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FAM122A promotes acute myeloid leukemia cell growth through inhibiting PP2A activity and sustaining MYC expression

Running title: FAM122A supports AML cell growth and survival

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Acute myeloid leukemia (AML) is a heterogeneous clonal disorder of hematopoietic stem and progenitor cells (HSPCs), characterized by uncontrolled proliferation, differentiation blockage and reduced apoptosis. Progressive advance has been made in AML treatment during previous decades, however, 5-year survival rates of AML patients are still low, especially for elderly patients (1, 2). Therefore, it is necessary to understand the molecular pathological mechanisms and develop more effective treatment strategies for this disease. The importance of protein phosphatase 2A (PP2A) as a tumor suppressor and promising target for the therapy is recently highlighted due to its frequent inactivation in AML cases (3). The loss of PP2A activation occurs at different levels in AML, either with the mutation and down-regulation of PP2A subunits or overexpression of PP2A inhibitors SET, CIP2A and SET interacting protein (4, 5). Pharmacological restoration of PP2A activity by its activator (FTY720) effectively antagonizes leukemogenesis (6). It was shown that FAM122A (family with sequence similarity 122a), a highly conserved protein among a variety of mammalian species, interacts with PP2A-A\(\alpha\) and -B55\(\alpha\) (a scaffold and regulatory subunit of PP2A complex) and promotes the polyubiquitination and degradation of its C\(\alpha\) subunit (7), and FAM122A sumoylation increases the degradation of PP2A-C\(\alpha\) protein together with the reduced phosphatase activity of PP2A (8). Recently, we demonstrate that FAM122A maintains the growth of hepatocellular carcinoma cells through promoting MAPK/AKT signaling (9). However, the normal function and pathophysiological significance of FAM122A are still largely unknown so far. Herein we investigated whether FAM122A has a role for the growth of AML cells and AML development.

Besides FAM122A, the FAM122 family also has other two members, FAM122B and FAM122C, both sharing 60% and 30% amino acid sequence identity respectively with FAM122A. We examined the mRNA expression levels of three FAM122
members in a panel of AML and acute lymphoblastic leukemia (ALL) patient samples as well as human CD34+ HSPCs by analyzing the public RNA-seq database (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE48173). As shown in Figure 1A, the mRNA expression level of FAM122A, rather than FAM122B and FAM122C, is significantly increased in AML and ALL patients compared to normal HSPCs. The similar higher expression pattern of FAM122A is also found in bone marrow cells (BMs) of four subtypes of AML with different karyotypes, that is, t(8;21), t(15;17), inv(16)/t(16;16) and t(11q23)/MLL, compared with hematopoietic stem cells (HSCs) enriched for Lin−CD34−CD38−CD90−CD45RA− in normal BMs (Supplemental Figure 1A) (http://servers.binf.ku.dk/hemaexplorer/). TCGA datasets also showed that AML patients with high FAM122A mRNA expression have shorter overall survival (OS) (Figure 1B), suggesting the potentially oncogenic role of FAM122A in AML. Moreover, FAM122A protein expression was also higher in AML cell lines, compared to those of peripheral blood mononuclear cells (PBMCs) from 3 normal persons (Figure 1C).

To assess the function of FAM122A in AML, three human AML cell lines, including NB4 (a t(15;17)-carrying acute promyelocytic leukemia cell line), U937 and THP1 with MLL-AF9 fusion gene were respectively transfected with lentiviruses expressing short hairpin RNAs (shRNAs) targeting two distinct regions of FAM122A mRNA (designated shFAM122A#1 and shFAM122A#2) together with a non-specific shRNA (shScramble). The results showed that FAM122A could be effectively knocked down with these two pairs of shRNAs in all three AML cells (upper panels, Figure 1D), and knockdown of FAM122A caused a substantial decrease in cell growth compared to shScramble-expressing NB4 cells (bottom panels, Figure 1D), which could be significantly rescued by re-expression of FAM122A (Figure 1E). Meanwhile,
FAM122A silencing could also suppress the growth of normal CD34+ cells to a degree (Supplemental Figure 1B-D), suggesting there is a differential requirement of FAM122A on the growth of normal HSPCs and AML cells.

