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Prognostic Values of the Autophagy-Related Proteins; ATG16L and LC3B Expression in Oropharyngeal and Oral Cavity Squamous Cell Carcinoma (OSCC); an Immunohistochemical Study

Samar M Abd-El Raouf[1*], Randa Mohamed Kaf[1], Rehab Hemeda[2], Doaa Mandour[2], Rham Z Ahmed[3], Gamal Osman[4] and Ramadan Mahmoud[4]

*[*1]Department of Pathology, Faculty of Medicine, Zagazig University, Egypt*

[2]Department of Clinical Oncology and Nuclear Medicine, Faculty of Medicine, Zagazig University, Egypt

[3]Department of Medical Oncology, Faculty of Medicine, Zagazig University, Egypt

[4]Department of General Surgery, Faculty of Medicine, Zagazig University, Egypt.

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ABSTRACT

Background: Autophagy has a paradoxical role in cancer. Autophagy-related 16-like 1 (ATG16L1) and the light chain 3 B (LC3B) are proteins that form an essential part of the membranes of the autophagosomes. To date, the significance of ATG16L and LC3B expression in OSCC has not been fully clarified.

Aim: Aim of the current study was to investigate ATG16L and LC3B tissue protein expression levels in tissues which were taken from OSCC patients then correlate our results with pathological and clinical patients' data.

Methods: ATG16L and LC3B expression was assessed in 40 patients with OSCC using immunohistochemistry. We followed the patients for 5 years for progression and recurrence of the disease and for survival.

Results: High ATG16L1 and LC3B expression was correlated with higher grade (p=0.021 and 0.049, respectively), advanced stage of the tumor (p=0.003 and 0.005, respectively), presence of lymph node metastases (p=0.007 and 0.033, respectively), distant metastases (p=0.045 and 0.040, respectively), poor response to therapy (p=0.018 and 0.050, respectively), higher incidence of tumor recurrence, worse 5 year DFS and OS rates (p<0.001).

Conclusion: ATG16L and LC3B overexpression are markers of poor prognosis in OSCC patients.

Keywords: ATG16L, LC3B, OSCC, Autophagy, Immunohistochemistry, Prognosis

INTRODUCTION

Squamous cell carcinoma (OSCC) which arises from the oropharynx and from the oral cavity is the commonest head and neck cancer and when combination of their incidence is made together they ranked as the 8th commonest cancer worldwide which occupies more than 90% of oral cavity malignancies [1]. This serious cancer frequently presented with metastasis, high rate of recurrence and dismal outcome due to late diagnosis and relative resistance to chemotherapy. Although there is improvement in early diagnosis and management of such type of cancer; the outcome of patients is still dismal with high incidence of local recurrence and distant metastasis which lead to unfavorable survival rates [2]. Thus, exploring novel methods of diagnosis and novel molecular markers which could predict the prognosis of patients with OSCC for is urgently needed for adequate management of OSCC [3]. Autophagy, with its subtype; macro-autophagy, is a catabolic cellular process which is responsible for the degradation of un-needed cellular components which helps in the maintenance of cellular integrity during stressful conditions. It was recently found that, dysfunctional autophagy is observed in different types of cancer [4], additionally; autophagy plays paradoxical roles in both cancer promotion and inhibition. Autophagy has a cancer-

Corresponding author: Samar M Abd-El Raouf, Department of Pathology, Faculty of Medicine, Zagazig University, Zagazig-44519, Egypt, E-mail: olaharb2015@gmail.com

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promoting role through protecting cancer cells from the harmful effects of chemotherapy or by being a source of energy for cancer cells to live under hypoxic and acidic conditions despite the lack of mature vessels. On the other hand, autophagy inhibition could increase the frequency of spontaneous cancer occurrence [5]. There are about 30 autophagy-related proteins (Atgs) that have participated in the autophagic process. Most of these proteins are involved in the phases of autophagosome formation; proteins which play a role in autophagosomes formation were proved to give the ability to detect autophagy in tissue samples. Autophagy-related 16-like 1 (ATG16L1) is considered a part of a huge protein complex that proved to participate at elongation autophagosomal membranes [6]. The microtubule-associated protein 1 light chain 3 B (LC3B), is transformed to the lipidated LC3B-II and it forms an integral part of the autophagosomes membranes [4]. The role of autophagy in cancer progression remains controversial because it has both cancer promoting and inhibiting properties [1]. There are many clinical trials which were investigating the efficacy of using autophagy inhibitors as novel therapies in addition to the currently used targeted therapies, immunotherapies and chemotherapeutic agents have been started since 2010 and produced encouraging results [7]. To date, the significance of autophagy related biomarkers; ATG16L and LC3B expression in OSCC has not been completed studied.

