CD33, CD96 and Death Associated Protein Kinase (DAPK) Expression Are Associated with the Survival Rate and/or Response to the Chemotherapy in the Patients with Acute Myeloid Leukemia (AML)

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Background: Leukemia stem cells (LSC) are involved in the incidence, drug resistance, and relapse of leukemia while LSC-related antigen CD33, CD96, and DAPK expression in AML and its prognosis is still unclear. This study explored LSC-related antigens expression in acute myeloid leukemia (AML) and its prognosis.

Material/Methods: A total of 156 cases of AML patients were enrolled in the experiment. The expression of CD33, CD96, and DAPK in CD34+CD38–CD123+ LSC were tested by flow cytometry. The survival curve was established using the Kaplan-Meier method.

Results: Among different subtypes of AML, the positive rate of CD33 was M3> M5> M1> M2> M4; for CD96 it was M5> M4> M2> M3> M1; and for DAPK it was M3> M2> M5> M4> M1. After chemotherapy, the response rate in CD33 and CD96 high expression groups, and DAPK low expression group was significantly lower than the groups with CD33 low expression, CD96 low expression, and DAPK high expression. The median survival time in the CD33 high expression group was markedly lower than the CD33 low expression group (36.5 months). The CD96 high expression group exhibited obviously shorter median survival time than the CD96 low expression group. The DAPK high expression group exhibited longer median survival time than the DAPK low expression group.

Conclusions: CD33 and CD96 overexpression, and DAPK downregulation in the LSC of AML patients were associated with poor chemotherapy effect and prognosis, and higher recurrence rate.

MeSH Keywords: Butylscopolammonium Bromide • Core Binding Factor Alpha 2 Subunit • Prognosis

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Background

Leukemia is a group of heterogeneous hematopoietic system malignant tumor caused by the differentiation failure, apoptosis disorder, and malignant proliferation of hematopoietic stem/progenitor cells at different differentiation stages. It is a kind of acquired hematopoietic stem cell malignant clonal disease [1,2]. At present, the priority treatment of leukemia is chemotherapy [3]. Following medical science development, the emergence of various new chemotherapy drugs significantly increased the remission rate of leukemia [4]. However, most patients may relapse over a period of time after treatment, which can seriously affect life and health, quality of life, and prognosis [5]. Numerous studies have shown a group of cells with trace amount in the blood, only accounting for 0.1~1% of all leukemia cells, that are called leukemia stem cells (LSC). These cells usually lead to increased incidence of leukemia and disease relapse, and exhibited a reduced sensitivity to chemotherapy drugs and drug resistance. Similar to the differentiation grade in normal hematopoietic cells, leukemia cells also have classification, such as stem/progenitor cells, immature differentiated cells, and mature cells [6]. LSCs are derived from normal hematopoietic stem cell gene mutation during the differentiation development process, and they have the ability to produce differentiated blood cells that can have induce leukemia incidence, development, and recurrence [7]. Acute myeloid leukemia (AML) is a set of malignant diseases originated from myeloid hematopoietic stem/progenitor cells. It is characterized by abnormal proliferation of original and immature myeloid cells in the bone marrow and peripheral blood. The clinical manifestations of AML include anemia, bleeding, infection, fever, organs infiltration, and metabolic abnormalities of the skin, gingiva, liver, spleen, lymph nodes, and joints. Most cases present as acute and heavy state, and the prognosis is poor [8]. The immune phenotypes of LSC differs among different types of leukemia and individuals, and is related to clinical characteristics, therapeutic response, survival, and prognosis. Immune phenotype is one of the most commonly used indicators to identify LSC. The recognized immune phenotype of LSC in acute is CD34+CD38−CD123+ [9]. CD33 is also considered as a marker of LSC [10]. However, a variety of studies found that CD33 was not expressed on all the surface of myeloid LSC [11]. As a member of the immunoglobulin superfamily, CD96 expresses on the majority of CD34+CD38− acute myelogenous leukemia stem cells. It was reported that CD96 expression was correlated with the function of LSC [12]. Death associated protein kinase (DAPK) was also reported to be express in LSC with the phenotype of CD34+CD38−, while it was not detected in normal hematopoietic stem cells [13]. LSC-related antigen CD33, CD96, and DAPK expression in AML and their relationship with prognosis is still unclear. This study investigated LSC-related antigen CD33, CD96, and DAPK expression in AML, and the relationship with curative effect and prognosis.

