

Biology of DNMT3 and LSD1 inhibition in acute myeloid leukaemia (AML)

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Abstract

Epigenetic dysregulation plays a critical role in the pathogenesis of acute myeloid leukaemia (AML). The enzymes DNA methyl-transferase (DNMT3a) and Histone Lysine Demethylase (LSD1 or KDM1) are key molecules involved in transcription, DNA repair and differentiation of myeloid cells. In fact, DNMT3a is one of the most frequently mutated genes in acute myeloid leukaemia (AML) and LSD1 is essential in retinoic acid induced differentiation of myeloid cells. However, despite extensive investigation, it is not yet known their precise role in the evolution of AML clones. This information may be relevant for the successful management of the disease. In fact, it is unclear whether, mutated DNMT3a in AML clones has a dominant negative function and disrupts the formation of transcriptional complexes leading to aberrant methylation. Also, it is not known how LSD1 inhibition impacts on growth, differentiation and chemo-resistance of leukemic myeloid cells. In this review, we focus on the emerging evidences that correlate LSD1 and DNMT3a activity in acute myeloid leukaemia, their clinical relevance and how a deeper understanding of their biological function could lead to the discovery of new specific targets, some of which are currently tested in mechanism-based clinical trials.

Introduction

Acute leukaemia are clonal disorders of hematopoiesis in which leukemic stem cells (LSCs) develop unlimited self-renewal capacity, enhanced proliferation and impaired hematopoietic differentiation programs [1,2]. Recent advances in leukaemia biology come from studies that investigated on genetic and epigenetic abnormalities in leukaemic cells [3-7]. Leukaemic stem cells (LSCs) are a functionally defined multipotent entity that can undergo self-renewal, the origin of which has been the subject of considerable research in recent years. During normal developmental progression from stem cell to progenitors and then mature cells, mutations may potentially occur at any stage giving rise to a malignant entity. Both DNMT3A and LSD1 are known to regulate hematopoietic stem cell (HSC) differentiation [8]. LSD1 mediated repression of the regulatory elements of hematopoietic stem and progenitor cell (HSPC) genes is required to fully silence these genes for proper hematopoietic maturation [9], and loss of DNMT3A in HSCs results in the retention of multipotency gene expression during differentiation [10]. Inhibition of DNA methylation at PpG enhancers in LSD1 inhibitor-treated cells is likely due to its effect on DNMT3A activity. It is possible that a similar epigenetic crosstalk mechanism may regulate enhancer repression and stable silencing of multipotency genes, thus maintaining the fidelity of differentiation during hematopoiesis [11]. DNA methyl transferases and Lysine-specific histone demethylase 1 are recognized cancer targets of particular importance in the pathogenesis of acute myeloid leukaemia [1,10,12-15]. Since the expression of both enzymes has been found altered mainly in hematological malignancies [10,13,14,16-18], a great number of comprehensive whole genome sequencing, exome sequencing, and targeted 56 sequencing studies have been performed in AML and myeloid neoplasms in the last decade. Nevertheless, this information remains essentially descriptive [4,5,19-21]. Many of the newly identified recurrently mutated genes are involved in the epigenetic regulation of transcription [Network CGAR 2013]. Epigenetic modifiers include

proteins involved in modifications of DNA cytosine residues (e.g., methylation) or post-translational modifications of histones such as methylation or acetylation. Mutations in these genes often lead directly to aberrant gene expression in AML [22]. Currently, these mutations represent a major focus of interest, and several novel epigenetic therapies are in preclinical testing phases or have entered clinical trials. In this review, we focus on recent discoveries elucidating the impact of aberrant DNA methylation and/or de-methylation on acute myeloid leukaemia with an emphasis on the role of cytosine-modifying enzymes and histone demethylase. We further discuss recent efforts to selectively inhibit these enzymes in acute myeloid leukaemia AML, which remains a deadly disease.

DNMT3

CpG methylation is mediated by a family of DNA methyltransferases (DNMTs) that regulate both maintenance (DNMT1) and de novo (DNMT3A/B) methylation in the genome [23]. During replication, DNMT1 adds methyl groups to the newly synthesized DNA strand, ensuring the preservation of DNA methylation patterns during proliferation [24,25]. De novo methyltransferases, DNMT3A and DNMT3B, establish new DNA methylation patterns [26]. Mutations in DNMT3A occur in 20-30% of *de novo* AML patients [27-30] and often co-occur with NPM1 mutations and FLT3-ITD and confer adverse risk [29,31]. Although mutations can occur in different functional domains, almost 60% of patients display a heterozygous substitution of arginine

