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Original Article

Clinical significance of *BECLIN1* and *ATG5* expression in acute myeloid leukemia patients

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Abstract: Introduction: In acute myeloid leukemia (AML), it has been found that harnessing the autophagy process has led to leukemia cell death and had synergistic effects with chemotherapy. *BECLIN1* and *ATG5* are vital upstream regulators in the macroautophagy signaling pathway. Therefore, we explored the expression levels of *BECLIN1* and *ATG5* in AML patients and investigated their prognostic value, that of other clinical features. Methods: Real-time quantitative PCR was used to investigate the mRNA levels of *BECLIN1* and *ATG5* in 101 newly diagnosed leukemia patients. Results: AML samples with *CEBP*α or *c-KIT* mutations showed lower *BECLIN1* expression levels compared with those without mutations (P=0.044 and P=0.036) and those with the *c-KIT* mutation showed lower *ATG5* expression (P=0.040). Overexpression of *BECLIN1* and *ATG5* was related to a shorter overall survival (OS; P=0.02 and P=0.035) but not to disease-freesurvival (DFS). In multivariate analysis, the clinical characteristics exhibited no statistically significant differences in OS, except for the*FLT3-ITD* mutation (P=0.001) and age of the patients (P=0.032). Conclusion: Our results indicate that high levels of *BECLIN1* and *ATG5* are associated with poor disease outcome. However, they are not independent risk factors for AML and further studies are needed to verify the underlying mechanism.

Keywords: Acute myeloid leukemia, autophagy, BECLIN1, ATG5, real-time quantitative PCR, mRNA level

Introduction

Acute myeloid leukemia (AML) is a common malignancy in the hematological system. It is characterized by stagnation of hematopoietic progenitor cell differentiation at different periods, accumulation of abnormal hematopoietic cells in the bone marrow and peripheral blood, and finally restriction of normal hematopoiesis [1]. Although chemotherapy and allogeneic stem cell transplantation are beneficial for prolonging the life span of patients, relapse or refractory leukemia remain a major challenge.

Autophagy is a catabolic process that leads to the elimination of abnormal components to protect cells from various stressors including hypoxia, nutritional deficiencies, and energy depletion [2]. Three main pathways have been discovered: microautophagy, chaperone-mediated autophagy, and macroautophagy. Macroautophagy is thought to be the core mechanism of autophagy, so henceforth, macroautophagy will be referred to simply as autophagy [3]. The mTOR pathway is the most classic one in macroautophagy and *BECLIN1* and *ATG5* are involved in this pathway. When the signaling protein mTOR is inhibited by unhealthy conditions, the ULK-ATG13-FIP200 complex is formed. This complex recruits additional ubiquitin-like proteins, including ATG12-ATG5-ATG16. The BECLIN1-CLASS III PI3K (phosphoinositide 3-kinase) complex is indispensable for this recruitment process. Through a series of processes, the autophagosome generates and presents its digestion contents to the lysosomes becoming autolysosomes [4].

Emerging data has shown that autophagy can suppress tumor initiation. Some scholars have considered that the appearance of hematopoietic stem cells (HSCs) can increase autophagic flux, which is essential for normal hematopoiesis [5]. However, when a tumor had already

Table 1. Clinical characteristics of AML patients based on BECLIN1 and ATG5 expression level