Material and Methods

Objects

A total of 156 AML patients seen at the Second Affiliated Hospital of Harbin Medical University between January 2013 and June 2015 were enrolled, including 86 males and 70 females with mean age of 38.2±10.5 (15–49) years old. According to classification criterion published by FAB [14], there were 27 cases of M1, 35 cases of M2, 25 cases of M3, 40 cases of M4, and 29 cases of M5. All the enrolled patients conformed to the WHO diagnostic classification criterion as analyzed by immunophenotyping and karyotype.

Main reagents and materials

CD45, CD33, CD34, CD38, CD123, and CD96 antibodies were purchased from BD Pharmingen. DAPK antibody was procured from Santa Cruz. Mononuclear cell separating medium Ficoll-Paque PREMIUM was purchased from GE Healthcare.

Immunophenotyping detection

A total of 2 to 5 mL bone marrow was extracted with EDTA anticoagulation from AML patients before chemotherapy. The marrow was treated by density gradient centrifugation in Ficoll-Paque PREMIUM to isolate mononuclear cells. Four colors (FITC, PE, PerCP, and APC) fluorescent antibodies were used to label the cells. FSC/SSC was set to exclude debris, SSC/CD45 was set to select white blood cells, and CD34+CD38− was set to select hematopoietic stem cells. Furthermore, CD123+ was set among CD34+CD38- hematopoietic stem cells to identify LSC. CD33, CD96, and DAPK expression was analyzed in CD34+CD38−CD123+ LSC. Positive cell rate ≥20% was considered as positive expression in LSC.

Treatment method

AML patients except acute promyelocytic leukemia (APL) were treated by induction therapy based on harringtonine (HA, 2–3 mg/day, 5–7 days) or mitoxantrone (NA, 10 mg/day, 3 days), followed by cytarabine (Ara-C, 150–300 mg/day, 5–7 days) for consolidation therapy. APL patients were mainly treated by arsenite induction, or arsenite and retinoic acid dual induction therapy. In addition to chemotherapy, all patients received blood transfusion, anti-infection, and comprehensive support therapy according to the state of illness. Curative effect was evaluated based on the blood disease diagnosis and standard therapy criterion [15]. (1) Complete remission (CR): no anemia, bleeding, infection, or leukemia cells infiltrating performance; hemoglobin >90 g/L, white blood cells normal or reduce, without immature cells, platelets >100×10⁹/L; primitive cells and early infant stage cells (or immature cells) in the bone marrow <5%,
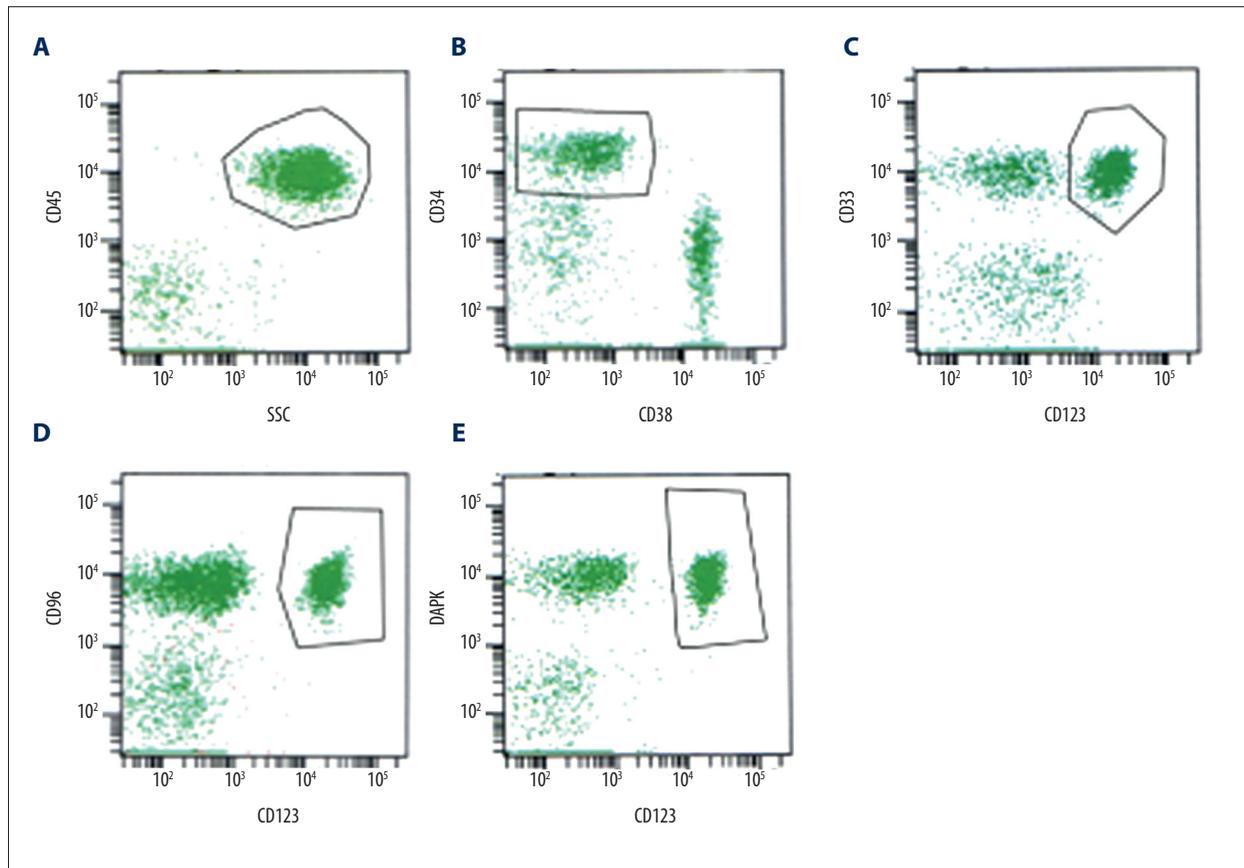
Figure 1. Flow cytometry detection of CD33, CD96, and DAPK expression in LSC. (**A**) SSC/CD45 set for white blood cells. (**B**) CD34+CD38– set for hematopoietic stem cells. (**C**) CD33 expression in CD123+ LSC. (**D**) CD96 expression in CD123+ LSC. (**E**) DAPK expression in CD123+ LSC.

