Comprehensive Expression and Prognosis Analyses of ITGB1 in AML

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Abstract

Background

Acute myeloid leukemia (AML) is a dangerous type of leukemia. The emergence of multidrug resistance (MDR) and recurrence limits the prognosis and survival of patients. In recent studies, we have known that the bone marrow microenvironment was closely related to the poor prognosis of AML. However, the underlying mechanisms are still far from fully understood. By utilizing the bioinformatics analysis, we screened out integrin $\beta 1$ (ITGB1) as the hub gene, which is associated with the bone marrow microenvironment mediated changes of AML cells, with expression profile GSE73157 downloaded from National Center for Biotechnology Information-Gene Expression Omnibus (NCBI-GEO) database.

Methods

R studio software was used to screen out candidate hub genes and further visualize the differential expression. R package “limma” was to find out differentially expressed genes (DEGs). Gene Ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway were conducted by R package “cluster Profiler”. Furthermore, protein-protein interaction (PPI) network was also performed by online tool STRING and software Cytoscape. Last but not least, online tool PrognoScan and GEPIA was utilized for the evaluation of clinical significance of the selected hub gene. P and Cox p value <0.05 was considered to be statistical significance.

Results

ITGB1 was filtrated as the only hub gene in this profile. We found that patients with high expression of ITGB1 had significantly longer overall survival (OS) than those with low expression (COX p value= 0.016730). Besides, the expression of the ITGB1 gene in AML patients is lower than that in normal people significantly (p value <0.01).

Conclusion

We identified ITGB1 as a key gene in the bone marrow microenvironment mediated poor prognosis in AML. The down-regulated expression of ITGB1 was related to AML patients’ poor outcome. ITGB1 may be a potential marker for predicting and guiding AML treatment.

Background

Leukemia is a malignant clonal disease of the hematopoietic system, which accounts for 4% of all cancers[1]. Among them, acute myeloid leukemia (AML) is a common and dangerous type of leukemia. Currently, chemotherapy is still the most important option for the treatment of AML. However, the emergence of multidrug resistance (MDR) and recurrence greatly limits the effects of chemotherapy drugs. Therefore, the research on MDR of AML cells has always been a research hotspot in hematology.

In recent years, studies have found that the bone marrow microenvironment not only plays an important role in the hematopoietic process, but is closely related to the occurrence and development of tumors. The bone marrow microenvironment contains multiple cell types, such as osteoblasts, mesenchymal stem cells, endothelial cells, neurons and so on. In normal humans, the bone marrow microenvironment affects hematopoietic stem cells mostly through the regulation of blood oxygen partial pressure and the secretion of cytokines[2]. However, in cancer patients, the occurrence of MDR and recurrence is inseparable from the role of bone marrow microenvironment. Leukemia and leukemia stem cells (LSCs) interact with the bone marrow microenvironment in a different way from hematopoietic stem cells to affect the development of leukemia[3]. LSCs can hide in the bone marrow niche to escape the killing effect of chemotherapy. As a result, the bone marrow niche has also become a key part of the recurrence and MDR of leukemia[4-6]. According to recent researches, we have known that MDR mediated by the bone marrow microenvironment might stem from the following several mechanisms: 1. The influence of cytokines[7, 8]; 2. Changes in adhesion of leukemia cells [9, 10]; 3. The regulation of expression of MDR-related genes[11]; 4. The adjustment of cell cycle and metabolism[12]. In addition, many factors can also be contributed to the recurrence of leukemia, such as the secretion of cytokines or the changes with certain pathways.

As the emergence and rapid development of high-throughput platforms and microarray, numerous molecular heterogeneity on AML has been acknowledged. In order to find potential key biomarker of AML related to the bone marrow microenvironment-mediated poor prognosis, through the use of biological information technology and the analysis of high-throughput sequencing chip data, we screen out the differentially expressed genes (DEGs) of AML cells cultured alone and co-cultured with stromal cells, which were all treated with different concentrations of Arsenic trioxide (ATO). Furthermore, we pick up ITGB1 as a hub gene that can be considered as the predictor of prognosis. We systematically reveal the occurrence and development of AML on the level of molecule and provide a potential guidance for targeted therapy.