Characteristics	All cases	Beclin1^{high}	Beclin1^{low}	*P*	ATG5^{high}	ATG5^{low}	*P*
Age (years), median (range)	(20~86)	60 (20~86)	50.5 (24~83)	0.382	60.5 (20~86)	60 (24~83)	0.368
Sex, male/female	50/51	27/24	23/27	0.485	27/23	23/28	0.371
WBC (*10^9/L), median (range)	15.53 (0.224~497.85)	14.63 (0.224~401)	17.44 (1.2~497.8)	0.465	11.5 (0.224~401.8)	25.83 (1.4~497.8)	0.110
Hb (g/L), median (range)	84 (40~126)	80 (43~126)	90.5 (40~126)	0.238	80 (43~126)	90 (40~126)	0.341
PLT (*10^9/L), median (range)	38.5 (5~619)	32 (6~619)	42.5 (5~489)	0.831	38 (6~619)	40 (5~489)	0.934
BM blasts(%), median (range)	59.2 (4~98)	52.4(4~98)	66 (6~95.2)	0.228	54.2 (4~98)	64 (6~95.2)	0.372
NPM1 (+)	16.9% (14/83)	11.9% (5/42)	22.0% (9/41)	0.222	12.2% (5/41)	21.4% (9/42)	0.261
CEBPα (+)	14.8% (12/81)	7.1% (3/42)	23.1% (9/39)	**0.044**	7.3% (3/41)	22.5% (9/40)	0.054
FLT3-ITD (+)	11.8% (10/85)	15.9% (7/44)	7.3% (3/41)	0.219	16.3% (7/43)	7.1% (3/42)	0.191
C-KIT (+)	4.9% (4/82)	0% (0/42)	9.8% (4/40)	**0.036**	0% (0/41)	9.8% (4/41)	**0.040**
2016 WHO classification							
Therapy-related myelodysplasia neoplasms (number)	1	0	1		0	1	
AML with recurrent genetic abnormalities (number)	33	13	20		13	20	
AML with myelodysplasia-related changes (number)	16	10	6		9	7	
AML without maturation M1 (number)	15	11	4		11	4	
AML with minimal differentiation M2 (number)	24	13	11		14	10	
AML monoblastic/monocytic M4 (number)	5	3	2		2	3	
AML myelomonocytic M5 (number)	7	1	6	0.099	1	6	0.108
Prognosis risk group							
Favorable	17	7	10		7	10	
Intermediate	46	23	23		22	24	
Unfavorable	22	12	10	0.705	12	10	0.707
Cytogenetic risk group							
Favorable	5	3	2		3	2	
Intermediate	85	41	44		42	43	
Unfavorable	11	6	5	0.824	6	5	0.864
CR rate		62.5%	78.9%	0.316	63.3%	77.5%	0.317

Abbreviations: BM = bone marrow; Hb = hemoglobin; PLT = platelets; WBC = white blood cells;CR: complete remission.

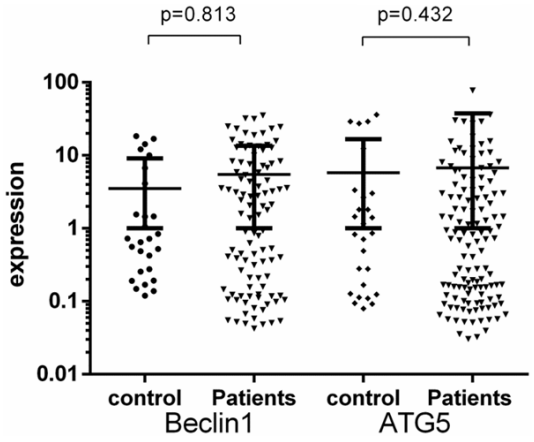

Figure 1. mRNA levels of Beclin1 and ATG5 in AML patients (n=101) and healthy controls (n=25).

been formed, autophagy benefited the cancer cell survival [6]. This viewpoint was confirmed by several *in vitro* studies. S100A8, an S100 calcium-binding protein family member, induced the formation of the BECLIN1-PI3KC3 complex, resulting in autophagy and chemoresistancein leukemia cells [7]. Liu et al. found that removal of *ATG5* before transplantation could extend the survival time of recipient MLL-AF9-driven mice [8]. As previously known, *BECLIN1* and *ATG5* are crucial regulator genes in the upstream pathway of autophagy. However, data regarding *BECLIN1* and *ATG5* expression has varied for different types of cancer. In non-small cell lung cancer, lower expression of *BECLIN1* was associated with shorter survival [9]. Overexpression of *ATG5* was associated with better DFS in breast cancer patients [10]. However, *BECLIN1* expression was highly positive in malignant melanoma [11] and cutaneous squamous cell carcinoma [12], as was overexpression of *ATG5* in prostate cancer cells [13]. Decreased expression of *BECLIN1* has been referred to as a favorable prognostic factor for gastric cancer and chronic lymphocytic leukemia [14, 15]. The roles of these two genes in newly diagnosed AML patients are poorly understood. Therefore, we set out to study the detailed roles of expression of *BECLIN1* and *ATG5* and explore their prognostic significance in AML.

Materials and methods

Patients

In this study, 101 newly diagnosed AML patients were enrolled, diagnosed, and treated according to the 2016 WHO criterion at the Department of Hematology, the First Affiliated Hospital of Nanjing Medical University from September 2013 to January 2016. Twenty-seven healthy controls were brought into this study. Clinical characteristics and molecular biological features including age, gender, white blood cell counts, hemoglobin concentration, platelet counts, cytogenetics, and molecular markers (e.g. *NPM1, FLT3-ITD, CEBP*α, and *c-KIT*) that have been proven to predict the outcome of AML were available. Bone marrow (BM) samples from all participants in this cohort were analyzed. Approval of the Institutional Review Board of the First Affiliated Hospital of Nanjing Medical University was obtained. All participants freely gave written informed consent.

