML.

Reference 146 (Odenike O., et al., Clin Ca Res, 2008): showing that patients with CBF AML were particularly susceptible to the antileukemic effects of the HDI romidepsin (depsipeptide), and this was associated with upregulation of AML1-ETO target genes. This work provided evidence to support the hypothesis that reversal of transcriptional repression mediated by AML1 fusion genes can be achieved *in vivo* with the use of a HDI.

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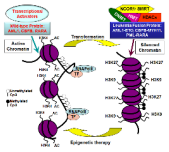

Figure 1. Leukemia fusion proteins and epigenetic deregulation

Oncogeneic fusion proteins such as AML1-ETO, CBFB-MYH11, and PML-RARA recruit transcriptional co-repressor complexes (including NCOR1 and SMRT) which result in the loss of histone acetylation and the acquisition of repressive histone modification marks such as histone H3 lysine 9 (H3K9) methylation and H3K27 trimethylation, as well as DNA methylation, and thereby a closed chromatin structure. This leads to transcriptional silencing of various target genes including genes that are critical for hematopoietic differentiation. Epigenetic or transcriptional therapy (targeting the fusion proteins, components of the corepressor complexes or downstream effectors such as miRNAs) has the potential to reverse these changes leading to histone acetylation, and acquisition of active marks such as H3K4 methylation, an open chromatin structure with subsequent transcriptional activation and differentiation of the leukemic clone. HDACs, histone deacetylases; DNMT, DNA methyltransferases; HMT, histone methyltransferases; NCOR1, nuclear receptor co-repressor 1; SMRT, the silencing mediator of retinoic acid and thyroid hormone receptor, also known as NCOR2; TF, transcription factor; Ac, histone acetylation; RNApolII, RNA polymerase II.

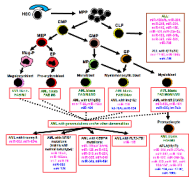

Figure 2. Involvement of miRNAs in acute leukemia

miRNAs that are up-regulated (in purple) or down-regulated (in blue) in a subtype of acute leukemia are shown. Some miRNAs are associated with specific leukemia subtypes and thereby may be able to serve as biomarkers for classification and diagnosis of these subtypes. For example, miR-126 in CBF leukemia (leukemias with t(8;21) or inv(16))[89, 104], *mir-17-92* in *MLL*-associated leukemia (those with t(11q23))[89, 90], miR-196b in *MLL*-associated leukemia[89, 108, 109] and in AML with *NPM1* mutations[104], miR-224, miR-382 and miR-376 family in APL (t(15;17))[89, 104, 117], miR-10a and b in AML with *NPM1* mutations[104, 106], and miR-155 in AML with *FLT3*-ITD[104, 105] are likely to be such markers. AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; FAB, French-American-British (FAB) classification; FAB, French American British Classification, MO, Acute myeloid leukemia with minimal differentiation; M1 Acute myeloid leukemia without differentiation; M2, Acute myeloid leukemia with maturation, M3, Acute promyelocytic leukemia; M4, Acute myelomonocytic leukemia, M5, Acute monoblastic leukemia; M6, Acute erythroleukemia; M7, acute megakaryoblastic leukemia; HSC, hematopoietic stem cell; MPP, multipotent progenitor cell; CMP, common myeloid progenitor; CLP, common lymphoid progenitor; MEP, megakaryocyte-erythrocyte progenitor; GMP, granulocyte-macrophage progenitor; EP, erythroid progenitor; Meg-P, megakaryocyte progenitor; GP, granulocyte progenitor; MP, monocyte progenitor.

Table 1

Acute leukemia and chromosome abnormalities*

Malignancy (FAB subtype)†	Chromosomal abnormality	Molecular alterations (genes involved)§	Frequency in AML§	Frequency in ALL§	Risk group assignment
AML (M2)	t(8;21)(q22;q22)	*AML1-ETO*	5–12%	NA	favorable
AML (M4eo)	inv(16)(p13q22)/t(16;16)(p13;q22)	*CBFB-MYH11*	3–10%	NA	favorable
AML (M3)	t(15;17)(q22;q21)	*PML-RARA*	6–15%	NA	favorable
AML(M4, M5), Or, ALL	t(11q23)	*MLL*-various	5–8%	7–10%	unfavorable, or, intermediate
AML	−5/del(5q)	NA	1–11%	NA	unfavorable
AML	−7/del(7q)	NA	1–7%	NA	unfavorable
AML	+8	NA	3–10%	NA	unfavorable
AML, or, ALL	Normal karyotype (NK)	NA	30–50%	20–45%	favorable, or, intermediate
AML, or, ALL	t(9;22)(q34;q11.2)	*BCR-ABL*	1–2%	5–20%	unfavorable
ALL (L1 or L2)	t(12;21)(p13;q22)	*TEL-AML1*	NA	10–25%	favorable
ALL (L1 or L2)	t(1;19)(q23;p13.3)	*TCF3-PBX1*	NA	2–5%	favorable, or, intermediate
ALL	t(17;19)(q22;p13)	*TCF3-HLF*	NA	1%	unfavorable
ALL	t(8;14); t(2;8); t(8;22)	*MYC*-various	NA	1–2%	unfavorable
ALL (L1 or L2)	Hyperdiploidy (>50 chromosomes)	NA	NA	10–25%	favorable
ALL	Hypodiplody (<45 chromosomes)	NA	NA	1–5%	Unfavorable, or, intermediate

