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Clinical Relevance of LC3B, CXCL10, and Bcl-2 in Breast Cancer

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Abstract

Autophagy is a highly conserved catabolic process, and its dysfunction has been associated with a variety of human diseases including cancer. The role of autophagy in cancer is complex showing a double-edged sword activity. The chemokine ligand CXCL10 has divergent roles in tumors, either promoting or inhibiting tumor progression. In addition, chemokines appear to modulate senescence and cell survival. This study is aimed at exploring the complex role of autophagic marker microtubule-associated protein light chain 3B (LC3B) and chemokine CXCL10 among breast cancer patients with a focus on understanding the relation between LC3B and the anti-apoptotic marker Bcl-2. The study was conducted on sixty females who were classified into forty-five breast cancer patients with different stages and fifteen healthy females as the control group. Venous blood samples were obtained for the quantification of LC3B mRNA transcripts using real time PCR technique. The serum was separated for the detection of CXCL10 and Bcl-2 levels using enzyme-linked immunosorbent assay (ELISA). The results showed a significant increase in the mean of LC3B expression, CXCL10 and Bcl2 concentrations in patients. Moreover, the LC3B expression and Bcl2 level were negatively associated with age, tumor stage, lymph-node involvement, and vascular invasion, while a positive association was observed between CXCL10 and both tumor stage and vascular invasion. Furthermore, a positive correlation was observed between LC3B expression and the Bcl2 level. The current study concludes that both LC3B and CXCL10 may act as potential prognostic indicators for breast cancer patients. In addition, the modulation of autophagy and apoptosis could be promising targets for the treatment of breast cancer.

Keywords: Autophagy, Microtubule-associated protein light chain 3B (LC3B), Chemokine ligand CXCL10, Bcl-2 and breast cancer

1. Introduction

Breast cancer is most common among women in both the developed and developing countries. It is the leading cause of cancer-related deaths in women worldwide, accounting for 25 % of all cancer cases and 15 % of total cancer deaths among females (Torre, *et al.,* 2015). In Egypt, it is also the most common cancer among women, accounting for approximately 37.7 % of cancers with a peak incidence in the age group of 40–59 years and a mortality rate of 29.1% (Ahmed, *et al.,* 2013).

Circulating tumor cells (CTCs) are cancer cells which dissociate from the primary tumor and circulate within the peripheral blood, initiating metastasis at a distant location. CTCs represent a “non-invasive, liquid biopsy” that must undergo a phenotypic shift acquiring the ability to survive anoikis within the blood stream and evade immune surveillance in order to form metastases (Mohme, *et al.*, 2017).

Physiologically, autophagy is a homeostatic and a survival-promoting pathway that captures, degrades, and recycles intracellular proteins and organelles in lysosomes preserving organelle function, preventing the toxic buildup of cellular waste products, and providing substrates to sustain metabolism during stress (Frankel, *et al.,* 2017). Microtubule-associated protein light chain 3B (LC3B) has an important role in the formation of autophagosome, and its amount is correlated with the autophagy level, therefore, it is used as a marker of autophagy (Huang and Brumell, 2014). Autophagy showed a critical role in longevity; however, the relationship between autophagy and human aging remains complex. It has been observed that autophagy decreases with age. On the other hand, the elevated production of reactive oxygen species (ROS) during aging upregulates autophagy to eliminate impaired organelles.

The role of autophagy in cancer is complex where it shows a double-edged sword functional activity, since it can act as a tumor suppressor or tumor promoter. It is likely dependent on tumor type, stage, and genetic context (White, 2015). It has been suggested that autophagy can constrain tumor initiation by regulating DNA damage and oxidative stress, while in established tumors, it can also be required for tumor maintenance, allowing tumors to survive environmental stress and providing intermediates for cell metabolism (Singh, *et al*., 2018).

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B-cell Lymphoma 2 (Bcl-2) is an antiapoptotic protein belonging to the Bcl-2 family. The role of Bcl-2 differs depending on its interaction with other members of the Bcl-2 family (Bouchalova, *et al.,* 2014). The expression of Bcl-2 protein should inhibit apoptosis *in vivo* which, therefore, means a worse outcome for the patients. However, it has been revealed that Bcl-2 positive patients had better prognosis since Bcl-2 not only inhibits apoptosis, but also has an inhibitory effect on cell proliferation (Dawson, *et al.,* 2010).

The chemokine interferon-γ inducible protein CXCL10 is a member of the CXC chemokine family which binds to the CXCR3 receptors to exert its biological effects. It is associated with a variety of human diseases including infectious diseases, chronic inflammation, immune dysfunction, tumor development, progression and metastasis (Wightman, *et al.,* 2015). It has been shown that CXCR3/CXCL10 expression in tumors has divergent roles, either promoting or inhibiting tumor progression (Cerny, *et al.,* 2015).

