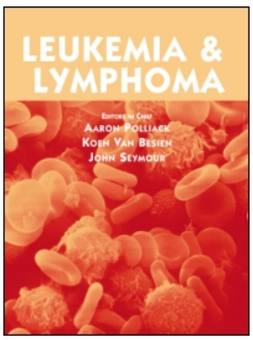

Leukemia & Lymphoma

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/ilal20

Tumor suppressors in acute myeloid leukemia

Jacqueline Wallwitz, Petra Aigner & Dagmar Stoiber

To cite this article: Jacqueline Wallwitz, Petra Aigner & Dagmar Stoiber (2021): Tumor suppressors in acute myeloid leukemia, Leukemia & Lymphoma, DOI: 10.1080/10428194.2021.1907372

To link to this article: https://doi.org/10.1080/10428194.2021.1907372

Published online: 30 Apr 2021.

LEUKEMIA & LYMPHOMA
https://doi.org/10.1080/10428194.2021.1907372

Taylor & Francis
Taylor & Francis Group

REVIEW

Tumor suppressors in acute myeloid leukemia

Jacqueline Wallwitz[a], Petra Aigner[b] and Dagmar Stoiber[a]

[a]Department Pharmacology, Physiology and Microbiology, Division Pharmacology, Karl Landsteiner University of Health Sciences, Krems, Austria; [b]Institute of Pharmacology, Center for Physiology and Pharmacology, Medical University of Vienna, Vienna, Austria

ABSTRACT

Acute myeloid leukemia (AML) is a very heterogeneous type of blood cancer, which presents with a high rate of mortality especially in elderly patients. Better understanding of critical players, such as molecules with tumor suppressive properties, may help to fine-tune disease classification and thereby treatment modalities for this detrimental disease. Here, we summarize well-known and established tumor suppressors as well as emerging tumor suppressors, including transcription factors (TCFs) and other transcriptional regulators, such as epigenetic modulators. In addition, we look into the versatile field of miRNAs also interfering with tumorigenesis and progression, which offer new possibilities in AML diagnosis, prognosis, and therapy.

ARTICLE HISTORY
Received 27 September 2020
Revised 2 March 2021
Accepted 17 March 2021

KEYWORDS
Acute myeloid leukemia; tumor suppressor; transcription factor; miRNA; epigenetic regulator

Introduction and classification of AML

Acute myeloid leukemia (AML) is the most common acute leukemia in adults, with a higher prevalence in older patients (>65 years). It is a complex and heterogeneous malignancy, characterized by the clonal expansion of immature myeloid stem cells with abnormal proliferation and differentiation, resulting in impaired hematopoiesis, and bone marrow failure [1]. AML has long been associated with chromosomal rearrangements like translocations [1], chromosomal aneuploidies with deletions [2,3], monosomies [4], and polysomies [4–6] of chromosomes, but over the last decade, it was determined that beside cytogenetic abnormalities gene mutations play also a prominent role in tumorigenesis [7]. Only 3% of patients with AML do not harbor a known driver mutation [8]. Moreover, individual leukemias harbor multiple mutations with even more diverse mutationally composed subclones, rendering each patient's AML genetically unique [9].

A first attempt to distinguish AML subtypes was established in 1976 with the French-American-British (FAB) classification system [10], which was complemented by the World Health Organization (WHO) classification in 2008 [11]. The 2016 revision of the WHO classification of myeloid neoplasms and acute leukemias attempts to include the advancements in clinical, prognostic, morphologic, immunophenotypic, and genetic research to define new entities of clinical significance [12]. So far, there are four WHO subclasses for AML defined: AML with recurrent genetic abnormalities (AML-RGA), AML with myelodysplasia-related changes (MDS-AML), therapy-related myeloid neoplasms (tAML), and AML not otherwise specified (AML-NOS) (Figure 1).

Recently, Song et al. have conducted a retrospective study of the United States National Cancer Institute Surveillance, Epidemiology, and End Result (SEER) database that covers approximately 29% of the US population to determine the distribution of AML subtypes [13]. The lowest incidence with 0.2% refers to therapy-related AML, followed by 6.4% MDS-AML (Figure 1). A higher percentage of AML cases (12.4% of reported cases) were associated with WHO classification AML-RGA. Surprisingly, by far the largest group was AML-NOS with over 80%, including 50.9% of patients diagnosed with AML that were not associated with WHO classification and therefore included in the AML-NOS group.

Although the focus still lies on specifying significant cytogenic and genetic subgroups to further define AML disease entities, such as AML harboring *BCR-ABL1* fusion gene or mutated runt-related transcription factor 1 (*RUNX1*), which are provisionally included since 2016, the update of classification systems is slower

CONTACT Dagmar Stoiber dagmar.stoiber@kl.ac.at Department Pharmacology, Physiology and Microbiology, Division Pharmacology, Karl Landsteiner University of Health Sciences, Dr.-Karl-Dorrek-Straße 30, Krems 3500, Austria

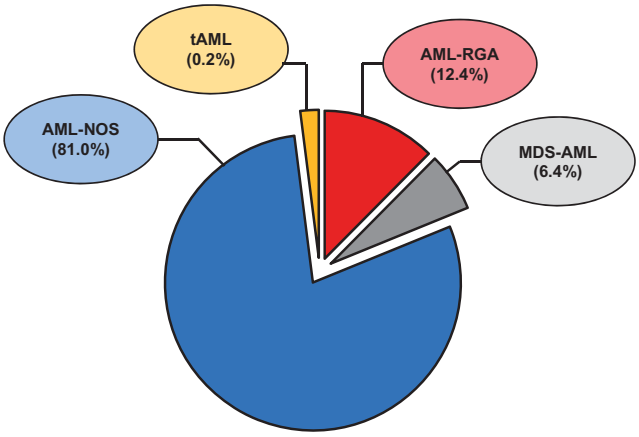

Figure 1. Distribution of AML WHO subtypes in SEER database between year 2001 and 2013 (based on data from [13]). AML-NOS: acute myeloid leukemia not otherwise specified; MDS-AML: AML myelodysplasia-related changes; tAML: therapy-related myeloid neoplasms; AML-RGA: AML with recurrent genetic abnormalities; percentages indicate the prevalence.

