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RESEARCH ARTICLE

High fibroblast growth factor 19 (FGF19) expression predicts worse prognosis in invasive ductal carcinoma of breast

Abdelbaset Buhmeida · Ashraf Dallol · Adnan Merdad · Jaudah Al-Maghrabi · Mamdooh A. Gari · Muhammad M. Abu-Elmagd · Adeel G. Chaudhary · Adel M. Abuzenadah · Taoufik Nedjadi · Eramah Ermiah · Fatima Al-Thubaity · Mohammed H. Al-Qahtani

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Abstract Metabolic diseases like diabetes and obesity are major risk factors for breast cancer. Aberrant expression of metabolic effectors such as fibroblast growth factor 19 (FGF19) could be therefore associated with the disease. The expression of FGF19 was examined in 193 archival breast tumor samples by immunohistochemistry and evaluated semi-quantitatively by determining the staining index and correlating it with clinicopathological parameters using Fisher's exact test. The correlation between FGF19 expression and 5-year disease-specific survival rate was determined using the univariate Kaplan–Meier analysis. The prognostic value of FGF19 expression was evaluated using the multivariate Cox regression analysis. Of the 193 tumors analyzed, 40 % were classified with low FGF19 expression, whereas 60 % were categorized as tumors with high FGF19 expression. There was a highly significant correlation between high FGF19 expression and patients' age ($p=0.008$) as well as 5-year disease-specific survival ($p=0.001$). However, FGF19 expression did not show any significant correlations with other clinicopathological parameters, including hormonal status, tumor grade, tumor size, or lymph node status. Univariate Kaplan–Meier log rank analysis showed that patients with high FGF19 expression exhibited a significantly shorter disease-specific 5-year survival ($p=0.007$). This effect was exacerbated by lymph node metastasis ($p=0.001$), negative estrogen receptor (ER) status ($p=0.002$), or old age ($p=0.013$). Multivariate analysis showed that high FGF19 expression could be an independent prognostic marker of disease-specific survival in breast cancer patients ($p=0.030$). Quantification of FGF19 expression appears to provide valuable prognostic information in breast cancer, particularly in older patients with lymph node metastasis and negative ER status.

Keywords FGF19 expression · Breast cancer · Lymph node positive · IHC · Prognosis · Targeted therapy

A. Buhmeida · A. Dallol (✉) · M. A. Gari · M. M. Abu-Elmagd · A. G. Chaudhary · A. M. Abuzenadah · M. H. Al-Qahtani
Center of Excellence in Genomic Medicine Research, King Abdulaziz University, P.O. Box 80216, Jeddah 21589, Kingdom of Saudi Arabia
e-mail: adallol@kau.edu.sa

A. Dallol · A. M. Abuzenadah
KACST Technology Innovation Center in Personalized Medicine, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia

A. Merdad · F. Al-Thubaity
Department of Surgery, Faculty of Medicine, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia

J. Al-Maghrabi
Department of Pathology, Faculty of Medicine, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia

M. A. Gari · A. G. Chaudhary · A. M. Abuzenadah · M. H. Al-Qahtani
Faculty of Applied Medical Sciences, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia

T. Nedjadi
King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia

E. Ermiah
Department of Oncology, National Cancer Institute, Sabratha, Libya

M. M. Abu-Elmagd
Zoology Department, Faculty of Science, Minia University, Minia, Egypt

Introduction

Breast cancer (BC) is a leading cause of cancer-related death worldwide and is particularly spreading at an alarming rate in

developing countries [1]. Many BC patients from these countries exhibit a young age of onset and have advanced-stage disease when presented to the clinic. They also have elevated mortality rate associated with early disease recurrence, despite advances in BC management [2–5]. Therefore, it is important to secure good cancer control through two different strategies, first by improvements in early detection and second to find prognostic factors, which applied with traditional factors to predict the outcome of the individual patient and allow selection of appropriate therapy [6]. Hence, the patient's outcome of BC disease in terms of local recurrence, distant metastases and progression, is the major focus of several studies aimed to identifying the most reliable prognostic factors [7–9].