Accumulating evidence showed the controversial action of autophagy in cancer without clarifying the expression of LC3B in circulating tumor cells (Gallagher, *et al.,* 2016). In addition, the role of CXCL10 in breast cancer has not been well-characterized. Furthermore, a recent study has demonstrated the relation between autophagy and other chemokines (Fang, *et al.,* 2015). In contrast, little is known about the relation between autophagy and CXCL10.

Accordingly, this study is aimed at investigating the role of autophagic marker LC3B, apoptotic marker Bcl-2 and chemokine CXCL10 in the peripheral blood of patients with different stages of breast cancer. In addition, all parameters were correlated with each other and with the clinicopathological features of the disease.

2. Material and Methods

The current study has been conducted on sixty females who were classified into forty-five breast cancer patients with different stages of the disease and fifteen age-matching healthy females as the control group. Patients were recruited from the Surgery Department outpatient clinic or ward and Cancer Management and Research Department, Medical Research Institute, Alexandria University during the period from February to May 2018.

The patients' samples were taken before any intervention (surgery or chemotherapeutic intervention); females who were above sixty years old were excluded from the study. Venous blood samples were obtained from all subjects under study, and were divided as follows: 3 ml in heparin vacutainers for the isolation of peripheral blood mononuclear cells (PBMCs) and the quantification of LC3B mRNA transcripts using real time PCR technique, and 2 ml in plain vacutainers for serum separation that was divided into aliquots for detection of CXCL10 and Bcl-2 levels using enzyme-linked immunosorbent assay (ELISA) technique.

2.1. Isolation of Peripheral Blood Mononuclear Cells (PBMCs)

This was carried out on a fresh heparinized blood sample using density gradient centrifugation over Ficoll-Hypaque (1077) purchased from Sigma-Aldrich Chemical Company (Fuss, *et al.,* 2009). The diluted sample was over-layered carefully over half of its volume of Ficoll-Hypaque (1077) by the side wall of the tube that was centrifuged at 1800 rpm speed for thirty minutes at room temperature. After centrifugation, the interface cells (white ring) were carefully aspirated and washed twice with a sterile phosphate buffer saline (PBS). They were pelleted and resuspended in 1 ml PBS, and were stored at -80 until use for RNA isolation.

2.2. RNA Isolation and cDNA Synthesis

Genomic RNA was isolated using GeneJET RNA Purification Kit (Thermo Scientific®) (Boom, *et al*., 1990) following the manufacturer's instructions (www.thermo-scientific.com/ onebio). cDNA is reverse transcribed from the total RNA samples using high capacity cDNA Reverse Transcription Kit (Applied Biosystems®). The reaction conditions for reverse-transcription were as follows: 10 µL of total RNA was mixed with 10 µL of reverse transcription (RT) master mix in the reaction tubes that were centrifuged and incubated for five minutes on ice. Then, the tubes were loaded into the thermal cycler to be run at 25 °C for ten minutes and at 37°C for two hours. The reaction was then heated at 85°C for five minutes and stored at -80°C until use.

2.3. Quantitative Real-Time Reverse Transcription-PCR

The level of mRNA expression of LC3B was determined using the TaqMan gene expression Assay (Thermo-Fisher Scientific®) together with the TaqMan Universal PCR Master Mix II (Applied Biosystems®) according to the manufacturer's instructions.

The amplification program included an initial 95°C Taq activation stage for ten minutes followed by forty cycles of 95 °C denaturation for fifteen seconds and 60°C anneal for sixty seconds. After amplification, a melting curve analysis was performed by collecting fluorescence data. GAPDH was chosen as an internal control. The relative amount of target gene was calculated using the 2-DDCT ($2^{-\Delta\Delta CT}$) method (Livak and Schmittgen , 2001).

2.4. Chemokine CXCL10 and Anti-apoptotic Marker Bcl-2 Serum Levels Assessment using Enzyme Linked Immunosorbent Assay (ELISA) Technique

Serum CXCL10 and Bcl-2 concentrations were quantified with the commercially available ELISA kits (Han, *et al.,* 2016; Kosacka, *et al.,* 2016, respectively) according to manufacturer's instructions (Cat. No : E0412Hu Human CXCL10 Bioassay Technology Laboratory ELISA Kit) (Cat.No : E1832Hu Human Bcl-2 Bioassay Technology Laboratory ELISA Kit). The peripheral blood samples taken from the study population were collected in plain vacutainers and were left for twenty minutes at 37°C to clot followed by one hour at 4°C to retract blood clots. Finally, the blood samples were centrifuged at 1800 rpm for ten minutes. The sera were collected and stored in aliquots at -80°C until used.