Therefore, we aimed in the current study to investigate ATG16L and LC3B tissue protein expression levels in tissues which were taken from OSCC patients then correlate our results with pathological and clinical patient's data.

PATIENTS AND METHOD

The current prospective cohort study is a study where we have included sections from formalin fixed paraffin embedded 40 tissue blocks that collected from samples of 40 cases of OSCC that have been surgically operated in General Surgery Hospitals, Oncology unit, Faculty of Medicine, Zagazig University. Specimens were sent to Pathology department Faculty of Medicine, Zagazig University for further processing, accurate diagnosis, grading and staging. We followed our patients for 5 years till death or their most recent medical examination for response to therapy, progression, and recurrence of the disease after successful therapy and survival of patients in Clinical Oncology and Nuclear Medicine Department and in Medical Oncology Department, Faculty of Medicine, Zagazig University for 5 years in the period between December 2013 to December 2018. We include all cases of OSCC and the World Health Organization classification was used for pathologic grading. The study complied with the guidelines of the Local Ethics Committee of Faculty of Medicine, Zagazig University.

Immunohistochemical analysis

Formalin-fixed paraffin-embedded blocks from malignant tissues were cut into 4 μm sections, deparaffinized and rehydrated in xylene and graded alcohols, respectively. Antigen retrieval was done by heating in a microwave in citric acid buffer; we incubated the sections with 3% H_2O_2 for half an hour at room temperature so as to antagonize endogenous peroxidase activity. We have incubated all sections with normal serum for half an hour then incubated them with the primary antibodies; rabbit polyclonal anti-ATG16L1 antibody and anti-LC3B (Novus Biologicals, CO, Littleton) at a dilution of 1:250 for 30 min at room temperature.

Evaluation of the ATG16L and LC3B cytoplasmic immunoreactivity in OSCC tissues

Immunoreactivity score (IRS), was obtained by multiplying the scores of stain intensity and scores of stain extent. Regarding stain intensity, negative cytoplasmic staining was scored as 0, weakly positive as 1, moderately positive as 2 and strongly positive as 3. Regarding extent score, negative stains were scored as 0, 1-10%, as 1, 11-50% as 2 and as 3>50%. We assigned the final IRS as ranges from 0 to 9, with 0-4 were defined as being low expression and 6-9 as high expression. Cervical adenocarcinoma cells and breast cancer cells were used as positive control for ATG16L and LC3B, respectively. Negative control was obtained by replacing the primary antibody with non-immune serum [9,10].

All slides were evaluated independently by 2 senior pathologists who were blinded to the patients' clinical information and follow-up data.

STATISTICAL ANALYSIS

The categorical variables were compared through using Fisher's exact test or Pearson's Chi-square test when was appropriate. Trend of change between ordinal data were compared using Chi-square test for trend. Strength of relationship between IHC of ATG16L1 and LC3B were determined by computing phi coefficient with (+) sign was indicator for direct relationship and (-) sign was indication for inverse relationship. Overall Survival (OS) was calculated as the time from diagnosis to death or the most recent follow-up. Disease Free Survival (DFS) was calculated as the time from starting treatment to date of relapse or the most recent follow-up contact that patient was known as relapse free. Stratification of OS and DFS was done according to ATG16L1 and LC3B. These time-to-event distributions were estimated using the method of Kaplan-Meier plot. A p-value <0.05 has been considered a significant value. Statistics have been done by using SPSS 22.0 for windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Patient characteristics

40 samples collected from OSCC patients. The clinical characteristics of the 40 patients with OSCC that are included in the study are present in **Table 1**.

The 40 OSCC cases were divided into 35 (87.5%) males and 5 (12.5%) females. Demographic data of our patients were included in **Table 1**.

Table 1. Clinicopathological features, immunohistochemical markers and outcome of 40 patients with oral cavity and oropharyngeal carcinoma.

