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Original Articles

ADAM28 promotes tumor growth and dissemination of acute myeloid leukemia through IGFBP-3 degradation and IGF-I-induced cell proliferation

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ABSTRACT

ADAM28 has been shown to relate with tumor proliferation and prognosis. The expression of ADAM28 is up-regulated in acute myeloid leukemia (AML). However, the mechanism by which ADAM28 regulates the leukemic cell and the prognostic relevance with AML remain unknown. Here, we found that the expression level of ADAM28 was significantly elevated in AML patients suffering a relapse compared with those remaining in complete remission (CR). ADAM28 promoted the proliferation, migration and invasion in leukemic cells in vitro. Additionally, the increased expression of ADAM28 led to more IGFBP-3 degradation and IGF-I-induced cell proliferation. In a xenotransplantation mouse model, knockout of ADAM28 alleviated HL-60 cells growth and dissemination. The cumulative incidence of relapse (CIR) was significantly higher in patients with high ADAM28 expression. When separately considering the impact of ADAM28 on prognosis within the risk stratifications, patients with high ADAM28 expression levels had a significantly higher CIR in the favorable and intermediate-risk group but not in poor-risk group. Taken together, these data suggest a pivotal role for ADAM28 in regulating the proliferation and invasion of leukemic cells and in the prediction of relapse in AML patients.

1. Introduction

Acute myeloid leukemia (AML) is the most common type of acute leukemia in adults [1,2] and is fatal as a result of primary refractoriness, relapse, or treatment-related mortality [1,3]. Although the majority of patients with AML enter remission upon induction chemotherapy, the risk of relapse is considerable [4]. Transplantation regimens can be curative, but it remains challenging to identify high-risk patients suitable for early transplantation. The current risk assignment uses age, genetic subtype and response to initial therapy to stratify patients. Notably, many relapses occur in patients who initially present with favorable prognostic features [4,5]. New prognostic biomarkers may fine-tune risk assessment in adult AML and understanding their roles in leukemia may facilitate the selection of treatment options and benefit patients. Therefore, there is a clear need to improve the identification of patients at increased risk of relapse, particularly those currently stratified as favorable risk, for whom more intensive treatments are already available.

ADAM28 is one of the metalloproteinase-type A disintegrin and metalloproteinases (ADAMs), and it is expressed in human lymphocytes and the spleen and to a lesser extent in peripheral leukocytes [6]. It is involved in various biological events, including cell adhesion, cell

Abbreviations: AML, acute myeloid leukemia; ADAM, metalloproteinase-type A disintegrin and metalloproteinases; HSCT, hematopoietic stem cell transplantation; CR, complete remission; CIR, cumulative incidence of relapse; RFS, relapse-free survival; EFS, event-free survival; IGFBP-3, insulin-like growth factor binding protein-3; KO, knock out; ROC, receiver operating characteristic; CNS, central nervous system; CNSL, CNS leukemia

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fusion, membrane protein shedding, and proteolysis [6,7]. ADAM28 in malignant cells is up-regulated by multiple mechanisms, including the cleavage of von Willebrand factor (vWF), insulin-like growth factor binding protein-3 (IGFBP-3) and connective tissue growth factor (CTGF), as well as the promoting PSGL-1/P-selectin-mediated cell adhesion. Studies show that ADAM28 is highly expressed in several human tumors, such as lung, breast and bladder cancers, and chronic lymphocytic leukemia, and its tissue expression levels correlate with cancer metastasis. Recently, our previous study [8] suggested that the ADAM28 protein expression level of relapsed B-cell acute lymphoblastic leukemia (B-ALL) patients is significantly higher than that in the patients in remission; a high expression of ADAM28 is an independent risk factor for relapse-free survival (RFS) and event-free survival (EFS). Additionally, our findings showed that ADAM28 expression is also elevated in de novo AML patients, suggesting the potential prognostic relevance of ADAM28 in AML. To date, there has been no information available on the function and the prognostic relevance of increased ADAM28 levels in adult AML patients. Therefore, we sought to investigate how ADAM28 expression acts in AML and analyzed the relevance between ADAM28 expression and the prognosis of AML patients.

2. Materials and methods

2.1. Subjects

Bone marrow samples were obtained from adults with AML (N = 107) and normal individuals (N = 24) recruited at the Hematology Department of Peking University People's Hospital between May 2013 and May 2014 after providing written informed consent. The mononuclear cells (MNCs) from the bone marrows were collected by density gradient centrifugation.

ADAM28 expression levels in the leukemic blast populations of AML patients were studied by 7-color flow cytometry using an ADAM28 antibody (Proteintech,USA). Distinct cell populations (clusters) were identified based on any combination of forward and orthogonal light scatter properties and on fluorescence intensity with various antibody combinations. Immunophenotypic abnormalities within leukemic blast populations were determined on the basis of deviations from normal myeloid development. Expression levels of ADAM28 were compared with the degree of fluorescence of the same specimen stained with the isotypic control antibody [9]. The concentration of ADAM28 in serum was detected by ADAM28 ELISA kit. Complete clinical and laboratory data were available for 107 subjects. Subjects were followed until death, loss to follow-up or June 2017. Details of the treatment regimens have previously been described [10]. In total, 27 subjects (25%) received an allo-hematopoietic stem cell transplantation (HSCT) [11,12]. Complete remission (CR), refractory disease, relapse and risk-stratification were defined as previously described [13]. Cumulative incidence of relapse (CIR) was determined from the date of first CR to the date of first relapse. Event-free survival (EFS) was determined from the date of first CR to the date of first relapse or death. OS was determined from the date of diagnosis to the date of death. The study design was summarized in Supplemental Fig. 1. The study was approved by the Ethics Committee of Peking University People's Hospital, and informed consent was obtained according to the Declaration of Helsinki.

2.2. Primary AML cells

Bone marrow samples were collected from 20 newly diagnosed AML patients and subjected to a density gradient centrifugation. Then the ADAM28 expression of MNCs were assessed by flow cytometry. The 8 highest and the 8 lowest ADAM28 expression samples were assigned into 2 group and cultured in RPMI 1640 medium containing 10% fetal calf serum (FBS), penicillin and streptomycin (all from Sigma-Aldrich) at 37 °C with 5% CO_2 in a humidified incubator for subsequent experiments.