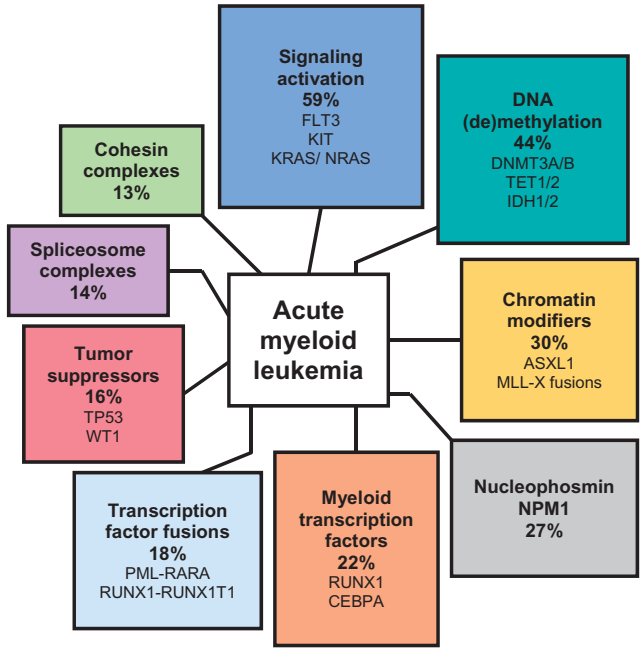

Figure 2. Categories of genetic mutations and their frequency of occurrence (modified from [15]). As in most cases of AML patients at least two genes are genetically mutated the number in % indicates the prevalence of occurrence.

than desired. Obviously more than 50% of diagnosed AML cases in the SEER database were not classified according to WHO. In addition, the European LeukemiaNet (ELN) published recommendations for diagnosis and management of AML in 2010 [14] and updated these in 2017 [7]. Three disease classes have emerged in ELN compared with WHO: AML with chromatin and RNA-splicing regulator mutations, AML with *TP53* mutations and/or chromosomal aneuploidies, and AML with *IDH2*^{R172} mutations [7].

The Cancer Genome Atlas Research Network 2013 and recently Grimwade and colleagues have described nine categories of genes that are frequently mutated in AML patients and influence core cellular processes. In average the number of mutated genes per patient was 13, with the highest frequency of mutations in genes contributing to signaling activation pathways (Figure 2) [9,15].

For further classification of the various genetic mutations resulting in AML a two-hit model of leukemogenesis was proposed [16], where class I mutations activate pro-proliferative pathways and simultaneously class II mutations affect transcription factors (TCFs) impairing normal hematopoietic differentiation and apoptosis [17,18]. Mutations in Fms-related tyrosine kinase 3 (*FLT3*) [19], *TP53* [20], proto-oncogene *c-Kit* [21], and Janus kinase 2 (*JAK2*) [22] are well-described class I mutations resulting in worse prognosis and survival in AML patients. Classical class II mutations include chromosomal aberrations which generate fusion transcripts (e.g. *RUNX1/RUNX1T1* and *CBFbeta/MYH11*) and mutations in TCFs like CCAAT/enhancer-binding protein alpha (*C/EBPA*) and nucleophosmin (*NPM1*) [15]. Advances in research over the last decade have given rise to a third class of mutations that involve epigenetic regulators, e.g. the DNA-methylation-related genes ten-eleven translocation 2 (*TET2*), DNA methyltransferase 3A (*DNMT3A*), and isocitrate dehydrogenase 1 and 2 (*IDH1* and *IDH2*) [8,23,24].

Although there has been great progress in cytogenetic, karyotypic, and genetic classification over the last decades, the underlying molecular mechanisms of occurrence and development of AML are still incompletely understood. Many of the mutated genes used by WHO and ELN for classification of AML are tumor suppressors. Classical tumor suppressors are proteins, which negatively regulate cell growth, and loss-of-function mutations in their genes result in uncontrolled proliferation of cells representing the function of a main driver in oncogenesis. Thus, this review will focus on the role of tumor suppressors in disease progression and prognosis of AML and additionally highlight newly emerging tumor suppressor classes like microRNAs.

Tumor suppressors used for AML classification

P53 is a well-defined and deeply studied tumor suppressor in AML (reviewed by [20]) and mutations of *TP53* are already used for classification of AML

Table 1. Tumor suppressors in AML and their role in risk stratification and prognosis.

	Risk group stratification				
	Favorable	Intermediate	Adverse	Prognosis	References
Classification of AML					
PML	●				[26,27]
RUNX1		●	●	Shorter OS	[28–30]
TP53			●	Absence of clinical remission, poor OS, and poor DFS;	[20,25]
Transcription factors					
C/EBPdelta	●				[31]
FOXN3				Lower complete remission (CR) rate and shorter OS	[32]
KLF5				Poor OS	[33]
NR4A				Poor OS	[34,35]
STAT3				High STAT3β/STAT3α mRNA ratio correlates with better OS and DFS	[36]
TCF21			●		[37]
WT1				Poor OS	[38,39]
Epigenetic modulators					
ASXL1/2			●	Poor OS	[24,25,40]
DNMT3A			●	Resistance to chemotherapy and disease relapse	[24,30]
TET2			●	Poor OS	[25]
miRNAs					
miR-155				Poor OS	[41,42]
miR-206			●		[43]
miR-29b				Poor OS	[43,44]
miR-3151				Shorter OS and DFS	[45]

OS: overall survival; DFS: disease-free survival.

according to ELN guidelines. In general, dysregulation of the p53 pathway leads to altered cell cycle arrest, apoptosis, senescence, DNA repair, metabolism, and autophagy. According to the two-hit model, Ohgami et al. recently showed that *TP53* mutations are associated with a complex karyotype in AML and may represent prognostic significance with worse overall survival (OS) and disease-free survival (DFS) [25] (Table 1). Such mutations are common with therapy-related AML or AML with myelodysplastic-related changes. Also, AML patients who carry intact *TP53* alleles may exhibit decreased p53 activity due to inactivating processes in the p53 pathway like *Mdm2* overexpression, or miR-3151 and miR-125b overexpression [45]. Therefore, small-molecule activators were developed that reactivate p53 and harness its physiological tumor suppressive function. Targeting upstream molecules like HDM2 and WIP1/PPM1D or altering post-translational modification like acetylation and phosphorylation of p53 is another approach to enhance therapeutic intervention success (reviewed by [26]).

The promyelocytic leukemia (*PML*) gene has also been described as a tumor suppressor, which is involved in cell cycle regulation, growth inhibition, and apoptosis; it was also reported to be responsible for the acetylation of p53 [46]. In line, PML can suppress the transcription of anti-apoptotic proteins. When fused with retinoic acid receptor alpha (RARα) due to chromosomal translocation it inhibits differentiation of hematopoietic cells. The PML-RARA fusion protein can be detected in 97% of APL patients and is therefore used for diagnosis and WHO classification [47].