*Acquired (`somatic') clonal karyotype abnormalities are detected in 55–80% of acute leukemia patients, while the remaining 20–45% have a normal karyotype[177–182]. Recurrent genetic abnormalities have prognostic and therapeutic implications, and also provide insights into the mechanisms of leukemogenesis[178, 179, 182, 183].

†ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; FAB, French-American-British (FAB) classification; M, subtype of AML; L, subtype of ALL.

§*AML1*, runt-related transcription factor 1 *(RUNX1); ETO*, runt-related transcription factor 1 *(RUNX1T1); CBFB*, core-binding factor, beta subunit; *MYH11*, myosin, heavy chain 11, smooth muscle; *PML*, promyelocytic leukemia; *RARA*, retinoic acid receptor alpha; *MLL*, mixed lineage leukemia; *BCR*, breakpoint cluster region; *ABL*, c-abl oncogene 1, receptor tyrosine kinase *(ABL1); TEL*, ets variant 6 *(ETV6); TCF3*, transcription factor 3 (also known as *E2A*); *PBX1*, pre-B-cell leukemia homeobox 1; *HLF*, hepatic leukemia factor; NA, not applicable.

Table 2

Examples of oncogenic and tumor suppressor miRNAs in leukemogenesis

miRNA	Function in normal hematopoiesis	Function in acute leukemia	Regulator	Known targets	References
let-7	Repress megakaryocytopoiesis	Lower in ALL than in AML; Down-regulated in CBF leukemias; Up-regulated in AMLs bearing *NPM1* mutations	LIN28	*NRAS*, *KRAS*, *HMGA2*	93, 104, 106, 111, 112, 120, 184
miR-15/16	Promote erythropoiesis	Up-regulated in APL cells after ATRA treatment		*BCL2*, *MYB*	100, 113–116, 185, 186
the *mir-17-92* cluster	Down-regulated during monocytopoiesis and megakaryocytopoiesis; Repress monocytopoiesis and megakaryocytopoiesis; Promote the transition from pro-B to pre-B cell stage	Overexpressed in *MLL*-rearranged leukemia; Significantly downregulated in APL; Enhance cell proliferation and block cell differentiation; Promote leukemogenesis	MYC, E2F1, E2F2, E2F3	*BIM* (also known as *BCL2L11)*, *PTEN*, *E2F1*, *E2F2*, *E2F3*, *RASSF2*, *APP*, *CDKN1A* (which encodes p21), *AML1* (also known as *RUNX1)*	89–99, 121, 187
miR-155	Repress both megakaryopoiesis and erythropoiesis; Important miRNA in lymphopoiesis and immune response (for both B and T cells)	Significantly upregulated in AMLs carrying *FLT3-ITD*; Overexpressed in a subset of AML (particularly, AML-M4 and M5); Sustained expression in HSCs caused a myeloproliferative disorder	FOXP3	*MAP3K7IP2* (also known as *TAB2)*, *INPP5D* (also known as *SHIP)*, *CEBPB*	93, 100–107, 188–193
miR-196a/b	Within the hematopoietic lineage, reaches a peak in STR-HSC and then decreased as cells became more differentiated; Significantly downregulated during the transition from CMPs to GMPs	Upregulated in AMLs bearing *NPM1* mutations; Upregulated in *MLL*-rearranged leukemia; Significantly downregulated in APL; Enhance cell proliferation and block differentiation	GFI1	*HOXB8*, *HOXC8*, *HOXD8*, *HOXA7*	89, 104, 108–110, 194

Note: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; APL, Acute promyelocytic leukemia; *APP*, amyloid beta (A4) precursor protein; *ATRA*, all-trans retinoic acid; *CDKN1A*, cyclin-dependent kinase inhibitor 1A; *CEBPB*, C/EBP beta; *FOXP3*, forkhead box P3; *HOX*, homeobox; *INPP5D*, inositol polyphosphate-5-phosphatase; *MAP3K7IP2*, MAPK kinase kinase 7 interacting protein 2; *PTEN*, phosphatase and tensin homolog; CMP, common myeloid progenitors; STR-HSC, short-term repopulating hematopoietic stem cell.