2.5. Statistical Analysis

Data were analyzed using SPSS software. The results are expressed as mean ± SD. Analysis of data was done using t test, Mann- Whitney U test, Chi square and ANOVA test (F test). Correlation was tested between variables of interest. The results were considered significant at $P<0.05$.

3. Results

3.1. LC3B Expression in Different Studied Groups and its Association with Clinicopathological Parameters (age, stage, grade, lymph node involvement and vascular invasion)

The present study showed that a significant increase in the mean of LC3B expression of patients compared to healthy individuals (p<0.001). In addition, a significant negative association was observed between LC3B expression and different parameters including age (p= 0.005), stage (p<0.001) and lymph-node involvement (p<0.001). However, there was no association with the tumor grade (p= 0.416) or vascular invasion (p= 0.172) (Table 1)

Table 1. Relationship between LC3B expression and different parameters in the cases' groups.

Different parameter	N	LC3B expression			p
		Min. – Max.	Mean ± SD.	Median	
Age (years)					
≤50	21	0.44 – 6.36	3.46 ± 1.68	3.53	0.005*
>50	24	0.01 – 5.82	1.92 ± 1.82	1.29	
Stage					
I	14	2.64 – 6.36	4.84 ± 1.01	4.84	
II	15	1.07 – 4.29	2.65 ± 0.99	2.75	
III	15	0.01 – 1.99	0.76 ± 0.61	0.51	
IV#	1	-	0.03	-	<0.001*
Grade					
I	3	2.64 – 4.92	3.97 ± 1.19	4.35	
II	33	0.01 – 6.36	2.64 ± 1.95	2.66	0.416
III	9	0.37 – 5.31	2.20 ± 1.88	1.40	
Lymph node involvement					
N0	16	2.64 – 6.36	4.34 ± 1.22	4.44	
N1	24	0.01 – 5.82	1.79 ± 1.52	1.38	<0.001*
N2	3	0.41 – 4.29	1.81 ± 2.15	0.73	
N3	2	0.37 – 0.66	0.52 ± 0.21	0.52	
Vascular invasion					
No	10	0.18 – 4.92	3.37 ± 1.40	3.52	0.172
Yes	35	0.01 – 6.36	2.43 ± 1.99	1.75	

*: Statistically significant at $p \leq 0.05$; #: Excluded from the comparison

3.2. CXCL10 Concentration in Different Studied Groups and its Association with Different Parameters (stage, lymph node involvement, grade, vascular invasion and LC3B expression)

The present study showed a significant increase in the mean of CXCL10 serum concentration in patients compared to healthy individuals (p <0.001). Moreover, Table 2 illustrates that CXCL10 serum concentration was positively associated with stage (p<0.001), lymph node involvement (p<0.001), and vascular invasion (p=0.041). On the other hand, there was no association between the CXCL10 concentration and tumor grade (p=0.095). Furthermore, a negative correlation was observed between the CXCL10 concentration and LC3B expression in patients (p <0.001) (Figure 1).

Table 2. Relationship between chemokine (CXCL10) concentration and different parameters in the cases' groups.

Different parameters	N	CXCL10 concentration (pg/ml)			p
		Min. – Max.	Mean ± SD.	Median	
Stage					
I	14	650.0–911.0	765.3±79.13	765.0	
II	15	734.0–1049.0	878.7±81.83	875.0	
III	15	1130.0–1377.0	1235.6±75.61	1255.0	
IV#	1	-	1445.0	-	<0.001*
Lymph-node involvement					
N0	16	650.0 – 017.0	798.75 ± 101.10	790.50	
N1	24	734.0 – 445.0	1049.63 ± 20.81	1089.50	<0.001*
N2	3	894.0 – 283.0	1106.33 ± 96.94	1142.0	
N3	2	1206.0 –1377.0	1291.50 ± 20.92	1291.50	
Grade					
I	3	673.0 – 815.0	725.67 ± 77.78	689.0	
II	33	650.0 – 1445.0	976.97 ± 226.05	894.0	0.095
III	9	734.0 – 1377.0	1050.67 ± 13.37	1017.0	
Vascular invasion					
No	10	673.0 –1204.0	865.70 ±161.42	844.0	0.041*
Yes	35	650.0 – 1445.0	1006.17 ± 234.0	916.0	

*: Statistically significant at $p \leq 0.05$; #: Excluded from the comparison

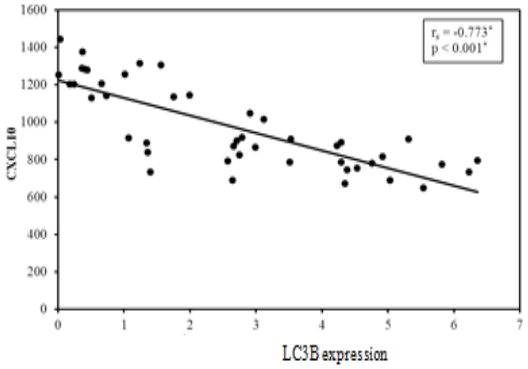

Figure 1. Correlation between LC3B expression and CXCL10 concentration in breast cancer patients.

