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ORIGINAL ARTICLE

Influence of the C5a–C5a receptor system on breast cancer progression and patient prognosis

Takahisa Imamura[1] · Mutsuko Yamamoto-Ibusuki[2] · Aiko Sueta[2] · Tatsuko Kubo[1] · Atsushi Irie[3] · Ken Kikuchi[4] · Toru Kariu[5] · Hirotaka Iwase[2]

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Abstract

Background Emerging evidence has shown activation of the complement system in cancer tissues and anaphylatoxin C5a release from C5 by cancer cells, suggesting C5a as a component in the cancer microenvironment. We revealed aberrant expression of C5a receptor (C5aR) in various human cancers and C5a-elicited enhancement of C5aR-expressing cancer cell invasion.

Methods To explore an influence of the C5a–C5aR system in breast cancer (BC), we investigated BC C5aR expression in relation to clinicopathological parameters of the patients and an effect of C5a on BC cell proliferation.

Results BC cell C5aR expression was observed immunohistochemically in 22 of 171 patients (13 %) and related to larger tumor size, higher nuclear grade and Ki-67 labeling index, presence of lymph node metastasis and advanced clinical stages. Interestingly, BC cells were C5aR-negative in all patients with BC in situ and C5aR-positive rate was high (38 %) in patients with hormone receptor-negative, namely triple-negative BC. For BC cells in metastasized lymph nodes, 12 of 22 patients (55 %) were C5aR-positive and included 7 patients with C5aR-negative BC in the primary site. Survival rate of patients with C5aR-positive BC was lower than that of patients with C5aR-negative BC. C5a enhanced proliferation of C5aR-expressing triple-negative BC cells in a C5aR-dependent manner.

Conclusion Relation of BC C5aR expression to tumor development and poor prognosis of the patients and proliferation enhancing effect of C5a on C5aR-expressing BC cells suggest that the C5a–C5aR system is closely associated with BC progression. This system may be a new target to treat BC patients, particularly with triple-negative BC.

Keywords C5a · C5a receptor · Prognosis · Lymph node metastasis · Proliferation

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Abbreviations

BC	Breast cancer
C5	The fifth complement component
C5aR	C5a-receptor
TNBC	Triple-negative breast cancer
RFS	Relapse-free survival
DRFS	Distant relapse-free survival
BCSS	Breast cancer-specific survival

Introduction

Breast cancer (BC) is the most common female cancer, accounting for 29 % of all new cancer patients, and ranks first among women aged 20–59 years as cause of cancer death in the United States [1]. It is proposed that BC is a

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two-component mixture of two main cell types of origin [2]; basal-like BCs are initiated within the basal/myoepithelial cell compartment and human epidermal growth factor receptor 2 (HER2)-enriched and luminal intrinsic molecular subtypes are initiated from the luminal cell compartment. The former subtype is defined by triple-negativity of immunohistochemical staining for estrogen and/or progesterone receptors and HER2, and represents 10–20 % of all BCs [2–5]. Compared with other subtypes, triple-negative BC (TNBC) has a more aggressive clinical course with a high incidence of distant recurrence and death within 5 years from diagnosis despite adjuvant chemotherapy has been performed [6]. Due to benefit of chemotherapy and newer biologic and targeted therapies, mortality of BC patients has declined; however, incidence trends to increase and death rate is still placed at the second highest among the rates of female cancers [1]. Thus, further development of treatments is needed for patients with BC.

Anaphylatoxin C5a is a fragment of the fifth complement component (C5) and is released by C5-convertase formed in the process of complement activation [7]. Through binding to C5a receptor (C5aR), C5a acts as a leukocyte chemoattractant and inflammatory mediator [8, 9]. There is emerging evidence that the complement system is activated in cancer tissues in human specimens [10, 11] and animal models [12, 13]. Moreover, cancer cells can release C5a from C5 by their own proteases on the cell membrane [13, 14]. C5a is likely a component in the cancer microenvironment. We previously showed aberrant expression of C5aR in various human cancers and C5a-elicited enhancement of C5aR-expressing cancer cell invasion [15]. In addition to this direct effect on cancer cells C5a recruits myeloid-derived suppressor cells for anti-tumor $CD8^+$ T cells, indirectly fostering cancer cells [12]. The presence of C5a in cancer tissues and cancer promoting activity of C5a suggest that C5aR-expressing cancer cells are potentially aggressive, leading to unfavorable prognosis of the patients. Accordingly, C5aR expressed on cancer cells may be predictive of poor outcomes of the patients, furthermore become a therapeutic target.