Materials And Methods

2.1 Analysis and screening of differential expressed genes (DEGs)

The GEO database (<https://www.ncbi.nlm.nih.gov/geo/>) of the National Center for Biotechnology Information was used to select and download the gene express profile GSE73157[13]. This profile was generated on the platform of GPL17077 (Agilent-039494 SurePrint G3 Human GE v2 8*60K Microarray 039381). In order to reduce false positive results, the online analysis tool GEO2R in the GEO database was used to process the data profile. The screen criteria were adjusted p value < 0.05 and the use of Benjamini-Hochberg. Then, R package “limma” was used to clarify the DEGs between NB4 cultured alone and co-cultured with stromal cells after the treatment of ATO. The screen criteria were adjusted p value < 0.05 and $[\log_2\text{FoldChange}(\log_2\text{FC})] > 1$ [14]. All genes were visualized by volcanic maps and top 50 dramatically differentially expressed genes were selected to draw a heatmap by R package “ggplot2”[15].

2.2 Functional enrichment analysis and pathway analysis of DEGs

R package “clusterProfiler” was used to conduct Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis in order to perform functional enrichment and pathway analysis of DEGs[16]. Biological process (BP), molecular function (MF) and cellular component (CC) are included in GO enrichment. Analysis results were illustrated as figures by R package “GOplot”[17]. A p value less than 0.05 was considered statistically significant. Also, the ClueGo plug-ins of Cytoscape software 3.8.1 was utilized to display the relationship between pathways[18]. A p value less than 0.05 was considered statistically significant.

2.3 Screening candidate genes through protein-protein interaction (PPI) network

The PPI prediction website STRING (<https://string-db.org>) constructed protein interaction network[19]. And Cytoscape software 3.8.0 was used to visualize the interactions of DEGs[18]. Further, we used “cytoHubba” to degree the interaction of hub-gene clustering by the method of Maxima Clique Centrality (MCC). Overlapped genes were separated by online tool Bioinformatics & Evolutionary Genomics (<http://bioinformatics.psb.ugent.be/webtools/Venn/>) and were displayed by Venn diagram.

2.4 Selecting candidate genes and conducting survival analysis

In order to evaluate the prognostic value of candidate genes in AML patients, we utilized the PrognoScan database (<http://dna00.bio.kyutech.ac.jp/PrognoScan/>) and online tool Gene Expression Profiling Interaction Analysis (<http://gepia.cancer-pku.cn/index.html>) to conduct the survival analysis[20, 21]. The results are displayed with hazard ratio (HR) and Cox p value from a Log-rank test. Cox p value < 0.05 was considered statistically significant. Besides, according to online tool Gene Expression Profiling Interaction Analysis (GEPIA), we compared the expression of candidate genes in AML patients and normal people. The screen criteria were adjusted p value < 0.01 and $[\log_2\text{FoldChange}(\log_2\text{FC})] > 1$

Results

3.1 Analysis and screening of differential expressed genes (DEGs)

The goal of this study is to screen out the hub gene of AML related to the bone marrow microenvironment-mediated MDR (Figure 1). Perform statistical analysis on GEO database, we found that the data set GSE73157 contains 32080 DEGs in AML cultured alone groups and co-cultured groups (All the following are referred to as alone groups and co-culture groups, respectively). By using R package “limma”, DEGs were classified as up-regulated genes (marked as red plots) and down-regulated genes (marked as green plots) (Figure 2a). Besides, a heatmap illustrated top 50 DEGs (Figure 2b).

3.2 Functional enrichment analysis and pathway analysis of DEGs

Go and KEGG enrichment analysis was performed on all DEGs with R package “clusterProfiler”. The results of GO include three subontologies referred to as biological process (BP), cellular components (CC),

molecular function (MF). The GO results show that angiogenesis pathway, cell adhesion molecule binding pathway and collagen-containing extracellular matrix pathway were selected as the most significant pathway in each subontologies, respectively (Figure 3a-c). And we chose angiogenesis pathway as our further analysis pathway as a result of comparison of p values. 177 genes were found enriched in this GO term. In addition, PI3K-Akt signaling pathway was the top enriched pathway of the DEGs in KEGG enrichment analysis (Figure 3d). After we used the ClueGo to further analyze the interrelation of the enriched pathways and the DEGs, PI3K-Akt signaling pathway was still the most significant pathway, and involved 120 DEGs (Figure 3e, f). Finally, we selected two pathways for our further research, which involved 177 and 120 DEGs, respectively (Table1). Then, after taking the intersection of the DEGs included in the two pathways, we could find that there are 26 genes included both in the two pathways (Figure 3g). They were PDGFRB, FLT1, NGFR, EFNA1, FGFR1, ITGB1, VEGFC, BRCA1, PDGFRA, PGF, EPHA2, EFNA3, PIK3CG, SYK, EREG, PTK2, ITGB8, FGF2, THBS1, FGF18, THBS2, AKT3, IL6, ANGPT1, FN1, ANGPT2.

