Menoufia Medical Journal

PRINT ISSN: 1110-2098 - ONLINE ISSN: 2314-6788

journal hompage: www.menoufia-med-j.com

Volume 35 | Issue 4 Article 6

3-4-2023

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Recommended Citation

Khalifa, Khaled A.; Osman, Nahla F.; Gebba, Rania T. H.; and Elshenawy, Salama M. (2023) "Study of autophagy genes BECN1, PIK3C3 and surface SLAMF1 expression in chronic lymphocytic leukaemia," *Menoufia Medical Journal*: Vol. 35: Iss. 4, Article 6.
DOI: https://doi.org/10.4103/mmj.mmj_202_22

Study of autophagy genes BECN1, PIK3C3 and surface SLAMF1 expression in chronic lymphocytic leukaemia

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Received 15 June 2022
Revised 20 July 2022
Accepted 24 July 2022
Published 04 March 2023

Menoufia Medical Journal 2022, 35:1647–1654

Background
Chronic lymphocytic leukemia (CLL) is the most common leukemia among adults in the Western world. Autophagy is a self-degradative process that is essential for balancing the energy sources at critical times and in response to stress. BECN1 and phosphoinositide 3-kinase C3 (PIK3C3) genes are crucial for the initiation of autophagy process. SLAMF1 is a costimulatory molecule and microbial sensor involved in genetic pathways that adjust chemotaxis and autophagy.

Objectives
To study the role of BECN1 and PIK3C3 gene expression and assess SLAMF1 surface expression in CLL and correlate with disease characteristics.

Patients and methods
In this case–control study, 40 newly diagnosed patients with CLL were included as well as 10 age-matched and sex-matched apparently healthy control. RT-PCR was used to measure PIK3C3 and BECN1 genes expression. SLAMF1 surface expression was measured by flow cytometry.

Results
Patients with CLL had lower BECN1 and PIK3C3 gene expressions than the control group ($P < 0.001$). PIK3C3 showed a statistically significant correlation with CD38 ($P = 0.012$), whereas BECN1 showed a statistically significant difference with different Binet stages ($P = 0.017$). Otherwise, there was no significant correlation between expression of both genes and different clinical parameters. SLAMF1 surface expression was statistically lower in the patient group than in the control group ($P < 0.001$). In addition, it was found to be an independent prognostic marker in CLL, with higher expression being associated with a good prognosis.

Conclusion
Autophagy genes and SLAMF1 represent promising candidates for future studies with respect to their role in autophagy in CLL, and they may represent targets of treatment.

Keywords:
autophagy, BECN1, chronic lymphocytic leukemia, phosphoinositide 3-kinase C3, SLAMF1

Menoufia Med J 35:1647–1654

1110-2098

Introduction

Chronic lymphocytic leukemia (CLL) is a mature B-cell neoplasm, presently an incurable disease, representing the most common form of leukemia. CLL represents how dysregulation of the cell death pathways leads to malignancy [1]. Egypt has one of the highest incidences of lymphoproliferative neoplasm. The epidemiological studies in Egypt showed that CLL is the commonest type of leukemia. The etiology of CLL is unknown, but many factors may be contributing to its development [2].

The promotion of cell survival is vital for cancer development. The course of tumorigenesis involves many genetic alterations that collectively lead to cellular transformation and tumor growth [3].

Autophagy is an evolutionarily conserved cellular recycling process in cell homeostasis and stress adaptation. It confers protection and promotes survival in response to metabolic/environmental stress and is upregulated in response to nutrient deprivation, hypoxia, and chemotherapies [4].

Macroautophagy, hereafter autophagy, can be divided into three phases: initiation mainly by PIK3 core complex, transport of the autophagosome to the lysosome, and degradation [5]. The process of autophagy was initially described as a strategy for nutrition supply under stressed conditions, but it may also lead to programmed cell death by excessive activation of its self-degrading system [6].

The phosphoinositide 3-kinase (PI3K) pathway plays an important role in the cell cycle and cancer development [7]. PI3Ks are divided into three subclasses (classes I–III) according to their regulation and function. Class III predominantly regulates membrane trafficking and has an important role in the autophagy pathway as it participates in the initiation core complex [8].