At present, prognosis is predicated on the basis of clinicopathological parameters such as histological type, tumor size, lymph node status, tumor stage, and nuclear grade. In fact, these are powerful independent prognosticators [10–12] but may be only crude measures of the biological behavior of a tumor. Moreover, some of these parameters may be influenced by the subjectivity of the pathologist and, consequently, limited in their prognostic value [13, 14]. Thus, it is valuable to find other prognostic markers, which can be measured reliably to support these traditional factors, and can then be used to help in evaluating patient's risks and selection of treatment [15, 16].

Fibroblast growth factor 19 (FGF19) and its murine ortholog (FGF15) are members of the FGF19 subfamily which also includes FGF21 and FGF23. Unlike other FGFs, members of the FGF19 subfamily function as endocrine factors or hormones regulating various cellular processes such as regulation of glucose, lipid, and vitamin D metabolisms as well as bile acid synthesis [17, 18]. Several studies have shown that both FGF19 mRNA and protein are widely distributed in human tissues where they play an important role in cell proliferation, differentiation, and motility [18–20]. In disease state, high levels of FGF19 have been associated with poor outcome in prostate cancer [21] and hepatocellular carcinoma patients [22, 23]. FGF19 is located at chromosomal region 11q13, a region known to be amplified in breast cancer [24]. Furthermore, Nicholes et al. [25] have shown that ectopic expression of FGF19 in transgenic mice led to the development of liver carcinoma suggesting that targeting FGF19 could be of therapeutic value [26]. The functional activity of FGF19 in BC has, nevertheless, remained unidentified.

Mechanistically, it has been reported that effects of FGF19 are mediated through FGFR4 and are associated with the activation of a number of downstream signaling pathways including mitogen-activated protein kinase (MAPK) and beta-catenin pathways [27, 28]. Interestingly, recent findings revealed that FGFR4 requires a glycoprotein named KLOTHO which acts as a co-receptor to potentiate the binding of the FGF19 to the corresponding FGFR4 receptor [29–31]. It has been demonstrated that KLOTHO expression appears to control the action of several fibroblast growth factors, mainly FGF19 and FGF23 [32, 33].

In this study, we report the analysis of FGF19 expression in a cohort of 193 patients of BC. To do so, we carried out an immunohistochemical staining using FGF19 antibody in primary breast tumors. We, in addition, evaluated the correlation of FGF19 expression with patients' clinicopathological variables including survival outcome as part of our systematic search for prognostic markers in breast cancer.

Patients and methods

The study was performed on female BC patients, diagnosed with invasive ductal carcinoma, at the Department of Pathology, King Abdulaziz University Hospital, Jeddah, Saudi Arabia and the National Oncology Institute, Sabratha, Libya between 2000 and 2009. Sample collection procedures followed were in accordance with the local ethical guidelines. The pertinent clinicopathological features (age, menopausal status, stage, grade, and lymph node status) and the follow-up and survival data were collected from patient's files and summarized in Table 1. The mean age at the time of diagnosis was 48 years (range, 26–94 years).

Treatment and follow-up

The patients were seen at 3–6-month intervals until death or end of follow-up (FU) which was mid-August 2009. The mean FU time for the whole series was 47 months (range, 1–125 month). During the FU period, 46 (24 %) patients developed recurrence and 36 (19 %) patients died of disease. Five-year disease-specific survival (DSS) and disease-free survival (DFS) were calculated as the time from diagnosis to death (due to disease) or to the date last seen alive within 60 months following diagnosis, and time from diagnosis to the appearance of recurrent disease or date last seen disease-free within 60 months following diagnosis, respectively. In calculating DSS, patients who died of other or unknown causes were excluded. Those cases were included in univariate Kaplan–Meier analysis but censured. During the FU, patients were subjected to clinical examination every 6–12 months, and bone isotope scan, chest, and abdominal-pelvic CAT scan were performed whenever needed. In most instances, the causes of death were obvious on clinical grounds alone. Autopsy was not performed in any case. Almost all patients were subjected to surgery in form of lumpectomy, radical or modified radical mastectomy with axillary node clearance. Post-operative early adjuvant systemic therapy in the form of chemotherapy, radiotherapy, and hormonal therapy was given inclusively to patients, respectively. Most patients with known treatment history have received all forms of adjuvant therapy (48.9 %, $n=133$). Thirty two patients (24 %, $n=133$)