DEGs	Genes
Angiogenesis pathway	ABL1, ACVR1, ACVRL1, ADM, AKT3, AMOTL2, ANG, ANGPTL4, ANGPTL6, ANXA2, AQP1, ARHGAP22, C3, CARD10, CAV1, CCBE1, CCL2, CD40, CDH13, CELA1, CHI3L1, CHRNA7, COL18A1, COL8A2, CYP1B1, DCN, DDAH1, DLL4, E2F7, ECM1, EFNA1, EFNA3, EGFL7, ELK3, EMP2, ENPP2, EPAS1, EPHA2, EPHB2, EREG, ESM1, FAP, FGF2, FLT1, FN1, FZD5, GATA6, GBX2, GPNMB, GPR4, GREM1, HDAC9, HEY1, HLA-G, HMOX1, HOXB13, HPSE, HTATIP2, ID1, IL18, IL1A, IL1B, IL6, ITGAX, ITGB1, ITGB2, ITGB8, KLF5, LEF1, LOXL2, LRG1, MAPK14, MDK, MFGE8, MMP14, MMP2, NFATC4, NGFR, NOTCH3, NRP1, NRP2, PARVA, PDGFRA, PDGFRB, PGF, PGK1, PLCD1, PLK2, PPARG, PRKD2, PTGS2, PTK2, PTN, PTPRM, RAMP3, RBM15, RHOB, RNF213, RORA, RRAS, SASH1, SAT, SERPINE1, SERPINF1, SFRP1, SFRP2, SMAD1, SOX17, SPARC, SRPX2, SYNJ2BP, TGFB1, THBS1, THBS2, THY1, TIE1, TNFAIP3, TNFSF12, TWIST1, VASH1, VEGFC, WNT5A, ADM2, AGGF1, ANGPT1, ANGPT2, BRCA1, CCR2, CX3CL1, CXCR2, CYBB, DAB2IP, DAG1, ECSCR, EFNB2, EMILIN1, EPHA1, F3, FBXW7, FGF18, FGFBP1, FGFR1, FOXJ2, GATA2, GPLD1, HHEX, HOXA7, IHH, JAG1, JUN, KRIT1, LEMD3, MAPK7, MED1, NCL, NOTCH4, NPPB, NRAR, PDE3B, PIK3CG, PKNOX1, POFUT1, PRKX, ROCK2, SETD2, SIRT1, SOX18, SPRED1, SPRY2, SRF, STAB1, SYK, TBX1, TERT, TGFB1, THSD7A, UBP1
PI3K-Akt signaling pathway	TCL1B, GNGT2, OSMR, GNG11, GNG10, IGF2, IGF1R, LAMB2, LAMB3, BCL2, GNG7, F2R, LAMA1, COL9A3, MYB, LAMA4, EIF4EBP1, LAMB1, ITGA11, THBS1, PDGFRA, THBS2, TNC, GNG12, pik3ap1, INSR, FGF18, LPAR1, RXRA, MET, HRAS, LPAR5, HSP90B1, AREG, KIT, CCND2, PDGFC, FLT3, RPTOR, FLT1, PDGFRB, LPAR4, PIK3CG, PIK3R1, CCNE2, ITGA1, IKBKB, FN1, AKT3, SOS2, IRS1, CREB3, CDC37, KITLG, NRAS, IL3RA, Ifna4, CDK6, IL2RB, NGFR, CDK4, BRCA1, IL2RA, JAK2, THEM4, JAK3, EREG, ITGA2B, ITGA3, TLR4, ITGB4, MAPK3, ITGB5, CREB3L2, ITGB7, PIK3R5, IL6R, ANGPT1, TLR2, ANGPT2, EFNA1, GYS2, IL6, CDKN1A, TSC2ITGB1, PGF, YWHAB, PTK2, EFNA3, NFKB1, SPP1, SYK, VEGFC, CSF3, GNG2, FGF2, LAMC3, IFNB1

Table 1: DEGs identified from selected pathways of GO and KEGG

3.3 Screening candidate genes through protein-protein interaction (PPI) network

In order to further understand the DEGs, we displayed the protein-protein interaction (PPI) network including all the DEGs in the two selected pathways mentioned above in STRING (Figure 4a, b). Then,

cytoHubba plug-ins in Cytoscape was also used to screen out top 30 candidate hub genes of each pathway according nodes rank (Figure 4c, d), and all the top hub genes has been listed in Table 2. In this part, we identified 4 common genes in the two sets of top 30 hub genes, including FN1, THBS1, ITGB1 and THBS2 as candidate hub genes.

