Original Research Papers

Diagnostic significance of Beclin-1 and Transforming growth factor β (TGF-β) in Breast Cancer

ABSTRACT

Background: The main cause of cancer deaths amongst women breast cancer remains a clinical and social challenge, and a serious public health problem. On a worldwide level, it continues to be a devastating disorder.BECN1 is a tumor suppressor gene implicated in the initiation of autophagy. It encodes beclin-1 protein that inhibits cancer growth. There is wide disputation concerning its role in initiation, promotion of tumor and predictive importance of autophagic molecules. Transforming growth factor β (TGF-β) induces process of epithelial-mesenchymal transition (EMT) keeping, epithelial cells more motile and invasive resulting in cancer progression and metastasis.

Aim: Detection of beclin1 level in metastatic and non-metastatic breast cancer patients and study its role in tumorigenesis of breast cancer through attainable association with the inflammatory cytokine, TGF-β.

Methods: Expression levels of beclin-1and TGF-β were assessed in 70 breast cancer female patients and 20 controls using quantitative real-time PCR.

Results: Beclin-1 expression levels as well as TGF-β were significantly higher in metastatic breast cancer patients and non-metastatic patients compared to controls. Positive correlation was found between beclin-1 expression and TGF-β in breast cancer patients.

Conclusion: Our results indicated that expression of both beclin-1 and TGF-β was associated with aggressive clinical outcomes of breast cancer patients. Beclin-1 over-expression, as well as TGF-β over-expression, contributed to tumor growth. These findings suggest that beclin-1 and TGF-β are associated with tumorigenesis of breast cancer.

Keywords: autophagy, BECN1, TGF-β, breast cancer.

1. INTRODUCTION

Breast carcinoma is the y main cancer that influences females accounting for 37.7% of their overall malignancies in Egypt. Moreover, it is important reason of cancer related mortality in Egypt, constituting about 29.1% of all most cancer associated mortality [1]. Autophagy is a cellular degradation or "self-eating" pathway extremely preserved throughout all existence kingdoms. [2]**.** Considering the truth that autophagy is implicated in numerous cellular processes, and keeping in mind the complexness of the molecular mechanisms of tumor initiation and development, it is no longer shocking that the interruption of autophagy plays role in tumor inatition and progression. Indeed, cancer was the primary disease allied with disturbed autophagy besides, the first for which medical trials in humans were performed [3].

Beclin-1 (BECN1) is a rate-limiting element of autophagy and a haplosufficient cancer suppressor that is fundamental for embryonic development. Moreover, this protein is a core factor essential for autophagosome formation [4].Transforming growth factor-beta (TGF-β) is a protein that essential regulator in homeostasis in various organs [5]. **(**As a signaling molecule, TGF-β has a variety of biological function and participates in several physiological and pathological processes [6].

2. MATERIAL AND METHODS

2.1.Subjects:

The study included 70 Egyptian women with breast cancer at distinctive stages, their age ranged from (23-76 years). They were collected from Kasr Alainy hospital. Patients were diagnosed by clinical examination and affirmed by mammography and surgical biopsies. Twenty clinically normal adjoining specimens were moreover collected from adjacent healthy breast tissue of non-metastatic breast cancer cases .The participants in this study were provided us with informed permission after ethical committee approval from Medical Biochemistry Department, Faculty of Medicine, Cairo University. All specimens were subjected to estimation of expression levels of beclin-1 and TGF-β in breast tissue.

The studied subjects were separated into three groups as following:

Group I: (n=26) metastatic breast cancer female patients with distant metastasis (bone, liver, and lung).

Group II: (n=44) non-metastatic breast cancer female patients.

Group III: (n=20) healthy adjacent normal tissues of non-metastatic breast cancer cases as a control group.

2.2.Tissue sampling and RT PCR analysis

Total RNA was extracted from breast tissues using Qiagen tissue extraction kit (Qiagen, USA) according to instructions of manufacture. The first strand cDNA was derived from total RNA (0.5µg) and oligo (dT) using the Reverse Transcription System (The QuantiTect Reverse Transcription Kit). For real-time quantitative RT-PCR, gene specific primers and QuantiTect SYBR Green PCR Kit were used. GAPDH was used to normalize the quantity of specific mRNA. The amplification efficiency determined for both target and housekeeping genes was equal. Relative expression levels were calculated by the $2^{-\Delta\Delta Ct}$ method.