2.3. Leukemia cell lines

Human leukemia cell lines, including NB4, OCI-AML2/3, BALL-1, THP-1, KG-1, HL60, BV173 and K562 were cultured in RPMI 1640 medium containing 10% FBS, penicillin and streptomycin (all from Sigma-Aldrich) at 37 °C with 5% CO_2 in a humidified incubator. OCI-AML2 and 3 were gifts from the lab of Prof. Qian-Fei Wang (Chinese Academy of Sciences) and the rest were purchased from American Type Culture Collection (ATCC, Manassas, VA).

2.4. Lentiviral transduction

HL-60 cells were infected with CRISPR/Cas9 lentivirus targeting human ADAM28 and the control lentivirus (GeneChem, Shanghai, China, MOI = 100). Media containing lentiviral particles were replaced with complete medium at 12 h post-infection, and stably transfected HL-60 cells were selected with 0.5 μg/ml puromycin dihydrochloride (Genechem, Shanghai, China) post-infection. ADAM28 expression levels were confirmed by RT-qPCR and western blot assay.

2.5. Western blot analyses

Western blot was performed as previously described [14]. Antibody ADAM28 were purchased from Proteintech, USA and IGFBP3 were purchased from Cell Signaling Technology, USA. GAPDH was used as a loading control.

2.6. Cell proliferation assay

Cell proliferation was determined with the Cell Counting Kit-8 (CCK8, Dojin Laboratories, Kumamoto, Japan) assay. Briefly, the cells were seeded onto 96-well plates at a density of 4×10^4 and treated according to the manufacturer's protocol. The plates were scanned by a microplate reader at 450 nm at the indicated time points. Experiments were conducted 3 independent times in triplicate. Proliferation was further assessed by flow cytometry using Ki-67 antibody (CST, USA) and PCNA antibody (CST, USA).

2.7. Cell migration and invasion assay

The cells were seeded into the upper chamber of a transwell insert (pore size, 8 μm) in RPMI-1640 supplemented with 1% FBS. The upper chamber was then placed into the transwell containing medium supplemented with 10% FBS in the lower chamber. For the invasion assay, a matrigel coating (BD systems, San Jose, CA, USA) was used. After 24 h, the cells in the upper chamber or lower chamber were centrifuged and counted under microscope, respectively. Experiments were conducted 3 independent times in triplicate.

2.8. IGF-I stimulation

For IGF-I stimulation assay, cells were seeded onto 96-well plates at a density of 4×10^4 with or without the treatment of IGF-I (BioVision,No. 7507–20). CCK-8 assays were performed at the indicated time points. The IGF-I induced proliferation were calculated as

$$\frac{\text{proliferation of IGF–I treated cells} - \text{proliferation of control cells}}{\text{proliferation of control cells}} * \%$$

2.9. Tumor xenograft mouse model

Male 8-week-old NOD/SCID mice (Beijing HFK Bioscience Co., Ltd.; Beijing, China) were sublethally irradiated (200 cGy) and intraperitoneal injected with CD122 at a dosage of 10ug/g 12 h before

transplantation. They were anesthetized with isoflurane 3% inhalation, and intrafemorally injected with 1 × 10^6 HL-60 cells suspended in 30ul PBS.

Peripheral blood samples were collected in EDTA-coated tubes from the facial vein using lancets, and complete blood counts were analyzed using a Hemavet Model HV950 hematology analyzer (Drew Scientific, UK). Mouse tibias, spleens, livers, kidneys and brains were collected at the indicated time and fixed in 4% paraformaldehyde. Bones were decalcified in 20% paraformaldehyde at 4 °C for 7 days. samples were then embedded in paraffin. Sections (4 μm thick) were mounted on slides, deparaffinized and stained with hematoxylin and eosin (HE) or anti-CD45 antibody. All mice were maintained under standard conditions, in accordance with institutional animal care guidelines. Animal experiments were approved by the Animal Ethics Committee of Peking University People's Hospital.

2.10. Statistics

The numerical data are presented as the mean ± standard deviation of the mean. The 2-tailed *t*-test was used for statistical comparisons of ADAM28 expression between two subgroups of the cohort of patients. Univariate and multivariate Cox regression analyses were performed for the time to CIR, EFS and OS, and all factors with $P < 0.5$ were retained in the multivariable model. Receiver operating characteristic (ROC) curves were analyzed to assess the most appropriate cut-off values for the ADAM28 expression levels in the BM between the relapse patients and the control groups. All of the statistical analyses were performed using the SPSS software program (version 19) and GraphPad Prism software (version 5). A P-value of < 0.05 indicated statistical significance.

3. Results

3.1. Overexpression of ADAM28 in the test group

The characteristics of the patients were summarized in Table 1. The relative expression level of ADAM28 in the bone marrow cells of the testing group was significantly higher than that of the control BM cells (0.98 ± 0.09 vs 0.49 ± 0.04; $p < 0.01$, Fig. 1A). But the ADAM28 level in serum of the testing group was not differed significantly from that of controls (1.52 ± 0.31 ng/ml vs 0.86 ± 0.10 ng/ml; $p = 0.227$, Fig. 1B). Expression of ADAM28 in patients with M3 was significantly lower than that in patients with M2 and M5 (Fig. 1C). Further, no additional significant correlations between ADAM28 and clinical data including age, WBC, platelet count, hemoglobin value, lactate dehydrogenase (data not shown) and cytogenetic stratification (Fig. 1D) were found.

Table 1
Characteristics of de novo Patients with AML.

Patients	Total	Testing Group	Validation Group
N	107	32	75
Male/female	62/45	17/15	45/30
Age (years) mean (range)	41.0 (17–77)	39.5 (17–75)	41.6 (18–77)
WBC (× 10^9/l) mean (range)	27.8 (0.6–199.7)	43.5 (1.5–199.7)	19.1 (20.1–86.9)
Hemoglobin (g/dl) mean (range)	83.9 (1.9–138.0)	85.3 (33.0–115.0)	83.2 (1.9–138.0)
Platelet count (× 10^9/l) mean (range)	67.9 (1.0–354.0)	58.3 (7.0–248.0)	73.0 (1.0–354.0)
Risk groups[a]			
favorable	14	7	7
Intermediate	51	12	39
poor	21	8	13
Treatment outcome			
Relapse	33	11	22
Death	43	11	32

[a] Patients with M3 were excluded.

Interestingly, the expression levels of ADAM28 in patients suffering relapse were significantly higher than that in patients remaining in CR (1.23 ± 0.12 vs 0.76 ± 0.07; $p < 0.01$ Fig. 1E). Furthermore, the ADAM28 level in cerebrospinal fluid (CSF) of patients with central nervous system leukemia (CNSL) was significantly higher than that without CNSL (2.87 ± 0.81 vs 0.50 ± 0.08, $p < 0.001$, Fig. 1F). These data suggested the up-regulated expression of ADAM28 might be correlated with relapse and dissemination of AML.

