Cooperation of Dnmt3a R878H with Nras G12D promotes leukemogenesis in knock-in mice: a pilot study

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Abstract

Background: DNMT3A R882H, a frequent mutation in acute myeloid leukemia (AML), plays a critical role in malignant hematopoiesis. Recent findings suggest that DNMT3A mutant acts as a founder mutation and requires additional genetic events to induce full-blown AML. Here, we investigated the cooperation of mutant DNMT3A and NRAS in leukemogenesis by generating a double knock-in (DKI) mouse model harboring both Dnmt3a R878H and Nras G12D mutations.

Methods: DKI mice with both Dnmt3a R878H and Nras G12D mutations were generated by crossing Dnmt3a R878H knock-in (KI) mice and Nras G12D KI mice. Routine blood test, flow cytometry analysis and morphological analysis were performed to determine disease phenotype. RNA-sequencing (RNA-seq), RT-PCR and Western blot were carried out to reveal the molecular mechanism.

Results: The DKI mice developed a more aggressive AML with a significantly shortened lifespan and higher percentage of blast cells compared with KI mice expressing Dnmt3a or Nras mutation alone. RNA-seq analysis showed that Dnmt3a and Nras mutations collaboratively caused abnormal expression of a series of genes related to differentiation arrest and growth advantage. Myc transcription factor and its target genes related to proliferation and apoptosis were up-regulated, thus contributing to promote the process of leukemogenesis.

Conclusion: This study showed that cooperation of DNMT3A mutation and NRAS mutation could promote the onset of AML by synergistically disturbing the transcriptional profiling with Myc pathway involvement in DKI mice.

Keywords: Acute myeloid leukemia, DNMT3A mutation, Nras G12D, Myc activation

Background

DNA methyltransferase 3A (DNMT3A), a member of DNA methyltransferases family, is responsible for de novo DNA methylation, which is essential for genome regulation and development [1]. DNMT3A mutations have been identified in various hematologic malignancies, with frequencies of 20–25% in AML [2–6]. The hotspot mutation of DNMT3A in AML occurs at the residue Arginine 882 (R882) [2, 7]. Dnmt3a knock-out mice showed increased self-renewal and impaired differentiation of Hematopoietic stem cells (HSCs) [8–10]. Mouse models established through retroviral transduction system showed that DNMT3A R882H alone did not develop frank AML, but were susceptive to AML development upon acquisition of additional genetic mutations [11, 12]. Dnmt3a R878H which is homologous with human DNMT3A R882H, only induced moderate AML with an average of 20% immature cells in the bone marrow (BM) and a relatively long latency in the conditional knock-in mice model [13]. DNMT3A mutations were proved to play a key role in clonal hematopoiesis at premalignant stages [14, 15], whereas activated signaling genes including RAS and FLT3 mutations occur in the subsequent process of malignant development [16]. Large scale sequencing of specimens from AML patients has discovered that DNMT3A mutations...
often coexist with other gene abnormalities, such as FLT3, IDH1/2, NPM1 and RAS [2, 7, 17]. These findings suggest that abnormal DNMT3A acts as a founder mutation and requires additional genetic events to induce an aggressive full-blown AML.

RAS is mutated in ~25% of human cancers including AML and other myeloid malignancies [18, 19]. Mutations in NRAS have been identified in AML and coexist with DNMT3A mutations in a portion of AML patients [20, 21]. Mouse models showed that NRAS mutation alone was not sufficient to cause AML [22, 23]. Loss of Dnmt3a and endogenous Kras G12D cooperated to promote myeloid leukemogenesis in mice [24]. Besides, a previous report showed co-expression of DNMT3A R878H and NRAS G12D could induce mouse AML by using a retroviral transduction system, in which the expression of mutant DNMT3A and mutant NRAS were driven by a retroviral promoter instead of the endogenous promoter/enhancer [12, 25]. However, the cooperation of DNMT3A mutation with NRAS mutation under the control of endogenous promoters in inducing AML in mice which mimics human leukemic features and the underlying mechanism remains elusive. In this work, we report that Dnmt3a R878H cooperates with Nras G12D to develop frank AML by establishing a DKI mouse model.

