Overexpression of *FRAT1* protein is closely related to triple-negative breast cancer

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Purpose: Triple-negative breast cancer (TNBC) is an aggressive subtype of breast cancer with a poor prognosis and a lack of targeted therapy. Overexpression of *FRAT1* is thought to be associated with this aggressive subtype of cancer. Here, we performed a comprehensive analysis and assessed the association between overexpression of *FRAT1* and TNBC.
Methods: First, using different web-based bioinformatics platforms (TIMER 2.0, UALCAN, and GEPIA 2), the expression of *FRAT1* was assessed. Then, the expression of the *FRAT1* protein and hormone receptors and HER2 status were assessed by immunohistochemical analysis. For samples of tumors with equivocal immunoreactivity, we performed silver in situ hybridization of the HER2 gene to determine an accurate HER2 status. Next, we used the R package and bc-GenExMiner 4.8 to analyze the relationship between *FRAT1* expression and clinicopathological parameters in breast cancer patients. Finally, we determined the relationship between *FRAT1* overexpression and prognosis in patients.
Results: The expression of *FRAT1* in breast cancer tissues is significantly higher than in normal tissue. *FRAT1* expression was significantly related to worse overall survival (P < 0.05) and was correlated with these clinicopathological features: T stage, N stage, age, high histologic grade, estrogen receptor status, progesterone receptor status, Her-2 status, TNBC status, basal-like status, CK5/6 status, and Ki67 status.
Conclusion: *FRAT1* was overexpressed in breast cancer compared to normal tissue, and it may be involved in the progression of breast cancer malignancy. This study provides suggestive evidence of the prognostic role of *FRAT1* in breast cancer and the therapeutic target for TNBC.
[Ann Surg Treat Res 2022;103(2):63-71]

Key Words: *FRAT1*, Prognosis, Triple negative breast neoplasms

INTRODUCTION

Breast cancer is the most common cancer and is the leading cause of cancer death among women [1]. According to Korean National Cancer Registration data, the incidence rate of breast cancer is increasing annually [2]. Breast cancer is a heterogeneous disease in terms of histology, therapeutic response, and patient outcomes [3]. Triple-negative breast cancer (TNBC) is a highly heterogeneous and clinically aggressive breast cancer that accounts for 15%–20% of all breast cancers and has the highest death rate of all breast cancer subtypes [4-6]. Because of its heterogeneity, various combined therapeutic modalities are implemented. Clinicopathological factors such as TNM stage, histological grade, estrogen receptor (ER), progesterone receptor (PR) expression, and human epidermal growth factor receptor 2 (HER-2) gene amplification

are considered prognostic factors which provide the key for therapeutic strategies. Therefore, it is essential to define specific tumor markers to reflect the prognosis.

FRAT1 (frequently rearranged in advanced T-cell lymphomas 1), a member of the Frat family, is located on human chromosome 10q24.1, encoding a 29-kDa protein comprising 279 amino acids [7]. *FRAT1* has been considered to be a positive activator of the Wnt/beta-catenin (β-catenin) signaling pathway, which is a family of proteins that are involved in many vital cellular functions such as regeneration and organogenesis [8-10]. *FRAT1* has been reported to be overexpressed in several tumors, such as non-small-cell lung cancer (NSCLC) [8], gliomas [11], ovarian cancers [12], esophageal squamous cell carcinomas [13], and colon cancer [14]. This data suggests that *FRAT1* is associated with an aggressive phenotype. The relevance of the expression status of *FRAT1* in breast cancer has not been determined, and its role in biological behavior is also unknown.

In the present study, we investigated the prognostic validity of *FRAT1* expression and analyzed correlations with clinicopathological parameters and overall survival (OS) in breast cancer patients. These results may have a great contribution to the prediction of prognosis.

METHODS

Patients and clinicopathologic data

This study includes not only the breast cancer dataset from The Cancer Genome Atlas (TCGA) database (http://dna00.bio. kyutech.ac.jp/PrognoScan/index.html) [15], but we also collected 226 cases of invasive breast cancer at Konkuk University Medical Center between January 2009 and December 2011. Clinicopathological data such as age, tumor size, nodal stage, histologic grade, ER status, PR status, HER-2 amplification status, and CK5/6 expressions were obtained from the patients' medical records and pathological reports. All patients were of the female gender, and their treatment was either modified radical mastectomy or breast-conserving surgery, and tumors were completely removed by surgery with pathological evaluation. After the completion of the surgery, adjuvant treatment with radiotherapy, chemotherapy, and hormone therapy was administered based on international guidelines. All the patients were staged according to the TNM stage, which was based on the criteria of American Joint Committee on Cancer, 8th edition. The patients were classified into 4 molecular subtypes, as follows: luminal A: ER-positive (ER+) and/or PR-positive (PR+), HER-2-negative (HER-2−) and low Ki67 level (<14.0%); luminal B: ER+ and/or PR+, Ki67 ≥14.0%, HER-2− or HER-2-positive (HER-2+); HER-2: ER-negative (ER−) and PR-negative (PR−), HER-2+; and TNBC: ER−, PR−, and HER-2−. We also analyzed the survival times of patients. The median follow-up time was 24 months.