In mammals, the core complex is composed of BECN1, also named autophagy-related gene 6, phosphoinositide 3-kinase C3 (PIK3C3), and phosphoinositide 3-kinase R4, with BECN1 being the most crucial for regulation and induction of autophagy [9]. This complex serves as a binding site for several proteins that are either promoters or inhibitors of autophagy [10].

The signaling lymphocytic activation molecule SLAMF1 (CD150) is a costimulatory molecule and microbial sensor ~70 kDa. CD150 is a transmembrane glycoprotein expressed by T and B lymphocytes, natural killer, and dendritic cells.

Its expression is rapidly upregulated on these cells upon activation [11]. Loss of SLAMF1 expression modulates genetic pathways that regulate chemotaxis and autophagy [12,13].

In cancer, autophagy can participate in both tumor suppression and progression. Dependent on the cellular context, it enables tumor cells to survive chemotherapy-mediated stress. On the contrary, it can maintain cellular homeostasis by removing damaged organelles and preventing the genomic damage that leads to cancer [14].

The aim of the study was to study the role of BECN1 and PIK3C3 gene expression and assess SLAMF1 surface expression in CLL and correlate with disease characteristics.

Patients and methods

We selected 40 patients with new diagnosis of CLL based on the WHO criteria. These patients presented to the hemato-oncology unit in Menoufia University Hospital in the period between December 2018 and December 2019. The Institutional Review Board (IRB) of the Menoufia Faculty of Medicine approved the study (ID: 4/2016.CPATH). The research work was performed in accordance with the Declaration of Helsinki. A written patient consent was taken from all participants involved in the study. Patients with other small B-cell NHL, other diffuse large B-cell lymphoma, or other hematological disease and those who had treatment for CLL were excluded. In addition, if the diagnosis was not clear or the investigations were incomplete. Of our patients; 55% had Binet stage A, 25% stage B, and 20% stage C. The patient group included 26 males and 14 females and the median age was 62.5 (range, 58.0–67.5) years. A total of 10 age-matched and sex-matched apparently healthy participants were included as a control. The median age of normal controls was 60 years, with a range of 56.0–62.0. None of these controls had previously been diagnosed with any type of malignancy or had a chronic illness.

All groups were subjected to full history taking, clinical examination, and complete blood picture. Complete blood count (CBC) was carried out using XT-1800i hematology analyzer (SYSMEX, Kobe, Japan). Peripheral blood smears were obtained. Blood chemistry, including tumor burden markers and prognostic markers, was done using Beckman AU480 Chemistry analyzer (Beckman Instruments Inc., Fullerton, California, USA), Abbott AXSYM system (Minnesota, USA).

Blood samples were collection for total RNA extraction and analysis of BECN1 and PIK3C3 by RT-PCR.

Blood collection and RNA extraction: 2 ml of blood samples was collected in EDTA tubes and processed within 24 h of collection. RNA extraction was done manually using an RNeasy Mini Kit (Catalog No. 52314) (Qiagen Company, Hilden, Germany).

RT-PCR: all extracted total RNA from all patients and controls was converted to cDNA by reverse transcription using Reverse Transcription Kits (Catalog No. 205313) (Qiagen Company).

In brief, the reverse transcription reaction was performed in 20 μl of mixture containing 4 μl RT Buffer, 1 μl RT Primer Mix, 1 μl Reverse Transcriptase, and 14 μl Template RNA. The 20-μl reaction volume was then stored at −20°C.

Real-time RT-PCR gene expression: the expression levels of the target genes BECN1 and PIK3C3 were detected by real-time PCR using Quantitect SYBR Green Master Mix (Catalog No. 204141, Applied Biosystem, Foster City, California, USA) (Qiagen Company) and real-time cycler 7500 device (Applied Biosystem, USA).

The 25-μl PCR mixture included 12.5 μl of Quantitect SYBR green PCR master mix, 1.25 μl of forward primer, 1.25 μl of reverse primer, 5 μl of RNase-free water, and 5 μl cDNA. PCR program conditions were incubation at 95°C for 15 min, followed by 45 cycles of 15 s at 94°C, 30 s at 55°C, and 30 s at 72°C. Gene expression levels, measured as the threshold cycle (Ct)

values, were normalized to the reference gene GAPDH using the normalization tool Δ Ct = Ct target gene-Ct reference gene. Relative gene expression levels for each patient were calculated using 2-ΔΔCt.