RUNX1 is a TCF mainly involved in proliferation, hematopoietic stem cell differentiation, and apoptosis (reviewed by [48]). Various frameshift and missense mutations have been identified in the *RUNX1* gene, and the RUNX family exhibits a dual role acting as oncogenes or tumor suppressors. Mutated *RUNX1* is reported in about 10% of *de novo* AML, frequently occurring in the intermediate cytogenetic risk group, and resulting in shorter OS and chemoresistance to standard therapy [28–30]. Of note, mutated *RUNX1* has been a provisional entity in the WHO AML classification system since 2016.

In line, identification of novel tumor suppressors in AML could provide additional tools to classify this heterogeneous disease and thereby improve therapeutic approaches.

Transcription factors as tumor suppressors in AML

There are several key TCFs besides RUNX1 that have been found to be inactivated or deleted in AML and are thus considered important tumor suppressors for this type of leukemia [49]. One example is *NR4A3* (*NOR-1*), a direct target gene of *RUNX1*. A reduction of *NR4A3* expression correlated with increased proliferation and clonogenic potential in hematopoietic stem cells [50]. NR4A3, as well as its highly homologous NR4A family member NR4A1 (Nur77), are nuclear orphan receptors acting as intracellular TCFs that were previously described as tumor suppressors in AML. The deletion or silencing of both NR4A factors results in rapid development of AML in mice and mutations are also commonly found in AML patients showing

poorer OS [34,35,51,52]. Functionally, NR4A1 and NR4A3 suppress hyperproliferation and DNA damage in hematopoietic stem cells through direct activation of C/EBPA and suppression of NFκB activation [53]. NR4A factors can furthermore repress *c-MYC* and *c-MYC*-target gene transcription in AML cells [54].

Another transcriptional regulator inhibiting NFκB signaling is promyelocytic leukemia protein 4 (PML4). PML4 is one isoform of PML (see also previous chapter) that was reported to influence several apoptosis and cell cycle pathways including downregulation of survivin and destabilization of c-MYC [55,56]. Additionally, PML4 plays an important role in hematopoietic differentiation and is implicated as a tumor suppressor in leukemia [27]. The Krueppel-like transcription factors (KLF) were previously described to be involved in cellular differentiation as well [57]. KLF expression levels are low in cells derived from AML patients compared to differentiated myeloid cells [58]. KLF5 in particular is considered a tumor suppressor in AML and was found to be significantly reduced in AML patients [33]. It is involved in myeloid differentiation as well as hematopoiesis and hematopoietic stem cell proliferation and localization [59]. In addition, Forkhead box protein N3 (FOXN3) plays a key role in cell cycle regulation and *FOXN3* expression levels have been shown to negatively correlate with leukocyte numbers in AML patients. In line, FOXN3 levels are frequently decreased in AML patients and cell lines [32,60].

Constitutively active signal transducer and activator of transcription 3 (STAT3) was reported to have an oncogenic role in AML patients by increasing proliferation and protecting against apoptosis [61,62]. However, it was recently shown that the alternatively spliced STAT3β isoform has a tumor-suppressive role [36]. Furthermore, a higher mRNA ratio *STAT3β* to *STAT3α* correlates with favorable prognosis and a better OS in AML patients (Table 1). Another TCF with different isoforms involved in the regulation of cell survival, proliferation, and differentiation is Wilms tumor 1 (WT1). It was shown that it is overexpressed in the majority of AML patients, thereby associated with resistance to therapy and a poor OS [38]. Also, several mutations of WT1 have been identified. However, the different roles of the four isoforms are not completely understood, but seem to have oncogenic, as well as tumor-suppressive functions [39].

Somatic mutations of *PHF6,* a member of the plant homeodomain (PHD)-like finger (PHF) family, have also been reported to frequently occur in myeloid malignancies such as MDS and AML [63,64]. Such *PHF6* mutations are usually found in more aggressive types of myeloid malignancies and are often linked with mutations of *RUNX1* or *IDH1 [65]*.

Many TCFs like C/EBPdelta, chromodomain helicase DNA binding protein 5 (CHD5), or B-cell factor 3 (EBF3) are tumor suppressors that are influenced by epigenetic modulation like hypermethylation of promoter regions. In contrast to other C/EBP family members, C/EBPdelta expression is diminished in more than 35% of AML patients due to hypermethylation of its promoter regions. Based on those findings, the authors concluded that C/EBPdelta is a novel tumor suppressor in AML [31]. However, a correlation between disease stage and *C/EBPdelta* methylation/silencing could not be demonstrated [66]. In addition, CHD5 is frequently found to be silenced by promoter methylation in AML. Similar to its described role in other cancers, CHD5 controls cell proliferation and suppresses leukemogenesis in AML [67]. In pediatric AML patients, early EBF3 is repressed by promoter hypermethylation based on its role in apoptosis regulation, which renders also EBF3 tumor suppressor in AML [68].

Transcription factor 21 (TCF21) is another TCF, which is epigenetically inactivated in a variety of human cancers, including AML, and therefore, proposed as a candidate tumor suppressor gene in MDS and AML. *TCF21* hypermethylation is associated with adverse risk karyotype in AML and might be used as a potential biomarker for risk stratification [37].

Many TCFs and their reduced expression in AML seem to correlate with an adverse disease outcome. Therefore, the reactivation of their TCF function or the activation/repression of their downstream targets may be a beneficial therapeutic approach.

Epigenetic modulators involved in AML

The tumor-suppressive role of many TCFs is influenced by epigenetic modulation, but there are also epigenetic regulators acting as tumor suppressors in AML. Epigenetic regulation involves factors impacting DNA cytosine modifications and those mediating posttranslational modifications of histones. One representative of the former group is *DNMT3A*, which is one of the most frequently mutated genes in AML (accounting for about 23% of all AML patients and up to 36% of cytogenetically normal AML patients) [69]. *DNMT3A* mutations are linked to chemotherapy resistance, disease relapse and to adverse risk mainly in *FLT3*-ITD mutant intermediate-risk AML patients (classified according to their karyotype) [24,30]. Mutations in

DNMT3A represent nonsense, frameshift, or missense alterations; of those a single recurrent mutation at Arginine 882 accounts for approximately half of all *DNMT3A* mutations [70,71]. While mutations of the *DNMT3A* gene in general seem to be loss-of-function mutations, the effect of the heterozygous Arg882 mutation is unclear [24].

Another gene that is affected by loss-of-function mutations, but also copy-number loss, is *TET2*. The methylcytosine dioxygenase TET2 plays a key role in DNA demethylation and its loss/loss-of-function mutation has been associated with poor overall prognosis of patients with AML [72]. The frequency of such mutations in AML is enriched in patients over 60 years of age relative to younger patients [24]. Similar to *DNMT3A* mutations, mutations in *TET2* are associated with adverse OS in patients with intermediate-risk AML or CN-AML, with 18–23% mutational frequency. However, in contrast to *DNMT3A*, the effect of *TET2* mutations is independent of a *FLT3*-ITD mutation in the subgroup of intermediate-risk AML. Interestingly, loss of *Tet2* alone does not result in AML in mice [24].