Table 1 Clinicopathological characteristics of the BC cases included in this study

Clinicopathological features	Number of patients (%)
Age	
<50	104 (54 %)
≥50	89 (46 %)
Hormonal status (ER)	
Positive	98 (51 %)
Negative	45 (23 %)
Unknown	50 (26 %)
Hormonal Status (PR)	
Positive	72 (38 %)
Negative	51 (26 %)
Unknown	70 (36 %)
Histological type	
IDC	167 (87 %)
Others	26 (13 %)
Tumor size	
<2 cm	28 (14 %)
2–5 cm	88 (46 %)
>5 cm	53 (28 %)
Unknown	24 (12 %)
Lymph node status	
N0	57 (30 %)
N1	136 (70 %)
Metastasis	
M0	112 (58 %)
M1	14 (7 %)
MX	67 (35 %)
Stage	
I/II	78 (40 %)
III/IV	73 (38 %)
Unknown	42 (22 %)
Grade	
I	38 (20 %)
II	85 (44 %)
III	66 (34 %)
Unknown	4 (2 %)
Recurrence	
No	126 (65 %)
Yes	46 (24 %)
Unknown	21 (11 %)
Status at end point	
Alive	144 (75 %)
Died of disease	36 (19 %)
Unknown	13 (6 %)
Treatment response	
OR	116 (60 %)
NR	17 (9 %)
Unknown	60 (31 %)

are not known to have received radiotherapy. Only ten patients (7.5 %, $n=133$) did not receive any form of chemotherapy.

FGF19 immunohistochemistry staining

Immunohistochemistry staining analysis was performed using the Bench-Mark XT automated system (Ventana Medical Systems, Inc., Tucson, AZ, USA). This is a fully automated processing of bar code-labeled slides that included baking of the slides, solvent-free deparaffinization, antigen retrieval in a cell conditioning buffer CC1 (mild: 36 min conditioning, and standard: 60 min conditioning), incubation with the monoclonal anti-FGF19 (W12) antibody (Santa Cruz Biotechnology, INC, SC-73984), for 32 min, at 37 °C, and application of ultraView™ Universal DAB Inhibitor, ultraView Universal DAB Chromogen, ultraView Universal DAB H_2O_2, and ultraView Universal DAB Copper. Counterstaining with hematoxylin (2021) and post-counter staining with bluing reagent (2037) were performed for a maximum of 4 min. After staining, the sections were dehydrated in ethanol, cleared in xylene, and covered with Mountex and cover slips.

Evaluation of FGF19 expression

The evaluation of staining of all tissue slides was performed in a blind fashion to the patients' clinicopathological parameters with an upright light microscope at ×40 magnification. The typical expression patterns of FGF19 are illustrated in Fig. 1. Staining was graded into two categories: (1) no/weak (low) expression and (2) moderate/strong (high) expression. In calculating staining index, the intensity of staining and the fraction of positively stained cells were taken into account, using the following formula:

$$I = 0 \times f0 + 1 \times f1 + 2 \times f2 + 3 \times f3$$

where I is the staining index and $f0$–$f3$ are the fractions of the cells showing a defined level of staining intensity (from 0 to 3). Theoretically, the index could vary between 0 and 3 [34, 35]

Statistical analysis

Statistical analyses were performed using the IBM SPSS® Statistics (IBM Company, New York, NY, USA) software package (IBM SPSS Statistics for Mac, version 21). Fischer's exact test (two-sided) was used to assess the significance of the associations between the categorical variables. Univariate survival analysis for the outcome measures (DSS, DFS) was based on Kaplan–Meier method, with log-rank (Mantel–Cox) comparison test. To assess the value of FGF19 expression as an independent predictor, multivariate survival analysis was performed, using the Cox proportional hazards regression model including the following variables:

high FGF19 expression, age, estrogen receptor status, and lymph node status as well as tumor grade. In all tests, the values $p < 0.05$ were regarded as statistically significant.