The study was approved by the Institutional Review Board of Konkuk University Medical Center (No. KUH1210054).

The expression of *FRAT1* was determined using TIMER 2.0

TIMER (Tumor Immune Estimation Resource) 2.0 (http:// timer.cistrome.org) is a comprehensive resource for systematical analysis of immune infiltrates across diverse cancer types [16]. In this study, TIMER 2.0 was used to analyze the expression of *FRAT1* in breast cancer tissues and normal tissues across all TCGA datasets.

UALCAN analysis

UALCAN (http://ualcan.path.uab.edu) is a website for the online analysis and mining of cancer data based mainly on the analysis of relevant cancer data in the TCGA database [17]. In this study, we used it to assess *FRAT1* expression in breast cancer tissues and normal breast tissues.

GEPIA 2 web analysis

GEPIA (Gene Expression Profiling Interactive Analysis) 2 (http://gepia2.cancer-pku.cn/#index) is a web server for analyzing the RNA sequencing expression data of tumors and normal samples from the TCGA and GTEx (Genotype-Tissue Expression) projects, using a standard processing pipeline [18]. The GEPIA box-plot tool was used for tumor/normal differential expression analysis for *FRAT1* in breast cancers.

Immunohistochemical assessment of *FRAT1*

Four-micrometer sections were made from the formalin-fixed, paraffin-embedded blocks, which were then deparaffinized in xylene and rehydrated in a graded series of alcohol solutions. For antigen retrieval, paraffin tissue sections were cooked with 10-mM sodium citrate buffer, pH 6.0, at a sub-boiling temperature for 15 minutes and cooled for 20 minutes at room temperature. The sections were washed twice with Tris-buffered saline for 10 minutes. The antibody incubations were carried out at room temperature for 1 hour. *FRAT1* was detected with standard avidin-biotin-peroxidase techniques using diaminobenzidine as the chromogen. Afterward, the slides were briefly counterstained with hematoxylin, dehydrated, and mounted. The immunostained samples were evaluated by 2 pathologists. The expression of *FRAT1* was graded in terms of the percentage of cytoplasmic membrane staining in each block as well as the intensity of staining. However, there is no common cutoff value for the patterns of staining. In this study, the grade was designated according to the following criteria: negative, staining in less than 1% and positive, staining in more than 1% of tumor cells (Table 1).

Immunohistochemical assessment of hormone receptors and HER-2

ER and PR statuses were determined by immunohistochemistry (IHC) using the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines. Tumors with >1% positively stained tumor cells were classified as positive for ER and PR. HER-2 status was also determined by IHC. HER-2 positive tumors were defined as 3+ and HER-2 negative tumors were defined as 0 or 1+, using the ASCO/CAP guidelines as the criteria for immunohistochemical staining. We performed silver *in situ* hybridization (SISH) to determine an accurate HER-2 status in tumors with equivocal immunoreactivity (2+).

Overexpression of *FRAT1* survival analysis

We used PrognoScan (http://dna00.bio.kyutech.ac.jp/PrognoScan/index.html) to analyze the overexpressed *FRAT1* and OS in breast cancer. The Kaplan-Meier plotter, and R2: Kaplan-Meier scanner with the log-rank test was used to examine the data. The cohorts from GSE (GSE9893) and TCGA were divided into 2 groups according to *FRAT1* expression (high, >67%; low, ≤33%). All statistical tests were 2-sided.

Breast Cancer Gene-Expression Miner ver. 4.8 analysis

The Breast Cancer Gene-Expression Miner ver. 4.8 (bc-GenExMiner 4.8, http://bcgenex.ico.unicancer.fr/BC-GEM/GEM-Accueil.php?js=1) is developed as a web-based tool including a MySQL relational database [19]. It was used to analyze the relationship between *FRAT1* expression and clinicopathological parameters in breast cancer patients.

Statistical analysis

R software ver. 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria) was used to perform the statistical analysis. Differences between groups were compared using the Wilcoxon rank-sum test or Student t-test, as appropriate. The chi-square test was used to analyze the possible correlations between *FRAT1* expression and clinicopathologic factors. OS was defined as the time from surgery until the time of death. OS

Table 1. Antibodies and antigen retrieval techniques used

Antibody	Clone	Source	Buffer	Antigen retrieval	Incubation time (min)	Dilution
FRAT1	Polyclonal	Epitomics	EDTA buffer pH 8.6	Mild	32	1:10,000
ER	SP1	Neomarker	EDTA buffer pH 8.6	Standard	60	1:10,000
PR	SP2	Neomarker	EDTA buffer pH 8.6	Standard	80	1:10,000
HER2	Polyclonal	DAKO	EDTA buffer pH 8.6	Mild	60	1:10,000