3.3. Bcl-2 Concentration in Different Studied Groups and its Association with Different Parameters (age, stage, grade, Lymph node involvement, vascular invasion, CXCL10 and LC3B expression)

The present study showed a significant increase in the mean of Bcl-2 serum concentration in patients compared to healthy individuals (p <0.001). The results demonstrated that serum Bcl-2 concentration was negatively associated with age (p=0.012), stage (p<0.001), lymph-node involvement (p<0.001) and vascular invasion (p=0.034), whereas there was no association with tumor grade (p=0.178) (Table 3). Moreover, it was observed that Bcl-2 concentration correlated negatively with the CXCL10 concentration in breast cancer patients (rs = -0.780, p<0.001) (Figure 2), while it correlated positively with LC3B expression (rs =0.823, p<0.001) (Figure 3).

Table 3. Relationship between Bcl-2 concentration and different parameters in the cases' groups.

Different parameters	N	Bcl-2 concentration (U/mL)			*p*
		Min. – Max.	Mean ± SD.	Median	
Age (years)					
≤50	21	29.22 – 73.65	51.40 ± 13.67	48.0	0.012*
>50	24	21.0 – 66.0	41.80 ± 10.06	40.67	
Stage					
I	14	43.0 – 73.65	61.37 ± 8.79	62.0	
II	15	40.33 – 50.0	45.35 ± 2.99	45.0	
III	15	29.22 – 41.0	34.81 ± 3.32	35.70	
IV#	1	-	21.0	-	<0.001*
Grade					
I	3	43.0 – 70.50	58.17 ± 13.97	61.0	
II	33	21.0 – 71.0	44.60 ± 12.03	43.50	0.178
III	9	36.90 – 73.65	48.46 ± 13.78	44.0	
Lymph-node involvement					
N0	16	42.0 – 73.65	56.30 ± 10.89	56.0	
N1	24	21.0 – 66.0	40.89 ± 10.90	39.17	<0.001*
N2	3	37.26 – 48.0	42.09 ± 5.45	41.0	
N3	2	36.90 – 37.30	37.10 ± 0.28	37.10	
Vascular invasion					
No	10	31.90 – 73.65	53.73 ± 14.33	48.30	0.034*
Yes	35	21.0 – 69.0	44.15 ± 11.55	42.0	

*: Statistically significant at $p \leq 0.05$; #: Excluded from the comparison

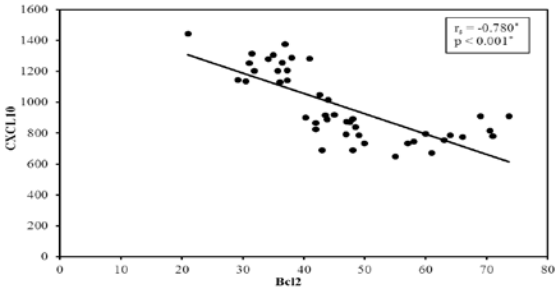

Figure 2. Correlation between CXCL10 and Bcl-2 concentration in breast cancer patients.

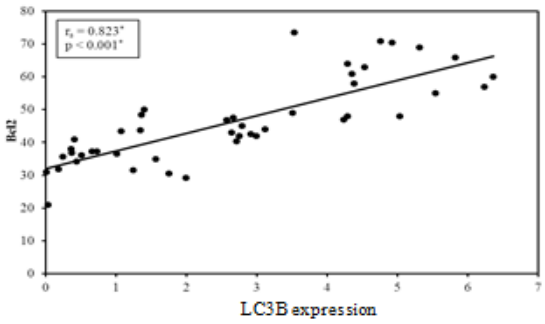

Figure 3. Correlation between LC3B expression and Bcl-2 concentration in breast cancer patients.

4. Discussion

The role of autophagy in cancer is complex and dynamic which suggests that it is dependent on the tumor stage, cell type, and/or genetic factors. The present study shows that the mean of LC3B expression was significantly increased in patients compared to healthy individuals. In agreement with this result, Suman, *et al.* (2014) revealed that LC3B is highly expressed in breast tumors compared with normal tissues. This may be due to the fact that autophagy is maintained at a basal level to serve its housekeeping function under normal circumstances where it acts as an intracellular quality-control mechanism recycling long-lived or misfolded/aggregate-prone proteins and damaged organelles. However, autophagy is upregulated in order to generate building blocks that are necessary for cellular survival under different forms of stress (e.g. nutrient and growth factor deprivation, hypoxia and so on) (Choi, *et al.*, 2013) .