Angiogenesis pathway			PI3K-Akt signaling pathway		
Rank	Name	Score	Rank	Name	Score
1	FN1	1.68E+08	1	PTK2	7.42E+14
2	CCL2	1.67E+08	2	ITGB1	7.42E+14
3	MMP2	1.65E+08	3	ITGA3	7.42E+14
4	FGF2	1.58E+08	4	ITGA1	7.42E+14
5	SERPINE1	1.38E+08	5	ITGA2B	7.42E+14
6	THBS1	1.33E+08	6	ITGB5	7.42E+14
7	IL6	1.24E+08	6	ITGA11	7.42E+14
8	VEGFC	1.21E+08	8	ITGB7	7.42E+14
9	FLT1	1.07E+08	9	ITGB4	7.40E+14
10	PGF	1.07E+08	10	ITGB8	7.40E+14
11	ANGPT2	1.04E+08	10	ITGA10	7.40E+14
12	ANGPT1	1.00E+08	12	COL1A1	7.18E+14
13	PTGS2	6.38E+07	13	COL1A2	7.17E+14
14	COL18A1	4.98E+07	14	COL6A1	7.17E+14
15	IL1B	4.08E+07	15	COL6A3	7.15E+14
16	JUN	3.63E+07	16	COL6A2	7.14E+14
17	HMOX1	3.31E+07	17	LAMA4	3.83E+14
18	MMP14	2.35E+07	18	LAMB1	3.82E+14
19	PPARG	2.18E+07	19	THBS2	3.58E+14
20	CAV1	1.86E+07	20	COL9A3	3.56E+14
21	IL18	1.73E+07	21	LAMB2	2.37E+13
22	MAPK14	1.58E+07	22	LAMA1	2.36E+13
23	ITGB1	1.34E+07	23	LAMC3	2.10E+13
24	PDGFRB	1.30E+07	24	LAMB3	2.09E+13
25	SIRT1	1.10E+07	25	THBS1	3.95E+12
26	F3	7622520	26	SPP1	1.58E+12
27	ANG	7258344	27	FN1	1.43E+12

28	THBS2	6250378	28	TNC	9.39E+10
29	IL1A	5449080	29	PIK3CG	6.24E+09
30	CYBB	3774960	30	PIK3R1	6.24E+09

Table 2: The top 30 genes with the highest score of each pathway through the Cytoscape “cytoHubba” module analysis

3.4 Selecting candidate genes and conducting survival analysis

Subsequently, the correlation between hub genes and prognosis of AML patients was analyzed through PrognoScan and GEPIA online survival website. The results showed that except for ITGB1 gene, as the result of no survival significance, the other genes were removed. We found that patients with high expression of ITGB1 had significantly longer overall survival (OS) than those with low expression (COX p value= 0.016730) (figure 5a, b). Besides, through the online website GEPIA, we performed a visual analysis of the expression of hub gene ITGB1 in the TCGA database in AML patients and normal people, showing that the expression of the ITGB1 gene in AML patients is lower than that in normal people significantly (p value<0.01) (Figure 5c).

Discussion

With the elucidation of molecular mechanisms and the emergence of targeted therapies, the treatment of AML has made great progress, and the overall survival rate of patients has been significantly improved. However, the resistance of leukemia cells and the recurrence after treatment are still two major problems that plague us. We now believe that the protective effect of leukemia stem cells (LSCs) mediated by the bone marrow microenvironment is also an important factor for the refractory and recurrence of leukemia. Recent studies have shown that there is a complex interaction between leukemia cells and the bone marrow microenvironment. The two induce and support each other, which together lead to the occurrence and development of the disease.

In this study, we analyzed 32080 DEGs between cultured alone and co-cultured with stomal cells AML cells based on GSE73157 dataset. Then, we used GO enrichment analysis and KEGG pathway analysis to detect the dataset to explore the interaction between DEGs. GO analysis showed that these genes mainly reacted with the process of angiogenesis, collagen-containing extracellular matrix production and cytokine binding, suggesting that these DEGs may be involved in improving the ability of tumor cell to migrate and invade, promoting metastasis, recurrence and multiple drug resistance of cancer[22, 23]. KEGG analysis illustrated that 120 DEGs mainly affect the AML cells through PI3K-Akt signaling pathway. As we all known, phosphatidylinositol 3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/Akt/mTOR) signaling pathway is over activated in hematological malignancies, and the development of inhibitors of PI3K/Akt/mTOR signaling pathway has become a common concern for hematology researchers in recent years[24]. Excessive activation of this pathway may promote the tumor proliferation and weaken the immune monitoring of tumor cells[25]. At the same time, we utilized the

