Complement component 1, q subcomponent binding protein is a marker for proliferation in breast cancer

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Abstract

Complement component 1, q subcomponent binding protein (C1QBP), is a multi-compartmental protein with higher mRNA expression reported in breast cancer tissues. This study evaluated the association between immunohistochemical expression of the C1QBP protein in breast cancer tissue microarrays (TMAs) and clinicopathological parameters, in particular tumor size. In addition, an *in vitro* study was conducted to substantiate the breast cancer TMA findings. Breast cancer TMAs were constructed from pathological specimens of patients diagnosed with invasive ductal carcinoma. C1QBP protein and proliferating cell nuclear antigen (PCNA) immunohistochemical analyses were subsequently performed in the TMAs. C1QBP immunostaining was detected in 131 out of 132 samples examined. The C1QBP protein was predominantly localized in the cytoplasm of the breast cancer cells. Univariate analysis revealed that a higher C1QBP protein expression was significantly associated with older patients ($P = 0.001$) and increased tumor size ($P = 0.002$). Multivariate analysis showed that C1QBP is an independent predictor of tumor size in progesterone-positive tumors. Furthermore, C1QBP was also significantly correlated with expression of PCNA, a known marker of proliferation. Inhibition of C1QBP expression was performed by transfecting *C1QBP* siRNA into T47D breast cancer cells, a progesterone receptor-positive breast cancer cell line. *C1QBP* gene expression was analyzed by real-time RT-PCR, and protein expression by Western blot. Cell proliferation assays were also performed by commercially available assays. Down-regulation of C1QBP expression significantly decreased cell proliferation and growth in T47D cells. Taken together, our findings suggest that the C1QBP protein could be a potential proliferative marker in breast cancer.

Keywords: Breast cancer, tissue microarray, C1QBP, immunohistochemistry, gene modulation, proliferation

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Introduction

Breast cancer accounts for an estimated 1.38 million of total new cancer cases globally, making this disease the most common cancer diagnosed in the female population.¹ Early detection and diagnosis of breast cancer has remarkably improved the treatment options and survival of patients. However, recurrence of the disease (with a high rate of metastasis) occurs in approximately 30% of patients with early stage breast cancer.² Hence, breast cancer is a cause for concern as this dreaded disease is still the primary cause of cancer death among women.¹

Adjuvant therapy such as chemotherapy has effectively lead to a sustained decline of breast cancer mortality over the decades. However, as breast cancer is a heterogeneous

disease, systemic chemotherapy may only provide absolute benefit for a subset of breast cancer patients. Therefore, there is a need to identify novel biomarkers which are able to determine the progression of the disease in breast cancer patients, and to develop potential efficacious therapeutic interventions. As such, there has been a search for specific molecular targets in breast cancer with the aim of developing targeted cancer therapy,³ which could possibly reduce the adverse side effects induced by systemic chemotherapy or radiotherapy. Thus far, established molecular targets in breast cancer include hormonal receptors (in particular the estrogen receptor) and human epidermal growth factor receptor 2 (HER2).³

Complement component 1, q subcomponent binding protein (C1QBP), also documented as hyaluronic acidic

binding protein 1 (HABP1) and gC1QR, was first observed to bind C1q to the mitochondrial membrane of a baboon's heart.⁴ C1QBP is known to be a multi-compartmental protein which is predominantly localized in the mitochondria.⁵ Subsequent studies have shown the involvement of C1QBP in the regulation of complement activation,⁶ pre-mRNA splicing,⁷ and mitochondrial function.^{8,9} Notably, previous studies also verified that C1QBP is a participant in tumorigenesis. For instance, expression of C1QBP in prostate cancer was significantly correlated with a higher Gleason score, prostate specific antigen relapse time after surgery and lower prostate specific antigen recurrence-free survival rate.¹⁰ Similarly, C1QBP expression was associated with lymph node metastasis, cisplatin-resistance, and poor survival rate in serous ovarian cancer patients.¹¹ Higher C1QBP mRNA levels were observed in tissues from breast cancer patients with lower survival rate and lymph node metastasis.¹² In addition, the expression of C1QBP has been associated with higher proliferation and motility in breast cancer *in vitro*.¹³ Recently, the C1QBP protein has also been linked with distant metastasis in breast cancer.¹⁴ The same investigators showed that protein kinase C ζ , an interacting partner of C1QBP, could activate chemotaxis in breast cancer cells.