Characteristics	All patients (N=40)	
	No.	%
Age (years)		
Mean ± SD	54.60 ± 9.73	
Median (Range)	56 (33- 69)	
≤ 60 years	29	72.5%
>60 years	11	27.5%
Sex		
Male	35	87.5%
Female	5	12.5%
History of smoking		
Non-smoker	10	25%
Smoker	30	75%
Site		
Oral cavity	29	72.5%
Grade		
Grade I	12	30%
Grade II	19	47.5%
CIS		
Absent	22	55%
Present	18	45%
PNI		
Absent	26	65%
Present	14	35%
p16		
Negative	18	45%
Positive	22	55%
T stage		
T1	10	25%
T2	10	25%
T3	12	30%
T4a	4	10%
T4b	4	10%
N stage		

N0	18	45%
N1	2	5%
N2	9	22.5%
N3	11	27.5%
M stage		
M0	33	82.5%
M1	7	17.5%
AJCC 2017 stage group		
Stage I	8	20%
Stage III	14	35%
Stage IVA	3	7.5%
Stage IVB	3	7.5%
Prognostic group		
Early disease	9	22.5%
Locally advanced resectable	9	22.5%
Locally advanced unresectable	15	37.5%
Metastatic	7	17.5%
ATG16L1		
Low	19	47.5%
High	21	52.5%
LC3B		
Low	21	52.5%
High	19	47.5%
Response to treatment (N=26)		
CR	8	30.8%
PR	8	30.8%
SD	5	19.2%
PD	5	19.2%
OAR	16	61.5%
NR	10	38.5%
Follow-up duration (months)		
Mean ± SD	33.50 ± 11.46	
Median (Range)	34.50 (12-55)	
Recurrence (N=24)		
Absent	11	45.8%
Present	13	54.2%
Mortality (N=40)		
Alive	19	47.5%
Died	21	52.5%

Immunohistochemical results

ATG16L1expression and its correlation clinico-pathological features of OSCC patients: High ATG16L1 expression was found in 21 (52.5%) of cases of OSCC and its expression was significantly positively correlated with higher grade (p=0.021), advanced stage of the tumor (p=0.003), presence of lymph node metastases (p=0.007), distant metastases (p=0.045), LVI (p=0.028), PNI (p=0.015), poor prognostic group (p=0.004), and higher incidence of P16 positivity (p=0.005). No significant correlation was found between ATG16L1 expression with initial site of the tumor, age or sex of our patients, history of smoking or presence of adjacent foci of CIS **(Table 2 and Figure 1)**.

Table 2. Correlation between clinicopathological features and ATG16L1 and LC3B expression in oral cavity and oropharyngeal carcinoma patients (N=40).

Characteristics	All		ATG16L1				p-value	LC3B				p-value
	(N=40)		Low (N=19)		High (N=21)			Low (N=21)		High (N=19)		
	No.	(%)	No.	(%)	No.	(%)		No.	(%)	No.	(%)	
Age												
≤ 60 years	29	(72.5%)	15	(51.7%)	14	(48.3%)	0.385‡	15	(51.7%)	14	(48.3%)	0.873‡
>60 years	11	(27.5%)	4	(36.4%)	7	(63.6%)		6	(54.5%)	5	(45.5%)	
Sex												
Male	35	(87.5%)	17	(48.6%)	18	(51.4%)	1.000‡	19	(54.3%)	16	(45.7%)	0.654‡
Female	5	(12.5%)	2	(40%)	3	(60%)		2	(40%)	3	(60%)	
History of smoking												
Non-smoker	10	(25%)	5	(50%)	5	(50%)	1.000‡	5	(50%)	5	(50%)	1.000‡
Smoker	30	(75%)	14	(46.7%)	16	(53.3%)		16	(53.3%)	14	(46.7%)	
Site												
Oral cavity	29	(72.5%)	16	(55.2%)	13	(44.8%)	0.115‡	18	(62.1%)	11	(37.9%)	0.049‡
Oropharynx	11	(27.5%)	3	(27.3%)	8	(72.7%)		3	(27.3%)	8	(72.7%)	
Grade												
Grade I	12	(30%)	7	(58.3%)	5	(41.7%)	0.021§	7	(58.3%)	5	(41.7%)	0.049§
Grade II	19	(47.5%)	10	(52.6%)	9	(47.4%)		11	(57.9%)	8	(42.1%)	
Grade III	9	(22.5%)	2	(22.2%)	7	(77.8%)		3	(33.3%)	6	(66.7%)	
CIS												
Absent	22	(55%)	11	(50%)	11	(50%)	0.726‡	12	(54.5%)	10	(45.5%)	0.775‡
Present	18	(45%)	8	(44.4%)	10	(55.6%)		9	(50%)	9	(50%)	
LVI												
Absent	16	(40%)	11	(68.8%)	5	(31.2%)	0.028‡	11	(68.8%)	5	(31.2%)	0.043‡
Present	24	(60%)	8	(33.3%)	16	(66.7%)		10	(41.7%)	14	(58.3%)	
PNI												
Absent	26	(65%)	16	(61.5%)	10	(38.5%)	0.015‡	17	(65.4%)	9	(34.6%)	0.026‡
Present	14	(35%)	3	(21.4%)	11	(78.6%)		4	(28.6%)	10	(71.4%)	