Methods

Generation of DKI mice

All mouse experiments were performed according to the guide of laboratory animal care and use standards, and were approved by the animal use committee of Shanghai Jiao Tong University School of Medicine. Animals were maintained with sterilized water and food in the specific pathogen free circumstance in Research Center for Experimental Medicine at Rui Jin Hospital Affiliated to Shanghai Jiao Tong University School of Medicine. Mx1-Cre; Dnmt3a R878H KI C57 mouse model was established as described in our previous work [13]. Mx1-Cre; Nras G12D KI C57 mice were generously provided by Ren Lab from Shanghai Institute of Hematology. The Mx1-Cre; Dnmt3a R878H KI mice were crossed with the Mx1-Cre; Nras G12D KI mice to obtain DKI mice harboring both Dnmt3a R878H and Nras G12D mutations. Cre expression was induced through intraperitoneal injection of 250 μg Polyinosinic-polycytidylic acid (pIpC) every other day for two times at 4 weeks old. The mice were monitored for leukemia development and sacrificed for phenotypic analysis 4 months after pIpC injection. The mice were sacrificed after the study. The method of euthanasia used to sacrifice the mice was cervical dislocation.

Flow cytometric analysis

Peripheral blood (PB) was obtained from the tail vein of mice, red blood cells (RBCs) were lysed by RBC Lysis Buffer prior to staining. BM cells were flushed out from the tibias and femurs, and suspended in PBS buffer with 2% fetal bovine serum (FBS). The spleen cells were suspended as a single cell suspension in PBS buffer with 2% FBS. Cells were washed and resuspended in PBS buffer containing 1% FBS and subsequently stained with fluorochrome-conjugated antibodies (Biolegend) as following: PE anti-mouse Gr-1, APC anti-mouse Mac-1, BV421 anti-mouse B220, FITC anti-mouse CD3, FITC anti-mouse Lineage, PE anti-mouse Sca-1, APC anti-mouse c-Kit, APC-Cy7 anti-mouse CD48, BV421 anti-mouse CD150, PE-Cy7 anti-mouse Sca-1, BV786 anti-mouse c-Kit, APC anti-mouse CD16/32, PE anti-mouse CD34. Flow cytometry was performed on LSRFortessa (BD), and data were analyzed by using FlowJo software (Tree Star, Ashland, OR).

Bone marrow transplantation (BMT)

BM cells were isolated from the tibias and femurs of diseased DKI mice and mice at the same age of the other groups including Dnmt3a R878H/+, Nras G12D/+ and WT mice (CD45.2+), respectively. BMT was then performed by injecting 4 × 10^5 BM cells suspended in PBS into the tail vein of sublethally irradiated (350 cGy) recipient mice (CD45.1+) at 2–3 months old.

Cell sorting

All the BM cells isolated from the various genotype mice were suspended in PBS buffer containing 1% FBS and subsequently stained with PE-conjugated Gr-1 antibody for half an hour. The cells were then washed and resuspended in 1–2 ml PBS buffer with 2% FBS. Gr-1+ cells were sorted by FACS AriaII (BD).

RNA-seq

RNA was extracted from Gr-1+ cells using TRIzol-isopropanol precipitation. The quality of the RNA was checked using Nano drop, Qubit and Agilent 2100 Bioanalyzer. The mRNA of the qualified sample was enriched with mRNA Capture Beads, and then the fragmentation of mRNA was realized by the action of high temperature and metal ions. Using mRNA as template, a single chain cDNA was synthesized by six base random primers, followed by two strand cDNA synthesis reaction, then VAHTSTM DNA Clean Beads was used to purify double chain cDNA. The purified double strand cDNA was first repaired (poly A was added and sequenced), and VAHTSTM DNA Clean Beads was used to resize the fragment size. Finally, PCR amplification was carried out and the PCR products were purified by VAHTSTM DNA Clean Beads. The obtained library was then checked using Agilent High Sensitivity DNA Reagent with a 2100 Bioanalyzer, and 200-bp paired-end sequencing was carried out on an Illumina HiSeq.
Quantitative real-time RT-PCR
The cDNA was synthesized using M-MLV reverse transcriptase (Invitrogen). RT-PCR was performed as described using reagents according to instructions of the manufacturer (Hieff TM qPCR SYBR® Green Master Mix; Yeasen). Reactions were performed on ABI PRISM 7500 Fast Real-Time PCR System or Applied Biosystems ViiA™ 7 Real-Time PCR System. Data were analyzed using formula \(2^{-\Delta\Delta Ct}\).

Western blot analysis
Total BM cells were lysed by RIPA Lysis Buffer (Beyotime). Protein assay was performed on Tecan infinite 200 Microplate Reader by BCA Protein Assay Kit (Beyotime). Antibodies were purchased from Cell Signaling Technology (anti-β-actin) and Abcam (anti-cMyc, anti-p62-cMyc).