ER, estrogen receptor; PR, progesterone receptor; EDTA, ethylenediaminetetraacetic acid.
Epitomics: Burlingame, CA, USA; Neomarker: Fremont, CA, USA; DAKO: Glostrup, Denmark.
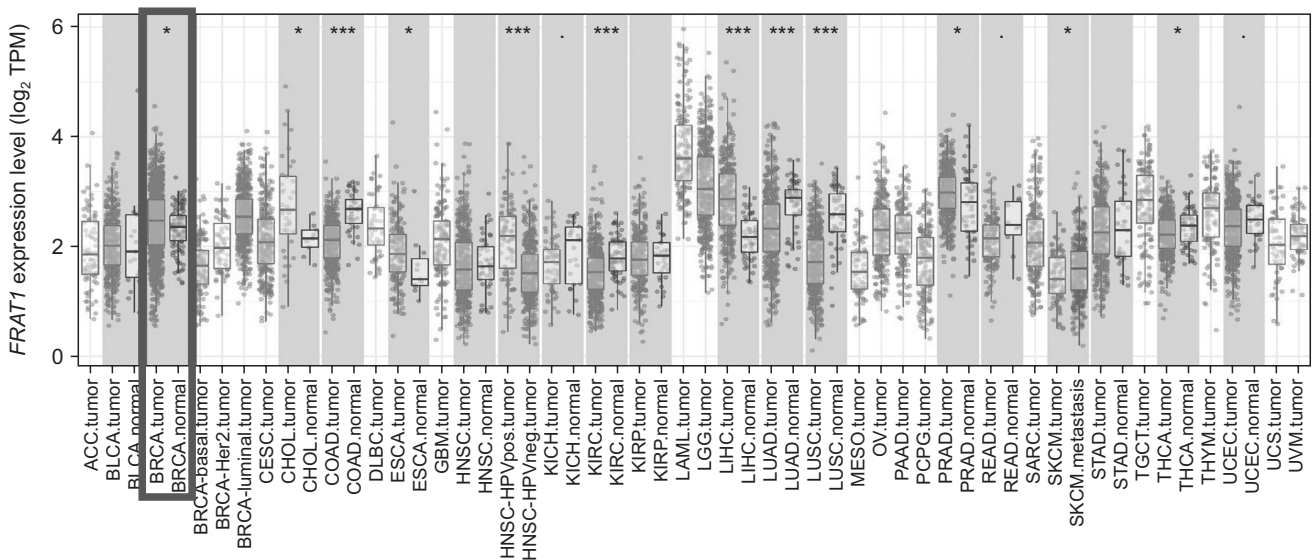
Fig. 1. The expression of *FRAT1* messenger RNA in Pan-cancer. Human *FRAT1* expression levels in different tumor types from TCGA database were determined by TIMER 2.0 (http://timer.cistrome.org). TCGA, The Cancer Genome Atlas; TIMER, Tumor Immune Estimation Resource. ***P < 0.001, **P < 0.01, *P < 0.05.

was estimated using the PrognoScan, Kaplan-Meier plotter, and Kaplan-Meier scanner. A P-value of <0.05 was considered statistically significant. IBM SPSS Statistics ver. 20.0 (IBM Corp, Armonk, NY, USA) was used for statistical analyses.

RESULTS

The differential expression of *FRAT1* in human breast cancer

TIMER 2.0 was used to analyze the messenger RNA (mRNA) levels of *FRAT1* in tumor tissues and normal tissues. The results showed that *FRAT1* was overexpressed in breast cancer as compared to its expression levels in normal tissues, P < 0.05 (Fig. 1). To further evaluate the differential expression of *FRAT1*, we compared its expression level in the TCGA dataset using UALCAN. As shown in Fig. 2A, the expression of *FRAT1* in breast cancer tissues is significantly higher than in normal tissue, P < 0.001 (Fig. 2A). In addition, it was discovered that the expression of *FRAT1* mRNA in breast cancer tissues was significantly higher than that in normal tissues using GEPIA2 web analysis (P < 0.01) (Fig. 2B).

Clinicopathologic characteristics

The median age was 56 years (range, 27–79 years). Among them, 66 patients underwent modified radical mastectomy, and 130 underwent breast-conserving surgery. The distribution of tumor and node stage was as follows: T1 in 104, T2 in 77, T3 in 12, N0 in 124, N1 in 39, N2 in 24, and N3 in 4 cases.
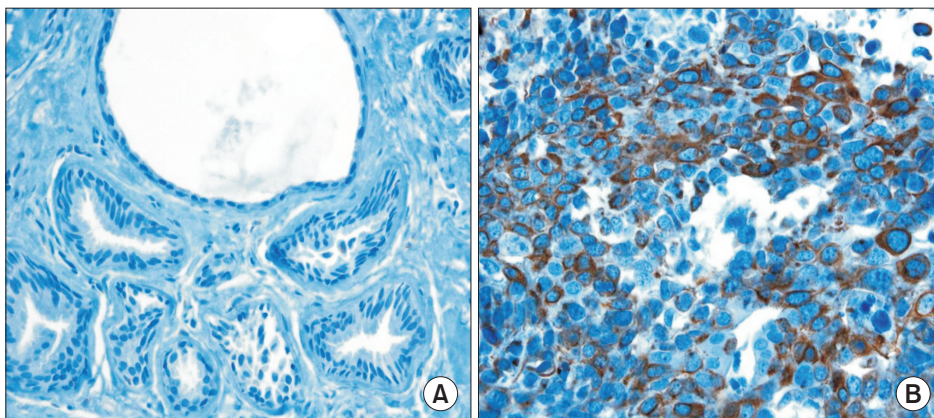
Immunohistochemistry

Immunohistochemical analysis of *FRAT1* in normal breast tissue showed no reaction in the ductal and myoepithelial cells (Fig. 3A). In contrast, 21 out of 196 (10.7%) demonstrated cytoplasmic and membranous expression of *FRAT1* in breast cancer tissues (Fig. 3B).