3.2. The effect of ADAM28 on the proliferation, migration and invasiveness of leukemia cells

Given the indication that high ADAM28 expression might be associated with relapse in patients with AML, we next investigated whether ADAM28 influenced the proliferation, migration and invasiveness of AML cells. Primary blast cells were isolated from AML patients and were divided into high ADAM28 expression group and low ADAM28 expression group according to the expression level (Fig. 2A). Cells in high ADAM28 group displayed a better proliferation capacity (Fig. 2B). The expression of Ki-67 and the PCNA level in the high ADAM28 group were higher than those in the low ADAM28 group (Fig. 2C and D). We next further confirmed the effect of ADAM28 by knocking out its expression in the AML cell line HL-60 which had an abundant expression of ADAM28 (Fig. 2E) by lentivirus.

Blocking the expression of ADAM28 resulted in a significant decrease in cell proliferation (Fig. 2F), as well as the Ki-67 and PCNA positive rate (Fig. 2G and H). Additionally, the number of migrating cells in the ADAM28 knock out group was notably reduced when compared with the vector control group (Fig. 2I). Furthermore, blocking the expression of ADAM28 significantly inhibited cell invasion through a matrigel-coated transwell (Fig. 2J).

3.3. ADAM28 is involved in IGFBP-3 degradation

Previous study indicated that ADAM28 is involved in IGF-I-induced cell proliferation. We next investigated the association between ADAM28 and the IGF-I-induced proliferation which relies on the degradation of IGFBP3 and the release of IGF-I. First, we sought to investigate the effect of ADAM28 on primary cells derived from AML patients. The degradation of IGFBP-3 tended to be stronger in the ADAM28 high expression group (Fig. 3A). The addition of IGF-I promoted the proliferation of primary cells. The IGF-I-induced proliferation was significantly stronger in the ADAM28 high expression group when compared with the low expression group at 48 h ($p = 0.014$) and 72 h ($p < 0.001$) (Fig. 3B). Consistent with the data of primary cells, in ADAM28 knock out HL-60 cells, the fragment formation of IGFBP3 was inhibited (Fig. 3C) and the IGF-I induced proliferation was attenuated When stimulated with IGF-I in the culture medium (Fig. 3D and E).

3.4. Knock out of ADAM28 limits leukemia cell growth and dissemination after xenotransplantation

We used the well-established NOD/SCID xenotransplantation assay to test the effect of ADAM28 on AML growth and dissemination in vivo. In the experiments, the recipients were intrafemorally injected with HL-60 cells with or without knockout of ADAM28. The counts of peripheral leukocytes were significantly lower in mice receiving ADAM28-KO cells compared with those in mice with control cells after 3 weeks of injection (Fig. 4A). In addition, a Kaplan-Meier plot in Fig. 4B demonstrates that mice bearing ADAM28-KO cells survived longer compared with control mice ($P = 0.017$). The leukemia burden was further characterized by spleen size. As shown in Fig. 4C, slighter splenomegaly was observed in mice with ADAM28-KO cells compared with that of the

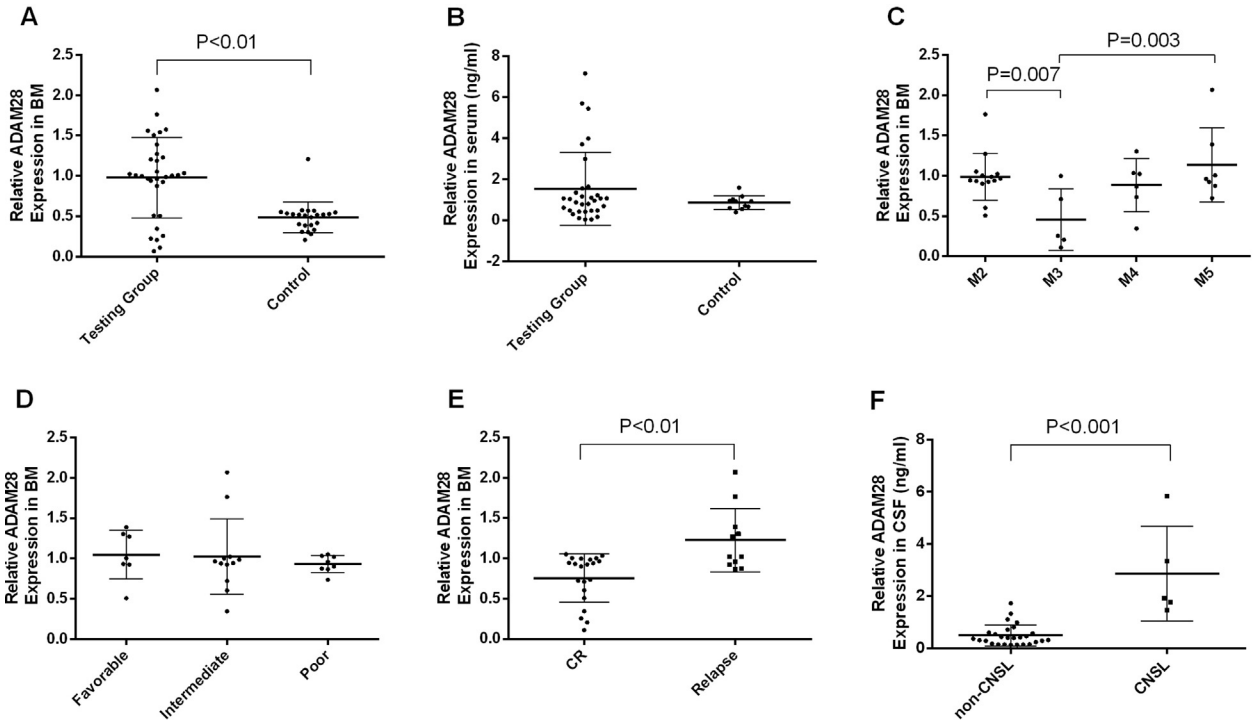

Fig. 1. ADAM28 expression in the test group. A. ADAM28 expression in the leukemic blasts of the AML patients and healthy controls was measured by flow cytometry. B. ADAM28 expression in the serum of the AML patients and healthy controls was measured by ELISA. C. ADAM28 expression in the bone marrow of the patients with different FAB morphology types. D. ADAM28 expression in the bone marrow of the patients with different cytogenetic risk stratifications. E. ADAM28 expression in the bone marrow differed significantly between the CR and relapsed patients with AML in the test group. F. ADAM28 expression in the CSF was higher in the CNSL patients than that in the non-CNSL patients in the test group as detected by ELISA. BM, bone marrow; CR, complete remission; CSF, cerebrospinal fluid; CNSL, central nervous system leukemia.

control mice. Histological studies (Fig. 4D) and detection of human CD45 $^{+}$ cells in bone marrow by flow cytometry (Fig. 4E) showed a lower leukemia burden in the bone marrow. Furthermore, histological study confirmed a lower leukemia dissemination in spleens, livers and kidney of mice bearing ADAM28-KO HL-60 cells compared with controls (Fig. 4F). These results suggested that knock out of ADAM28 in leukemic cells can suppress the growth of AML cells in the hematopoietic tissue and the dissemination into the non-hematopoietic tissues in a xenotransplantation mouse model.