We continued to investigate whether FAM122A knockdown induces cell death and/or cell cycle distribution. The results showed that less living cells were seen under light microscope in NB4 cells after transduction with shFAM122A-lentivirus for 4 days, compared with their control cells (upper panels, Figure 1F). NB4 cells with infection by shFAM122A#2 and especially shFAM122A#1 displayed significant increases in AnnexinV+/PI+ cells without significant alteration of the distribution of cell cycle (bottom panels of Figure 1F and data not shown), indicating FAM122A silencing led to cell apoptosis. The similar results were also observed in U937 and THP1 cells (Figure 1G), although these effects were obvious after transduction for 6 days. The role of FAM122A in leukemic cell growth in vivo was further investigated by AML mouse model driven by MLL-AF9, which is produced by t(9;11)(p22;q23) frequently found in infant AML and associated with M5 subtype AML (10). MLL-AF9 leukemic cells with YFP tag from BMs of leukemic mice were engineered to express either GFP tagged shControl or shRNAs that target murine Fam122a (designated shFam122a#3 and shFam122a#4) with lentivirus system (Figure 2A). GFP+/YFP+ cells in each condition purified by sorting were assessed for infection efficiency and colony formation, as well as AML development through injecting the sorted leukemic cells into lethally irradiated mice (all mice used and cared according to SJTU-SM’s and national guides). The efficient silencing effects of FAM122A were confirmed in either shFam122a#3- or #4-expressing cells and FAM122A inhibition significantly abrogated colony forming capacity in methylcellulose (Figure 2B-C). The purified MLL-AF9 leukemic cells expressing two Fam122a-shRNAs together with shControl at equal numbers (2000 or
5000 cells), were respectively transplanted into lethally irradiated recipient mice. As seen in Figure 2D, the recipient mice transplanted with shFam122a#3 and #4 cells displayed significantly longer disease latency and extended survival, as determined by mean survival days (50 or 53 days in shFam122a#3 or #4 mice versus 25 days in mice with 2000 cells; 43 or 50 days in shFam122a#3 or #4 mice versus 22 days in controls with 5000 cells). FACS analysis of hematopoietic organs and peripheral (PB) further showed that mice transplanted with both Fam122a-shRNAs leukemic displayed significantly lower percentages of YFP+ cells in PB, BM and spleen, compared with those of shControl counterparts (Figure 2E). The sizes and weights of livers and spleens from FAM122A silencing recipients appeared much smaller than control recipients (Figure 2F-G), accompanied with less leukemic cell infiltrations (Figure 2H). Collectively, these observations strongly suggest FAM122A is a critical regulator of AML cell growth and development in vitro and in vivo.

Many key substrates of PP2A are involved in the regulation of cell growth and apoptosis, including pro-proliferation regulators AKT, ERK, MYC (11) and antiapoptotic factors Bcl-2 (12) and BAD (13). Considering FAM122A is identified as a PP2A inhibitor previously, we further examined the phosphorylation states of a of PP2A substrates. FAM122A silencing could significantly decrease the phosphorylations of several PP2A substrates, including MYC at Ser62, ERK at Thr202/Tyr204, BAD at Ser112 and Bcl-2 at Ser70, rather than the substrate of phosphatase SHP-1 such as JAK2 (Figure 3A). The decreased phosphorylation could be abolished when re-expression of FAM122A in shFAM122A#1 cells, indicating the altered phosphorylation events are specifically regulated by FAM122A, although we didn’t find the alteration of AKT phosphorylation. FAM122A also reduced total MYC and Bcl-2 protein levels (Figure 3A). Okadaic acid (OA, a
PP2A inhibitor) treatment could significantly abrogated FAM122A silencing-decreased phosphorylations of ERK and BAD, but not phosphorylated MYC, suggesting FAM122A maintained AML cell growth and survival possibly by modulating PP2A activity (Figure 3B). Indeed, PP2A-Cα silencing could partially rescue FAM122A knockdown-triggered apoptosis (Supplemental Figure 2).

MYC is essential for AML occurrence and progression (14), and mounting evidence showed that Ser62 phosphorylation is critical for maintaining the stability of MYC protein (15). However, we found OA treatment or PP2A-Cα knockdown (Supplemental Figure 2B) didn’t affect the phosphorylated MYC in FAM122A knockdown cells, suggesting that reduced phosphorylated MYC was not mediated by PP2A directly. FAM122A knockdown resulted in decreased MYC mRNA level (Figure 3C), but did not affect its protein stability or degradation (Figure 3D-E). AF9-induced leukemic cells with FAM122A silencing also demonstrated the decrease in MYC protein (Figure 2B). These results strongly implied that FAM122A promotes AML cell growth also by maintaining MYC transcriptional expression.