Real-time qRT-PCR gene expression analysis

Total RNA was extracted from participant bone marrow mononuclear cells (BMNCs). RNA was then reverse transcribed to complementary DNA (cDNA) using the PrimeScript™ 1st Strand cDNA Synthesis Kit. Each reaction mixture included 5 μL of cDNA, 12.5 μL of fluorescent dye SYBR GREEN I PCR Master Mix (Applied Biosystems), 6 μL of deionized water, and 0.5 μLof primers either for *ATG5* (forward: 5'-TGCATCAAGTTCAGCTCTTCCT-3', reverse: 5'-GCAATCCCATCCAGAGTTGC-3'), *BECLIN1* (forward: 5'-CCAGGAACTCACAGCTCCATT-3', reverse: 5'-ATGAATCTGCGAGAGACACCA-3') or *GAPDH* (for reference, forward: 5'-TGGGTGGAATCATATTGGAAC-3', reverse: 5'-TCAACGGATTTGGTCGTATTG-3'). Reactions of the qPCR complex were performed in triplicate using the Applied Biosystems ABI 7300 Real Time PCR system (Applied Biosystems software: SDSv2.0). Thermal cycle conditions were 30 s at 95°C, 40 cycles of 5 s at 95°C and then 30 s at 60°C, and lastly 1 cycle of 15 s at 95°C, 1 min at 60°C, and then 15 s at 95°C. Expression levels for each sample were defined as the threshold cycle (Ct), which was normalized to expression of the reference gene *GAPDH*. The relative expression level was calculated by the $2^{-\Delta\Delta Ct}$ method.

Cytogenetics and mutational analysis

As the International System for Human Cytogenetic Nomenclature (ISCN) guidelines described, BM cells were cultured after 24-48 hours of unstimulated culture, metaphase chromosomes were banded using the Giemsa R-banding method and cultured using an

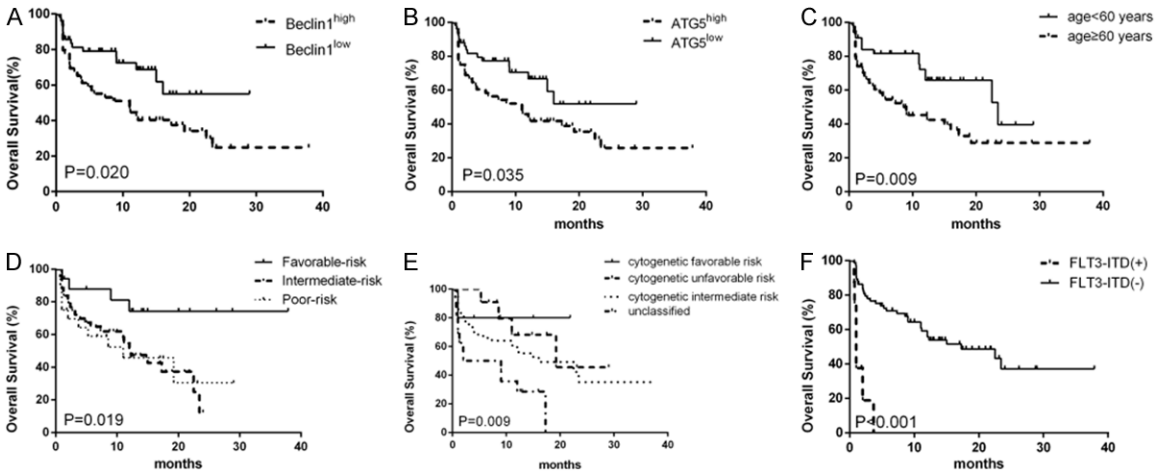

Figure 2. Kaplan-Meier estimate of OS for AML patients are shown according to BECLIN1 and ATG5 expression level, age, risk group, cytogenetic, FLT3-ITD. *P* value was obtained using the log-rank test.

Table 2. Univariate COX proportional hazards regression analysis with patients

Characteristic	OS		DFS	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Age (*<60 years VS ≥60 years*)	0.456 (0.251-0.825)	0.009	1.958 (0.750-5.110)	0.170
Gender	1.521 (0.874-2.649)	0.138	0.548 (0.208-1.441)	0.222
WBC	1.003 (0.999-1.006)	0.105	1.003 (0.996-1.010)	0.403
Hb	0.998 (0.986-1.011)	0.789	1.013 (0.992-1.034)	0.233
PLT	0.999 (0.996-1.002)	0.508	1.000 0.996-1.004)	0.873
BM blast (%)	0.846 (0.316-2.263)	0.739	0.696 (0.113-4.299)	0.696
Prognostic risk group	0.260 (0.084-0.800)	0.019	2.010 (0.970-4.167)	0.061
Cytogenetic risk group	1.418 (1.090-1.844)	0.009	1.157 (0.929-2.447)	0.096
NPM1, wild type VS mutated	0.908 (0.404-2.043)	0.816	0.039 (0.000-24.004)	0.323
CEBPA, wild type VS mutated	0.463 (0.165-1.300)	0.144	0.825 (0.229-2.966)	0.768
FLT3-ITD, wild type VS mutated	6.909 (3.031-15.750)	<0.001	0.048 (0.000-16.340)	0.924
c-kit, wild type VS mutated	1.861 (0.255-13.556)	0.540	0.045 (0.000-762.682)	0.533
Beclin1 expression, high VS low	0.491 (0.270-0.892)	0.020	1.001 (0.382-2.624)	0.999
ATG5 expression, high VS low	0.529 (0.293-0.955)	0.035	1.020 (0.388-2.679)	0.968