The current results showed that lower LC3B expression was significantly associated with advanced stages. In agreement with this result, Ladoire *et al.* (2016) stated that the presence of cytoplasmic dots positive for microtubule-associated protein light chain 3B (LC3B) in human breast cancer cells indicates an enhanced autophagic flux and favorable prognosis. This may be explained on the basis of the protective autophagy response due to its catabolic roles, by degrading and/or recycling cell components to protect cells against the deleterious effects of reactive oxygen species (ROS). It also hinders the proliferation of cells with cancer-linked mutations suppressing tumorigenesis by facilitating senescence (biological aging) (Jiang and Mizushima, 2014). Moreover, autophagy's role in innate and adaptive immune responses is implicated in immuno-surveillance for pre-malignant cells. Furthermore, it has been reported that autophagy can promote cell death in the cases of severe stress through excessive self-digestion and degradation of essential cellular constituents (Yonekawa and Thorburn , 2013). On the contrary, Wei *et al.* (2012) demonstrated that the suppression of autophagy inhibits mammary tumorigenesis and progression by both impairing tumor-cell proliferation and inducing increased immune surveillance, suggesting a pro-tumorigenic role of autophagy.

Autophagy has a prominent role in determining the lifespan of many model organisms beyond its function in the adaptation of individual cells or organs to changing conditions. The study demonstrated that LC3B expression decreased with age. The exact mechanisms involved in the decreased autophagy with aging remain unclear. However, it has been demonstrated that aging is associated with the hyperactivation of mTOR that exerts an inhibitory effect on autophagy (Cornu, *et al.*, 2013). Moreover, transcriptional down-regulation of key autophagy genes such as *ATG5* and *ATG7* was detected in human brains during normal aging either due to a general failure to maintain *ATG* gene expression or because of the reduced upstream signaling (Lipinski. *et al.*, 2010).

The chemokine interferon-γ inducible protein CXCL10 is a member of the CXC chemokine family which binds to the CXCR3 receptors to exert its biological effects. It has been associated with cancer; however, it shows a paradoxical role, either promoting or inhibiting tumor progression (Cerny, *et al.*, 2015). The present study showed a significant increase in the mean of CXCL10 serum concentration in the patients compared to the healthy individuals. In agreement with this result, Jafarzadeh, *et al (*2016) stated that higher CXCL10 levels in patients with breast cancer may represent the role of chemokine in tumor development. The possible mechanism that may explain this is that Ras induces CXCL10 overexpression in human breast-cancer cell lines through Raf and PI3 kinase signaling pathways, promoting breast cancer growth (Liu, *et al.*, 2011).

The results revealed that CXCL10 serum concentration was significantly increased with advanced stages. In addition, a significant association was observed between serum CXCL10 concentration and clinicopathological parameters including lymph-node involvement and vascular invasion. In concordance, Wightman, *et al.* (2015) stated that CXCL10/CXCR3 signaling has a role in promoting tumor cell growth, motility, and metastasis. This may be explained on the basis that CXCL10 binding increases tumorigenicity and metastasis by acting directly on the tumor cells to promote their tumor-supporting characteristics (Mulligan, *et al.*, 2013). In addition, tumor expression of CXCL10 is associated with lymphocytic infiltration including Tregs that block the stimulation of T effector cells and NK cells (Lunardi, *et al.*, 2014). On the contrary, Kajitani, *et al* (2012) demonstrated the antitumor role of CXCL10 by which IFN-γ secreted from NK cells likely promotes the production of CXCL10 from breast cancer cells, which, in turn, accelerates the migration of CXCR3-expressing NK cells into the tumor site.

Autophagy plays critical roles in inflammation through influencing the transcription, processing and secretion of a number of cytokines**,** in addition to being regulated by cytokines. Regarding the relation between autophagy and CXCL10, the results of this study showed that serum CXCL10 concentration is negatively correlated with the LC3B expression in patients. In agreement with this result, Zhang, *et al.* (2017) showed that CXCL10 impaired autophagy. This may be explained on the basis that CXCL10 contributes to the impairment of autophagosome-lysosome system by impairing lysosomal acidification that is critical for the degradation of autophagosomal cargos for the maintenance of autophagic flux. Moreover, it has been reported that CXCL10 was found to be upregulated in *BECN1*− tumor cells (Mgrditchian, *et al.*, 2017).

