Overexpression of lncRNA ITGB2-AS1 Predicts Adverse Prognosis in Acute Myeloid Leukemia

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Research

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Abstract

Background

In recent years, lncRNA *ITGB2-AS1* has been found to play important roles in the occurrence and development of human solid tumors. However, its role in hematological diseases, especially acute myeloid leukemia (AML), remains unclear. Therefore, the aim of this study was to identify the expression pattern of *ITGB2-AS1* in AML patients and to further explore its clinical significance.

Methods

ITGB2-AS1 expression was analyzed in public datasets (including TCGA and GSE63270) and further validated in our cohort of 109 AML patients using real-time quantitative PCR (RQ-PCR).

Results

The level of *ITGB2-AS1* was up-regulated among two independent cohorts (TCGA, P<0.05; GSE63270, *P*<0.05), which was confirmed by our own data (*P*<0.05). Clinically, high *ITGB2-AS1* expression was associated with older age (*P*=0.023) and lower complete remission (CR) rate (*P*=0.005). Multivariate analysis identified that high *ITGB2-AS1* expression was an independent prognostic factor not only for CR rate (*P*=0.027) but also for overall survival (OS) time (*P*=0.011). *ITGB2-AS1* was found positively correlated with *ITGB2* expression in both TCGA (R=0.74, *P*<0.001) and our own data (R=0.881, *P*<0.001). Similarly, high *ITGB2* expression was also associated with older age (*P*=0.02) and lower CR rate (*P*=0.015). Moreover, high *ITGB2* expression also predicted worse OS (*P*=0.028).

Conclusion

ITGB2-AS1 is overexpressed in AML and predicts poor prognosis in AML.

1. Introduction

Acute myeloid leukemia (AML) is a highly heterogeneous disease in cytogenetics and molecular biology, which is characterized by poor differentiation and uncontrolled proliferation of immature myeloid progenitor cells [1]. At present, genetic abnormalities such as chromosomal aberrations and gene mutations are considered as the most powerful prognostic information [2]. However, AML patients with moderate cytogenetic risk perform differently in terms of chemotherapy consolidation, which means that more new markers involved in leukemogenesis and molecular stratification need to be further improved in order to better classify risks and ultimately find better treatments [3].

With the application of next-generation sequencing technology, thousands of LncRNA have been discovered in many solid tumors. Recent evidence suggests that these LncRNAs play a crucial role in gene regulation, thereby affecting various aspects of cell homeostasis, including proliferation, survival, migration, or genome stability [4]. LncRNA *ITGB2-AS1*, up-regulated in pancreatic cancer, breast cancer,

osteosarcoma, and ovarian cancer, plays an important role in promoting the proliferation, invasion, migration and metastasis of cancer cells, and is associated with poor prognosis [5-10]. *ITGB2*, as a gene on the *ITGB2-AS1* complementary chain, has also been found to be involved in tumor adhesion, invasion, angiogenesis and specific immune response [11]. In addition, beta 2-integrin-derived signaling has been revealed to induce survival and proliferation of neonatal AML cells by activating the Syk/STAT signaling axis [12]. However, the expression and clinical significance of *ITGB2-AS1* and *ITGB2* remain unknown in AML. Herein, this study was aimed to explore the expression pattern and clinical impact of *ITGB-AS1* and *ITGB2* in the context of known molecular prognosticators in AML, and found that *ITGB2-AS1* can serve as a biomarker for prognosis prediction.

2. Materials And Methods

2.1 Patients and samples

To analyze the prognostic impact of *ITGB2-AS1* in AML patients, three independent cohorts with survival information were included in this study: (1)TCGA datasets from GEPIA (http://gepia.cancer-pku.cn/detail.php), OncoLnc (http://www.oncolnc.org) and cBioPortal (http://www.cbioportal.org/); (2) The validation cohort consisted of 62 AML patients and 42 normal controls (GSE63270). Detailed information of the two cohorts are described in Additional Methods. (3) An independent cohort of 109 AML patients enrolled and treated in the People's Hospital affiliated to Jiangsu University from 2005 to 2016. All participants provided informed consents, and the study was approved by the Institutional Review Board of the Affiliated People's Hospital of Jiangsu University. BM mononuclear cells (BMMNCs) were isolated using lymphocyte separation medium (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China).

2.2 RNA isolation and reverse transcription

Total RNA was extracted from BMNC using Trizol reagent (Invitrogen Life Technologies, USA) as described previously [13-17]. 2 ug of total RNA were reverse transcribed into cDNA using 10 umol/l random primers, 200 U MMLV reverse transcriptase, 0.5 mmol/l dNTP, 10 mmol/l dithiothreitol, and 25 U RNase inhibitors.

2.3 RT-qPCR

ITGB2-AS1 expression was detected by real-time quantitative PCR (RT-qPCR) using AceQ qPCR SYBR Green Master Mix (Vazyme Biotech Co., Piscataway, NJ, USA) on a 7500 Thermo cycler (Applied Biosystems, CA). PCR primers were designed using Primer Premier 6 (Premier Biosoft, Palo Alto, CA, USA). The primers for *ITGB2-AS1* expression were 5'- TTGCTGTCAAAGCATGCCAC -3' (forward) and 5'- AAGGCAGCCCACACTTTTCT -3' (reverse). The primers for *ITGB2* expression were 5'- GATGACGGCTTCCATTTCGC -3' (forward) and 5'- TGGGGATGATCTCGGTGAGT -3' (reverse). Reaction conditions and PCR cycling were conducted as previously described [13-17] except for the optimized

primer annealing temperatures (60 °C). The relative quantification was calculated using the ΔΔCT method and normalized to the ABL1 housekeeping gene.

2.4 Karyotype and gene mutation detection

By conventional R-banding method, karyotype was analyzed at the time of initial diagnosis. Risk classification based on the karyotype findings has been done as previously described [13-17]. Mutations in C-KIT, NPM1, DNMT3A, N/K-RAS, and U2AF1 were detected by high-resolution melting analysis, whereas FLT3-ITD and CEBPA mutations were detected by direct DNA sequencing.

2.5 Statistical and bioinformatics analyses

SPSS software version 20.0 (IBM Corporation, Armonk, NY, USA) was used to carry out the statistical analysis. Receiver operating characteristic (ROC) curve and area under the ROC were applied to assess the value of *ITGB2-AS1* and *ITGB2*'s expression. Besides, Pearson's chi-squared analysis was conducted to determine the difference of categorical variables between *ITGB2-AS1* high group and *ITGB2-AS1* low group. Through Kaplan–Meier method and Cox regression analysis, the effect of *ITGB2-AS1* expression on prognosis was analyzed. Logistic regression analysis was used to identify the independent risk factors on complete remission (CR). In all tests, $P<0.05$ was defined as statistically significant. R script was used for plotting gene volcano maps co-expressed with *ITGB2-AS1*. Details of bioinformatics and receiver operating characteristic (ROC) curve were shown in Additional Methods.

3. Results

3.1 Expression pattern of *ITGB2-AS1* and *ITGB2* in AML

By using the GEPIA data (http://gepia.cancer-pku.cn/detail.php), we found that the expression of *ITGB2-AS1* was significantly increased in AML patients compared with normal BM samples (Fig. 1a, $P<0.001$). A similar result was also found in another dataset (GSE63270; Fig. 1b, $P<0.001$). In order to confirm the results, we further analyzed the expression of *ITGB2-AS1* in our cohort of 109 AML patients and 31controls. *ITGB2-AS1* expression was consistently up-regulated in whole cohort AML, Non-M3-AML and CN-AML compared with controls (Fig. 1c; $P<0.0003$, $=0.001$, and <0.0001, respectively). Next, we identified the positive correlation between *ITGB2-AS1* and 37 genes from 18107 genes using cBioPortal (Fig. 3a, $R>0.7$, $P<0.05$). Among them, *ITGB2* attracted our attention because of its special position on chromosome. We analyzed the expression of *ITGB2* in the GSE63270 dataset (Figure. 2b, $P<0.001$), the online website GEPIA (Fig. 2a, $P<0.001$) as well as our cohorts (Fig. 2c, $P=0.0009$, $=0.0014$, and <0.0001, respectively), and found that ITGB2 was also upregulated in AML. Furthermore, the positive correlation was confirmed between *ITGB2* and IGB2-AS1 expression in our cohort (Fig. 3b).

3.2 Association between *ITGB2-AS1* expression and clinical characteristics

109 AML patients of our cohort were divided into two subgroups (*ITGB2-AS1*^{high} and *ITGB2-AS1*^{low}) according to the median level of *ITGB2-AS1* transcript. The comparison of clinical/laboratory characteristics between the two subgroups was shown in Table1. No significant differences were observed in peripheral blood counts, BM blasts, FAB classification, cytogenetics, and common gene mutations (P>0.05). However, *ITGB2-AS1*^{high} patients were older than those *ITGB2-AS1*^{low} patients (P=0.023). Moreover, CR rate was significantly lower in *ITGB2-AS1*^{high} patients than in *ITGB2-AS1*^{low} patients (P=0.005).

3.3 Association between *ITGB2* expression and clinical characteristics

The whole cohort of AML patients was also divided into two subgroups according to the median level of *ITGB2* transcript (*ITGB2*^{high} and *ITGB2*^{low}) (Table2). Consistent with the results of *ITGB2-AS1*, significant differences in age and CR rate were also revealed between the two subgroups (P=0.015 and =0.02, respectively).

