Chapter 8

Triple-Negative Breast Cancer, Cisplatin and Calpain-1

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Additional information is available at the end of the chapter

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Abstract

Chemo-resistance of breast cancer is a major obstacle for successful treatment and is mainly represented as a defect in apoptosis. The differential effects of platinum-based drugs (PBDs) were assessed on breast cancer cell ultrastructure. Three representative cells, including triple-negative breast cancer (TNBC), were treated with different concentrations and timings of cisplatin, carboplatin, and oxaliplatin. Changes on cell surface and ultrastructure were detected by scanning electron microscope (SEM) and transmission electron microscope (TEM). In addition, using advanced techniques in molecular biology, we demonstrated that calpain-1 plays an essential role in modulating breast cancer cell sensitivity to cisplatin-induced apoptosis. We also showed that the correlation of its expression to the proliferating/apoptotic index using immunohistochemical staining in TNBC tissue was variable. Exploring new pathways will help in overcoming chemo-resistance in breast cancer cells.

Keywords: triple-negative breast cancer, platinum-based drugs, cisplatin, calpain-1, apoptosis

1. Introduction

Breast cancer is ranked second as one of the leading cause of deaths among women worldwide [1]. It is characterized by heterogeneity displaying a wide scope of morphological features, different immunohistochemical profiles, and unique histopathological subtypes. According to immunohistochemical phenotypes [i.e., presence or absence of estrogen receptor (ER), progesterone receptor (PgR), and epidermal growth factor receptor 2 (HER2)], breast cancer can be classified into five subtypes. These are luminal A, luminal B, HER2 overexpression, basal-like, and normal-like subtypes, each of which has distinct clinical outcomes [2]. Luminal A accounts for 50% of invasive breast cancers and are ER/PgR positive or HER2 negative. Luminal B category represents 20% of invasive breast cancers. The ER/PgR is positive, while

HER2 expression is variable (positive or negative). Luminal B tumors have higher proliferation and poorer prognosis than luminal A tumors. HER2 overexpression group accounts for 15% of all invasive breast cancers and the tumor usually tends to be ER/PR negative. The basal class is typically ER/PR negative and HER2 negative, hence the name TNBC [3]. It comprises about 15% of all invasive breast cancers and have a fairly poor prognosis. Normal-like tumors account for 7.8% of all breast cancer cases in a lymph-node negative cohort. It is positive for ER and PgR but negative for HER2 [4, 5].

Due to this heterogeneity, the treatment is complicated and the therapeutic strategies should be selected carefully. To overcome the disease, it is imperative that each patient be treated individually according to the morphological classification with molecular parameters and sensitivity to available therapy. Treatment of breast cancer includes surgery, radiation therapy, hormone-modification therapy and chemotherapy (anticancer drugs). Chemotherapy treatment has markedly reduced the risk for recurrence and mortality after primary treatment of breast cancer and have increased the 5- and 10-year survival rates [6].

One of the major modes of action of chemotherapeutic drugs may be the activation of apoptosis (programmed cell death) [7]. Hence, anticancer drugs are associated with the activation of proapoptotic genes and the suppression of antiapoptotic genes. The attenuation of proapoptotic genes and increases in antiapoptotic genes causes resistance to apoptosis [8]. Hence, in order to increase the therapeutic effect of chemotherapy, there is a need to assess the molecular mechanisms of apoptosis induced anticancer drugs. This may lead to new strategies for the enhancement of the antitumor effect against target organs.

In this chapter, we hope to summarize three attempted molecular biology studies on breast cancer that have contributed to further knowledge in this field. We have compared the effects of platinum based-chemotherapeutic drugs such as cisplatin, carboplatin and oxaliplatin on the ultrastructure of the three human breast cancer cell lines representing the most diagnosed types; MDA-MB-231, MCF-7 and BT-474 [9]. We have particularly demonstrated the role of cisplatin in inducing apoptosis in MDA-MB-231 via the endoplasmic reticulum- mediated calpain-1 pathway [10]. At the same time, we have assessed the expression of calpain-1 as a potential prognostic factor in TNBC tissues [11]. Understanding the pathways by which platinum-based drugs induce apoptosis and how these pathways are altered in chemoresistance can provide valuable information necessary to target specific cell death pathways in the treatment of clinically resistant breast cancer.