Another group of epigenetic regulators that includes tumor suppressors in AML comprises polycomb group family members, which modulate histone posttranslational modifications. The best studied in the context of AML is additional sex combs-like 1 (ASXL1), which regulates histone H3 lysine 27 methylation. This polycomb group gene was found to be frequently somatically deleted or point-mutated in the entire spectrum of myeloid malignancies. In AML this refers to loss-of-function mutations and copy number loss [24]. More specifically, the leukemia-associated mutations in *ASXL1* most frequently are nonsense as well as frameshift mutations in the 5′ end of the last exon [40]. The incidence of these mutations positively correlates with advancing age [24]. *ASXL1* mutation appears to be a novel biomarker of adverse OS in patients with MDS and AML [40]. An enrichment of *ASXL1* mutations in patients with AML having a history of MDS was observed [73]. Interestingly, *ASXL1* loss was shown to cooperate with oncogenic mutated *NRas* (NRasG12D) [40].

Moreover, the related transcriptional regulator additional sex combs-like protein 2 (ASXL2) has been demonstrated to be also crucial during hematopoietic stem cell self-renewal and its loss can promote leukemogenesis. Thus, ASXL2 can also be considered as a tumor suppressor in AML [74]. *ASXL2* was reported to be frequently mutated in *RUNX1-RUNX1T1* AML patients [75,76].

The most frequently mutated epigenetic regulator in MDS and AML is UTX (*KDM6A*). Recurrent presence of mutations, which result in the loss of H3K27me3 in myeloid malignancies (including *ASXL1, EZH2, SUZ12*, and *EED* deletions and somatic mutations) suggest a rationale for inhibiting demethylation of H3K27 in these disorders [24]. Preclinical studies have evaluated therapeutic potential of an UTX/JMJD3 inhibitor [77], however, so far this inhibitor has not reached clinical application. Interestingly, although *UTX* was originally identified as cancer driver gene (*via* TCGA analysis), more recently it was also described to have tumor-suppressive function, as loss of UTX occurs in different cancer types. It represses genes by increasing the levels of H3K27me3 marks, thereby enhancing proliferation, clonogenicity, adhesion, and tumorigenicity in myeloid among other tumors [78]. In line, the majority of myeloid-specific *Utx* knock-out mice developed AML as well [79].

Mutations in epigenetic regulators mainly result in adverse risk and poor OS in AML patients. Thus, better understanding of epigenetic abnormalities in AML might also provide novel therapeutic targets.

MicroRNAs as an emerging class of tumor suppressors

MicroRNAs (miRNAs, miRs) are small single-stranded non-coding RNAs of 19–25 nucleotides that can act tumor suppressive (also called anti-oncomiRs) or tumor inducive (oncomiRs) by altering expression or mRNA degradation of target genes. Their roles are complex, as one miRNA may target many different mRNAs, and in turn also various miRNAs can affect the same mRNA [80]. Over the last years, a variety of miRNAs has been associated with AML pathogenesis. On the one hand increased expression of oncomiRs like miR-9, miR-17-92 cluster, miR-22, miR-125b, miR-126, miR-155, and miR-196b were shown to be involved in AML disease progression [43], whereas on the other hand downregulation of anti-oncomiRs and therefore overexpression of their target genes also influences AML progression by epigenetic modifications, cell proliferation, growth, differentiation, and apoptosis (Figure 3) [81].

Aberrant epigenetic modifications are recognized in acute leukemias and these include also deregulation of miRNAs [82]. In addition, miRNAs like the miR-29 family are involved in epigenetic regulation and DNA methylation. miR-29b regulates the balance of DNA methylation and demethylation by repressing the activities of DNA methyltransferases (DNMTs) like the

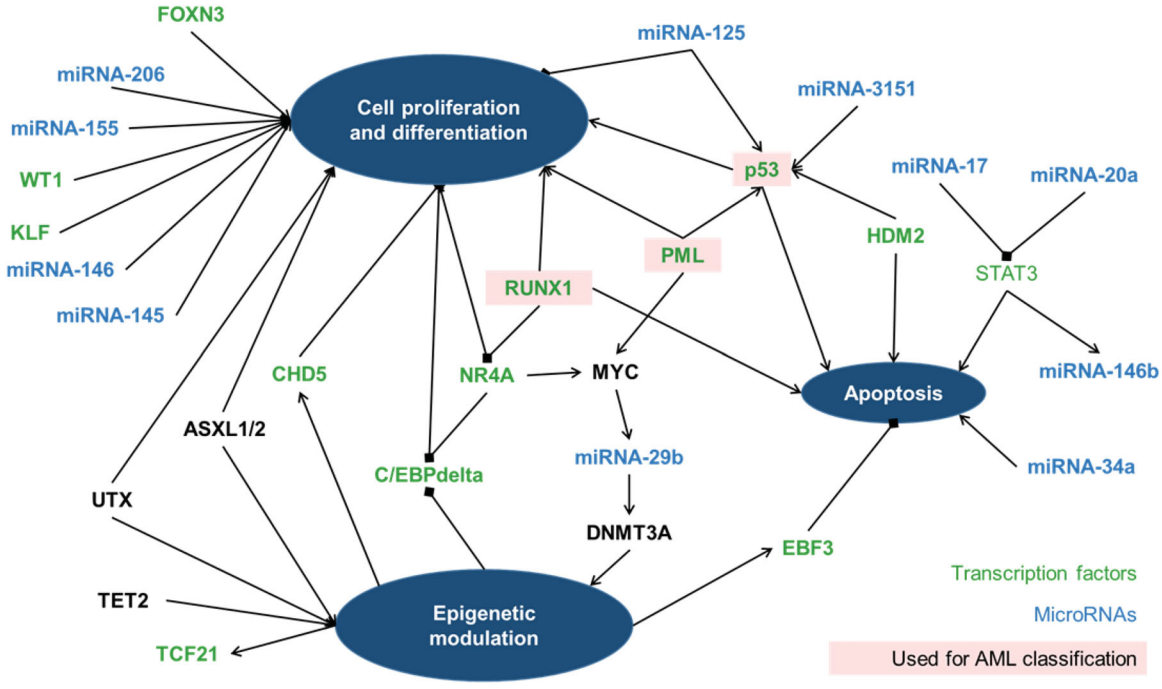

Figure 3. Tumor suppressors involved in AML development regulate vital cellular functions. All described transcription factors (green), micro RNAs (blue), and other proteins (black) acting as tumor suppressors in AML and their complex interplay on cell proliferation and differentiation, epigenetic modulation, and apoptosis is displayed. Proteins used for AML classification are marked with red shape fill.

previously discussed DNMT3 and DNA demethylases. It was shown that its expression is suppressed in several cancers including AML by c-Myc, NFκB, and transforming growth factor-beta (TGF-β) signaling [43,44]. Decreased miR-29b levels are associated with poor OS [43]. NFκB signaling is involved in proliferation, survival and apoptosis, and its activation can lead to malignancies like AML [83]. Both miR-145 and miR-146a were shown to be negative regulators of the NFκB pathway by inhibiting the activator protein interleukin-1 receptor-associated kinase 1 (IRAK1), and TNF receptor-associated factor 6 (TRAF6). Thus, they act as tumor suppressors, and their decreased expression could be detected in AML [84].