To study the C5a–C5aR system in BC, we investigated relation of BC C5aR expression to clinicopathological parameters of the patients and an effect of C5a on BC cell proliferation.

Materials and methods

Patients and breast specimens

Breast tumor specimens from 171 female patients with invasive breast carcinoma, who were treated at Kumamoto University Hospital from 2001 to 2009, were included. These patients were from a consecutive series and patients with other malignancies or bilateral BC were excluded. We performed adjuvant treatment and neoadjuvant treatment for patients according to the recommendations of the St. Gallen international expert consensus on the primary therapy of early BC. We evaluated patient disease risk by tumor biology (expression of ER, PgR, and HER2) and clinical stage for the treatments. After operation, patients were followed every 3 months. The median follow-up period was 66 months (range 15–144 months). In addition, 31 BC in situ was investigated but not included in the total BC number. Written permission for using cancer and adjacent noncancerous tissues and clinicopathological records was obtained from all the patients. Ethics committee of Kumamoto University Graduate School of Medical Sciences approved the use of the samples and records for the present study.

Immunohistochemistry

Immunohistochemical analysis was performed by the methods described previously [15, 16]. Deparaffinized 3-µm-thick tissue sections were pretreated with 0.3 % H_2O_2 in methanol for 20 min, followed by Protein Block, Serum-Free (Dako Cytomation) treatment, for 20 min. To match cells, mirror sections were prepared for immunostaining for C5aR and estrogen receptor, and for C5aR and HER2. Sections were incubated with the primary mouse antibody against human C5aR (2 µg/ml; Hycult Biotechnology) or nonspecific mouse IgG (2 µg/ml; Dako Cytomation) at room temperature for an hour, followed by staining using EnVision+ solution (Dako Cytomation) and 3,3′-diaminobenzidine tetrahydrochloride solution containing 0.006 % H_2O_2, according to the manufacturer's instructions. Nuclei were counterstained with hematoxylin. Mouse monoclonal antibodies against ERα (SP1, Ventana Japan, Tokyo, Japan), progesterone receptor (PgR) (1E2, Ventana Japan) and Ki-67 (MIB1, Dako Japan, Tokyo, Japan), and a polyclonal antibody against HER2 (Dako Japan, 1:200) were used for immunostaining against respective antigens. Staining was carried out in the NexES IHC Immunostainer (Ventana Medical Systems, Tucson, AZ, USA), according to the manufacturer's instructions. ER and PgR status were evaluated based on the percentage of stained nuclei and nuclear staining of ≥ 1 % was considered positive. HER2 was evaluated by the HercepTest method (Dako), scoring cell membrane staining from 0 to 3+. Tumors with scores of 3+ or with a ≥ 2.2-fold increase in HER2 gene amplification determined by fluorescence in situ hybridization were considered to be positive for HER2 overexpression. Ki-67 labeling index was determined by the percentage of nuclear-stained cells out of all

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cancer cells along the invasive front of the tumor in 400× high-power fields and stained ratio ≥20 % was considered to be high expression.

Quantitative real-time polymerase chain reaction (qRT-PCR)

BC cell lines MDA-MB-231, MDA-MB-453, MDA-MB-468, MCF-7 and T47D were provided by Dr. Masahiro Nakano, Department of Molecular Genetics, Kumamoto University. For quantitative analysis of the C5aR transcripts by quantitative real-time PCR, RNA was extracted from cells using ISOGEN II reagent (NIPPON GENE, Tokyo, Japan). Removal of genomic DNA and synthesis of 1st strand cDNA was carried out using PrimeScript™ RT reagent Kit with gDNA Eraser (Takara Bio Inc., Otsu, Shiga, Japan). The expression of C5aR gene was quantitated by ViiA™ 7 Real-Time PCR System (Thermo Fisher Scientific Inc., Waltham, MA, USA) with KAPA SYBR Fast qPCR Kit (KAPA biosystems, Wilmington, MA, USA) and primers for *β-actin*, 5′-AGCACTGTGTTGGCG TACAG-3′ and 5′-AGCACTGTGTTGGCGTACAG-3′ and for C5aR gene, 5′-AAGCTGGACTCCCTGTGTGT-3′ and 5′-GCGTGAATGACTTGCTCTCC-3′. C5aR transcripts were normalized to human *β-actin* levels by $2^{-\Delta\Delta CT}$ method [17].

