Int J Clin Exp Pathol 2017;10(6):6544-6551
www.ijcep.com /ISSN:1936-2625/IJCEP0052131

Original Article

Clinical implications of TUFT1 protein expression and correlation with RelA protein in breast cancer

Weiguang Liu[1*], Jian Du[2*], Min Zhao[1], Lei Zhang[1], Zining Jin[1], Bo Chen[1]

[1]*Department of Breast Surgery, The First Hospital of China Medical University, Shenyang, Liaoning Province, China;* [2]*Department of Hepatobiliary Surgery, The First Affiliated Hospital of Dalian Medical University, Dalian, Liaoning Province, China.* [*]*Equal contributors and co-first authors.*

Received March 3, 2017; Accepted May 3, 2017; Epub June 1, 2017; Published June 15, 2017

Abstract: Tuftelin 1 (TUFT1), which plays an important role in the initial stages of ectodermal enamel mineralization, is widely expressed in various embryonic and adult tissues, and in some tumor cells. The aim of the study was to investigate the clinical and prognostic implications of TUFT1 in breast cancer. We assessed TUFT1 expression levels in 180 cases of invasive breast cancer by immunohistochemical methods. The positive expression levels of TUFT1 protein in invasive breast were significantly higher than adjacent normal tissues ($P = 0.000$) and TUFT1 mRNA expression levels were higher in MCF-7, T-47D, and MDA-MB-231 breast cancer cell lines than in the MCF-10A breast cell line ($P < 0.05$). The positive expression of TUFT1 protein was related to tumor size, histological type, and lymph node metastasis ($P = 0.007$, $P = 0.027$, and $P = 0.000$, respectively). Univariate and multivariate analyses revealed that positive TUFT1 expression was significantly associated with HER-2 and RelA expression ($P < 0.05$). Disease-free survival (DFS) analysis and survival analysis showed that TUFT1-positive tumors also exhibited a significantly higher postoperative recurrence, distant metastasis rate and poor survival compared with TUFT1-negative tumors ($P = 0.000$, $P = 0.012$, respectively, log-rank test). In addition, TUFT1 expression was increased in a dose-dependent manner when RelA was inhibited by JSH-23. In summary, TUFT1 might be a potential new biomarker for breast cancer.

Keywords: Breast cancer, TUFT1, biomarker, RelA, survival

Introduction

Tuftelin (TUFT1) was originally identified and characterized in developing and mature extracellular enamel, the unique and highly mineralized epithelial tissue that covers vertebrate teeth [1]. It has now also been shown to be expressed in the morula; embryonic stem cells; various normal soft tissues such as the brain (specifically neurons), kidneys, adrenal gland, liver, and testis; and several tumor cells. Therefore, TUFT1 may be involved in the function of mesenchymal stem cells, as well as the differentiation of neural cells mediated by nerve growth factor [2, 3]. Furthermore, one study found that 1% O_2 in human HepG2 and MCF-7 cell lines allowed TUFT1 mRNA to adapt to hypoxia, and this adaptation to a hypoxic environment is necessary for tumorigenesis [4]. This suggests that TUFT1 may not only play a role in tooth development and mineralization, but is also likely to have a wider range of functions in the body that can promote the development of certain diseases, and even the occurrence and development of tumors. The expression and biological function of TUFT1 in tumors remains understudied. From the Cancer Genome Atlas database, we previously analyzed differentially expressed genes (cancer and adjacent tissues) using large clinical samples of breast cancer and found that TUFT1 expression was significantly increased in breast cancer tissues. However, whether TUFT1 plays a role in breast cancer is unclear. Therefore, in current study, we further investigated the expression of TUFT1 by immunohistochemical methods and predicted an unfavorable prognosis in breast cancer.