The expression of the tumor-suppressive STAT3 target miR-146b was also found to be decreased in tumor cells [85,86], whereas the STAT3 upstream miRNA-17 and miRNA-20a both inhibit the expression of STAT3 at the post-transcriptional level leading to impaired apoptosis and thereby inducing leukemogenesis [87].

One miRNA, which plays a crucial role in antitumorigenesis, is miR-628. It could be shown recently that there is an inverse correlation of decreased miR-628 levels with the increased expression of insulin-like growth factor receptor (*IGF-1R*) in AML patients, suggesting that the tumor-suppressive effect of miR-628 in AML is mediated by regulating the PI3K/AKT pathway [88]. Downregulation of miR-34a was also reported to be common in AML patients resulting in diminished cell apoptosis and suppressed autophagy [89].

In some reports, miRNAs are related to therapy and mainly cytarabine sensitivity in AML cell lines and OS of patients [90]. For example, miRNA-25 was correlated with an improved response rate in AML patients [90]. But besides their tumor suppressive and oncogenic role or their influence on therapy response, miRNAs are more frequently used as biomarkers for the diagnosis and prognosis of different malignancies including AML. Quirico et al. have recently summarized that miR-155-3p, miR-181-5p, miR-150, miR-342, miR-10-5p, miR-210, miR-155, miR-10b, and miR-203 could be used as diagnostic or prognostic markers [41], whereas especially miR-155 is associated with poor OS in AML patients [42]. For many more miRNAs like miR-92a, miR-143, miR-193b, and miR-342 a reduced expression could be associated with AML, indicating a tumor-suppressive role, but their mechanism of action as well as up-stream regulators and/or down-stream targets are not identified yet [91,92].

A lot of research is ongoing in the field of microRNAs and AML, but due to their complexity (and

the promiscuity in targets) they require thorough understanding before using them in AML therapy. However, the most important potential application of miRNAs for AML in the clinics is rather their usage as biomarkers [43].

Tumor suppressors in AML and their (potential) implications for diagnosis, risk stratification, prognosis, and treatment

By definition the expression or functionality of tumor suppressors is reduced in malignant diseases. Therefore, tumor suppressors mostly are not directly targeted by treatment, but their mutation and hence presence or absence is rather used for diagnosis, risk stratification, and/or prognosis of cancer (sub)types.

Three widely accepted and established risk categories – favorable, intermediate, and adverse – for patients diagnosed with AML were defined in 2017 by ELN [7]. The genetic background of disease is basis for this classification, however, additional prognostic markers are strongly required for further risk stratification. Therefore, multiple studies aim to identify differentially expressed proteins to predict the outcome of OS, DFS and complete remission in AML patients [93,94]. Table 1 summarizes the tumor suppressors discussed here serving to optimize assignments to risk groups and prediction of overall prognosis within AML patients.

Altered expression or mutation of tumor suppressors is also known to influence therapeutic efficacy. Mutations of *DNMT3A* and *RUNX1* for instance correlate with resistance to chemotherapy and disease relapse [30]. Regarding epigenetic modulators, nonselective DNMT-inhibitors, such as azacitidine and decitabine have been in the clinic for a long time, leading to clinical response primarily in hematopoietic cancers (MDS and AML). Besides them, specific targeting of epigenetic regulators in cancer therapy is widely investigated, but focusing on tumor drivers [95].

As the expression of tumor suppressors is frequently reduced or even lost in AML patients, efforts are undertaken to reactivate them, or to use tumor suppressor mimics as therapeutics. It was recently shown that miRNA-29b and miRNA-206 mimics in such context could positively influence the outcome of AML models *in vitro* and *in vivo* [43]. But these promising molecules need further validation in pre-clinical and clinical studies. By contrast, a phase I clinical trial was successfully assessed for the first tumor-targeted miRNA-34 mimic MRX34. It reactivates the synergistic effect with the tumor suppressor p53. Other small molecule wild-type p53 activators have been investigated since decades. One example is the selective MDM2:p53 inhibitor RG7338 (idasanutlin) already in clinical phase III study [96]. Additionally, extensive effort is undertaken to find small molecules targeting and reactivating mutant p53, with the challenge to target a variety of mutations [96].

A new aspect in AML therapy may be opened by recently published large prospective cohort studies that arose dealing with early identification of clonal hematopoiesis of indeterminate potential (CHIP) associated with malignant diseases including AML [97]. Abelson et al. could show that putative driver mutations in tumor suppressors, including *DNMT3A*, *TET2*, *ASXL1*, *TP53*, and *RUNX1* (in descending order), were more common in individuals subsequently developing AML compared with matched controls [98]. Also, the simultaneous number of these mutations was strongly associated with the risk of progression to AML [98]. Comparable results were published by Desai et al., who found a correlation of individuals developing AML within a median of 9.6 years with already existing mutations in the tumor suppressors *DNMT3A*, *TET2, TP53*, and *ASXL1* [99]. These studies show that early mutations, *inter alia* in tumor suppressors, are associated with subsequent AML progression and that individuals at high risk to develop AML could be identified years before they are actually diagnosed with the disease. This could be a starting point for early preventive treatment concepts using, e.g. tumor suppressor mimics. However, these highly interesting findings need to be more thoroughly investigated and validated in further prospective cohorts as well as in pre-clinical and clinical studies. These strategies hold promise to be of use in the near future for improved disease diagnosis, prognosis, and/or treatment, maybe even before AML manifestation.

Conclusion

AML is a complex and heterogeneous human malignancy characterized by cytogenetic, karyotypic, and genetic variations. This review focused on genetic mutations in tumor suppressors as well as epigenetic modulators and miRNAs with tumor-suppressive function in AML (Figure 3). Mutations in tumor suppressors might be of predictive but also therapeutic value. Such alterations have been intensely studied over the last decades and resulted in great progress of understanding the deeply interwoven molecular mechanisms of occurrence, development, progression, and potential therapeutic targets of AML. However, further

studies are still required in this field to elucidate the exact roles of tumor suppressors in these processes which could allow improved prediction and novel personalized treatment options in AML.