normal RBC and megakaryocyte system. (2) Partial remission (PR): one or two of three aspects of clinical, blood, and bone marrow failed to meet CR criteria; primitive cells and early infant cells in bone marrow <20%. (3) Not remission (NR): three aspects of clinical, blood, and bone marrow failed to meet CR criteria; primitive cells, and early infant cells in bone marrow >20%, including invalid patients.

Statistical analysis

All data was input and analyzed by SPSS 18.0 software. Enumeration data was presented as percentage and compared by chi-square test, while measurement data was depicted as mean ± standard deviation (SD) and compared by rank sum test. Patients' survival curve was established by Kaplan-Meier method. Progress free survival (PFS) was compared by Log-rank test; $p < 0.05$ was considered as statistical significance.

Results

LSC immunophenotyping analysis in AML patients with different subtypes

The detection flow chart of CD33, CD96, and DAPK expression in CD34+CD38–123+ LSC was showed in Figure 1. SSC/CD45 was set to select white blood cells (Figure 1A), and CD34+CD38– was set to select hematopoietic stem cells (Figure 1B). Moreover, CD33 (Figure 1C), CD96 (Figure 1D), and DAPK (Figure 1E) expression in CD123 positive LSC were detected. Among all AML patients, CD33 presented the highest positive rate (80.8%), followed by CD96 (50.6%), and DAPK (40.7%). A total of 126 cases from 156 AML patients showed CD33 positive, with the expression quantity as 5.1–98.7% and median value at 47.2%. CD96 positive rate in CD34+CD38–123+ LSC was 50.6%, with the expression quantity as 3.1%–82.5% and median value at 40.7%. There were 31 cases exhibited DAPK positive in CD34+CD38–123+ LSC (19.8%), with the expression quantity as 4.9%–78.8% and median value at 39.2% (Table 1).

Table 1. LSC related antigens expression in AML with different subtypes.

Phenotype	M1	M2	M3	M4	M5	Total
CD33 in LSC Positive rate (%)	25.9% (7/27)	20.0% (7/35)	44.0% (11/25)	10.0% (4/40)	31.0% (9/29)	24.4% (38/156)
CD33 in LSC Expression (median, %)	42.5 (9.4~65.7)	38.8 (6.6~56.8)	55.6 (18.4~98.7)	35.3 (5.1~43.2)	50.6 (16.3~80.6)	47.2 (5.1~98.7)
CD96 in LSC Positive rate (%)	25.9% (7/27)	51.4% (18/35)	32.0% (8/25)	62.5% (25/40)	72.4% (21/29)	50.6% (79/156)
CD96 in LSC Expression (median, %)	21.8 (3.1~45.8)	37.2 (3.6~56.1)	33.8 (4.4~65.9)	45.5 (5.2~80.4)	52.1 (7.9~82.5)	40.7 (3.1~82.5)
DAPK in LSC Positive rate (%)	11.1% (3/27)	22.8% (8/35)	36.0% (9/25)	15.0% (6/40)	17.2% (5/29)	19.8% (31/156)
DAPK in LSC Expression (median, %)	18.6 (3.9~25.8)	48.1 (5.6~62.7)	56.2 (6.4~78.8)	27.4 (4.5~36.3)	41.6 (4.8~49.1)	39.2 (3.9~78.8)

Table 2. The relationship of CD33, CD96, and DAPK expression with curative effect.