Materials and methods

Human specimens and cell lines

We evaluated 180 patients with histologically confirmed invasive breast cancer that under-

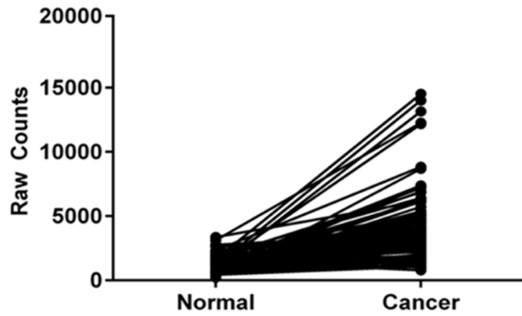

Figure 1. TUFT1 expression in TCGA Breast. Total samples: 106 pairs; Samples unchanged: 33 pairs; Samples up: 72 pairs; Samples down: 1 pairs (P = 7.77E-55). The horizontal axis represents cancer and adjacent normal tissues, the vertical axis represents the raw data of expression levels of each sample. Each sample is a broken line, the trend of broken line shows out the changes of the gene in all samples.

went radical operations in the Surgical Oncology Department at the First Affiliated Hospital of the China Medical University between January 2010 and September 2011. The inclusion criteria were as follows: (i) curative operations were performed; (ii) resected specimens were pathologically examined; (iii) more than 10 lymph nodes were pathologically examined postoperatively, and (iv) a complete medical record was available. The study protocol was approved by the Ethics Committee of China Medical University.

Human breast cancer cell line MCF-7, T-47D, MDA-MB-231 and Human breast cell line MCF-10A, were obtained from Chinese Academy of Sciences (China), American Type Culture Collection (USA), American Type Culture Collection (USA), Chinese Academy of Sciences (China), respectively. Three cancer cells were cultured in RPMI-1640 with 10% fetal calf serum (FCS), MCF-10A was cultured in DMEM/F12 (Invitrogen) with 5% horse serum, and incubated in a 37°C in an atmosphere containing 5% CO_2.

Immunohistochemistry

A polyclonal rabbit antihuman TUFT1 antibody (dilution 1:100) was obtained from RayBiotech. Monoclonal mouse antihuman estrogen receptor (ER) antibody (dilution 1:100), monoclonal mouse antihuman RelA antibody (dilution 1:100), and monoclonal mouse anti-HER2/neu antibody (dilution 1:100) were purchased from ABCAM. All cases of breast cancer tissues were de-waxed with xylene, gradually hydrated with gradient alcohol, and then washed with phosphate buffer saline (PBS). The sections were incubated overnight at 4°C with the primary antibody after incubating with 3% H_2O_2 for 10 min at room temperature. Following washings with PBS, the secondary antibody (poly peroxidase-anti-mouse/rabbit immunoglobulin; Maixin Biotech Co. Ltd.) was then applied to the sections for 30 min at 37°C. The immunoreactive products were visualized by catalysis of 3,3-diaminobenzidine following extensive washings. Sections were then counterstained in Gill's hematoxylin and dehydrated in ascending grades of ethanol before clearing in xylene and mounting under a coverslip. A negative control was prepared by substituting the primary antibody over PBS.

Expression of TUFT1, ER, PR and RelA was classified semi-quantitatively according to the following criteria: 0, if < 1% of the cells were stained; 1+, if < 10% of the cells were stained; 2+, if 10-50% of the cells were stained; and 3+, if > 50% of the cells were stained. We considered grades 1+, 2+, and 3+ as positive. Expression of HER-2 was scored according to the standardized criteria established by Wolff et al. [5]; in this study, only a score of 3+ (defined as uniform intense membrane staining of 30% of invasive tumor cells) was considered positive. Fluorescence in situ hybridization was conducted after primary immunohistochemistry testing yielded an immunostaining score of 2+ [6]. Two independent pathologists, both blinded to the patients' clinical status, made these judgments.

RNA extraction and quantitative real-time PCR

Total RNA of three breast cancer cell lines and one breast cell line was extracted with Trizol (Invitrogen), which was followed by reverse transcription, according to the manufacturer's instructions (Invitrogen). Quantitative real-time PCR was used to determine TUFT1 expression levels using the following primers: TUFT1, forward: TCA GAC TGT GTG GCT TTT GAG, reverse: GTC AGC ATT GTT GCT CCG AAG; GAPDH, used as a control, forward: TGA CTT CAA CAG CGA CAC CCA, reverse: CAC CCT GTT GCT GTA GCC AAA.

