Fibroblast Growth Factor Receptor 4 as A Prognostic Indicator in Triple-Negative Breast Cancer

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Abstract

Background: Triple-negative breast cancer (TNBC) is one of the most aggressive breast cancers, and patients with this subtype usually have a poor prognosis. Early analyses identified that fibroblast growth factor receptor 4 (FGFR4) was involved in breast cancer, but its prognostic effect on TNBC is unknown. In the present study, we investigated the association between FGFR4 and TNBC prognosis.

Methods: FGFR4 protein expression was detected in 282 TNBC patients using immunohistochemistry.

Results: FGFR4 was highly expressed in TNBC patients. Lymph node metastasis (LNM) ($P=0.033$) and p53 status ($P=0.019$) were associated with high FGFR4 expression. Univariate analysis identified high FGFR4 expression ($P=0.016$) as a prognostic predictor, and multivariate analysis found that high FGFR4 expression ($P=0.016$) was an independent prognostic factor. The Kaplan-Meier survival curve showed that high FGFR4 protein expression was correlated with poorer overall survival.

Conclusions: The results of our present study show that FGFR4 protein expression is correlated with a worse prognosis in TNBC.

Background

Triple-negative breast cancer (TNBC) is one of the four major molecular subtypes of breast cancer (DeSantis, Ma et al. 2017). It is characterized as loss of expression of estrogen receptors (ERs), progesterone receptors (PRs) and human epidermal growth factor receptor 2 (HER2), and it is one of the most aggressive breast cancer subtypes and is more prone to metastasize compared with other subtypes (Foulkes, Smith et al. 2010, Metzger-Filho, Tutt et al. 2012, Zeng, He et al. 2018). Although several genes and proteins have been identified as prognostic indicators or therapeutic targets in breast cancer, there is still a lack of therapeutic targets for TNBC (Olopade, Grushko et al. 2008, Sun, Jiang et al. 2012, Li, Xu et al. 2018). TNBC patients usually have a poor prognosis and a high rate of recurrence after chemotherapy (Anders and Carey 2009, Cinkaya, Akin et al. 2016). Thus, prognostic indicators or therapeutic targets of TNBC still need to be identified.

Fibroblast growth factor receptor 4 (FGFR4) is a member of the FGFR family, which is part of the receptor tyrosine kinase (RTK) family (Kato and Nakagama 2014). Previous studies have shown that FGFR4 may be involved in the carcinogenesis and progression of many cancers (Inokuchi, Murase et al. 2017, Joshi, Coffey et al. 2017, Motylewska, Stepień et al. 2018, Quintanal-Villalonga, Ojeda-Marquez et al. 2018). FGFR4 has also been implicated in breast cancer. FGFR4 can increase glucose metabolism and lead to chemoresistance (Xu, Chen et al. 2018), and the FGF19/FGFR4 axis can enhance basal-like breast cancer cell survival and might be an effective strategy to suppress cancer development, progression and metastasis (Tiong, Tan et al. 2016, Zhao, Xu et al. 2018). There have been almost no studies on the prognosis of FGFR4 in TNBC, so in the present study, we investigated the association between FGFR4 and TNBC prognosis through immunohistochemistry analyses.

Methods

Subjects

A total of 282 primary breast cancer patients from November 2008 to March 2011 were included. Patients with sporadic breast cancer underwent initial diagnosis and resection at Harbin Medical University Cancer Hospital. Patients did not receive any chemotherapy or radiotherapy before surgery. Routine testing for estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), p53 and Ki67 was performed for every patient. TNBC patients were included with invasive ductal carcinomas histological type. Written informed consent was obtained from every participant. This study was approved by the ethics committee of Harbin Medical University and carried out in accordance with approved guidelines.

Patient follow-up

Patient follow-up was conducted as our previous study(Wei, You et al. 2018). It was conducted on a scheduled basis until patient death or the end of the observation period (May 1st, 2016). Examinations were carried out every 6 months for the first 2 years and every 12 months thereafter. All patients were followed regularly for at least 5 years at Harbin Medical University Cancer Hospital. Every patient was contacted by telephone after terminal treatment. Survival time was calculated in months. Overall survival (OS) was used to assess prognosis.

Immunohistochemistry (IHC)

A total of 282 samples from patients were randomly selected for immunohistochemistry analyses. FGFR4 IHC was performed using a rabbit anti-FGFR4 monoclonal antibody (1:200 dilution, Abcam: ab41948) as previously described (Wei, You et al. 2018).