p16												
Negative	18	(45%)	13	(72.2%)	5	(27.8%)	0.005‡	13	(72.2%)	5	(27.8%)	0.024‡
Positive	22	(55%)	6	(27.3%)	16	(72.7%)		8	(36.4%)	14	(63.6%)	
T stage												
T1	10	(25%)	9	(90%)	1	(10%)	0.004§	9	(90%)	1	(10%)	0.008§
T2	10	(25%)	4	(40%)	6	(60%)		4	(40%)	6	(60%)	
T3	12	(30%)	4	(33.3%)	8	(66.7%)		6	(50%)	6	(50%)	
T4a	4	(10%)	2	(50%)	2	(50%)		2	(50%)	2	(50%)	
T4b	4	(10%)	0	(0%)	4	(100%)		0	(0%)	4	(100%)	
N stage												
N0	18	(45%)	13	(72.2%)	5	(27.8%)	0.007§	13	(72.2%)	5	(27.8%)	0.033§
N1	2	(5%)	0	(0%)	2	(100%)		0	(0%)	2	(100%)	
N2	9	(22.5%)	4	(44.4%)	5	(55.6%)		5	(55.6%)	4	(44.4%)	
N3	11	(27.5%)	2	(18.2%)	9	(81.8%)		3	(27.3%)	8	(72.7%)	
M stage												
M0	33	(82.5%)	18	(54.5%)	15	(45.5%)	0.045‡	20	(60.6%)	13	(39.4%)	0.040‡
M1	7	(17.5%)	1	(14.3%)	6	(85.7%)		1	(14.3%)	6	(85.7%)	
AJCC 2017 stage group												
Stage I	8	(20%)	7	(87.5%)	1	(12.5%)	0.003§	7	(87.5%)	1	(12.5%)	0.005§
Stage II	11	(27.5%)	6	(54.5%)	5	(45.5%)		6	(54.5%)	5	(45.5%)	
Stage III	14	(35%)	5	(35.7%)	9	(64.3%)		7	(50%)	7	(50%)	
Stage IVA	3	(7.5%)	1	(33.3%)	2	(66.7%)		1	(33.3%)	2	(66.7%)	
Stage IVB	3	(7.5%)	0	(0%)	3	(100%)		0	(0%)	3	(100%)	
Stage IVC	1	(2.5%)	0	(0%)	1	(100%)		0	(0%)	1	(100%)	
Prognostic group												
Early disease	9	(22.5%)	8	(88.9%)	1	(11.1%)	0.004§	8	(88.9%)	1	(11.1%)	0.010§
Locally advanced resectable	9	(22.5%)	4	(44.4%)	5	(55.6%)		4	(44.4%)	5	(55.6%)	
Locally advanced unresectable	15	(37.5%)	6	(40%)	9	(60%)		8	(53.3%)	7	(46.7%)	
Metastatic	7	(17.5%)	1	(14.3%)	6	(85.7%)		1	(14.3%)	6	(85.7%)	

