

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

5
7

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-1 and 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 complexity** of the molecular mechanisms of tumor initiation and development, it is no longer

shocking that the interruption of autophagy plays role in tumor **ination initiation** 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 **is** 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 follows:

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 (0.5 μ g) 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 \pm 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 clinicopathological 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

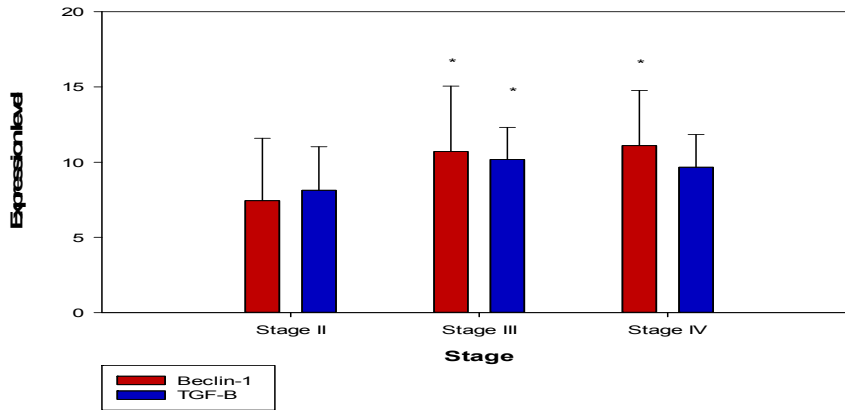


Figure (1): Expression levels of beclin-1 and TGF-β according to TNM stage.

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	Metastatic 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

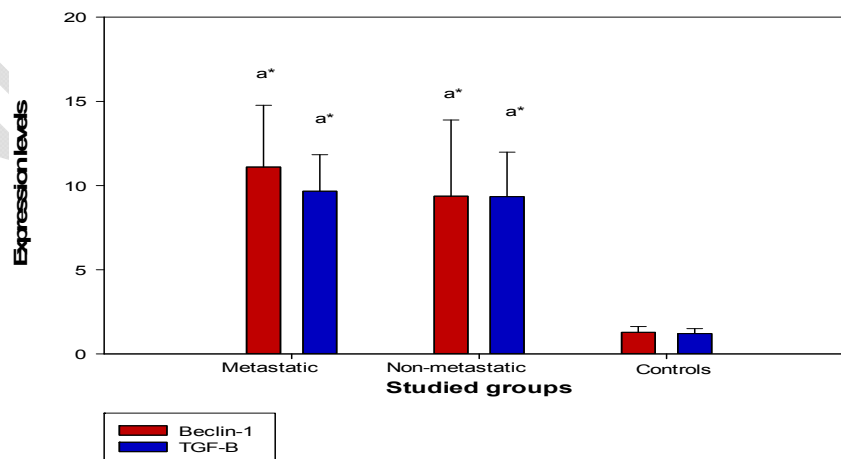


Figure (2): Mean ± SD of beclin-1 and TGF-β expression levels for the studied groups