3.5. Inhibition of ADAM28 decreases the incidence of CNS involvement

Leukemia dissemination into the CNS is a rare but serious complication and an impediment to disease-free remission in AML. Previous data indicated that the ADAM28 level in the CSF of patients with CNSL is significantly higher than that in those without CNSL. We therefore tested the effect of ADAM28 on the development of CNSL in vivo. To determine the neurologic involvement, mice were sacrificed 60 days after injection of HL-60 cells. Immunohistochemistry study detected the CNS infiltration by human CD45 staining in 1 out of 12 mice in ADAM28-KO group, which was a significantly lower rate than that of control group (3 out of 10) (Fig. 5A). And the CNS infiltration tended to be alleviated in mice bearing ADAM28-KO cells compared with that of control group (Fig. 5B). Together, these results suggested that the inhibition of ADAM28 reduced AML infiltration in the CNS in the mice model of xenotransplantation.

3.6. Prognostic analysis of ADAM28 expression in AML patients

We use the ROC curve analyses to establish a cut-off value in test group to determine overexpression. The cut-off value of ADAM28 protein level in BM based relapse was 0.828.

To validate the effect of ADAM28 protein level on the prognosis of AML patients, we extended ADAM28 expression level detection to a validation group of 75 patients with AML (Table 1). The ADAM28 expression level of the validation group in bone marrow between patients with or without CR had the same trend with the testing group (Fig. 6). The prognostic analysis was performed according to the study design (Supplemental Fig. 1).

Prognostic analysis revealed that in the enrolled 87 patients (patients with APL were excluded), the CIR was significantly higher, and the OS and EFS were worse in the ADAM28 high expression group ($p = 0.003$, 0.021 and 0.017 respectively, Fig. 7A–C). Multivariable analysis including ADAM28 expression level, treatment, risk stratification, WBC count and age further confirmed that high expression level of ADAM28 is an independent risk factor of relapse in patients with AML (Table 2). But the prognostic analysis in patients with M3 did not differed significantly (Supplemental Fig. 2).

Notably, when separately analyzing the impact of ADAM28 expression level on prognosis within the clinically defined favorable (Fig. 7D–F), intermediate (Fig. 7G–I) and poor-risk groups (Fig. 7J-L), patients with high ADAM28 expression levels had a significantly higher CIR (Fig. 7D) and worse EFS (Fig. 7F) in the favorable-risk group, and a significantly higher CIR (Fig. 7G) in the intermediate-risk group, but this discrimination was not observed in the poor-risk group (Fig. 7J-L). Because the patients with favorable risk were predominantly inclined to chemotherapy, patients with high ADAM28 expression presented a significantly higher CIR than the low expression patients within the chemotherapy subgroup (Supplemental Fig. 3), whereas the relapse didn't differ significantly with the ADAM28 expression level in patients receiving hematopoietic stem cell transplantation (HSCT) (Supplemental Fig. 3).

Interestingly, when the median (Supplementary Figs. 4–5) or arithmetic average (Supplementary Figs. 6–7) was taken as the cut-off

