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[Review]

Cellular carcinogenesis in preleukemic conditions : drivers and defenses

Koki Ueda and Kazuhiko Ikeda

Department of Blood Transfusion and Transplantation Immunology, Fukushima Medical University, Fukushima, Japan

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Abstract

Acute myeloid leukemia (AML) arises from preleukemic conditions. We have investigated the pathogenesis of typical preleukemia, myeloproliferative neoplasms, and clonal hematopoiesis. Hematopoietic stem cells in both preleukemic conditions harbor recurrent driver mutations ; additional mutation provokes further malignant transformation, leading to AML onset. Although genetic alterations are defined as the main cause of malignant transformation, non-genetic factors are also involved in disease progression. In this review, we focus on a non-histone chromatin protein, high mobility group AT-hook2 (HMGA2), and a physiological p53 inhibitor, murine double minute X (MDMX). HMGA2 is mainly overexpressed by dysregulation of microRNAs or mutations in polycomb components, and provokes expansion of preleukemic clones through stem cell signature disruption. MDMX is overexpressed by altered splicing balance in myeloid malignancies. MDMX induces leukemic transformation from preleukemia via suppression of p53 and p53-independent activation of WNT/β-catenin signaling. We also discuss how these non-genetic factors can be targeted for leukemia prevention therapy.

Keywords : acute myeloid leukemia, HMGA2, leukemia stem cell, MDMX, myeloproliferative neoplasms, preleukemia

Introduction

Acute myeloid leukemia (AML) includes a diverse spectrum of neoplasms with a variety of genetic abnormalities and variable responses to treatment. AML arises from hematopoietic cells harboring chromosomal translocations and/or somatic/germline mutations in recurrently affected genes[1-4]. Genetic screening of healthy individuals has shown that about 10% of non-diseased adults over 65 years old have leukemia-related mutations in hematopoietic cells, manifesting as clonal hematopoiesis (CH)[5-7]. In addition, myelodysplastic syndromes (MDS) and myeloproliferative neoplasms (MPN) are well-characterized "preleukemic" myeloid diseases with variable degrees of severity. Somatic mutations are uniquely distributed in these diseases but show significant overlap with those in AML[8-10]. The onset of AML from preleukemia is more frequent than in age-matched individuals without hematopoietic cell mutations. The transformation rate can be predicted from risk factors, such as the type of mutated genes, number of mutations, variant allele frequency (VAF) of mutated genes, and loss of heterogeneity (LOH) in affected genes[11-13].

In comparison with genetic alterations, the effect of non-genetic factors such as the transcriptome during preleukemic to leukemic transition has not been well elucidated. Before the next-generation sequencing (NGS) era, gene expression profiling by microarray was an attractive tool to classify normal-karyotype AML, in which mutation status was largely unknown[14-16]. Studies with NGS have revealed many recurrent mutations in AML, including normal-karyotype AML. The classification of AML is

Corresponding author : Koki Ueda E-mail : kouki@fmu.ac.jp

being redefined by mutations in combination with karyotypes rather than gene expression profiles obtained by microarray. Although gene expression profiles in bulk AML samples are now less important than previously, single-cell RNA sequencing (scRNAseq) techniques reveal that gene expression status – especially in the hematopoietic stem/progenitor cell (HSPC) fraction – is still important to clarify mechanisms of how normal hematopoiesis can be disrupted and give rise to leukemic transformation[17,18]. Abnormal expression of various genes could be the result of mutations in genes such as epigenetic modifiers, splicing factors, kinases, and transcription factors (TFs)[19]; however, there remain many unsolved mechanisms of abnormal transcriptome processing in leukemogenesis.

Expression changes in TFs are the best-studied elements in hematopoiesis and leukemogenesis. Especially, frequent dysregulation of CEBPA[20,21], GATA1/2[22,23], HOX families[24], PU.1[25], and RUNX1[26] by both mutational and non-mutational mechanisms have been reported in myeloid malignancies. The transcriptional regulation of TFs is complicated, due to their reciprocal actions, temporospatial regulations, and stochasticity in expression levels[27-30]. Single-molecule fluorescence in situ hybridization (SmFISH)[31,32] together with scRNAseq has been utilized to analyze these intricate mechanisms, and ongoing projects are expected to shed more light in this field[33].