3.4 Effect of *ITGB2-AS1* and *ITGB2* expression on chemotherapy response in AML

Among our cohort, 88 patients had available follow-up data. We found that *ITGB2-AS1*^{high} patients had a lower CR rate (P=0.005, Table 1). Additionally, clinical characteristics in patients with and without CR were further compared. Significant differences were found in *ITGB2-AS1* expression, age, WBCs, platelets, BM blast, and risk group (P<0.05, Table 3). However, there was no significant difference in the expression of *ITGB2* in patients with and without CR (P=0.065, Table 3). Logistic regression analysis including the most predictive factors was further performed, which revealed that *ITGB2-AS1* expression was an independent risk factor affecting CR in whole-cohort AML patients (P=0.027, Table4).

3.5 Association between *ITGB2-AS1* expression and prognosis in AML patients

To further explore the prognostic relevance of *ITGB2-AS1* expression, we investigated the correlation between *ITGB2-AS1* expression and clinical outcomes in two independent AML cohorts. *ITGB2-AS1*^{high} patients in TCGA dataset had significantly reduced OS (Fig. 4a, P=0.012), which was validated in our cohort (Fig. 4b, 4c and 4d). Our data also demonstrated that *ITGB2-AS1*^{high} patients had significantly reduced LFS (Fig. 4e, P=0.043). Moreover, Cox regression analysis also confirmed that *ITGB2-AS1* expression independently affected the OS (P=0.019, Table5; P=0.026, Table6) and leukemia-free survival (LFS) (P=0.005, Table7) in our cohort.

3.6 Association between *ITGB2* expression and prognosis in AML patients

Similar results were shown in AML patient with *ITGB2* over expression, though the high expression of *ITGB2* was not an independent prognostic risk factor (P=0.589, Table5), it tended to indicate poor prognosis. By using the OncoLnc, we found that *ITGB2*^{high} patients in TCGA dataset had significantly reduced OS (Fig. 5a, P<0.010), which was confirmed in our whole patient cohort (Fig. 5b, P=0.020), but

not in non-M3 AML (Fig. 5c, *P*=0.106) and CN-AML (Fig. 5d, *P*=0.094). In addition, the expression of *ITGB2* in AML patients had the trend affecting on LFS (Fig. 5e, *P*=0.078).

4. Discussion

Over the past decades, the importance of non-coding RNA has received increasing attention [18]. Numerous studies have found that lncRNAs play important roles in the proliferation, differentiation and apoptosis of cells [19-23]. For example, the expression of *PVT1* can induce apoptosis and necrosis of AML cells by downregulating the expression of c-Myc [24]. Recent studies have shown that lncRNA *UCA1* and *CRNDE* also play an important role in the proliferation and differentiation of AML cells [25, 26]. The study of Zhang et al. showed that *HOTAIRM1* affects the differentiation and maturation of myeloid cells by regulating the expression level of the annexin gene, and downregulation of *HOTAIRM1* expression will prevent all-trans retinoic acid (ATRA)-induced granulocyte differentiation [27].Therefore, gaining insight into the role of lncRNA in AML may provide opportunities for early diagnosis and therapeutic targeting of AML.

The roles of *ITGB2-AS1* in tumorigenesis has just been explored in a few solid tumors [5-10]. Initially, *ITGB2-AS1* was found overexpressed while its promoter was highly methylated in pancreatic cancer [5]. At a similar time, Liu et al identified that *ITGB2-AS1* is upregulated and associated with poor survival in breast cancer [6]. Their further studies disclosed that *ITGB2-AS1* could induce *ITGB2* expression in breast cancer cells and then promote the migration and invasion. Then, upregulation of *ITGB2-AS1* and prognostic relevance was discovered in osteosarcoma, ovarian cancer and pancreatic cancer [7-9].

As far as we know, this is the first study on *ITGB2-AS1* in leukemia. We found that and *ITGB2-AS1* was significantly up-regulated in AML patients compared to controls. Moreover, we revealed that *ITGB2-AS1* overexpression may have an adverse impact on chemotherapy response, which was confirmed by the lower CR rate. Furthermore, we also confirmed the adverse effect of *ITGB2-AS1* overexpression on survival. All these results indicate that *ITGB2-AS1* can add additional prognostic information by stratifying molecularly defined patients into more homogeneous groups and help to select better treatment strategies. Without doubt, prospective studies are needed to confirm the prognostic prediction of *ITGB2-AS1* overexpression before it can be clinically used in AML.

Integrin family has been shown to be involved in leukemogenesis [28, 29]. Our previous studies disclosed the clinical relevance of two members of integrin family, *ITGA2* and *ITGBL1* [30, 31]. In this study, we demonstrated for the first time that *ITGB2* is also overexpressed in AML patients. In addition, we also found the positive correlation between *ITGA2-AS1* with *ITGB2* in AML. The effects of *ITGB2* overexpression on chemotherapy response and survival were found by univariate analysis, but not by multivariate analysis. More cases should be investigated to reveal the significance of *ITGB2* aberration in AML. Further functional studies of *ITGB2* and *ITGB2-AS1* in leukemia are also needed.

In conclusion, our results indicate that *ITGB2-AS1* is overexpressed in AML and is an independent poor prognostic factor in AML. Furthermore, *ITGB2* expression is also upregulated in AML and is associated

with *ITGB2-AS1* expression.

Abbreviations

lncRNA: long non-coding RNA

AML: acute myeloid leukemia

BM: bone marrow

PB: peripheral blood

OS: overall survival

LFS: leukemia-free survival time

CR: complete remission

RT-qPCR: real-time quantitative PCR

TCGA: The Cancer Genome Atlas

BMMNCs: Bone Marrow Mononuclear Cells

WBC: white blood cell

CN: cytogenetically normal

Declarations

Ethics approval and consent to participate

The study was approved by the Clinical Research Ethics Committee of the Affiliated People's Hospital of Jiangsu University.

Consent for publication

Written informed consent was obtained from all enrolled individuals before their participation.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' Contributions

QJ and Y-l Z designed the study and wrote the paper; Y-l Z performed all experiments; Y-l Z and Z-j X analyzed the data; Z-j X, J-d Z, and T-j Z were involved in the delivery of the clinical data; J L, J-c M,J Q and D-m Y offered technique and language support. All authors read and approved the final manuscript.

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Tables

Table 1. Association between *ITGB2-AS1* expression and clinical characteristics

Patient's parameters	*ITGB2-AS1* expression		
	High (n=54)	Low (n=55)	P-value
sex, male/female	28/26	35/20	0.241
Median age, years (range)	58(20-84)	52(24-84)	0.023
Median WBC, ×10 9/l (range)	51.4(0.3-528)	34.4(0.3-207.9)	0.239
Median hemoglobin, g/l (range)	80.70(49-135)	84.02(34-141)	0.411
Median platelets, ×109/l (range)	58 (7-382)	52(3-415)	0.328
BM blasts, % (range)	46.6(1-97.5)	46.6(3-92)	0.958
Cytogenetics			0.578
Normal	30(55.6%)	25(45.5%)	
t(15,17)	9(16.7%)	9(16.4%)	
t(8,21)	1(1.9%)	6(10.9%)	
Inv(16)	1(1.9%)	0	
+8	2(3.7%)	2(3.6%)	
-7/del(7)	1(1.9%)	0	
Others	4(7.4)	6(10.9)	
Complex	4(7.4%)	4(7.4%)	
No data	2(3.7%)	2(3.6%)	
FAB class classifications			0.152
M0	0	1(1.8%)	
M1	0	1(1.8%)	
M2	15(27.8%)	22(40.0%)	
M3	10(18.5%)	10(18.2%)	
M4	15(27.8%)	6(10.9%)	
M5	5(9.3%)	6(10.9%)	
gene mutation			
CEBPA (+/−)	4/35	5/32	0.466
NPM1 (+/−)	1/38	4/33	0.194
FLT3-ITD (+/−)	2/37	5/32	0.256
C-KIT (+/−)	1/38	2/35	0.610

N/K-RAS (+/−)	3/29	1/27	0.616
IDH1/2 (+/−)	1/38	0/37	-
U2AF1 (+/−)	1/38	1/37	1.000
DNMT3A (+/−)	2/37	4/33	0.425
CR (−/+)	27/17	13/31	0.005

Abbreviations: BM, bone marrow; CR, complete remission; FAB, French-American-British; WBC, white blood cells.