Statistical analysis
Kaplan-Meier survival analysis was performed and survival differences between groups were assessed with the Log-rank test, assuming significance at \(P < 0.05\). Unpaired 2-tailed Student’s t-test was used to determine the significance between two data sets, assuming significance at \(P < 0.05\).

Results
Dnmt3a R878H cooperates with Nras G12D to shorten the lifespan of DKI mice
To discover the pathogenicity of DNMT3A and NRAS mutations in an endogenous expression environment, we crossed the Dnmt3a R878H KI mice (Dnmt3aR878H/+) with the Nras G12D KI mice (NrasG12D/+). The conditional KI mouse line Nras G12D and Dnmt3a R878H have been generated as previously described, respectively [13, 26, 27]. The DKI mouse model (Mx1-Cre; Dnmt3aR878H/+; NrasG12D/) was established by crossing Mx1-Cre; Dnmt3aR878H/+ mice with Mx1-Cre; NrasG12D/+ mice (Fig. 1a). Their descendants’ genotypes were identified by PCR at genomic DNA level (Fig. 1b). Ptcp was administered by intraperitoneal injection in KI and DKI mice to induce Cre-mediated gene expression. Nine weeks after ptpc induction, we found that white blood cells (WBCs) in PB of DKI mice began to significantly increase (Fig. 1c) compared with the Dnmt3aR878H/+ mice which also had gradually increased WBCs, whereas WBCs did not obviously changed in the NrasG12D/+ group, thus suggesting cooperation of DNMT3A and NRAS mutations could promote proliferative potential of hematopoietic cells. Interestingly, the DKI mice showed a significantly shortened survival (median survival time 189 days) compared with Dnmt3aR878H/+ mice.
mice (median survival time 243 days) and Nras<sup>G12D/+</sup> mice (Fig. 1d).

**Dnmt3a R878H cooperates with Nras G12D to induce a full-blown AML**

Routine blood test revealed elevated counts of WBCs, and reduced value of hemoglobin (Hb) and RBCs in Dnmt3a<sup>R878H/+</sup> mice and DKI mice while Nras<sup>G12D/+</sup> mice showed no obvious changes compared with wild-type (WT) 4 months after pIpC injection (Fig. 2a). Of note, DKI mice showed obviously increased amount of WBCs compared with Dnmt3a<sup>R878H/+</sup> group (Fig. 2a). BM cells cytospin with Wright-Giemsa staining revealed higher proportion of immature cells in DKI mice (average, 32.5%) than in Dnmt3a<sup>R878H/+</sup> mice (average, 20%) and Nras<sup>G12D/+</sup> mice (average, 9.5%) (Fig. 2b). The flow cytometry analysis of BM cells showed an increase of myelomonocyte and decrease of lymphocyte with varying degrees in knock-in mice (Fig. 2c). The most extraordinary inversion of the proportion of myelomonocytes and lymphocytes was observed in DKI mice (Fig. 2c). DKI mice also showed significantly increased Lin<sup>-</sup> Sca-
1 c-Kit+ (LSK) cells in contrast with Dnmt3a^R878H/+ or Nras^G12D/+ KI mice (Fig. 2d). Among the LSK cells, the quantity of lineage-restricted progenitors (LRPs) of both DKI and Dnmt3a^R878H/+ mice remarkably expanded and DKI mice manifested more LRPs than Dnmt3a^R878H/+ mice. And there was an obvious growth of multipotent progenitors (MPPs) in DKI mice whereas other groups remained unperturbed. We also observed some perturbation in the downstream progenitors of LSK cells with a notable raise of common myeloid progenitors (CMPs) in all the three groups of KI mice and a prominent increase of megakaryocyte-erythroid progenitors (MEPs) in DKI compared with WT mice (Fig. 2d). These data suggest that Dnmt3a R878H mutation with additional Nras G12D mutation could induce much more severe AML with distinct abnormal hematopoietic stem cells and progenitors.