Phenotype	CR+PR (cases, %)		NR (cases, %)		χ^2	P
CD33					7.561	0.006
Low expression	53	(67.9%)	25	(32.1%)		
High expression	36	(46.1%)	42	(53.9%)		
CD96					13.839	<0.001
Low expression	56	(71.8%)	22	(28.2%)		
High expression	33	(42.3%)	45	(57.7%)		
DAPK					9.444	0.002
Low expression	35	(44.9%)	43	(55.1%)		
High expression	54	(69.2%)	24	(30.8%)		
CD33 CD96 DAPK					11.982	<0.001
CD33^{Low}/CD96^{Low}/DAPK^{High}	20	(90.9%)	2	(9.1%)		
Others	69	(51.5%)	65	(48.5%)		

Among different subtypes of AML, the positive rate of CD33 was highest in M3 (96.0%) and lowest in M4 (67.5%). The sequence of CD33 was M3 >M5 >M1 >M2 >M4, for CD96 it was M5 >M4 >M2 >M3 >M1, and for DAPK it was M3 >M2 >M5 >M4 >M1.

The relationship of LSC-related antigens expression with curative effect and recurrence

The patients were divided into two groups upon the median antigen expression of CD33, CD96, and DAPK in LSC to compare the chemotherapy effect. After two courses of chemotherapy, there were 36 cases (46.1%) obtained remission (CR + PR) and 42 cases

(53.9%) showed no remission in CD33 high expression group. Among CD33 low expression group after chemotherapy, 53 cases (67.9%) presented remission (CR + PR) and 25 cases (32.1%) exhibited no remission (χ^2=7.561, p=0.006). The response rate (CR + PR) was 42.3% in CD96 high expression group, which was significantly lower than that of the CD96 low expression group (71.8%) (χ^2=13.839, p<0.001). On the contrary, the response rate (CR + PR) of DAPK high expression group (69.2%) was obviously higher than that of DAPK low expression group (44.9%) (χ^2=9.444, p=9.444). The response rate in patients with CD33^{Low}/CD96^{Low}/DAPK^{High} was 90.9%, which was significantly higher than that in other patients (51.5%) (χ^2=11.982, p<0.001) (Table 2).

Table 3. The relationship of CD33, CD96, and DAPK expression with relapse.

Phenotype	Total cases	Relapse cases (%)		χ^2	P
CD33				5.179	0.023
Low expression	53	14	(26.4%)		
High expression	36	18	(50.0%)		
CD96				10.647	0.001
Low expression	56	13	(23.2%)		
High expression	33	19	(57.6%)		
DAPK				5.998	0.014
Low expression	35	18	(51.4%)		
High expression	54	14	(25.9%)		
CD33 CD96 DAPK				4.919	0.027
CD33^{Low}/CD96^{Low}/DAPK^{High}	20	3	(15.0%)		
Others	69	29	(42.0%)		

In 88 cases of CR + PR patients, there were 53 cases in CD33 low expression group with 14 cases of relapse (26.4%), whereas 36 cases in CD33 high expression group with 18 cases of relapse (50.0%) (χ^2=5.179, p=0.023). Upon CD96, 13 cases (23.2%) exhibited relapse among the 56 cases in low expression group, while 19 cases (57.6%) of 33 cases in high expression group presented relapse (χ^2=10.647, p=0.001). The relapse rate was 51.4% (18/35) in DAPK low expression group, while it was 25.9% (14/54) in DAPK high expression group (χ^2=5.998, p=0.014). The relapse rate in patients with CD33^{Low}/CD96^{Low}/DAPK^{High} was 15.0%, which was significantly lower than that in other patients (42.0%) (χ^2=4.919, p=0.027) (Table 3).

