

# Relation of autophagy to apoptosis and progesterone receptor expression in endometrial carcinoma type I

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## Background

The incidence of endometrial carcinoma among Egyptian women has increased in the past few years. This work was performed to evaluate the role of autophagy marker Beclin-1 in endometrial carcinoma type I and correlate it with progesterone receptor (PR) and Bcl-2 expression.

## Materials and methods

This retrospective study included 30 cases of endometrial carcinoma type I, eight cases of endometrial hyperplasia with atypia, and seven cases of endometrial hyperplasia without atypia. Cases were collected from archives of Pathology Department and Early Cancer Detection Unit, Faculty of Medicine, Benha University, through the years 2011 to 2017. Beclin-1, Bcl-2, and PR immunohistochemical staining was performed on all cases, and the patterns of expression were analyzed.

## Results

Beclin-1 was expressed in 36.7, 62.5, and 71.5% of endometrial carcinoma, endometrial hyperplasia with atypia, and hyperplasia without atypia, respectively. This showed a statistically significant difference ( $P < 0.05$ ). Bcl-2 expression was positive in 53.3, 25, and 14.5% of endometrial carcinoma, endometrial hyperplasia with atypia, and hyperplasia without atypia, respectively, with statistically significant difference ( $P < 0.05$ ). Positive PR immunoreactivity was reported in 40% of endometrial carcinomas, 75% of endometrial hyperplasia with atypia, and 85.5% of hyperplasia without atypia ( $P < 0.05$ ). Both Beclin-1 and PR expressions were significantly increased in correlation with decreased Bcl-2 expression in carcinoma cases ( $P < 0.01$ ).

## Conclusion

Combined estimation of PR and Beclin-1 is a good prognostic marker for endometrial carcinoma type 1. Combination of progestins with proautophagic substances could be used in resistant cases to improve results of treatment.

## Keywords:

apoptosis, autophagy, Bcl-2, Beclin-1, endometrial carcinoma, PR

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## Introduction

Autophagy is the mechanism by which tissues regulate homeostasis and survival through degrading damaged proteins and organelles to sustain metabolism. It is considered a defense mechanism against toxic substances released from injured cellular organisms. Therefore, autophagy has gained a vital role in keeping proteins in high quality (Mizushima and Komatsu, 2011).

Autophagy is also a mechanism by which removal of pathogens occurs, and the cells that undergo apoptosis get engulfed. However, the role of autophagy in cancer is not fully recognized. Evidence supports that sustained cellular survival is maintained by autophagy. However, unopposed autophagy leads to cell death through organelle consumption (Mathew *et al.*, 2007).

Beclin-1 was the first autophagy gene identified in mammals. In many studies, autophagy was considered to be a tumor-suppression mechanism (White, 2015). Studies included hepatocellular carcinoma, ovarian carcinoma, and laryngeal carcinoma revealed low level of Beclin-1 protein expression, whereas other studies found the opposite regarding colorectal carcinoma, gastric, and pancreatic ductal adenocarcinoma (Hu *et al.*, 2016).

Beclin-1 was initially isolated as an interactor of the oncogenic antiapoptotic protein Bcl-2 (Valente *et al.*, 2014). Bcl-2 serves as an autophagy inhibitor for its

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ability to bind to Beclin-1 (Blagosklonny, 2014). According to previous studies, Bcl-2 may also promote tumor proliferation and increases the ability to develop cancer and drug resistance (Oh *et al.*, 2011).

Endometrial cancer accounts for the fourth most common malignant tumor in women and the most common cancer of the gynecological system (Zhang *et al.*, 2015). In Egypt, it is the eighth most common cancer in women in the rank, representing 4%. Most malignant tumors of uterine body are endometrial carcinomas (85.2%). Endometrioid carcinoma represented 92.6% of them (Helal *et al.*, 2015).

Endometrial adenocarcinoma arises from endometrial glands. Higher incidence is related to prolonged unopposed estrogen action. Cases complaining of advanced or recurrent cancer showed good response to progestins and regressed. Although many of them have already undergone surgical procedures, chemotherapy, or both, progestin therapy may be an alternative mainly in cases with progressive disease or old aged patients presenting with multiple comorbidities (Kim and Chapman-Davis, 2010).