Table 2. Association between *ITGB2* expression and clinical characteristics

Patient's parameters	*ITGB2* expression		
	High (n=55)	Low (n=54)	P-value
Sex, male/female	28/27	35/19	0.176
Median age, years (range)	58 (20-84)	51(22-84)	0.015
Median WBC, ×10 9/l (range)	49.9 (0.3-528)	35.0(0.3-207.9)	0.292
Median hemoglobin, g/l (range)	80(49-135)	85(34-141)	0.105
Median platelets, ×109/l (range)	56 (4-383)	52 (3-415)	0.355
BM blasts, % (range)	45.6(1-97.5)	46.6(3-92)	0.457
Cytogenetics			0.092
Normal	31□56.4%□	24(44.4%)	
t(15,17)	0	9(16.7%)	
t(8,21)	2(3.6%)	5(9.3)	
Inv(16)	1(1.8%)	0	
+8	2(3.6%)	2(3.7%)	
Others	4(7.3%)	6(11.1%)	
Complex	4(7.3%)	4(7.4%)	
No data	2(3.6)	2(3.7%)	
FAB class classifications			0.160
M0	0	1(1.9%)	
M1	0	1(1.9%)	
M2	15(27.3%)	22(40.7%)	
M3	10(18.2%)	10(18.5%)	
M4	15(27.3%)	6(11.1%)	
M5	5(9.1%)	6(11.1%)	
gene mutation			
CEBPA (+/−)	5/35	4/32	1.00
NPM1 (+/−)	1/39	4/32	0.184
FLT3-ITD (+/−)	2/38	5/31	0.246
C-KIT (+/−)	1/39	2/34	0.601

N/K-RAS (+/−)	3/29	1/27	0.616
IDH1/2 (+/−)	1/39	0/36	-
U2AF1 (+/−)	1/39	1/36	1.00
DNMT3A (+/−)	2/38	4/32	0.414
CR (−/+)	19/26	29/14	0.02

Abbreviations: BM, bone marrow; CR, complete remission; FAB, French-American-British; WBC, white blood cells

Table3. Comparison of clinical manifestations and laboratory features between CR and non-CR in AML patients receiving induction therapy

Patient's parameters	**Complete remission**		
	Yes (n=48)	No (n=40)	P-value
ITGB2-AS1 expression	13.92(0.08-194.46)	18.84(0.17-23.26)	0.033
ITGB2 expression	14.86(0.10-104.88)	14.44(0.69-142.17)	0.065
sex, male/female	23/25	27/13	0.085
Median age, years (range)	50(24-78)	59(20-77)	0.001
Median WBC, ×10 9/l (range)	34.8(0.3-528)	59.2(0.4-207.5)	0.001
Median hemoglobin, g/l (range)	83(34-135)	82(49-121)	0.899
Median platelets, ×109/l (range)	40(3-192)	64(9-415)	0.018
BM blasts, % (range)	39.7(1-97.5	52.2(6.5-92.0)	0.032
Cytogenetics			0.034
Normal	21(43.8%)	21(52.5%)	
t(15,17)	13(27.1%)	3(7.5%)	
t(8,21)	7(14.6%)	0	
Inv(16)	0	1(2.5%)	
+8	1(2.1%)	2(5%)	
-7/del(7)	0	1(2.5%)	
Others	2(4.2%)	4(10%)	
Complex	3(6.3%)	4(10%)	
No data	1(2.1%)	3(7.5%)	
FAB class classifications			0.034
M0	0	1	
M1	0	1	
M2	19	16	
M3	14	4	
M4	8	13	
M5	4	5	
gene mutation			
CEBPA (+/−)	4/27	5/32	1.000
NPM1 (+/−)	2/29	2/35	1.000

FLT3-ITD (+/−)	3/28	3/34	1.000
DNMT3A (+/−)	3/28	3/34	1.000

AML, acute myeloid leukemia; BM, bone marrow; CR, complete remission; WBC, white blood cell.

Table 4. Univariate and multivariate analyses of variables for complete remission in whole-cohort AML patients

Variables	CR			
	Univariate analysis		Multivariate analysis	
	OR (95% CI)	P-Value	OR (95% CI)	P-Value
Age	0.195(0.076-0.497)	0.001	0.337(0.121-0.939)	0.037
WBC	0.994(0,987-1.002)	0.143	0.638(0.209-1.952)	0.431
ITGB2-AS1 expression	0.264(0.109-0.641)	0.003	0.330(0.123-0.880)	0.027
ITGB2 expression	0.353(0.148-0.842)	0.019	492849290.2(0.000-)	0.999
Cytogenetic risk	0.387(0.194-0.770)	0.007	0.464(0.220-0.978)	0.043
CEBPA mutation	1.055(0.257-4.324)	0.941	-	-
NPM1 mutation	0.829(0.110-6.251)	0.855	-	-
FLT3-ITD (+/−)	0.824(0.154-4.404)	0.820	-	-
DMT3A (+/−)	0.824(0.154-4.404)	0.820	-	-

Notes: Variables including WBC (≥30×109 vs 60 years), *ITGB2-AS1* expression (low vs high), *ITGB2* expression (low vs high), risk classification (favorable vs intermediate vs poor), and gene mutations (mutant vs wild type). Multivariate analysis includes variables with $P<0.200$.

AML, acute myeloid leukemia; CR, complete remission; OR, odds ratio; WBC, white blood cell.

Table 5. Univariate and multivariate analyses of prognostic factors for overall survival in whole-AML patients

Variables	OS			
	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P-Value	HR (95% CI)	P-Value
Age	2.772(1.587-4.843)	0.000	0.923(0.366-2.325)	0.864
WBC	1.001(0.999-1.004)	0.217	-	-
ITGB2-AS1 expression	2.121(1.326-3.391	0.010	2.317(1.149-4.670)	0.019
ITGB2 expression	1.861(1.076-3.218)	0.026	0.668(0.154-2.887)	0.589
Cytogenetic risk	2.327(1.629-3.323)	0.000	2.229(1.429-3.475)	0.000
CEBPA mutation	1.346(0.558-3.250)	0.508	-	-
NPM1 mutation	1.101(0.338-3.591)	0.873	-	-
FLT3-ITD (+/−)	0.880(0.312-2.483)	0.809		
C-KIT (+/−)	0.047(0.000-62.202)	0.404	-	-
N/K-RAS (+/−)	2.988(1.036-8.618)	0.043	21.444(0.333-6.261)	0.623
IDH1/2 (+/−)	6.958(0.870-55.647)	0.067	6.021(0.730-49.632)	0.095
U2AF1 (+/−)	5.674(1.272-25.318)	0.023	5.538(1.222-25.102)	0.026
DNMT3A (+/−)	1.526(0.593-3.923)	0.381	-	-

Notes: Variables including WBC (≥30×109 vs 60 years), *ITGB2-AS1* expression (low vs high), *ITGB2* expression (low vs high), risk classification (favorable vs intermediate vs poor), and gene mutations (mutant vs wild type). Multivariate analysis includes variables with $P<0.200$.

AML, acute myeloid leukemia; HR, hazard ratio; OS, overall survival; WBC, white blood cell.

Table 6. Univariate and multivariate analyses of prognostic factors for overall survival in non-M3 AML patients

Variables	OS			
	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P-Value	HR (95% CI)	P-Value
Age	1.693(0.974-43.027)	0.076	0.765(0.308-1.902)	0.565
WBC	1.736(0.973-3.099)	0.062	0.765(0.308-1.905)	0.565
ITGB2-AS1 expression	1.871(1.040-3.365)	0.036	2.231(1.100-4.522)	0.026
ITGB2 expression	1.586(0.886-2.838)	0.120	0.795(0.169-3.745)	0.772
Cytogenetic risk	1.914(1.259-2.993)	0.003	2.301(1.470-3.603)	0.000
CEBPA mutation	1.138(0.467-2.774)	0.776	-	-
NPM1 mutation	0.880(0.267-2.899)	0.834	-	-
FLT3-ITD (+/−)	0.910(0.313-2.646)	0.863		
C-KIT (+/−)	0.045(0.000-32.094)	0.355	-	-
N/K-RAS (+/−)	2.821(0.967-8.233)	0.058	1.487(0.342-6.464)	0.597
IDH1/2 (+/−)	8.824(1.031-75.542)	0.047	6.097(0.737-50.410)	0.093
U2AF1 (+/−)	6.441(1.398-29.678)	0.017	5.573(1.266-25.340)	0.026
DNMT3A (+/−)	1.265(0.488-3.282)	0.629	-	-

Notes: Variables including WBC (≥30×109 vs 60 years), *ITGB2-AS1* expression (low vs high), *ITGB2* expression (low vs high), risk classification (favorable vs intermediate vs poor), and gene mutations (mutant vs wild type). Multivariate analysis includes variables with $P<0.200$.

AML, acute myeloid leukemia; HR, hazard ratio; OS, overall survival; WBC, white blood cell.

Table7. Univariate and multivariate analyses of prognostic factors for leukemia-free survival in whole-AML patients

Variables	LFS			
	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P-Value	HR (95% CI)	P-Value
Age	2.528(1.413-4.488)	0.002	1.148(0.526-2.506)	0.728
WBC	2.136(1.215-3.754)	0.008	1.200(0.611-2.356)	0.597
ITGB2-AS1 expression	2.052(1.161-3.628)	0.013	2.724(1.347-5.509)	0.005
ITGB2 expression	1.825(1.038-3.206)	0.037	0.793(0.175-3.588)	0.764
Cytogenetic risk	1.981(1.381-2.842)	0.000	1.960(1.281-3.000)	0.002
CEBPA mutation	1.133(0.472-2.717)	0.780	-	-
NPM1 mutation	1.024(0.315-3.333)	0.968	-	-
FLT3-ITD (+/−)	0.998(0.355-2.906)	0.997	-	-
C-KIT (+/−)	0.046(0.00-71.024)	0.412	-	-
N/K-RAS (+/−)	2.457(0.847-7.127)	0.098	2.145(0.729-6.310)	0.166
IDH1/2 (+/−)	2.235(0.304-16.441)	0.430	-	-
U2AF1 (+/−)	2.283(0.541-9.631)	0.261	-	-
DNMT3A (+/−)	1.357(0.530-3.471)	0.525	-	-

Notes: Variables including WBC (≥30×109 vs 60 years), *ITGB2-AS1* expression (low vs high), *ITGB2* expression (low vs high), risk classification (favorable vs intermediate vs poor), and gene mutations (mutant vs wild type). Multivariate analysis includes variables with $P<0.200$.

AML, acute myeloid leukemia; HR, hazard ratio; LFS, leukemia-free survival; WBC, white blood cell.