Immunophenotypic scoring system was obtained to differentiate CLL cases from other lymphoproliferative disorders. CLL cells were defined as CD19 +ve, CD23 +ve, CD5 +ve, CD20 dim, Fmc7 –ve, or weekly +ve.

Flow cytometric detection of SLAMF1, CD19, and CD38 in the peripheral blood was done. Measurement of CD38 was done using human CD38 PE-conjugated monoclonal antibody kit (Catalog No. 12-0389-42) (Thermo Fisher Scientific, Waltham, Massachusetts, USA), SLAMF1 using human SLAM/CD150 fluorescein-conjugated antibody (Catalog No. FAB1642F) (R and D Systems, Minneapolis, Minnesota, USA), and CD19 using antihuman CD19 PE antibodies (Catalog No. 11-0199) (eBioscience, USA).

Overall, 2 ml of blood was added gently to an equal volume of Ficoll and centrifuged for 20 min at 1200 rpm. Samples were analyzed within 24 h from sampling. The separated cells were washed three times with phosphate-buffered saline and centrifugation for 5 min at 2000 rpm. The supernatant was gently aspirated and discarded. The pellet was resuspended in 500 μl of phosphate-buffered saline. Three polystyrene test tubes (12 × 75 mm) were prepared. Dual stains with 10 μm of SLAMF1 FITC and 10 μm of CD19 PE were placed in the first tube, and 10 μm of CD38 PE in the second tube. The third tube was used as a negative control. Overall, 100 μl of the suspended cells was added to each tube and mixed gently with a vortex mixer. The tubes were incubated for 30 min in the dark. Analysis was performed on a FACS Calibur flow cytometer (Becton Dickinson Immunocytometry system, San Jose, California, USA) after gating on the lymphocyte area. The number of events to be analyzed was adjusted to be 10 000. The sample was mixed properly before acquisition.

Statistical analysis

Data were fed to the computer and analyzed using IBM SPSS software package, version 20.0. (IBM Corp., Armonk, New York, USA). Categorical data were represented as numbers and percentages. χ^2 test was applied to investigate the association between the categorical variables. Alternatively, Monte-Carlo correction test was applied when more than 20% of the cells have expected count less than 5. For continuous data, they were tested for normality by the Shapiro–Wilk test. Quantitative data were expressed as range (minimum and maximum), mean, SD, and median. Student *t* test was used to compare two groups for normally distributed quantitative variables, whereas analysis of variance was used for comparing the different studied groups, followed by post-hoc test (Tukey) for pairwise comparison. On the contrary, Mann–Whitney test was used to compare two groups for not normally distributed quantitative variables, whereas Kruskal–Wallis test was used to compare different groups for not normally distributed quantitative variables, followed by post-hoc test (Dunn's for multiple comparisons test) for pairwise comparison. Spearman coefficient was used to correlate between not normally distributed quantitative variables. Significance of the obtained results was judged at the 5% level.

Results

Patients with CLL showed a significant reduction in expression levels of BECN1, PIK3C3, and SLAMF1, with a mean expression of 0.79 ± 0.99, 0.87 ± 0.19, and 33.12 ± 18.94, in comparison with the control group (1.08 ± 0.05, 1.14 ± 0.07, and 87.12 ± 4.97, respectively) ($P < 0.001$) (Table 1).

BECN1 gene expression showed a significant difference among different Binet stages ($P = 0.017$), with a mean of 0.41 ± 0.27 and a median of 0.45 in stage A compared with a mean of 1.37 ± 1.72 and a median of 0.78 in stage B and a mean of 1.10 ± 0.63 and a median of 0.93 in stage C (Table 2).

SLAMF1 expression showed a statistically significant difference among different Binet stages ($P < 0.001$), with higher expression in stage A, with a mean of 44.12 ± 14.57 and a median of 44.50, compared with more advanced stages, with a mean of 30.50 ± 9.90 and a median of 28.0 in stage B and a mean of 6.15 ± 3.77 and a median of 5.55 in stage C (Table 2).

On the contrary, there was no significant difference in PIK3C3 expression among the different Binet stages ($P = 0.533$) (Table 2).

There was no statistically significant correlation between BECN1 gene expression and different CBC parameters, including; hemoglobin (Hb), total lymphocytic count (TLC), absolute lymphocytic count (ALC), and platelet count or with markers of tumor burden, lactate dehydrogenase (LDH) and B2 microglobulin ($P = 0.142$, 0.183, 0.121, 0.523, 0.437, 0.127, respectively). In addition, there was no significant correlation between BECN1 expression and CD38 ($P = 0.142$) (Table 3).