unstimulated method for at least 20 metaphases. According to the National Comprehensive Cancer Network (NCCN) guidelines of AML (Version 2, 2016), all patients were divided into different cytogenetic risk groups. The favorable-risk group was comprised of patients with the chromosomal abnormalities t(15, 17), inv 16/t(16, 16), and t(8, 21). The poor-risk group included AML patients with t(3,3)/inv(3), t(6,9), t(9,22), -5/5q-, -7/7q-, 11q23-non t(9, 11), or complex aberrations (≥3 clonal chromosomal abnormalities) monosomal karyotype. The intermediate-risk group was comprised of patients with normal karyotypes +8, t(9; 11), and other non-classified chromosomal abnormalities.

Genomic DNA was isolated from BM mononuclear cells. Relevant mutation (*NPM1, FLT3-ITD, CEBP*α, and *c-KIT*) analyses were conducted as described previously [16-19]. All PCR products were sequenced by using a BigDye Terminator V1.1 cycle sequencing kit (Applied Biosystems, Foster City, CA, USA).

Statistical analyses

The Statistical Package for Social Sciences software (SPSS Version 16.0) was used to analyze the data in this study. The cut-off values adopted for *BECLIN1* and *ATG5* were the median value of all AML samples. For comparing incidence of the clinical characteristics between two groups, the nonparametric Mann-Whitney