Figures

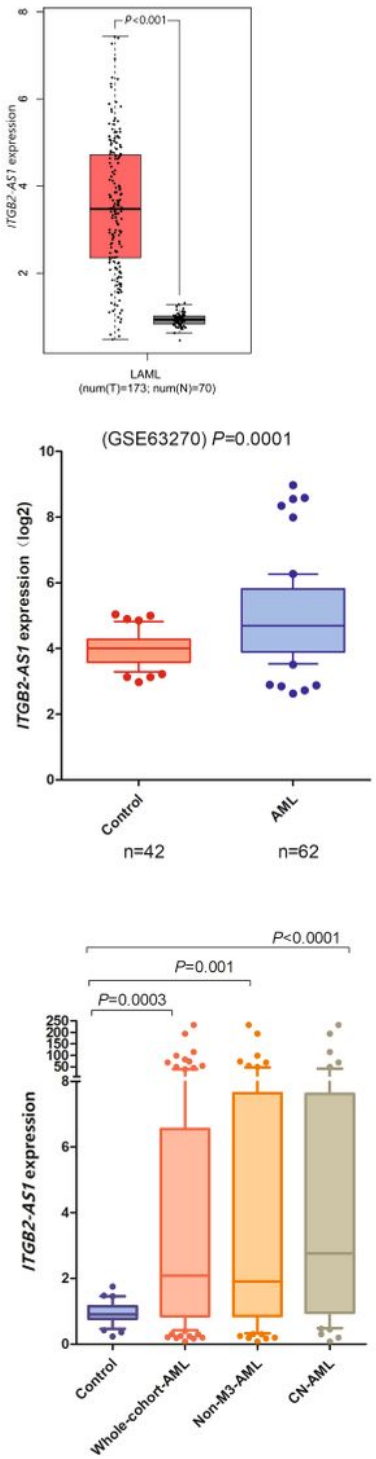
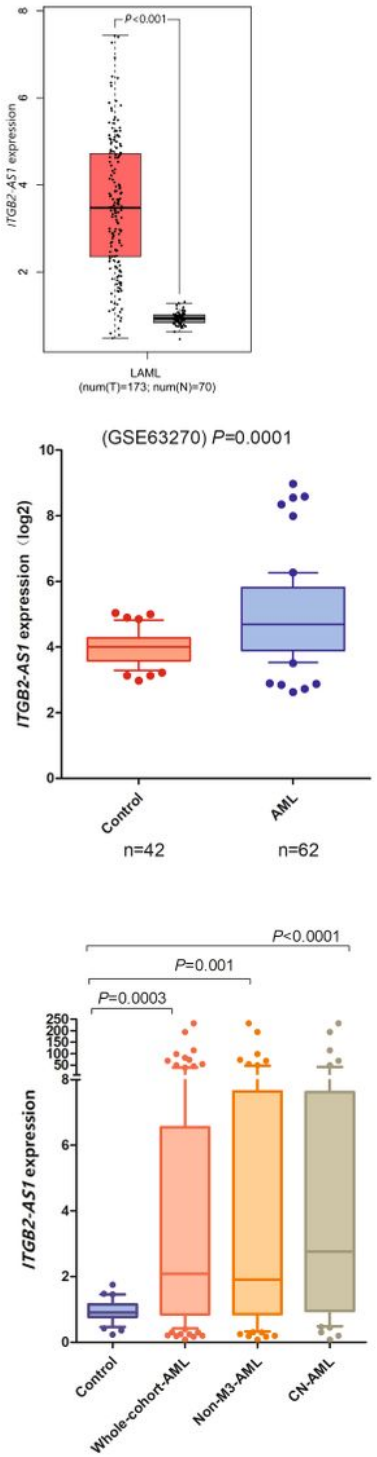


Figure 1

ITGB-AS1 over expression in AML a. ITGB2-AS1 expression in controls and AML patients from TCGA datasets using the GEPIA (http://gepia.cancer-pku.cn/detail.php). b. Box plot showing ITGB2-AS1 expression differences in normal controls and AML patients, from GEO: GSE63270, calculated using the Mann-Whitney test. c. ITGB2-AS1 expression differences in normal controls, whole-cohort AML patients, non-M3 AML patients, and CN-AML patients, calculated using the Mann-Whitney test.



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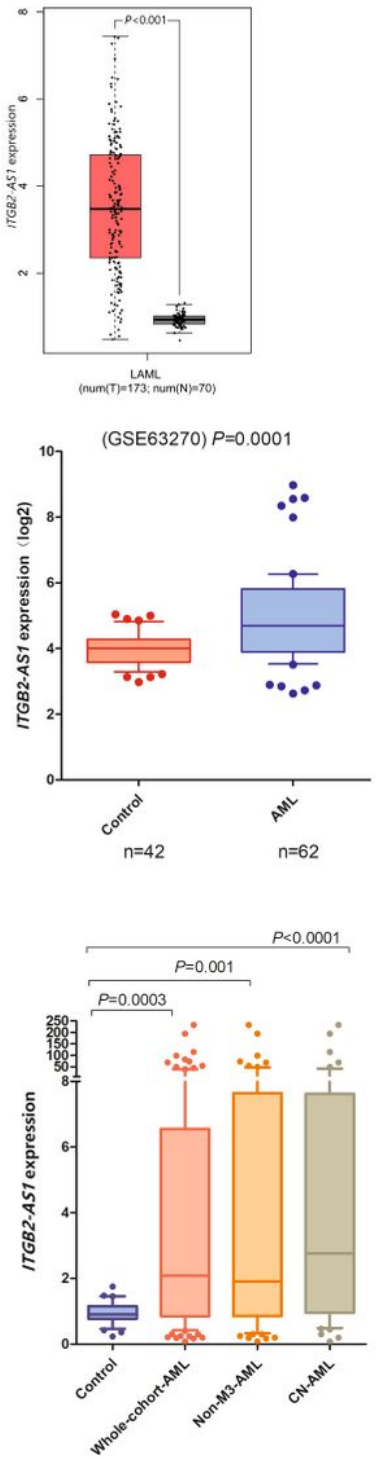

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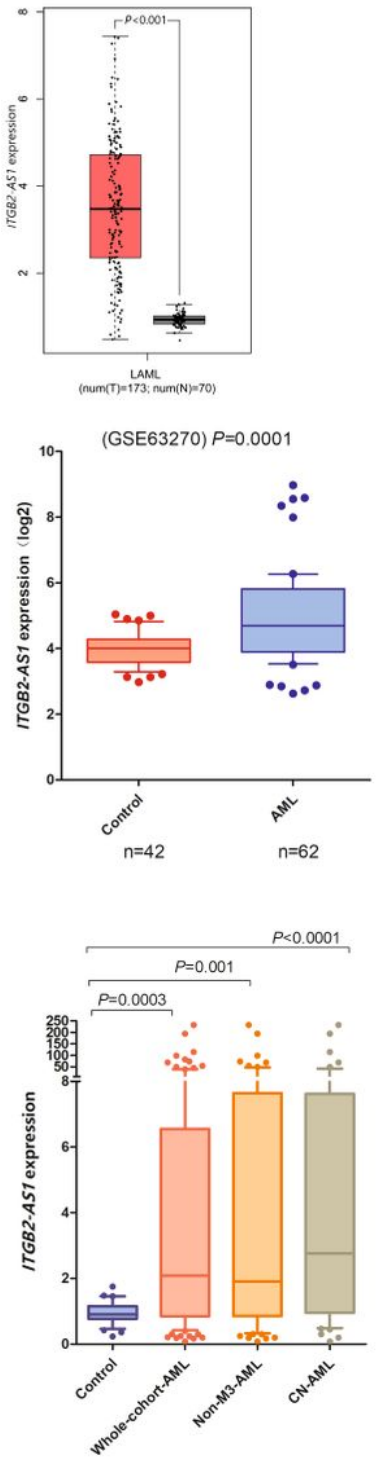

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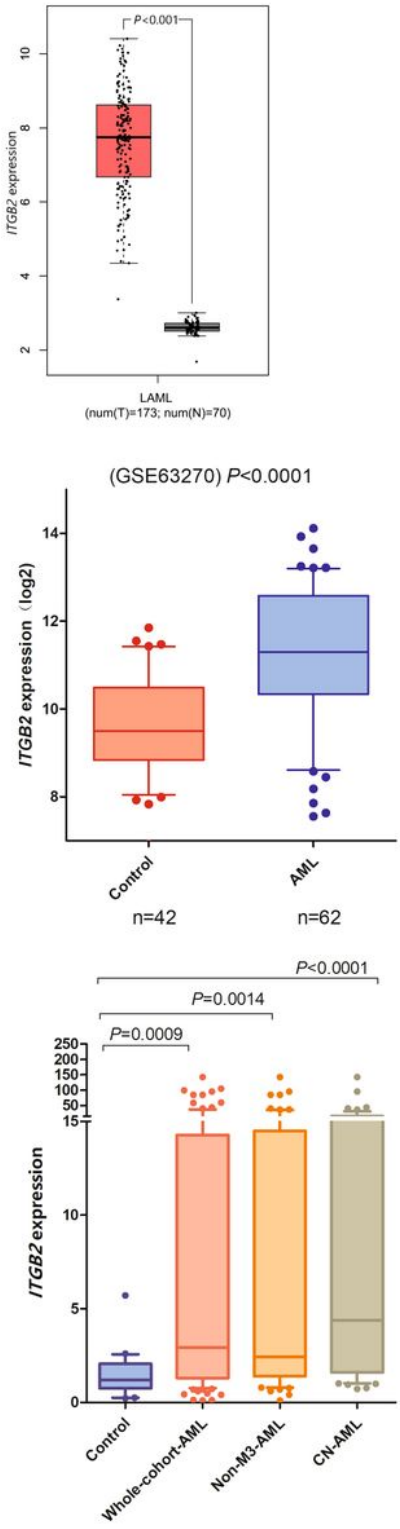