Results

Description of FGF19 expression pattern

The expression pattern of FGF19 was predominantly cytoplasmic in the tumor area with faint nuclear signal apparent in the stromal cells such as fibroblast, inflammatory, and adipose cells (Fig. 1). Based on the intensity of FGF19 expression, the tumors were stratified into two categories, a low FGF19 expression category (78 cases out of 193, 40 %) and a high FGF19 expression category (115 cases out of 193, 60 %). Cases exhibiting a negative or weak staining intensity were classified into the low FGF19 expression category (Fig. 1b). In contrast, cases exhibiting moderate to strong staining intensity were classified into the high FGF19 expression category (Fig. 1a). In addition, heterogeneous expression pattern was also observed in some cases in which the tumors near to the adipose tissue showed a markedly stronger expression compared to the tumor tissue core (Fig. 1c–d).

FGF19 expression and clinicopathological features

High FGF19 expression showed a strong association with patients' age ($p = 0.008$). Out of 103 patients below 50 years old, 71 (69 %) of them showed high FGF19 expression intensity. In contrast, only 44/89 (49 %) of older patients (above 50 years old) showed high FGF19 expression intensity. A weak association between FGF19 expression levels and ER status was detected ($p = 0.07$). However, this association became much more statistically significant ($p = 0.006$) when patients were stratified according to their lymph node status. In patients with lymph node metastasis, high FGF19 expression was associated with negative ER status ($p = 0.006$) (Table 2). FGF19 expression showed a highly significant ($p = 0.001$) correlation with DSS in that in 83 % (30/36) patients who died of disease, their tumors showed high FGF19 expression, as compared to 17 % (6/36) whose tumor tissues showed low expression of FGF19. There were no statistically significant associations between FGF19 expression and other clinicopathological parameters including tumor size, lymph node status, histological stage, and grade or treatment response (Table 2).

FGF19 expression and survival

Univariate Kaplan–Meier survival analysis showed that there is no significant association between DFS and FGF19 expression in breast cancer. On the other hand, high FGF19 expression was significantly associated with poor survival when 5-year DSS was evaluated ($p = 0.007$, Fig. 2a). Univariate Kaplan–Meier survival analysis also showed that high FGF19 expression compounds the poor prognosis of older age ($p = 0.013$, Fig. 2b), negative ER status ($p = 0.002$,