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882 in the catalytic domain that abrogates methyltransferase activity and DNA binding in vitro [6,32]. The R882 mutation in AML patients correlates with global hypomethylation, especially at CpG islands, shores and promoters [33], although promoter hypermethylation has also been described [6,12,34]. Mutant Dnmt3A - predominantly mutant R882 - has been shown to interact with wild-type Dnmt3A and Dnmt3B in a dominant negative manner inhibiting the wild-type methyltransferase activity of the tetrameric complex [10,33]. Dnmt3A is required for the normal self-renewal capacity of HSCs in adult mice and for maintaining the differentiation potential of serially transplanted HSCs in wild-type recipients [35]. Conditional deletion of DNMT3A in murine HSC causes a higher self-renewal capacity and reduced differentiation resulting in an accumulation of HSC in the bone marrow [10,12]. In two studies, patients with DNMT3A mutations had higher survival rates when treated with high-dose daunorubicin compared to standard-dose daunorubicin [36,37] although this has not been studied in other, well-annotated clinical trial cohorts. The effect of DNMT3a mutations in AML is elusive because AML patients with the mutant DNMT3a did not express a methylation signature that discriminates them from patients with wild-type enzyme and in many cases display aberrant methylation [31,38]. Although there is a tight association between the AML patients with DNMT3a mutations and the prognosis of AML, the mechanism by which this occurs is not known yet [27,39]. Previous work suggested that R882 is important for the oligomerization of DNMT3A because it is located at the surface of protein-protein interaction of the enzyme, and the oligomer form of the enzyme is essential for its function [40,41]. More recently, Dnmt3a R878H conditional knockin mice revealed significant changes in gene expression and epigenetic regulatory patterns that cause differentiation arrest and growth advantage [42]. DNMT3a mutations exert a dominant negative effect because the enzyme is a tetramer and the mutated subunit may function as dominant negative variant that mislocalizes the enzyme across the genome [33].

DNMT3 Inhibitors: To date, a targeted agent is not available against DNMT3A mutations, which are thought to arise in the preleukemic hematopoietic stem cell population [43]. The DNMT inhibitors (DNMTis) azacitidine and decitabine, are generic hypomethylating agents (HMAs), nucleoside analogs that integrate into DNA and inhibit DNMTs [44]. They represent a reasonable treatment option for low blast count AML patients who are unfit for intensive induction chemotherapy with CRc rates around 20–30% [45]. Guadecitabine (SGI-110) is a second generation HMA formulated as a dinucleotide of decitabine and deoxyguanosine, which increases the half-life of decitabine by protecting it from deamination [46,47]. Two studies reviewed in Saygin, *et al.* [48] report ongoing multicenter studies on AML patients, who were ineligible for intensive chemotherapy, no marginal difference in OS benefit [49]. A randomized phase 3 trial has been initiated to compare guadecitabine vs treatment choice (i.e., azacitidine, decitabine, or LDAC) in patients with previously untreated AML (NCT02348489). Moreover, guadecitabine has also been investigated in R/R AML patients, and a recently reported long-term 3 follow-up of phase 2 studies demonstrated a slight ameliorate [49] based on this, a phase 3 randomized, open-label study of guadecitabine vs treatment choice in R/R AML has been initiated (NCT02920008). DNA methylation profiling identified biologically distinct subtypes of AML, and certain methylation profiles were associated with adverse outcome [50,51]. Furthermore, differentially methylated regions of DNA at baseline distinguished patients who responded to HMA from non-responders in different myeloid malignancies [52,53]. Specific methylation signatures may predict responsiveness to treatment with

guadecitabine and offer an opportunity to improve management of elderly or R/R AML patients. Guadecitabine has demonstrated clinical activity in first-line and R/R settings, and two ongoing phase 3 studies for these patient populations may provide evidence to justify its use over LDAC and first-generation HMAs.

LSD1

FAD-dependent amine oxidase, lysine-specific demethylase 1 (LSD1) is a unique protein with the ability to catalyse the demethylation of H3K4me2 and H3K9me2, and therefore act as a transcriptional repressor or activator, respectively [54,55]. LSD1 was first reported to demethylate H3K4me2 and repress transcription with the CoREST complex. However, LSD1 also catalyses the demethylation of H3K9me2 with the androgen or oestrogen receptor, and acts as a transcriptional activator [55,56]. In addition, LSD1 has since been identified in a number of activating complexes. When recruited by these complexes to target genes, LSD1 demethylates the repressive H3K9me2 mark leading to activation of gene expression [55]. Aberrant expression of LSD1 has been shown in many types of cancers and high LSD1 expression confer poor prognosis [15,18,57]. The role of LSD1 is a little better understood in acute leukaemia. Particularly, in a subset of Acute Myeloid Leukaemia, LSD1 is crucial for the function and maintenance of the leukemic stem cells, a subset of malignant cells that may induce relapse in those patients. LSD1 inhibition, when coupled with all-trans retinoic acid (ATRA) therapy, induce differentiation and suppress leukaemia engraftment in AML cell lines that otherwise were insensitive to ATRA [15]. These studies provided the initial preclinical rationale to develop LSD1 inhibitors, either as monotherapy or in combination with ATRA, as a potential approach for patients with AML. Recent studies have identified a functional link between LSD1 and leukaemia maintenance. Expression analysis suggests that LSD1 might regulate a subset of genes that activate the oncogenic program associated with MLL-AF9 leukaemia and chromatin immunoprecipitation and next generation sequencing, (ChIP-Seq), showed that H3K4me2 increase is the only detectable change at MLL-AF9 promoters following LSD1 silencing. These results clearly demonstrate that in AML, demethylation by LSD1 is associated with activation of LSC associated oncogenic target genes [18]. Pharmacologic inhibition of LSD1 induced differentiation of mouse AML cells and impaired the ability of these cells to cause leukaemia in recipient mice.