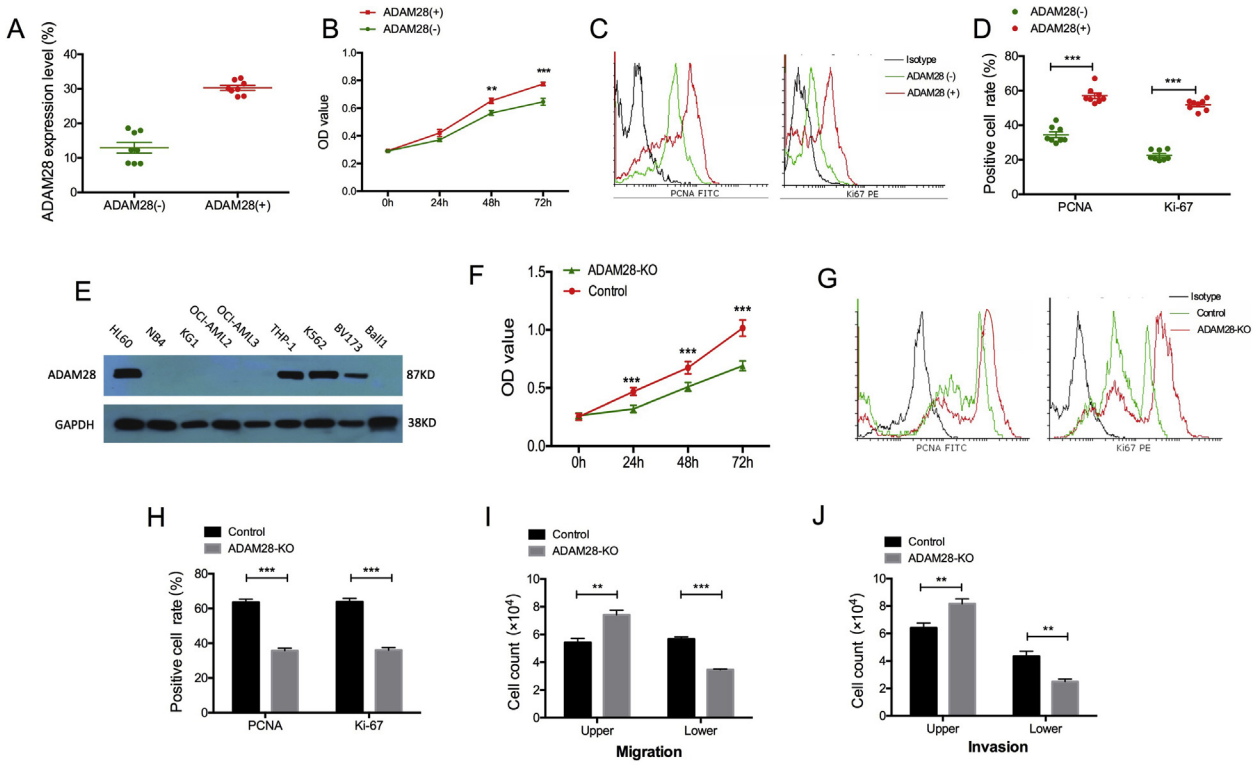

Fig. 2. The effect of ADAM28 on the proliferation, migration and invasion of leukemic cells. A. The expression of ADAM28 in primary leukemia cells detected by flow cytometry. B. Proliferation of primary leukemia cells detected by CCK-8. C. Representative image of the PCNA and Ki-67 expression in the primary leukemia cells detected by flow cytometry. D. Positive cell rate of PCNA and Ki-67 in the primary leukemia cells (n = 8 in each group). E. Detection of ADAM28 expression in the leukemia cell lines by western blot. F. Proliferation of the vector control and ADAM28-KO HL-60 cells detected by CCK-8 assay. Knock out of ADAM28 decreased the proliferation rate of the HL-60 cell as determined by Ki-67 and PCNA staining (G, H). A transwell assay confirmed the decreased migration and invasion ability after knocking out ADAM28 expression in the HL-60 cells (I, J). **, $P < 0.01$; ***, $P < 0.001$. KO, knock out.

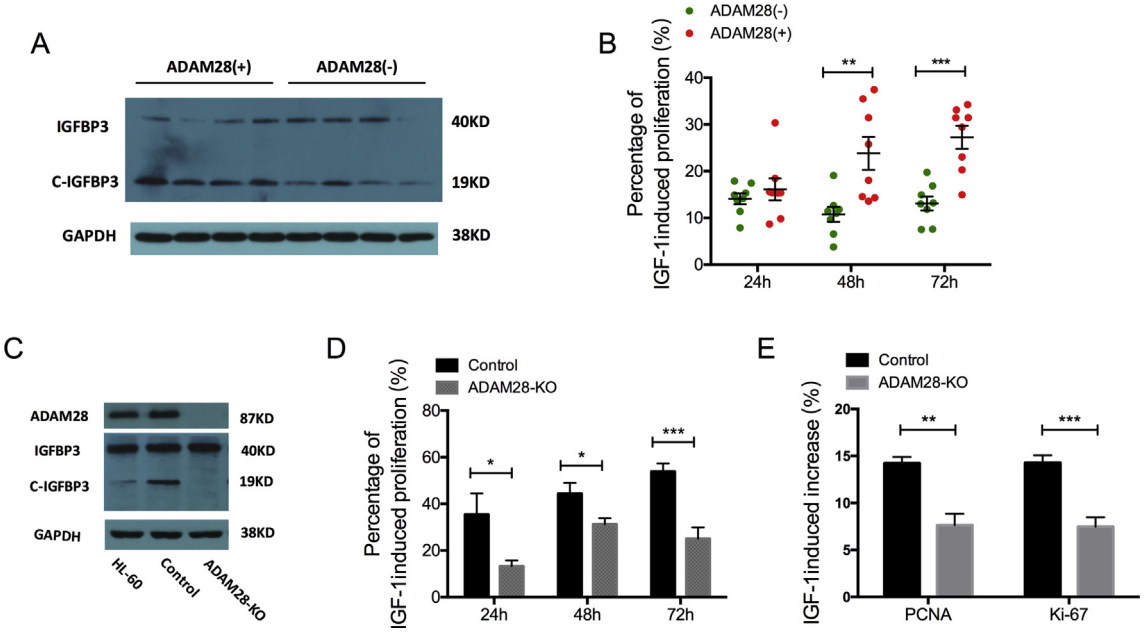

Fig. 3. ADAM28 is involved in IGFBP-3 degradation. A. The cleavage of IGFBP3 in primary leukemia cells. B. The increased proliferation upon IGF-I treatment. C. The IGFBP-3 fragment decreased after blocking ADAM28 in the HL-60 cells. D. The knockout of ADAM28 inhibited the IGF-I-stimulated proliferation in HL-60 cells. E. The knockout of ADAM28 inhibited the IGF-I-stimulated increase in PCNA and Ki-67 positive HL-60 cells. IGF-I-stimulated proliferation was calculated as $\frac{\text{proliferation of IGF-I treated cells} - \text{proliferation of control cells}}{\text{proliferation of control cells}} * 100\%$. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. KO, knock out.