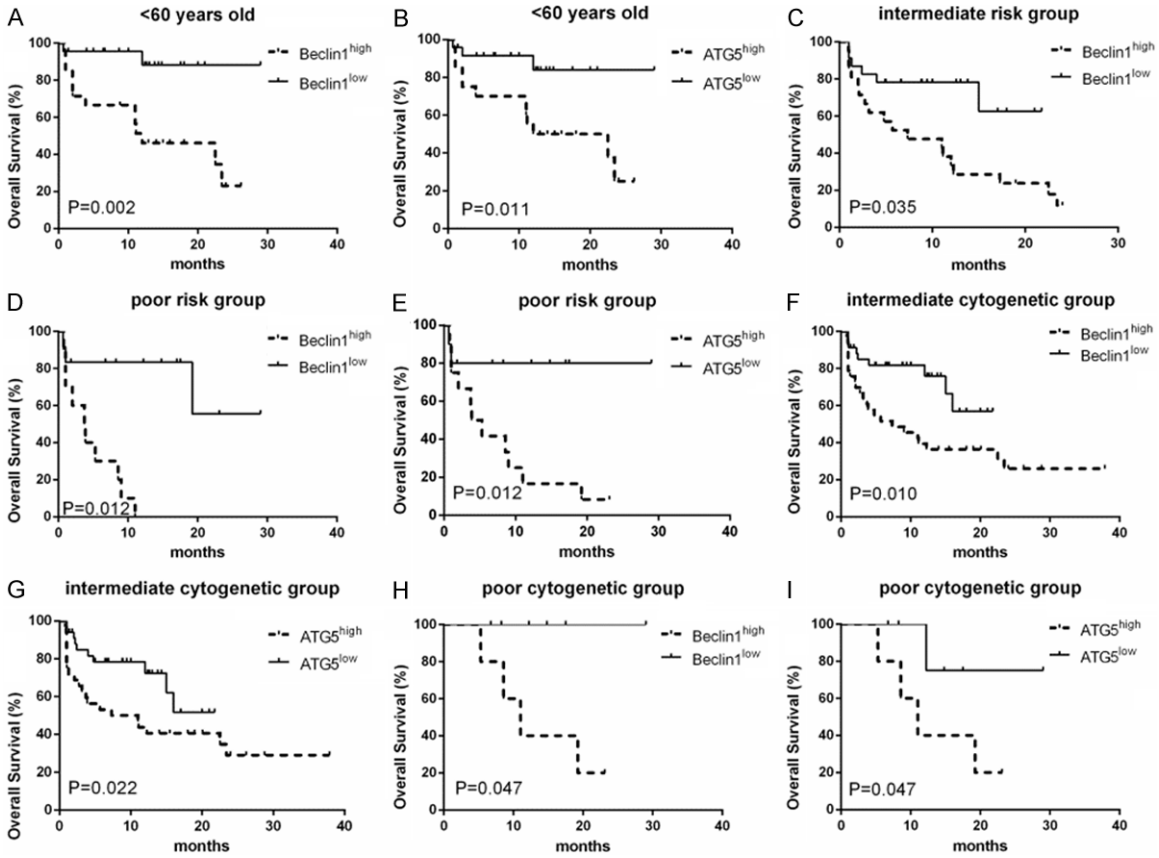

Figure 3. In young age group, intermediate-risk or poor-risk group, intermediate-cytogenetic or poor-cytogenetic group, FLT3-ITD (-) group, respectively, Kaplan-Meier estimate of OS for AML patients is shown according to BECLIN1 and ATG5 expression level. However, no statistical significance was observed in the intermediate-risk group between $ATG5^{high}$ and $ATG5^{low}$ groups (P=0.084). *P* value was obtained using the log-rank test.

U-test, an independent Simples t-Test, and Pearson's χ^2 test were applied. The overall survival (OS) was defined from diagnosis to death or the last follow up. The disease-free survival (DFS) was measured from complete remission to relapse or death or the last follow up. The Kaplan-Meier estimator was applied to estimate the OS and DFS curves and the log-rank test was used for group comparisons. Multivariate Cox proportional hazards models was employed to analyze the prognostic effect of *BECLIN1* and *ATG5* levels and other clinical data. *P*-values <0.05 were considered statistically significant.

Results

Beclin1 and ATG5 expression correlation with patient characteristics

The detailed clinical characteristics from 101 patients are listed in **Table1**. The median age of enrolled participants was 60 years (range, 20-86 years). Approximately half of the patients were over 60 years old. The male to female ratio was 1:1. Most of the patients were classified into the intermediate prognosisrisk group and intermediate cytogenetic risk group. There were no significant differences between the expression of *BECLIN1* or *ATG5* in relation to age, gender, white blood cell count, hemoglobin level, blood platelet count, bone marrow blast, various risk groups, different cytogenetic risk groups, or WHO classification (P>0.05). No significant differences were found between newly diagnosed patients and normal control subjects for *BECLIN1* or *ATG5* expression levels ($P_{BECLINE1}$=0.813, P_{ATG5}=0.432, **Figure 1**). The $BECLIN1^{high}$ group and $ATG5^{high}$ groups showed higher CR rates than those in the low groups (*BECLIN1* CR rate, 62.5% *vs* 78.9%; *ATG5* CR rate, 63.3% vs 77.5% for the high and low groups, respectively). However, these differences were not statistically significant. *BECLIN1* RNA expression was significantly different in

Table 3. Multivariate Cox regression analysis with patients

Characteristic	OS	
	HR (95% CI)	*P*
Age (*<60 years VS ≥60 years*)	2.080 (1.066-4.061)	0.032
Prognostic risk group	1.194 (0.676-2.108)	0.541
Cytogenetic risk group	1.294 (0.82-2.041)	0.269
FLT3-ITD, wild type VS mutated	5.732 (1.990-16.455)	0.001
BECLIN1 expression, high VS low	2.453 (0.465-12.946)	0.290
ATG5 expression, high VS low	0.648 (0.125-3.358)	0.605

patients with the *CEBPα* mutation (P=0.044) and *c-KIT* mutation (P=0.036). The *ATG5* expression level was just significantly different in patients with *c-KIT* mutation (P=0.040). The patients in the *BECLIN1*^{low} and *ATG5*^{low} groups were more likely to have *CEBP*α and *c-KIT* mutations. Expression levels of *BECLIN1* and *ATG5* in the patients cohort manifested a highly significant positive correlation (r=0.998, P<0.01), the same as in healthy control (r=0.929, P<0.01).