LSD1 Inhibitors: Data from mouse model of AML show that co-treatment of the LSD1 inhibitor 22509 and panobinostat showed synergistic lethality of primary AML blasts and prolonged survival in xenograft AML mouse models compared to either agent alone [58]. *In vitro* data on IMG-98, a novel LSD1 inhibitor that irreversibly binds to LSD1's essential cofactor FAD, show that leads to its inactivated enzyme form. Exposure of AML cell lines to IMG-98 has been shown to promote differentiation and growth inhibition of AML blasts, especially in combinations with ATRA. The first clinical trials with an optimized drug closely related to IMG-98 are expected to start in early 2016. In leukaemia cell lines, there appears to be synergism between HDAC and LSD1 inhibitors which supports a clinical trial for further exploration [15]. More recently, a study describes a novel, specific, reversible lysine-specific demethylase 1 inhibitor JL1037 that induce apoptosis in cell lines as well as primary cells from AML patients [60]. Preclinical data have prompted multiple phase I trials using either irreversible LSD1 inhibitors such as GSK2879552 as a single agent or tranlycypromine in combination with ATRA (NCT02177812 and NCT02261779 at <https://clinicaltrials.gov/>, respectively). LSD1 is also overexpressed in solid tumors [52,57]. Given that the mechanisms by

which LSD1 inhibition slows cancer cell proliferation remain unclear, a genetic or epigenetic signature that correlates with response would be extremely useful to help inform ongoing and future clinical trials. The selective tranylcypromine derivative LSD1 inhibitors ORY-1001 developed by Oryzon Genomics (EudraCT number 2013- 002447-29) and GSK2879552 developed by GlaxoSmithKline (NCT02177812) have entered early phase clinical trials in patients with relapsed and refractory acute leukaemia. A phase I clinical study of ATRA and tranylcypromine for adult patients with AML and MDS (NCT02273102), and a phase I/II trial of ATRA and tranylcypromine in patients with relapsed or refractory AML and no intensive treatment possibility (NCT02261779) are currently recruiting.

Conclusions

Despite significant progress in understanding the pathogenesis of AML, therapeutic options remain quite limited. DNA methyltransferases (DNMT) and Lysine-specific histone demethylase 1 (LSD1) are recognized cancer targets of particular importance in the pathogenesis of acute myeloid leukaemia. However, current pharmacological approaches are based on catalytic inhibition of these enzymes and surprisingly the molecular mechanism remains unknown. Unveiling the mechanisms of action by which these two enzymes regulate pathogenesis may be clinically relevant for the management of patients with leukaemia with poor prognosis. Further epigenetic modifiers are continuously investigated in current literature leading to sketchy data on mechanism [61]. Epigenetic treatments approved by the FDA include the DNMT inhibitors azacytidine and decitabine, non-specific hypomethylating agent. The sensitivity to this de-methylating drug is relevant, because the 5-AzaC effects on AML clones are rather heterogeneous [62] and it is not known whether DNMT3a genetic background may influence the final response to the therapy. Particularly, the prognostic importance of DNMT3A mutated gene is not clear and, to date, not defines a clinic-pathologic entity. Conversely, LSD1 has a number of substrates, including both histone and non-histone substrates [39,42] and has been suggested to have multiple mechanisms of action to regulate gene expression [54,55]. Moreover, under many circumstances, LSD1 inhibitors alone only have minimal to modest therapeutic efficacies, which are greatly enhanced in combination with other therapies [18].

Pre-clinical studies have suggested that combination therapies of two or more epigenetic drugs, or a combination of an epigenetic drug combined with a kinase inhibitor, may have additional synergistic effects. Critical ongoing efforts include further accurate pre-clinical models to elucidate how mutations in epigenetic modifiers interact with other AML disease alleles, and clinical studies to assess the efficacy of epigenetic therapies alone or in combination with other anti-leukemic agents. It is unlikely that any single approach employing the agents described above, including the most targeted, will be successful in eradicating AML cells due to the related problems of intrinsic or acquired forms of resistance [63]. Indeed, dual inhibition of DNMTs and LSD1 in acute myeloid leukaemia by synergistic re-activation of epigenetically silenced genes has been reported [64]. Moreover, the speculation that the LSD1 inhibition suppress DNMT3A activity is supported by fluorescence polarization and isothermal titration calorimetry experiments [52,65] Work by Petell and coworkers shows that LSD1-facilitated interaction of Dnmt3a with histone tails, which is reduced by LSD1 inhibitor treatment [11]. An innovative approach could interfere effectively without blocking the target enzymatic activity by using peptide against specific region of the two enzymes to provide novel, specific, low-toxicity treatment agents to supplement

current treatment protocols [66-84].

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