In the present study, C1QBP protein expression in tissue microarrays (TMAs) of breast cancer patients diagnosed with invasive ductal carcinoma were correlated with clinicopathological parameters, in particular tumor size. In addition, the functional role of C1QBP in breast cancer proliferation was investigated by modulation of the *C1QBP* gene *in vitro*.

Materials and methods

Clinical materials

TMAs consisted of 1 mm core from paraffin embedded tissue samples from 132 patients diagnosed with breast invasive ductal carcinoma. The samples were collected between 2004 and 2007 and archived in the Department of Pathology of the Singapore General Hospital, with ethics approval granted by the Institutional Review Board. The age of the patients ranged from 23 to 88 years with 56 years as the mean age. Tumor sizes of patients ranged from 10 mm to 140 mm with mean of 41 mm. Details of the clinicopathological parameters are provided in Supplementary Table 1.

Immunohistochemical staining

Immunohistochemical staining was performed using the Leica BOND-MAXTM System according to the manufacturer's protocol. The primary antibody used was rabbit polyclonal anti-C1QBP antibody, which was raised against the recombinant C1QBP-His₆ protein and then purified through a protein G Sepharose column.¹⁵ The antibody produced a single band when probed against HeLa cell lysate, as shown in Supplementary Figure 1. This anti-C1QBP antibody was used at a dilution of 1:500. Staining of proliferating cell nuclear antigen (PCNA) was done using anti-PCNA antibody (1:10,000 dilution; Sigma Aldrich, St. Louis, MO).

Tissue sections were treated with epitope retrieval solution 2 for 20 min with a standard protocol F from the BOND-MAXTM System. The sections were counterstained using hematoxylin. A negative control was included by omitting the primary antibody to ensure non-specific binding did not exist. The TMA slides which had been stained were viewed at 100X and 400X magnifications under a light microscope. Only the percentage and intensity of immunopositive staining in malignant epithelial cells were recorded. The different intensities of the staining were categorized as 1+, 2+, and 3+ for weak, moderate, and strong staining, respectively. The weighted average intensity (WAI) score was calculated as the ratio of the sum of each intensity multiplied by its respective percentage, to the total percentage of immunopositive staining. The scores were validated independently by two researchers, one of whom is a pathologist.

Cell culture

Progesterone receptor-positive T47D and ZR-75-1 breast cancer cells, together with the progesterone receptor-negative MDA-MB-231 breast cancer line were obtained from the American Type Culture Collection. RPMI-1640 medium containing 10% fetal bovine serum was used to maintain the breast cancer lines in a humidified 37°C CO₂ incubator.

Real-time RT-PCR

The concentration and purity of total mRNA extracted from the cells were determined by the ratio of the absorbance readings at 260 nm to 280 nm (where the acceptable range for the absorbance readings was from 1.9 to 2.1). cDNA obtained from conversion of the mRNA, was analyzed by real-time PCR using the ABI Prism 7500 Thermocycler (Applied Biosystems Inc., Foster City, CA). The following specific primers were used:

C1QBP forward: 5'-AGAAGCGAAATTAGTGCGGAA-3';
C1QBP reverse: 5'-CCACGAAATTGGGAGTTGATGTC-3';
GAPDH forward: 5'-GAAGGTGAAGGTCGGAGTCAACG-3';
GAPDH reverse: 5'-TGCCATGGGTGGAATCATATTGG-3'.

GAPDH was used as the housekeeping gene. The cycling parameters were optimized and relative fold change was computed as $2^{-\Delta\Delta C_t}$, where $\Delta\Delta C_t = [(C_{t_{\text{target}}} - C_{t_{\text{GAPDH}}}) \text{ of target group}] - [(C_{t_{\text{target}}} - C_{t_{\text{GAPDH}}}) \text{ of control group}]$.

Western blotting

Extraction of cellular proteins was performed using the mammalian protein extraction reagent (M-PER) comprising HaltTM protease inhibitor cocktails and 0.5 mol/L of EDTA (Pierce, Rockford, IL). Twenty micrograms of protein were loaded onto each lane for SDS-PAGE electrophoresis. After electrophoresis, proteins were transferred to a polyvinylidene fluoride membrane and incubated with 5% non-fat milk for an hour, to block non-specific sites.