2. Platinum-based drugs and breast cancer cells

Platinum-based drugs (PBDs) are used for adjuvant chemotherapy to reduce mortality from breast cancer with reversible side-effects [12]. A key feature of platinum based drugs is that once platinum salts enter cells, they can bind to DNA to form Platinum-DNA adducts that can cause damage to the DNA. Following DNA damage, cell cycle checkpoints are activated to repair either the damaged DNA or induce apoptosis (cell death) [13, 14]. Thus, the ultimate goal in the application of platinum-based chemotherapy is to shift the dynamics away from

cell growth and survival in favor of cell differentiation and apoptosis. This will in turn reduce and eliminate tumor progression and malignancy [15].

Although PBDs are initially effective, their efficacy is limited by the occurrence of resistance, which is attributed to alterations in cellular pathways such as DNA repair, drug transport, drug metabolism and apoptosis [16]. Several studies have explored the cellular and molecular pathways involved in the mechanism of PBDs resistance to breast cancer [13, 16–18]. However, only a few ultrastructural studies on the intracellular organelles of breast cancer cells have been performed to determine the effectiveness of these drugs.

2.1. Surface structure of breast cancer cells differ from normal breast cells

We used SEM to compare the surface morphology between three models of breast cancer cells, each of which is characterized with a distinct immunohistochemical profile. The MCF-7 cell line was used to represent the luminal A breast cancer [19], the BT-474 cell line, the luminal B tumor [20] and the MDA-MB-231 cell line, the basal-like subtype, TNBC [3].

Normal breast cells, MCF-10A, revealed round shape cells characterized by short lamellipodia, whereas, the breast cancer cells had a semiflattened surface structure containing microvilli with extending lamellipodia. Lamellipodia consist of protrusive filamentous actin and signaling proteins, which play a role in cell migration and cell–cell communication. These surface protrusions are important in enhancing movement and adhesion to the surrounding stroma [21]. They appeared to be lesser in number and finer in shape for both MCF-7 and BT-474 cells but higher in number and thicker for MDA-MB-231 cells. Since MDA-MB-231 cells are advanced cancer cells with metastatic characteristics, therefore it is not surprising for these cells to contain higher numbers of lamellipodia on their cell surface. This is indicative of their importance of cell shape modifications in their invasiveness process unlike the normal breast cells. These distinct features of TNBCs *in vivo* models might demonstrate their aggressiveness and give them a metastatic potential [21–23]. TEM micrographs revealed the absence of nuclei in the MDA-MB-231 cells whereas more than one nucleus were detected in MCF-7 and BT-474 cells.

2.2. Effect of PBDs on the cell membrane of breast cancer cells

Treatment with cisplatin, carboplatin and oxaliplatin, using two concentrations of 10 and 20 μm with the time period of 15 minutes, the initial response of the treated breast cancer cells started with the formation of pores on the cell membranes indicating the active process of drug influx/efflux. The pores on the surface of the MDA-MB-231 cells were deeper and wider due to the high number of lamellipodia, unlike the two cell types; MCF-7 and BT-474. Subsequently the lamellipodia retracted causing the cells to shrink and change their shape to semioval and to round shape. This was more evident to a higher extent in the MDA-MB-231 cells.

When we treated all the three types of breast cancer cells for 12 hours with the three types of PBDs, SEM revealed the early stages of apoptosis presented by convoluted membrane, membrane blebs and apoptotic bodies. The membrane blebbing is caused by deep cytoskeleton rearrangement as result of alterations in organelle distribution and cell shape, a pattern of apoptosis. Differences on the response of the cells to the three types of PBDs were detected

for BT-474 and MCF-7 cells. BT-474 cells sensitivity response was maximal for Carboplatin whereas MCF-7 cells sensitivity response was maximal for cisplatin. However, MDA-MB-231 cells response was similar for all the PBDs. Hence, cell mediated drug response is dependent on the cellular characteristic and the drug action.

2.3. Effect of PBDs on the intracellular organelles of breast cancer cells

We then used TEM to gain further insight into the ultrastructural alterations induced by PBDs and to study how the drug cytotoxicity differentially caused these alterations. Other distinct morphological characteristics of apoptosis consistent with the literature were evident such as shrinkage of the cytoplasm, microvilli retraction, fragmentation and condensation of the nucleus and swelling of both the mitochondria and endoplasmic reticulum [24, 25]. Splitting of apoptotic cells characterizes the final stage of apoptosis [24]. In addition to apoptosis, TEM micrographs also revealed the necrotic type of death. Changes identified on plasma membrane shows incoherence, causing cell swelling and organelles disruption. Occasionally, apoptotic cells, *in vitro*, undergo a late process of secondary necrosis. Necrosis was considered to be a physical process of cell death that was not regulated. However, emerging evidence suggests that it is as another form of apoptosis and an independent genetically encoded cell death pathway [25, 26]. Overall, treated cells with the three types of PBDs exhibited similar ultrastructural changes exhibiting distinct features such as the increased number of vacuoles portraying as a defense mechanism for cell survival and this is consistent with other studies in other types of cancers [27–29]. PBD deposits were mainly attracted to the fat droplets of the cells suggesting an active role of cellular lipids in the potentiation of PBDs to induce apoptosis.