The primary treatment of endometrial cancer is surgical. There are no studies examining the involvement of autophagy regarding the treatment of endometrial carcinoma. This work discusses the role of autophagy marker Beclin-1, the antiapoptotic protein Bcl-2, and progesterone receptor in endometrial carcinoma type I and if they have a role in treatment.

## Materials and methods

This retrospective study was conducted on 30 cases diagnosed as endometrial carcinoma type I (endometrioid type). Staging of cases was considered according to TNM classification of the American Joint Committee on International Union against Cancer AJCC (2010). In addition, there were seven cases of endometrial hyperplasia without atypia and eight cases of endometrial hyperplasia with atypia. Cases were collected from archives of Pathology Department and Early Cancer Detection Unit, Faculty of

Medicine, Benha University, through the years 2011–2017. Sections were prepared from paraffin blocks. Hematoxylin and eosin sections were reviewed by two pathologists to confirm diagnosis.

## Immunohistochemical study

Three formalin-fixed, paraffin-embedded, 4- $\mu$ m tissue sections were prepared on positive charges slides. For immunohistochemical analysis, streptavidin–biotin technique was used following the manufacturer's instructions. Antibodies and antigen retrieval are shown in Table 1. For the secondary developing reagents, a labeled streptavidin–biotin kit (Neomarker; LabVision, Fremont, California, USA) was used. The sections were stained with 0.02% diaminobenzidine solution. Finally, hematoxylin was used as counterstain. Neglecting the step of primary antibody and replacing it with normal rabbit serum IgG was used as a negative control for every marker.

## Beclin-1 interpretation

Beclin-1 was detected in the cytoplasm or membrane of glandular epithelial cells. The index of immunoreactivity was evaluated according to intensity and proportion, as reported by Huang *et al.* (2010).

## BCL-2 interpretation

Bcl-2 was detected as cytoplasmic staining of glandular epithelial cells. Positivity is considered when more than 10% of tumor cells are stained, whereas negativity is considered if no staining or less than 10% of tumor cells are stained (Geng *et al.*, 2013).

## PR interpretation

Nuclear staining of the cells was considered positive. According to Mohammed *et al.* (2012), an Allred score (0 or 2–8) is recorded for each case and is defined as positive when at least 3.

## Statistical analysis

Data were collected, and analysis was done using SPSS version 16 software (SPSS Inc., Chicago, Illinois, USA). To determine the association between studied markers, Pearson correlation test was used. The

**Table 1 Antibodies used in this study**

Antibody	Source	Cat. no.	Dilution	Incubation period	Positive control	Staining pattern
Beclin-1	Novus Biological (Littleton, Colorado, USA)	NB500-249	1 : 200	Overnight at room temperature	Benign tonsillar tissue	Cytoplasmic/membranous
Bcl-2	Lab Vision/Thermoscientific (USA)	A15764	1 : 200	60 min at room temperature	Lymph nodes	Cytoplasmic
PR	Novus Biological, (Littleton, Colorado, USA)	NBP1-87774	1 : 200	60 min at room temperature	Breast cancer tissue samples	Nuclear

PR, progesterone receptor.

accepted level of significance in this work was stated at 0.05 ( $P < 0.05$  was considered significant).

## Results

### Immunohistochemical results

#### Beclin-1 expression

Beclin-1 was expressed in 11/30 (36.7%) of endometrial carcinoma cases. Its expression was lower than comparison groups [5/8 (62.5%) and 5/7 (71.5%), respectively], and this was statistically significant ( $P < 0.05$ ; Table 2 and Fig. 1a). Beclin-1 showed significant relation with lower grade, lower FIGO stage, and absence of distant metastasis ( $P < 0.05$  each; Table 3).

#### Bcl-2 expression

Bcl-2 showed positivity in 16/30 (53.3%), 2/8 (25%), and 1/7 (14.5%) of endometrial carcinoma, endometrial

hyperplasia with atypia, and hyperplasia without atypia, respectively ( $P < 0.05$ ; Table 2 and Fig. 1b). A significant relation was detected with higher tumor grades ( $P < 0.05$ ), higher FIGO stage ( $P < 0.05$ ) and presence of distant metastases ( $P < 0.01$ ; Table 3).