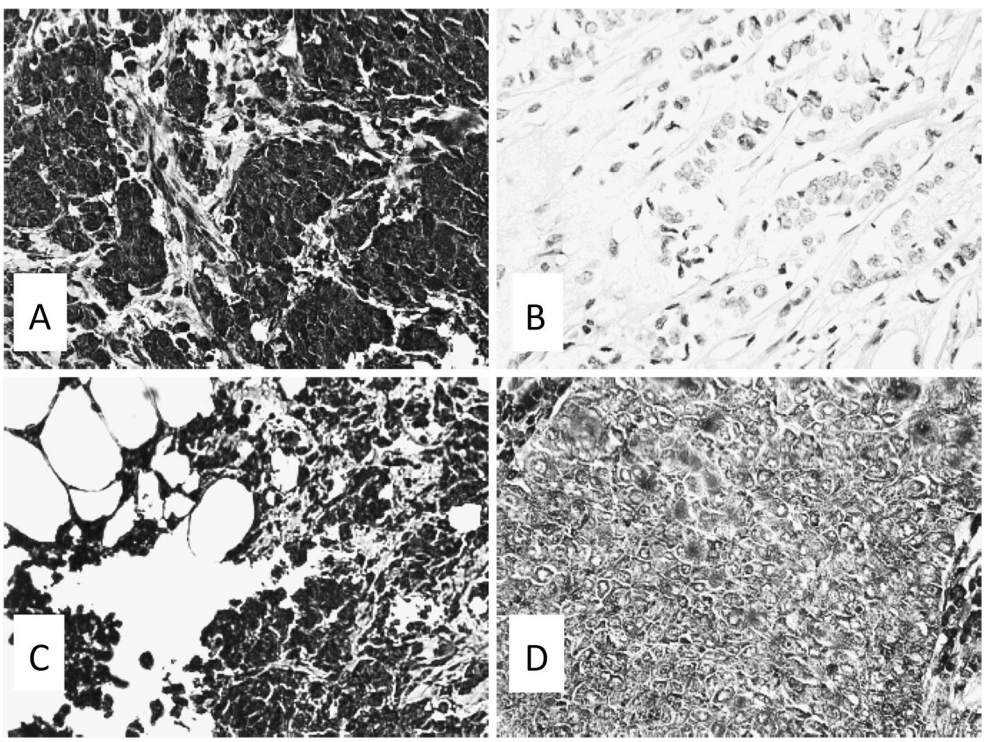

Fig. 1 Description of FGF19 expression pattern in invasive ductal carcinoma of breast tissues. **a** Strong expression of FGF19. **b** Example of FGF19 expression scored as no/weak (low). **c** Strong expression of FGF19 near to adipose tissues. **d** Weak expression of FGF19 of the same sample (**c**) within the core of the tumor tissue

Table 2 Correlation of FGF19 expression and clinicopathological features of BC in our cohort

Features	*N*	FGF19 cytoplasmic expression		*P*
		Low (%)	High (%)	
Age group (years)	192			**0.008**
<50		32 (16.7 %)	71 (37.00 %)	
>50		45 (23.4 %)	44 (22.9 %)	
ER status	143			0.07
Negative		8 (5.6 %)	37 (25.9 %)	
Positive		33 (23.1 %)	65 (45.4 %)	
PR status	123			0.84
Negative		15 (12.2 %)	36 (29.3 %)	
Positive		19 (15.4 %)	53 (43.1 %)	
Histological types	193			0.83
IDC		67 (34.7 %)	100 (51.8 %)	
Others		11 (5.7 %)	15 (7.8 %)	
Tumor size	169			0.98
<2 cm		12 (7.1 %)	16 (9.5 %)	
2–5 cm		38 (22.5 %)	50 (29.6 %)	
>5 cm		22 (13 %)	31 (18.3 %)	
Lymph node status	193			0.20
Negative		19 (9.8 %)	38 (19.7 %)	
Positive		59 (30.6 %)	77 (39.9 %)	
Metastasis	126			1.00
M0		35 (27.8 %)	77 (61.1 %)	
M1		4 (3.2 %)	10 (7.9 %)	
Stage	151			0.54
I/II		21 (13.9 %)	57 (37.7 %)	
III/IV		23 (15.3 %)	50 (33.1 %)	
Grade	189			0.87
I		14 (7.4 %)	24 (12.7 %)	
II		34 (18 %)	51 (27 %)	
III		28 (14.8 %)	38 (20.1 %)	
Recurrence	172			0.23
No		57 (33.1 %)	69 (40.1 %)	
Yes		16 (9.3 %)	30 (17.5 %)	
Status at end point	180			**0.001**
Alive		67 (37.2 %)	77 (42.8 %)	
Died of disease		6 (3.3 %)	30 (16.7 %)	
Treatment response	133			0.87
OR		32 (24.1 %)	84 (63.2 %)	
NR		5 (3.7 %)	12 (9 %)	

Data in bold highlights the most important result according to the *p* value

Fig. 2c), and lymph node metastasis ($p=0.001$, Fig. 2d) Performing this analysis on the metastasis-free (M0) patients only did not show any significant difference from the overall cohort ($p=0.033$, $n=66$). As shown in Table 3, multivariate Cox regression analysis shows that high FGF19 expression ($p=0.030$) as well as ER status ($p=0.023$) are independent prognostic markers for 5-year DSS of breast cancer patients (Table 3).