Flow cytometry

MDA-MB-468 cells of basal subtype BC [18] were treated for 30 min with a murine monoclonal FITC-conjugated anti-C5aR antibody (Serotec Ltd., Oxford, UK) or a FITC-conjugated isotype matched control antibody (Serotec Ltd.), followed by washing with PBS twice. C5aR antigen was quantified with FACScan (BD Biosciences).

Cancer cell proliferation assay

Cell proliferation promoting activity was determined by measuring cell increase using Cell Counting Kit-8 (Dojindo Laboratories, Kumamoto, Japan). MDA-MB-468 cells were seeded in RPMI-1640 containing 10 % FBS in a 96-well plate and cultured at 37 °C for 24 h. After washing with PBS, cells (5×10^3 cells/100 µl) were cultured in a well for 24 h in FBS-free RPMI-1640 supplemented with C5a in the presence or absence of C5aR antagonist W-54011 (1 µM) (Calbiochem, San Diego, CA, USA). Then, WST-8 reagent solution (10 µl) was added to each well, followed by incubation for 2 h at 37 °C. Absorbance at 450 nm in a well was measured with an EMax Precision Microplate Reader (Molecular Devices Japan, Tokyo, Japan). Values were expressed as averages ± SD in triplicate assay.

Statistical analyses

Chi-square (χ^2) test was used for analysis of relation of BC C5aR expression to clinicopathological parameters except patients' age and pathological tumor size which were analyzed using unpaired Student's t test. Relapse-free survival (RFS), distant relapse-free survival (DRFS) and BC-specific survival (BCSS) curves were calculated according to the Kaplan–Meier method and verified by the log-rank test. Cox proportional hazards model was used to calculate the hazard ratio, 95 % confidential interval and P value for univariate and multivariate analyses. The effect of C5a on cancer cell proliferation was analyzed using unpaired Student's t test. JMP software version 10.0.0 for Windows (SAS institute Japan, Tokyo, Japan) was used for all statistical analyses. All statistical significance was defined as $P < 0.05$.

Results

C5aR expression of BC cells

First, cancer tissues from primary sites were examined for C5aR expression. Cancer cells were found to express C5aR in 22 patients among 171 BC patients. This C5aR-positive ratio 12.9 % was similar to the ratio obtained previously for a smaller BC number of patients (14.3 %, 3 for 21) and the lowest among the ratios of cancers from various organs examined [15]. C5aR was predominantly present on the BC cell membrane (Fig. 1a, c, e), which was consistent with C5aR localization on the cell surface of other cancer cells [15]. In some patients tumor tissues included C5aR-positive cancer cells expressing together estrogen receptor (Fig. 1a, b) or HER2 (Fig. 1c, d). Interestingly, cancer cell C5aR expression was found in some TNBC cases (Fig. 1e). Noncancerous mammary gland cells and adjacent cells did not express C5aR (Fig. 1f). These results indicated that malignant transformation of mammary gland cells is involved but not sufficient for C5aR expression.

Relation of BC C5aR expression to clinicopathological parameters

Next, BC cell C5aR expression was investigated in association with clinicopathological parameters of the patients. Tumor size of C5aR-positive BC was larger than that of C5aR-negative BC. BC C5aR expression ratio was significantly high in high nuclear grade and Ki-67 labeling index, and in patients with lymph node metastasis and at advanced clinical stages (Table 1). The analysis by Wilcoxon method revealed that median Ki-67 labeling index of C5aR-positive or -negative BC cells were 45 and 22 %,