90 **3.3 Receiver operating characteristics (ROC) curves**

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

92 **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

93 **ROC curve of beclin 1**

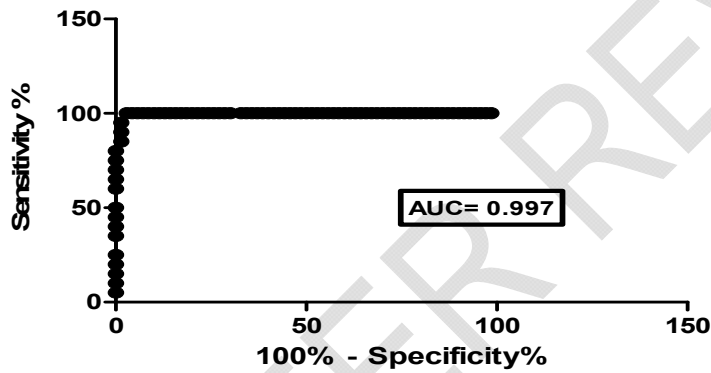


Figure (3): ROC curve of beclin1

ROC curve of TGF- β

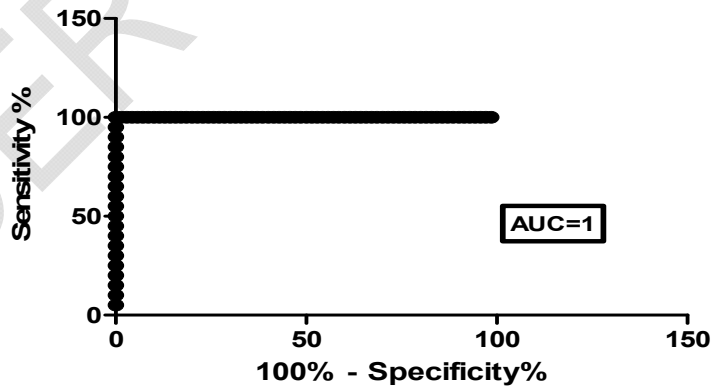


Figure (4): ROC curve of TGF- β

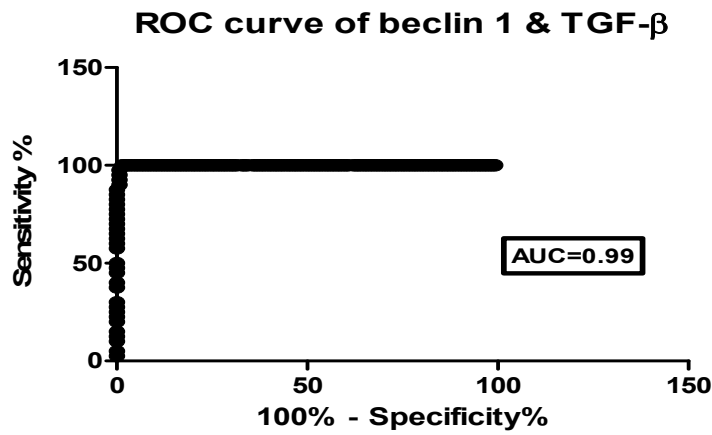


Figure (5): ROC curve of beclin1 and TGF-β

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**

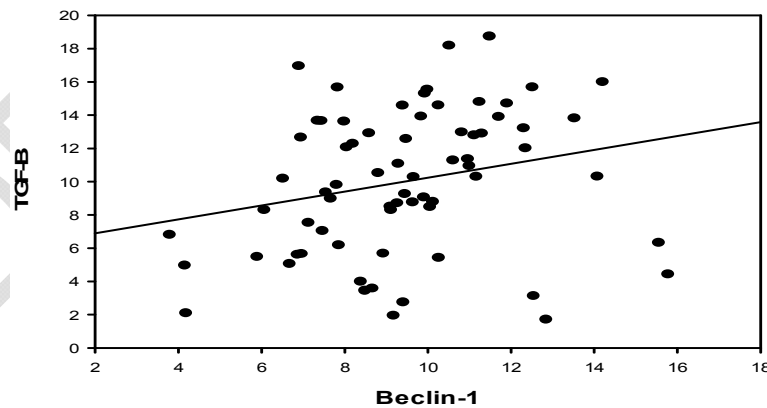


Figure (6): Correlation between beclin-1 and TGF-β expression levels.