Table 1 Comparison between the two studied groups according to different parameters

	Cases (*n*=40)	Control (*n*=10)	Test of significance	*P*
Sex [*n* (%)]				
Male	26 (65.0)	7 (70.0)	χ^2=0.089	^{FE}P=1.000
Female	14 (35.0)	3 (30.0)		
Age (years)				
Mean±SD	62.33±7.13	60.0±5.27	*t*=0.965	0.340
Median (min.-max.)	62.5 (48-81)	60 (52-70)		
TLC (×10³/µl)				
Mean±SD	58.52±31.06	7.66±1.31	*U*=0.0*	<0.001*
Median (min.-max.)	54.85 (15.7-125)	7.85 (5.40-10.0)		
ALC (×10³/µl)				
Mean±SD	48.80±28.20	2.50±0.58	*U*=0.0*	<0.001*
Median (min.-max.)	45.75 (11.16-119.0)	2.39 (1.80-3.50)		
Hb (g/dl)				
Mean±SD	11.0±1.18	13.49±0.59	*t*=6.447*	<0.001*
Median (min.-max.)	11.0 (8.40-14.30)	13.65 (12.50-14.30)		
PLT (×10³/UI)				
Mean±SD	146±57.8	229±84.3	*t*=3.703*	0.001*
Median (min.-max.)	137 (43-298)	204 (154-432)		
CD38				
Mean±SD	31.7±18.9	42.1±6.6	*U*=111.5*	0.030*
Median (min.-max.)	29.0 (2.52-74.0)	42.25 (30.4-50.2)		
LDH				
Mean±SD	695±185	348±67.3	*t*=9.592*	<0.001*
Median (min.-max.)	667 (320-1098)	350 (245-450)		
B2 microglobulin				
Mean±SD	2006±1068	627±194	*U*=0.0*	<0.001*
Median (min.-max.)	1774 (970-6923)	631 (330-910)		
Beclin1				
Mean±SD	0.79±0.99	1.08±0.05	*U*=61.0*	<0.001*
Median (min.-max.)	0.53 (0.05-4.53)	1.07 (1.01-1.17)		
PIK3C3				
Mean±SD	0.87±0.19	1.14±0.07	*t*=7.207*	<0.001*
Median (min.-max.)	0.84 (0.52-1.40)	1.14 (1.03-1.24)		
SLAMF1				
Mean±SD	33.12±18.94	87.12±4.97	*U*=0.0*	<0.001*
Median (min.-max.)	33.50 (1.50-65.0)	86.50 (80.20-95.0)		

χ^2, χ^2 test; ALC, absolute lymphocytic count; FE, Fisher exact; Hb, hemoglobin; LDH, lactate dehydrogenase; max., maximum; min., minimum; PIK3C3, phosphoinositide 3-kinase C3; PLT, platelet; *t*, Student *t* test; TLC, total lymphocytic count; *U*, Mann-Whitney *U* test. *Statistically significant.

Table 2 Relation between clinical stage by Binet system with SLAMF1, Beclin 1, and phosphoinositide 3-kinase C3 in patients with chronic lymphocytic leukemia (*n*=40)

Studied variables	Clinical stage by Binet system			Test of significance	*P*
	Stage A (*n*=22)	Stage B (*n*=10)	Stage C (*n*=8)		
SLAMF1					
Mean±SD	44.12±14.57	30.50±9.90	6.15±3.77	*K*=22.391*	<0.001*
Median (min.-max.)	44.5 (15-65)	28 (18-48)	5.55 (1.5-12)		
Beclin1 [*n* (%)]					
Low	15 (68.2)	3 (30.0)	2 (25.0)	χ^2=6.279*	^{MC}P=0.034*
High	7 (31.8)	7 (70.0)	6 (75.0)		
Mean±SD	0.41±0.27	1.37±1.72	1.10±0.63	*K*=8.179*	0.017*
Median (min.-max.)	0.45 (0.06-0.95)	0.78 (0.05-4.53)	0.93 (0.47-2.07)		
PIK3C3 [*n* (%)]					
Low	12 (54.5)	4 (40.0)	4 (50.0)	χ^2=0.661	^{MC}P=0.910
High	10 (45.5)	6 (60.0)	4 (50.0)		
Mean±SD	0.86±0.18	0.93±0.27	0.83±0.12	*F*=0.640	0.533
Median (min.-max.)	0.82 (0.54-1.20)	0.86 (0.52-1.40)	0.84 (0.63-0.98)		

χ^2, χ^2 test; *F*, analysis of variance test; *K*, Kruskal-Wallis test; max., maximum; MC, Monte-Carlo; min., minimum; PIK3C3, phosphoinositide 3-kinase C3. *Significant.