Primary antibody incubation (anti-C1QBP; 1:500) was performed at 4°C, overnight. The membrane was then probed with horseradish peroxidase-conjugated goat anti-rabbit antibody (1:5000 dilution) for 1 h at room temperature. Enhanced chemiluminescence was performed using the SuperSignal West Pico Chemiluminescent Substrate (Pierce) and developed on X-ray films. β -Actin was used as the loading control. The optical density (OD) ratio of the bands was determined with the β -actin band as the denominator.

Transfection of small interfering RNA targeting C1QBP into T47D cells

T47D cells were seeded in either 6-well plates or 24-well plates with cell densities of 2.5×10^5 cells/well or 0.625×10^5 cells/well, respectively, and incubated at 37°C, 5% CO₂ overnight. Twenty nanomole per liter of human C1QBP ON-TARGETplus SMARTpool or non-targeting siRNA (Dharmacon, Chicago, IL) were transfected into T47D cells using the transfection reagent, DharmaFECT1 (Dharmacon) as described in the manufacturer's protocol. The transfection medium was replaced with 10% fetal bovine serum (FBS) RPMI-1640 medium 24 h post-transfection.

Cell proliferation assay

AlamarBlue® assay. Cell growth curve was obtained using alamarBlue® Cell Viability Reagent (Invitrogen™, Life Technologies, Carlsbad, CA). The alamarBlue® reagent was introduced from 0 h to 168 h after transfection at 24 h

intervals. Fluorescence intensity was measured at 530 nm excitation and 590 nm emission wavelengths.

MTS assay. The CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay (MTS) (Promega, Madison, WI) was also used to analyze proliferation at 96 h post-transfection as a second method to validate the results. Cells were fasted for 24 h by replacing FBS-containing medium with pure RPMI-1640 medium. MTS solution was introduced at 96 h post-transfection. The absorbance was then read at 490 nm using a microtiter plate reader.

Statistical analyses

Univariate and multivariate analyses were carried out to examine the association of C1QBP's protein expression with patients' clinicopathological data. The STATA version 10 software was used for the analyses (STATA Corp LP, TX). Univariate analysis was done using chi-square test and statistical significance was set at $P < 0.05$. For the multivariate analysis, tumor size, grade of tumor, associated ductal carcinoma *in situ* (DCIS) grade, associated DCIS extent, tubule formation, pleomorphism, and mitotic index were considered the outcome of interest. The potential confounders were identified as age, estrogen receptor status, progesterone receptor status, and HER2 status. Logistic regression was used to model the association between outcome and the main predictors, corrected for confounders. For this model, the outcomes of interest were categorized into binary variables—tumor size (≤ 41 mm and > 41 mm);

Figure 1 Immunohistochemical staining of C1QBP with (a) weak, (b) moderate, and (c) strong staining. Scale bar: 100 μ m. (d) Negative control of C1QBP immunohistochemistry was performed by omitting primary antibody. Images were taken at 400X magnification. (A color version of this figure is available in the online journal.)

grade (≤ 1 and > 1); associated DCIS grade (low and intermediate/high); associated DCIS extent (none and minimal/extensive); tubule formation scores (≤ 2 and 3); nuclear pleomorphism scores (1 and ≥ 2); mitotic scores (1 and ≥ 2). The steps in the model of logistic regression used were as previously described.¹⁶ For *in vitro* experimentation, statistical analyses for comparing two variables were done using the Student's *t*-test and one-way ANOVA was used for comparison of three or more groups. Two-way ANOVA was used for comparison of the growth curves. *P* value < 0.05 is considered as statistically significant.

Results

C1QBP-immunopositive staining ranged from 0% to 95% in all available samples. Only one sample was observed to have no immunopositive staining. The C1QBP-

immunostaining was mainly observed to be localized in the cytoplasm of the cancer cells and no staining was noted in the stroma. The vast majority of the epithelial cells had granular cytoplasmic staining with rare membrane accentuation although a distinct membrane staining was absent. The different intensities of cytoplasmic C1QBP staining in the breast cancer cells are depicted in Figure 1a-c. There was no staining seen in the negative control, reflecting the specificity of the staining (Figure 1d).

The immunohistochemical expression of C1QBP was represented by the WAI score. The mean WAI score of 1.5 was used as the cut-off point to stratify the C1QBP immunostaining into two groups. Univariate analysis using the chi-square test indicated that C1QBP was significantly associated with patients' age ($P=0.001$) and tumor size ($P=0.002$) (Table 1). Higher expression of C1QBP was observed to be associated with tumor size of more than 41 mm. The expression of C1QBP was not significantly associated with grade, tubule formation, mitotic index, estrogen and progesterone receptor status, associated DCIS extent and grade, pleomorphism, and c-erbB2 status.