Discussion

This study, in fact, is a continuation of our efforts to further elucidate the biology of BC and to identify more effective prognostic factors than the traditional staging system to aid therapeutic decision making [36–39]. The main aim of the present study is to cast further light on the issues related to prognostication of BC, while assessing the value of quantitative FGF19 expression profile as an independent prognostic factor. Our current study, to the best of our knowledge, is the first to systematically assess the expression of FGF19 in human BC, as related to clinical data and disease outcome. In our cohort, BC tissues often showed cytoplasmic expression of FGF19 with the majority of cases (60 %) showing high levels of FGF19 expression. Moreover, an interesting heterogeneous expression pattern was also observed in some cases in that tumor cells adjacent to fatty tissues revealed a very strong cytoplasmic FGF19 expression as compared to those tumor cells located at the epithelial tumor tissue core. The possibility that FGF19 activity requires the co-expression of both fibroblast growth factor receptors 4 (FGFR4) and β-Klotho (KLB) could explain this heterogeneous expression. Interestingly, unlike the broad distribution of FGFR4, KLB is highly expressed in adipose tissue [21]. Interestingly, high FGF19 expression was more common among younger than older patients. This observation could be explained in the context that tumors derived from old patients have lower lipid proportion compared to young patients [40, 41]. This in turn could contribute to reduced access to KLB and thus diminished FGF19 activity [21].

Interestingly, in our univariate (Kaplan–Meier) survival analysis, FGF19 expression was a significant predictor of DSS, particularly in BC cases exhibiting lymph node metastasis and lacking estrogen receptor or in BC patients above 50 years old. Age alone did not contribute towards DSS. As expected, negative ER status as well as lymph nodes metastasis are themselves poor prognosis indicators for DSS. However, when combined with high FGF19 expression, the significance of this association was markedly increased (p value of 0.002 compared to 0.012 in terms of ER status alone and p value of 0.001 compared to 0.018 for lymph node status alone).

Understanding the role of the FGF19–FGFR4 signaling axis in breast cancer is important because FGF19 inhibition is an attractive potential therapeutic strategy for cancer [23]. Recent studies [26, 28, 42] determined the importance of FGF19 in tumor growth and development by generating anti-FGF19 and FGFR4 blocking antibodies that selectively inhibit the cross-talk between FGF19 and FGFR4 via inhibiting MAPK and Wnt β-catenin signaling in cell lines