In summary, our study shows that FAM122A is critical for maintaining AML cell growth and survival by modulating PP2A activity and sustaining MYC expression, which is warranted to further investigate its therapeutic significance for AML treatment.

**Conflicts of interest**

All authors declare no conflict of interest.

**Grant support**

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Author contributions

MHL: performed the most experiments and organized data; JC: contributed reagents and experimental materials; YSY and YQW: animal breeding and analyzed some data; YZ: provided human cord bloods; GQC: revised the manuscript; YH: designed the experiments and wrote the paper.

Acknowledgements

We thank Professors Cheng-Cheng Zhang (University of Texas Southwestern Medical Center) and Jun-ke Zheng (SJTU-SM) for kindly providing MLL-AF9 plasmid. pINDUCER20 was kindly provided by Professor Stephen J Elledge (Howard Hughes Medical Institute).

References


Figure legends

Figure 1. FAM122A is required for the growth and survival of AML cells. (A) The expression levels of \textit{FAM122A}, \textit{FAM122B}, and \textit{FAM122C} mRNA were compared among HSPCs, ALL, and AML patient samples in RNA-seq data from GEO dataset (GSE48173). Each point presented the related gene expression of individual patients with horizontal line indicated the median expression level in each group. Symbols * and *** were respectively indicated p < 0.05 or p < 0.001, compared with HSPCs. Symbol “ns” indicated no significance when comparing two groups. (B) Kaplan-Meier plot showed the correlation between \textit{FAM122A} expression and overall survival of AML patients from TCGA database. The patients were stratified into a high- and low-expression group by the median of \textit{FAM122A} expression. Log-rank test was performed to compare the survival curves of these two groups, with p < 0.01 indicating a significant difference. (C) FAM122A protein was compared with western blot between three normal human peripheral blood mononuclear cells (PBMCs) and indicated AML cell lines. (D) Effects of FAM122A knockdown in AML cells were confirmed by western blot (upper panels). The cellular growth rates of FAM122A knockdown cells (designated shFAM122A#1 and shFAM122A#2) and control cells (shScramble) were monitored and analyzed by a cellular counter (lower panels). (E) Re-expression of FAM122A with shRNA-resistant plasmid carrying 6 mutations in shFAM122A#1-expressing NB4 cells (designated shFAM122A#1/FAM122A-6m) was confirmed by western blot (upper panel). The growth of indicated cells was also monitored by a cellular counter (lower panel). (F) The photograph of FAM122A knockdown NB4 cells were observed and captured under microscopy (upper panel, scale bar = 100 μm) after transduction with lentiviruses expressing shRNAs for 4 days, together with apoptosis analysis by calculating AnnexinV+/PI+ cells with flow.
cytometry (bottom panel). (G) FAM122A knockdown U937 and THP1 cells, together with their corresponding control cells, were respectively subjected to apoptosis assay after infection with shRNAs viruses for 6 days. The analyzed quantification data in (F-G) were shown on the right panels of FACS data. Symbol * indicated $p < 0.05$, while symbols ** and *** were respectively presented $p < 0.01$ or $p < 0.001$, compared with corresponding shScramble cells. Symbols # indicated $p < 0.01$, compared with shFAM122A#1-expressing cells.