Correlation between *FRAT1* expression and clinicopathological variables

The expression of *FRAT1* in breast cancer tissue was correlated with the patients' clinicopathologic features. The clinicopathological characteristics of the patients according to *FRAT1* positivity and negativity are shown in Table 2. In this study, *FRAT1* was positively and markedly correlated with an
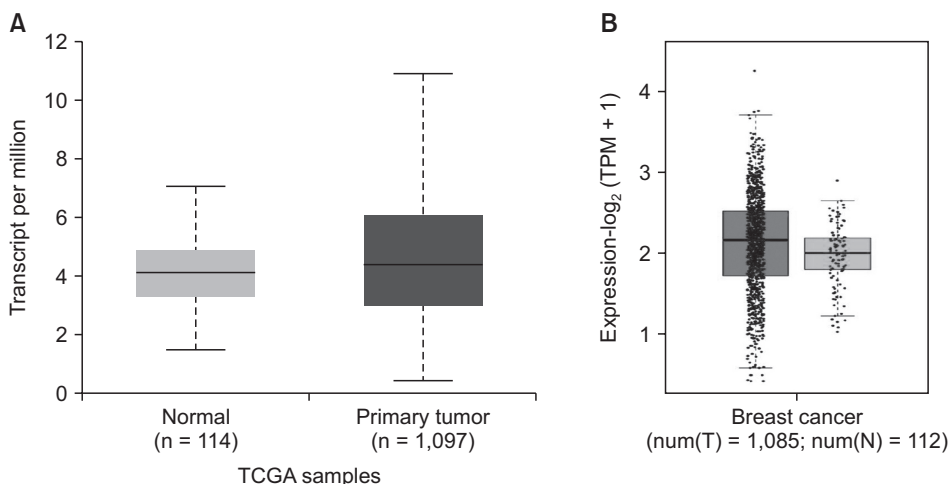
Fig. 2. The expression of *FRAT1* messenger RNA in breast cancer. (A) Expression of *FRAT1* in breast cancer based on sample types by UALCAN (http://ualcan.path.uab.edu/index.html), P < 0.001. (B) The Expression of *FRAT1* in breast cancer tissue and normal tissue generated by GEPIA 2 web (http://gepia2.cancer-pku.cn/#index; TCGA database), P < 0.01. GEPIA, Gene Expression Profiling Interactive Analysis; TCGA, The Cancer Genome Atlas.

Fig. 3. Immunohistochemical analysis of *FRAT1* expression. (A) Negative reactivity of *FRAT1* in the normal ductal and myoepithelial cells (×200). (B) Cytoplasmic and membranous expression of *FRAT1* in breast cancer (×400).

increase in the TNM stages. *FRAT1* positivity was correlated with tumor size (P = 0.004) and nodal status (P = 0.008). The *FRAT1* positivity group had an association with a high histologic grade (P < 0.001) compared to the normal group. The expression of *FRAT1* was shown to be increased with histological grade. Interestingly, CK5/6 is the most useful and important marker for the identification of the basal subgroup of TNBC [20]. Eighteen out of 21 cases with positive staining of CK5/6 showed *FRAT1* expression, which is statistically significant (P < 0.001). Furthermore, the *FRAT1* positivity group also had an association with ER negativity (P < 0.001) and

PR negativity (P < 0.001). Although there was no statistically significant result, *FRAT1* positivity was observed more in HER-2 negativity (P = 0.363). There was a significant difference in the subtype and the *FRAT1* positivity group had more TNBC (11 out of 21). There was no significant correlation between *FRAT1* expression and age and HER-2 status. Kaplan-Meier survival analysis demonstrated that the group with *FRAT1* expression showed significantly worse OS than the one without *FRAT1* expression (Fig. 4).