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

121 was to assess expression levels of beclin-1 and TGF- β in breast carcinoma Egyptian women compared with normal
122 controls and to determine the potential value of beclin-1 and TGF- β as molecular biomarkers for diagnosis of breast
123 cancer in Egyptian women. In the present study, we detected expression of beclin-1 and TGF- β in breast cancer tissue.
124 We found, compared with the expression in normal breast tissue, that beclin-1 and TGF- β were over-expressed in tumor
125 tissue. Moreover, the over-expression of beclin-1 and TGF- β in breast cancer was considerably associated with TNM
126 stage. These findings may suggest that beclin-1 and TGF- β have direct influence on development and progression of
127 breast carcinoma. In the current study, beclin-1 level was significantly increased in breast cancer groups in comparison to
128 healthy subjects (P value < 0.01). Our results are supported with different previous studies presented by **Hamurcu, et**
129 **al.**, [8] who have studied the relative concentrations of beclin-1 in the tissue of patients with breast cancer and
130 metastatic disease and healthy women who stated that expression levels of beclin-1 was higher in highly aggressive
131 and metastatic and noninvasive cancer cells compared to non-tumorigenic normal human breast cell. The function of
132 autophagy is exist different opinion in solid cancers including breast cancer [8]. In another study **Wang, et al.**, [9] founded
133 a significant increase of beclin-1 expression in eighteen breast carcinoma patients which was associated with tumor
134 progression. Also **Park, et al.**, [10] revealed that the expression of beclin-1, protein was increased in tumor cells relative
135 to normal-appearing and adjacent colonic mucosa in all cases. Similarly **Chen, et al.**, [11] reported an increased
136 expression of beclin-1 in tumor tissue compared with under-expression in normal gallbladder specimen (p < 0.05). The
137 concept that autophagy represents a mechanism that promotes tumor growth is based on the need of tumor cells to adjust
138 to ischemia in the surroundings that are hypoxic, besides growth factors and supplemental deprivation. Consistent with
139 this aspect, autophagy is activated in hypoxic environment of tumors [12]. Beclin-1 was firstly to be found to have tumor
140 suppressor role. However, elevated expression of beclin-1 was related with cancer progression in some carcinoma [13]. In
141 contrast to our study, decreased beclin-1 expression was observed in seventy percent of the breast tumors, and the
142 protein levels were co-related to the mRNA levels [14]. However, **Claude-Taupin, et al.**, [15] reported that no major
143 difference in Beclin-1 mRNA expression between tumor and healthy adjacent tissues. The current study revealed that
144 TGF- β was significantly increased in breast carcinoma patients compared to control group (P value < 0.001) and this
145 finding are affirmed by **Ciftci, et al.**, [16] who have studied the expression level of TGF- β and established that the mean
146 serum TGF- β level of breast cancer patients was considerably higher than controls. There was no considerable difference
147 according to known disease-related clinicopathological parameters. Our finding is supported by **El-Aziz et al.**, [17] who
148 revealed that the level of TGF- β was considerably higher in malignant groups than normal control group with p value
149 (<0.0001). The results of the present study was supported by **Scherer et al.**, [18] who detect the level of TGF- β in
150 breast cancer patients; finding that its level was increased. In the current study, ROC curve of beclin-1 showed 95%
151 sensitivity and 98.5% specificity and this is supported by **Harb, et al.**, [19] who reported that the sensitivity of beclin-1 as a
152 predictor for advanced stage of IDC was 85.5% and the specificity was 98.5%. Also ROC curve of TGF- β showed 100%
153 sensitivity and 98.5% specificity and this is supported by **El Hussein, et al.**, [20] who reported that 98.2% sensitivity and
154 100% specificity demonstrating the diagnostic power of this studied marker in differentiating between breast cancer
155 patients and controls. TGF- β is connected with expanded cancer progression, higher cell movement, cancer invasiveness,
156 and metastasis. It is furthermore included in cancer surrounding medium alteration and advancement of migration and
157 invasiveness [21]. TGF- β elevated the mRNA levels of beclin-1, and other protein kinase implicated in death process.
158 Moreover, TGF- β evoked autophagy in some mammary carcinoma cell. These findings illustrate that TGF- β signaling
159 pathway activates autophagy in certain human cancer cells and that induction of autophagy is a novel aspect of biological
160 role of TGF- β [22]. Concerning clinicopathological factors, the over-expression of beclin-1 was considerably related with

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

171 5. CONCLUSION

172 This study affirmed that up-regulation of beclin-1 expression is present in breast carcinoma tissues. Our results
173 demonstrated that of beclin-1 and TGF- β high levels were associated with forceful clinical outcomes of breast cancer
174 patients. Beclin-1 over-expression, as well as TGF- β over-expression, contributed to tumor development. Additional study
175 on a larger scale will be essential to clarify the diagnostic importance of beclin1 in the process of tumorigenesis.
176