**Figure 2. FAM122A promotes AML development in vivo.** (A) Schematics of the experimental process of bone marrow transplantation assay. MLL-AF9-expressing mouse leukemic cells were respectively infected with GFP tagged shControl or shFam122a lentiviruses. Flow-sorted GFP$^+$ cells were subjected to verify the knockdown effect and colony forming ability. Subsequently, 2000 or 5000 GFP$^+$ cells, mixed with $2 \times 10^5$ normal bone marrow cells, were transplanted into lethally irradiated recipient mice for survival calculation. (B-C) GFP$^+$ MLL-AF9 leukemia cells from each shRNA condition, were assessed for FAM122A knockdown effects (B) and colony formation (scale bar = 100 $\mu$m) (C). (D) Kaplan-Meier survival curve analysis of mice transplanted with 2000 or 5000 purified MLL-AF9 cells expressing GFP tagged shControl or shFam122a (n=5; log-rank test). (E-H) After transplantation with 2000 AML cells for 21 days, the percentages of GFP$^+$YFP$^+$ positive cells in peripheral blood (PB), bone marrow (BM) and spleen (SP) from indicated recipient mice were analyzed by flow cytometry (E). The spleens (F) and livers (G) of indicated mice were photographed and weighed. HE images of liver and spleen from indicated mice (H) were observed by microscopy (scale bar = 100 $\mu$m). Symbols ** and *** were respectively presented $p < 0.01$ or $p < 0.001$, compared with
shControl-expressing AML mice.

**Figure 3. The effect of FAM122A silencing on phosphorylations of PP2A substrates and MYC expression.** (A) AML cells with FAM122A silencing and reexpression (shFAM122A#1-FAM122A-6m) were subjected to western blot for detection of indicated proteins. (B) The indicated cells were treated with or without 50 nM okadaic acid (OA) for 6 hours in NB4 cells, or 20 nM OA for 12 hours in U937 cells, followed by performing western blot to examine indicated protein levels. (C) The MYC mRNA expression level was examined in FAM122A knockdown AML cells. Data indicated means with bar as SD in an independent experiment. Symbol *** indicated $p < 0.001$, compared with that of shScramble cells. (D-E) FAM122A knockdown NB4 cells, together with shScramble cells, were respectively treated with or without 20 μM MG132 for 4 hours (D), or treated with cyclohexamide (CHX) at 100 μg/ml for different time points (E), followed by western blot to examine the indicated proteins.
Liu MH et al, Figure 3
Online Supplemental Materials and Figures for
the role of FAM122A in AML

Material and methods

Public data collection and analysis

RNA expression profiling of *FAM122A* gene in AML cohorts were obtained from Gene Expression Omnibus (GEO) (http://www.ncbi.nlm.nih.gov/geo/) and HemaExplorer (http://servers.binf.ku.dk/hemaexplorer/). GEO dataset (GSE48173) includes 17 CD34⁺ human cord blood samples (n=5 of CD34⁺; n=12 of CD34⁺CD45RA), 43 AML, and 12 ALL patients. HemaExplorer dataset includes 8 normal hematopoietic stem cells from human bone marrow (HSC-BM) samples, 39 AML with t(8;21)/AML1-ETO, 37 AML with t(15;17)/PML-RARα, 28 AML with inv(16)/t(16;16) and 38 AML with t(11q23)/MLL patient samples. These two datasets were respectively used to compare the expression levels of *FAM122A* mRNA between AML with different subtypes and normal HSPC or HSCs. The correlation of *FAM122A* mRNA expression level and overall survival of AML patients (from TCGA database) were analyzed with PROGgeneV2 online software, in which a group of AML patients were divided into a high-expression group with 79 patients and a low-expression group with 78 patients according to the median expression level of *FAM122A*.

Plasmid construction and viral production

Human and mouse complementary oligonucleotides specifically against FAM122A were synthesized, annealed and ligated into pLKO.1 vector according to the
Addgene’s pLKO.1 protocol. pLKO.1-GFP vector was constructed from pLKO.1-puro by replacing puro with GFP. The target sequences for FAM122A (FAM122A shRNAs) were shown respectively as following: 5’-TTGCACCAGATCAAACAAGAA-3’ for shFAM122A #1, 5’-GATTGCCTCCAAGCCCTATTC-3’ for shFAM122A#2, 5’-CAC CAGATCAACAGGAGGAG-3’ for shFam122a#3, 5’-GAGGAAAGTTTCAGCCT GA-3’ for shFam122a#4, and 5’-CAACGAAGGAATTCTTCG GCC-3’ for shScramble or shControl. Human FAM122A cDNAs amplified from HEK293T cells by reverse transcription-PCR, were first cloned into pENTR1A donor vector (containing attL sites), followed by recombination with pINDUCER20 destination vector (containing attR sites) to generate pINDUCER20-FAM122A expression plasmid using Gateway LR Clonase II Enzyme mix kit (Invitrogen, USA). pINDUCER20-FAM122A-6m shRNA-resistant plasmid was generated using Quick change Lightning Site-Directed Mutagenesis Kit (Stratagen, USA) by introducing six mutations (-CATCAAATTAAGCAGGAG-) into the pINDUCER20-FAM122A plasmid. For virus packaging, lentivirus constructs pLKO.1-GFP-shRNAs, pINDUCER20-FAM122A-6m, or control vectors, were respectively mixed with pSPAX2 and pMD2.G (4:3:1), while retroviral construct MSCV-MLL-AF9-IRES-YFP was mixed with PCL-ECO (2:1), followed by transfecting into 293T cells using Lipofectamine 2000 (Invitrogen, USA). Virus-containing supernatants were collected at 48-72 hour post-transfection and used for infection.