Table 2. Relationship between *FRAT1* and clinicopathological factors in breast cancer tissue

Characteristic	FRAT1		
	Negative	Positive	P-value
Age (yr)			
≥40	153	16	0.141
<40	22	5	
T stage			
1	96	8	0.004
2	69	8	
3	9	3	
X	2	1	
N stage			
0	111	13	0.008
1	36	3	
2	22	2	
3	4	0	
X	3	2	
H grade			
0	4	1	<0.001
1	40	2	
2	86	8	
3	45	10	
ER status			
Negative	46	14	<0.001
Positive	129	7	
PR status			
Negative	95	17	0.015
Positive	80	4	
HER2 status			
Negative	161	15	0.363
Positive	43	7	
CK5/6			
Negative	139	3	<0.001
Positive	36	18	
Subtypes			
Luminal A	119	6	<0.001
Luminal B	14	2	
Her2	17	2	
TNBC	25	11	

ER, estrogen receptor; PR, progesterone receptor; TNBC, triple-negative breast cancer.
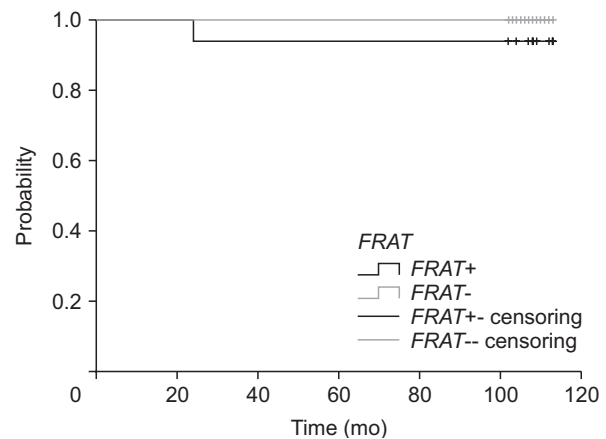

Fig. 4. Kaplan-Meier survival analysis. Kaplan-Meier survival analysis demonstrated that the group with *FRAT1* expression showed significantly worse overall survival than the one without *FRAT1* expression.

Table 3. Clinicopathological relationship of *FRAT1* messenger RNA expression in breast cancer (TCGA database)

Characteristic	Low expression of FRAT1	High expression of FRAT1	P-value
No. of patients	541	542	
T stage			0.005
T1	128 (11.9)	149 (13.8)	
T2	341 (31.6)	288 (26.7)	
T3	54 (5.0)	85 (7.9)	
T4	17 (1.6)	18 (1.7)	
N stage			0.013
N0	284 (26.7)	230 (21.6)	
N1	162 (15.2)	196 (18.4)	
N2	57 (5.4)	59 (5.5)	
N3	32 (3.0)	44 (4.1)	
M stage			0.985
M0	476 (51.6)	426 (46.2)	
M1	10 (1.1)	10 (1.1)	
Age (yr)	57.0 (48.0–66.0)	59.5 (49.3–68.0)	0.023

Values are presented as number only, number (%), or median (interquartile range).
TCGA, The Cancer Genome Atlas.
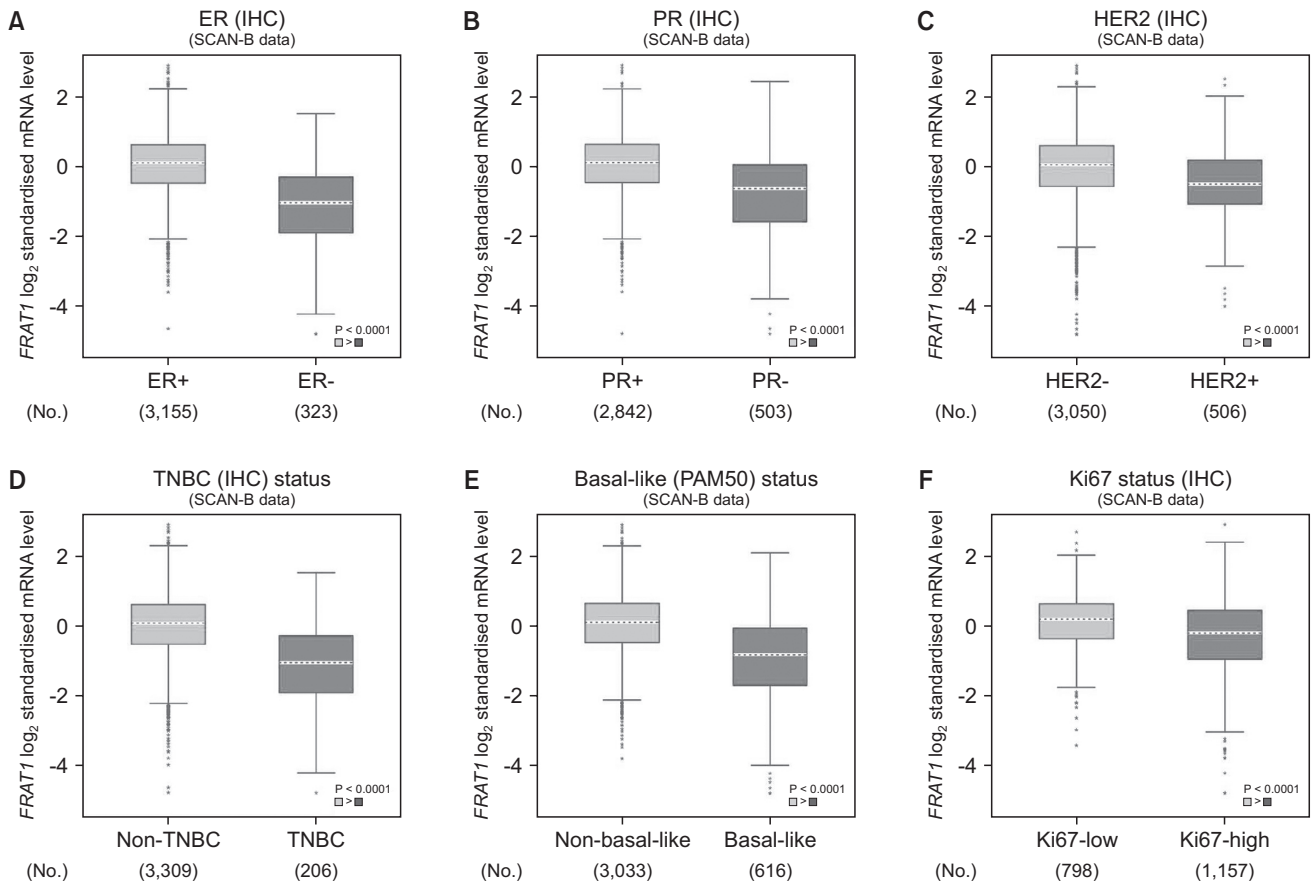
Fig. 5. Relationship between *FRAT1* expression and clinicopathological parameters in breast cancer patients. The box plot was retrieved from the Breast Cancer Gene-Expression Miner ver. 4.8 (http://bcgenex.ico.unicancer.fr/BC-GEM/GEM-Accueil. php?js=1). Global significant difference between groups was assessed by Welch t-test to generate P-values, along with the Dunnett-Tukey-Kramer test. ER, estrogen receptor; PR, progesterone receptor; HER2, HER2 receptor; TNBC, triple-negative breast cancer; IHC, immunohistochemistry; mRNA, messenger RNA.