**DKI mice develop more aggressive AML than Dnmt3a^R878H/+ mice**

Both moribund DKI mice and Dnmt3a^R878H/+ mice had distended abdomens, indicating characteristics of splenomegaly. Confirmed by the analysis of dissection, diseased DKI mice and Dnmt3a^R878H/+ mice showed significant increased weight of spleen, while DKI mice displayed much heavier spleen compared with Dnmt3a^R878H/+ mice (Fig. 3a). Pathological section revealed a more grievous effacement of splenic architecture as a result of extensive infiltration of leukemic cells in DKI mice in comparison to Dnmt3a^R878H/+ mice and Nras^G12D/+ mice (Fig. 3b). Confirmed by flow analysis of spleen cells, Gr-1+ Mac-1+ myeloid cells were significantly increased in DKI group comparison with Dnmt3a^R878H/+ mice and Nras^G12D/+ mice (Fig. 3c). These results showed that Dnmt3a R878H synergize with Nras G12D to transform cells into leukemic cells with a strong ability of infiltration.

**Leukemic cells from DKI mice show significant advantage of proliferation**

To further investigate the cooperation between Dnmt3a R878H and Nras G12D, we performed secondary transplantation by transplanting freshly isolated BM cells from terminally diseased DKI mice and mice at the same age of Dnmt3a^R878H/+, Nras^G12D/+, and WT mice (with CD45.2 marker) into sublethally irradiated (350 cGy) recipient mice (with CD45.1 marker), respectively. The percentage of CD45.2+ cells in PB of recipient mice were then detected 5 weeks after transplantation by flow cytometry. There were almost no CD45.2+ cells detectable in the PB of Nras^G12D/+ and WT mice during the first 5 weeks post transplantation, while Dnmt3a^R878H/+ mice showed an average of 13.7% CD45.2+ cells. In contrast, recipient mice of DKI showed an average of 30% CD45.2+ cells (Fig. 4a). During the next 15 weeks, CD45.2+ cells in PB from DKI mice continuously grew to an average of 80.6%, whereas there was only a moderate increase of CD45.2+ cells from mice with single mutation (an average of 28.2% for Dnmt3a^R878H/+ mice
19.2% for Nras\(^{G12D/+}\) (Fig. 4a). These results revealed that cooperation of Dnmt3a R878H and Nras G12D could synergistically promote the proliferation of transformed hematopoietic cells. Phenotype analysis at 24 weeks after transplantation showed that the recipient mice of DKI developed an AML phenotype similar to Nras\(^{G12D/+}\) alone.

**Fig. 4** Dnmt3a R878H and Nras G12D-induced leukemia is transplantable with advantage of myeloid proliferation. **a** The percentage of CD45.2\(^+\) cells in PB of each group after BMT. (Left panel) Fold line diagram of the change of CD45.2\(^+\) cells. (Right panel) Representative flow cytometric results. **b** Count of WBCs in PB of each group 24 weeks after BMT. **c** Morphological analysis of BM cells with Wright's staining of each group. **d** Macroscopic appearance of spleen of each group at the same time point. **e** Flow cytometric analysis of CD45.2\(^+\) cells and hematopoietic cell populations in BM of each group. CD45.2\(^+\) cells were gated for analysis of Gr-1\(^+\), B220\(^+\), CD3\(^+\) and c-Kit\(^+\) cells. \(n = 5\) for each group. Horizontal bars correspond to Mean ± SEM values. * \(P < 0.05\), ** \(P < 0.01\), *** \(P < 0.001\), significance was determined by two-tailed Student's \(t\) test.
that observed in primary mice. The recipient mice harboring two kinds of mutations showed a significant increase of WBCs compared with Dnmt3a R878H or Nras G12D mutation alone (Fig. 4b). Compared to the single mutation recipient mice, mice with double mutations displayed much more immature cells in BM and significantly enlarged spleen (Fig. 4c and Fig. 4d). Flow cytometry analysis of CD45.2+ cells in the BM revealed a remarkable altered granulocyte/lymphocyte ratio and increased c-Kit+ cells in DKI recipient mice (Fig. 4e). These data suggest that cooperation of Dnmt3a R878H with additional Nras G12D mutation could accelerate the onset of AML and worsen disease progression.