‡ Chi-square test; § Chi-square test for trend; $p<0.05$ is significant

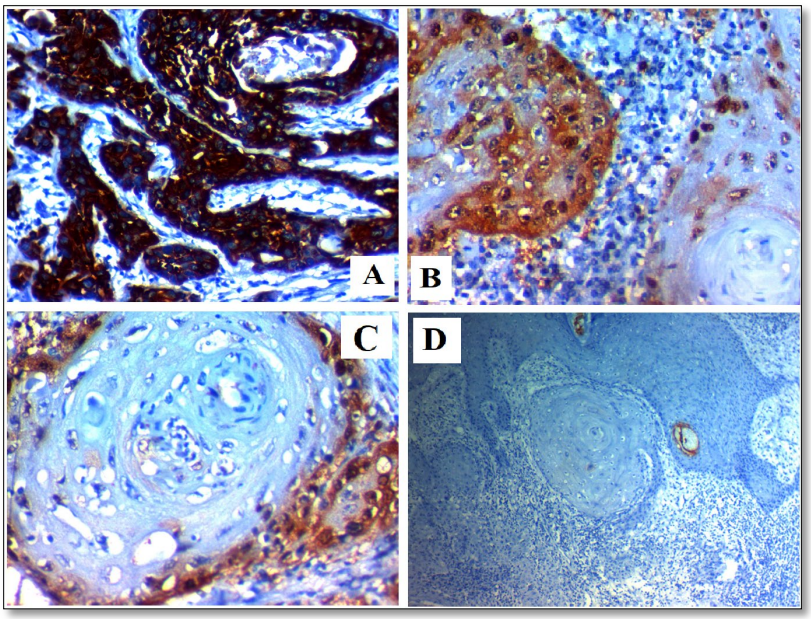

Figure 1. ATG16L1 expression in oral cavity and oropharyngeal carcinoma (OSCC): (A) High cytoplasmic expression of ATG16L1 in poorly differentiated OSCC stage IV 400x. (B) High cytoplasmic expression of ATG16L1 in moderately differentiated OSCC, stage III 400x. (C) Low cytoplasmic expression of ATG16L1 in well differentiated OSCC stage II 400x. (D) Negative cytoplasmic expression of ATG16L1 in well differentiated OSCC; stage I 400x.

LC3B expression and its correlation clinico-pathological features of OSCC patients: High LC3B was found in 19 (47.5%) of cases of OSCC and its expression was significantly positively correlated with higher grade (p=0.049), advanced stage of the tumor (p=0.005), presence of lymph node (p=0.033), distant metastases (p=0.040), LVI (p=0.043), PNI (p=0.026), poor prognostic group (p=0.010) and higher incidence of P16 positivity (p=0.024), no significant correlation was found between LC3B expression with initial site of the tumor, age or sex of our patients, history of smoking or presence of adjacent foci of CIS **(Table 2 and Figure 2)**.

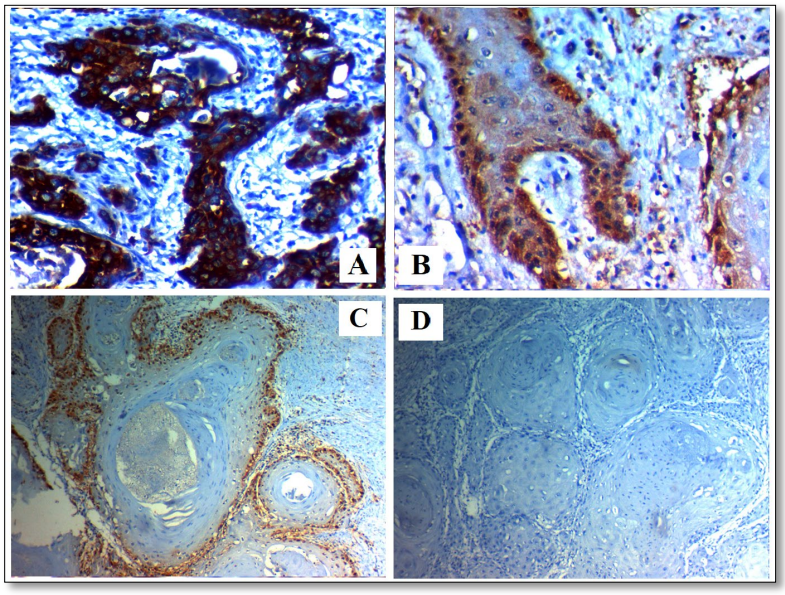

Figure 2. LC3B expression in oral cavity and oropharyngeal carcinoma (OSCC): (A) High cytoplasmic expression of LC3B in poorly differentiated OSCC stage IV 400x. (B) High cytoplasmic expression of LC3B in moderately differentiated OSCC, stage III 400x. (C) Low cytoplasmic expression of LC3B in well differentiated OSCC stage II 400x. (D) Negative cytoplasmic expression of LC3B in well differentiated OSCC; stage II 400x.

ATG16L, LC3B expression are positively correlated with each other phi coefficient r= +0.905 (p<0.001).

Follow-up and survival results

After a median follow-up period of 38.79 months with range (33.98-43.61) months, OS rates were 97.5%, 82.5%, 59% and 37% in the 1st, 2nd, 3rd and 4th years, respectively. 16 (61.5%) patients showed response to therapy, 13 (54.2%) of patients showed recurrence of the disease.