Clinicopathological relationship of *FRAT1* the messenger RNA expression in breast cancer

In the TCGA database, differential expression of *FRAT1* in breast cancer was correlated with T stage, lymph node stage, and age (Table 3). In Fig. 5, we used bc-GenExMiner 4.8 to compare the transcription levels of *FRAT1* among groups of patients according to different clinicopathological characteristics. The results showed that the overexpression of *FRAT1* was significantly related to ER (IHC) status, PR (IHC) status, Her-2 (IHC) status, TNBC (IHC) status, basal-like (PAM 50) status, and Ki67 (IHC) status, P < 0.001 (Fig. 5).

Overexpression of *FRAT1* survival analysis

Next, the prognostic value of *FRAT1* mRNA expression in human breast cancer was further evaluated, using these online databases, including PrognoScan, and R2: Kaplan-Meier scanner. As shown in Fig. 6 (PrognoScan), we found that overexpression of *FRAT1* was associated with poorer OS (P = 0.010) (Fig. 6). The result of R2: Kaplan-Meier scanner showed that overexpression of *FRAT1* was associated with a poor prognosis for patients
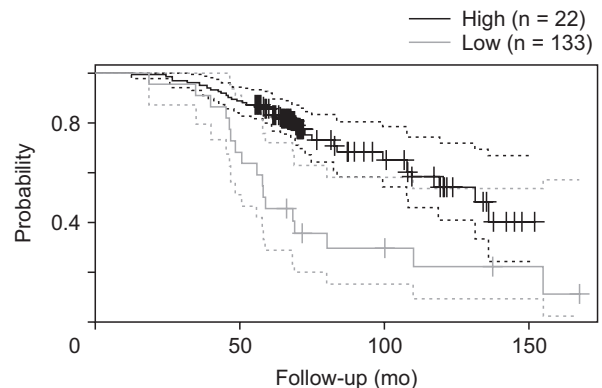
Fig. 6. Relationship between *FRAT1* messenger RNA expression and clinical outcomes in breast cancer patients in PrognoScan database (http://dna00.bio.kyutech.ac.jp/ PrognoScan/index.html). Breast cancer; GSE9893; overall survival; hazard ratio, 1.33 (1.10–1.61), P = 0.010.

with breast cancer (P = 0.025) (Fig. 7). The results revealed that overexpression of *FRAT1* was significantly associated with poor prognosis. All these findings indicate that *FRAT1* may serve as
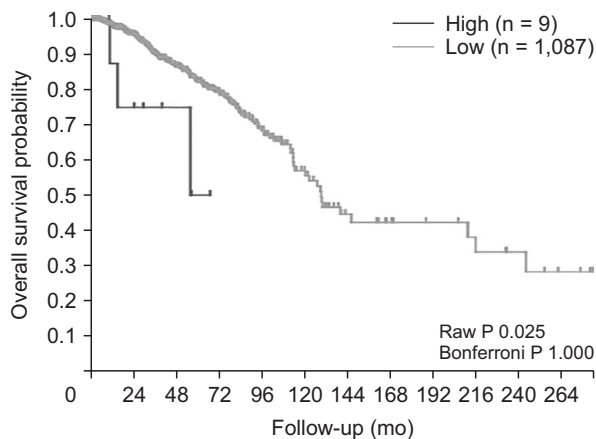
Fig. 7. Relationship between *FRAT1* messenger RNA expression and clinical outcomes in breast cancer patients in R2: Kaplan-Meier scanner. Tumor breast invasive carcinoma; The Cancer Genome Atlas (TCGA); overall survival, P = 0.025.

a poor predictor of the prognosis of patients with breast cancer.