**Cell lines, infection and reagents**

NB4, U937, and THP1 cells were grown in RPMI-1640 medium (Life Technologies, USA) supplemented with 10% FBS (Sigma, USA) and 1%
penicillin/streptomycin (New Cell & Molecular Biotech CO. Ltd, China). Three cell lines were obtained from Shanghai Institute of Hematology. MLL-AF9 primary bone marrow cells were grown in StemSpan Serum-Free Expansion Medium (StemCell Technologies, Canadian) with 2% FBS, 1% penicillin/streptomycin, 20 ng/ml SCF, 20 ng/ml IL-3, 10 ng/ml IL-6 (all cytokines from PeproTech, USA). These cells were grown in a humidified incubator at 37 °C and 5% CO₂/95% air (v/v). NB4, U937 and THP1 cells were respectively infected with virus supernatants (1×10⁵ cells/ml) with 6 μg/ml polybrene (TR-1003, Sigma, USA) and centrifuged at 1200 g for 2 hours at 32°C, followed by replacing with fresh culture medium for incubating another 4 hours. After 48 hours of infection, GFP⁺ cells expressing shRNAs were flow-sorted for subsequent experiments. PINDUCER20-transduced NB4 cells were induced by doxycycline (1μg/ml, D9891, Sigma, USA) for efficient expression. Okadaic acid was purchased from Sigma-Aldrich (459616). Cycloheximide was purchased from CST (2112) and MG132 was purchased from Calbiochem (474790). PBMCs (peripheral blood mononuclear cells) were bought from Shanghai Miao-tong Biological Technology (PB010C, TPCS), with informed consents obtained from all patients in accordance with the Declaration of Helsinki. Puromycin (540411, MERCK, Germany) and G418 (345810, Sigma, USA) were purchased.

**CD34⁺ HSPCs’ isolation and lentiviral transduction**

Total mononuclear cells were isolated from cord bloods of healthy donors from Obstetrics and Gynecology Department of Shanghai Ren-Ji Hospital using Ficoll (p9011, Solarbio, Beijing, China), and HSPCs were enriched using human CD34 positive selection kit (18056, Stem Cell Technologies, Vancouver, Canada). The CD34⁺ HSPCs were cultured in StemSpan SFEM medium (09655, StemCell
Technologies, Vancouver, Canada) supplemented with 100 ng/ml human SCF, 100 ng/ml human FLT3-L, 50 ng/ml human TPO, and 1% penicillin/streptomycin. All these cytokines were purchased from Novoprotein (Shanghai, China). The experiments related to human cord bloods were approved by the Ethics Committee for Medical Research (IRB) at SJTU-SM.

CD34+ HSPCs were transfected with lentiviruses carrying FAM122A pLKO.1-GFP-shRNAs as used in AML cells. Briefly, 12-well plates were pre-coated with 40 µg/ml fibronectin (CA82, Novoprotein, Shanghai, China), followed by added with freshly collected virus supernatants and centrifuged at 2000 g for 2 hours at 32°C. Afterwards, supernatants in plates were removed and added CD34+ HSPCs with culture medium for 18 hours. After that, the transduction cells were washed and cultured in vitro for assessing the cell growth in CD34+ HSPCs with FAM122A silencing. For this purpose, the percentages of GFP+ cells and total cell numbers were monitored at days 2, 5 and 8 by flow cytometry (CytoFLEX S, Beckman Coulter, USA), and GFP+ cells were sorted for confirming FAM122A silencing effects with qRT-PCR.