High ATG16L1 expression was significantly associated with poor response to therapy (p=0.018), higher incidence of tumor recurrence, worse 5-year DFS and OS rates (p<0.001). High LC3B expression was significantly associated with poor response to therapy (p=0.050), higher incidence of tumor recurrence, worse 5 year DFS and OS rates (p<0.001) **(Table 3 and Figure 3)**.

Table 3. Correlation between ATG16L1, LC3B expression and outcome of oral cavity and oropharyngeal carcinoma patients (N=40).

Outcome	All		ATG16L1				p-value	LC3B				p-value
			Low		High			Low		High		
	No.	(%)	No.	(%)	No.	(%)		No.	(%)	No.	(%)	
Response to treatment	(N=26)		(N=10)		(N=16)			(N=12)		(N=14)		
CR	8	(30.8%)	4	(40%)	4	(25%)	0.505‡	4	(33.3%)	4	(28.6%)	0.140‡
PR	8	(30.8%)	4	(40%)	4	(25%)		6	(50%)	2	(14.3%)	
SD	5	(19.2%)	1	(10%)	4	(25%)		1	(8.3%)	4	(28.6%)	
PD	5	(19.2%)	1	(10%)	4	(25%)		1	(8.3%)	4	(28.6%)	
OAR	16	(61.5%)	8	(80%)	8	(50%)	0.018‡	10	(83.3%)	6	(42.9%)	0.050‡
NR	10	(38.5%)	2	(20%)	8	(50%)		2	(16.7%)	8	(57.1%)	
Recurrence	(N=24)		(N=13)		(N=11)			(N=15)		(N=9)		
Absent	11	(45.8%)	11	(84.6%)	0	(0%)	<0.001‡	11	(73.3%)	0	(0%)	0.001‡
Present	13	(54.2%)	2	(15.4%)	11	(100%)		4	(26.7%)	9	(100%)	
Disease Free Survival												
Mean (months) (95%CI)	43.95 months (39.23-48.67)		52 months (48.16-55.83)		32.33 months (30.04-34.62)		<0.001†	52 months (48.16-55.83)		32.33 months (30.04-34.62)		**<0.001†**
1 year DFS	100%		100%		100%			100%		100%		
2 year DFS	100%		100%		100%			100%		100%		
3 year DFS	59.1%		92.3%		11.1%			92.3%		11.1%		
4 year DFS	50%		84.6%		----			84.6%		----		
Mortality	(N=40)		(N=19)		(N=21)			(N=21)		(N=19)		
Alive	19	(47.5%)	16	(84.2%)	3	(14.3%)	<0.001‡	17	(81%)	2	(10.5%)	**<0.001‡**
Died	21	(52.5%)	3	(15.8%)	18	(85.7%)		4	(19%)	17	(89.5%)	
Overall Survival												
Mean (months) (95%CI)	38.79 months (33.98-43.61)		49.36 months (43.33-55.38)		29.79 months (25.75-33.83)		<0.001†	48.46 months (42.57-54.35)		29.33 months (24.96-33.71)		**<0.001†**
1 year OS	97.5%		94.7%		100%			95.2%		100%		
2 year OS	82.5%		89.5%		76.2%			90.5%		73.7%		
3 year OS	59.8%		89.5%		32.4%			84.4%		33.1%		
4 year OS	37%		82%		----			77.4%		----		