Few but prominent differences between the three types of breast cancer cells were detected when treated with PBDs. These included the following;

1. Carboplatin did not cause any swelling and disarrangement of the mitochondria on the BT-474 and the MDA-MB-231 cells as opposed to the MCF-7 cells.
2. Carboplatin-treated cells exhibited more lamellar bodies compared to cisplatin or oxaliplatin treated cells. Lamellar bodies are specialized lipid storage or secretory organelles, which have a core composed of multilamellar structure and can be surrounded by a membrane [30]. It is possible that PBDs induce lipidosis in cancer cells and cause accumulation of lamellar bodies.
3. Carboplatin, cisplatin and oxaliplatin caused apoptosis in all the three types of breast cancer cell lines, however, it is possible that apoptosis independent of DNA damage could have contributed to the way some of the enucleated cells of the MDA-MB-231 cells die. This will be discussed further in Section 3.

3. Cisplatin-induced calpain-1 activation by endoplasmic reticulum in TNBC cells

Cisplatin has been shown to induce apoptosis in enucleated cells [31, 32]. It does this by initially acting on the endoplasmic reticulum causing an increase in cytosolic calcium (Ca^{2+}),

leading to the activation of calpain-1 [33]. Calpains belong to a family of Ca^{2+}-dependent proteases which play many roles in basic cellular processes including cell proliferation and apoptosis, through activation of the caspase pathways. Calpain-1 and calpain-2, encoded by CAPN1 and CAPN2, respectively, are the most abundant isoforms within their family [31]. Although we, and others, have shown that cisplatin-induced apoptosis occurs by way of the calpain-1 dependent pathway, [34–36]; however, information in TNBC cells is limited. This prompted us to investigate the role of the calpain-1 pathway by way of the endoplasmic reticulum in the apoptotic death of TNBC cells induced by cisplatin.

3.1. Cisplatin caused calcium release in TNBC cells

Using Von Koss staining, we were able to represent the variation of Ca^{2+} deposits between the cisplatin-treated and untreated TNBC cells. Ca^{2+} deposits in the cytoplasm increased with increasing cisplatin concentration (0, 20 and 40 μm) in the cisplatin-treated cells with no significant deposits observed in the untreated cells.

3.2. Cisplatin caused structural changes in the endoplasmic reticulum of TNBC cells

Several studies have concentrated on the investigation of non-nuclear pathways in the apoptosis of cancer cells induced by cisplatin [31, 32, 34]. Such studies contribute to the understanding of the causes of sensitivity and resistance to cisplatin [31, 37]. The endoplasmic reticulum is involved in the regulation of cellular responses to stress and alterations in Ca^{2+} homeostasis [38]. Alterations in Ca^{2+} homeostasis and accumulation of misfolded proteins in the endoplasmic reticulum caused endoplasmic reticulum stress resulting in apoptosis [39]. Using TEM, we detected the intracellular deposits of cisplatin and its structural changes on the endoplasmic reticulum in TNBC cells. TEM micrographs revealed that cisplatin induced clear structural changes in both the endoplasmic reticulum and the mitochondria. This phenomenon represented swelling of the lumen and disarrangement of their internal folding as compared to the control cells without treatment which appeared as well-defined structures. Hence, these findings were consistent with a study conducted by Mandic et al. who demonstrated that the endoplasmic reticulum is the non-nuclear target of cisplatin [31].

3.3. Location of calpain-1 in TNBC cells

Studies have reported that calpain-1 is mainly located in the cytoplasm of breast cancer cells [40, 41]. We also used immunohistochemical staining to confirm this finding. The staining intensity of calpain-1 in the cytoplasm increased with increasing concentrations (0, 20 and 40 μm) of cisplatin.

3.4. Cisplatin activated calpain-1 and induced apoptosis through the endoplasmic reticulum-mediated pathway

The results of some experiments attempted to investigate the role of calpain-1 in the apoptotic death of TNBC cells induced by cisplatin by way of the endoplasmic reticulum are summarized in **Table 1**.