The relationship between LSC related antigens expression and prognosis

The complete follow-up information was obtained from 146 of the total 156 patients, with the follow-up rate at 93.6% and the follow-up period at 8–37 months. The patients were divided into two groups upon the median antigen expression of CD33, CD96, and DAPK in LSC. There were 71 cases in CD33 high expression group, 75 cases in CD33 low expression group, 74 cases in CD96 high expression group, 72 cases in CD96 low expression group, 76 cases in DAPK high expression group, and 70 cases in DAPK low expression group, 20 cases with CD33^{Low}/CD96^{Low}/DAPK^{High}, and 126 cases for others. One-year survival rate of CD33 high expression and low expression group was 80.8% and 91.2%, respectively. Three-year survival rate was 28.4% and 49.6%, respectively. The median survival time in CD33 high expression group (29.8 months) was markedly lower than the low expression group (36.5 months) (χ^2=5.552, p=0.018) (Figure 2A). CD96 high expression patients

showed one-year and three-year survival rate as 78.5% and 25.5%, while CD96 low expression group presented one-year and three-year survival rate as 87.4% and 45.4%. CD96 high expression group exhibited obviously shorter median survival time (30.6 months) than the low expression (35.7 months) (χ^2=4.689, p=0.030) (Figure 2B). DAPK high and low expression groups presented the one-year survival rate as 73.6% and 88.7%, while the three-year survival rate as 26.1% and 50.3%. DAPK high expression group revealed longer median survival time (37.2 months) than the low expression group (30.3 months) (Figure 2C). One-year survival rate was 92.8% for patients with CD33^{Low}/CD96^{Low}/DAPK^{High} and 82.5% for other patients, and three-year survival rate was 85.1% for patients with CD33^{Low}/CD96^{Low}/DAPK^{High} and 24.9% for other patients with statistical significance of survival time (Figure 2D).

Discussion

AML has the high morbidity and mortality in China. It is of great significance to explore the pathogenesis and prognosis associated factors of AML for clinical diagnosis and treatment [16,17]. Several studies showed that the pathogenesis of AML was closely related to LSC. LSC is a type of small amount of cell group with self-renewal ability in the leukemia. They can produce heterogeneity leukemia cell group. LSC can cause and maintain the leukemia both in the long-term *in vitro* cell culture model and animal model [17,18]. Similar to other cancer stem cells, LSC plays an important role in leukemia cell proliferation, survival, metastasis, and recurrence [19]. Although the proportion and quantity are small, LSC stays in relative dormant state for a long time. They express a variety
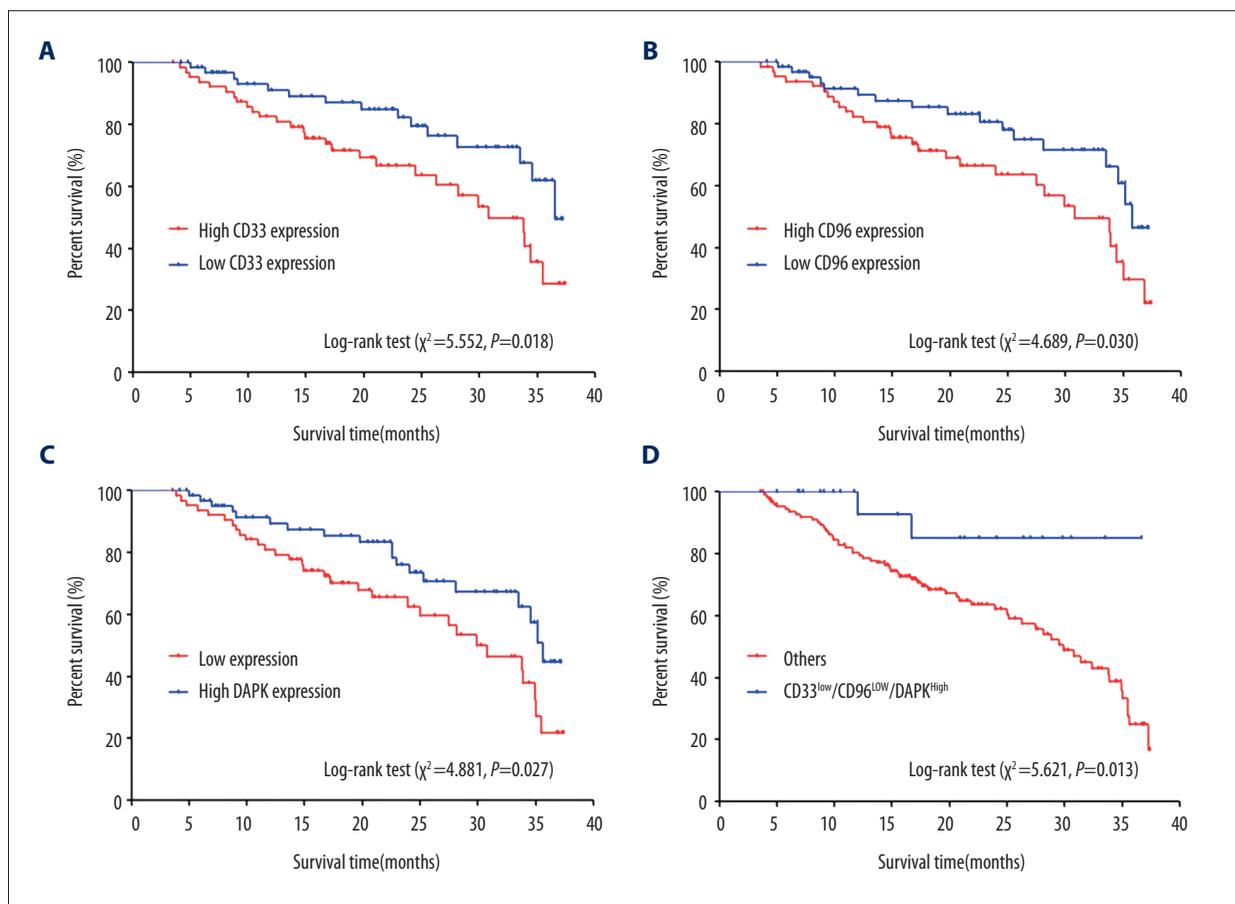
Figure 2. The relationship between LSC related antigens expression and prognosis. (**A**) Survival curve analysis of CD33. (**B**) Survival curve analysis of CD96. (**C**) Survival curve analysis of DAPK. (**D**) Survival curve analysis of CD33^{Low}/CD96^{Low}/DAPK^{High}.