‡ Chi-square test; † Log rank test; p<0.05 is significant

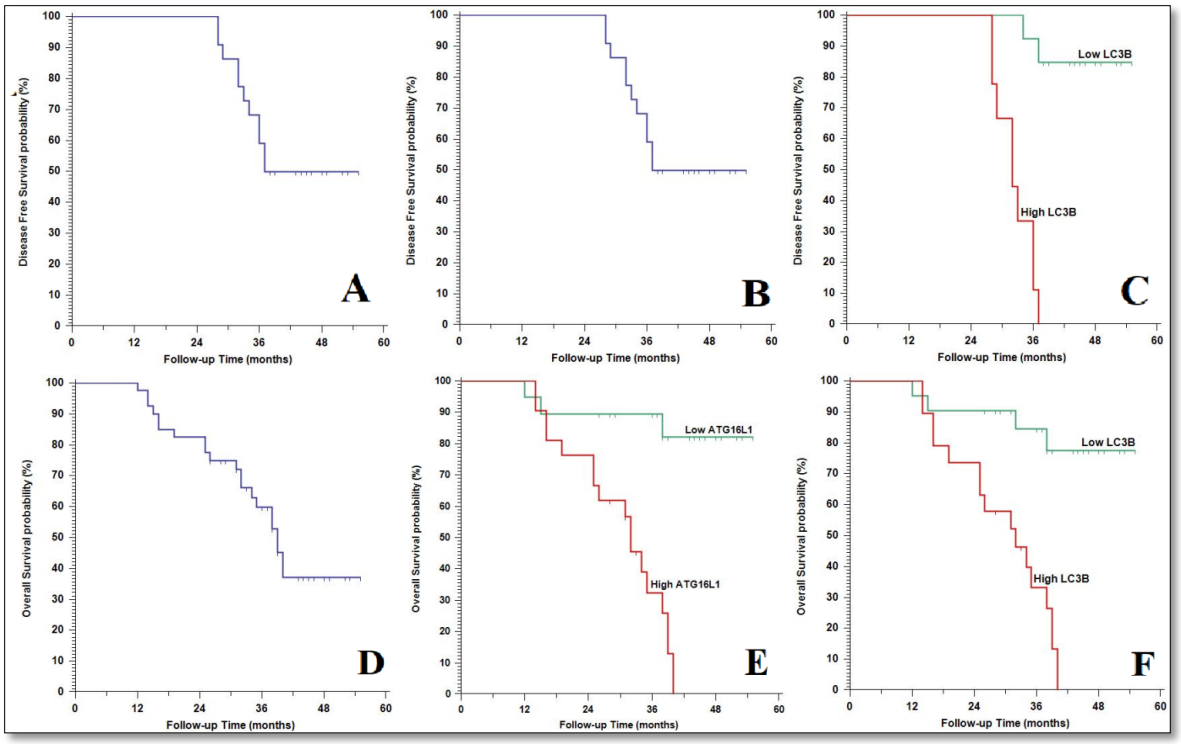

Figure 3. Kaplan Meier plot, (A) Disease Free Survival (DFS) for all studied patients, (B) DFS Stratified by ATG16L1, (C) DFS Stratified by LC3B, (D) Overall Survival for all studied patients, (E) OS Stratified by ATG16L1, (F) DFS Stratified by LC3B.

DISCUSSION

OSCC is the most serious cancer which leads to marked disfigurement and it is considered a serious health problem, particularly in the developing countries. OSCC pathogenesis is complex and had many factors for its pathogenesis. Previous reports have pointed to that autophagy dysregulation could alter the metabolic process of the cells and it has significant consequences which is related to plethora of human cancer, including OSCC [8,11], the role of autophagy in cancer progression is found as there is altered expression of many ATGs has been reported in many human malignancies [8]. The dysregulation of different autophagy related proteins have been found to correlate with clinico-pathological parameters and cancer patients outcomes. Such findings highlighted the vital roles of autophagy in carcinogenesis. Despite numerous studies which assessed the associations between autophagy and cancer progression; the role of autophagy in cancer remains controversial as it has cancer inhibiting and stimulating properties [1]. As cancer initiating agent, autophagy gives amino acids as an alternating source of energy for proliferation of the malignant cells and it provides cancer cells resistance toward radiotherapy and chemotherapy [12].

In the current study we tried to clarify the role of expression of 2 proteins which have association with autophagy in OSCC; ATG16L and LC3B.

We have noticed that ATG16L1 is considered one of the least clarified autophagy related proteins in human oncogenesis. ATG16L1 is a part of a large protein complex which is essential for autophagy [13]. We have proved that overexpression of ATG16L1 was significantly associated with advanced stage and higher grade of the tumor. Moreover, we found that ATG16L1 was increased in OSCCs with the presence of lymph node metastasis. Regarding the Kaplan-Meier plots analysis showed that patients with ATG16L1 overexpression had a poor OS and DFS. All these results were similar to Tang et al. [8], who proved similar results in OSCC and CHEN et al. [13], in osteosarcoma.

Moreover, the results of our current study are in line with previous results proved by Nomura et al. [14] who proved that increased ATG16L1 expression was associated with presence of lymph node metastasis in patients with OSCC. Tang et al. [8] study clarified the role of overexpression of ATG16L1 in OSCC pathogenesis in the survival and recurrence of patients of OSCC. Our present study and results of previous studies provided additional evidences of dysregulation of autophagy in OSCC which suggested that overexpression of ATG16L1 have prognostic significance in OSCC and raised the benefit of considering autophagy in the targeted therapy against OSCC. Previous studies have demonstrated that autophagy is mostly activated as a protective mechanism in cancer cells against several chemotherapeutics [8], which explain our results regarding

the association between autophagy activation and OSCC progression. Autophagy is responsible for degradation of intracellular components so as to regenerate metabolites for energy and growth by the lysosomal machinery [15]. But, the molecular mechanisms which are underlying autophagy-mediated resistance to chemotherapy in cancer cells remain unknown [13].