177 REFER/ENCES

- 181 1. Morsy M M, Raafat A, Mohamed M, Fayed A. (2018). Prognostic Value of Percentage of Positive to Total Excised
182 Axillary Lymph Nodes in Egypt with Triple Negative Breast Cancer : Multiple-Centers Experience. *Int. J. of Life*
183 *Sciences*, 6(3), 719–732.
- 184 2. Arakawa S, Honda H, Yamaguchi H, Shimizu S. (2017). Atg12 Conventional autophagy ATG5-ATG12 LC3-PE
185 Beclin-1 Alternative autophagy Rab-9. *Proceedings of the Japan Academy, Series B*, 93(6), 378–385.
- 186 3. Abraham NM, Kirubel, MM, and Abraham DA. (2018). Autophagy as a Possible Target for Cancer Therapy.
187 *Journal of Orthopedic Oncology*, 04(01), 1–9. <https://doi.org/10.4172/2472-016X.1000124>.
- 188 4. Morris DH, Yip CK, Shi Y, Chait BT, Wang QJ. (2015). BECLIN 1-VPS34 COMPLEX ARCHITECTURE:
189 UNDERSTANDING THE NUTS AND BOLTS OF THERAPEUTIC TARGETS. *Front. Biol. (Beijing)*, 10(5), 398–
190 426. <https://doi.org/10.1038/s41556-018-0061-z>.
- 191 5. Furler RL, Nixon DF, Brantner CA, Popratiloff A, Uittenbogaart CH. (2018a). TGF- β sustains tumor progression
192 through biochemical and mechanical signal transduction. *Cancers*, 10(6), 1–18.
193 <https://doi.org/10.3390/cancers10060199>.
- 194 6. Wang ., Gong X, Xu J, Xie R. (2018). The Role of TGF- β in Gastrointestinal Cancers. *Journal of Cancer Science*
195 *& Therapy*, 10(11), 345–350. <https://doi.org/10.4172/1948-5956.1000566>.
- 196 7. Mizushima N. (2017). The exponential growth of autophagy-related research: from the humble yeast to the Nobel
197 Prize. *FEBS Letters*, 591(5), 681–689. <https://doi.org/10.1002/1873-3468.12594>.
- 198 8. Hamurcu Z, Delibaşı N, Geçene S, Şener EF, Dönmez-Altuntaş H, Özkul Y, et al, (2018). Targeting LC3 and
199 Beclin-1 autophagy genes suppresses proliferation, survival, migration and invasion by inhibition of Cyclin-D1 and
200 uPAR/Integrin β 1/ Src signaling in triple negative breast cancer cells. *Journal of Cancer Research and Clinical*
201 *Oncology*, 144(3), 415–430. <https://doi.org/10.1007/s00432-017-2557-5>.
- 202 9. Wang MC, Wu AG, Huang Y, Shao, GL, Ji SF, Wang RW, et al, (2015). Autophagic regulation of cell growth by
203 altered expression of Beclin 1 in triple-negative breast cancer. *International Journal of Clinical and Experimental*
204 *Medicine*, 8(5), 7049–7058.
- 205 10. Park JM, Huang S, Wu TT, Foster NR, Sinicrope FA (2013). Prognostic impact of Beclin 1, p62/sequestosome 1
206 and LC3 protein expression in colon carcinomas from patients receiving 5-fluorouracil as adjuvant chemotherapy.
207 *Cancer Biology and Therapy*, 14(2), 100–107. <https://doi.org/10.4161/cbt.22954>.
- 208 11. Chen Y, Yan J, Yu S, Wang X, Zheng Q. (2014). Over-expression of beclin-1 in gallbladder carcinoma and its
209 relationship with prognosis. *Wspolczesna Onkologia*, 18(3), 171–176. <https://doi.org/10.5114/wo.2014.41395>.
- 210 12. Ávalos Y, Canales J, Bravo-Sagua R, Criollo A, Lavandero S, Quest AFG, et al, (2014). Tumor Suppression and
211 Promotion by Autophagy. *BioMed Research International*, 2014, 1–15. <https://doi.org/10.1155/2014/603980>.
- 212 13. Parkhitko A, Myachina F, Morrison TA, Hindi KM, Auricchio N, Karbowiczek M, Henske EP. (2011).
213 Tumorigenesis in tuberous sclerosis complex is autophagy and p62/sequestosome 1 (SQSTM1)-dependent.
214