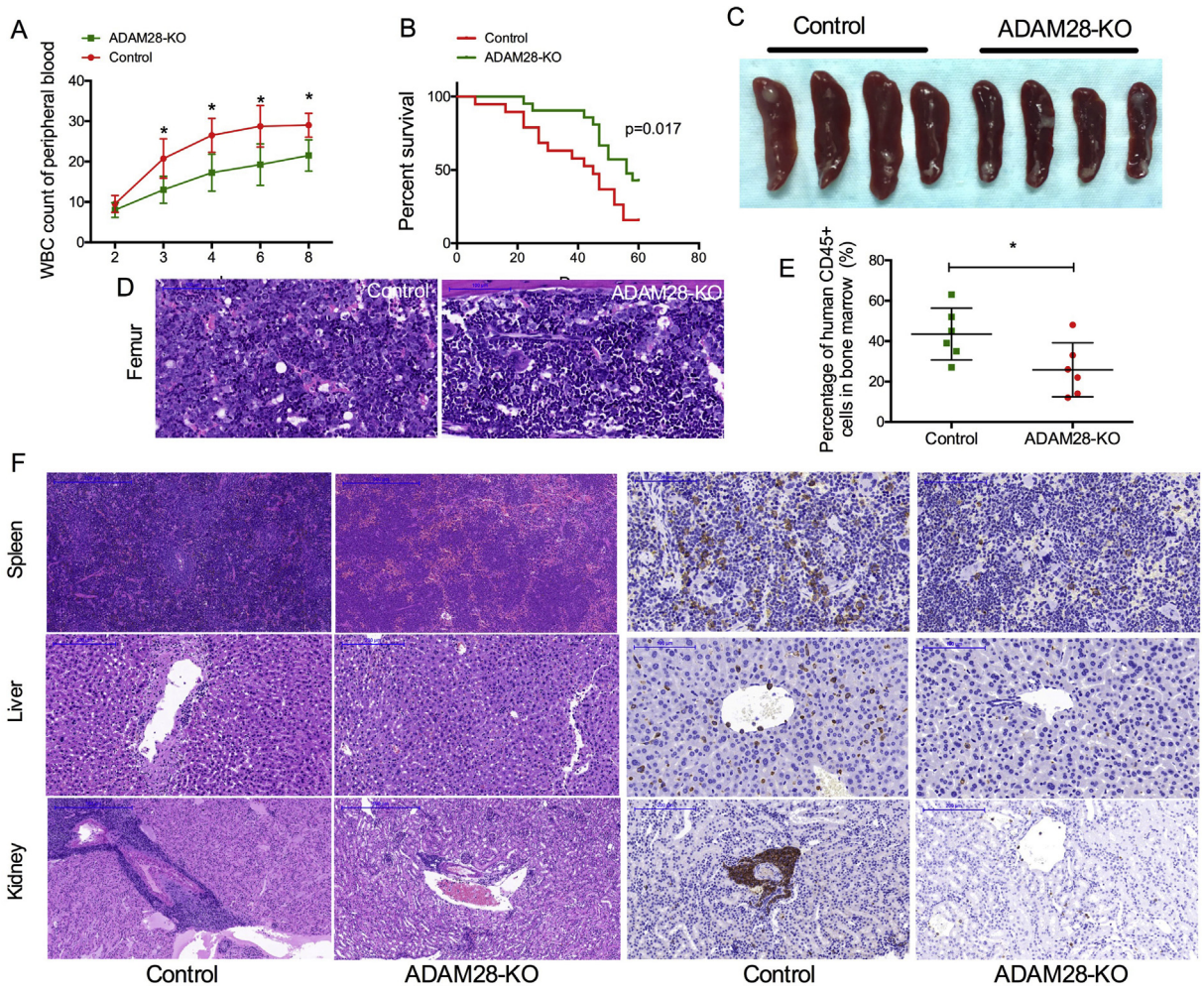

Fig. 4. Knock out of ADAM28 limits AML growth and dissemination after xenotransplantation. A. The peripheral count of leukocytes detected in the ADAM28-KO and control group. B. Mice bearing the ADAM28-KO HL-60 cells had a better survival than those treated with the control cells. Mice in each group were sacrificed 30 days after the HL-60 administration to assess the splenomegaly (C), leukemia infiltration in bone marrow (D, E). The leukemia dissemination in the spleen, liver and kidney was assessed by HE and human CD45 staining (F). *, $P < 0.05$. KO, knock out.

value of ADAM28 expression, the result of survival analyzes seemed to have a similar trend in the favorable-risk group, which might indicate that ADAM28 was a valuable prognostic marker.

4. Discussion

In the study, we evaluated the relationship between ADAM28 expression and prognostic outcomes of AML patients. The up-regulated ADAM28 expression was found in the AML patients with relapse. ADAM28 promotes cell proliferation, migration and invasion in leukemic cells by the cleavage of IGFBP-3, which improved the IGF-I-induced proliferation. In vivo, ADAM28 accelerates the growth and dissemination of leukemic cells. ADAM28 high expression is a risk factor of CIR of AML and specifically predicts poor clinical outcomes in favorable-risk patients.

ADAM28 is an important member of the ADAM family that is involved in various biological events, including cell adhesion, proteolysis, growth and metastasis of solid tumors and hematological malignancies [7,15] [6,16]. Studies have shown that ADAM28 is highly expressed in several human tumors, such as lung [17], breast [18] and bladder cancers [19], chronic lymphocytic leukemia [20] and B-cell acute lymphoblastic leukemia [8], and its tissue expression levels correlate with cancer metastasis.

Here, we found that the expression levels of ADAM28 were up-regulated in de novo AML patients and differed significantly between patients suffering a relapse and those remaining in CR. Furthermore, the ADAM28 levels in the CSF of patients with CNSL were significantly higher than those in patients without CNSL. These data suggested that ADAM28 levels might be related to the incidence of relapse in patients with AML.

We further investigated whether ADAM28 impacts the proliferation, migration and invasiveness of leukemic cells in vitro.

In the study, primary AML cells with high ADAM28 expression levels had better proliferation, migration and invasion capacities than those with low ADAM28 expression levels. Knocking out ADAM28 with a CRISPR/Cas9 lentivirus significantly inhibited the proliferation, migration and invasion in leukemic cells, suggesting that ADAM28 played a crucial role in the proliferation, migration and invasion of AML cells.

ADAM28-mediated cancer cell proliferation has been reported to be regulated by the cleavage of IGFBP-3 [15,18,21,22]. The cellular action of IGFs is strictly regulated by IGFBPs because the affinities of IGFs to IGFBPs are higher than those to IGF receptors [23]. Therefore, the proteolysis of IGFBPs directly controls the bioavailability of IGFs to the IGF receptors and thereby indirectly modulates cell proliferation [23]. Although the IGFBP family is composed of six proteins with a high affinity to IGFs, the major IGF transport function is attributed to IGFBP-3, which is the most abundant circulating IGFBP species synthesized by the liver and locally produced by cancer tissues [24]. Proteolytic cleavage is shown for IGFBP-3 and has gained wide acceptance as the predominant mechanism for IGF release from the IGF/IGFBP-3 complex