The Bcl-2-protein family regulates both cell death and proliferation. High Bcl-2 levels have been detected in a variety of tumor types (Dai, *et al.*, 2016). The present study showed a significant increase in the mean of Bcl-2 serum concentration in patients compared to healthy individuals. This result is in agreement with Hwang, *et al.* (2012), who demonstrated that the expression of Bcl-2 was frequent in breast cancer patients compared to healthy blood donors. The possible mechanisms that may explain this upregulation include Bcl-2 gene rearrangement and hypomethylation. It has been reported that Bcl-2 is upregulated by estrogens in breast cancer, through a direct consequence of transcriptional induction.

The results demonstrated that higher serum Bcl2 concentration was associated with early stages. In agreement with this result, Eom, *et al.* (2016) observed an increase in Bcl-2 level in early stages of breast cancer, and its upregulation was related to favorable prognosis. Although this is contradictory to the anti-apoptotic function of Bcl-2, this discrepancy may be explained by the interactions of various proteins involved in apoptotic pathways such as p53, Fas and so on. In addition, an inverse association was observed between Bcl-2 and the proliferation marker (Ki-67) expression in tumors of various types. Bcl-2 is known *in vitro* to inhibit cell cycling independent of apoptosis and activate a program of premature senescence in human carcinoma cells (Wang, *et al.*, 2014). Furthermore, it is postulated that the tumor suppressive effect of Bcl-2 is more prominent in breast cancer, and that its expression was significantly associated with ER and PR positivity (Eom, *et al.*, 2016).

As far as age is concerned, there was a negative association between Bcl2 and age. In concordance with this finding, Zhang, *et al.* (2016) demonstrated that the decreased $CD3^+$, $CD4^+$ and $CD8^+$ T cells in aged mice were highly associated with the reduced expression of Bcl-2.

The connection between autophagy and apoptosis or other forms of cell death is an emerging area of research. The current results revealed that Bcl2 correlated positively with LC3B expression. It has been shown that autophagy degrades selectively the active caspase-8, and inhibits the TRAIL (tumor necrosis factor related apoptosis inducing ligand) induced apoptosis (Ojha, *et al.*, 2015). Furthermore, inhibition of autophagy by degradation of ATG proteins was reported by apoptotic proteins such as caspases and calpains (Luo, *et al.*, 2012). This is, however, contradictory to the role of Bcl-2 in inhibiting autophagy indirectly through its interaction with beclin-1. The possible mechanism that may explain this contradiction is that nutrient starvation activates JNK1 (C-Jun N-terminal protein kinase 1), which phosphorylates the regulatory loop of Bcl-2 and then releases the interaction between Bcl-2 and beclin-1 (Yang and Yao, 2015). However, the molecular connections between autophagy and cell death are multifaceted, complex, and still poorly understood.

The present study showed a negative correlation between Bcl-2 and CXCL10 in breast cancer patients. This negative correlation is in agreement with Razmkhah, *et al.* (2014) who revealed that Bcl-2 transcripts showed a lower expression in Adipose Derived Stem Cells (ASCs) transfected with CXCL10 compared to non-transfected cells. This may be explained on the basis that CXCL10 increased the Ca^{2+} uptake by the mitochondria, which released cytochrome causing apoptosis. In addition, it has been reported that CXCL10-induced TLR4 activation was involved in apoptosis where CXCL10 induces long-term Akt and JNK activation, which switches towards apoptosis by caspase-3 and PAK-2 cleavage (Sahin, *et al.*, 2013). This is in line with the finding that the knockout of either CXCL10 or TNF-α reduced germ cell apoptosis in the co-cultures of germ cells and Sertoli cells in response to the infection (Jiang, *et al*., 2017).

5. Conclusion

It can be concluded from the previous results that both LC3B and CXCL10 may serve as potential prognostic biomarkers for breast cancer. In addition, the modulation of autophagy and apoptosis could be promising targets for the treatment of breast cancer.

References

Ahmed AZ, Mohamed R, Ayman AG and Fatma MT. 2013. Clinico-pathological features of breast carcinoma in elderly Egyptian patients: A comparison with the non-elderly using population-based data. *J Egypt Natl Canc Inst*, **25**:5 –11.

Boom R, Sol CJ, Salimans MM, Jansen CL, Wertheim-van Dillen PM and van der Noordaa J. 1990. Rapid and simple method for purification of nucleic acids. *J Clin Microbiol*, **28**(3):495-503**.**

Bouchalova K, Kharaishvili G, Bouchal J, Vrbkova J, Megova M and Hlobilkova A. 2014. Triple negative breast cancer: BCL2 in prognosis and prediction. *Curr Drug Targets*, **15**:1166–1175.