Acknowledgments

The authors thank Sophie Rosa Edtmayer, Agnieszka Witalisz-Siepracka, Bernhard Moser, and Andreas Birbach for critical reading of the manuscript.

Disclosure statement

The authors report no conflict of interest.

Funding

This work was supported by the Austrian Science Fund (FWF) under Grant P32693 to D.S.

References

[1] De Kouchkovsky I, Abdul-Hay M. Acute myeloid leukemia: a comprehensive review and 2016 update. Blood Cancer J. 2016;6(7):e441.
[2] Naarmann-de Vries IS, Sackmann Y, Klein F, et al. Characterization of acute myeloid leukemia with del(9q) - Impact of the genes in the minimally deleted region. Leuk Res. 2019;76:15–23.
[3] Nagarajan L. Chromosomal deletions in AML. Cancer Treat Res. 2010;145:59–66.
[4] Rucker FG, Schlenk RF, Bullinger L, et al. TP53 alterations in acute myeloid leukemia with complex karyotype correlate with specific copy number alterations, monosomal karyotype, and dismal outcome. Blood. 2012;119(9):2114–2121.
[5] Moosavi L, Bowen J, Coleman J, et al. Acute myelogenous leukemia with trisomy 8 and concomitant acquired factor VII deficiency. J Investig Med High Impact Case Rep. 2019;7:2324709619872657.
[6] Hemsing AL, Hovland R, Tsykunova G, et al. Trisomy 8 in acute myeloid leukemia. Expert Rev Hematol. 2019;12:947–958.
[7] Dohner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood. 2017;129(4):424–447.
[8] Papaemmanuil E, Gerstung M, Bullinger L, et al. Genomic classification and prognosis in acute myeloid leukemia. N Engl J Med. 2016;374(23):2209–2221.
[9] Grimwade D, Ivey A, Huntly BJ. Molecular landscape of acute myeloid leukemia in younger adults and its clinical relevance. Blood. 2016;127(1):29–41.
[10] Krause JR. Morphology and classification of acute myeloid leukemias. Clin Lab Med. 2000;20(1):1–16.
[11] Vardiman JW, Thiele J, Arber DA, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. Blood. 2009;114(5):937–951.
[12] Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391–2405.
[13] Song X, Peng Y, Wang X, et al. Incidence, survival, and risk factors for adults with acute myeloid leukemia not otherwise specified and acute myeloid leukemia with recurrent genetic abnormalities: analysis of the surveillance, epidemiology, and end results (SEER) database, 2001–2013. Acta Haematol. 2018;139(2):115–127.
[14] Dohner H, Estey EH, Amadori S, et al. Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European Leukemia net. Blood. 2010;115(3):453–474.
[15] Cancer Genome Atlas Research N, Ley TJ, Miller C, et al. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. N Engl J Med. 2013;368(22):2059–2074.
[16] Gilliland DG, Griffin JD. The roles of FLT3 in hematopoiesis and leukemia. Blood. 2002;100(5):1532–1542.
[17] Takahashi S. Current findings for recurring mutations in acute myeloid leukemia. J Hematol Oncol. 2011;4:36.
[18] Kihara R, Nagata Y, Kiyoi H, et al. Comprehensive analysis of genetic alterations and their prognostic impacts in adult acute myeloid leukemia patients. Leukemia. 2014;28(8):1586–1595.
[19] Leick MB, Levis MJ. The future of targeting FLT3 activation in AML. Curr Hematol Malig Rep. 2017;12(3):153–167.
[20] Barbosa K, Li S, Adams PD, et al. The role of TP53 in acute myeloid leukemia: challenges and opportunities. Genes Chromosomes Cancer. 2019;58(12):875–888.
[21] Malaise M, Steinbach D, Corbacioglu S. Clinical implications of c-Kit mutations in acute myelogenous leukemia. Curr Hematol Malig Rep. 2009;4(2):77–82.
[22] Benton CB, Boddu PC, DiNardo CD, et al. Janus kinase 2 variants associated with the transformation of myeloproliferative neoplasms into acute myeloid leukemia. Cancer. 2019;125(11):1855–1866.
[23] Dhall A, Zee BM, Yan F, et al. Intersection of epigenetic and metabolic regulation of histone modifications in acute myeloid leukemia. Front Oncol. 2019;9:432.
[24] Abdel-Wahab O, Levine RL. Mutations in epigenetic modifiers in the pathogenesis and therapy of acute myeloid leukemia. Blood. 2013;121(18):3563–3572.
[25] Ohgami RS, Ma L, Merker JD, et al. Next-generation sequencing of acute myeloid leukemia identifies the significance of TP53, U2AF1, ASXL1, and TET2 mutations. Mod Pathol. 2015;28(5):706–714.
[26] Ladds M, Lain S. Small molecule activators of the p53 response. J Mol Cell Biol. 2019;11(3):245–254.
[27] Wu J, Zhou LQ, Yu W, et al. PML4 facilitates erythroid differentiation by enhancing the transcriptional activity of GATA-1. Blood. 2014;123(2):261–270.
[28] Gaidzik VI, Teleanu V, Papaemmanuil E, et al. RUNX1 mutations in acute myeloid leukemia are associated