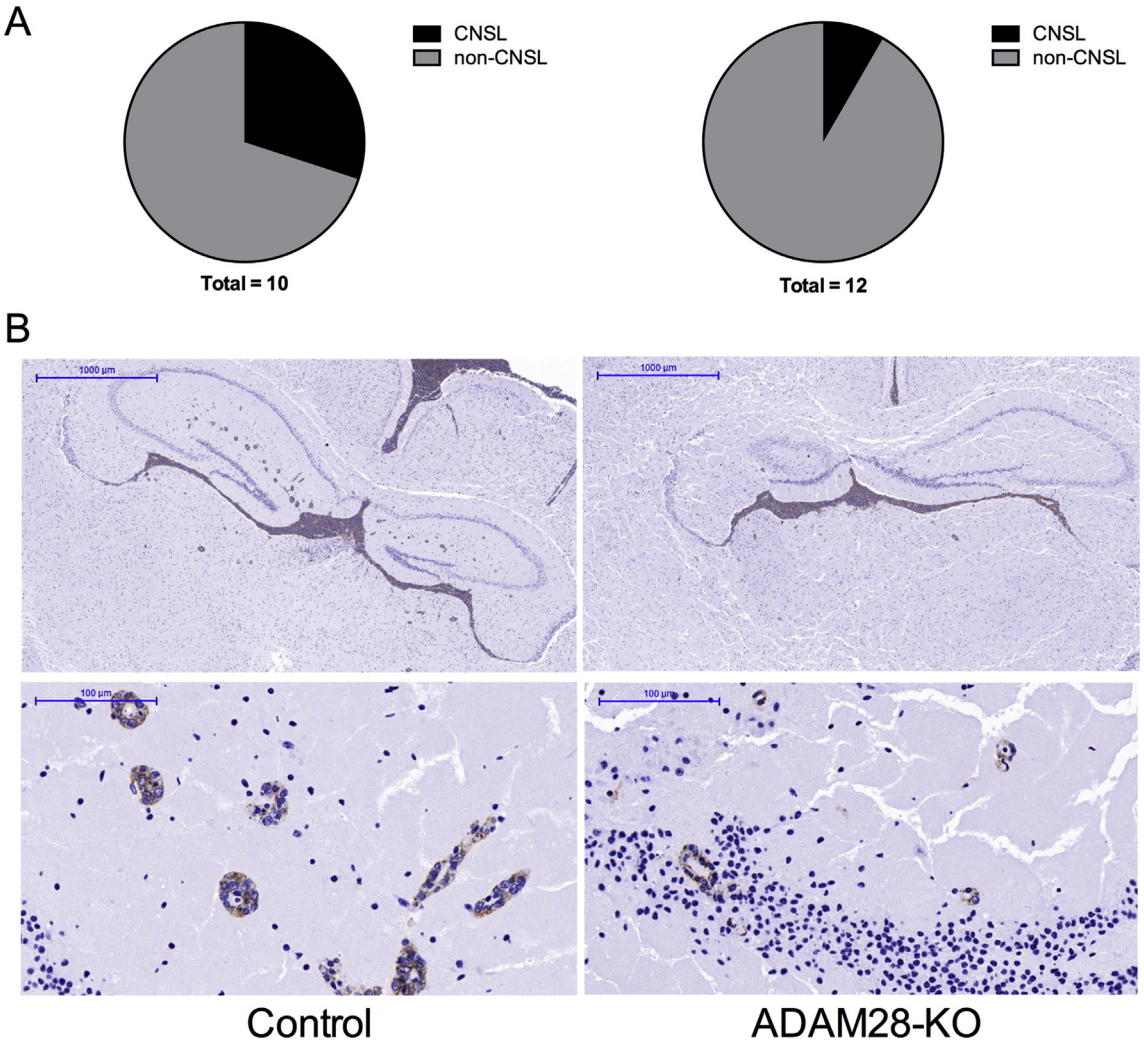

Fig. 5. ADAM28-KO and the infiltration of CNS. A. the ratio of mice with CNS infiltration detected by Immunohistochemistry. B. Immunohistochemistry of brain stained with human CD45 in mice bearing vector control and ADAM28-KO HL-60 cells. KO, knock out; CNSL, central nervous system leukemia.

[25]. It is reported that ADAM28 releases IGF-I from the IGF-I/IGFBP-3 complex through the selective cleavage of IGFBP-3 [26]. In the present study, the data strongly suggest that in leukemia cells, ADAM28 is involved in the degradation of IGFBP-3 and IGF-I-induced proliferation in the leukemic cells.

The overexpression of ADAM28 is implicated in the poor prognosis of many cancers. Our previous study revealed that ADAM28 expression in B-ALL patients was significantly increased. Patients experiencing disease relapse exhibited significantly increased ADAM28 expression compared with those with favorable outcomes. In addition, ADAM28 overexpression was associated with lower probabilities of relapse-free (RFS) and EFS, suggesting that ADAM28 may serve as a prognostic factor in B-ALL [8]. Compared to B-ALL, AML is a more common hematologic malignant disease and is also with a risk of relapse [27]. To date, there is no information available on the prognostic and relapse relevance of increased ADAM28 levels in adult AML patients. We

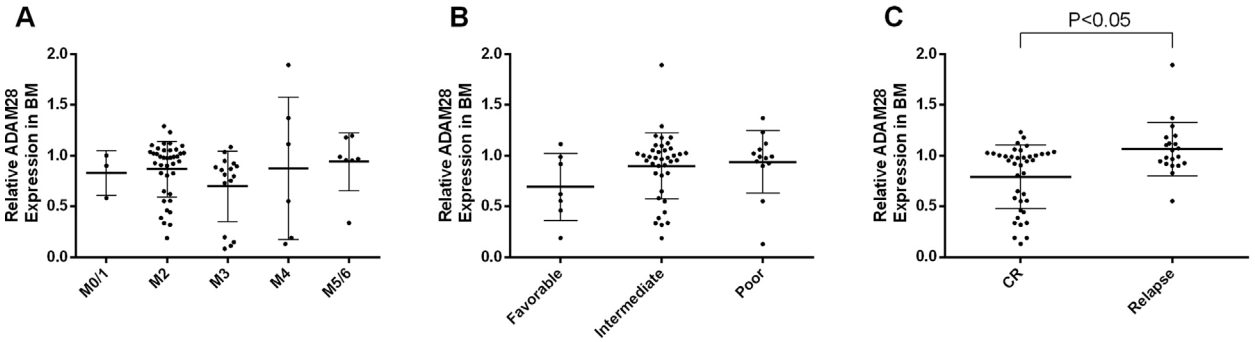

Fig. 6. ADAM28 expression in the validation group. A. ADAM28 expression in the bone marrow of the patients with different morphology types. B. No significant difference was detected for ADAM28 expression in the risk stratification. C. ADAM28 expression in the bone marrow differed significantly between the CR and relapsed patients with AML in the validation group. BM, bone marrow; CR, complete remission.