However, regarding gene expression changes in myeloid malignancies as candidate therapeutic targets, TFs may be difficult to target because most of them are essential for physiological cellular activities. Therefore, focusing on non-TFs that are overexpressed in myeloid malignancies could be a better choice to generate new target therapy. Especially, non-TFs which broadly regulate other genes without transcription activity and are highly expressed in myeloid malignancies – while indispensable to normal hematopoiesis – could be good candidate targets. The role of overexpression of non-TFs in leukemogenesis might be simpler than that of TFs, but has been less studied thus far. In this review, we focus on the role of two non-TF proteins, HMGA2 and MDMX, in the development and progression of myeloid malignancies. Both HMGA2 and MDMX are overexpressed at the hematopoietic stem cell level during the transition from moderate or asymptomatic hematopoietic conditions (e.g., CH and low-risk MDS, MPN, and MDS/MPN overlap neoplasms) to fatal myeloid malignancies (e.g., high-risk MDS and MPN, AML). We discuss how these proteins are overexpressed in HSPCs and provoke disease progression, and how can we target them as tumor stem cell-directed therapy.

1. The role of HMGA2 in myeloid malignancies

The canonical function of HMGA2

The high mobility group (HMG) proteins are non-histone chromatin-associated nuclear proteins that regulate gene expression and chromatin structure[34,35]. Among three HMG superfamily members (HMGA, HMGB, and HMGN), the HMGA family consists of two members, HMGA1 and HMGA2. The HMGA family mainly functions to bind AT-rich regions in the minor groove of DNA[36], change chromatin structures, and help DNA binding of TFs in cooperation with protein-protein interactions induced by the acid domain of HMGA2[37-40]. HMGA2 is widely expressed in normal tissues during development, with expression levels decreasing in late development to adulthood[41,42]. Except for the development period, the role of HMGA2 in normal tissues is limited to the maintenance of stem cells and mesenchymal cells[43-47]. However, HMGA2 is re-expressed in various cancers and is associated with the progression of the disease[48,49].

HMGA2 in myeloid malignancies

Numerous mechanisms of overexpression, roles in tumor progression, and target genes of HMGA2 have been reported in various cancers, and appear to be context-dependent[48,49]. In this review, we focus on the overexpression of HMGA2 in myeloid malignancies. A schematic cause-and-effect diagram of HMGA2 overexpression in myeloid malignancies is presented in Figure 1.

Overexpression of *HMGA2* transcripts is reported in the whole blood and hematopoietic stem cell fractions of patients with MPNs, and the frequency of *HMGA2* overexpression in patients with primary myelofibrosis (PMF), which is the most severe subtype of MPN, reaches nearly 100%[50-55]. In addition, overexpression of *HMGA2* has been reported in some patients with myeloid malignancies such as chronic myeloid leukemia (CML)[56-58], MDS[59], AML[56,60], as well as paroxysmal nocturnal hemoglobinuria (PNH)[61,62], which is a benign but acquired clonal hematologic disease.

HMGA2 has a long 3'UTR, and it is targeted by various microRNAs (miRNAs)[49,63]. Disruption of

