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Expression of autophagy-related proteins ATG5 and FIP200 predicts favorable disease-free survival in patients with breast cancer

Lei Wang [a, b, 1], Ling Yao [a, b, 1], Yi-Zi Zheng [a, b], Qing Xu [c], Xiu-Ping Liu [d], Xin Hu [a, b, ***], Peng Wang [e, f, **], Zhi-Ming Shao [a, b, g, *]

[a] Department of Breast Surgery, Key Laboratory of Breast Cancer in Shanghai, Fudan University Shanghai Cancer Center, Fudan University, Shanghai, China
[b] Department of Oncology, Shanghai Medical College, Fudan University, Shanghai, China
[c] Department of Finance and Control Sciences, School of Mathematical Sciences, Fudan University, Shanghai, China
[d] Department of Pathology, School of Basic Medical Sciences, Fudan University, Shanghai, China
[e] Key Laboratory of Systems Biology, Shanghai Advanced Research Institute, Chinese Academy of Sciences, Shanghai, China
[f] School of Life Science and Technology, ShanghaiTech University, Shanghai, China
[g] Institutes of Biomedical Sciences, Fudan University, Shanghai, China

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ABSTRACT

Autophagy is a self-digesting process that is primarily responsible for the removal and recycling of long-lived proteins and damaged organelles to maintain the homeostasis of the cell. Recent studies have indicated dual roles for autophagy in cancer: suppression of tumor progression and promotion of survival. In this study, we sought to investigate the prognostic value of two autophagy-related proteins, autophagy-related gene 5 (ATG5) and FAK family kinase-interacting protein of 200 kDa (FIP200), in patients with operable breast cancer. More specifically, the expression of ATG5 and FIP200 was evaluated by immunohistochemistry (IHC) in surgical specimens collected from 200 patients who were diagnosed with histologically proven invasive ductal breast cancer. A stepwise Cox multivariate analysis was then performed to construct a risk prediction model. In this retrospective cohort study, both ATG5 (HR = 0.465, 95% CI 0.247–0.872, $P = 0.017$) and FIP200 (HR = 0.521, 95% CI 0.278–0.979, $P = 0.043$) correlated with prolonged disease-free survival (DFS). In a receiver operating characteristic (ROC) analysis, the addition of ATG5 and FIP200 expression led to a significantly improved area under the time-dependent ROC curve (AUC) at 3 years (0.748 versus 0.680, $P < 0.001$) and 5 years (0.756 versus 0.699, $P < 0.001$). Collectively, our findings established the prognostic significance of ATG5 and FIP200 in patients with breast cancer.

1. Introduction

Adjuvant systemic therapy has been proven to be an effective and comprehensive approach to reducing the risk of recurrence and mortality in patients with operable breast cancer. The selection of a regimen should be individualized based on the risk recurrence category. Accordingly, numerous risk models for the development of breast cancer have been created, incorporating clinical factors with or without biomarker assays [1,2]. However, no risk prediction models are currently widely used in clinical practice. Thus, the identification of novel biomarkers that can provide additional valuable risk assessment of personalized treatment modalities is urgent.

In recent years, studies of autophagy, a conserved cellular catabolic process characterized by the self-digestion of organelles,

* Corresponding author. Department of Breast Surgery, Fudan University Shanghai Cancer Center, 270 Dong-An Road, Shanghai, 200032, PR China. Fax: +86 21 64434556.
** Corresponding author. Key Laboratory of Systems Biology, Shanghai Advanced Research Institute, Chinese Academy of Sciences, 100 Haike Road, Shanghai, 200032, PR China. Fax: +81 21 20320869.
*** Corresponding author. Key Laboratory of Breast Cancer in Shanghai, Fudan University Shanghai Cancer Center, Fudan University, 270 Dong-An Road, Shanghai, 200032, PR China. Fax: +86 21 64175590.
E-mail addresses: xinhu.nlorg@gmail.com (X. Hu), wangpeng@sari.ac.cn (P. Wang), zhimingshao@yahoo.com (Z.-M. Shao).
[1] These authors contributed equally to this work.

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have provided insight into the adaptation process for cells, which either allows tolerance of adverse conditions or triggers cell suicide mechanisms [3]. Emerging evidence regarding autophagy in cancer suggests a double-edged sword: autophagy can enable tumor cells to survive stress, including hypoxia, starvation, chemotherapy and radiotherapy, or can be tumor suppressive through the elimination of oncogenic protein substrates, toxic unfolded proteins and damaged organelles [4]. Recent studies have suggested that autophagy participates in broad crosstalk with multiple pathways that determine cell fate, including apoptosis [5].