Experiments			Results	
	Control (μm/nM)	Treatment after 24 hours		P value of apoptosis
Cisplatin to induce endoplasmic reticulum stress (calcium release) and activate calpain-1 was assessed as activation of endoplasmic reticulum downstream effectors; α-fodrin and caspase-12. Calpain-1, α-fodrin and caspase-12 protein content (total and cleaved) was measured by Western blotting.	Cisplatin (0 μm)	Cisplatin (20 μm)	Cisplatin activated calpain-1 as reflected in cleavage of α-fodrin and caspase-12 and induced apoptosis in TNBC cells. Although cisplatin had no effect on calpain-1 content, it significantly caused cleavage of α-fodrin and caspase-12 and induced apoptosis in a dose-dependent manner [10].	P < 0.001 vs. control
	Cisplatin (0 μm)	Cisplatin (40 μm)		P < 0.001 vs. control
Cisplatin to activate calpain-1 by way of endoplasmic reticulum using CPA treatment was assessed as activation of endoplasmic reticulum downstream effectors; GRP78, calmodulin, α-fodrin and caspase-12, were measured using immunoblotting.	Cisplatin (0 μm) + CPA (50 μm)	Cisplatin (20 μm) + CPA (50 μm)	CPA significantly enhanced upregulation of cisplatin-induced, calpain-1 activation and apoptosis compared with the controlled group [10].	P < 0.001 vs. CPA Control
Cisplatin to activate calpain-1 by way of endoplasmic reticulum using siRNA treatment was assessed as activation of α-fodrin. The effect of calpain-1 siRNA on its content and activation (indicated by α-fodrin cleavage) was measured using immunoblotting	Cisplatin (0 μm) + calpain-1 siRNA (150 nM)	Cisplatin (20 μm) + calpain-1 siRNA (150 nM)	Calpain-1 small interfering RNA (siRNA) significantly attenuated cisplatin-induced apoptosis in TNBC cells by downregulating calpain-1 in TNBC cells [10].	P < 0.01 vs. Calpain-1 siRNA Control
Apoptosis was measured by Hoechst staining using fluorescent microscopy.				

Table 1. Summary of results of experiments attempted to investigate the role of calpain-1 in the apoptotic death of TNBC cells induced by cisplatin by way of the endoplasmic reticulum.

We have shown in this study the effect of cisplatin on calpain-1 protein and its activation in TNBC cells. This has also been reported by others in other types of cancer cells [34, 35]. The finding that the increase in both calcium deposits and upregulation of endoplasmic reticulum

stress indicator proteins such as GRP78 and calmodulin suggest the involvement of endoplasmic reticulum stress-dependent Ca^{2+} release in the cellular mechanism of action of cisplatin. The ability of cisplatin-induced apoptosis by way of endoplasmic reticulum stress has been shown to involve calpain-mediated activation of caspase-12 [42]. Caspase-12 is localized to the endoplasmic reticulum and may be activated by the disturbance of intracellular calcium homeostasis [43]. Cyclopiazonic acid (CPA) is a selective Ca^{2+} ATPase inhibitor, which depletes the endoplasmic reticulum (ER) of Ca^{2+} and therefore, activates Ca^{2+} – dependent proteases such calpain. For that reason, the activity of calpain-1 was enhanced by CPA through the endoplasmic reticulum-mediated pathway which further increased the TNBC cells response to cisplatin-induced apoptosis. In contrast, the sensitivity was attenuated by calpain-1 inhibition using the exogenous inhibitor, calpain-1 siRNA. These findings support the role of calpain-1 responsible for the pro-apoptotic effects of cisplatin in TNBC cells by way of endoplasmic reticulum. Hence, targeting calpain-1 activity with specific inhibitors could be a novel approach in limiting development of primary tumors and formation of metastases.

4. Calpain-1 as a potential prognostic factor in TNBC

TNBC has been reported to have a clinical and pathological aggressive pattern due to its heterogeneous characteristic [44]. The ineffectiveness of hormonal and targeted therapies and poor prognosis for this subtype requires developing alternative therapeutic strategies such as biomarkers. The expression of a number of proteins has been shown to be associated with clinical outcome in TNBC patients [40, 45, 46]. Hence, there is a need to identify additional biomarkers to allow personalized treatment for patients with TNBC. For this reason, we explored the role of calpain-1 as a potential prognostic factor for TNBC therapy. We also evaluated the proliferation and apoptotic index for their potential use as possible prognostic factors since the biological behavior of tumor growth is a result of a balance between the proliferative activity and the number of cells dying by apoptosis [47]. Thus, they are considered as dominant histopathologic features in tumors. Several studies have also shown that calpain-1 expression significantly associated with tumor grade [40], proliferation [48, 49] and apoptosis [50]. Therefore, we also assessed the association between calpain-1 expression, cell proliferation and apoptosis in TNBC tissues.