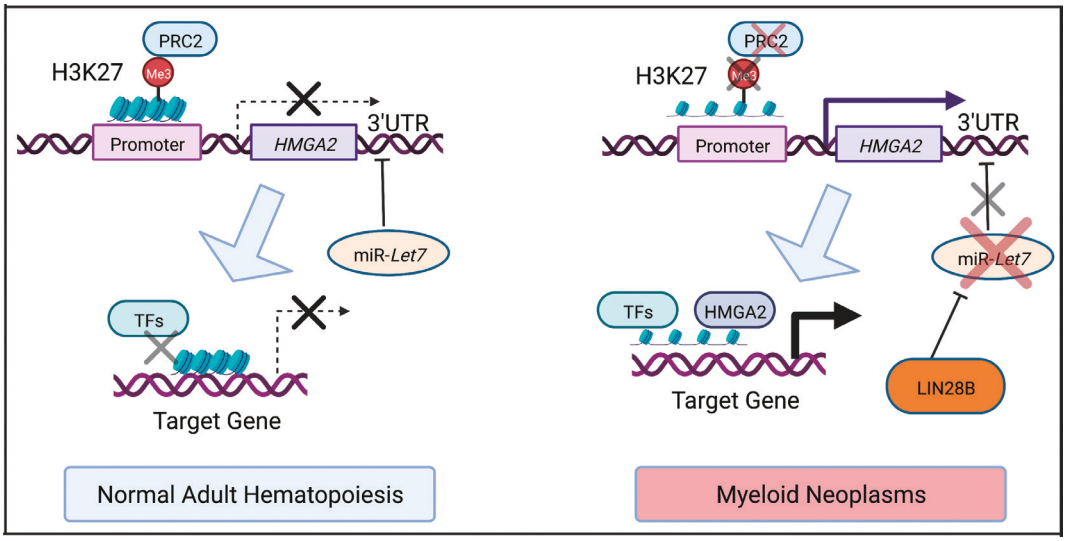

Fig. 1. The implication of HMGA2 on myeloid malignancies.
Schematic cause-and-effect diagram of HMGA2 overexpression in myeloid malignancies. In normal hematopoietic cells, expression of *HMGA2* is repressed mainly by PRC2 via H3K27 tri-methylation of its promoter and/or miR-Let7-mediated silencing. Those suppressors are altered in myeloid malignancies leading to overexpression of HMGA2 followed by upregulation of its target genes. (Me3 : tri-methylation, PRC2 : polycomb repressive complex 2, TFs : transcription factors, UTR : untranslated lesion)

miRNAs is implicated in the development and progression of hematopoietic malignancies as well as other cancers[64,65]. Among these miRNAs, family members of *miR-Let7* are the most powerful degraders of HMGA2, as there are eight predicted *miR-Let7* target sequences on the 3'UTR of *HMGA2*[66,67]. Reduced expression of *miR-Let7* and other microRNAs targeting *HMGA2*, or deletion of the 3'UTR of *HMGA2*, which includes target sequences of microRNAs, are implicated in overexpression of HMGA2 in hematological diseases[53,54,62,68-75].

Also, LIN28B, the negative regulator of *miR-Let7*, is frequently overexpressed in progressive cancers[76], and activation of the LIN28B-*Let7*-HMGA2 axis contributes to the progression of various cancers[77]. The LIN28B-*Let7*-HMGA2 axis is also an essential regulator in the development of hematopoiesis but is inactivated in adulthood[78,79]. Reactivation of this pathway in HSPC could be the cause of malignant transformation, but this requires further evidence about any relationships between LIN28B and myeloid malignancies.

Moreover, abnormal splicing contributes to overexpression of *HMGA2* in patients without genetic amplification or translocation in *HMGA2* coding lesion[53,59,61], indicating that mutations or dysfunction of splicing factors may be associated with dysregulation of HMGA2 expression.

In addition to the mechanisms mentioned above, mutations in epigenetic modulators are associated with overexpression of HMGA2 in patients with MPNs[55]. MPNs are derived from HSPCs with constitutive activation of JAK-STAT signaling, which is provoked by driver mutations such as *JAK2*-V617F[80,81], *MPL*-W515L/K[82], and *CALR*-exon9-indels[83,84]. *EZH2* mutation is one of the most frequent co-occurring mutations in *JAK2*-mutated MPNs and is associated with poor prognosis[85,86]. EZH2 is the catalytic component of the polycomb repressive complex 2 (PRC2), and loss of *EZH2* is associated with overexpression of HMGA2[87,88].

Murine models with HMGA2 overexpression

To clarify how HMGA2 contributes to disease progression, we and other groups generated several murine models that express external *Hmga2* transgenes[89,90] or re-express endogenous HMGA2 by deletion of *Ezh2*[87,88,91]. A model of transgenic mice with a truncated murine *Hmga2* (*Hmga2*-Tg, also described as Δ*Hmga2*) shows about 3- to 5-fold increased expression of HMGA2 compared to wild-type (WT) controls in hematopoietic tissues. This model shows moderate elevation of white blood cell (WBC), red blood cell (RBC), and platelet (PLT) counts, increased number and repopulating capacity of HSPCs as well as increased megakaryopoiesis ; however, it does not develop lethal myeloid disease[89]. *Hmga2* conditional knock-in mice reported from elsewhere were phenotypically similar to those with *Hmga2*-Tg[90]. Of note, another model

of *HMGA2*-Tg mice that overexpresses human *HMGA2* has been reported to develop very aggressive acute lymphoid leukemia[92]. This might reflect the "context-dependent" oncogenic activity of HMGA2. Differences in species, promotors, and expression levels may lead to differences in which hematopoietic lineage would be the most affected.