with distinct clinico-pathologic and genetic features. Leukemia. 2016;30(11):2160–2168.
[29] Haferlach T, Stengel A, Eckstein S, et al. The new provisional WHO entity 'RUNX1 mutated AML' shows specific genetics but no prognostic influence of dysplasia. Leukemia. 2016;30(10):2109–2112.
[30] DiNardo CD, Cortes JE. Mutations in AML: prognostic and therapeutic implications. Hematol Am Soc Hematol Educ Program. 2016;2016(1):348–355.
[31] Agrawal S, Hofmann WK, Tidow N, et al. The C/EBPdelta tumor suppressor is silenced by hypermethylation in acute myeloid leukemia. Blood. 2007;109(9):3895–3905.
[32] Zhang J, Wang Y, Mo W, et al. The clinical and prognostic significance of FOXN3 downregulation in acute myeloid leukaemia. Int J Lab Hematol. 2020;42(3):270–276.
[33] Diakiw SM, Kok CH, To LB, et al. The granulocyte-associated transcription factor Krüppel-like factor 5 is silenced by hypermethylation in acute myeloid leukemia. Leuk Res. 2012;36(1):110–116.
[34] Mullican SE, Zhang S, Konopleva M, et al. Abrogation of nuclear receptors Nr4a3 and Nr4a1 leads to development of acute myeloid leukemia. Nat Med. 2007;13(6):730–735.
[35] Boudreaux SP, Duren RP, Call SG, et al. Drug targeting of NR4A nuclear receptors for treatment of acute myeloid leukemia. Leukemia. 2019;33(1):52–63.
[36] Aigner P, Mizutani T, Horvath J, et al. STAT3β is a tumor suppressor in acute myeloid leukemia. Blood Adv. 2019;3(13):1989–2002.
[37] Gu Y, Zhou JD, Xu ZJ, et al. Promoter methylation of the candidate tumor suppressor gene TCF21 in myelodysplastic syndrome and acute myeloid leukemia. Am J Transl Res. 2019;11(6):3450–3460.
[38] Rampal R, Figueroa ME. Wilms tumor 1 mutations in the pathogenesis of acute myeloid leukemia. Haematologica. 2016;101(6):672–679.
[39] Luna I, Such E, Cervera J, et al. WT1 isoform expression pattern in acute myeloid leukemia. Leuk Res. 2013;37(12):1744–1749.
[40] Abdel-Wahab O, Dey A. The ASXL-BAP1 axis: new factors in myelopoiesis, cancer and epigenetics. Leukemia. 2013;27(1):10–15.
[41] Quirico L, Orso F. The power of microRNAs as diagnostic and prognostic biomarkers in liquid biopsies. Cancer Drug Resist. 2020;3(2):117–139.
[42] Narayan N, Bracken CP, Ekert PG. MicroRNA-155 expression and function in AML: an evolving paradigm. Exp Hematol. 2018;62:1–6.
[43] Wallace JA, O'Connell RM. MicroRNAs and acute myeloid leukemia: therapeutic implications and emerging concepts. Blood. 2017;130(11):1290–1301.
[44] Yan B, Guo Q, Fu FJ, et al. The role of miR-29b in cancer: regulation, function, and signaling. Onco Targets Ther. 2015;8:539–548.
[45] Prokocimer M, Molchadsky A, Rotter V. Dysfunctional diversity of p53 proteins in adult acute myeloid leukemia: projections on diagnostic workup and therapy. Blood. 2017;130(6):699–712.
[46] Pearson M, Carbone R, Sebastiani C, et al. PML regulates p53 acetylation and premature senescence induced by oncogenic Ras. Nature. 2000;406(6792):207–210.
[47] Bernardi R, Pandolfi PP. Role of PML and the PML-nuclear body in the control of programmed cell death. Oncogene. 2003;22(56):9048–9057.
[48] Otalora-Otalora B, Henriquez B, Lopez-Kleine L, et al. RUNX family: oncogenes or tumor suppressors (review). Oncol Rep. 2019;42(1):3–19.
[49] Heidari N, Abroun S, Bertacchini J, et al. Significance of inactivated genes in leukemia: pathogenesis and prognosis. Cell J. 2017;19(1):9–26.
[50] Bluteau D, Gilles L, Hilpert M, et al. Down-regulation of the RUNX1-target gene NR4A3 contributes to hematopoiesis deregulation in familial platelet disorder/acute myelogenous leukemia. Blood. 2011;118(24):6310–6320.
[51] Wenzl K, Troppan K, Neumeister P, et al. The nuclear orphan receptor NR4A1 and NR4A3 as tumor suppressors in hematologic neoplasms. Curr Drug Targets. 2015;16(1):38–46.
[52] Shimizu R, Muto T, Aoyama K, et al. Possible role of intragenic DNA hypermethylation in gene silencing of the tumor suppressor gene NR4A3 in acute myeloid leukemia. Leuk Res. 2016;50:85–94.
[53] Freire PR, Conneely OM. NR4A1 and NR4A3 restrict HSC proliferation *via* reciprocal regulation of C/EBPa and inflammatory signaling. Blood. 2019;131:1081–1093.
[54] Boudreaux SP, Ramirez-Herrick AM, Duren RP, et al. Genome-wide profiling reveals transcriptional repression of MYC as a core component of NR4A tumor suppression in acute myeloid leukemia. Oncogenesis. 2012;21:e19.
[55] Xu ZX, Zhao RX, Ding T, et al. Promyelocytic leukemia protein 4 induces apoptosis by inhibition of survivin expression. J Biol Chem. 2004;279(3):1838–1844.
[56] Buschbeck M, Uribesalgo I, Ledl A, et al. PML4 induces differentiation by Myc destabilization. Oncogene. 2007;26(23):3415–3422.
[57] Chambers SM, Boles NC, Lin KY, et al. Hematopoietic fingerprints: an expression database of stem cells and their progeny. Cell Stem Cell. 2007;1(5):578–591.
[58] Humbert M, Halter V, Shan D, et al. Deregulated expression of Kruppel-like factors in acute myeloid leukemia. Leuk Res. 2011;35(7):909–913.
[59] Shahrin NH, Diakiw S, Dent LA, et al. Conditional knockout mice demonstrate function of Klf5 as a myeloid transcription factor. Blood. 2016;128(1):55–59.
[60] He H, Zhang J, Qu Y, et al. Novel tumor-suppressor FOXN3 is downregulated in adult acute myeloid leukemia. Oncol Lett. 2019;18(2):1521–1529.
[61] Redell MS, Ruiz MJ, Alonzo TA, et al. Stat3 signaling in acute myeloid leukemia: ligand-dependent and -independent activation and induction of apoptosis by a novel small-molecule Stat3 inhibitor. Blood. 2011;117(21):5701–5709.
[62] Steensma DP, McClure RF, Karp JE, et al. JAK2 V617F is a rare finding in de novo acute myeloid leukemia, but STAT3 activation is common and remains unexplained. Leukemia. 2006;20(6):971–978.