**Cell growth assay**

FAM122A shRNA GFP+ cells were sorted by flow cytometry one day post-infection, and subsequently 1×10^5 (NB4, U937) or 2 × 10^5 (THP1) cells were seeded in 12-well plates. Cell numbers were calculated by a cellular counter (Beckman Coulter Vi-CELL XR Automated Cell Viability Analyzer, USA) on culturing days 2, 4 and 6 with triplicate wells.

**Cell apoptosis and cell cycle assays**
Cell apoptosis assay were performed according to the manufacturer’s instructions (56547, BD, USA). Briefly, cells were infected with FAM122A shRNAs for 48 hours. Subsequently, the cells were cultured for another 1 day for NB4 or another 4 days for U937 and THP1 cells. Then, the cells were collected and washed twice with cold PBS followed by resuspending in 100 µl of 1 × Binding Buffer with 5 µl of FITC AnnexinV and 5 µl PI. After incubation for 15 minute at RT (25°C) in the dark, each sample was added with 200 µl of 1 × Binding Buffer and analyzed by flow cytometry (BD FACSCalibur, USA) within 1 hour. For cell cycle analysis, NB4 cells were collected after virus infection for 48 hours, and U937 or THP1 cells were collected at 4 days after infection, followed by fixation in 70% ethanol. The fixed cells were washed and resuspended in 0.5 ml of FxCycle™ PI/RNAse Solution (1985233, Invitrogen, USA). After incubation for 15-30 minutes at room temperature in the dark, samples were analyzed by flow cytometry (BD FACSCalibur, USA).

**MLL-AF9 mouse leukemia model study**

Leukemia mice were generated by infection with recombinant retroviruses expressing MLL-AF9. Specifically, Lin<sup>-</sup> fetal liver cells were isolated and infected with MLL-AF9 retroviruses with 4 µg/ml polybrene and centrifuged at 1200 g for 2h at 32°C. Cells were cultured overnight, followed by another round of spin infection. Infected cells were transplanted into lethally irradiated (10 Gy) C57BL/6 mice by retro-orbital injection to generate MLL-AF9 mice. Bone marrow cells from leukemia mice were further infected with pLKO.1-GFP lentiviruses expressing FAM122A shRNAs with 4µg/ml polybrene and centrifuged at 1200 g for 2 h at 32°C. For transplantation assays, FACS sorting GFP<sup>+</sup> cells (2000 or 5000 cells/mouse) together with 2×10<sup>5</sup> normal mouse bone marrow cells were transplanted into lethally irradiated
recipient mice at 24 hours post-infection. For colony formation assays, 500 purified GFP⁺ leukemia cells were cultured in 1 ml of methylcellulose supplemented with cytokines (M3434, StemCell Technologies, Canadian) for 5-7 days. For HE staining, liver and spleen tissues were fixed in 4% paraformaldehyde and embedded in paraffin. Sections were stained with HE for the analysis of the infiltration of leukemia cells. C57BL/6 mice (Shanghai SLAC Laboratory Animal Co. Ltd., China) were housed in the SPF facility of Rui-Jin Hospital affiliated to SJTU-SM.

**Western blot**

Whole cell lysates were extracted in PBS plus 2×SDS, equally loaded on 10-15% SDS-polyacrylamide gels, and subsequently transferred to nitrocellulose membrane (162-0115, Bio-Rad, USA). After blocking in 5% skim milk in TBST at room temperature for 1 hour, the membranes were incubated with indicated primary antibodies overnight at 4°C, followed by HRP-linked secondary antibodies (7074, CST, USA) at room temperature for 1 hour. The signals were detected by reacting with chemiluminescent HRP substrate (WBKLS0500, Millipore, USA) and visualized using a chemiluminescent detector (LAS-4000, FUJIFILM, Japan). The antibodies include as following: anti-FAM122A (customized by Abclone, China), anti-phospho-Akt (Thr308) (13038, CST, USA), anti-phospho-Akt (Ser473) (4060, CST, USA), anti-Akt (10176-2-AP, proteintech, China), anti-MYC (10828-1-AP, proteintech, China), anti-phospho-MYC (ser62) (11311, Signalway Antibody, USA), anti-phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (4370, CST, USA), anti-p44/42 MAPK (Erk1/2) (4695, CST, USA), anti-phospho-Bad (Ser112) (5284, CST, USA), anti-Bcl-2 (60178-1-lg, proteintech, China), anti-phospho-Bcl-2 (Ser70) (2827, CST, USA), anti-phospho-Jak2 (Tyr1007/1008) (3771, CST, USA) and
anti-HRP-conjugated alpha-tubulin (HRP-66031, proteintech, China).