Impact of Beclin1 and ATG5 expression on survival

The median follow up time of patients was 8.8 months (range 0.4 to 37.9 months). The Kaplan-Meier survival function showed that OS was significantly longer in the *BECLIN1*^{low} and *ATG5*^{low} groups ($P_{BECLIN1}$=0.020, P_{ATG5}=0.035; **Figure 2A**, **2B**). Old age (≥60 years old, P=0.009; **Figure 2C**), being in the high-risk group (P=0.019; **Figure 2D**), being in the poor cytogenetic-risk group (P=0.009; **Figure 2E**), and the FLT3-ITD mutation (P<0.001; **Figure 2F**) were correlated with shorter OS (**Table 2**). We analyzed *BECLIN1* and *ATG5* expression in the subgroups and some results are shown in **Figure 3**. In the younger group (age<60 years old), overexpression of these two genes showed significant association with shorter OS ($P_{BECLIN1}$=0.002, P_{ATG5}=0.011; **Figure 3A**, **3B**) but no statistical significance was shown in the older group. In the respective risk status groups and cytogenetic groups, we found trends toward shorter OS for those with high *BECLIN1* and *ATG5* expression levels: (Intermediate-risk group: $P_{BECLIN1}$=0.035; **Figure 3C**, P_{ATG5}=0.084; poor-risk group: $P_{BECLIN1}$=0.012, P_{ATG5}=0.012; **Figure 3D**, **3E**; intermediate-cytogenetic group: $P_{BECLIN1}$=0.010, P_{ATG5}=0.022; **Figure 3F**, **3G** poor-cytogenetic group: $P_{BECLIN1}$=0.047, P_{ATG5}=0.047; **Figure 3H**, **3I**). There were no clinical characteristics significantly associated with DFS, possibly due to the small cohort.

Prognostic significance of BECLINE1 and ATG5 expression in AML patients

We applied multivariate Cox regression analysis to evaluate the prognostic impact of those parameters that were significantly associated with OS in the Kaplan-Meier analyses, as shown in **Table 2**. The multivariate analysis found that those clinical parameters, including *BECLIN1* and *ATG5* expression, exhibited no statistically significant differences in OS, except the *FLT3-ITD* mutation (P=0.001) and age (P=0.032; **Table 3**). We failed to confirmthe *BECLIN1* and *ATG5* genes as independent risk factors.

Discussion

Numerous studies have elucidated autophagy gene expression levels in distinct tumors as previously described. The results of our study indicate that most of the patient clinical characteristics are not related with *BECLIN1* and *ATG5* expression levels. However, *CEBP*α and *c-KIT* mutations were frequently observed in the *BECLIN1*^{low} group and the *c-KIT* mutation was also readily found in the *ATG5*^{low} group. The co-occurrence of *CEBP*α biallelic mutation and *ATG5/BECLIN1* low is interesting, as *CEBP*α biallelic mutation has been associated with better outcomes [20]. Researchers have previously found that a lower level of autophagy leads to the accumulation of abnormal mitochondria in human AML blasts [21]. It seems to be that low expression of *BECLIN1* and *ATG5* has a positive influence on outcomes. A larger sample is needed to confirm this hypothesis. Few studies have been carried out to assess the relationship between *CEBPα* and *c-KIT* mutations and autophagy in AML. More effort is needed to discover the underlying mechanisms. A Chinese group discovered that *BECLIN1* levels are also higher in acute leukemia patients than in normal controls [22] but we probably did not observe this same result because of a small cohort of healthy control subjects. Our experiments also demonstrate that decreased expression of *BECLIN1* and *ATG5* is associated with higher CR rate, even if it is not significantly different.

Overexpression of *BECLIN1* and *ATG5* related to shorter OS, which was in accord with findings from Sumitomo etal. showing that inhibition of autophagy by removing of *ATG5* or *ATG7* prolonged the life span of leukemic mice and reduced functional leukemia-initiating cells (LIC) [23]. Sujan Piya and team found that knockdown of *ATG7* obviously induced apoptosis in AML cellstreated with Ara-c and idarubicin as well as prolonged the OS of themice with leukemia [24]. An age over 60 years, a poor prognosis, a poor karyotype, and the *FLT3-ITD* mutation were associated with shorter OS. Further stratified analysis on these subgroups followed. In the younger group, classification in the intermediate or poor prognosis-risk groups and classification in the intermediate- or poor-cytogenetic groups that showed excessive expression were correlated with shorter OS. However, age and *FLT3-ITD* mutation are independent risk factors for AML. The *FLT3-ITD* mutation is generally known as a poor prognostic factor. This karyotype was not previously regarded as an independent risk factor, possibly because some patients with an intermediate karyotype but an unfavorable molecular marker were classified in the poor-risk group. We believe, along with Iranian scholars, that *BECLIN1* expression shows no statistical difference in the favorable risk group and favorable cytogenetic-risk group [25].