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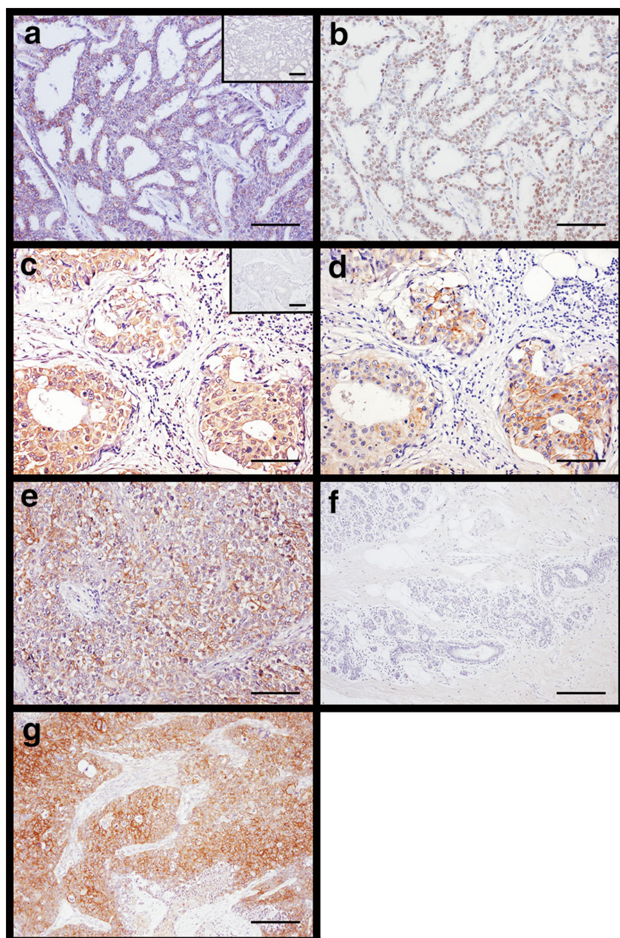

Fig. 1 Immunohistochemical staining of primary BC tissues for C5aR (**a**, **c**, **e**, **f**), estrogen receptor (**b**), HER2 (**d**), or control IgG (**a** *inset*, **c** *inset*). Immunohistochemical staining of BC-metastasized lymph nodes for C5aR (**g**). *Scale bars* 100 μm for **a**–**e** and 200 μm for **f** and **g**

respectively. The result that 31 cases of ductal carcinoma in situ were all C5aR-negative suggested connection of C5aR expression to BC invasion. C5aR-positive BC patient ratio was significantly high in HER2-positive patients, in contrast, was low in hormone receptor-positive patients, which agreed with low C5aR-positive ratio in estrogen-receptor-positive/HER2-negative BC subtype. It should be noted that TNBC patients showed a considerably high C5aR-positive ratio (37.5 %) and C5aR-positive TNBC patients accounted for 27.3 % of whole C5aR-positive patients. These results indicated relation of C5aR expression to progression of BC, particularly hormone receptor-negative cancer subtypes.

Relation of BC C5aR expression to lymph node metastasis

The result that lymph node metastasis ratio of C5aR-positive BC patients (26.7 %: 12/45) was higher than C5aR-negative BC patients (9.6 %: 10/104) (Table 1) may show high metastatic potential of C5aR-positive BC cells. Thus, for 22 lymph node metastasis-positive patients, C5aR expression of BC cells in metastasized lymph nodes was investigated. BC cells in metastasized lymph nodes expressed C5aR (Fig. 1g). C5aR was positive in all 5 patients with C5aR-positive cells and 7 of 17 patients with C5aR-negative cells in the primary sites (Table 2). A high C5aR-positive BC ratio (55 %: 12/22) in metastasized lymph nodes suggested a relationship between BC C5aR expression and metastasis promotion. Statistical analysis revealed a significant lymph node metastasis increase of C5aR-positive BC with 2.4-fold high risk in comparison with C5aR-negative BC.

Relation of BC C5aR expression to patients' survival

The relation of C5aR expression with BC progression and metastasis (Tables 1, 2) may suggest poor prognosis of the patients, thus survival rate was compared between patients with C5aR-positive and -negative BC. Survival rates of C5aR-positive BC patients were significantly low in the three parameters in comparison with those of C5aR-negative patients (Fig. 2). By univariate analysis, both relapse-free survival (Table 3) and breast cancer-specific survival (Table 4) of patients with C5aR-positive BC were significantly shorter than patients with C5aR-negative BC, but these survival parameters were not significant by multivariate analysis. Low survival rates of C5aR-positive BC patients indicated that BC C5aR expression was a possible prognostic marker for poor outcome of the patients.