IHC for ER, PR, HER2, Ki67 and p53 (ZSBG-BIO: ZM-0104, ZM-0215, ZM-0065, ZM-0165, ZM-0405) was performed similarly. Staining for ER and PR was considered negative if < 1% of tumor cell nuclei were stained (Hammond, Hayes et al. 2010). The expression of HER2 was evaluated with the HercepTest kit (Dako) and scored as 0, 1+, 2+ and 3+. Scores of 0 and 1+ were considered to be negative. Scores of 2+ were insufficient to determine positive or negative status; thus, HER-2/neu status confirmed by fluorescence in situ hybridization (FISH) was added (Hsu, Ho et al. 2002). Ki67 scores of 30% or above were considered positive (Coates, Winer et al. 2015). p53 status was defined as positive when more than 10% of the tumor cells stained positive (Sun, Liang et al. 2015).

The expression of FGFR4 was evaluated by multiplying the intensity by the percent reactivity extension values. The intensity of staining was scored as no staining (0), weak staining (1), moderate staining (2) and strong staining (3), while the percent reactivity extension value was scored as a continuous variable (<10%=0, 10-30%=1, 30-50%=2, >50%=3). A cut-off value of 4 was used to categorize FGFR4 expression into high and low (Huang, Feng et al. 2015). All staining was scored by the original two pathologists and a senior pathologist.

Statistical analysis

We performed statistical analyses with SPSS software version 22.0 (SPSS, Chicago, IL). We used the chi-square test to conduct the association analysis between FGFR4 protein expression and clinicopathological variables. A Cox regression model was performed for univariate and multivariate survival analyses, and the Kaplan-Meier method was employed to estimate the OS of TNBC patients. $P < 0.05$ was considered statistically significant.

Results

Patient characteristics

A total of 282 TNBC patients were enrolled in the present study. Patient characteristics: The mean age of the patients was 49.6 ± 10.2 . A total of 218 patients (77.3%) and 64 patients (22.7%) were classified as stage I/II and stage III, respectively. There were 249 patients (88.3%) whose tumor diameters were less than or equal to 2 cm, whereas 33 (11.7%) had tumor diameters greater than 2 cm. Other detailed clinicopathological features of the patients are shown in Table 1.

Associations between FGFR4 protein expression and clinicopathological features in TNBC

The expression of FGFR4 protein is shown in Figure 1. In total, 154 (54.6%) patients had high FGFR4 expression, and the remaining 128 (45.4%) had low FGFR4 expression. As indicated in Table 2, statistically significant associations between high expression of FGFR4 and LNM and p53 status were noted. Patients with high FGFR4 expression were more likely to have LNM ($P=0.033$, $R=0.127$) and p53-positive status ($P=0.019$, $R=0.140$). Nevertheless, the associations between high FGFR4 status and other clinicopathological characteristics, such as pTNM stage, tumor size, pathological grade, vessel cancer embolus and status of Ki67, were not significant.

Univariate and multivariate analyses of the prognostic value of FGFR4 expression in TNBC

We conducted univariate and multivariate analyses to evaluate the clinical prognostic value of FGFR4 in patients with TNBC (Table 3). The univariate analysis was performed first, and the results showed that pTNM stage ($P < 0.001$), tumor size ($P=0.03$), LNM ($P=0.002$), Ki67 status ($P=0.007$) and FGFR4 expression ($P=0.016$) were significant prognostic predictors in the present population. There was no prognostic value of other features. Furthermore, the statistically significant factors ($P < 0.05$) were selected for a final model to perform multivariate analysis on the same group of patients. pTNM stage ($P=0.004$), Ki67 status ($P=0.017$) and FGFR4 expression ($P=0.016$) were found to be independent prognostic factors, whereas tumor size and LNM were not.

Kaplan-Meier survival analysis

Kaplan-Meier analysis was used to evaluate the survival of TNBC patients. The survival information for the patients is shown in Figure 2. TNBC patients with high FGFR4 expression were likely to have

significantly poorer OS ($P=0.015$). It was suggested that high FGFR4 expression was associated with worse OS in TNBC patients.