After adjusting for potential confounders such as age, HER2 status, estrogen receptor status, and progesterone receptor status, C1QBP was found to be an independent predictor of tumor size in progesterone receptor-positive patients but not progesterone receptor-negative patients (Table 2). It was ascertained that having a WAI score of more than 1.5 in progesterone receptor-positive patients increased the risk of having a tumor size of more than 41 mm by 10.8 times.

As tumor size is a parameter in tumor proliferation, the correlation of C1QBP with PCNA, an established marker of cell proliferation, was determined. PCNA staining was observed in 5–100% of invasive ductal carcinoma sections (Figure 2). A significant correlation was observed between the expression of PCNA and C1QBP in the tissue samples (Pearson's $r=0.2863$; $P=0.0229$) (Figure 3).

The mRNA expression of C1QBP in ZR-75-1 breast cancer cell line was found to be lowest compared to MDA-MB-231 breast cancer cells and T47D cells which showed relatively higher expression of C1QBP

Table 1 Univariate analysis for C1QBP immunostaining with clinicopathological parameters

Clinicopathological parameters [#]	WAI ≤ 1.50 N (%)	WAI > 1.50 N (%)	P value
Age (years)			
≤56	48 (70%)	18 (30%)	0.001**
>56	29 (40%)	37 (60%)	
Histological grade			
1	6 (75%)	2 (25%)	0.312
2 and 3	67 (57%)	51 (43%)	
Nuclear pleomorphism			
1	4 (100%)	0 (0%)	0.086
2 and 3	69 (57%)	52 (43%)	
Tubule formation			
1 and 2	15 (45%)	18 (55%)	0.079
3	58 (63%)	34 (37%)	
Mitotic index			
1	14 (70%)	6 (30%)	0.251
2 and 3	59 (56%)	46 (44%)	
Associated DCIS extent			
None/minimal	57 (60%)	38 (40%)	0.494
Extensive	12 (52%)	11 (48%)	
Associated DCIS grade			
Low	2 (50%)	2 (50%)	0.676
Intermediate/High	49 (60%)	32 (40%)	
Tumor size			
≤41 mm	57 (68%)	27 (32%)	0.002**
>41 mm	18 (39%)	28 (61%)	
Estrogen receptor status			
Negative	25 (51%)	24 (49%)	0.200
Positive	50 (63%)	30 (38%)	
Progesterone receptor status			
Negative	34 (55%)	28 (45%)	0.465
Positive	41 (61%)	26 (39%)	
HER2 status			
Negative	54 (64%)	31 (36%)	0.085
Positive	21 (48%)	23 (52%)	

**Statistical significance at $P < 0.01$.

[#]Number of unavailable data for each parameter is reported in Supplementary Table 1.

Table 2 Multivariate analysis

Parameters	Odds ratio	Standard error	P value	95% CI
Tumor size				
PR-positive patients				
C1QBP	10.7919	0.6571	$< 0.001^{***}$	(2.9771, 39.1203)
PR-negative patients				
C1QBP	1.3572	0.5166	0.591	(0.4931, 3.7357)
Mitotic index				
ER	0.1342	0.7665	0.0088**	(0.0299, 0.6030)
HER2	3.3525	0.6595	0.0666	(0.9204, 12.2108)
Associated DCIS extent				
PR status	2.6690	0.3898	0.0118*	(1.2432, 5.7300)

* Statistical significance at $P < 0.05$.

**Statistical significance at $P < 0.01$.

***Statistical significance at $P < 0.001$.

Figure 2 Immunohistochemical staining of PCNA with (a) high and (b) low percentage of immunopositive cells. Scale bar: 100 μ m. (A color version of this figure is available in the online journal.)

Figure 3 Correlation between percentage of immunopositivity of C1QBP and PCNA. A significant correlation was observed with Pearson's $r = 0.2863$ ($*P = 0.0229$)

(Figure 4a). The C1QBP protein expression of the breast cancer cell lines are also demonstrated in Figure 4b. The proliferation status of each cell line was measured, with MDA-MB-231 breast cancer cells having the highest percentage of cell growth compared to ZR-75-1 breast cancer cells at 48 h post seeding (Figure 4c). However, since MDA-MB-231 breast cancer cells are known to be progesterone receptor-negative, further experiments were performed with progesterone receptor-positive T47D breast cancer cells.