4.1. Patient characteristics

We tested calpain-1 protein expression and the proliferative/apoptotic index on paraffin-embedded tissues from a cohort of 55 patients with TNBC. The main histological type was infiltrative ductal carcinoma in 96.4% (53 of 55), infiltrative lobular carcinoma in 1.8% (1 of 55) and micropapillary carcinoma 1.8% (1 of 55). Patients were females with a median age of 47 years (19–74). A total of 34 cases (61.8%) were premenopausal with no family history of breast cancer. Based on the disease indexing system, half (50.9%) of the patients were defined as stage III or IV at the time of diagnosis. Almost half of the patients (n = 26, 47.3%)

received neoadjuvant treatment and 5 (19.2%) achieved complete pathological response. Anthracyclines and taxanes were the most commonly used chemotherapeutic agents as front-line treatment. Breast cancer related overall survival (OS) was defined as the time interval (in months) from the date of diagnosis until death from breast cancer. Similarly, recurrence-free survival (RFS) was defined as the time interval (in months) between the start of primary treatment and date of cancer relapse.

4.2. Calpain-1 expression in TNBC tissues

Immunostained tissues with calpain-1 were significantly expressed and demonstrated cytoplasmic and membranous staining with some granularity and heterogeneity between adjacent tumor cells varying from weak to intense staining in which low staining was detected in 32.7% (18 of 55), intermediate staining in 38.2% (21 of 55) and high staining in 29.6% (16 of 55) of the cases analyzed. The cut off value was determined by screening the stained tissue under light microscope where the staining intensity of calpain-1 in tumor cells was assessed as none (0), weak (1), medium (2), and strong (3) using an immunohistochemical *H*-score. The *H*-scores were calculated by multiplying the percentage area by the intensity grade (*H*-score range 0–300).

4.3. Correlation between calpain-1 expression and clinicopathological variables and outcome of TNBC patients

In order to investigate the possibility of using calpain-1 protein as a prognostic biomarker in TNBC, its expression was assessed for association with a number of clinicopathological variables. We determined that calpain-1 expression displayed a significant positive association to the lymph node status ($P = 0.02$) but not with other clinicopathological variables. Kaplan–Meier survival curves were plotted with significance determined using the log-rank test in order to determine the relationship between calpain-1 protein expression in the recurrence-free survival (RFS) and in the overall survival (OS) patients. The expression of calpain-1 in the triple-negative tissues was not significantly associated with breast cancer RFS ($P = 0.71$) or OS ($P = 0.88$) in which the median RFS was 18 months (3–77 months) and OS was 41 months (0–105 months) in the total patient cohort.

TNM classifies lymph node status as a tumor-related prognostic factor, therefore, our results suggest that calpain-1 might be used as a prognostic factor in TNBC. Calpain-1 was also found to be associated with lymph node status in other types of cancer, such as renal cell carcinoma [51]. The observation of the lack of association of calpain-1 with other clinicopathological variables is consistent with a study conducted by Storr et al. in which they demonstrated a correlation between calpain-1 expression and tumor grade but not with other clinicopathological variables [40].

The variations among the presence or absence of association with lymph node status or tumor grade which are essential in determining its prognosis can be explained by several theories; (i) the majority of patient samples were of intermediate grade tumor and therefore calpain-1 activity may have started at later stages as suggested by its correlation with the lymph node status, (ii) the lack of wide range of sample collection in regards to tumor grades may have

created a diversion in the statistical analysis, (iii) the insufficiency of samples might have contributed to lack of significant correlations, (iv) the possibility of genetic differences between the populations in the current study and the ones already published may be the cause of differences on the expression of calpain-1 in breast cancer cells [40] and finally (v) the presence or absence of the hormonal receptors such as ER, PR, and HER2 that determine breast cancer behavior and thus treatment can influence the outcome. Storr *et al*. (2011) reported that there was no association between the expression of calpain-1 in HER2-positive breast cancer patients treated with trastuzumab following adjuvant chemotherapy with any of the clinicopathological variables [52]. Hence, their observation is consistent with our data but may differ in terms of the positivity of HER2.