[63] Haferlach T, Nagata Y, Grossmann V, et al. Landscape of genetic lesions in 944 patients with myelodysplastic syndromes. Leukemia. 2014;28(2):241–247.
[64] Papaemmanuil E, Gerstung M, Malcovati L, et al. Clinical and biological implications of driver mutations in myelodysplastic syndromes. Blood. 2013; 122(22):3616–3627.
[65] Przychodzen BP, Gu X, You D, et al. PHF6 somatic mutations and their functional role in the pathophysiology of myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML). Washington (DC): American Society of Hematology; 2016.
[66] Lowe SW, Sherr CJ. Tumor suppression by Ink4a-Arf: progress and puzzles. Curr Opin Genet Dev. 2003; 13(1):77–83.
[67] Zhao R, Meng F, Wang N, et al. Silencing of CHD5 gene by promoter methylation in leukemia. PLoS One. 2014;9(1):e85172.
[68] Tao YF, Xu LX, Lu J, et al. Early B-cell factor 3 (EBF3) is a novel tumor suppressor gene with promoter hypermethylation in pediatric acute myeloid leukemia. J Exp Clin Cancer Res. 2015;34:4.
[69] Marcucci G, Metzeler KH, Schwind S, et al. Age-related prognostic impact of different types of DNMT3A mutations in adults with primary cytogenetically normal acute myeloid leukemia. J Clin Oncol. 2012;30(7): 742–750.
[70] Ley TJ, Ding L, Walter MJ, et al. DNMT3A mutations in acute myeloid leukemia. N Engl J Med. 2010;363(25): 2424–2433.
[71] Yan XJ, Xu J, Gu ZH, et al. Exome sequencing identifies somatic mutations of DNA methyltransferase gene DNMT3A in acute monocytic leukemia. Nat Genet. 2011;43(4):309–315.
[72] Kosmider O, Gelsi-Boyer V, Cheok M, et al. TET2 mutation is an independent favorable prognostic factor in myelodysplastic syndromes (MDSs). Blood. 2009; 114(15):3285–3291.
[73] Boultwood J, Perry J, Pellagatti A, et al. Frequent mutation of the polycomb-associated gene ASXL1 in the myelodysplastic syndromes and in acute myeloid leukemia. Leukemia. 2010;24(5):1062–1065.
[74] Micol JB, Pastore A, Inoue D, et al. ASXL2 is essential for haematopoiesis and acts as a haploinsufficient tumour suppressor in leukemia. Nat Commun. 2017;8: 15429.
[75] Micol JB, Duployez N, Boissel N, et al. Frequent ASXL2 mutations in acute myeloid leukemia patients with t(8;21)/RUNX1-RUNX1T1 chromosomal translocations. Blood. 2014;124(9):1445–1449.
[76] Faber ZJ, Chen X, Gedman AL, et al. The genomic landscape of core-binding factor acute myeloid leukemias. Nat Genet. 2016;48(12):1551–1556.
[77] Kruidenier L, Chung CW, Cheng Z, et al. A selective jumonji H3K27 demethylase inhibitor modulates the proinflammatory macrophage response. Nature. 2012; 488(7411):404–408.
[78] Chang S, Yim S, Park H. The cancer driver genes IDH1/2, JARID1C/ KDM5C, and UTX/ KDM6A: crosstalk between histone demethylation and hypoxic reprogramming in cancer metabolism. Exp Mol Med. 2019; 51(6):1–17.
[79] Gozdecka M, Meduri E, Mazan M, et al. UTX-mediated enhancer and chromatin remodeling suppresses myeloid leukemogenesis through noncatalytic inverse regulation of ETS and GATA programs. Nat Genet. 2018;50(6):883–894.
[80] He C, Luo B, Jiang N, et al. OncomiR or antioncomiR: role of miRNAs in acute myeloid leukemia. Leuk Lymphoma. 2019;60(2):284–294.
[81] Panuzzo C, Signorino E, Calabrese C, et al. Landscape of tumor suppressor mutations in acute myeloid leukemia. J Clin Med. 2020;9(3):802.
[82] Agirre X, Martinez-Climent JA, Odero MD, et al. Epigenetic regulation of miRNA genes in acute leukemia. Leukemia. 2012;26(3):395–403.
[83] Zhou J, Ching YQ, Chng WJ. Aberrant nuclear factor-kappa B activity in acute myeloid leukemia: from molecular pathogenesis to therapeutic target. Oncotarget. 2015;6(8):5490–5500.
[84] Starczynowski DT, Kuchenbauer F, Argiropoulos B, et al. Identification of miR-145 and miR-146a as mediators of the 5q- syndrome phenotype. Nat Med. 2010; 16(1):49–58.
[85] Xiang M, Birkbak NJ, Vafaizadeh V, et al. STAT3 induction of miR-146b forms a feedback loop to inhibit the NF-κB to IL-6 signaling axis and STAT3-driven cancer phenotypes. Sci Signal. 2014;7(310):ra11.
[86] Walker SR, Xiang M, Frank DA. STAT3 activity and function in cancer: modulation by STAT5 and miR-146b. Cancers (Basel). 2014;6(2):958–968.
[87] He M, Wang QY, Yin QQ, et al. HIF-1α downregulates miR-17/20a directly targeting p21 and STAT3: a role in myeloid leukemic cell differentiation. Cell Death Differ. 2013;20(3):408–418.
[88] Chen L, Jiang X, Chen H, et al. microRNA-628 inhibits the proliferation of acute myeloid leukemia cells by directly targeting IGF-1R. Onco Targets Ther. 2019;12: 907–919.
[89] Huang Y, Zou Y, Lin L, et al. Identification of serum miR-34a as a potential biomarker in acute myeloid leukemia. Cancer Biomark. 2018;22(4): 799–805.
[90] Bhise NS, Chauhan L, Shin M, et al. MicroRNA-mRNA pairs associated with outcome in AML: from in vitro cell-based studies to AML patients. Front Pharmacol. 2015;6:324.
[91] Fayyad-Kazan H, Bitar N, Najar M, et al. Circulating miR-150 and miR-342 in plasma are novel potential biomarkers for acute myeloid leukemia. J Transl Med. 2013;11(1):31.
[92] Elhamamsy AR, El Sharkawy MS, Zanaty AF, et al. Circulating miR-92a, miR-143 and miR-342 in plasma are novel potential biomarkers for acute myeloid leukemia. Int J Mol Cell Med. 2017;6(2):77–86.
[93] Huang S, Zhang B, Fan W, et al. Identification of prognostic genes in the acute myeloid leukemia microenvironment. Aging (Albany, NY). 2019;11(22): 10557–10580.
[94] Prada-Arismendy J, Arroyave JC, Röthlisberger S. Molecular biomarkers in acute myeloid leukemia. Blood Rev. 2017;31(1):63–76.
[95] Mohammad HP, Barbash O, Creasy CL. Targeting epigenetic modifications in cancer therapy:

erasing the roadmap to cancer. Nat Med. 2019;25(3): 403–418.
[96] Nguyen D, Liao W, Zeng SX, et al. Reviving the guardian of the genome: small molecule activators of p53. Pharmacol Ther. 2017;178:92–108.
[97] Sellar RS, Jaiswal S, Ebert BL. Predicting progression to AML. Nat Med. 2018;24(7):904–906.
[98] Abelson S, Collord G, Ng SWK, et al. Prediction of acute myeloid leukaemia risk in healthy individuals. Nature. 2018;559(7714):400–404.
[99] Desai P, Mencia-Trinchant N, Savenkov O, et al. Somatic mutations precede acute myeloid leukemia years before diagnosis. Nat Med. 2018;24(7): 1015–1023.