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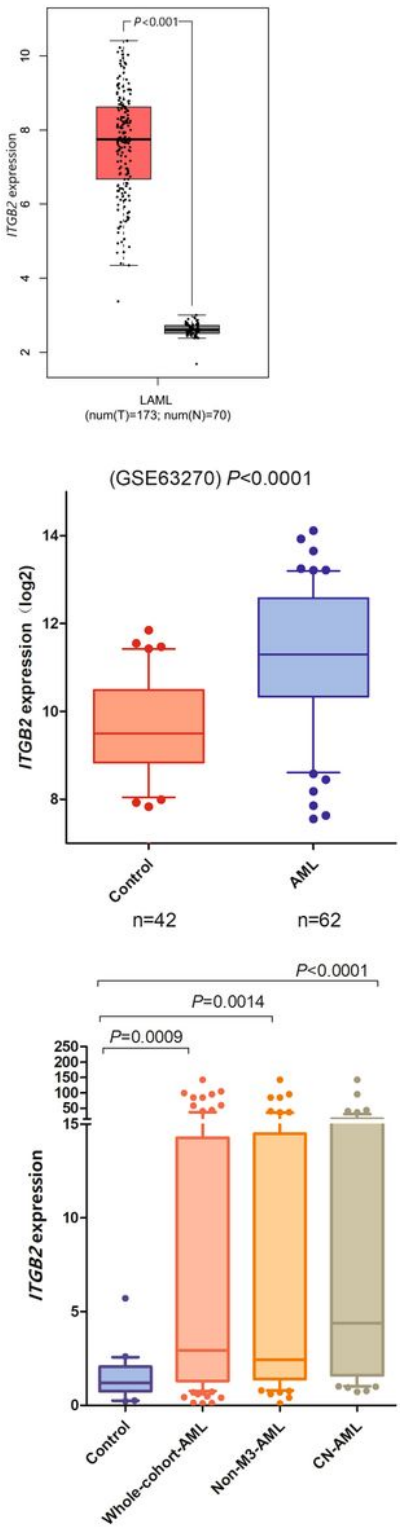

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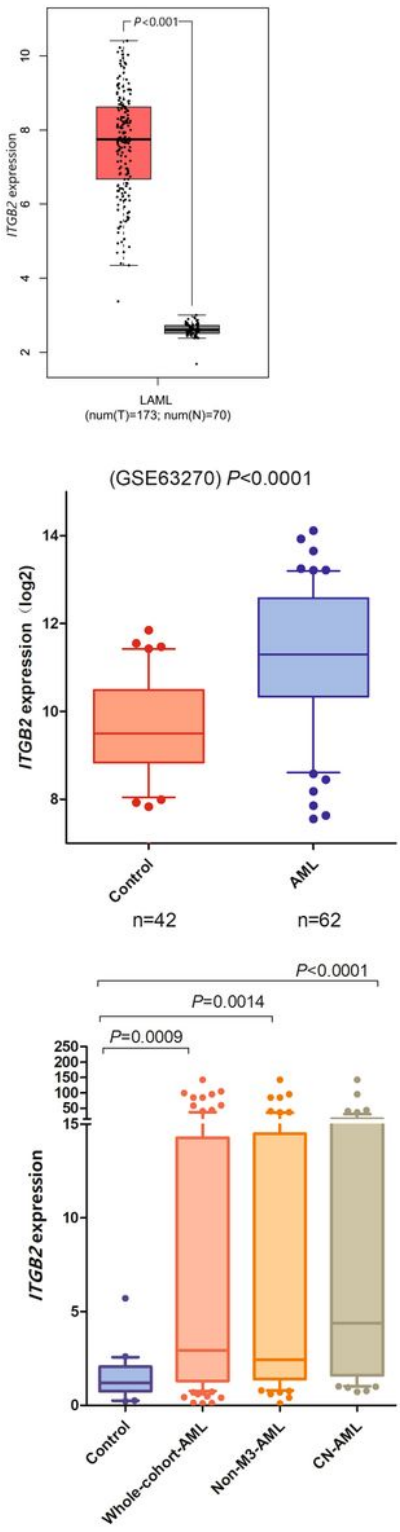

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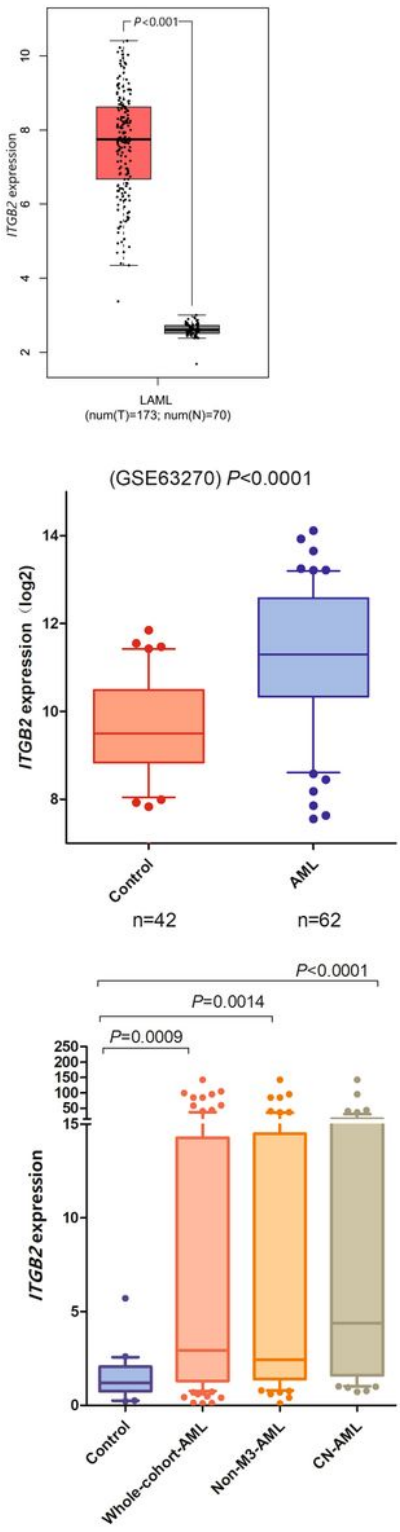