DISCUSSION

TNBC is a heterogeneous and deadly disease in women with limited therapy choices, necessitating the development of novel targeted therapies [21]. The *FRAT1* mapped to human chromosome 10q24.1, which is a human homolog of mouse proto-oncogene *FRAT1* [7]. Proto-oncogene *FRAT1* was first identified as a T-cell lymphoma proto-oncogene that encodes a 29KD protein. *FRAT1* regulates Wnt/β-catenin signaling through inhibiting the acidification of GSK3 on β-catenin and translocation into the nucleus, where it binds to the TCF/LEF signal transduction pathway [13,22]. Dysregulation of Wnt/β-catenin signaling has been thought to play an important role in different kinds of cancers. Extensive studies have shown that the Wnt signal transduction pathway is associated with different kinds of human cancers, such as colon cancer, acute leukemia, lung cancer, and glioma [12,23]. *FRAT1* has been identified as a component of the Wnt signaling pathway in previous research [24,25]. *FRAT1* is a positive regulator of the Wnt/β-catenin pathway [12]. Overexpression of *FRAT1* leads to β-catenin stabilization through dissociation of GSK-3β from Axin and inhibition of β-catenin phosphorylation [25,26]. High *FRAT1* expression is associated with increased malignancy and a higher clinical stage [27]. *FRAT1* was associated with invasion, metastasis, and malignant phenotypes. In this study, we observed *FRAT1* overexpression in breast cancer. *FRAT1* positivity was correlated with tumor size, nodal status, histologic grade, and CK5/6. Furthermore, the *FRAT1* positivity group also had an association with ER negativity and PR negativity. There was a significant difference in the subtype and the *FRAT1* positivity group had more TNBC. TNBC

is one of the subtypes of malignant breast cancer with the worst prognosis [28]. Most patients have been diagnosed at an advanced stage or experience a recurrence within a short period of time. Thus, a special approach to treatment and strategy are needed. Importantly, *FRAT1* positivity is correlated with poor OS in breast cancer patients. Furthermore, we assessed the expression pattern of *FRAT1* in human breast cancer by bioinformatics validation. Then, the prognostic significance of *FRAT1* expression in breast cancer was also analyzed. Higher expression of *FRAT1* indicated poorer OS in breast cancer in different kinds of online databases. Furthermore, previous studies on different types of malignancies have reported the role of high expression of *FRAT1* protein but not in breast cancer. Interestingly, previous studies demonstrated that Wnt7a protein expression was significantly correlated with ER expression, and loss of Wnt7a expression was associated with poor disease-free survival in breast cancer patients [29]. Wnt7a is a member of the Wnt family, which is regulated by *FRAT1*. The exact function that *FRAT1* plays in the pathway in controlling carcinogenesis is unknown. Therefore, further research will be needed. In conclusion, *FRAT1* protein is abnormally expressed in breast cancer. *FRAT1* might be involved in the malignancy progression of breast cancer. High expression of *FRAT1* is correlated with clinical and pathological stages and poorer OS. Therefore, positive *FRAT1* expression is a biomarker for cancer severity and a predictive marker of poor prognosis in breast cancer.

Conflict of Interest
No potential conflict of interest relevant to this article was reported.

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REFERENCES

1. Althuis MD, Dozier JM, Anderson WF, Devesa SS, Brinton LA. Global trends in breast cancer incidence and mortality 1973-1997. Int J Epidemiol 2005;34:405-12.

2. Schnitt SJ. Classification and prognosis of invasive breast cancer: from morphology to molecular taxonomy. Mod Pathol 2010;23 Suppl 2:S60-4.

3. Polyak K. Heterogeneity in breast cancer. J Clin Invest 2011;121:3786-8.

4. Liao C, Zhang Y, Fan C, Herring LE, Liu J, Locasale JW, et al. Identification of BBOX1 as a therapeutic target in triple-negative breast cancer. Cancer Discov 2020;10:1706-21.

5. Anders CK, Carey LA. Biology, metastatic patterns, and treatment of patients with triple-negative breast cancer. Clin Breast Cancer 2009;9(Suppl 2):S73-81.

6. Metzger-Filho O, Tutt A, de Azambuja E, Saini KS, Viale G, Loi S, et al. Dissecting the heterogeneity of triple-negative breast cancer. J Clin Oncol 2012;30:1879-87.

7. Saitoh T, Katoh M. FRAT1 and FRAT2, clustered in human chromosome 10q24.1 region, are up-regulated in gastric cancer. Int J Oncol 2001;19:311-5.