2.3. Statistical analysis:

Statistical analysis of data was performed by Sigma Plot version 12.5 and Graphpad Prism 5. Data was illustrated as mean ± standard deviation. Differences among groups were analyzed by Shapiro-Wilk test and t-test. Post-hoc testing was performed by the Tukey test to compare the difference among the groups. Simple linear correlation (Pearson correlation coefficient test) (r) was also done to test for linear relations between beclin-1 and TGF-β with other variables. P-value is considered significant if < 0.05. Receiver operating characteristics curves (ROC curves) were utilized to assess indicative performance of the studied parameters**.**

3. RESULTS

3.1 levels of beclin-1 and TGF-β in different clinicopathological factors in patients with breast cancer

The association between the levels of beclin-1 and TGF-β with clinicopathological factors were analyzed. The levels of beclin-1 and TGF-β were increased with TNM stage ($p< 0.01$) but showed no significant correlation with the other factors **(table 1 and figure1).**

Table (1): Correlation between beclin-1 and TGF-β with clinicopthological factors in the malignant groups

Clinicopathological Factors	Malignant patients (70)	Beclin-1 P value	TGF-β P value
Metastasis	26 (37.1 %)	0.05>	0.05>

Metastatic	**44 (62.9%)**		
Non-metastatic			
Family history		**0.05>**	**0.05>**
No family history	**54 (77.1%)**		
Family history	**16 (22.9%)**		
Menopause		**0.05>**	**0.05>**
Pre-menopause	**47 (67.1%)**		
Post-menopause	**23 (32.9%)**		
Site		**0.05>**	**0.05>**
Right	**35 (50%)**		
Left	**32 (45.7%)**		
Bilateral	**3 (4.3%)**		
Pathology		**0.05>**	**0.05>**
IDC II	**58 (82.8%)**		
Medullary	**1 (1.4%)**		
IDC III	**6 (8.6%)**		
IDC + ILC	**3 (4.3%)**		
ILC	**2 (2.9 %)**		
ER sensitivity		**0.05>**	**0.05>**
Positive	**49 (70%)**		
Negative	**21 (30%)**		
PR sensitivity		**0.05>**	**0.05>**
Positive	**42 (60%)**		
Negative	**28 (40%)**		
HER sensitivity		**0.05>**	**0.05>**
Positive	**29 (41.4%)**		
Negative	**41 (58.6%)**		
Molecular diagnosis		**0.05>**	**0.05>**
HER 2 enriched	**29 (41.4%)**		
Triple negative	**11 (15.7 %)**		
Luminal B	**26 (37.15%)**		
Luminal A	**4 (5.75%)**		
Stage		**0.05**<**	**0.05**<**
Stage II	**18 (25.7%)**		
Stage III	**26 (37.15%)**		
Stage IV	**26 (37.15%)**		

a* significant from stage II

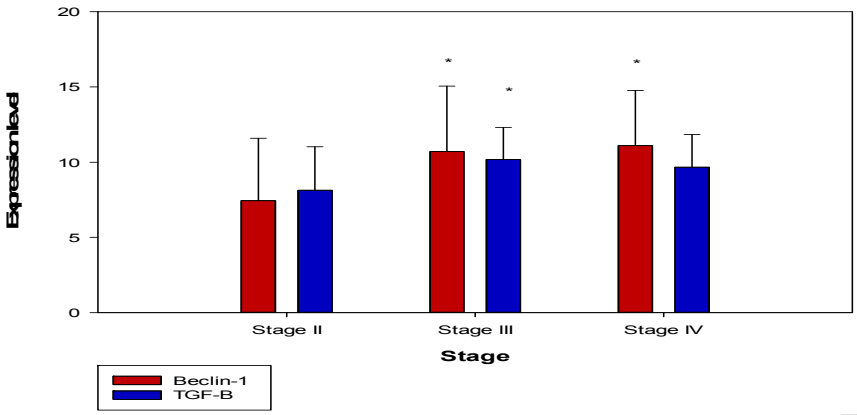

Figure (1): Expression levels of beclin-1 and TGF-β according to TNM stage.

3.2 Levels of beclin-1 and TGF-β in patients with breast cancer compared to control group

Levels of beclin-1 and TGF-β expression assessed by real time PCR in the studied groups were demonstrated in **table (2)** and **figure (2)**.