The relationship between TUFT1 and RelA confirmed by western blot

JSH-23 (RelA inhibitor, purchased from MedChemexpress LLC) was added into MDA-MB-

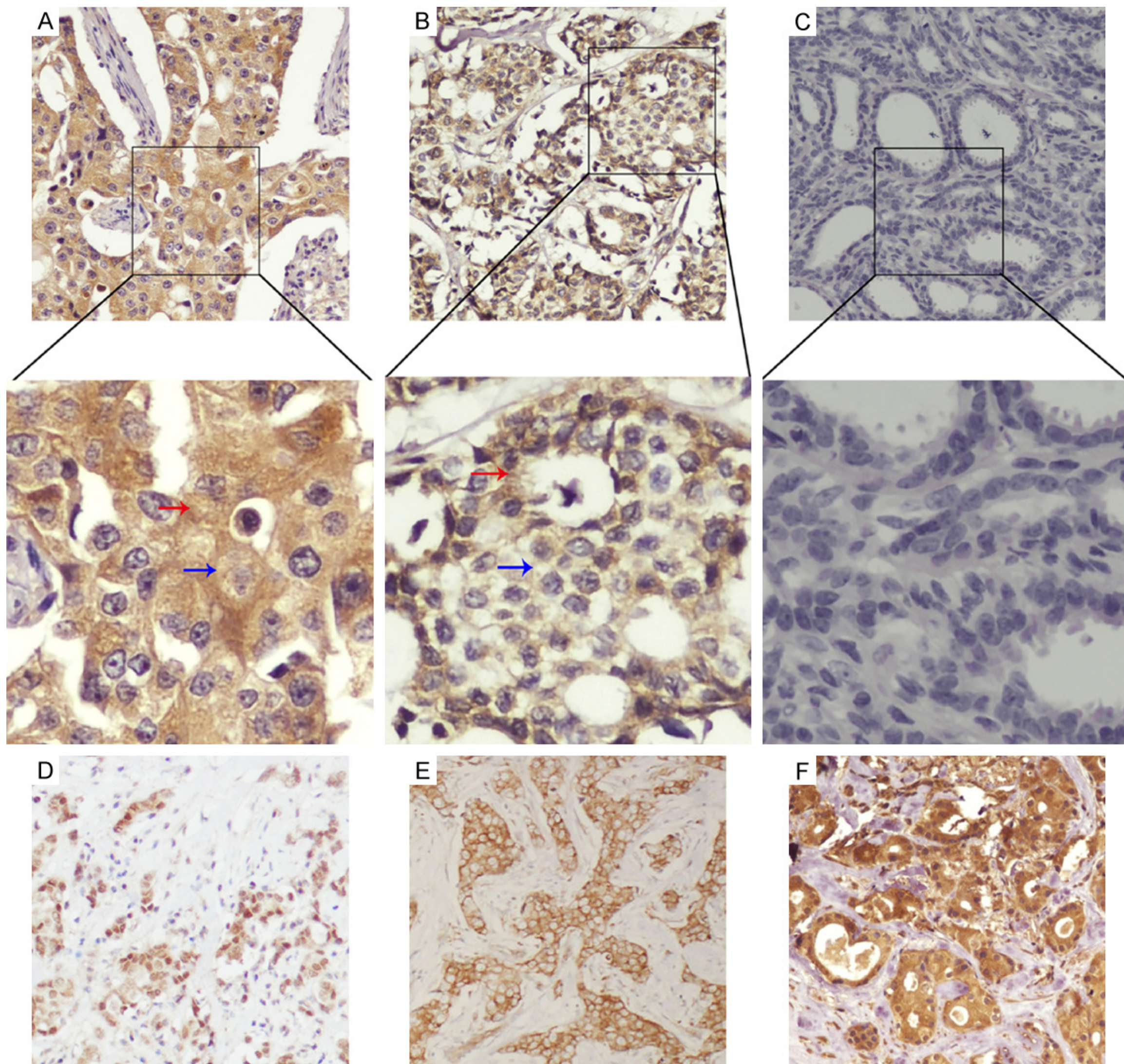

Figure 2. Strong, medium and negative expressions of TUFT1 are shown in (A-C), respectively. Blue arrow represents TUFT1 staining in the cytomembrane, red arrow represents TUFT1 staining in the cytoplasm. The expression of ER, HER2 and RelA in serial sections is presented in (D-F), respectively.