Similarly, we did not demonstrate a significant correlation between PIK3C3 expression and different CBC parameters or with LDH and B2 microglobulin (P = 0.712, 0.183, 0.383, 0.503, 0.098, and 0.108, respectively).

However, PIK3C3 expression showed a statistically significant correlation with CD38 (P = 0.012) (Table 4).

There was a significant correlation between SLAMF1 and Hb level, platelet count, LDH, B2 microglobulin, and CD38 (P < 0.001 for all). This correlation was not demonstrated with TLC or ALC (P = 0.846 and 0.629, respectively) (Table 5).

Discussion

CLL is a monoclonal disorder characterized by a progressive accumulation of CD5+/CD19+ small lymphocytes [15].

Autophagy is a highly conserved evolutionary process that is responsible for degrading and recycling of intracellular components by lysosomal system. Under physiological conditions, autophagy is maintained at basal levels, which contributes to the successive degradation of superabundant, abnormal, and damaged organelles [16]. Thus, efficient and strict regulation of autophagy is crucial for safeguarding cell homeostasis. Nonetheless, aberrant autophagy may underlie different human pathological conditions, as demonstrated by several studies [17].

BECN1 and PIK3C3 genes regulate autophagy, and they are a component of the PI3K complex, which mediates a vesicle-trafficking process as they participate in the initiation core complex.

The signaling lymphocytic activation molecule SLAMF1 (CD150) is costimulatory molecule and microbial sensor expressed by T and B lymphocytes, natural killer cells, and dendritic cells. Loss of SLAMF1 expression modifies genetic pathways that adjust chemotaxis and autophagy [12].

Defects in autophagy have been implicated in several diseases. The discovery that 40–75% of human breast, ovarian, and prostate tumors had monoallelic disruption of BECN1 put autophagy on the map as having tumor-suppressive function [18]. High autophagy activity was observed in pancreatic cancer primary tumors and cell lines, and in fact, it was observed that these tumors depend on autophagy for growth [19]. Autophagy likely has both tumor-suppressive and tumor-promoting functions. By maintaining cellular homeostasis, autophagy may prevent conditions that lead to cancer, but in already established tumors, it serves to enhance the survival of tumor cells [20].

In the present study of well-characterized CLL cases, expression levels of BECN1 and PIK3C3, which

Table 3 Relation between Beclin 1 and different parameters in patients with chronic lymphocytic leukemia (n=40)

Studied variables	Beclin 1		Test of significance	P
	Low (n=20)	High (n=20)		
TLC (×10³/μl)				
Mean±SD	67.35±37.33	49.70±20.56	U=150.5	0.183
Median (range)	71.25 (15.7-125)	49.35 (18.19-87.80)		
ALC (×10³/μl)				
Mean±SD	57.19±34.14	40.41±17.86	U=142.0	0.121
Median (range)	57.90 (11.16-119)	40.50 (13.43-77.50)		
Hb (g/dl)				
Mean±SD	11.28±1.13	10.73±1.19	t=1.499	0.142
Median (range)	11.10 (8.40-14.30)	10.60 (8.60-13.30)		
PLT (×10³/Ul)				
Mean±SD	152±64.6	140±51.0	t=0.644	0.523
Median (range)	133 (43.0-298.0)	145 (58-240)		
CD38				
Mean±SD	27.13±17.47	36.28±19.56	U=145.50	0.142
Median (range)	24.0 (2.52-69.0)	36.0 (4.30-74.0)		
LDH				
Mean±SD	672±189	718±183	t=0.785	0.437
Median (range)	606 (380-1098)	702 (320-1010)		
B2 microglobulin				
Mean±SD	1794±805	2217±1265	U=143.50	0.127
Median (range)	1600 (970-4170)	1915 (1035-6923)		

ALC, absolute lymphocytic count; Hb, hemoglobin; LDH, lactate dehydrogenase; PLT, platelet; t, Student t test; TLC, total lymphocytic count; U, U value for Mann-Whitney test.