The mean **±** SD of both beclin-1 and TGF-β in both malignant groups were significantly increased when compared to the control group.

Table (2): Non-parametric analysis of beclin-1 and TGF-β in the studied groups

Groups / Variables	Metastaic malignant group	Non-metastatic group	Control group	P value
Beclin-1 expression level	11±3.6 a*	9.3±4.5 a*	1.2±.34	<0.05**
TGF-β expression level	9.5±7.9 a*	9.3±7.7 a*	1.1±30	<0.05**

a* significant from control group

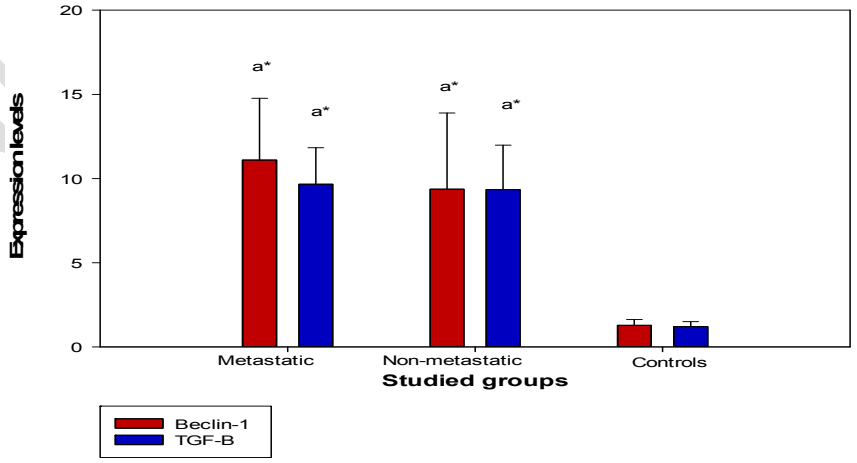

Figure (2): Mean ± SD of beclin-1 and TGF-β expression levels for the studied groups

3.3 Receiver operating characteristics (ROC) curves

Receiver operating characteristics curves were performed and demonstrated in **(table 3 and figures 3, 4&5).**

Table (3): Data of ROC curves of beclin-1 and TGF-β

Parameters	Sensitivity (%)	Specificity (%)	Cut-off	Accuracy (AUC)	P-value
Beclin-1	95	98.57	1.905	0.997	<0.0001
TGF-β	100	98.57	3.98	1	<0.0001
Beclin-1& TGF-β	97.5	99.29	1.91	0.99	<0.0001