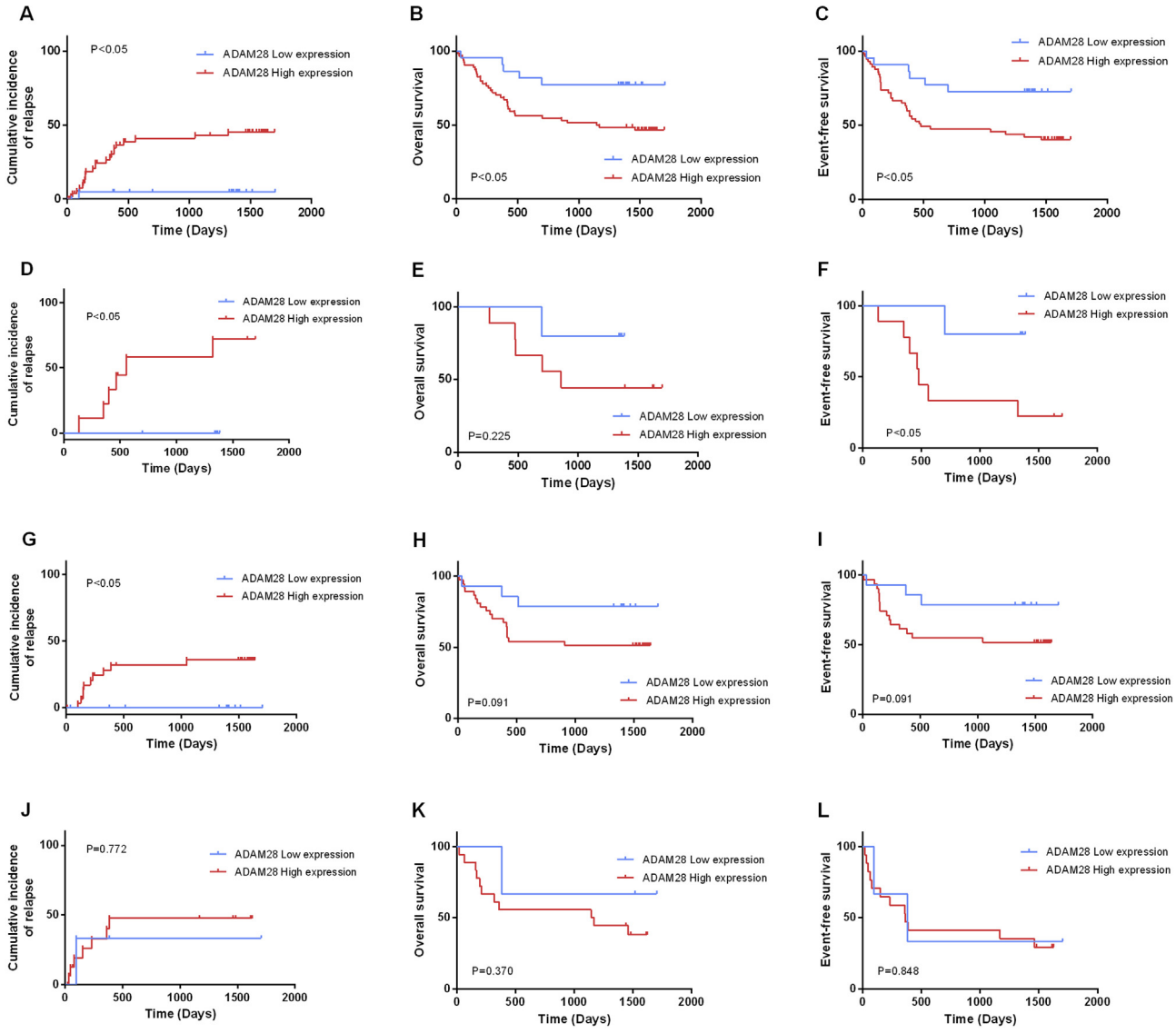

Fig. 7. Survival analysis of ADAM28 expression and outcomes of the AML patients except M3. CIR (A), OS (B) and EFS (C) differed significantly depending on the ADAM28 expression level in patients with AML. D: CIR in the favorable risk group. E: OS in the favorable risk group. F: EFS in the favorable risk group. G: CIR in the intermediate group. G: OS in the intermediate group. H: EFS in the intermediate group. I: CIR in the high-risk group. J: OS in the high-risk group. K: EFS in the high-risk group.

Table 2
Multivariable analysis of prognostic factors.

Covariate	CIR P	EFS P	OS P
ADAM28 expression (high vs low)	0.044	NS	NS
Treatment (HSCT vs CT)	0.003	0.000	0.002
Risk group (poor vs intermediate and favorable)	NS	NS	NS
WBC (≥20 vs < 20)	NS	NS	NS
Age (< 35 vs ≥ 35 years old)	NS	NS	NS

NS: Not Significant.

analyzed the prognosis relevance of ADAM28 in AML patients. Considering that APL is a particular subtype of AML, which has a distinct management strategy and prognosis, patient prognosis is analyzed without the inclusion of APL.

The CIR was significantly higher in the ADAM28 high expression group. This correlation was further confirmed in multivariate analysis. Risk stratification was not identified as independent prognostic factor in this study perhaps because patients with high risk of relapse were mostly allocated to receive more intensive treatment like HSCT. Also, subjects were not randomly-assigned to receive chemotherapy only or with an allotransplant so the cohorts may not be comparable for other predictive variables.

Moreover, when separately considering the impact of ADAM28 on prognosis within the risk stratifications, patients with high ADAM28 expression levels had a significantly higher CIR in the favorable and intermediate risk group and worse EFS in the favorable-risk group, but not in the poor-risk group. This indication is meaningful because it might be useful in identifying high risk of relapse in favorable risk group where there still is a subgroup of patients ended with poor prognosis.

Interestingly, the ADAM28 high expression patients presented a significantly higher CIR than the low expression patients in the chemotherapy subgroup, whereas the prognosis did not differ significantly with the ADAM28 expression level in the patients receiving a HSCT. These data suggest patients with high ADAM28 expression may benefit more from an allotransplant. Patients categorized as poor risk were directed to receive HSCT. The lack of impact of ADAM28 in poor risk group might also be attributed to the high rate of HSCT.

However, because of our non-random allocation to a transplant, this

conclusion needs validation.

With different treatment method and basic prognosis, the analysis of the patients with APL didn't showed a similar trend. Interestingly, when the median or arithmetic average was taken as the cut-off value for ADAM28 expression, the result of the CIR analyses showed a similar trend, which might be more convincible for the prognostic meaning of ADAM28 expression level in patients with AML.

There are several limitations to our study including small sample size. Also, subjects were not randomly-assigned to receive to chemotherapy only or with an allotransplant.

In summary, we demonstrated that ADAM28 improved the growth and dissemination of AML. ADAM28 expression levels also identified a new subgroup at a higher risk for relapse and with a poor prognosis in the favorable-risk AML patients, and this subgroup of patients, which were allocated to chemotherapy, might benefit more from HSCT..

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Peking University People's Hospital, and informed consent was obtained according to the Declaration of Helsinki.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.canlet.2018.10.028.

Conflicts of interest

The authors declare that they have no competing interests.

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