Table 3

Selected trials of agents targeting the epigenome in AML

Structural Class	Agent	Target	Phase of Study	Comments (References)
Single agent HDI trials				
Short chain fatty acids	Phenylbutyrate (PB)	HDAC I/II	I	Safety of PB established and hematologic improvement in platelet counts documented in the occasional patient with AML[195, 196]
	Valproate (+/− ATRA)	HDAC I/II	I/II	Hematologic improvement noted in one third of patients with MDS, including 1 patient with sAML/MDS[145]. Three of 11 patients with AML had CR/CRi[147], 5% response rate in AML[144]. Histone hyperacetylation demonstrated at therapeutic levels of VPA and 2 of 8 patients had HI associated with differentiation of the leukemic clone[142].
Cyclic tetrapeptides	Romidepsin (Depsipeptide)	HDAC I/II	I/II	No objective responses in AML but histone acetylation demonstrated in mononuclear cells[139]. Response in 1 of 9 patients with AML[143]. Antileukemic activity limited to CBF AML and associated with upregulation of AML1-ETO target genes[146].
Hydroxamic acids	Vorinstat (SAHA)	HDAC I/II	I	CR/CRi in 4 of 31 patients with AML, and antioxidant gene expression signature correlated with vorinostat resistance[140].
	†Panobinstat (LBH589)	HDAC I/II	I	Transient reductions in peripheral blasts, histone acetylation demonstrated in blast cells[197].
Benzamides	Entinostat (MS275)	HDAC I	I	No objective clinical responses, histone acetylation, *CDKN1A* induction and caspase 3 activation demonstrated in leukemic blasts[141].
	MGCD0103	HDAC I/IV	1	Two of 22 patients with AML had decline in bone marrow blasts to <5%[198].
Single agent DNMT inhibitor trials				
Nucleoside analog	Azacitidine	DNMT	II/III	Up to 48% of patients with clinical benefit[154]. Overall response rate including HI was 60%[162].
	Decitabine	DNMT	I	Eight of 35 patients with AML had a response, no correlation of baseline *CDKN2B* methylation with clinical activity[160].

Structural Class	Agent	Target	Phase of Study	Comments (References)
Combination DNMT+ HDI trials				
Nucleoside analog+Benzamide	Azacitidine+MS2 75	DNMT+HDAC	I	Responses were seen in 46% of patients with MDS or AML, no correlation of clinical response with reversal of methylation or gene expression[159].
Nucleoside analog+Hydroxamic acid	Azacitidine+SAHA	DNMT+HDAC	I/II	Responses occurred in 18 of 21 (86%) patients[199]
Nucleoside analog+Hydroxamic acid	Azacitidine+PXD 101	DNMT+HDAC	I	Responses in 7/21 patients, study is now in randomized phase[200].
Nucleoside analog + Short chain fatty acid	Azacitidine+PB	DNMT+HDAC	Pilot/I	Antileukemic effect in 2 of 8 patients with AML, no correlation of response with histone acetylation[161]. Responses in 11 of 36 patients with MDS or AML, significant correlation of response with *CDKN2B* or CDH-1 methylation reversal[158].
Nucleoside analog + Short chain fatty acid	Azacitidine+VPA+ATRA	DNMT+HDAC	I/II	Response rate was 42%, induction of histone acetylation, global DNA methylation and upregulation of *CDKN2B* and *CDKN1A* expression was observed which did not correlate with clinical response. Correlation of VPA levels with response[157].
Nucleoside analog + short chain fatty acid	Decitabine +VPA	DNMT+HDAC	I/II	9 of 48 patients with AML responded, patients with lower *CDKN2B* methylation had a significantly higher response rate, *CDKN2B* gene reactivation was not associated with clinical response. Correlation of VPA levels with response[156]. Response rate was 44%, induction of ER expression was associated with response. Addition of VPA did not appear to increase the response rate[155].
Histone methyltransferase antagonist				
Nucleoside analog	DZNep	HMT	Pre-clinical	Inhibits S-adenosyl-L-methionine dependent methyltransferases and leads to degradation of PRC2 HMTs and decrease in H3K27 methylation. Being investigated in leukemia cell lines and primary leukemia cells[174].
Hydroxamic acid	LAQ824	†HDAC/HMT	Pre-clinical	Degradation of HMTs such as EZH2 leading to decrease in H3K27 methylation and apoptosis in human primary leukemia cells[201].

Note: *CDKN2B*, encodes p15^{INK4b}; *CDKN1A*, encodes P21; HDAC, histone deacetylase; DNMT, DNA methyltransferase inhibitor, HMT, histone methyltransferase; VPA, valproic acid; DZNep, 3-deazaneplanocin A; PRC2, polycomb repressive complex 2; AML, acute myeloid leukemia, MDS, myelodysplastic syndrome.

†HDAC inhibitor with putative HMT inhibitory activity