Endogenous overexpression of HMGA2 by deletion of the PRC2 component *Ezh2* has been reported in MPN models, which harbor the *Jak2*-V617F mutation. These mice developed lethal myelofibrosis, and RNAseq/ChIPseq of HSPC showed elevated expression of *Hmga2* provoked by decreased H3K27 trimethylation in a promotor lesion[87,88]. To confirm that HMGA2 is responsible for disease progression in *JAK2*-V617F hematopoiesis, we crossed *JAK2*V617F-Tg mice[93] with *Hmga2*-Tg[89] (*JAK2*VF/*Hmga2*). *JAK2*VF/*Hmga2* mice reproduced the phenotypes of *JAK2*VF/*Ezh2*$^{-/-}$ mice and died with severe leukocytosis. Proliferated leukocytes were mature, and did not represent AML. Myelofibrosis was observed, but mice with *JAK2*VF alone also presented with severe myelofibrosis, so we could not determine whether overexpression of HMGA2 promotes fibrosis. HSPC of *JAK2*VF/*Hmga2* mice showed hematopoietic repopulating capacity after 3 iterations of competitive serial bone marrow transplantation, while *JAK2*VF alone was outcompeted by a WT competitor. Also, abnormal blood cell counts in *JAK2*VF/*Ezh2*$^{-/-}$ mice were partially recovered by a heterogenous knockout of *Hmga2*. We identified several oncogenes, *Lmo1*, *Gcat*, and *Prss16* as the upregulated genes in HSPC of both *JAK2*VF/*Hmga2* and *JAK2*VF/*Ezh2*$^{-/-}$. However, upregulation of myelofibrosis-related pathways such as TGFβ signaling was observed only in *JAK2*VF/*Ezh2*$^{-/-}$. Therefore, we concluded that HMGA2 contributes to the expansion of a *JAK2*-V617F mutated clone, and fibrosis is provoked by other targets of EZH2[55]. However, this last point is still controversial as other groups showed that overexpression of HMGA2 upregulates TGFβ signaling, which plays a critical role in bone marrow fibrosis[94,95], via overexpression of *Tgfbr2*[96]. They employed another *Jak2*-V617F mouse, and overexpressed *Hmga2* by lentivirus. Collectively, although its role in the progression of bone marrow fibrosis is controversial, we can conclude that HMGA2 enhances the fitness of *JAK2*-mutated clones, leading to disease progression (Figure 1).

Recently, HMGA1 – the sister protein of HMGA2 – has been reported to contribute to the progression of MPN. The investigators' murine model revealed that heterogeneous loss of *Hmga1* markedly improved myelofibrosis of *JAK2*-V617F-Tg mice[97]. They reported that targets of HMGA1 are proliferation pathways and GATA2, which is distinct from targets of HMGA2. This may suggest close collaboration between these sister genes in the pathogenesis of MPN and warrants further study.

Functional analysis of the CALR mutant, which is the second most frequent driver mutation in MPNs, has been insufficient, especially regarding its collaboration with other mutations. Recently, we generated *Calr*-del10 mice that lack 10 base pairs in exon 9 of *Calr*, mimicking type2-like *CALR* mutation in MPN patients ; these mice presented mild phenotypes of MPN[98]. Our preliminary data have shown that addition of *Hmga2*-Tg to *Calr*-del10 evokes progression of MPN phenotypes but not myelofibrosis or leukemic transformation, while deletion of *Ezh2* can provoke myelofibrosis or leukemic transformation after a long latency. From these observations, we speculate that HMGA2 just contributes to clonal expansion of MPN, and other targets of EZH2 contribute to myelofibrosis and clonal expansion, although this warrants further study.

Implications of HMGA2 in AML

Although HMGA2 is overexpressed in some of the patients with myeloid malignancies other than MPN[56-60], the role of HMGA2 in these diseases remains uncertain. Our model showed that HMGA2 expands *JAK2*-mutated clones and provokes lethal MPN, however, *JAK2*VF/*Hmga2* mice never develop overt AML[55]. A recent report showed that forced expression of *Hmga2* in *Tet2*-deficient HSPC activates IGF2BP2 and its targets, and transplantation recipients of these cells develop lethal MDS but not overt AML[99]. These findings suggest that overexpression of HMGA2 is insufficient for a complete transformation to AML. HMAG2 may be associated with a leukemia stem cell (LSC) signature rather than a leukemic transformation. AML cells with high HMGA2 expression have been reported to present more immature surface markers and higher LSC scores compared to HMGA2-low AML cells[100]. Immature phenotype should be associated with worse prognosis, therefore inhibition of HMGA2 may improve the treatment of AML.