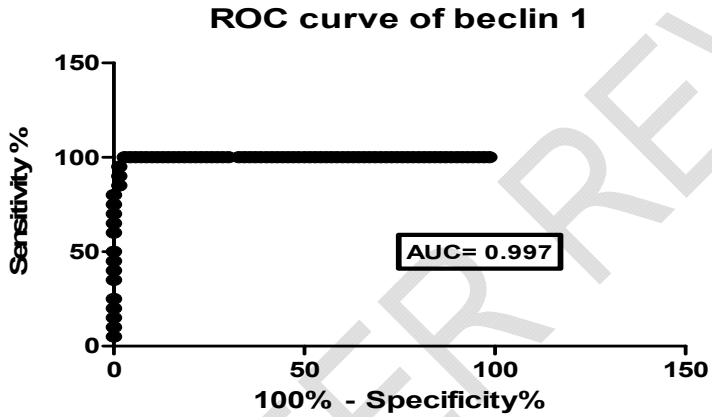

Figure (3): ROC curve of beclin1

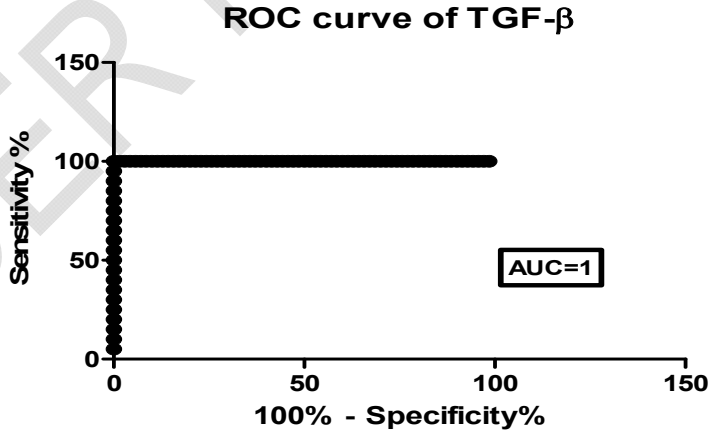

Figure (4): ROC curve of TGF-β

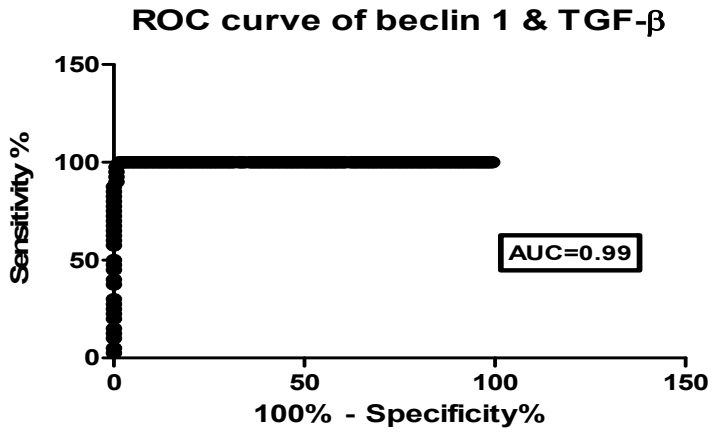

Figure (5): ROC curve of beclin1 and TGF-β

Correlation between expression level of beclin-1 and TGF-β was also shown in **table (4) and figure (6)** using pearson correlation revealing positive correlation between level of beclin-1 and TGF-β.

Table (4): Correlation between expression level of beclin-1 and TGF-β

Variable	R	P value
Beclin-1 with TGF-β	0.241	<0.05**

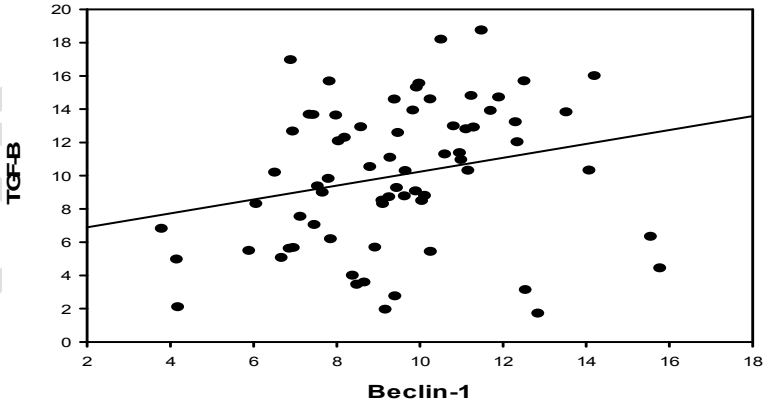

Figure (6): Correlation between beclin-1 and TGF-β expression levels.

4. DISSCUSSION

Autophagy is utilized by ordinary and cancer cells. However, the mechanism of autophagy in different cancers including breast cancer remains not clear in oncogenic/protumorigenic and tumor-suppressor feature [7]. The purpose of Our study