Cerny C, Bronger H, Davoodi M, Sharma S, Zhu L, Obana S, et al. 2015. The Role of CXCR3/Ligand Axis in Cancer. *Int Trends Immun*, **3**(2): 19-25.

Choi AM, Ryter SW and Levine B. 2013. Autophagy in human health and disease. *N Engl J Med*, **368**:651–662**.**

Cornu M, Albert V and Hall MN. 2013. mTOR in aging, metabolism, and cancer. *Curr Opin Genet Dev*, **23**(1):53–62.

Dai H, Meng XW and Kaufmann SH. 2016. BCL2 Family, Mitochondrial Apoptosis, and Beyond. *Cancer Transl Med*, **2**:7-20.

Dawson SJ, Makretsov N, Blows FM, Driver KE, Provenzano E, Le Quesne J, et al. 2010. BCL2 in breast cancer: a favourable prognostic marker across molecular subtypes and independent of adjuvant therapy received. *Br J Cancer*, **103**(5):668-675.

Eom YH, Kim HS, Lee A, Song BJ and Chae BJ. 2016. BCL2 as a Subtype-Specific Prognostic Marker for Breast Cancer. *J Breast Cancer*, **19**:252-260.

Fang WB, Yao M, Jokar I, Alhakamy N, Berkland C, Chen J, et al. 2015. The CCL2 chemokine is a negative regulator of autophagy and necrosis in luminal B breast cancer cells. *Breast Cancer Res Treat*, **150**:309-320**.**

Frankel LB, Lubas M and Lund AH. 2017. Emerging connections between RNA and autophagy. *Autophagy*, **13**:3-23.

Fuss IJ, Kanof ME, Smith PD and Zola H. 2009. Isolation of whole mononuclear cells from peripheral blood and cord blood. *Curr Protoc Immunol*, Chapter 7:Unit7.1.

Gallagher LE, Williamson LE and Chan EY. 2016. Advances in Autophagy Regulatory Mechanisms. *Cells*, **5**:24.

Han BK, Kuzin I, Gaughan JP, Olsen NJ and Bottaro A. 2016. Baseline CXCL10 and CXCL13 levels are predictive biomarkers for tumor necrosis factor inhibitor therapy in patients with moderate to severe rheumatoid arthritis: a pilot, prospective study. *Arthritis Res Ther*, **18**:93. Huang J and Brumell JH. 2014. Bacteria-autophagy interplay: a battle for survival. *Nat Rev Microbiol*, **12**:101-114.

Hwang KT, Woo JW, Shin HC, Kim HS, Ahn SK, Moon HG, et al. 2012. Prognostic influence of BCL2 expression in breast cancer. *Int J Cancer*, **131**:1109-1119.

Jafarzadeh A, Fooladseresht H, Nemati M, Assadollahi Z, Sheikhi A and Ghaderi A. 2016. Higher circulating levels of chemokine CXCL10 in patients with breast cancer: Evaluation of the influences of tumor stage and chemokine gene polymorphism. *Cancer Biomark*, **16**:545-554**.**

Jiang P and Mizushima N. 2014. Autophagy and human diseases. *Cell Res*, **24**(1):69-79**.**

Jiang Q, Wang F, Shi L, Zhao X, Gong M, Liu W, et al. 2017. C-X-C motif chemokine ligand 10 produced by mouse Sertoli cells in response to mumps virus infection induces male germ cell apoptosis. *Cell Death Dis*, **8**(10):e3146.

Jin WJ, Kim B, Kim D, Park Choo HY, Kim HH, Ha H, et al. 2017. NF-κB signalling regulates cell-autonomous regulation of CXCL10 in breast cancer 4T1 cells. *ExpMol Med*, **49**(2):e295**.**

Kajitani K, Tanaka Y, Arihiro K, Kataoka T and Ohdan H. 2012. Mechanistic analysis of the antitumor efficacy of human natural killer cells against breast cancer cells. *Breast Cancer Res Treat*, **134**(1):139-155**.**

Kosacka M, Porębska I, Korzeniewska A, Rubinsztajn R, Grabicki M, Jankowska R,et al. 2016. Serum levels of apoptosis-related markers (sFasL, TNF-a, p53 and bcl-2) in COPD patients. *Pneumonol Alergol Pol*, **84**(1):11-15.

Ladoire S, Enot D, Senovilla L, Chaix M, Zitvogel L and Kroemer G. 2016. Positive impact of autophagy in human breast cancer cells on local immunosurveillance. *Oncoimmunol.*, **5**(6): e1174801.

Lipinski MM, Zheng B, Lu T, Yan Z, Py BF, Ng A, et al. 2010. Genome-wide analysis reveals mechanisms modulating autophagy in normal brain aging and in Alzheimer's disease. *Proc Natl Acad Sci U S A*, **107**(32):14164–14169.