Effect of C5a on BC cell proliferation

Larger tumor size and higher tumor cell Ki-67 labeling index of C5aR-positive BC patients in comparison with C5aR-negative BC patients (Table 1) suggested an enhancing effect of C5a on BC cell proliferation. To explore this effect, we first examined C5aR mRNA expression of BC cell lines by qRT-PCR. C5aR mRNA expression was observed in all the five cell lines, and MDA-MB-468 and T47D cells expressed C5aR mRNA at higher levels (Fig. 3a). C5aR protein expression on the cell membrane was clearly shown in MDA-MB-468 cells of basal subtype BC [18] by flow cytometry (Fig. 3b). MFC7 cells also expressed C5aR but C5aR expression was not clear for MB-231, MB-453 and T47D cells. MDA-MB-468 cells increased in the presence of C5a in a dose-dependent manner, which was completely inhibited by C5aR antagonist W-54011 (Fig. 3c). It is likely that C5a enhanced BC cell proliferation via C5aR.

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Table 1 Relation of BC C5aR expression to clinicopathological parameters

Parameter	Patient number	C5aR expression		P
		(+)	(−)	
Age (years)		57.8 ± 2.72	59.7 ± 1.07	0.52
Menopause				
Pre-	41	6	35	0.7
Post-	130	16	114	
Pathological tumor size (cm)		3.90 ± 0.47	2.19 ± 0.17	0.001
Nuclear grade				
1	88	6	82	0.011[b]
2	40	5	35	
3	41	11	30	
Ki-67 labeling index				
<20 %	70	5	65	0.0038
≥20 %	95	17	78	
Nodular status				
–	149	45	104	0.028
+	22	12	10	
Clinical stages				
0[a]	31	0	31	
I	88	6	82	0.011[b]
II	40	5	35	
III	41	11	30	
Estrogen receptor (ER)				
–	34	10	24	0.026
+	135	12	123	
Progesterone receptor				
–	52	11	41	0.038
+	119	11	108	
HRE2				
–	141	9	132	0.0014
+	30	13	17	
Tumor subtype				
ER(+) + HER2(−)	118	7	111	<0.0001[c]
Any ER + HER2(+)	25	9	16	
Triple negative	22	6	16	

[a] Ductal carcinoma in situ, not included in the total BC patient number

[b] 0 vs. 1 + 2 + 3

[c] ER(+) + HER2(−) vs. any ER + HER2(+) + triple negative

Discussion

Breast cancer tumor diameter, histologic grade, presence of lymphovascular invasion, lymph node status, hormone receptor status and HER2 status are used to predict patients' outcomes and select appropriate systemic therapy for the patients [19]. Estrogen-focused therapies are major treatments for the receptor-positive BC patients; however, in locally recurrent or metastatic tumors, up to 50 % of the patients become resistant to first-line endocrine treatment despite an initial response [20]. HER2-targeted therapy is another option to treat BC patients, but about 70 % of patients with metastatic BC possess intrinsic resistance and nearly all become resistant to the therapy after initial responsiveness [21, 22]. To date there are no established first-line therapies specific for patients with TNBC. To develop BC treatments further discovery of new target molecules is an urgent problem. Here, we showed that survival rates of C5aR-positive BC patients were lower than those of C5aR-negative BC patients in all the three parameters (Fig. 2a–c), suggesting that C5aR expression is predictive of poor outcome of the patients. Poor prognosis

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Table 2 C5aR expression of BC cells in metastasized lymph nodes

C5aR expression	Primary site		$P^{\#}$
	(+)	(−)	
Lymph node			
(+)	5	7	0.040
(−)	0	10	
Total	5	17	
C5aR(+) risk	1	0.412	
Risk difference	0.588 (95 % CI 0.354–0.822)		
Risk ratio	2.4 (95 % CI 1.4–4.3)		

Lymph node metastasis cases were investigated for BC C5aR expression in lymph nodes and respective primary sites. (+) and (−) indicate C5aR expression positive and negative, respectively

[#] 2-sided Fisher's exact test

of patients with C5aR-positive BC agrees with significant relation of BC C5aR expression to large tumor size, high nuclear grades and Ki-67 labeling index, presence of lymph node metastasis and advanced clinical stages (Table 1). Relation of C5aR expression to poor prognosis of patients was recently reported regarding non-small cell lung cancer [23], nasopharyngeal carcinoma [24], urothelial carcinoma [25], ovarian cancer and lung squamous cell carcinoma [26]. This relation is possibly attributed to C5aR-mediated enhancement of cancer proliferation (Fig. 3b) and invasion [15] by C5a that is generated in the cancer microenvironment through activation of the complement system [10–13] and cancer-associated proteolysis of C5 [14].