HMGA2 inhibitors

So far, no clinical-grade HMGA2 inhibitor is available. Netropsin is a pan-AT hook-binding drug that has been used experimentally[101], but cannot be

administered to humans. Ciclopirox and tetrac are reported as direct inhibitors of HMGA2[102,103]. Small molecule inhibitors of LIN28 have also been tested, and are expected to induce an abundance of *Mir-Let7*, leading to degradation of HMGA2[104]. Further study is needed to develop selective HMGA2 inhibitors for human therapy.

Summary

In summary, HMGA2 is inadequately re-expressed by genetic amplification, loss of regulatory lesion of 3'UTR, alteration of the LIN28B-Let7-HMGA2 axis, loss-of-function mutation in EZH2, or disruption of splicing (Figure 1). Overexpression of HMGA2 contributes to the expansion of disease clones harboring driver mutations of myeloid malignancies by activating genes involved in stem cell signatures.

2. The role of MDMX in myeloid malignancies

Overview of the functions of MDMX

Murine double minute X (MDMX, also known as MDM4 and HDM4) and its homolog murine double minute 2 (MDM2, also known as HDM2) physiologically inhibit p53, which is overexpressed in various cancers, including myeloid malignancies[105]. However, overexpression of MDMX has an oncogenic role in p53-null and mutated backgrounds, suggesting functionality independent of p53[106,107]. Several p53-independent functions such as proteasomal degradation of p21[108], induction of genomic instability[109], and stabilization of TOP2A[110] have been reported. Also, we reported a novel p53-independent function of MDMX that activates WNT/β-catenin signaling via a reduced abundance of β-catenin degrader CK1α[111]. For the details of p53 dependent and independent functions of MDMX and its role in hematopoiesis, please refer to our newest review[112]. In this review, we focus on the role of MDMX in myeloid malignancies.

The mechanism of MDMX overexpression in myeloid malignancies

MDMX is overexpressed in bulk AML samples and LSCs, in contrast to other cancer samples and normal HSPCs[113-115]. Strong associations between expression levels of MDMX and mutation status have not been reported, except for relatively higher expression in AML with a complex karyotype[116].

The mechanism behind MDMX overexpression is largely unknown. Copy number alteration of *MDMX* has been reported in some cancers, but gene amplification is not the main cause of MDMX overexpression in myeloid malignancies[117-119]. Instead, the splicing balance between the oncogenic transcription variant, full-length *MDMX* (*MDMX*-FL), and the non-oncogenic transcription variant, short *MDMX* (*MDMX*-S), is frequently altered[120]. The skipping of exon 6 (exon 7 in murine *MDMX*) produces the *MDMX*-S transcript, which is unstable compared to *MDMX*-FL because the termination codon of *MDMX*-S is targeted by antisense-mediated decay[121,122]. Thus, expression of *MDMX*-S results in reduced protein expression of MDMX[123]. Inversely, the inclusion of exon 6 results in the expression of stable *MDMX*-FL transcripts, resulting in abundant MDMX protein. Exon inclusion/skipping is controlled by splicing factor SRSF families, arginine methyltransferase PRMT5, and another RNA-binding protein, Zmat3. Therefore, dysregulation of these factors may be associated with MDMX overexpression[120,124,125], although this warrants further investigation. Moreover, there is a feedback loop between p53 and *MDMX* expression. The constitutive promoter of *MDMX* located upstream of exon 1 (P1) is targeted by various TFs other than p53, while the second promoter located in intron 1 (P2) is p53-responsive. P2 promoter is active in specific conditions such as stress response, and the transcripts from P2 cooperate with MDM2 to degrade p53 more efficiently than transcripts from P1[126].