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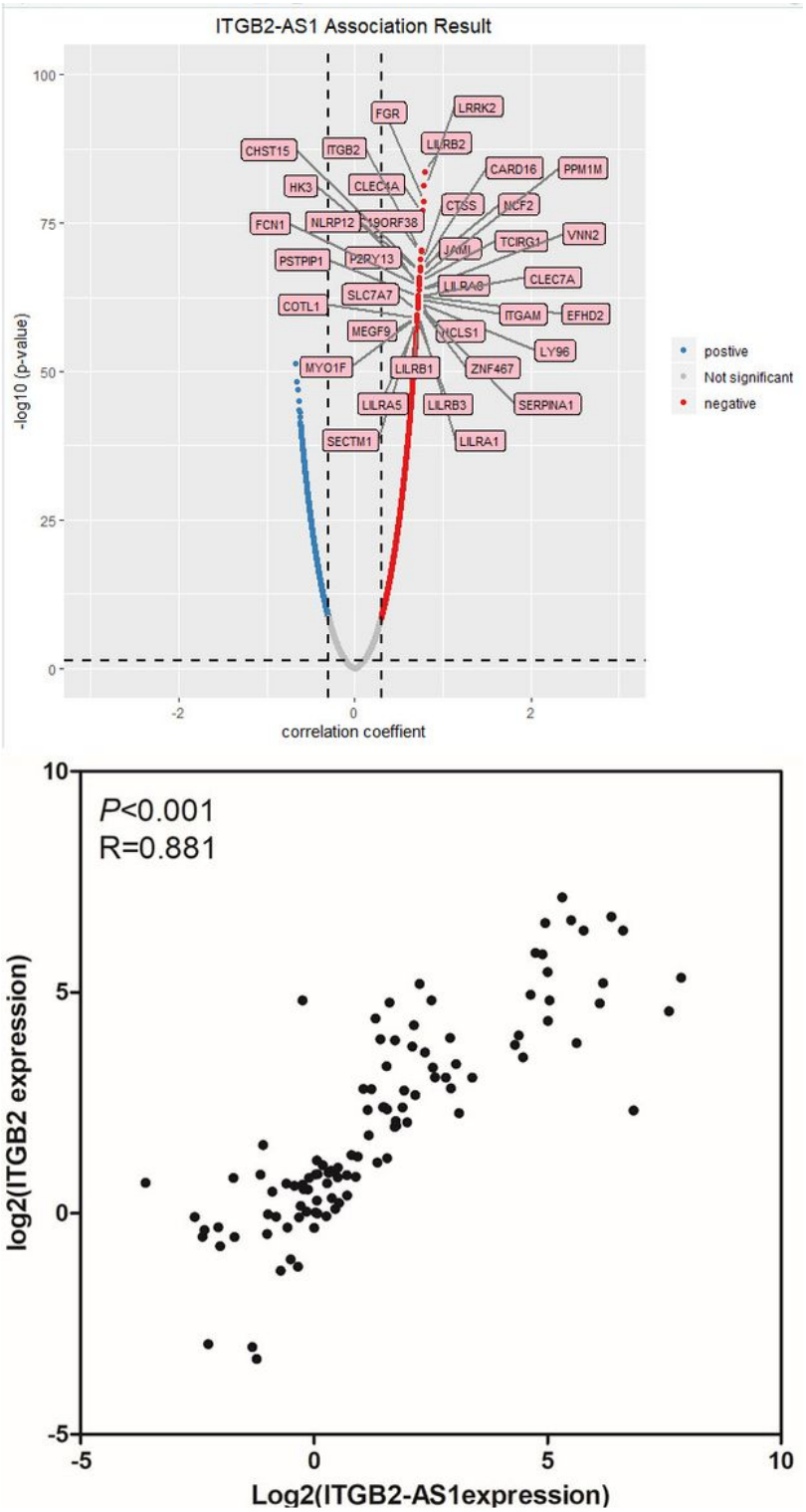
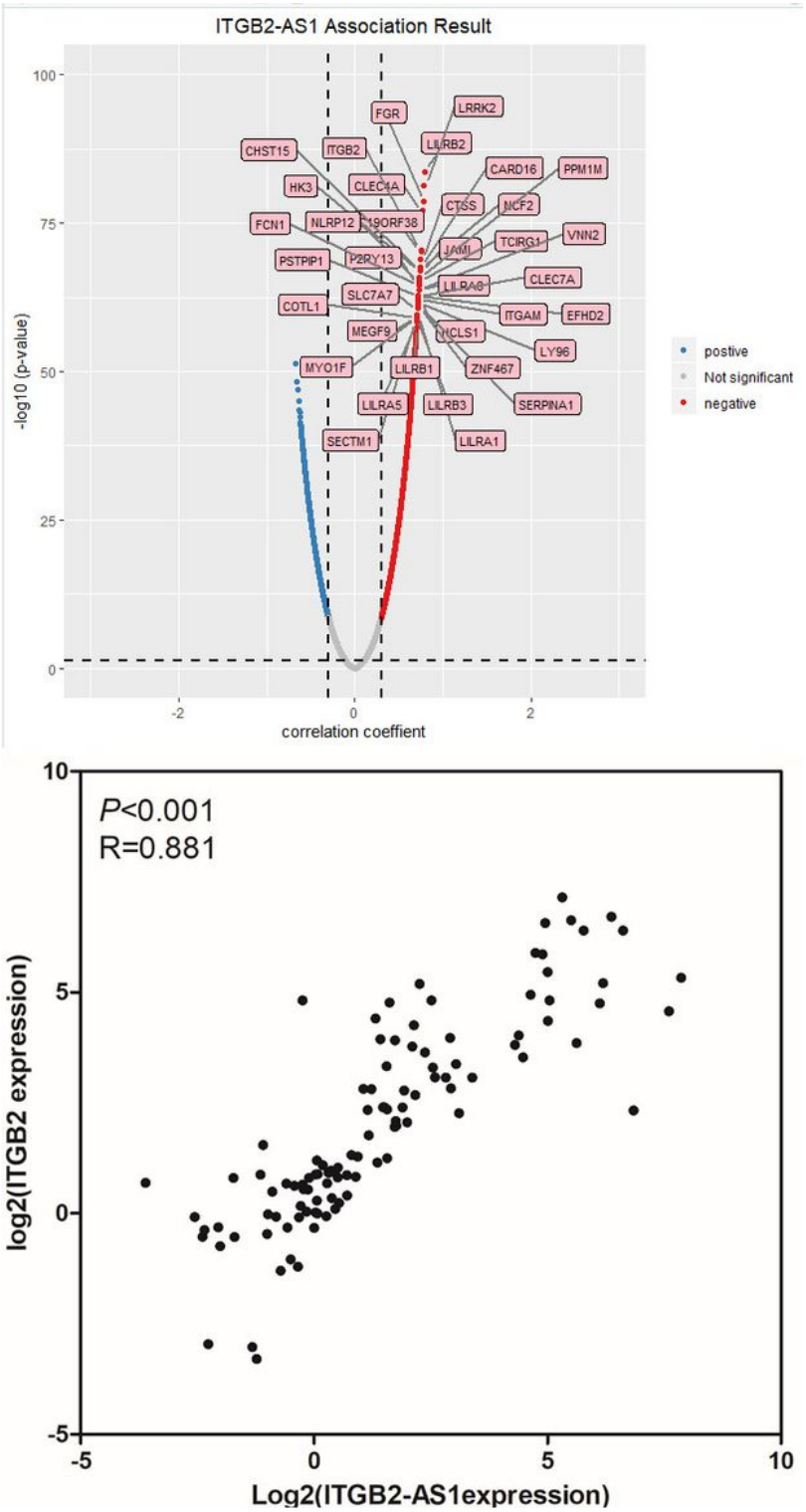


Figure 3

Genes related to ITGB2-AS1 a. Volcano Map showing the correlation between 18107 genes and ITGB2-AS1, data from TCGA (downloaded from website GEPIA), plotted used R script (details of programming code in additional files). The37 genes annotated in the graph were significantly positively correlated with ITGB2-AS1 (R>0.7, P<0.05). b. The positive correlation between ITGB2 and IGB2-AS1 expression in AML patients in our cohort (R=0.8811, P<0.01).



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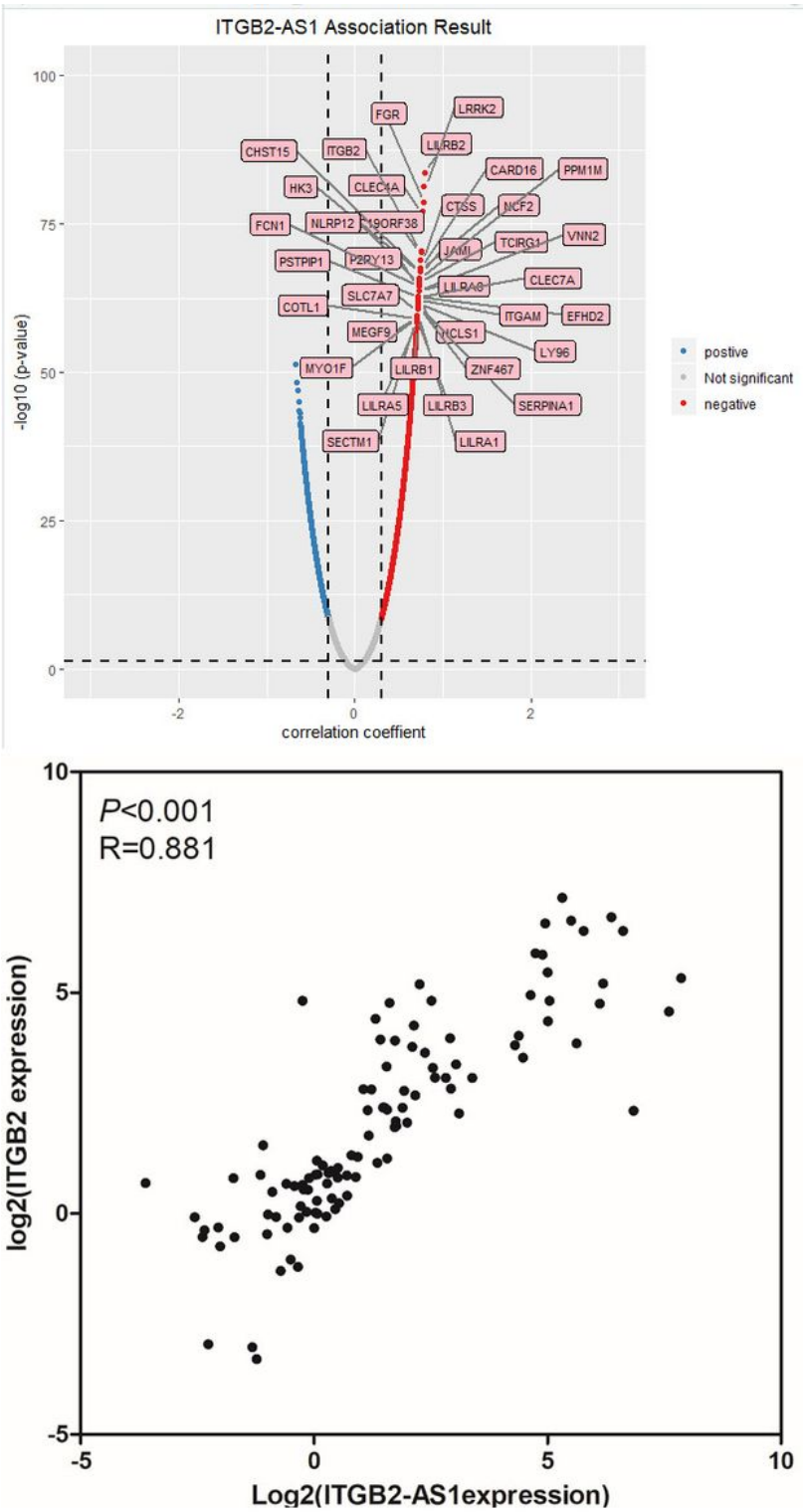