#### PR expression

Positive progesterone receptor (PR) immunoreactivity was reported in 12/30 (40%) of endometrial carcinoma specimens. Regarding endometrial hyperplasia with atypia, and hyperplasia without atypia, higher expression was detected [6/8 (75%) and 6/7 (85.5%)] ( $P < 0.05$ ; Table 2 and Fig. 1c). PR positivity showed a significant relation with lower grades of tumor and absence of distant metastasis ( $P < 0.05$  each) (Table 3).

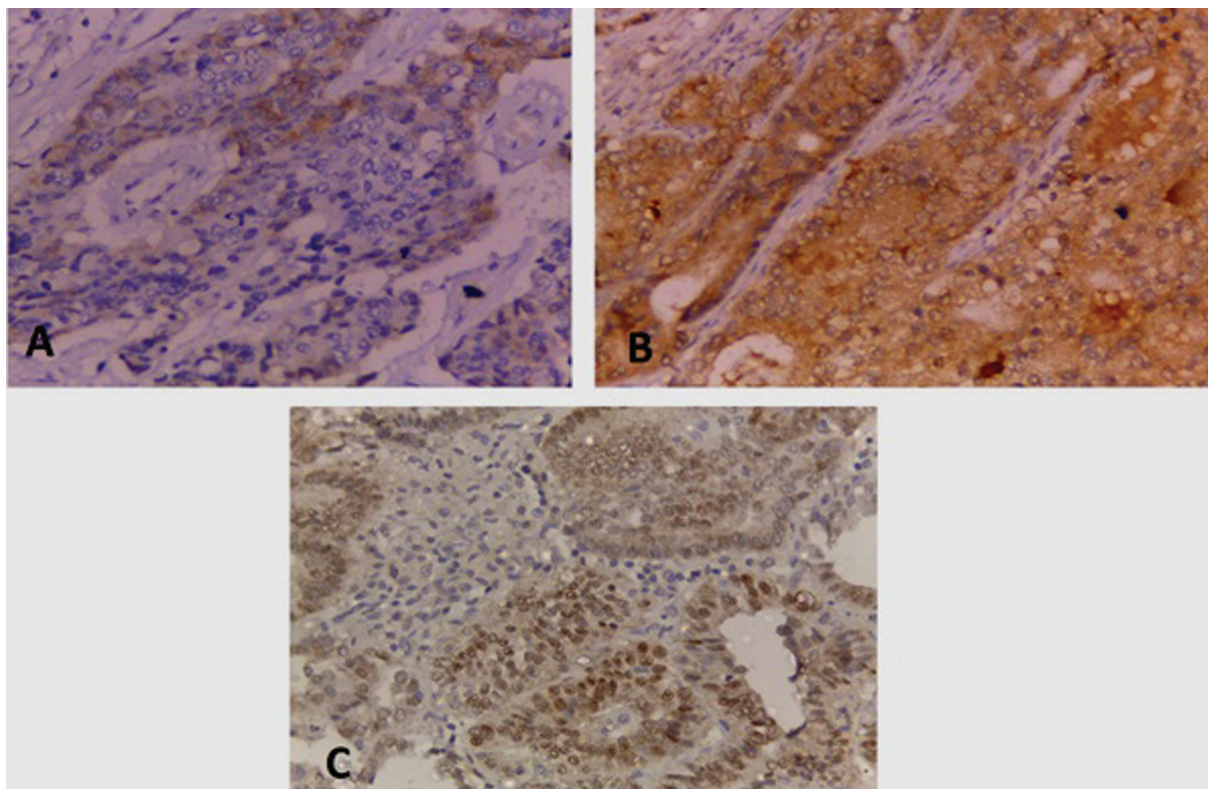
Regarding depth of tumor invasion and lymph node metastases, none of the used markers showed significant relation ( $P > 0.05$ ).

**Table 2** Beclin 1, BCL-2, and PR in studied case groups

Groups	N	Beclin-1		P	BCL-2		P	PR		P
		Negative	Positive		Negative	Positive		Negative	Positive	
Hyperplasia without atypia	7	2 (28.5%)	5 (71.5%)	<0.05	6 (85.8%)	1 (14.2%)	<0.05	1 (14.2%)	6 (85.8%)	<0.05
Hyperplasia with atypia	8	3 (37.5%)	5 (62.5%)		6 (75%)	2 (25%)		2 (25%)	6 (75%)	
Carcinoma	30	19 (63.3%)	11 (36.7%)		14 (46.7%)	16 (53.3%)		18 (60%)	12 (40%)	

PR, progesterone receptor.