To date, conventional chemotherapy drugs have demonstrated powerful benefits in AML treatments, however, relapse and resistance are becoming a difficult problem [21]. We assumed that autophagy inhibition could improve AML treatment and *in vitro* studies have confirmed this viewpoint. An autophagy inhibitor combined with cytosine arabino side induced Ara-C resistant acute leukemia cell death [26]. Combined treatment with AML1-ETO-targeting drugs and an autophagy inhibitor induced an accumulation of ubiquitinated proteins and potentiated the cytotoxicity of the treatment [27]. Bromodomain and extraterminal domain (BET) inhibitors were employed in tumors, including AML. Other groups discovered that the BET inhibitor JQ1 upregulated autophagy, producing a cytoprotective effect in resistant leukemia stem cells. An inhibitor of autophagy could increase the effect of JQ1 [28]. Other research has found that inhibition of autophagy by celecoxib [29] and momordica anti-human immunodeficiency virus protein of 30 kDa caused apoptosis in acute myeloid leukemia cells [30].

We infer that autophagy could serve as a new basis for AML treatment. Our study was incomplete in that we only tested the expression of two genes upstream in the autophagy pathway. We did not detect the downstream genes.Due to limitations of patient specimens, we did not examine the autophagy pathway protein levelsor conduct an *in vitro* experiment. More patients are needed to follow up on the present study.

In conclusion, our study assumed that high expression of *BECLIN1* and *ATG5* would be correlated with poor clinical results in AML patients. Although these two genes might play vital roles in the progression of AML and focusing on the autophagy pathway might be a beneficial strategy for improving AML treatments, additional investigation is required to confirm this hypothesis.

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Disclosure of conflict of interest

None.

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References

[1] Tenen DG. Disruption of differentiation in human cancer: aml shows the way. Nat Rev Cancer 2003; 3: 89-101.

[2] Levine B, Klionsky DJ. Development by self digestion molecular mechanisms and biological functions of autophagy. Dev Cell 2004; 6: 463-477.

[3] Lee J, Giordano S, Zhang J. Autophagy, mitochondria and oxidative stress: cross-talk and redox signalling. Biochem J 2012; 441: 523-540.

[4] Hurley JH, Schulman BA. Atomistic autophagy: the structures of cellular self-digestion. Cell 2014; 157: 300-311.

[5] Watson AS, Riffelmacher T, Stranks A, Williams O, De Boer J, Cain K, MacFarlane M, McGouran J, Kessler B, Khandwala S, Chowdhury O, Puleston D, Phadwal K, Mortensen M, Ferguson D, Soilleux E, Woll P, Jacobsen SE, Simon AK. Autophagy limits proliferation and glycolytic metabolism in acute myeloid leukemia. Cell Death Discov 2015; 1.

[6] Chen N, Karantza V. Autophagy as a therapeutic target in cancer. Cancer Biol Ther 2014; 11: 157-168.

[7] Yang M, Zeng P, Kang R, Yu Y, Yang L, Tang D, Cao L. S100a8 contributes to drug resistance by promoting autophagy in leukemia cells. PLoS One 2014; 9: e97242.

[8] Liu Q, Chen L, Atkinson JM, Claxton DF, Wang HG. Atg5-dependent autophagy contributes to the development of acute myeloid leukemia in an mll-af9-driven mouse model. Cell Death Dis 2016; 7: e2361.

[9] Wang X, Du Z, Li L, Shi M, Yu Y. Beclin 1 and p62 expression in nonsmall cell lung cancer-relation with malignant behaviors and clinical outcome. Int J Clin Exp Pathol 2015; 8: 10644-10652.

[10] Wang L, Yao L, Zheng YZ, Xu Q, Liu XP, Hu X, Wang P, Shao ZM. Expression of autophagy-related proteins atg5 and fip200 predicts favorable disease-free survival in patients with breast cancer. Biochem Biophys Res Commun 2015; 458: 816-822.

[11] Hara Y,Nakamura M. Overexpression of autophagy-related beclin-1 in advanced malignant melanoma and its low expression in melanoma-in-situ. Eur J Dermatol 2012; 22: 128-129.

[12] Okura R, Nakamura M. Overexpression of autophagy-related beclin-1 in cutaneous squamous cell carcinoma with lymph-node metastasis. Eur J Dermatol 2011; 21: 1002-1003.

[13] Kim MS, Song SY, Lee JY, Yoo NJ, Lee SH. Expressional and mutational analyses of atg5 gene in prostate cancers. APMIS 2011; 119: 802-807.

[14] Kristensen L, Kristensen T, Abildgaard N, Thomassen M, Frederiksen M, Mourits-Andersen T, Møller MB. High expression of pi3k core complex genes is associated with poor prognosis in chronic lymphocytic leukemia. Leuk Res 2015; 39: 555-560.

[15] Cao QH, Liu F, Yang ZL, Fu XH, Yang ZH, Liu Q, Wang L, Wan XB, Fan XJ. Prognostic value of autophagy related proteins ulk1, beclin 1, atg3, atg5, atg7, atg9, atg10, atg12, lc3b and p62 sqstm1 in gastric cancer. Am J Transl Res 2016; 8: 3831-3847.