231 cells with the dose of 0 umol, 1 umol, 5 umol when the cell planking density reached about 60% [7]. Then samples incubated for 24 h were collected when the cell density reached about 80%. Cells were collected following the trypsin digestion, washed with PBS, and lysed with radioimmunoprecipitation assay (RIPA) lysis buffer with phenylmethane sulfonyl fluoride (PMSF). Total protein levels were determined by using BCA Protein Assay Kit (Pierce). The same amount of protein was taken from each sample, the same volume of 2× loading buffer was added, and the sample was cooked in boiling water for 10 min. Proteins were separated on 10% SDS-PAGE, and transferred to polyvinylidene fluoride (PVDF) membranes (Amersham), which were blocked with Tris-buffered saline with Tween 20 (TBST) solution containing 5% skimmed milk for 1 h, and the membranes were incubated with primary antibodies overnight. PVDF membranes were incubated with the secondary antibodies for 2 h, and the results were visualized using the ECL Plus Western Blotting kit (Amersham).

Statistical analysis

All data were analyzed with SPSS statistics software (version 17.0). Relationships between tumor markers and other parameters, results

Table 1. The relationship between TUFT1 expression and the clinicopathological factors (n = 180)

Varible	n	TUFT1$^-$	TUFT1$^+$	P varible
Tissue				0.000
Cancer tissue	180	64	116	
Adjacent tissue	60	50	10	
Age				0.132
≥ 40	153	58	95	
< 40	27	6	21	
Tumor size				0.007
T1	65	30	35	
T2	98	33	66	
T3, 4	17	1	16	
Histological grades				0.027
I	42	20	23	
II	92	35	57	
III	46	9	36	
Lymph node metastasis				0.000
Negative	86	42	44	
Positive	94	22	72	

Table 2. Correlations between TUFT1 expression and immunohistochemical markers

Varible	n	TUFT1$^-$	TUFT1$^+$	P varible
ER				0.091
-	77	22	55	
+	103	42	61	
PR				0.381
-	81	26	55	
+	99	38	61	
HER2				0.032
-	145	57	88	
+	35	7	28	
RelA				0.001
-	73	15	58	
+	107	49	58	

ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; PR, progesterone receptor.

Table 3. Multivariate analysis of the factors related to TUFT1 expression

Characteristic	B	Exp (B)	95% CI for Exp (B)	P varible
ER	-0.124	0.884	0.351-2.222	0.792
PR	0.775	2.171	0.863-5.462	0.099
HER2	1.903	6.709	2.533-17.765	0.000
RelA	-0.704	0.495	0.280-0.873	0.015

CI, confidence interval.

from qPCR and western blot were studied using a chi-squared test, Fisher's extract test, or independent t-test. Spearman's correlation and logistic regression analyses were used to determine the correlation among ER, PR, HER-2, RelA, and TUFT1 protein expression. Disease-specific survival was analyzed using the Kaplan-Meier method. The log-rank test was used to analyze survival differences. A *P* value of less than 0.05 was considered statistically significant.

Results

Patient characteristics

We analyzed TUFT1 expression in large datasets from The Cancer Genome Atlas (TCGA) databases. The TCGA RNA Seq data showed that TUFT1 was significantly upregulated in over 67.92% of breast cancer tissues (*n* = 106) compared with adjacent normal breast tissues (P = 7.77E-55) (**Figure 1**).

TUFT1 staining occurred mainly in the cytoplasm and cytomembrane of the breast cancers, and the cytoplasmic staining was stronger than that observed in the membrane. Strong, medium, and negative expression of TUFT1 was shown in **Figure 2A-C**, respectively. ER and PR staining occurred in the nucleus of the breast cancers, while HER2 staining was located in the breast cancer membrane. RelA staining was located mainly in the cytoplasm and cytomembrane. Positive expression of ER, HER2 and RelA in serial sections was presented in **Figure 2D-F**.

The mean age of the 180 patients studied was 50.21 years (range 26-77 years). Immunohistochemistry revealed a TUFT1 positive rate of 64.4% (116/180) in breast cancers, which was significantly higher than 16.7% (10/60) in adjacent normal breast tissues (P = 0.000). Moreover, the positive expression of TUFT1 protein was associated with tumor size, histological grade, and lymph node metastasis of breast cancer (P = 0.007, P = 0.027, and P = 0.000, respectively, **Table 1**).