Liu M, Guo S and Stiles JK. 2011. The emerging role of CXCL10 in cancer. *Oncol Lett*, **2**(4):583-589.

Livak KJ and Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method. *Methods*, **25**:402-408.

Lunardi S, Jamieson NB, Lim SY, Griffiths KL, Carvalho-Gaspar M, Al-Assar O, et al. 2014. IP-10/CXCL10 induction in human pancreatic cancer stroma influences lymphocytes recruitment and correlates with poor survival. *Oncotarget*, **5**(22):11064-11080**.**

Luo S, Garcia Arencibia M, Zhao R, Puri C, Toh PP, Sadiq O, et al. 2012. Bim inhibits autophagy by recruiting Beclin 1 to microtubules. *Mol Cell*, **47**:359-370.

Mgrditchian T, Arakelian T, Paggetti J, Noman MZ, Viry E, Moussay E, et al. 2017. Targeting autophagy inhibits melanoma growth by enhancing NK cells infiltration in a CCL5-dependent manner. *Proc Natl Acad Sci USA*, **114**(44):9271-9279**.**

Mohme M, Riethdorf S and Pantel K. 2017. Circulating and disseminated tumour cells -mechanisms of immune surveillance and escape. *Nat Rev Clin Oncol*, **14**(3):155-167.

Mulligan AM, Raitman I, Feeley L, Pinnaduwage D, Nguyen LT, O'Malley FP, et al. 2013. Tumoral lymphocytic infiltration and expression of the chemokine CXCL10 in breast cancers from the Ontario Familial Breast Cancer Registry. *Clin Cancer Res*, **19**(2):336-346**.**

Ojha R, Ishaq M and Singh SK. 2015. Caspase-mediated crosstalk between autophagy and apoptosis: Mutual adjustment or matter of dominance. *J Can Res Ther*, **11**:514-524.

Razmkhah M, Jaberipour M and Ghaderi A. 2014. Downregulation of MMP2 and Bcl-2 in Adipose Derived Stem Cells (ASCs) following Transfection with IP-10 Gene. *Avicenna J Med Biotechnol*, **6**(1):27-37.

Sahin H, Borkham-Kamphorst E, do O NT, Berres ML, Kaldenbach M, Schmitz P, et al. 2013. Proapoptotic effects of the chemokine, CXCL 10 are mediated by the noncognate receptor TLR4 in hepatocytes. *Hepatol.*, **57**(2):797-805.

Singh SS, Vats S, Chia AY, Tan TZ, Deng S, Ong MS, et al. 2018. Dual role of autophagy in hallmarks of cancer. *Oncogene*, **37**(9):1142-1158.

Suman S, Das TP, Reddy R, Nyakeriga AM, Luevano JE, Konwar D, et al. 2014. The proapoptotic role of autophagy in breast cancer. *Br J Cancer*, **111**(2):309-317.

Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J and Jemal A. 2015. Global cancer statistics, 2012. *CA Cancer J Clin*, **65**(2):87-108.

Wang W, Wang D and Li H. 2014. Initiation of premature senescence by Bcl-2 in hypoxic condition. *Int J Clin Exp Pathol*, **7**(5):2446-2453

Wei H and Guan JL. 2012. Pro-tumorigenic function of autophagy in mammary oncogenesis. *Autophagy*, **8**(1):129-131**.**

White E. 2015. The role for autophagy in cancer. *J Clin Invest*, **125**(1):42-46.

Wightman SC, Uppal A, Pitroda SP, Ganai S, Burnette B, Stack M, et al. 2015. Oncogenic CXCL10 signaling drives metastasis development and poor clinical outcome. *Br J Cancer*, **113** (2):327-335.

Yang J and Yao S. 2015. JNK-Bcl-2/Bcl-xL-Bax/Bak Pathway mediates the crosstalk between matrine-induced autophagy and apoptosis via interplay with beclin 1. *Int J Mol Sci*, **16**(10):25744-25758**.**

Yonekawa T and Thorburn A. 2013. Autophagy and cell death. *Essays Biochem*, **55**:105-117.

Zhang X, Wu WK, Xu W, Man K, Wang X, Han J, et al. 2017. C-X-C Motif Chemokine 10 impairs autophagy and autolysosome formation in non-alcoholic steatohepatitis. *Theranostics*, **7**(11):2822-2836**.**

Zhang Y, Zhang M, Wang Y, Liu L and Sweeney C. 2016. Bcl-2-expression in Dys-regulated distribution of T and B lymphocytes in aged mice. *J Clin Cell Immunol*, **7**: 473.