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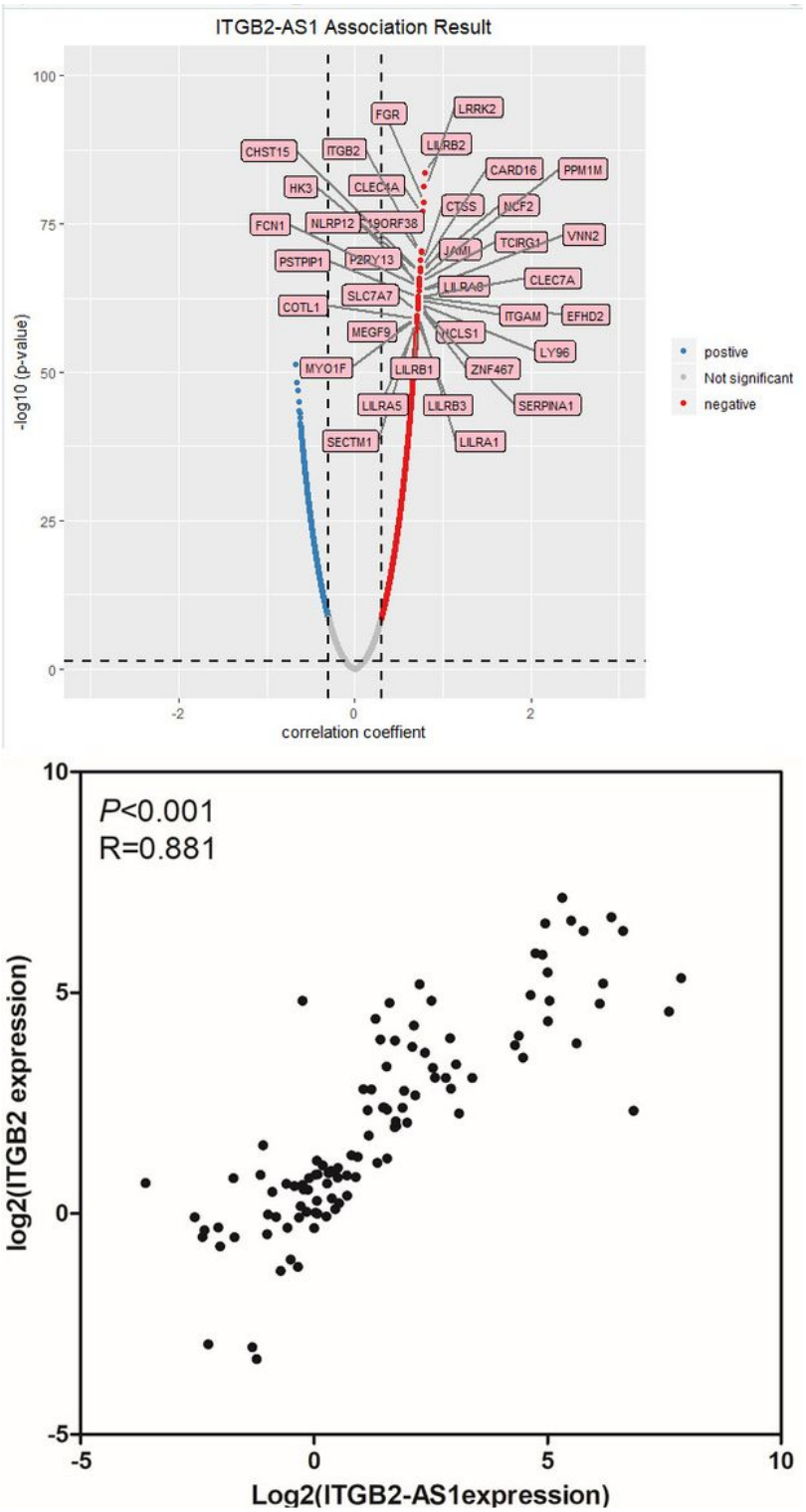

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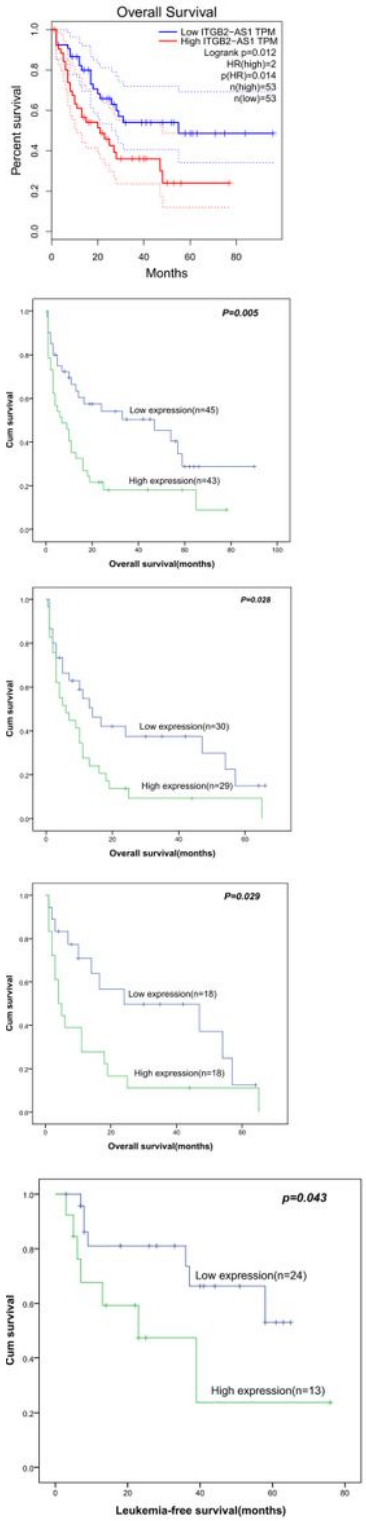

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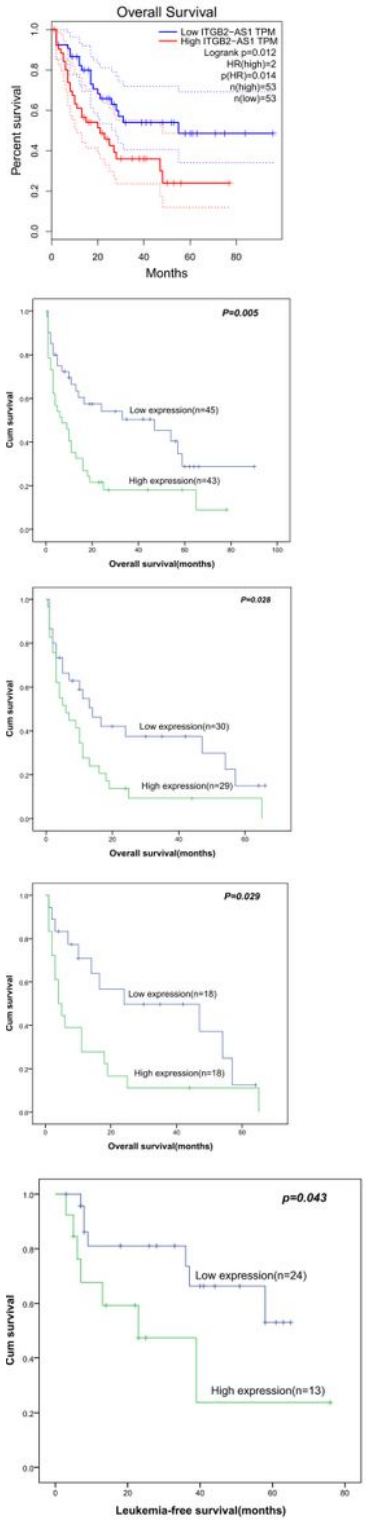

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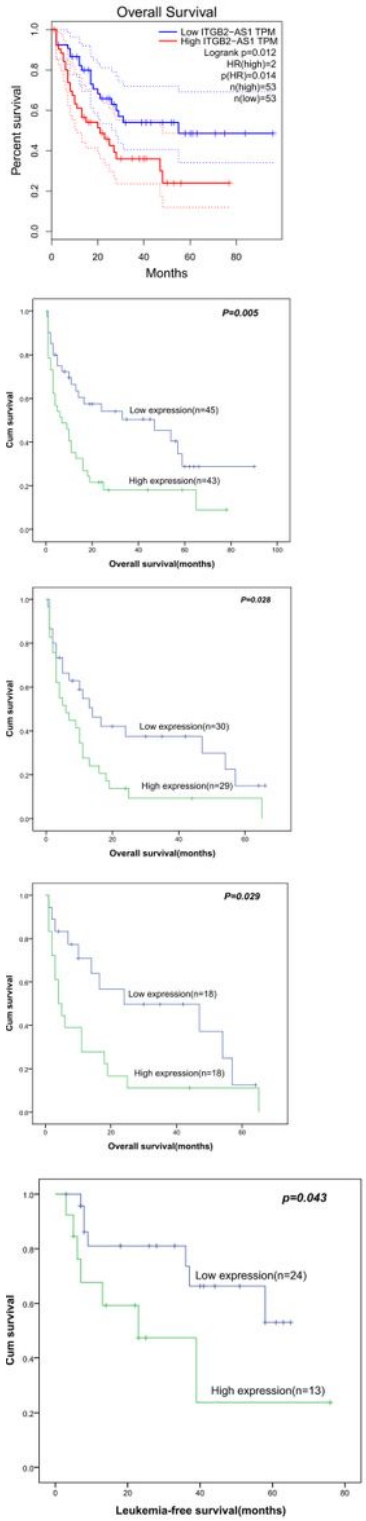

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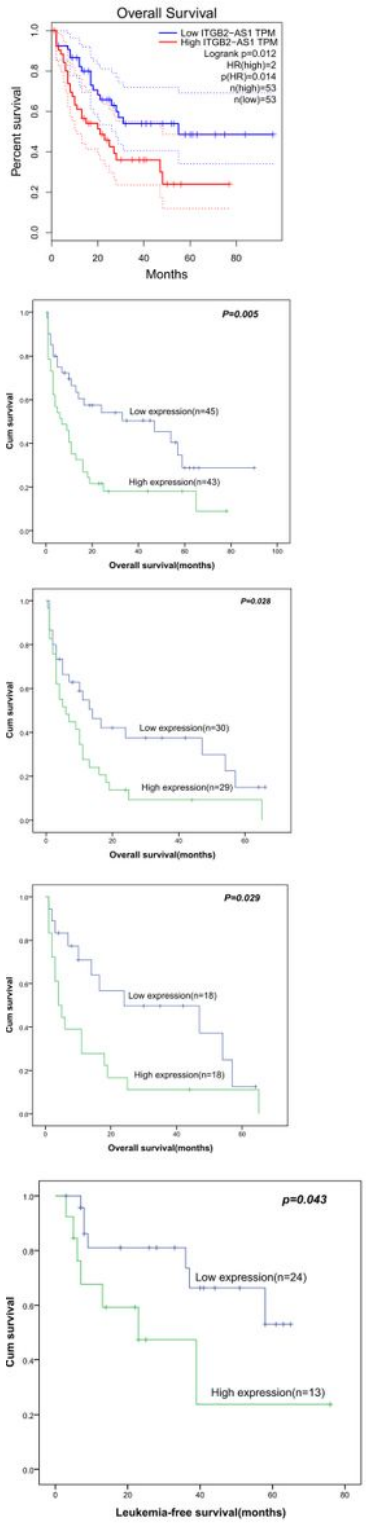