Murine models show a crucial role for MDMX in leukemogenesis

Because MDMX is overexpressed in the vast majority of myeloid malignancies, we have investigated whether MDMX overexpression directly induces the transformation of preleukemia to AML[111]. Although MDMX-overexpressing mice (*Mdmx*-Tg)[106,127] develop no myeloid disorders, HSCs of *Mdmx*-Tg mice present increased self-renewal and competitiveness over WT HSCs. RNA-sequencing and functional assays of HSCs reveal that activation of WNT/β-catenin signaling – rather than downregulation of p53 targets – was the main cause of proliferative HSCs in *Mdmx*-Tg mice. These results were reproduced in comparisons of *Trp53*$^{-/-}$ versus *Trp53*$^{-/-}$ with *Mdmx*-Tg, suggesting that upregulation of WNT/β-catenin signaling in MDMX-overexpressed mice is p53 independent.

Moreover, we crossbred *Mdmx*-Tg mice with preleukemic murine models, such as PU.1 knockdown mice (URE$^{-/-}$)[128], *Tet2*$^{-/-}$ mice, *Tet2*$^{-/+}$ mice[129],

and *Flt3*$^{ITD/WT}$ mice[130]. These mice develop preleukemic diseases of variable severity, but do not develop overt AML. The addition of *Mdmx*-Tg provoked overt AML in all these models. This provides evidence that overexpression of MDMX induces leukemic transformation. Unlike the comparison of WT and *Mdmx*-Tg, both downregulation of p53 targets and upregulation of WNT/β-catenin targets were detected in HSCs of URE$^{-/-}$ with *Mdmx*-Tg mice compared to URE$^{-/-}$ alone. Therefore, MDMX is presumed to induce leukemic transformation via both inhibition of p53 and upregulation of WNT/β-catenin. Because genetic alteration induces p53 activation[131-137], we speculate that preleukemic HSCs with MDMX overexpression are more dependent on p53 inhibition to survive, compared to HSCs with MDMX overexpression alone.

Molecular mechanism of p53-independent activation of WNT/β-catenin signaling by MDMX overexpression

To clarify the mechanism behind WNT/β-catenin activation, we screened proteins that interact with MDMX utilizing liquid chromatography-tandem mass spectroscopy (LC-MS/MS). Except for housekeeping proteins, CK1α (encoded by CSNK1a1) was the top binding partner[111]. The binding of CK1α and MDMX has been reported in non-hematopoietic contexts[138]. CK1α binds with MDMX's acidic domain, and releases intramolecular inhibition of MDMX's p53-binding domain, resulting in the binding of p53 and MDMX[139,140]. CK1α also binds with β-catenin, phosphorylates S45, and recruits the GSK3β/Axin/APC complex. GSK3β further phosphorylates β-catenin, triggering protease-mediated degradation of β-catenin[141].

According to these findings mentioned above, we hypothesized that overexpressed MDMX occupies CK1α, causing a reduced abundance of CK1α, resulting in the accumulation of β-catenin. We confirmed that MDMX overexpression induces increased β-catenin protein, and both inhibiter for β-catenin and exogenous overexpression of CK1α ameliorated proliferative phenotype of MDMX-overexpressing HSCs, while WT HSCs were not affected by these procedures[111]. Constitutive activation of WNT/β-catenin signaling leads to proliferation of HSCs[142] and is associated with various cancers including AML[143,144], while canonical WNT/β-catenin signaling is indispensable for normal adult hematopoiesis[145]. Thus, WNT/β-catenin signaling could be a potential therapeutic target to prevent leukemic transformation from preleukemia. The MDMX/CK1α/β-catenin axis was also confirmed by another group using pull-down and enzyme kinetic assays[146].

MDMX overexpression in preleukemic patients

In addition to overexpression of *MDMX* in AML[113-115], we have shown that patients with MDS whose HSCs express MDMX present with upregulation of WNT/β-catenin and a higher risk of leukemic transformation[111]. Overexpression of MDMX is also associated with leukemic transformation from MPN[118,147].

MDMX inhibitors for myeloid malignancies

Various MDM2/MDMX dual inhibitors (small molecules and peptides) are available in clinical settings[148,149]. Among these, the first-in-class structurally stabilized (stapled) peptide, ALRN-6924, has been tested in myeloid malignancies both in preclinical and clinical trials[114,150-152]. However, a phase 1 clinical trial of ALRN-6924 for AML (NCT02909972) revealed insufficient anti-AML effect, therefore no phase 2 trial has been conducted.