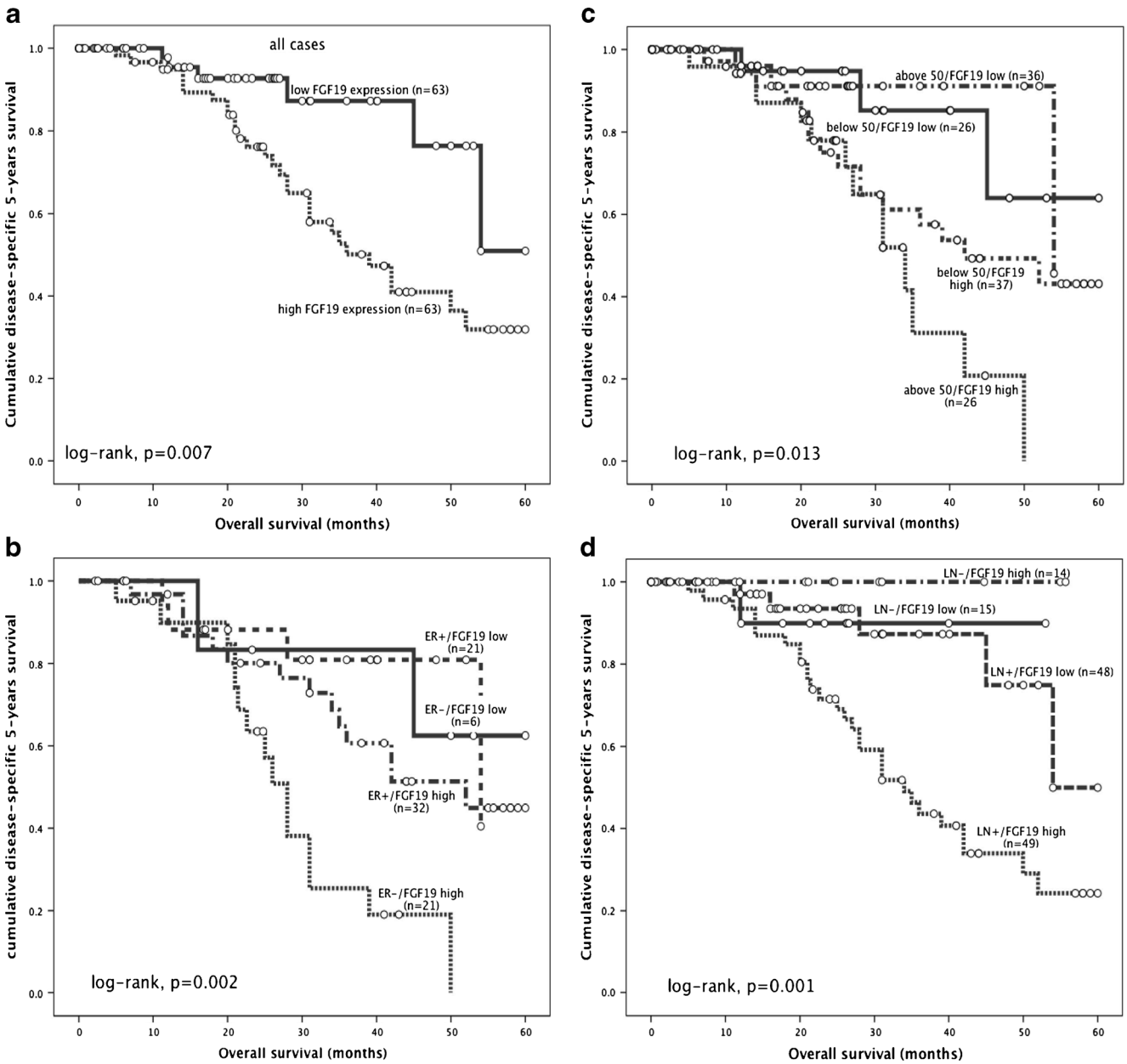

Fig. 2 FGF19 expression (low/high) as a determinant of DSS in univariate (Kaplan–Meier) analysis in all cases (**a**) or stratified according to age (**b**), ER status (**c**), or lymph node status (**d**)

Table 3 Multivariate Cox-regression analysis of various clinicopathological parameters and their use as prognostic markers for disease-specific 5-year survival in BC patients in our cohort

	P	SE	Relative risk	95 % CI
Age	0.076	0.362	1.903	0.936–3.869
ER status	**0.023**	0.353	0.449	0.225–0.897
Grade	0.787	0.299	1.084	0.603–1.949
Lymph node status	0.144	1.062	4.714	0.588–37.797
High FGF19 expression	**0.030**	0.468	2.755	1.101–6.890

and animal models limiting tumor growth. Unfortunately, functional analysis studies of FGF19 expression and its role in mammary gland tumors in both in vitro and in vivo models are still lacking.

In conclusion, the present results revealed high FGF19 expression, and this seems to be associated with less favorable long-term DSS as compared with low FGF19 expression tumors. Finally, there is a possibility to functionally target high FGF19 expression in BC tumors with anti-FGF19 antibody, and this could make the tumor cells susceptible to therapy. To reach this level, further intensive functional analysis approach with validation study in a larger BC cohort is highly recommended.

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Conflicts of interest None.

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