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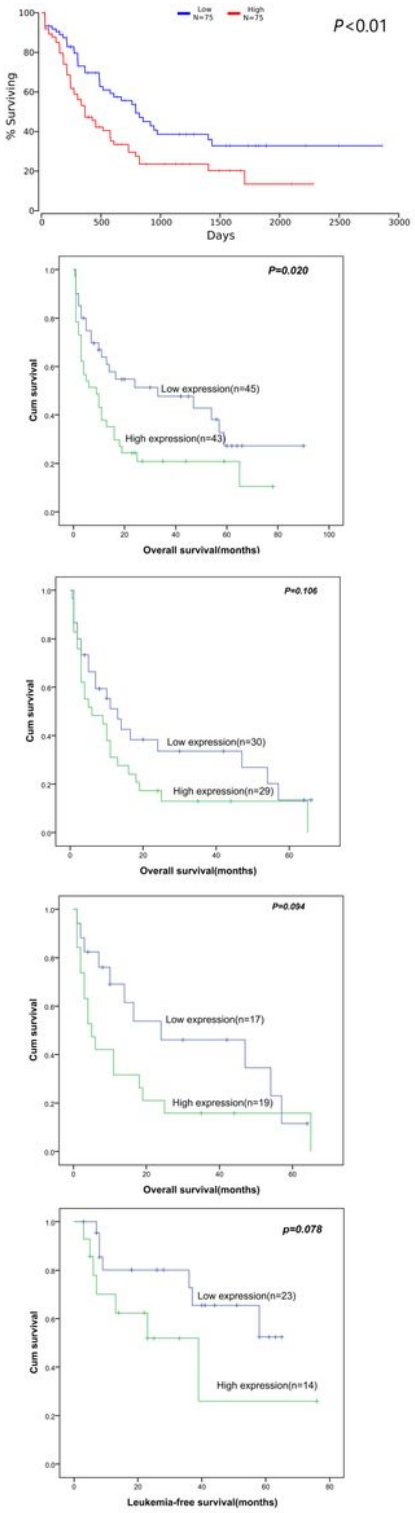

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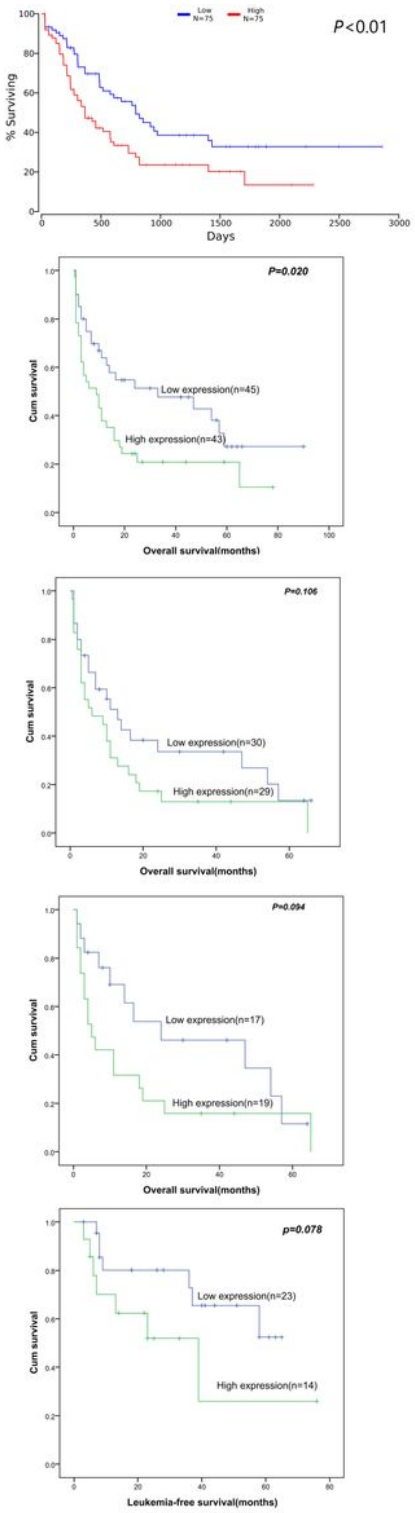

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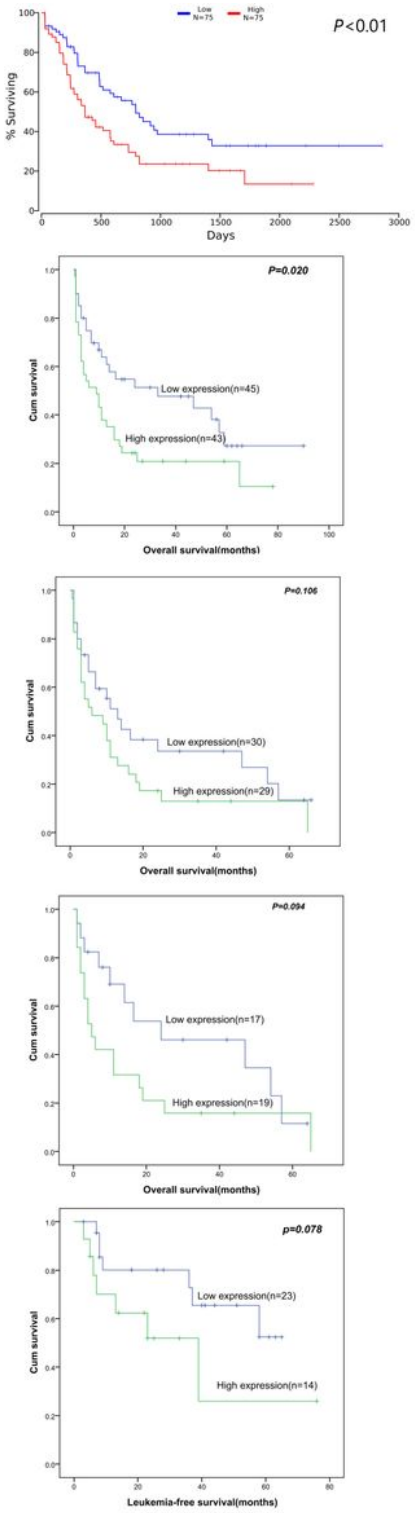

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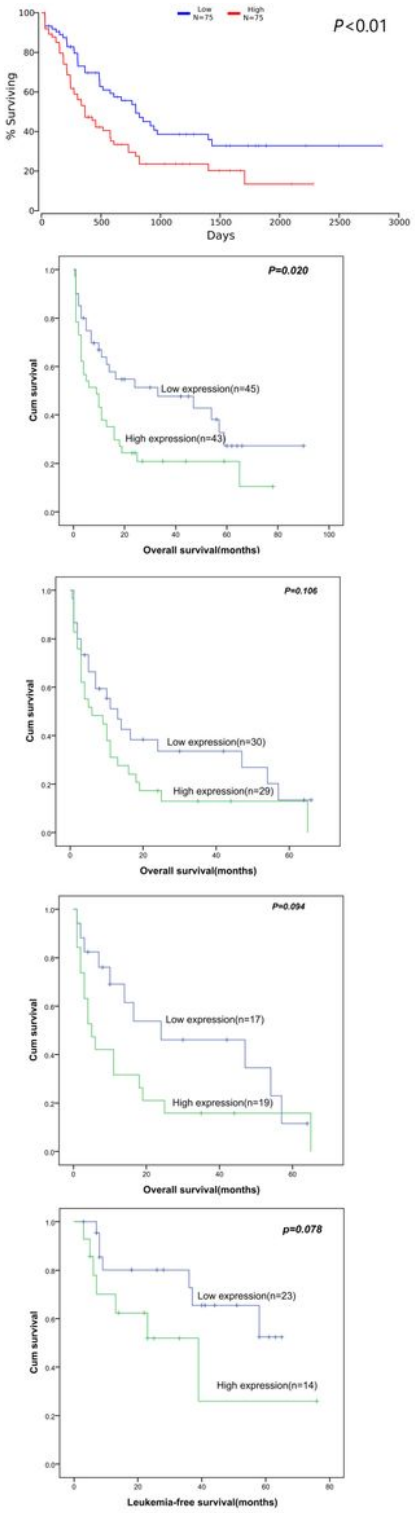

Figure 5

Prognostic value of ITGB2 expression in AML a. The impact of ITGB2 expression on overall survival of AML patients from TCGA datasets using the OncoLnc (http://www.oncolnc.org). b-d. Kaplan-Meier curves of overall survival (OS) in our cohort b. For whole-cohort AML c. For non-M3 AML d. For CN-AML e. Kaplan-Meier curves of leukemia-free (LFS) survival for whole-cohort AML in our cohort

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- AdditionalMethods.docx
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