C5aR is a seven transmembrane G protein-coupled receptor [27] like as chemokine receptor CXCR4 that is integral in actin polymerization and pseudopodia formation of BC cells [28]. Binding of the ligand CXCL12 to CXCR4 causes cell trafficking, migration, and proliferation, which are also induced by C5a via C5aR in bile duct cancer cells [15] and BC cells (Fig. 3). Signals via CXCR4 in human embryonic kidney cells (HEK-293) [29] and chondrosarcoma cells [30] and via C5aR in kidney cancer cells [31] activate the ERK pathway. CXCR4 is associated with BC cell invasion and lymph node metastasis [32] through PI3-kinase pathway [33]. This signaling pathway plays an important role in epithelial–mesenchymal transition [34], a crucial step in cancer dissemination, and is used for promotion of kidney cancer cell invasion by the C5a–C5aR axis [31]. Both receptors are involved in increased MMP expression of tumor cells [15, 30]. Thus, it is likely that signals via C5aR and CXCR4 induce similar cell activities. High CXCR4 expression was reported to correlate with nodal metastasis of human BC [35, 36]. We revealed a high lymph node metastasis rate of C5aR-positive BC (21 %) (Table 1) and a high C5aR-positive BC rate in

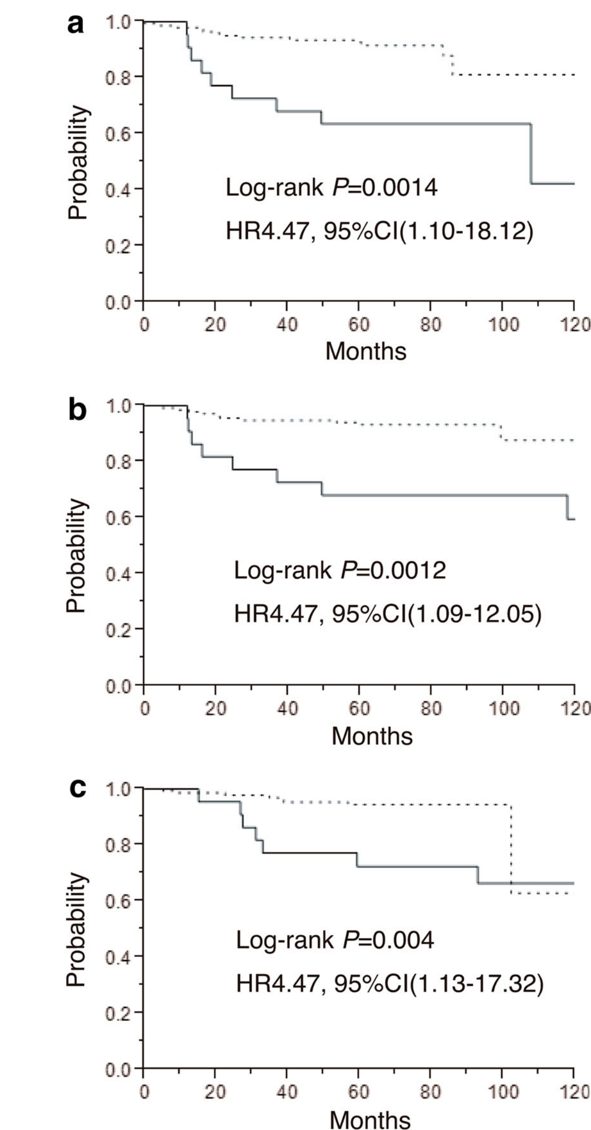

Fig. 2 Survival rates of patients with C5aR-positive BC ($n = 22$) (*solid line*) and C5aR-negative BC ($n = 149$) (*dashed line*). **a** Relapse-free survival, **b** distant relapse-free survival and **c** BC-specific survival

metastasized lymph nodes (55 %) (Fig. 1g; Table 2). Considering similar effects of signaling via C5aR and CXCR4 on cancer cells, it is probable that BC lymph node metastasis relates to expression of C5aR as CXCR4. Indeed, C5aR-positive rate was high in metastatic kidney cancer (96.7 %) in comparison with non-metastatic kidney cancer (50.5 %) [31]. The result that BC cells in metastasized lymph nodes were C5aR-positive in 7 of 17 patients with C5aR-negative BC in the primary sites suggests conversion of C5aR-negative cells to positive cells, which may facilitate lymph node metastasis of the cells. Taken together cancer cell C5aR expression presumably contributes to lymph node metastasis promotion.