Discussion

To investigate the role of FGFR4 in TNBC, we evaluated a substantially large patient cohort with long-term follow-up by analysis of FGFR4 protein expression and its association with clinicopathological features. A total of 282 TNBC patients were enrolled for evaluation via IHC. Our results revealed that high expression of the FGFR4 protein was associated with LNM and p53 status. Univariate analysis indicated that FGFR4 protein expression might be a prognostic predictor, and multivariate analysis showed that FGFR4 protein expression was an independent prognostic factor. Kaplan-Meier curves showed that high expression of FGFR4 protein was associated with worse outcomes. No significant correlation between FGFR4 expression and other clinical characteristics was found.

FGFR4 is encoded by the *FGFR4* gene, which is located at chromosome 5q35-qter (Jiang, Sun et al. 2015). Physiologically, FGFR4 is involved in embryonic development, angiogenesis and tissue differentiation (Thisse and Thisse 2005) and participates in regulating bile acid production, metabolism, muscle differentiation and tissue repair (Yu, Wang et al. 2000, Tomlinson, Fu et al. 2002, Yu, Zheng et al. 2004, Zhao, Caretti et al. 2006). FGFR4 is also involved in cancer development and progression.

Previous studies have shown that FGFR4 protein is highly expressed in many cancers, such as lung cancer, gastric cancer, colorectal cancer, and breast cancer (Penault-Llorca, Bertucci et al. 1995, Li, Zhang et al. 2014, Huang, Feng et al. 2015, Inokuchi, Murase et al. 2017). The present study is in line with those. FGFR4 was highly expressed in our TNBC patients, among whom 154 (54.6%) had high FGFR4 expression. Inokuchi M et al. (Inokuchi, Murase et al. 2017) and Murase H et al. (Murase, Inokuchi et al. 2014) found that high expression of FGFR4 was associated with LNM in gastric cancer, and the FGFR4 polymorphism Gly388Arg was reported to be correlated with LNM in many cancers (Jiang, Sun et al. 2015, Shim, Shin et al. 2016, Quintanal-Villalonga, Carranza-Carranza et al. 2017). We also found that high levels of FGFR4 expression had a relationship with LNM in our previous study (Wei, You et al. 2018). Consistent with these findings, our data revealed that high FGFR4 expression was correlated with LNM in TNBC patients. In addition, a significant correlation was observed between FGFR4 and the status of p53. However, there was no correlation between these factors in gastric cancer (Chen, Shen et al. 2015). The reasons for the opposite results might be different types of cancer or individual differences. Mutations in p53 are the most common mutations in TNBC, and approximately 60-88% of TNBC or basal-like breast cancers have p53 mutations (Cancer Genome Atlas 2012, Dumay, Feugeas et al. 2013). Many studies have reported that p53 status could affect chemotherapy responsiveness, but the findings were controversial. Bae SY et al. (Bae, Nam et al. 2018) reported that p53 positivity in TNBC was more sensitive to chemotherapy, but Giannakakou P et al. (Giannakakou, Poy et al. 2000) found that loss of functional p53 might facilitate the development of resistance. FGFR4 had a relationship with p53 in this study, which implies that FGFR4 may be involved in the chemotherapy responsiveness of TNBC. Thussbas C et al. suggested that the FGFR4 polymorphism Gly388Arg was associated with resistance to

chemotherapy in breast cancer (Thussbas, Nahrig et al. 2006). Tiong KH et al. found that FGFR4 and FGF19 autocrine enhanced basal-like breast cancer cell survival (Tiong, Tan et al. 2016). Xu M et al. found that high levels of FGFR4 increased glucose metabolism and led to chemoresistance in breast cancer (Xu, Chen et al. 2018).

The impact of FGFR4 on prognosis has been found in different cancers (Li, Zhang et al. 2014, Huang, Feng et al. 2015, Inokuchi, Murase et al. 2017), and we also confirmed that patients with high FGFR4 expression had worse outcomes (Wei, You et al. 2018). In the present study, TNBC patients with high FGFR4 expression tended to have shorter survival times than those with low FGFR4 expression (Figure 2). Our univariate analysis indicated that FGFR4 had prognostic value, and multivariate analysis indicated that FGFR4 was an independent prognostic indicator (Table 3). These findings suggest that FGFR4 may have important effects on TNBC.