Silencing of the *C1QBP* gene in T47D breast cancer cells with siRNA was achieved with a silencing efficiency of 85% and 81% at the mRNA and protein levels, respectively (Figure 5a and b). Knockdown of C1QBP expression in T47D breast cancer cell line significantly decreased cell proliferation after 96 h ($P < 0.01$; Figure 5c). In a separate experiment, a concomitant reduction of the growth rate was observed in the siC1QBP-transfected T47D breast cancer cells (Figure 5d).

Discussion

Previous reports have shown the expression of C1QBP in both *in situ* and invasive lobular and ductal carcinoma of the breast.^{14,17} Expression of C1QBP was noted to be higher in malignant tumors compared to their normal

counterparts.^{12,17} In this current study, protein expression of C1QBP in the breast cancer tissue samples was observed to be predominantly cytoplasmic, which is in agreement with published reports.¹⁷ The spotty cytoplasmic staining of C1QBP appears to be consistent with findings by Chen *et al.*, who described the staining as cytoplasmic brown particles.¹² C1QBP has been reported to be ectopically expressed at the cell membrane¹⁸ and such a phenomenon would have important implications for antibody targeted therapy.¹⁹ Furthermore, a recently developed antiangiogenic nanosystem, targeting C1QBP expressed on the cell surface of tumor cells in breast cancer, showed promising results in an orthotopic model of breast cancer.²⁰ However, no obvious membrane staining was detected in the present study, where aberrant and high expression of the C1QBP protein in the cytoplasm was found to be associated with age and tumor size. There was no correlation observed between grade, mitotic index, DCIS (grade and extent), c-erbB2 status, estrogen and progesterone receptor status, tubule formation and pleomorphism, and C1QBP.

Notably, multivariate analysis revealed that increased expression of C1QBP was an independent predictor of tumor size in progesterone receptor-positive tumors. C1QBP has been closely linked with metastasis and lymph node spread in breast cancer tissue samples but not breast cancer tumor size.^{12,14} Although Zhang *et al.* has reported a significant correlation between the expression of C1QBP and the TNM staging of breast cancer,¹⁴ the association of C1QBP with tumor size was not demonstrated. Cell proliferation, being one of the hallmarks of cancer,²¹ has been widely associated with tumor growth and progression. In addition, the efficacy of chemotherapeutics and radiotherapy is greatly influenced by proliferation. The treatment of breast cancer particularly of the luminal hormone receptor-positive subtype is largely based on the assessment of proliferation.²² Interestingly, a high rate of cell proliferation has also been associated with poor outcomes in male breast cancer.²²

We have also observed a correlation between the expression of C1QBP and PCNA, a proliferation marker that has been related to poor prognosis in breast cancer.²³ PCNA has been reported to be correlated with mitotic index and tumor size in breast cancer.²⁴ The cancer associated isoform of

Figure 4 (a) Relative *C1QBP* gene expression in ZR-75-1, T47D, and MDA-MB-231 breast cancer cell lines. Values are normalized to *GAPDH* and ZR-75-1 acted as the calibrator. Values are presented as mean $\pm$ SEM. * $P < 0.05$; **, $P < 0.01$. (b) C1QBP protein expression in ZR-75-1, T47D, and MDA-MB-231 breast cancer cell lines. Western blots of respective proteins and corresponding bar chart showing the relative optical densities of the protein bands. Values are presented as mean $\pm$ SEM. (c) Percentage of growth in breast cancer cell lines after 48 h incubation in RPMI supplemented with 10% FBS as compared to 0 h. Values are presented as mean $\pm$ SEM. * $P < 0.05$

PCNA has been reported to interact with DNA polymerase δ and involved in DNA replication in breast cancer.²⁵ In addition, phosphorylation of PCNA on tyrosine 211 has been associated with decreased overall survival in breast cancer²⁶ and binding of c-Abl to PCNA, which regulates cell growth.²⁷ The proliferation of breast cancer has also been measured by other markers, such as Ki-67, a protein expressed during the active stages of the cell cycle,^{28,29} which has been associated with tumor size.³⁰

For validation of the TMA study, silencing of the *C1QBP* gene in T47D cells, a progesterone receptor-positive breast cancer cell line, was performed. Decreased cell proliferation was demonstrated following siRNA-mediated silencing of the *C1QBP* gene, thereby verifying the results obtained from the immunohistochemical analysis. The *in vitro* results are also in accord with that reported by McGee *et al.* in MDA-MB-231 breast cancer cells.¹³ However, the molecular mechanism by which C1QBP modulates proliferation in breast cancer remains to be elucidated.