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Table 3 Univariate and multivariate analyses for relapse-free survival

Variables	Reference	Univariate analysis				Multivariate analysis			
		P	HR	95 % CI		*P*	HR	95 % CI	
				Lower	Upper			Lower	Upper
Menopause									
Pre vs. post	Pre	0.85	0.91	0.38	2.55				
Tumor size									
≦2 vs. >2 cm	≦2 cm	0.35	1.51	0.64	3.78				
Nodal status									
Posi. vs. nega.	Nega.	0.44	1.39	0.58	3.28				
Nuclear grade									
1 vs. 2, 3	1	0.011	3.32	1.33	8.36	0.78	1.18	0.33	3.93
ERα									
Posi. vs. nega.	Posi.	<0.0001	5.66	2.44	13.42	0.0091	4.13	1.41	12.9
PgR									
Posi. vs. nega.	Posi.	0.015	2.86	1.23	6.82				
HER2									
Posi. vs. nega.	Nega.	0.54	0.69	0.16	2.06				
Ki-67									
≦20 vs. >20 %	≦20 %	0.015	3.33	1.24	11.53	0.79	1.19	0.32	4.92
C5aR									
Posi. vs. nega.	Posi.	0.0048	3.95	1.56	9.48	0.15	2.09	0.76	5.51

P P value, *HR* hazard ratio, *CI* confidential interval, *ERα* estrogen receptor α, *PgR* progesterone receptor, *posi.* positive, *nega.* negative

Table 4 Univariate and multivariate analyses for breast cancer-specific survival

Variables	Reference	Univariate analysis				Multivariate analysis			
		P	HR	95 % CI		*P*	HR	95 % CI	
				Lower	Upper			Lower	Upper
Menopause									
Pre vs. post	Pre	0.68	0.77	0.17	2.44				
Tumor size									
≦2 vs. >2 cm	≦2 cm	0.15	2.21	0.75	7.96				
Nodal status									
Posi. vs. nega.	Nega.	0.58	1.33	0.45	3.81				
Nuclear grade									
1 vs. 2, 3	1	0.11	2.76	0.82	12.45				
ERα									
Posi. vs. nega.	Posi.	<0.0001	10.41	3.51	37.83	0.0022	6.15	1.87	25.81
PgR									
Posi. vs. nega.	Posi.	0.0012	5.86	1.98	21.29				
HER2									
Posi. vs. nega.	Nega.	0.14	0.28	0.016	1.43				
Ki-67									
≦20 vs. >20 %	≦20 %	0.0033	9.25	1.85	167.74	0.25	3.22	0.47	63.44
C5aR									
Posi. vs. nega.	Posi.	0.011	4.39	1.44	12.81	0.18	2.15	0.71	6.44

P P value, *HR* hazard ratio, *CI* confidential interval, *ERα* estrogen receptor α, *PgR* progesterone receptor, *posi.* positive, *nega.* negative