Table 4 Relation between phosphoinositide 3-kinase C3 and different parameters in patient with chronic lymphocytic leukemia (*n*=40)

Studied variables	PIK3C3		Test of significance	*P*
	Low (*n*=20)	High (*n*=20)		
TLC×(10^3/µl)				
Mean±SD	62.74±36.18	54.31±25.19	*U*=183.5	0.659
Median (range)	58.85 (15.70-125)	52.40 (18.95-121)		
ALC (×10^3/µl)				
Mean±SD	54.34±33.89	43.26±20.48	*U*=167.0	0.383
Median (range)	52.15 (11.70-119)	42.70 (11.16-97.4)		
Hb (g/dl)				
Mean±SD	11.07±1.21	10.93±1.18	*t*=0.371	0.712
Median (range)	11.0 (8.40-14.30)	11.10 (8.60-13.30)		
PLT (×10^3/µl)				
Mean±SD	152±63.4	140±52.4	*t*=0.677	0.503
Median (range)	148 (43-298)	128 (58-295)		
CD38				
Mean±SD	24.33±14.03	39.08±20.51	*U*=108.5*	0.012*
Median (range)	22.76 (2.52-52.0)	39.0 (4.30-74.0)		
LDH				
Mean±SD	646±190	743±172	*t*=1.694	0.098
Median (range)	598 (320-1010)	704 (515-1098)		
B2 microglobulin				
Mean±SD	1819±828	2192±1258	*U*=140.5	0.108
Median (range)	1695 (970-4170)	1985 (1109-6923)		

ALC, absolute lymphocytic count; Hb, hemoglobin; LDH, lactate dehydrogenase; PIK3C3, phosphoinositide 3-kinase C3; PLT, platelet; *t*, Student *t* test; TLC, total lymphocytic count; *U*, Mann-Whitney test. *Significant.

Table 5 Correlation between SLAMF1 and different parameters in patient with chronic lymphocytic leukemia (*n*=40)

Studied variables	SLAMF1	
	rs	*P*
TLC (×10^3/µl)	0.032	0.846
ALC (×10^3/µl)	0.079	0.629
Hb (g/dl)	0.584	<0.001*
PLT (×10^3/UI)	0.619	<0.001*
CD38	−0.691	<0.001*
B2 microglobulin	−0.731	<0.001*
LDH	−0.719	<0.001*

ALC, absolute lymphocytic count; Hb, hemoglobin; LDH, lactate dehydrogenase; PLT, platelet; r_s, Spearman coefficient; TLC, total lymphocytic count. *Significant.

encode the components of the PI3K core complex central to the initiation of autophagy, and the surface expression of SLAMF1 were all assessed, and their expression was correlated with the prognostic markers of the disease and the clinical outcome.

BECN1 expression level in patients with CLL was significantly lower than that in the control group. Similar results were reported by Kristensen *et al.* [21], with an overall threefold reduction. BECN1 through its main role in autophagy process by interacting with PIK3C3 and formation of autophagosome may explain why its low expression levels or deletion could contribute to incidence of many diseases including cancer and its suggestive role as tumor suppressor gene. Our study findings are in agreement with the findings of the study of Ghozlan *et al.* [22], which showed that BECN1 expression was low in adult Egyptian patients with do novo AML.

Our study results showed no statistically significant correlation between BECN1 expression and different CBC parameters (TLC, ALC, Hb, and platelet), as well as prognostic and tumor burden markers (CD38, LDH, and B2 microglobulin). These results are in agreement with Kristensen *et al.* [21], who showed no statistically significant correlation between BECN1 expression levels and any of the clinical characteristic markers.

Tandel *et al.* [23] reported that no significant differences were found between BECN1 expression in patients with AML in relation to age, Hb, platelets count, and bone marrow blasts.

According to the current study, BECN1 gene expression showed a statistically significant difference among different Binet stages, being lower in stage A compared with stage B and stage C. Lower gene expression was significantly associated with early-stage disease. In contrast, Kong *et al.* [24] reported significantly higher BECN1 mRNA levels in patients with Binet stage A compared with patients with stages B and C, suggesting that autophagy differs in CLL owing to the presence of heterogeneity. On the contrary, Kristensen *et al.* [21]

showed that there was no statistically significant difference of Beclin1 gene expression between different Binet stages of patients with CLL.