From the findings mentioned above, we speculate that overexpression of MDMX is required for transition from preleukemia to AML, rather than maintenance of AML. Therefore, an MDMX inhibitor could be used for preleukemic conditions. We also suppose that targeting the p53-independent oncogenic mechanism of MDMX mentioned above might enhance the effect of ALRN-6924. We tried β-catenin inhibitor in addition to ALRN-6924 and observed delayed disease onset in murine AML models[111].

Summary

The disruption of splicing is the main cause of the overexpression of MDMX, although its details have not been fully elucidated. MDMX induces leukemic transformation from preleukemic conditions via suppression of p53 and p53-independent activation of WNT/β-catenin signaling. Activation of WNT/β-catenin signaling is provoked by MDMX's occupation of CK1α which is the degrader of β-catenin (Figure 2). MDMX inhibition in a preleukemic stage shows promise as a strategy for leukemia prevention.

Discussion

As AML arises from preleukemic myeloid disorders, leukemia prevention therapy could be a reasonable choice for high-risk patients with CH, MDS, and MPN. However, intervention to prevent the

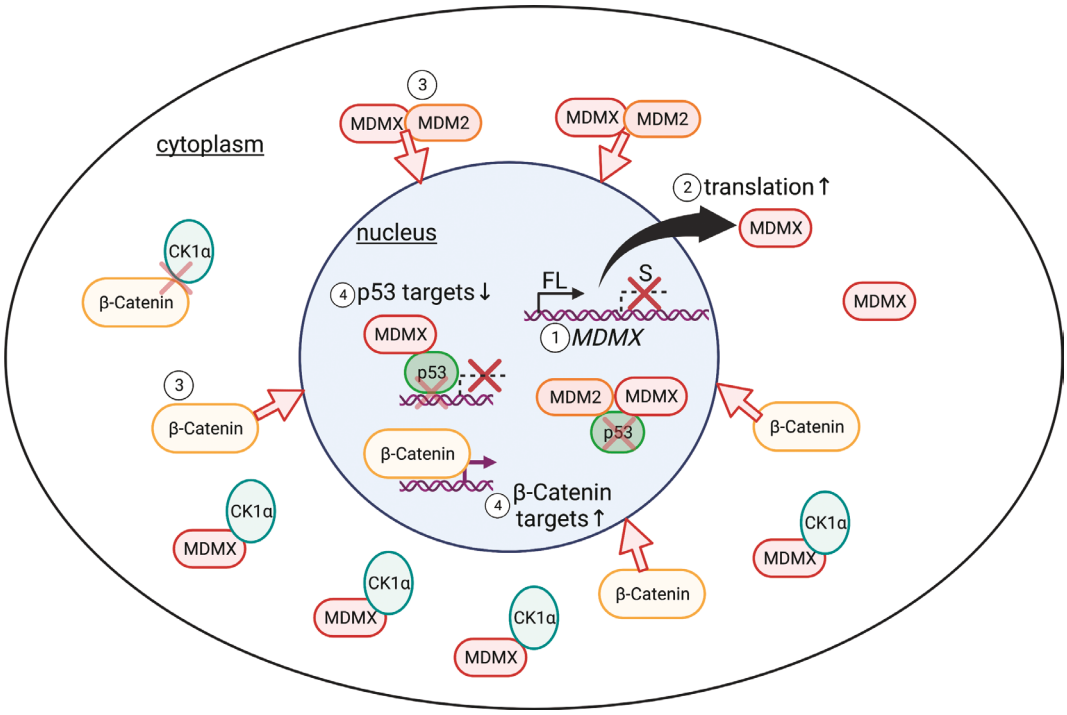

Fig. 2. The implication of MDMX on myeloid malignancies.
Schematic showing the mechanisms of MDMX overexpression and the resultant inactivation of p53 and activation of WNT/β-catenin signaling. Increased transcription of MDMX-FL compared to MDMX-S results in MDMX overexpression. MDMX shuttles into the nucleus when it binds with MDM2 and prevents p53 transactivation. In addition, MDMX in the cytoplasm binds with CK1α and induces its reduced abundance. It leads to the accumulation of β-catenin and increased nuclear transport.