**Fig. 1**



(a) Beclin-1 score 3 in endometrioid carcinoma grade III (ABC,  $\times 400$ ). (b) Bcl-2 showing positive cytoplasmic expression in endometrioid carcinoma grade III (ABC,  $\times 400$ ). (c) PR showing positive nuclear expression (score 7) in endometrioid carcinoma grade II (ABC,  $\times 400$ ).

**Table 3 Relation between Beclin-1, BCL-2, and PR expression and clinicopathological variables**

Clinicopathological variables	N	Beclin-1		P value	BCL-2		P value	PR		P value
		Negative	Positive		Negative	Positive		Negative	Positive	
Grade										
1	8 (26.7%)	1 (12.5%)	7 (87.5%)	<0.05	8 (100%)	0	<0.05	0	8 (100%)	<0.05
2	13 (43.3%)	9 (69.2%)	4 (30.8%)		5 (38.5%)	8 (61.5%)		11 (84.6%)	2 (15.4%)	
3	9 (30%)	9 (100%)	0		1 (11.1%)	8 (88.9%)		7 (77.8%)	2 (22.2%)	
Depth of invasion										
<50%	21 (70%)	12 (57.1%)	9 (42.7%)	>0.05	11 (52.4%)	10 (47.6%)	>0.05	12 (57.1%)	9 (42.7%)	>0.05
>50%	9 (30%)	7 (77.8%)	2 (22.2%)		3 (33.3%)	6 (66.7%)		6 (66.7%)	3 (33.3%)	
Figo stage										
Stage I	16 (53.3%)	7 (43.8%)	9 (56.2%)	<0.05	10 (62.5%)	6 (37.5%)	<0.05	9 (56.2%)	7 (43.8%)	>0.05
Stage II	8 (26.7%)	6 (75%)	2 (25%)		3 (37.5%)	5 (62.5%)		5 (62.5%)	3 (37.5%)	
Stage III	6 (20%)	6 (100%)	0		1 (16.7%)	5 (83.3%)		4 (66.7%)	2 (33.3%)	
Lymph node metastasis										
Present	6 (20%)	5 (83.3%)	1 (16.7%)	>0.05	3 (50%)	3 (50%)	>0.05	5 (83.3%)	1 (16.7%)	>0.05
Absent	24 (80%)	14 (58.3%)	10 (41.7%)		11 (45.8%)	13 (54.2%)		13 (54.2%)	11 (45.8%)	
Distant metastasis										
Present	9 (30%)	9 (100%)	0	<0.05	2 (22.2%)	7 (77.8%)	<0.01	9 (100%)	0	<0.05
Absent	21 (70%)	10 (47.6%)	11 (52.4%)		12 (57.1%)	9 (42.9%)		9 (42.9%)	12 (57.1%)	

PR, progesterone receptor.

**Table 4 Relations between Beclin-1, Bcl-2, PR expression in endometrioid carcinoma cases**

	N	Bcl-2		PR	
		Negative	Positive	Negative	Positive
Beclin-1					
Negative	19 (63.3%)	4 (21.1%)	15 (78.9%)	16 (84.2%)	3 (15.8%)
Positive	11 (36.7%)	10 (90.9%)	1 (9.1%)	2 (18.2%)	9 (81.8%)
			<i>P</i> <0.01		<i>P</i> <0.01
PR					
Negative	18 (60%)	5 (27.8%)	13 (72.2%)		
Positive	12 (40%)	9 (75%)	3 (25%)		
Total	30		<i>P</i> <0.01		

PR, progesterone receptor.

A significant relation ( $P<0.01$ ) between Beclin-1 and PR was detected. However, they showed a significant decrease in their levels with increased Bcl-2 expression in carcinoma cases ( $P<0.01$  each; Table 4).