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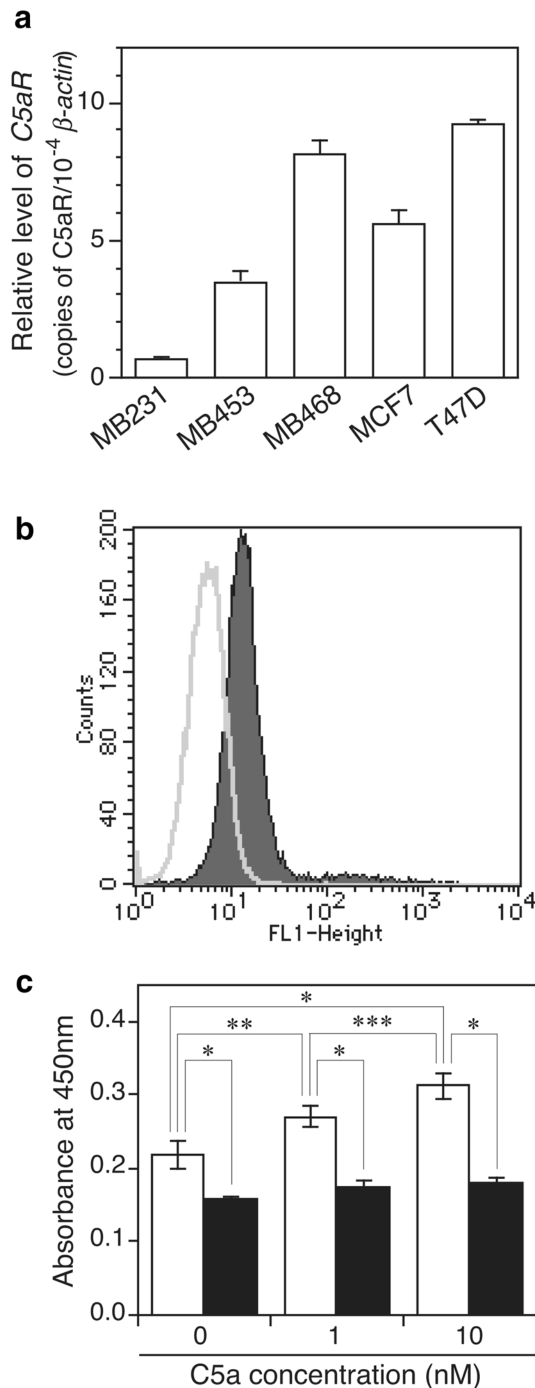

Fig. 3 Effect of C5a on BC cell proliferation. **a** C5aR mRNA levels of BC cell lines assessed by qRT-PCR. Levels relative to β-actin mRNA levels were shown and values were expressed the average ± SD ($n = 3$). **b** Flow cytometry of MDA-MB-468 cells using a murine monoclonal FITC-conjugated anti-C5aR antibody (*solid line*) or a FITC-conjugated isotype matched control antibody (*faint line*). **b** Cell proliferation assay. *Closed* and *open columns* indicate experiments performed in the presence or absence of C5aR antagonist W-54011 (1 μM). C5a concentration 0 indicates addition of PBS (C5a solvent) instead of C5a solution. $*P < 0.005$, $**P = 0.01$, $***P = 0.05$

The relation of BC C5aR expression to poor outcomes of patients (Fig. 2) together with cancer promoting activities of C5a (Fig. 3c) [15] indicates that the C5a–C5aR system is potentially a new target of BC therapy. In a cancer-bearing mouse model administration of CXCR4 antagonists reduced BC growth, invasion and metastasis and expression of vascular endothelial growth factor (VEGF) [37–39]. In addition, interfering the CXCL12/CXCR4 signaling with antagonist decreased infiltration of myeloid-derived CD11b-positive cells [37, 38] that induce anti-tumor $CD8^+$ T cell tolerance [40]. Taking account of signaling similarity between CXCR4 and C5aR, effective cancer control by CXCR4 antagonists suggests applicability of agents targeting C5a/C5aR signaling to cancer therapy. Certainly, like antibodies against C5a or C5aR that suppressed cancer cell invasion enhancement by C5a [14, 15], C5aR antagonist prevented C5a-elicited increase of BC cell proliferation (Fig. 3b). Furthermore, blocking of the C5a–C5aR signaling may hinder recruitment of tumor-associated macrophages (TAMs), myeloid-derived CD11b-positive cells and angiogenesis-mediating neutrophils [40]; all of these cancer-supporting leukocytes express C5aR [8, 12, 41], thereby inhibiting formation of the cancer-favorable microenvironment.

There are some limitations to our study. Data from patients who had heterogenous therapies were analyzed, which may affect prognostic evaluation for C5aR. Because the survey consisted of a homogenous ethnic group and in a relatively small patient number, the generalizability of the present results is potentially limited.

Conclusions

The present study provides proof-of-concept that C5aR expression is a prognostic marker candidate of BC patients and the C5a–C5aR system may be a feasible target for treating C5aR-positive BC patients. A high C5aR-positive patient rate of TNBC (Fig. 1e; Table 1) suggests that C5a/C5aR-targeted therapy can be applicable to treatments for TNBC patients at a high frequency, possibly improving prognosis of the patients.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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