of drug resistance molecules, thus are insensitive to physical and chemical factors stimulus and can escape the killing effect of radiation and chemotherapy. They can incubate for a long time in the body, even become the source of recurrence after leukemia cells are eliminated [20,21]. Following the development of targeted therapy, the clinical remission rate of leukemia patients largely increased. Discovery and in-depth investigation of LSC provide new prospect for the treatment of leukemia. A variety of biological therapies targeting LSC, such as CD33 monoclonal antibody [10], CD44 monoclonal antibody [22], and IL-3 cytokines coupling cytotoxin [23] achieved good clinical effect. LSC are generated from normal hematopoietic stem cell genetic mutation in the development process, thus having some of the phenotypes same to the normal hematopoietic stem cells, such as CD34+CD38–, HLA–DR–, and CD71–. However, they also have different phenotypes compared with hematopoietic stem cells, such as CD117 and CD90. Jordan et al. [9] explored LSC in AML patients and found that CD123 only exists on the surface of LSC but not the normal hematopoietic stem cells. Misaghian et al. [24] showed that CD34+CD38–CD123+ cells may induced and maintain leukemia in animal model, suggesting that this group of cells has

the feature of LSC, as CD34+CD38–CD123+ could be treated as the surface marker of LSC.

CD33 is a type of myelocyte differentiation antigen mainly distributed in the early stage of myeloid cells differentiation. CD33 could be detected in more than 90% of AML patients, while it was absent in normal hematopoietic stem cells, thus to be considered as a marker of LSC [10]. Hauswirth et al. [25] and Taussig et al. [11] reported that not all myeloid LSC expressed CD33 on the surface, suggesting that LSC expression had the heterogeneity. Hamann et al. demonstrated that monoclonal antibody targeting CD33 exhibited significant clinical effect. Combining the heterogeneity of CD33 expression on the surface of LSC, it indicated that the curative effect and prognosis of patients with different CD33 expression were different. CD96 is a kind of type I transmembrane protein belonging to immunoglobulin superfamily. It plays a critical role in mediating T cell and NK cell adhesion and immune inflammation reaction [26]. Hosen et al. [12] found that CD96 could be detected on most of CD34+CD38– AML stem cells, and CD96 expression was correlated with the function of LSC. Only CD96 positive LSC can induce AML in NOD/SCID mice, suggesting

that CD96 expression difference may affect the pathogenesis and prognosis of AML. Guzman et al. [13] discovered that tumor suppressor gene DAPK also expressed in LSC with the phenotype of CD34+CD38–, while DAPK cannot be detected in normal hematopoietic stem cells, revealing that DAPK was a marker related to LSC. The expression characteristics of CD33, CD96, and DAPK in AML, and their relationship with prognosis are still unclear. This study investigated LSC-related antigens CD33, CD96, and DAPK expression in AML and discussed their correlation with curative effect and prognosis.