Conclusion

In summary, we investigated the relationship between FGFR4 protein expression and TNBC prognosis, and we confirmed that FGFR4 had an effect on TNBC. FGFR4 was correlated with LNM, p53 status and a worse TNBC prognosis. Our findings suggest that FGFR4 may be used as a prognostic marker for TNBC. Because of the scale and method of our study, there are still many limitations. Therefore, more studies are needed to determine the detailed mechanism of action of FGFR4.

Abbreviations

TNBC, triple-negative breast cancer; FGFR4, fibroblast growth factor receptor 4; LNM, lymph node metastasis; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor; RTK, receptor tyrosine kinase; OS, overall survival; IHC, immunohistochemistry

Declarations

Ethics approval and consent to participate: This study was approved by the ethics committee of Harbin Medical University. All procedures performed in this study involving human participants were in accordance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Written informed consent was obtained from all individual participants included in the study.

Consent for publication: Not applicable.

Availability of data and materials: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare no conflict of interests.

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Authors' contributions: This work was designed and conceived by WW, XL and YJ. The experiment procedures and data analysis were carried out by JL, YW, QS, LA, SS and XZ. The manuscript was prepared by WW, DP and YJ. All authors read and approved the final manuscript.

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Tables

Table 1. Summary of patient characteristics.

Characteristics	Frequency(n[%])
Patients (n)	282
Age	49.6±10.2
pTNM Stage	
I, II	218(77.3%)
III	64(22.7%)
Tumour size (cm)	
≤2	249(88.3%)
>2	33(11.7%)
Pathological grade	
II	195(69.1%)
III	87(30.9%)
LN	
Negative	159(56.4%)
Positive	123(43.6%)
Vessel Cancer Embolus	
Negative	252(89.4%)
Positive	30(10.6%)
Ki67 status	
<30%	95(33.7%)
≥30%	187(66.3%)
P53 status	
Negative	137(48.6%)
Positive	145(51.4%)

Table 2. Correlation between FGFR4 expression and clinicopathological characteristics in TNBC.

Characteristics	Cases	FGFR4 protein expression		p-value
		High expression(%)	Low expression(%)	
pTNM stage				0.558
I, II	218	117(53.7)	101(46.3)	
III	64	37(57.8)	27(42.2)	
Tumour size (cm)				0.462
≤2	249	134(53.8)	115(46.2)	
>2	33	20(60.6)	13(39.4)	
Pathological grade				0.155
II	195	101(51.8)	94(48.2)	
III	87	53(60.9)	34(39.1)	
LNM				0.033
Negative	159	78(49.1)	81(50.9)	
Positive	123	76(61.8)	47(38.2)	
Vessel Cancer Embolus				0.811
Negative	252	137(54.4)	115(45.6)	
Positive	30	17(56.7)	13(43.3)	
Ki67 status				0.824
Negative	95	51(53.7)	44(46.3)	
Positive	187	103(55.1)	84(44.9)	
P53 status				0.019
Negative	137	65(47.4)	72(52.6)	
Positive	145	89(61.4)	56(38.6)	

Table 3. Prognostic factors in the Cox proportional hazards model.

Variables	Univariate analysis			Multivariate analysis		
	HR	95%CI	P	HR	95%CI	P
pTNM stage (I+II vs. III)	2.183	(1.425,3.343)	<0.001	1.897	(1.229,2.927)	0.004
Tumour size (≤2 cm vs. >2 cm)	1.786	(1.058,3.014)	0.03	1.614	(0.954,2.732)	0.074
Pathological stage (II vs. III)	1.153	(0.756,1.759)	0.509			
LNM (negative vs. positive)	1.899	(1.276,2.826)	0.002	1.226	(0.730,2.057)	0.441
Vessel Cancer Embolus (negative vs. positive)	1.286	(0.717,2.306)	0.398			
Ki67 status (negative vs. positive)	1.931	(1.201,3.103)	0.007	1.798	(1.112,2.909)	0.017
P53 status (negative vs. positive)	1.073	(0.721,1.595)	0.729			
FGFR4 expression (low vs. high)	1.660	(1.098,2.510)	0.016	1.665	(1.100,2.520)	0.016

Figures

Figure 1

Immunohistochemical staining of FGFR4 in TNBC tissues. Staining for each specimen is shown at two magnifications: left, 200×; right, 400×. FGFR4 protein low-expression specimens (a, b); FGFR4 protein high-expression specimens (c, d).

Figure 2

Kaplan-Meier analysis for overall survival (OS) of the TNBC patients included in this study based on the expression of the FGFR4 protein.