online tool STRING to display the functional interaction between proteins to discover the underlying mechanism of occurrence of development of AML. And then using the software Cytoscape 3.8.1 to analyze the protein interaction network diagram, we screened out FN1, THBS1, ITGB1, THBS2 as 4 candidate hub genes. The above 4 candidate genes not only interact closely with other genes, but may also determine the functions of other genes. After further survival analysis of the 4 selected genes, we found that only ITGB1 gene is statistically significant. We found that the expression of ITGB1 is negatively correlated with the prognosis of AML. Furthermore, compared with normal people, the expression of ITGB1 is significantly lower in AML patients. That is, ITGB1 may be a key gene for evaluating the prognosis and conducting targeted therapy of AML patients.

A huge amount of evidences show that integrin family molecules are involved in the occurrence and development of tumors, and play an important role in regulating tumor cell proliferation, invasion and metastasis and other biological behaviors[26]. Integrin $\beta 1$ (ITGB1) is a relatively important part of molecules in the cell adhesion molecule family, and is the main receptor that mediates the interaction between cells and extracellular matrix. This saying is consistent with our above analysis results: the bone marrow microenvironment may affect AML cells by regulating cell adhesion molecules and extracellular matrix. At present, our understanding of ITGB1 is limited to the primary stage, and its biological functions are not yet very clear. In the direction of solid tumors, there are certain studies on the direction of ITGB1, but there are few reports on the research of ITGB1 of hematological tumors in the world. In summary, ITGB1 may be a potential marker for predicting and guiding the treatment of AML, which requires further attention and research.

Conclusion

In summary, ITGB1 was a hub gene related to the bone microenvironment mediated poor prognosis in AML. The expression of ITGB1 was negatively correlated with prognosis of AML. This study used bioinformatics methods to analyze the relevant data of AML researches to obtain potential markers for predicting and guiding AML treatment, which provides a theoretical basis for a deeper understanding of the occurrence and development of AML, and also give the follow-up animal experiments and clinical trials research directions.

Abbreviations

AML: Acute myeloid leukemia

MDR: Multidrug resistance

ITGB1: Integrin $\beta 1$

NCBI-GEO: National Center for Biotechnology Information-Gene Expression Omnibus database

DEGs: Differentially expressed genes

GO: Gene Ontology

KEGG: Kyoto Encyclopedia of Genes and Genomes

PPI: Protein-protein interaction

OS: Overall survival

LSCs: Leukemia stem cells

ATO: Arsenic trioxide

BP: Biological process

MF: Molecular function

CC: Cellular component

MCC: Maxima Clique Centrality

HR: Hazard ratio

GEPIA: Gene Expression Profiling Interaction Analysis

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The dataset analysed during the current study are available in the NCBI-GEO repository, <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE73157>.

Competing interests

The authors report no conflicts of interest in this work.

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Authors' contributions

XYZ, NJ and BAC were responsible for confirming the topic, collecting the data and analyzing article. XYZ designed and wrote the paper, edited the figures, legends and tables. All authors read and approved the final manuscript.

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Figures

Figure 1

A schematic view of the procedure of the study with GSE73157

Figure 3

GO and KEGG enrichment analysis a. The GO biological process (BP) enrichment analysis. b. The GO molecular function (MF) analysis. c. The GO cellular component (CC) enrichment analysis. d. The KEGG enrichment analysis. e. The interrelation between pathways of KEGG. f. Venn diagram showed the common gene of candidate genes. g. Numbers of genes enriched in the certain pathway. GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

4a

4b

4c

4d

Figure 4

Protein-protein interaction (PPI) analysis a. Genes identified from angiogenesis pathway. b. Genes identified from PI3K-Akt signaling pathway. c. Analysis of interaction of top 30 genes from angiogenesis pathway. d. Analysis of interaction of top 30 genes from PI3K-Akt signaling pathway.

5a

5b

5c

Figure 5

Analysis of selected hub gene ITGB1. a. Kaplan-Meier survival curves comparing high and low expression of ITGB1 in AML patients with PrognScan (COX p value= 0.016730). b. Survival analysis curves comparing high and low expression of ITGB1 in AML patients with GEPIA (p value <0.05). c. Comparison the expression of ITGB1 of AML patients and normal people (p value <0.01).