Chen et al. [13] have stated that miR-410 could enhance the chemosensitivity by inhibition of autophagy through targeting ATG16L1 in osteosarcoma cells which provided an insight into a promising approach for future osteosarcoma treatment.

As the results of previous studies regarding prognostic role of ATG16L1 in OSCC we assessed the expression of another autophagy related marker which is LC3B that participates in elongation of autophagosome membrane formation, when it is activated it strongly binds to the membranes of the pre-autophagosomes and autolysosomes membranes [12,16] and we have proved that LC3B overexpression in OSCC was markedly linked to advanced disease stage, higher incidence of metastases of lymph node, and higher histological grade. Moreover, Kaplan- Meier plots showed that LC3B overexpression was related to poor patients' outcome in terms of shorter OS and DFS rates in OSCC patients.

LC3B is an autophagosome marker which was demonstrated to be an effective prognostic marker in many cancers including oral SCC [1,11,17]. Increased expression of LC3B is positively related to progression and poor prognosis of many cancers [18,19].

Liu et al. [17], proved that increased LC3B tissue expression was related to poor DFS in patients complaining with oral SCC which is similar to ours, but, a more definitive assessment of LC3B prognostic role in cancer could lead to establishment of its expression with the autophagy activity of cancer cells and thus assess the benefits of considering autophagy as a management strategy for OSCC patients.

Similarly, high LC3B expression is related to poor OS and DFS rates in locally advanced cancer breast and in TNBC [20,21]. In astrocytoma, marked increase of LC3B expression is related to dismal outcome and poor OS rate [22], additionally, in hepatocellular carcinoma and esophageal carcinoma high LC3B expression is related to advanced TNM stages, presence of vascular invasion, lymph node metastasis and poor OS rate [23,24]. In prostate adenocarcinoma, increased LC3B expression is associated with a high Gleason scores [25]. The present study showed that increased autophagy related proteins expression, which denotes activated autophagy, is correlated with dismal outcome of OSCC, but it was previously found that autophagy can be involved in either cancer promotion or inhibition. The importance of assessment of the role of autophagy in OSCC progression is that, when autophagy is considered in the therapeutic management strategies for these tumors.

The inhibition of the autophagy process by therapeutic specific inhibitors and RNA related interference of genes which are related to autophagy might lead to enhancement of malignant cells chemo-sensitivity and photosensitivity [12]. Recent clinical trials which investigated the values of using inhibitors of autophagy in combination with the currently used immunotherapies, targeted therapies and chemotherapeutic agents in malignant tumors have been used since 2010 and give encouraging primary results [7].

Moreover, LC3 gene deficiency could enhance the HCC cells sensitivity to Epirubicin [26]. Additionally, 3-Methyladenine (3-MA), that, inhibits autophagy by preventing formation of autophagosome through inhibition of phosphatidylinositol 3-kinase (PI3K), might enhance the cytotoxicity of many chemotherapy agents like; Cisplatin, Tamoxifen, 5-fluorouracil (5-FU), Camptothecin and Trastuzumab [8,9]. Radiotherapy (RT) could lead to induction of autophagy in cancer cells which has a huge role in radio-resistance. Suppression of autophagy by using CQ and/or RNAi could elevate the radio-sensitivity and chemo-radio-sensitivity in malignant cell lines [29,30]. Although the therapeutic significance of autophagy inhibition in response to RT is yet to be functionally tested on cells of OSCC, the activity of autophagy has been reported to be increased in irradiated OSCC cells [23]. The current study proved that autophagy is associated with progression of OSCC; it will be better that more recent studies should assess the benefits of inhibition of autophagy in OSCC cell lines in response to chemotherapy and radiotherapy which could help to classify other patient into subgroups for variable therapeutic approaches [31,32].

CONCLUSION

We have proposed that ATG16L and LC3B are considered prognostic markers for OSCC patients due to the marked association between their expressions and poor clinico-pathological criteria and survival outcomes in our patient cohort. As autophagy is involved in progression of OSCC, further studies in the future should evaluate the values of inhibition of autophagy in such tumors in response to radiotherapy and chemotherapy.

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