**Real-time quantitative reverse transcription polymerase chain reaction**

Total RNA was extracted by TRIZol reagent (molecular research center, USA), followed by treatment with FastQuant RT kit (TIANGEN, China) to wipe genomic DNA and complete reverse transcription of RNA to cDNA. RT-PCR was performed with SYBR select master mix (Applied Biosystem, USA) following the manufacturer’s instruction. Gene abundance was normalized against Ct of β-actin with the ΔΔCt method. The specific primers for real-time qPCR were given as following:

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<th>Genes</th>
<th>Forward primers</th>
<th>Reverse primers</th>
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<tr>
<td>FAM122A</td>
<td>CCGCTTGCAACGATCAAAC</td>
<td>CCGATTTTCTCCACGTCGTTG</td>
</tr>
<tr>
<td>MYC</td>
<td>GTCAAGAGGGCAGACACACAAAC</td>
<td>TTGGACGGACAGGATTGATGC</td>
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<tr>
<td>β-actin</td>
<td>CATCTCTACCCTGAAGTACC</td>
<td>AGCCTGATAGCAACGTACATG</td>
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**PP2A-Cα knockdown rescue study**

PP2A-Cα shRNA was generated and tested for the role of PP2A in FAM122A silencing-triggered cellular apoptosis. PP2A-Cα shRNA Oligos were cloned into PLKO.1-GFP vector and the target sequence is 5’-CCCATGTGTCTTATTTATT-3’. FAM122A shRNA Oligos were cloned into PLKO.1-puro vector. The PP2A-Cα- and FAM122A-shRNA viruses were packaged in the same batch, and then the virus supernatants were mixed to infect NB4 cells. Six experimental cell groups were generated as indicated. Three days after infection, the apoptosis of transduced NB4 cells were detected by flow cytometry and knockdown effects were examined by western blot.
**Statistical analyses**

Data are expressed as mean ± SD and analyzed by Student's t test, with $p < 0.05$ indicating significant difference. The Kaplan-Meier method was used to analyze the association between overall survival and *FAM122A* gene expression levels in AML patients, and the survival comparison was performed by log-rank test, $p < 0.05$ indicating significant difference. Data were analyzed using Graphpad Prism 7.0 software. All experiments were repeated at least three times.
Supplemental Figure legends

Supplemental Figure 1. The effect of FAM122A silencing on the growth of CD34+ cells. (A) The expression of FAM122A gene was compared among several subtypes of AML cells and HSCs in database from HemaExplorer (http://servers.binf.ku.dk/hemaexplorer). Each dot in the plot corresponded to the expression of FAM122A in a microarray, with horizontal lines representing the median expression for each class of cells. Symbols ** and * were presented p < 0.01 or p < 0.05, compared with HSCs. (B) Representative FACS plots were shown for GFP+ cell percentages in CD34+ HSPCs (cultured in vitro) after lentiviral transduction for 2, 5 and 8 days. (C) FAM122A knockdown efficiency was confirmed by qRT-PCR in GFP+ cells by sorting CD34+ HSPCs with lentiviral transduction for 5 days. (D) The relative growth rates of indicated cells were shown by absolute GFP+ cells after transduction for indicated days, which were calculated by the percentages of GFP+ cells multiplied with the total cell numbers against that of shScramble cells. Symbol * indicated p<0.05, and symbols ** and *** were respectively presented p<0.01 or p<0.001.

Supplemental Figure 2. The role of PP2A in FAM122A silencing-induced apoptosis. (A) Effects of FAM122A and/or PP2A Cα knockdown on cell apoptosis were examined in NB4 cells after lentiviral transduction for 3 days. Quantification of apoptosis was analyzed by AnnexinV+/7AAD+ cells. Symbol *** indicated p<0.001. (B) Phosphorylation states of several PP2A substrates were examined in FAM122A and/or PP2A-Cα knockdown cells by western blot.
Liu MH et al, Supplemental Figure 1
Liu MH et al, Supplemental Figure 2