[16] Zhang Y, Zhang SJ, Qiu HX, Qiao C, Sun HM, Li JY. Analysis of cebpa mutation in acute myeloid leukemia. Zhongguo Shi Yan Xue Ye Xue Za Zhi 2010; 18: 859-862.

[17] Dai D, Zhang SJ, Qiao C, Sun XM, Qian SX, Xu W, Xu YL, Li JY. Analysis of npm1 gene mutations in acute myeloid leukemia. Zhongguo Shi Yan Xue Ye Xue Za Zhi 2009; 17: 294-297.

[18] Wang D, Qiao C, Xiao M, Geng Z, Shang Z, He J, Huang M, Yang Y, Zhang N, Liu Y, Li J, Li C, Zhou J. Integrative analysis of prognostic factors in chinese core binding factor leukemia. Biochem Biophys Res Commun 2012; 428: 411-415.

[19] Zhang SJ, Shi JY, Zhu YM, Shi ZZ, Yan S, Gu BW, Bai XT, Shen ZX, Li JY. The investigation of mutation and single nucleotide polymorphism of receptor tyrosine kinases and downstream scaffold molecules in acute myeloid leukemia. Leuk Lymphoma 2009; 47: 2610-2616.

[20] Avellino R, Delwel R. Expression and regulation of c/ebpalpha in normal myelopoiesis and in malignant transformation. Blood 2017; 129: 2083-2091.

[21] Sehgal AR, Konig H, Johnson DE, Tang D, Amaravadi RK, Boyiadzis M, Lotze MT. You eat what you are: Autophagy inhibition as a therapeutic strategy in leukemia. Leukemia 2015; 29: 517-525.

[22] Hu XY, Bai H, Pan YZ, Wnag CB, Wu B, Zhao Q, Ai H, Chen Z, Han X. Expression of autophagy related gene beclin1 and maplc3 in bone marrow mononuclear cells isolated from acute leukemia patients and its significance. Zhongguo Shi Yan Xue Ye Xue Za Zhi 2011; 19: 598-601.

[23] Sumitomo Y, Koya J, Nakazaki K, Kataoka K, Tsuruta-Kishino T, Morita K, Sato T, Kurokawa M. Cytoprotective autophagy maintains leukemia-initiating cells in murine myeloid leukemia. Blood 2016; 128: 1614-1624.

[24] Piya S, Kornblau SM, Ruvolo VR, Mu H, Ruvolo PP, McQueen T, Davis RE, Hail N Jr, Kantarjian H, Andreeff M, Borthakur G. Atg7 suppression enhances chemotherapeutic agent sensitivity and overcomes stroma-mediated chemoresistance in acute myeloid leukemia. Blood 2016; 128: 1260-1269.

[25] Zare-Abdollahi DS, Safari S, Movafagh A, Ghadiani M, Tabarraee M, Riazi-Isfahani S, Gorji S,

Keyvan L, Gachkar L. Expression analysis of becn1in acute myeloid leukemia: association with distinct cytogenetic and molecular abnormalities. Int J Lab Hematol 2016; 38: 125-132.

[26] Kim Y, Eom JI, Jeung HK, Jang JE, Kim JS, Cheong JW, Kim YS, Min YH. Induction of cytosine arabinoside resistant human myeloid leukemia cell death through autophagy regulation by hydroxychloroquine. Biomed Pharmacother 2015; 73: 87-96.

[27] Torgersen ML, Engedal N, Bøe SO, Hokland P, Simonsen A. Targeting autophagy potentiates the apoptotic effect of histone deacetylase inhibitors in t(8; 21) AML cells. Blood 2013; 122: 2467-2476.

[28] Jang JE, Eom JI, Jeung HK, Cheong JW, Lee JY, Kim JS, Min YH. Ampk-ulk1-mediated autophagy confers resistance to bet inhibitor jq1 in acute myeloid leukemia stem cells. Clin Cancer Res 2017; 23: 2781-2794.

[29] Lu Y, Liu XF, Liu TR, Fan RF, Xu YC, Zhang XZ, Liu LL. Celecoxib exerts antitumor effects in hl-60 acute leukemia cells and inhibits autophagy by affecting lysosome function. Biomed Pharmacother 2016; 84: 1551-1557.

[30] Qian S, Sun L, Li J, Wu J, Hu G, Han Y, Yu K, Zhang S. Map30 inhibits autophagy through enhancing acetyltransferase p300 and induces apoptosis in acute myeloid leukemia cells. Oncol Rep 2016; 35: 3705-3713.