Autophagy is a double-face coin, as it can not only suppress tumor progression but also promote tumor survival. Our data showed that although low expression of BECN1 can contribute to tumorigenesis in CLL, it seems to be associated with slower progress likely through autophagy inhibition.

In addition, the discrepancy in the data from various studies can be owing to the variation of the disease characteristics, sample size, and associated molecular defects. Our study was carried out on newly diagnosed patients with CLL who had not received any treatment.

In the present study, PIK3C3 was significantly lower in patients with CLL than in the control group, which is unlike the study by Kristensen, *et al.* [21], which reported no significant difference between the two groups.

According to the current study results, no statistically significant correlation was found between PIK3C3 expression and different CBC parameters (TLC, ALC, Hb, and platelet) and tumor burden markers (LDH and B2 microglobulin). On the contrary, there was a significant correlation between PIK3C3 expression levels and CD38.

We did not demonstrate a significant correlation between the Binet and PIK3C3 in patients with chronic lymphocytic. This agrees with the data published by Kristensen *et al*. [21].

According to the current study results, SLAMF1 expression was significantly lower in patients with CLL than in the control group. There was great heterogeneity in the expression between patients with CLL, as it ranged from 1.50 to 65.0%. In agreement with the current study results, Elkholy and Allam [25] found that the expression of SLAMF1 was significantly decreased in patients with CLL compared with the control group.

These data agree with those reported by Coma *et al.* [26], who reported that the expression of SLAMF on B lymphocytes of patients with CLL was significantly lower than its expression on normal mature B cells.

Correlation of SLAMF1 expression with different CBC parameters revealed that there was a significant positive correlation between this marker and Hb level and platelet count. Similarly, Elkholy and Allam [25] reported that there was a statistically significant positive correlation between SLAMF1 and Hb level and platelets count.

According to this study, no significant correlation was demonstrated between SLAMF1 expression and TLC or ALC. This is similar to the data from Elkholy and Allam [25].

We demonstrated a significant negative correlation between SLAMF1 expression and LDH, B2 microglobulin, and CD38. Our results showed that loss of SLAMF1 expression directly contributes to poor prognosis for patients with CLL. SLAMF1 is a multifunctional type I transmembrane glycoprotein that belongs to the SLAM family, which regulates CLL response to chemokines and regulates autophagy, which plays a crucial role in removing misfolded proteins and damaged organelles, as their presence is very toxic to the cell [18]. So, loss of SLAMF1 in CLL cells makes them resistant to autophagy and autophagy-inducing drugs, such as B-cell CLL/lymphoma 2 inhibitors.

Similarly, Elkholy and Allam [25], in their study, demonstrated a significant negative correlation between marker of bad prognosis (CD38) and SLAMF1 expression. Bologna and Deaglio [12] also reported that CD150 expression (SLAMF1) negatively correlated with CD38 and CD49d expression.

Regarding the relation between clinical stage by Binet system and SLAMF1 in the current study, there was a highly statistically significant difference in SLAMF1 expression among different Binet stages, with higher expression in stage A compared with more advanced stages B and C. In concordance with the present study results, Bologna and Deaglio [12] reported that SLAMF1 expression decreased in advanced stages of CLL.

Conclusion

Autophagy genes BECN1 and PIK3C3 expression and surface expression of SLAMF1 were significantly lower in patients with CLL than that in the control group, which indicates a role in pathogenesis.

Autophagy genes BECN1 and PIK3C3 and surface SLAMF1 expression in CLL are promising candidates for more highly qualified larger sample studies examining the role of autophagy in CLL pathogenesis and may therefore represent possible targets for treatment.

Acknowledgements

The authors would like to thank the Central Laboratory, Faculty of Medicine, Menoufia University.

Funding: This study was supported by the scientific research budget of the General Organization for

Teaching Hospitals and Institutes and Menoufia University Hospitals.

Authors' contributions: R.T.H.G. collected the data, performed the research, analyzed the data, and wrote the manuscript. N.F.O., K.A.K., and S.M.E. designed the study and contributed to data analysis and writing of the manuscript.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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