Down-regulation of C1QBP in PC3 prostate cancer cells induced growth suppression due to a delay of G1 to S phase cell cycle progression along with disruption of cyclin D-cdk2 activity.¹⁰ Stable reduction of C1QBP in A549 lung adenocarcinoma cells has been reported to deregulate growth factor-induced signal transduction by depleting phosphorylation of Erk, Akt, insulin receptor, epidermal growth factor receptor, and insulin-like growth factor receptor (IGFR).³¹ The *in vitro* results in the same study were further substantiated when mice that were subcutaneously implanted with shC1QBP-treated A549 lung adenocarcinoma cells produced significantly smaller tumors compared to mice implanted with control cells, indicating a reduced tumorigenic ability. In addition, another study showed that injection of melanoma cells overexpressing C1QBP into mice induced an increased tumor mass and growth rate along with activation of matrix metalloproteinase 2 (MMP 2) and enhanced expression of membrane type 1-MMP.³² In liver cancer, increased C1QBP expression in HepG2 cells has been reported to have higher cell survival and cell proliferation, caused by activation of MAP kinase and AKT-mediated pathway which subsequently, increased downstream effectors like Ras, β -catenin and cyclin D1.³³ Furthermore, C1QBP is known to bind to hyaluronic acid and upregulation of C1QBP in HepG2 cells has been observed to increase the level of hyaluronic acid synthase-2, resulting in secretion of hyaluronic acid, thereby, causing cable-like hyaluronan structures which may increase cell adhesion.³⁴ The interaction of hyaluronan with CD44 and RHAMM (receptor of hyaluronan-mediated motility) has also been reported to induce tumor cell growth and survival.^{34,35} Expression of hyaluronan is known to be an independent prognostic factor in breast cancer patients.³⁶ A high expression of hyaluronan in stromal myxoid changes was significantly associated with tumor grade, lymphatic embolizations, and increased mortality.^{34,37}

In conclusion, this study has shown that the C1QBP protein is an independent marker of proliferation in breast cancer, supporting the role of C1QBP in breast carcinogenesis. Previous reports have shown that promoters of proliferation are important prognostic indicators and therapeutic

Figure 5 (a) Relative gene expression of *C1QBP* in T47D breast cancer cell line 96 h after treatment with siRNA targeting *C1QBP*. The expression of *C1QBP* was reduced by 85%. Values are presented as mean $\pm$ SEM. *** $P < 0.001$ (b) Western blot representation of *C1QBP* protein expression and *C1QBP* protein expression in T47D breast cancer cell line 96 hours after knockdown with siRNA targeting *C1QBP*. The protein expression of *C1QBP* was significantly reduced by 81%. Values are presented as mean $\pm$ SEM. *** $P < 0.001$. (c) Knockdown of *C1QBP* significantly decreased cell proliferation in T47D cells. Values are presented as mean $\pm$ SEM. ** $P < 0.01$. (d) siRNA-mediated silencing of the *C1QBP* gene reduced cell growth in T47D cells over 168 h. Values are presented as mean $\pm$ SEM. $P = 0.077$ (marginal significance)

targets in breast cancer. For instance, cyclins A, D1, B1, and E overexpression in breast cancer have been associated with poor outcomes in patients.^{38,39} IGF-1R has been observed to be upregulated in basal-like and luminal-B tumors.⁴⁰ A combined treatment regime targeting IGF-1R and HER2 has been shown to successfully inhibit the growth of tumors.^{41–43} The regulation of IGFR by C1QBP which has been reported in lung adenocarcinoma potentiates C1QBP as an additional target to inhibit tumor growth.³¹ Hence, the potential of C1QBP as a molecular target for breast cancer therapy, especially in progesterone receptor-positive patients should be further explored.

Author contributions: All authors participated in the design, interpretation of the studies and analysis of the data and review of the manuscript; OJS and YY conducted the experiments, AAT and PHT supplied the breast cancer TMAs and the scoring of the TMAs, KM supplied and characterized the C1QBP antibody, AS analyzed the data from the TMAs, OJS wrote the manuscript, BHB conceptualized and designed the study together with PHT, GWY and GHB revised the manuscript.

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