Our study found that their expression positive rate was CD96 >CD33 >DAPK in the LSC of AML patients. We detected the expression rate of CD96 was 50.6%, which was similar to the Hosen et al. report of 60% in LSC of AML patients [12]. As a kind of myelocyte antigen marker, CD33 positive rate is high in leukemia cells. However, its positive rate is lower in the LSC of leukemia. Larson et al. [27] suggested that only one-third of the LSC in AML patients expressed CD33. In this study, only 38 of 156 cases exhibited CD33 positive expression, accounting for 24.4%, which was similar to the results of Larson et al. [27]. Among different subtypes of AML, CD33, CD96, and DAPK expressions were various. The positive rate of CD33 was M3 >M5 >M1 >M2 >M4; for CD96 it was M5 >M4 >M2 >M3 >M1; and for DAPK it was M3> M2> M5> M4> M1. After chemotherapy, the response rate in the CD33 and CD96 high expression groups was significantly lower than the groups with CD33 and CD96 low expression, suggesting CD33 and CD96 elevation may reduce the sensitivity to chemotherapy and be related to poor prognosis. Further analysis demonstrated that the one-year survival rate, three-year survival rate, and median survival time in the CD33 and CD96 high expression groups were obviously lower than that in the groups with low CD33 or CD96 expression. Wang et al. [28] showed that the number of CD33+ LSC in AML patients obviously declined after chemotherapy. Compared with refractory and recurrent AML, AML patients with CR presented markedly lower CD33+ LSC

number, revealing the adverse impact of CD33 elevation on the effect of chemotherapy. Majeti et al. [22] revealed that antibody targeting CD96 on LSC exhibited curative effect on alleviating the disease and improving prognosis, suggesting the value of CD96 downregulation on AML targeted therapy. It also indicated that the role of CD96 elevation on the curative effect of AML patients, which may be the theoretical basis of lower curative effect and increased recurrence rate in patients with CD96 high expression. Du et al. [29] reported that the CR rate in AML patients with CD96 expression of <10% in CD34+CD38– LSC was markedly higher than in the patients with CD96 expression of >10%. DAPK is a kind of serine/threonine kinase regulated by calcium/calmodulin, participating in the multiple pathways mediated apoptosis, including p53 [30], TGF-β [31], TNF-α, and Fas ligand [32]. It plays a positive regulatory role in cell apoptosis, and is associated with the sensitivity of chemotherapy, radiotherapy, and endocrine therapy. Our study observed that the curative effect and prognosis of patients with DAPK low expression in LSC was significantly worse than for patients with DAPK high expression, whereas the recurrence rate was higher. It may be related to DAPK reduction on LSC and apoptosis declination.

Conclusions

Out study observed that CD33 and CD96 elevation, and DAPK reduction in LSC of AML patients declined the curative effect and prognosis, and increased the recurrence rate, suggesting CD33, CD96, and DAPK could be used as an indicator for evaluation of survival, curative effects, as well as relapse of patients with AML.

Disclosure of conflict of interest

The authors declare no competing financial or commercial interests in this manuscript.

References:

1. Vedi A, Santoro A, Dunant CF et al: Molecular landscapes of human hematopoietic stem cells in health and leukemia. Ann NY Acad Sci, 2016; 1370: 5–14

2. Li XL, Xue Y, Yang YJ et al: Hematopoietic stem cells: Cancer involvement and myeloid leukemia. Eur Rev Med Pharmacol Sci, 2015; 19: 1829–36

3. Wu S, Yang S, Zhu L et al: Prognosis of patients with *de novo* acute myeloid leukemia resistant to initial induction chemotherapy. Am J Med Sci, 2016; 351: 473–79

4. Slone WL, Moses BS, Evans R et al: Modeling chemotherapy resistant leukemia *in vitro*. J Vis Exp, 2016; (108): e53645

5. Printz C: Recurrence, poor survival more likely in patients with acute myeloid leukemia who have certain mutations. Cancer, 2015; 121: 4275

6. Chao MP, Seita J, Weissman IL: Establishment of a normal hematopoietic and leukemia stem cell hierarchy. Cold Spring Harb Symp Quant Biol, 2008; 73: 439–49

7. Najdi TS, Hatfield GW, Mjolsness ED: A 'random steady-state' model for the pyruvate dehydrogenase and alpha-ketoglutarate dehydrogenase enzyme complexes. Phys Biol, 2010; 7: 16016

8. Ryotokuji T, Yamaguchi H, Ueki T et al: Clinical characteristics and prognosis of acute myeloid leukemia associated with DNA-methylation regulatory gene mutations. Haematologica, 2016; 101(9): 1074–81

9. Jordan CT: Unique molecular and cellular features of acute myelogenous leukemia stem cells. Leukemia, 2002; 16: 559–62

10. Hamann PR, Hinman LM, Hollander I et al: Gemtuzumab ozogamicin, a potent and selective anti-CD33 antibody-calicheamicin conjugate for treatment of acute myeloid leukemia. Bioconjug Chem, 2002; 13: 47–58