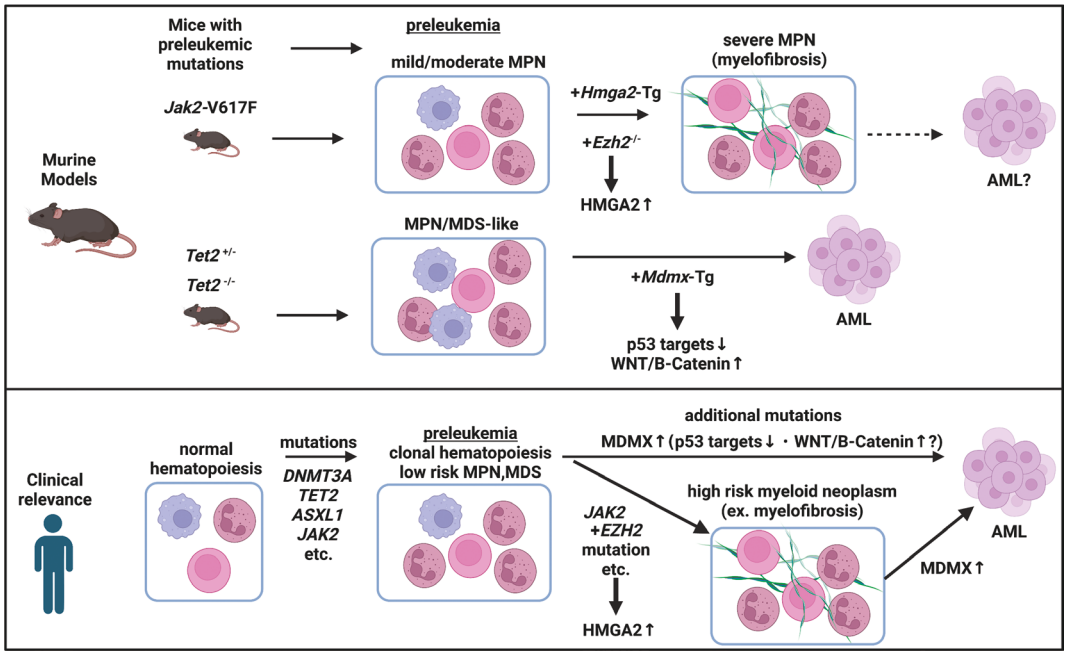

Fig. 3. The summary of the role of overexpressed HMGA2 and MDMX.
Schematic explanation of how overexpression of HMGA2 and MDMX is involved in the progression from preleukemic disease to fatal myeloid malignancies. AML : acute myeloid leukemia, MDS : myelodysplastic syndrome, MPN : myeloproliferative neoplasms.

transformation from preleukemia to AML has thus far not been successful. Although AML onset requires relatively small numbers of mutations compared to other cancers[153], AML is still a diverse disease manifesting with various genetic alterations[1–4]. Targeting every single mutation is thought to be impractical, especially in a preleukemic stage, because we cannot specify future leukemic clones during this period. Instead, targeting pathways commonly upregulated in transforming clones seems reasonable. To achieve this, we have to sequentially investigate human/murine preleukemic and leukemic hematopoietic cells, perhaps at a single-cell level.

With this in mind, HMGA2 and MDMX might be good candidates, although they require further study. Overexpression of HMGA2 induces expansion of malignant clones via alteration of stem cell signatures[55,100] (Figure 3). Also, because *Hmga2*-null mice show no hematological phenotypes[154], it may be indispensable for adult hematopoiesis. Therefore, we desire clinical-grade HMGA2 inhibitors for preleukemic patients. MDMX should be a more promising target because it is overexpressed in the majority of AML samples regardless of mutation status, and its overexpression directly induces leukemic transformation[111] (Figure 3). Deletion of MDMX induces hematopoietic defects via overactivation of p53, but spontaneous deletion of MDMX is not fatal for adult mice[155]. As well as its good tolerability in a phase 1 trial of MDMX inhibitor ALRN-6924 (NCT02909972), we speculate that inhibition of overexpressed MDMX should be a safe and attractive option for leukemia prevention, for which additional clinical trials are warranted.

Furthermore, we should investigate more mechanistic details in preleukemic to leukemic transitions. Although HMGA2 and MDMX are supposed to be common targets to prevent the transformation of preleukemia, a comprehensive analysis of preleukemic to leukemic human/murine models is needed.

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Declaration of interests

The authors have no conflicts of interest pertaining to this work.

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