TUFT1 expression and immunohistochemical markers

Univariate analysis showed that the TUFT1-positive expression rate was significantly high-

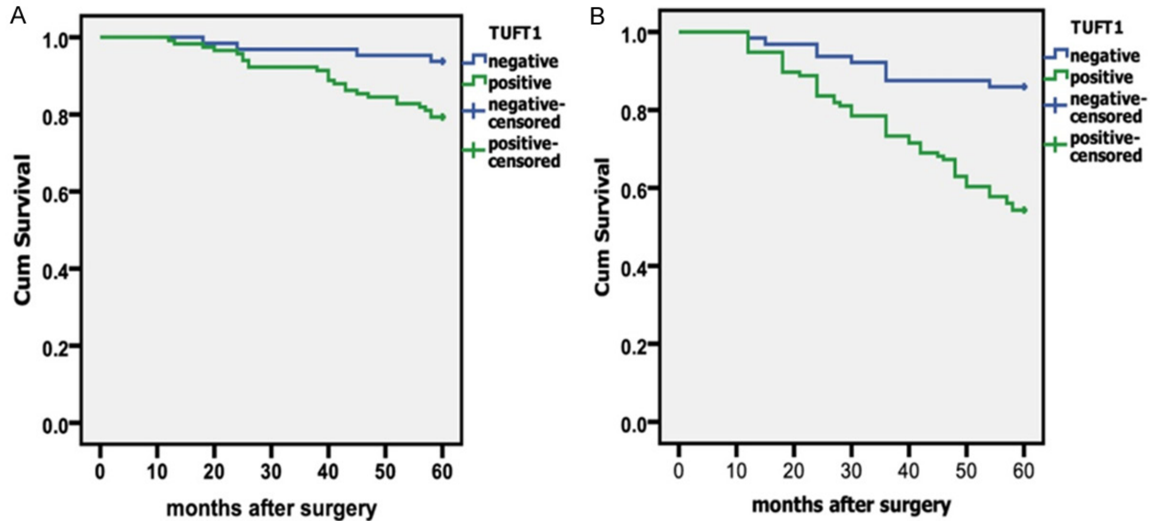

Figure 3. A. TUFT1 was Associated with Breast Cancer-specific Survival (P = 0.012, log-rank test); B. TUFT1 was Associated with Breast Cancer-specific Disease-free survival (P = 0.000, log-rank test).

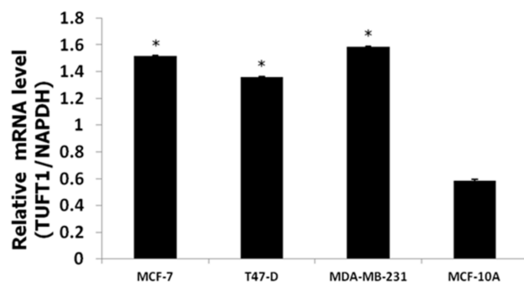

Figure 4. Levels of TUFT1 mRNA were analyzed by Real time-PCR in three breast cancer cell lines and the breast cell line. Data of TUFT1/NAPDH shown are the mean ± S.D. (*t test P < 0.05, n = 3).

er in HER2+ and RelA- cases than in HER2- and RelA+ cases (P = 0.032 and P = 0.001, respectively) (**Table 2**). However, there was no significant difference in TUFT1 expression in ER or PR cases (P = 0.091 and P = 0.381, respectively) (**Table 2**). We performed a logistic analysis on the above factors in order to exclude the effects of confounding factors. Multivariate analysis showed that ER and PR were not associated with TUFT1 expression (P = 0.792 and P = 0.099, respectively), but HER2 was positive associated with TUFT1 expression (Exp (B) = 6.709, P = 0.000), and RelA was negatively associated with TUFT1 expression (Exp (B) = 0.495, P = 0.015, respectively) (**Table 3**).