8. Zhang Y, Yu JH, Lin XY, Miao Y, Han Y, Fan CF, et al. Overexpression of Frat1 correlates with malignant phenotype and advanced stage in human non-small cell lung cancer. Virchows Arch 2011;459:255-63.

9. Hagen T, Cross DA, Culbert AA, West A, Frame S, Morrice N, et al. FRAT1, a substrate-specific regulator of glycogen synthase kinase-3 activity, is a cellular substrate of protein kinase A. J Biol Chem 2006;281:35021-9.

10. Krishnamurthy N, Kurzrock R. Targeting the Wnt/beta-catenin pathway in cancer: update on effectors and inhibitors. Cancer Treat Rev 2018;62:50-60.

11. Guo G, Mao X, Wang P, Liu B, Zhang X, Jiang X, et al. The expression profile of FRAT1 in human gliomas. Brain Res 2010;1320:152-8.

12. Wang Y, Hewitt SM, Liu S, Zhou X, Zhu H, Zhou C, et al. Tissue microarray analysis of human FRAT1 expression and its correlation with the subcellular localisation of beta-catenin in ovarian tumours. Br J Cancer 2006;94:686-91.

13. Wang Y, Liu S, Zhu H, Zhang W, Zhang G, Zhou X, et al. FRAT1 overexpression leads to aberrant activation of beta-catenin/TCF pathway in esophageal squamous cell carcinoma. Int J Cancer 2008;123:561-8.

14. Zhu K, Guo J, Wang H, Yu W. FRAT1 expression regulates proliferation in colon cancer cells. Oncol Lett 2016;12:4761-6.

15. Tomczak K, Czerwińska P, Wiznerowicz M. The Cancer Genome Atlas (TCGA): an immeasurable source of knowledge. Contemp Oncol (Pozn) 2015;19(1A):A68-77.

16. Li T, Fu J, Zeng Z, Cohen D, Li J, Chen Q, et al. TIMER2.0 for analysis of tumor-infiltrating immune cells. Nucleic Acids Res 2020;48(W1):W509-14.

17. Chandrashekar DS, Bashel B, Balasubramanya SA, Creighton CJ, Ponce-Rodriguez I, Chakravarthi BV, et al. UALCAN: a portal for facilitating tumor subgroup gene expression and survival analyses. Neoplasia 2017;19:649-58.

18. Tang Z, Li C, Kang B, Gao G, Li C, Zhang Z. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res 2017;45(W1):W98-102.

19. Jézéquel P, Campone M, Gouraud W, Guérin-Charbonnel C, Leux C, Ricolleau G, et al. bc-GenExMiner: an easy-to-use online platform for gene prognostic analyses in breast cancer. Breast Cancer Res Treat 2012;131:765-75.

20. Hashmi AA, Naz S, Hashmi SK, Hussain ZF, Irfan M, Bakar SM, et al. Cytokeratin 5/6 and cytokeratin 8/18 expression in triple negative breast cancers: clinicopathologic significance in South-Asian population. BMC Res Notes 2018;11:372.

21. Bianchini G, Balko JM, Mayer IA, Sanders ME, Gianni L. Triple-negative breast cancer: challenges and opportunities of a heterogeneous disease. Nat Rev Clin Oncol 2016;13:674-90.

22. Saitoh T, Mine T, Katoh M. Molecular cloning and expression of proto-oncogene FRAT1 in human cancer. Int J Oncol 2002;20:785-9.

23. Zhang Y, Han Y, Zheng R, Yu JH, Miao Y, Wang L, et al. Expression of Frat1 correlates with expression of β-catenin and is associated with a poor clinical outcome in human SCC and AC. Tumour Biol 2012;33:1437-44.

24. Yost C, Farr GH 3rd, Pierce SB, Ferkey DM, Chen MM, Kimelman D. GBP, an inhibitor of GSK-3, is implicated in Xenopus development and oncogenesis. Cell 1998;93:1031-41.

25. Thomas GM, Frame S, Goedert M, Nathke I, Polakis P, Cohen P. A GSK3-binding peptide from FRAT1 selectively inhibits the GSK3-catalysed phosphorylation of axin and beta-catenin. FEBS Lett

1999;458:247-51.

26. Li L, Yuan H, Weaver CD, Mao J, Farr GH 3rd, Sussman DJ, et al. Axin and Frat1 interact with dvl and GSK, bridging Dvl to GSK in Wnt-mediated regulation of LEF-1. EMBO J 1999;18:4233-40.

27. Walf-Vorderwülbecke V, de Boer J, Horton SJ, van Amerongen R, Proost N, Berns A, et al. Frat2 mediates the oncogenic activation of Rac by MLL fusions. Blood 2012;120:4819-28.

28. Jiang Y, Liu Y, Tan X, Yu S, Luo J. TPX2 as a novel prognostic indicator and promising therapeutic target in triple-negative breast cancer. Clin Breast Cancer 2019;19:450-5.

29. Yi K, Min KW, Wi YC, Kim Y, Shin SJ, Chung MS, et al. Wnt7a deficiency could predict worse disease-free and overall survival in estrogen receptor-positive breast cancer. J Breast Cancer 2017;20:361-7.