### Discussion

In this study, the expression of Beclin-1 was significantly lower in endometrial carcinoma than that in endometrial hyperplasia with and without atypia ( $P? 0.05$ ). These results suggested that decreasing expression of Beclin-1 had a role in the development of endometrial carcinoma. This agreed with the results of Yao *et al.* (2011) in breast carcinoma, Chen *et al.* (2009) in squamous cell carcinoma of esophagus, Wang *et al.* (2013) in non-small-cell

lung carcinoma, and Qiu *et al.* (2014) in hepatocellular carcinoma. In contrast, Ahn *et al.* (2007) suggested that increasing expression of Beclin-1 in malignant cells may have a role in colorectal and gastric oncogenesis. These conflicting results suggest that Beclin-1 may function in a tissue-specific manner, as reported by Hu *et al.* (2016).

This study also showed that lower expression of Beclin-1 was significantly related to poor tumor differentiation, advanced stage, and distant metastasis ( $P? 0.05$ ), which indicated that Beclin-1 has a role in progression of endometrial carcinoma. These results suggested that Beclin-1 could be considered as a valuable predictor for biological behavior of endometrial carcinoma.

Regarding Bcl-2 expression in this study, it was significantly higher in endometrial carcinoma compared with endometrial hyperplasia with and without atypia ( $P \leq 0.05$ ), indicating that upregulation of Bcl-2 has a role in tumorigenesis of endometrial carcinoma. These results agreed with Li *et al.* (2015) in colonic carcinoma and Sun *et al.* (2002) in pancreatic carcinoma. Bcl-2 expression was significantly related with tumor grade and stage. These results could suggest that Bcl-2 has an effective role in endometrial carcinoma progression.

This study revealed decreased Beclin-1 expression in relation to increased Bcl-2 expression in endometrioid carcinoma, and they were negatively related ( $P < 0.01$ ). These findings suggest that simultaneous decrease in autophagy and apoptosis is related to both initiation and progression of endometrioid carcinoma. These results were in agreement with Lin (2015) in gastric carcinoma, Cao *et al.* (2012) on their study on pancreatic cancer, and Kotsafti *et al.* (2012) in hepatocellular carcinoma. Bcl-2 overexpression is known in several human cancers and leads to increased chemoresistance and sometimes with aggressive tumor behavior. An interaction between Beclin-1 and Bcl-2 inhibits the role of the former in process of autophagy. Once Bcl-2 is phosphorylated, Beclin-1 is released and can induce cell cycle arrest (Oh *et al.* 2011; De Amicis *et al.* 2016).

This work also revealed significant decreased expression of PR from endometrial hyperplasia without atypia to endometrial hyperplasia with atypia to carcinoma ( $P \leq 0.05$ ). Another significant relation was found with decreased degree of differentiation and absence of distant metastases ( $P \leq 0.05$ ). These results agreed with Bender *et al.* (2011), Kreizman-Shefer *et al.* (2014) and Zhang *et al.* (2015), who also concluded that the higher the levels of PR the more favorable the prognosis.

In this work, Beclin-1 immunoreactivity was positively related with PR expression ( $P \leq 0.01$ ). This was in agreement with De Amicis *et al.* (2016), who reported that PR expression could significantly enhance the activity of proautophagic protein Beclin-1, which was also stimulated by progestin treatment in cells of breast cancer. In another study on ovarian cancer by Diep *et al.* (2013), nuclear PR was activated by progestins, inducing cell cycle arrest.

This work revealed that Beclin-1 and PR expressions were significantly decreased in relation with increased Bcl-2 expression among endometrial carcinoma cases

( $P < 0.01$ ). This could be explained by the results of Bender *et al.* (2011) who reported that in endometrial cancer cell lines, PR function induces cell aging, increases the sensitivity to apoptosis, and inhibit G1 to S cell cycle transition. De Amicis *et al.* (2016) also supported these results when they concluded that, in breast cancer cells, increasing the levels of functional Beclin-1 by progestins facilitates progression of autophagy, based on the release of Bcl-2 and causes irreversible cell cycle arrest.

## Conclusion

Combined estimation of PR and Beclin-1 is a good prognostic marker in endometrial carcinoma type 1. Combination of progestins with proautophagic substances could be used in resistant cases to improve results of treatment.

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## Conflicts of interest

There are no conflicts of interest.

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