Prognostic analysis

Overall, five (2.8%) patients received no further treatment after surgery, 45 (25%) patients received chemotherapy and radiotherapy as well as endocrine therapy, 58 (32.2%) patients received chemotherapy and endocrine therapy, 41 (22.8%) patients received chemotherapy alone, and the remaining four (2.2%) patients received endocrine therapy alone. Survival analysis revealed that the positive expression of TUFT1 was associated with breast cancer-specific survival in 180 cases (P = 0.012, log-rank test, **Figure 3A**). Furthermore, TUFT1 status was significantly associated with breast cancer disease-free survival (P = 0.000, log-rank test, **Figure 3B**).

TUFT1 expression in four breast cancer cell lines

TUFT1 mRNA expression levels were higher in three breast cancer cell lines, including MCF-7, T-47D, and MDA-MB-231, than in the MCF-10A breast cell line by real-time PCR (P < 0.05, **Figure 4**).

RelA inhibition can influence TUFT1 expression

TUFT1 expression was evaluated by western blot and we demonstrated that TUFT1 expression was increased in a dose-dependent manner when RelA was inhibited by JSH-23 (P < 0.05, **Figure 5**) and this revealed that RelA was a negative regulator of TUFT1.

Discussion

This study investigated the clinical and prognostic implications of TUFT1 in breast cancer

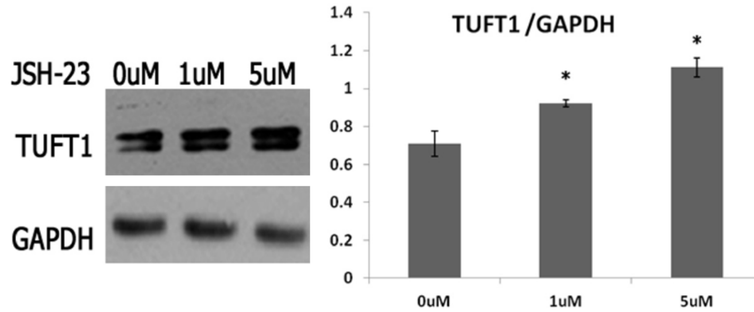

Figure 5. JSH-23 was added into MDA-MB-231 cells with the dose of 0 umol, 1 umol, 5 umol when the cell planking density reached about 60%. Then samples incubated for 24 h were collected when the cell density reached about 80% and confirmed by western blot. Data of TUFT1/NAPDH shown are the mean ± S.D. (*t test P = 0.016 as 1 uM group compared to 0 uM group, *t test P = 0.012 as 5 uM group compared to 1 uM group, n = 3, respectively).

using immunohistochemical methods. Breast cancer is a clinically heterogeneous disease. Histological type, grade, tumor size, lymph-node involvement, and ER and HER2 receptor status influence the prognosis and probability of response to systemic therapies, but do not fully capture the varied clinical course of breast cancer [8-10]. Endocrine therapy and trastuzumab adjuvant treatment have benefited patients with ER+ and/or PR+ cancers and HER2-overexpression [11, 12]. Therefore, the status of these proteins has prognostic ramifications in breast cancer. Consequently, much effort is focused on understanding the clinical significance of known markers, finding relationships between them, and discovering new ones. These efforts are aimed at optimizing the utilization of the available therapies and developing novel therapies based on improved cancer models.

TUFT1 is an acidic protein, first discovered, mapped, and cloned from a cDNA library of ameloblasts. In addition to TUFT1 expression in the developing and mineralizing enamel, partial TUFT1 cDNA sequences have also been detected in many normal soft tissues [1-3]. Moreover, Saarikoski et al. reported the induction of TUFT1 in cancer cells during hypoxia [4]. At present, few studies have investigated the role of TUFT1 in cancer. Bin Zhou [13] showed that TUFT1 is overexpressed in pancreas cancer (PC) tissues compared with adjacent normal pancreas tissues, and TUFT1 expression is significantly associated with lymph node metastasis and advanced PC stage. According to our results, TUFT1 protein levels were elevated in breast cancer tissues compared with adjacent normal tissues, and its mRNA levels were higher in MCF-7, T-47D, and MDA-MB-231 breast cancer cell lines, than in the MCF-10A breast cell line. In addition, we found that the positive TUFT1 expression was 16.7% in adjacent normal breast tissues, this revealed that TUFT1 could also express in normal breast tissues. However, our results showed that the positive staining intensity of adjacent normal breast tissues was mostly low (+), and the positive staining intensity of the tumor tissues was mostly medium or strong (++ or +++). These results indicate that TUFT1 could be a candidate marker assisting breast cancer diagnosis. Here we showed that TUFT1 overexpression was positively correlated with tumor size, histological grade, and lymph node metastasis. Disease-free survival analysis showed that TUFT1-positive tumors also exhibited a significantly higher postoperative recurrence and distant metastasis rate compared with TUFT1-negative tumors, and survival analysis revealed that TUFT1 was associated with breast cancer-specific survival. These results indicate that TUFT1 may be involved in the progression process of breast cancer and serve as a poor prognostic factor in breast cancer. Univariate and multivariate analyses revealed that TUFT1 expression was positively correlated with HER2 expression. This suggests that TUFT1 might play an important function in HER2 signaling pathway mediated breast carcinoma development and metastasis.