- 215 Proceedings of the National Academy of Sciences, 108(30), 12455–12460.
216 <https://doi.org/10.1073/pnas.1104361108>.
- 217 14. Li Z, Chen B, Wu Y, Jin F, Xia Y, Liu, X. (2010). Genetic and epigenetic silencing of the beclin 1 gene in sporadic
218 breast tumors. *BMC Cancer*, 10(98), 1–12. <https://doi.org/10.1186/1471-2407-10-98>.
- 219 15. Claude-Taupin A, Fonderflick L, Gauthier T, Mansi L, Pallandre JR, Borg et al, (2018). ATG9A Is Overexpressed
220 in Triple Negative Breast Cancer and Its In Vitro Extinction Leads to the Inhibition of Pro-Cancer Phenotypes.
221 *Cells*, 7(12), 1–17. <https://doi.org/10.3390/cells7120248>.
- 222 16. Ciftci R, Tas F, Yasasever CT, Aksit E, Karabulut S, Sen F, et al, (2014). Clinical significance of serum
223 transforming growth factor beta 1 (TGFB1) level in breast cancer. *Journal of Clinical Oncology*, 32(15), e11526.
224 <https://doi.org/10.1200/jco.2014.32.15>.
- 225 17. El-Aziz G, Kamel MM, Alkaffas M, Abdelhady EG, Rashed LA. (2018). Can Transforming Growth Factor Beta
226 Affect Breast Cancer by Targeting MicroRNA 195? *J Mol Cell Biochem*, 2(1), 1–4. Retrieved from
227 <http://www.imedpub.com/journal-molecular-cellular-biochemistry>.
- 228 18. Scherer SD, Bauer J, Schmaus A, Neumaier C, Herskind C, Veldwijk MR, Sleeman JP. (2016). TGF- β 1 is present
229 at high levels in wound fluid from breast cancer patients immediately post-surgery, and is not increased by
230 intraoperative radiation therapy (IORT). *PLoS ONE*, 11(9), 1–14. <https://doi.org/10.1371/journal.pone.0162221>.
- 231 19. Harb OA, Salem AA., Haggag R, El-shorbagy S, Gertallah LM. (2016). Immunohistochemical expressions of
232 SQSTM1 / p62 , Beclin-1 , and SOX4 in infiltrating duct carcinoma of the breast. *Egyptian Journal of Pathology*,
233 62, 95–103. <https://doi.org/10.1097/01.XEJ.0000484380.44411.44>.
- 234 20. EL-Husseini M, Hussein F, Bassily N, Abdelghany B. (2013). Clinical significance of TGF alpha , TGF beta1 and
235 VEGF in Sera of Egyptian patients with breast cancer Clinical Significance of TGF Alpha , TGF Beta1 and VEGF
236 in Sera of Egyptian Patients with Breast Cancer Abstract : The Egyptian Journal of Hospital Medicine July, 52,
237 555–565. <https://doi.org/10.12816/0000592>.
- 238 21. Zarzynska J. (2014). Two faces of TGF-beta1 in breast cancer. *Mediators of Inflammation*, 2014, 141747.
239 <https://doi.org/10.1155/2014/14174>.
- 240 22. Kiyono K, Suzuki H, Matsuyama H, Morishita Y, Komuro A, Kano MR, et al, (2009). Autophagy is activated by
241 TGF-beta and potentiates TGF-beta-mediated growth inhibition in human hepatocellular carcinoma cells. *Cancer*
242 *Research*, 69(23), 8844–8852.
- 243
- 244