11. Taussig DC, Pearce DJ, Simpson C et al: Hematopoietic stem cells express multiple myeloid markers: implications for the origin and targeted therapy of acute myeloid leukemia. Blood, 2005; 106: 4086–92

12. Hosen N, Park CY, Tatsumi N et al: CD96 is a leukemic stem cell-specific marker in human acute myeloid leukemia. Proc Natl Acad Sci USA, 2007; 104: 11008–13

13. Guzman ML, Upchurch D, Grimes B et al: Expression of tumor-suppressor genes interferon regulatory factor 1 and death-associated protein kinase in primitive acute myelogenous leukemia cells. Blood, 2001; 97: 2177–79

14. Ghannadan M, Wimazal F, Simonitsch I et al: Immunohistochemical detection of VEGF in the bone marrow of patients with acute myeloid leukemia. Correlation between VEGF expression and the FAB category. Am J Clin Pathol, 2003; 119: 663–71

15. Przepiorka D, Deisseroth A, Farrell AT: Acute myeloid leukemia response measures other than complete remission. J Clin Oncol, 2015; 33: 3675–76

16. Terwijn M, Zeijlemaker W, Kelder A et al: Leukemic stem cell frequency: A strong biomarker for clinical outcome in acute myeloid leukemia. PLoS One, 2014; 9: e107587

17. Garg S, Shanmukhaiah C, Ghosh K, Madkaikar M: Cell signaling in putative leukemic stem cells and blast population in acute myeloid leukemia. Leuk Lymphoma, 2016; 57(9): 2195–98

18. Lang F, Wojcik B, Rieger MA: Stem cell hierarchy and clonal evolution in acute lymphoblastic leukemia. Stem Cells Int, 2015; 2015: 137164

19. Hiraoka A: Leukemia cell lines require self-secreted stem cell growth factor (SCGF) for their proliferation. Leuk Res, 2008; 32: 1623–25

20. Fuchs D, Daniel V, Sadeghi M et al: Salinomycin overcomes ABC transporter-mediated multidrug and apoptosis resistance in human leukemia stem cell-like KG-1a cells. Biochem Biophys Res Commun, 2010; 394: 1098–104

21. Funayama K, Murai F, Shimane M et al: Adhesion-induced drug resistance in leukemia stem cells. Pharmacology, 2010; 86: 79–84

22. Majeti R: Monoclonal antibody therapy directed against human acute myeloid leukemia stem cells. Oncogene, 2011; 30: 1009–19

23. Feuring-Buske M, Frankel AE, Alexander RL et al: A diphtheria toxin-interleukin 3 fusion protein is cytotoxic to primitive acute myeloid leukemia progenitors but spares normal progenitors. Cancer Res, 2002; 62: 1730–36

24. Misaghian N, Ligresti G, Steelman LS et al: Targeting the leukemic stem cell: The Holy Grail of leukemia therapy. Leukemia, 2009; 23: 25–42

25. Hauswirth AW, Florian S, Printz D et al: Expression of the target receptor CD33 in CD34+/CD38–/CD123+ AML stem cells. Eur J Clin Invest, 2007; 37: 73–82

26. Fuchs A, Cella M, Giurisato E et al: Cutting edge: CD96 (tactile) promotes NK cell-target cell adhesion by interacting with the poliovirus receptor (CD155). J Immunol, 2004; 172: 3994–98

27. Larson RA, Sievers EL, Stadtmauer EA et al: Final report of the efficacy and safety of gemtuzumab ozogamicin (Mylotarg) in patients with CD33-positive acute myeloid leukemia in first recurrence. Cancer, 2005; 104: 1442–52

28. Wang J, Lai ZL, Chen XY et al: Effect of Compound Zhebei Granule () combined with chemotherapy on surface markers of leukemia stem cell in patients with acute myeloid leukemia. Chin J Integr Med, 2016; 22(6): 438–44

29. Du W, Hu Y, Lu C et al: Cluster of differentiation 96 as a leukemia stem cell-specific marker and a factor for prognosis evaluation in leukemia. Mol Clin Oncol, 2015; 3: 833–38

30. Wang WJ, Kuo JC, Yao CC, Chen RH: DAP-kinase induces apoptosis by suppressing integrin activity and disrupting matrix survival signals. J Cell Biol, 2002; 159: 169–79

31. Jang CW, Chen CH, Chen CC et al: TGF-beta induces apoptosis through Smad-mediated expression of DAP-kinase. Nat Cell Biol, 2002; 4: 51–58

32. Cohen O, Inbal B, Kissil JL et al: DAP-kinase participates in TNF-alpha- and Fas-induced apoptosis and its function requires the death domain. J Cell Biol, 1999; 146: 141–48