Activation of the NF-κB signaling pathway has been reported in breast cancer and other tumors [14]. The NF-κB family comprises five types of Rel-domain proteins, RelA/p65, c-rel, RelB, p50/NF-κB1, and p52/NF-κB2. The NF-κB signaling pathway includes cancerous and tumor suppressor activity. For its anti-cancer activity, NF-κB has been shown to mediate apoptosis in a variety of cell types [15]. The activation of the NF-κB pathway is significantly associated with p53-mediated apoptosis [16]

and individual expression of RelA can induce apoptosis in some types of cells [17]. Although the mechanism of pro-apoptosis of RelA is not fully clear in cancer, several studies have stated the pro-apoptosis function of RelA in some contexts. RelA could induce cytoplasmic nucleophosmin-mediated apoptosis by facilitating the mitochondrial accumulation of BAX [18] or by activating RelA-IRF1-CDK4 signaling axis to induce apoptosis [19]. Only one study showed that RelA might regulate promotion of TUFT1 to influence its function in H9c2 cells, but the mechanism was unclear [20]. Our univariate and multivariate analyses findings showed a significant negative correlation between TUFT1 and RelA expression, and western blot confirmed that TUFT1 expression was increased in a dose-dependent manner when RelA was inhibited by JSH-23 and this revealed that RelA was a negative regulator of TUFT1. However, the in-depth mechanism of interaction between TUFT1 and RelA remains unclear. These results may reveal a new transduction mechanism mediated by the NF-κB signaling pathway in breast cancer.

Acknowledgements

This work was supported by National Natural Science Foundation Project (No. 81372811).

Disclosure of conflict of interest

None.

Address correspondence to: Bo Chen, Department of Breast Surgery, The First Hospital of China Medical University, Shenyang 110001, Liaoning Province, China. E-mail: chbyxl@163.com

References

[1] Deutsch D, Palmon A, Fisher LW, Kolodny N, Termine JD, Young MF. Sequencing of bovineenamelin (“tuftelin”) a novel acidic enamel protein. J Biol Chem 1991; 266: 16021-16028.

[2] Deutsch D, Leiser Y, Shay B, Fermon E, Taylor A, Rosenfeld E, Dafni L, Charuvi K, Cohen Y, Haze A, Fuks A, Mao Z. The human tuftelin gene and the expression of tuftelin in mineralizing and nonmineralizing tissues. Connect Tissue Res 2002; 43: 425-434.

[3] Leiser Y, Blumenfeld A, Haze A, Dafni L, Taylor AL, Rosenfeld E, Fermon E, Gruenbaum-Cohen Y, Shay B, Deutsch D. Localization, quantification, and characterization of tuftelin in soft tissues. Anat Rec 2007; 290: 449-454.

[4] Saarikoski ST, Rivera SP, Hankinson O. Mitogen-inducible gene 6 (MIG-6), adipophilin and tuftelin are inducible by hypoxia. FEBS Lett 2002; 530: 186-190.

[5] Wolff AC, Hammond ME, Schwartz JN, Hagerty KL, Allred DC, Cote RJ, Dowsett M, Fitzgibbons PL, Hanna WM, Langer A, McShane LM, Paik S, Pegram MD, Perez EA, Press MF, Rhodes A, Sturgeon C, Taube SE, Tubbs R, Vance GH, van de Vijver M, Wheeler TM, Hayes DF; American Society of Clinical Oncology; College of American Pathologists. American society of clinical oncology college of American pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. J Clin Oncol 2007; 25: 118-45.