**Cooperation of Dnmt3a R878H and Nras G12D causes transcriptional alteration**

To clarify the mechanisms underlying the cooperation of Dnmt3a R878H and Nras G12D in leukemogenesis, we performed transcriptome profiling by RNA-seq of Gr-1+ BM cells harvested from diseased DKI mice as well as Dnmt3a R878H/+ and Nras G12D/+ mice, and WT mice at the same age. The Gr-1+ cells were sorted for RNA-seq due to an observation of distinct change of Gr-1+ cells in both DKI and Dnmt3a R878H induced AML mice which is consistent with our previous report [13]. There were more differentially expressed genes in DKI mice compared with Dnmt3a R878H/+ and Nras G12D/+ mice (Fig. 5a). In comparison to WT, DKI displayed 1129 significant differential genes, while Dnmt3a R878H/+ and Nras G12D/+ groups had 598 and 177 significant differential genes, respectively (Fig. 5b). Notably, 587 genes in DKI, 130 genes in Dnmt3a R878H/+ and 27 genes in Nras G12D/+ mice were up-regulated compared with WT (Fig. 5b). Gene ontology (GO) analysis revealed that the up-regulated genes in DKI mice compared with Nras G12D/+ mice were mainly related to positive of regulation of growth, negative regulation of differentiation, immune response and protein activation cascade (Fig. 5c upper panel). And the upregulated genes in DKI mice compared with Dnmt3a R878H/+ were mainly related to positive regulation of proliferation, negative regulation of apoptotic process, phosphorylation and immune response (Fig. 5c lower panel). Of note, the cooperation of Dnmt3a mutation and Nras mutation caused distinct dysregulated genes compared with Dnmt3a or Nras mutation alone. Venn analysis of differential genes also showed a dramatic amount of upregulated genes in DKI mice (Fig. 5d), including transcription factors Nme1 and Nme2, which were shown as potential molecular markers associated with prognosis in some studies [28, 29], and Bad gene involved in apoptotic process whose expression positively correlates with the disease stage [30]. In addition, Hoxa9 and Mef1 involved in development of myeloid leukemia were up-regulated [31, 32], while genes associated with differentiation, including Ptx5 and Sox1, were down-regulated in both DKI and Dnmt3a R878H/+ groups [33, 34] (Fig. 5d). Of note, the cooperation of Dnmt3a mutation and Nras mutation caused some newly dysregulated genes compared with the single mutation alone. These results indicate that Dnmt3a R878H plays a key role in regulating proliferation and differentiation, whereas the acquisition of Nras G12D provide an additional proliferation signal and negative regulation of apoptosis, thus contributing to promote leukemogenesis.

**Cooperation of Dnmt3a R878H and Nras G12D activates Myc pathway**

To further investigate the key upregulated genes in the pathogenesis of DKI mice, we performed gene set enrichment analysis (GSEA) of RNA-seq data. GSEA showed a significant enrichment of the gene sets related with negative regulation of differentiation and apoptotic signaling in the Gr-1+ cells of DKI groups compared with Dnmt3a R878H/+ mice (Fig. 6a). Interestingly, gene sets associated with Myc oncogenic signature and Myc targets were significantly enriched in DKI groups (Fig. 6b). The heat map displayed the dysregulated genes correspond to gene sets significantly affected according to GSEA (Fig. 6c). Compared with Dnmt3a R878H/+ mice and Nras G12D/+ mice, DKI mice showed obvious up-regulation of transcription factor Myc and a serial of Myc target genes [35], including Nme family genes, Cebpa, Npm1 and Bcl3 genes involved in the development of leukemia [36–38], Cdk4 and Bax genes related with cell cycle and apoptosis process [39, 40]. The upregulated-genes were validated by real-time qPCR (Fig. 6d). The oncogene cMyc is one of the most common genetic alterations in human cancers [41]. cMyc has been reported to be implicated in regulating a wide variety of biological activities, and particularly in balancing the self-renewal and differentiation of hematopoietic stem cell [42, 43]. Western blot showed that the expression of cMyc protein was increased in DKI mice compared with Dnmt3a R878H/+ or Nras G12D/+ single KI mice, and cMyc s62 phosphorylation, which was reported to increase cMyc protein stability and activity [44, 45], was also remarkably overexpressed in DKI mice. Therefore, up-regulation of Myc and Myc target genes related to proliferation and apoptosis may contribute to promote the process of Dnmt3a mutation-induced AML.

**Discussion**

As one of common epigenetic alterations in AML, Dnmt3A mutation has been demonstrated to play an important role in the pathogenesis of leukemia. Many studies have suggested that mutant DNMT3A requires additional gene mutations to cause full-blown AML. In this regard, a previous report showed the cooperation of
DNMT3A mutation and RAS mutation in leukemogenesis by using retroviral transduced mouse model [12]. However, the conditional KI approach is better than retroviral transduction system to recapitulate human leukemic features in mice. In this study, we created the first conditional DKI mouse model expressing Dnmt3a R878H and Nras G12D under the control of endogenous promoters by crossing Dnmt3a R878H/+ KI mice and Nras G12D/+ KI mice. By generating the conditional DKI mouse model, we discovered that cooperation of Dnmt3a R878H and Nras G12D could lead to a much earlier onset and more severe AML characterized by significantly increased WBCs, elevated immature cells, splenomegaly and shortened survival time, compared with Dnmt3a R878H/+ or Nras G12D/ + KI mice. In this pilot study, preliminary data derived from 10 mice per group were statistically analyzed.