4.4. Association between calpain-1 expression, cell proliferation and apoptosis in TNBC tissues

Calpains have been reported to be involved in the proliferation of breast cancer cells [48, 49]. However, the role of the calpain family in proliferation of TNBC cells has not been reported yet. Ki-67, a nuclear antigen is a protein encoded by Ki-67 on 10q25 and considered to be a proliferation marker for predicting tumor development [53]. It is expressed during all active phases of the cell cycle except the resting phase, thus being present only in dividing cells. Ki-67 is detected by monoclonal antibody MIB-1 which can be a useful marker of proliferation and of prognostic value [53]. The quantitative assessment of Ki-67 staining on paraffin embedded tumor sections has been reported as an accurate estimate of the proliferation index of individual tumors [53].

Therefore, proliferative fractions of paraffin embedded breast cancer tissues were determined by immunohistochemical staining for Ki-67 antibody. The cellular proliferative activity was estimated as the percentage of tumor cells stained per field ×40. Statistical analysis showed no significant correlation between calpain-1 expression and proliferation ($P = 0.29$). Possible theories of the presence and absence of the hormonal receptors, differences in the genetic makeup, and other members of calpains involvement may also influence the correlation with proliferation.

Cell proliferation along with cell death are both phenomena responsible for control of cell number in normal tissues and tumors. Since chemotherapy induces programmed cell death by apoptosis, hence, the apoptotic tumor cells can be morphologically identified using the conventional hematoxylin and eosin (H&E) method and cells are counted using light microscopy. Therefore, there has been interest in the application of the apoptotic index in malignant growths as a putative prognostic marker. The percentage of apoptotic cells in tumor sections may also be measured by a molecular-based approach, labeling of fragmented DNA breaks and calculating the apoptotic index (AI) using the terminal transferase-uridyl nick-end labeling (TUNEL) assay.

Therefore, in order to determine whether the frequency of apoptosis was related to tumorigenesis, two approaches; the conventional H&E staining method and the apoptotic TUNEL assay were both used to detect apoptotic cells and to prove that the two methods comparatively correlate with each other. H&E detects apoptosis in its degradation phase and can be subjective whereas the TUNEL assay detects apoptosis in its early phase and is more objective. Apoptotic cells were counted per 100 invasive tumor cells using ×40 objective. Apoptotic counts

using either method, were significantly correlated ($P < 0.001$, $r = 0.547$). Although both assays tested apoptosis from different aspects, but the results were the same, indicating the reliability of both assays. These findings were also consistent with a previous study by Watanabe et al. [54]. In addition, the relationship between apoptosis and proliferation was investigated in TNBC tissues. For all of the patients, high apoptotic counts significantly correlated with increased cell proliferation ($P = 0.045$). The positive correlation between proliferative and apoptotic indices seen in this study is also consistent with other types of cancers such as colorectal cancers [54].

In experimental models the calpain system has been shown to influence apoptosis in breast cancer [48, 55, 56]. The relationship between calpain-1 expression and apoptosis using the two methods, H&E-based apoptotic counts and apoptotic counts derived from the apoptotic TUNEL assay was investigated in the TNBC tissues. Interestingly, the data revealed that there were no significant association between the apoptotic indices when compared to calpain-1 expression ($P = 0.710$ and 0.100), respectively. Such results suggest that the TNBC cells undergo apoptosis via other members of the calpain family such as calpain-2.

Taken together, these data have clearly demonstrated the absence of correlation between calpain-1 expression and the proliferating/apoptotic index or clinicopathological variables except with the lymph node status of TNBC patients. Hence, calpain-1 could be a useful prognostic marker in TNBC. More studies should be conducted in the future to evaluate the prognostic value of calpain-1 in TNBC.

5. Conclusion

Breast cancer is the most leading cause of cancer death in females worldwide. Although its name is based on a single tissue of origin, this cancer is heterogeneous making it a complex disease. Compared to other subtypes of breast cancer, TNBC is more biologically aggressive and has higher recurrence rate, higher frequency of metastasis and worse survival. Challenges into identifying targets and treatments have led to advances in laboratory technology and research resulting into the expansion of our knowledge of tumor biology. Though no specific therapies currently exist for TNBC except for cytotoxic chemotherapy, there is ongoing research to identify potential targets for therapy. Therefore, the understanding of breast cancer subtypes and targeted drug therapies is a key to address resistance to current targeted drugs in order to pave the way for providing personalized breast cancer care.

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