[6] Phillips KA, Marshall DA, Haas JS, Elkin EB, Liang SY, Hassett MJ, Ferrusi I, Brock JE, Van Bebber SL. Clinical practice patterns and cost effectiveness of human epidermal growth receptor 2 testing strategies in breast cancer patients. Cancer 2009; 115: 5166-5174.

[7] Liu B, Sun L, Liu Q, Gong C, Yao Y, Lv X, Lin L, Yao H, Su F, Li D, Zeng M, Song E. A cytoplasmic NF-κB interacting long noncoding RNA blocks IκB phosphorylation and suppresses breast cancer metastasis. Cancer Cell 2015; 27: 370-381.

[8] Chen KT, Lee TW, Lo JM. In vivo examination of 188Re (I)-tricarbonyllabeled trastuzumab to target HER2-overexpressing breast cancer. Nucl Med Biol 2009; 36: 355-61.

[9] Jacquemier J, Charafe-Jauffret E, Monville F, Esterni B, Extra JM, Houvenaeghel G, Xerri L, Bertucci F, Birnbaum D. Association of GATA3, P53, Ki67 status and vascular peritumoral invasion are strongly prognostic in luminal breast cancer. Breast Cancer Res 2009; 11: R23.

[10] Vora HH, Patel NA, Rajvik KN, Mehta SV, Brahmbhatt BV, Shah MJ, Shukla SN, Shah PM. Cytokeratin and vimentin expression in breast cancer. Int J Biol Markers 2009; 24: 38-46.

[11] Ross JS, Slodkowska EA, Symmans WF, Pusztai L, Ravdin PM, Hortobagyi GN. The HER-2 receptor and breast cancer: ten years of targeted anti-HER-2 therapy and personalized medicine. Oncologist 2009; 14: 320-68.

[12] Ma CX, Sanchez CG, Ellis MJ. Predicting endocrine therapy responsiveness in breast cancer. Oncology 2009; 23: 133-42.

[13] Zhou B, Zhan H, Tin L, Liu S, Xu J, Dong Y, Li X, Wu L, Guo W. TUFT1 regulates metastasis of pancreatic cancer through HIF1-snail pathway induced epithelial-mesenchymal transition. Cancer Lett 2016; 382: 11-20.

[14] Singh S, Shi Q, Bailey ST, Palczewski MJ, Pardee AB, Iglehart JD, Biswas DK. Nuclear factor-kappaB activation: a molecular therapeutic target for estrogen receptor-negative and epidermal growth factor receptor family receptor-positive human breast cancer. Mol Cancer Ther 2007; 6: 1973-1982.

[15] Jing H, Lee S. NF-κB in cellular senescence and cancer treatment. Mol Cells 2014; 37: 189-195.

[16] Ryan KM, Ernst MK, Rice NR, Vousden KH. Role of NF-kappaB in p53-mediated programmed cell death. Nature 2000; 404: 892-897.

[17] Ricca A, Biroccio A, Trisciuoglio D, Cippitelli M, Zupi G, Del Bufalo D. relA over-expression reduces tumorigenicity and activates apoptosis in human cancer cells. Br J Cancer 2001; 85: 1914-1921.

[18] Khandelwal N, Simpson J, Taylor G, Rafique S, Whitehouse A, Hiscox J, Stark LA. Nucleolar NF-κB/RelA mediates apoptosis by causing cytoplasmic relocalization of nucleophosmin. Cell Death Diff 2011; 18: 1889-1903.

[19] Kochupurakkal BS, Wang ZC, Hua T, Culhane AC, Rodig SJ, Rajkovic-Molek K, Lazaro JB, Richardson AL, Biswas DK, Iglehart JD. RelA-induced interferon response negatively regulates proliferation. PLoS One 2015; 10: e0140243.

[20] Chen Y, Decker KF, Zheng D, Matkovich SJ, Jia L, Dorn GW 2nd. A nucleus-targeted alternately spliced Nix/Bnip3L protein isoform modifies nuclear factor κB (NFκB)-mediated cardiac transcription. J Biol Chem 2013; 288: 15455-15465.