Dnmt3a R878H/+ mice have been reported to develop moderate AML with considerably increased hematopoietic stem and progenitor cells especially LSK cells which were demonstrated to harbor leukemia-initiating cells [13]. Here, we...
showed that the LSK compartment was significantly enlarged in DKI AML mice compared with the diseased Dnmt3a R878H/+ mice, suggesting that DNMT3A mutation with acquisition of additional genetic abnormality such as NRAS mutation could significantly promote the leukemogenic transformation and proliferation of hematopoietic cells.

We investigated the mechanism underlying the leukemic phenotype induced by the cooperation of Dnmt3a R878H and Nras G12D. DKI mice showed much more up-regulated genes contributing to positive regulation of growth, negative regulation of differentiation and negative regulation of apoptosis process than Dnmt3a R878H/+ KI mice and Nras G12D/+ KI mice. Interestingly, the genes associated with phosphorylation and protein activation cascade were also up-regulated in DKI mice, indicating abnormal phosphorylation and protein activation caused by the cooperation of Dnmt3a and Nras mutation. We found that Dnmt3a mutation together with Nras mutation could not only increase the expression of Myc oncogene and Myc target genes, but also could induce the s62 phosphorylation of cMyc which has been reported to play a key role in increasing the protein stability and activating the Myc oncogenic signature [44, 45]. Therefore, the activation of Myc pathway may contribute to the pathogenesis of full-blown AML caused by the cooperation of Dnmt3a mutation and Nras mutation.

In this work, we generated a novel DKI mouse model to investigate the function and mechanism of Dnmt3a mutation under the presence of Nras mutation in inducing a full-blown AML. We discovered that Dnmt3a mutation and Nras mutation could cooperate to induce a much more severe AML than Dnmt3a or Nras mutation alone. In the mechanism study, we revealed that DKI mice showed significantly altered gene expression patterns with involvement of Myc pathway activation, indicating a potential therapeutic target in DNMT3A mutation-related leukemia which needs to be further investigated.

**Fig. 6** The activation of Myc oncogenic signature in DKI mice. a GSEA shows enrichment of differentiation and apoptosis gene sets in DKI groups compared with Dnmt3a R878H/+ group. b GSEA shows enrichment of Myc oncogenic signature and Myc target gene sets in DKI group compared with Dnmt3a R878H/+ group. NES, normalized enrichment score. c Heat map of up-regulated Myc and Myc target genes in DKI mice compared with WT, Dnmt3a R878H/+ and Nras G12D/+ mice. d The gene expression levels were validated by real-time RT-PCR. Primers were listed in Additional file 3: Table S3. e Western blot analysis of cMyc and p62-cMyc. Horizontal bars correspond to Mean ± SEM values. * P < 0.05, ** P < 0.01, ***P < 0.001, significance was determined by two-tailed Student's t test.
Conclusions
In conclusion, we discovered that cooperation of \textit{Dnmt3a} mutation with \textit{Nras} mutation could synergistically induce AML under the control of endogenous promoter/enhancer in mice, and activation of Myc pathway is one of the key players in the disease mechanism. Our study thus provides a unique mouse model that recapitulates many aspects of human AML and a potential therapeutic target for \textit{DNMT3A} mutation-related leukemia.

Supplementary information
Supplementary information accompanies this paper at https://doi.org/10.1186/s12885-019-6207-9.

Additional file 1: Table S1. Primers for identifying genotypes of Knock-in mice.
Additional file 2: Table S2. Genes with Different Expression Patterns of Gr-1 Positive Cells Between DKI and WT Mice.
Additional file 3: Table S3. Primers for validating the results of RNA-seq analysis.

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Authors’ contributions
XS, JM and YW designed the research. XS, YY, SS, SW, WZ, LP, TH and RZ performed the research. XS, YY, RR, and YW analyzed the data. XS and YW wrote the paper. YW and JM conceived and supervised the project. All authors read and approved the final manuscript.

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Availability of data and materials
All data generated or analyzed during this study are included in this published article.

Ethics approval and consent to participate
All mouse experiments were performed according to the guide of laboratory animal care and use standards, and were approved by the animal use committee of Shanghai Jiao Tong University.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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