was to assess expression levels of beclin-1 and TGF-β in breast carcinoma Egyptian women compared with normal controls and to determine the potential value of beclin-1 and TGF-β as molecular biomarkers for diagnosis of breast cancer in Egyptian women. In the present study, we detected expression of beclin-1and TGF-β in breast cancer tissue. We found, compared with the expression in normal breast tissue, that beclin-1 and TGF-β were over-expressed in tumor tissue. Moreover, the over-expression of beclin-1 and TGF-β in breast cancer was considerably associated with TNM stage. These findings may suggest that beclin-1 and TGF-β have direct influence on development and progression of breast carcinoma.In the current study, beclin-1 level was significantly increased in breast cancer groups in comparison to healthy subjects (P value< 0.01). Our results are supported with different previous studies presented by **Hamurcu, et al.,** [8] who have studied the relative concentrations of beclin-1 in the tissue of patients with breast cancer and metastatic disease and healthy women who stated that expression levels of beclin-1 was higher in highly aggressive and metastatic and noninvasive cancer cells compared to non-tumorigenic normal human breast cell. The function of autophagy is exist different opinion in solid cancers including breast cancer [8]. In another study **Wang, et a.l,** [9] founded a significant increase of beclin-1 expression in eighteen breast carcinoma patients which was associated with tumor progression. Also **Park, et al.,** [10] revealed that the expression of beclin-1, protein was increased in tumor cells relative to normal-appearing and adjacent colonic mucosa in all cases. Similarly **Chen, et al.,** [11] reported an increased expression of beclin-1 in tumor tissue compared with under-expression in normal gallbladder specimen ($p < 0.05$). The concept that autophagy represents a mechanism that promotes tumor growth is based on the need of tumor cells to adjust to ischemia in the surroundings that are hypoxic, besides growth factors and supplemental deprivation. Consistent with this aspect, autophagy is activated in hypoxic environment of tumors [12]. Beclin-1 was firstly to be found to have tumor suppressor role. However, elevated expression of beclin-1 was related with cancer progression in some carcinoma [13]. In contrast to our study, decreased beclin-1 expression was observed in seventy percent of the breast tumors, and the protein levels were co-related to the mRNA levels [14]. However, **Claude-Taupin, et al.,** [15] reported that no major difference in Beclin-1 mRNA expression between tumor and healthy adjacent tissues. The current study revealed that TGF-β was significantly increased in breast carcinoma patients compared to control group (P value< 0.001) and this finding are affirmed by **Ciftci, et al.,** [16] who have studied the expression level of TGF-β and established that the mean serum TGF-β level of breast cancer patients was considerably higher than controls. There was no considerable difference according to known disease-related clinicopathological parameters. Our finding is supported by **El-Aziz et al.,** [17] who revealed that the level of TGF-β was considerably higher in malignant groups than normal control group with p value (<0.0001). The results of the present study was supported by **Scherer et al.,** [18] who detect the level of TGF-β in breast cancer patients; finding that its level was increased.In the current study, ROC curve of beclin-1 showed 95% sensitivity and 98.5% specificity and this is supported by **Harb, et al.,** [19] who reported that the sensitivity of beclin-1 as a predictor for advanced stage of IDC was 85.5% and the specificity was 98.5%. Also ROC curve of TGF-β showed 100% sensitivity and 98.5% specificity and this is supported by **El Husseini, et al.,** [20] who reported that 98.2% sensitivity and 100% specificity demonstrating the diagnostic power of this studied marker in differentiating between breast cancer patients and controls. TGF-β is connected with expanded cancer progression, higher cell movement, cancer invasiveness, and metastasis. It is furthermore included in cancer surrounding medium alteration and advancement of migration and invasiveness [21]. TGF-β elevated the mRNA levels of beclin-1, and other protein kinase implicated in death process. Moreover, TGF-β evoked autophagy in some mammary carcinoma cell. These findings illustrate that TGF-β signaling pathway activates autophagy in certain human cancer cells and that induction of autophagy is a novel aspect of biological role of TGF-β [22]. Concerning clinicopathological factors, the over-expression of beclin-1 was considerably related with

TNM stage ($p < 0.05$) but it showed no noteworthy association with the other factors. The current results were similar to **Chen, et al.,** [12] **work** who declared that over expression of beclin-1 was essentially related with TNM stage but had no vital relationship with age, sex, lymphatic metastasis, or tumor differentiation. Also, the affiliation between hormonal status and TGF-β expression was examined for both Estrogen receptors (ER) and Progesterone receptors (PR) by **El-Aziz, et al.,** [17] concluding that no noteworthy difference of TGF-β expression neither between ER positive versus ER negative tumors, nor between PR positive and PR negative tumors and this is similar to our data. Concerning the relation between tissue tumor subtypes and TGF-β level, **El-Aziz et al.,** [17] reported no critical difference between TGF-β level and tissue tumor subtypes as we found.

5. CONCLUSION

This study affirmed that up-regulation of beclin-1 expression is present in breast carcinoma tissues. Our results demonstrated that of beclin-1 and TGF-β high levels were associated with forceful clinical outcomes of breast cancer patients. Beclin-1 over-expression, as well as TGF-β over-expression, contributed to tumor development. Additional study on a larger scale will be essential to clarify the diagnostic importance of beclin1 in the process of tumorigenesis.

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