We have previously reported the prognostic significance of the autophagy marker LC3B in neoadjuvant chemotherapy (NCT)-treated breast cancer [6]. However, in NCT-naïve specimens, the prognostic value of LC3B is confined to luminal A breast cancer [7], which prompted us to evaluate the survival correlations of other autophagy-related proteins. Autophagy involves the spatially and temporally coordinated activation of a number of molecules, among which both autophagy-related gene 5 (ATG5) and FAK family kinase-interacting protein of 200 kDa (FIP200) are key players; the deletion of either gene leads to an autophagy deficiency [8,9]. A preclinical study has shown that forced expression of ATG5 sensitizes tumor cells to anticancer drug treatment, whereas silencing ATG5 results in chemotherapy resistance [10]. However, the prognostic value of ATG5 in breast cancer has not been studied. FIP200, also known as RB1CC1, is an important component of the ULK1-FIP200-ATG13-ATG101 complex, which is functionally coupled to the negative autophagy regulator mTOR complex 1 (mTORC1) and which initiates autophagy. In particular, FIP200 inhibits G_1-S phase progression, proliferation and clonogenic survival in breast cancer cells [11]. Previous studies have demonstrated that the abnormal RB1CC1/RB1/p53 pathway is associated with poor long-term prognosis in breast cancer [12] and have revealed the prognostic significance of FIP200 in prostate cancer [13] and salivary gland cancer [14].

Our current knowledge about ATG5 and FIP200 has been largely derived from *in vitro* studies, and thus, the *in vivo* relevance of these proteins needs more rigorous investigation. In the present study, we investigated the prognostic value of ATG5 and FIP200 using the expression profiles of the two markers in 200 specimens from stage I to III breast cancer. We then developed a risk model for the development of recurrence and metastases in patients following breast cancer surgical resection, and we assessed the relationships between biomarkers, clinical patient characteristics, and disease-free survival (DFS). Our results demonstrated that ATG5 expression and FIP200 expression are strongly associated with DFS and could serve as novel prognostic markers in breast cancer.

2. Materials and methods

2.1. Breast cancer specimens

In this study, we collected clinical data and surgical specimens from 200 female patients who were diagnosed with stage I to III primary breast cancer at the Department of Breast Surgery in Fudan University Shanghai Cancer Center (FDUSCC, Shanghai, China) between August 2001 and March 2006. All patients in this cohort were histologically confirmed as having invasive ductal carcinoma and either underwent a mastectomy and axillary lymph node dissection or breast conservation surgery. DFS was calculated from the date of surgery to the date of disease relapse at a local, regional or distant site. Patients with a study end date or who were lost to follow-up were considered as censored. This study was approved by the Ethics Committee of FDUSCC, and written informed consent was provided by all patients.

2.2. Tissue microarray construction

The tissue microarrays (TMAs) were constructed as described before [15].

2.3. Immunohistochemistry

TMAs were subjected to immunohistochemical staining for the ATG5 and FIP200 proteins using a 2-step protocol (GTVision™ III). ATG5 was detected using the rabbit anti-ATG5 polyclonal antibody 10181-2-AP (1:50; Proteintech Group), and FIP200 was detected using the rabbit anti-FIP200 polyclonal antibody 16172-1-AP (1:100; Proteintech Group). The negative controls consisted of phosphate-buffered saline (PBS) instead of primary antibodies. Positive controls were established according to the instructions provided with the antibodies. Detailed methods for the immunohistochemistry (IHC) are presented in the Supplementary Materials.

2.4. Evaluation of immunostaining for ATG5 and FIP200

As in our previous study [15,16], the expression of ATG5 was semi-quantitatively classified according to the staining index (SI; range 0–9), which was calculated by multiplying the staining intensity by the proportion score. The staining intensities were classified according to four grades (0 denoting negative; 1, weak; 2, moderate; and 3, strong), and the proportion score was graded as the percentage of cells that were stained (1 denoting 0 to < 10% of cells; 2, between 10 and 50% of cells; and 3, staining of >50% of cells). In this study, SI $\geq$ 5 was defined as ATG5-positive staining, whereas SI < 5 was defined as negative staining. Additionally, as described by Ikebuchi et al. [17], the grades of FIP200 expression were classified into 3 categories: cytoplasm-/nuclei- (Grade I), cytoplasm+/nuclei- (Grade II), or cytoplasm + or -/nuclei+ (Grade III). Grade III FIP200 expression was defined as positive, whereas Grade I or II FIP200 expression was negative. The scores were evaluated independently by two experienced pathologists who were blinded to all clinical data on a case-by-case basis, and the score used in all subsequent analyses was the average across the available scores.

2.5. Statistical analysis

The Pearson χ^2 test was used to compare qualitative variables, and Fisher's exact test was performed when necessary. We constructed prognostic models for DFS events using univariate and multivariate Cox analyses. Risk scores and time-dependent receiver operating characteristic (ROC) curves were computed as described before [18]. $P < 0.05$ was considered to be statistically significant. All statistical analyses were completed using R 3.1.2 (R Development Core Team, Vienna, Austria).

3. Results

3.1. Expression patterns of ATG5 and FIP200 in breast cancer

The clinicopathological characteristics of the 200 participants, who had an average age of 52.2 years (SD 9.7, median 51.5, range 29–85), are presented in Table 1. After a median follow-up time of 7.8 years, 51 of the 200 cases have experienced recurrence or death. To investigate the clinical implications of ATG5 and FIP200 in breast cancer, we performed an immunohistochemical examination of the cohort (Fig. 1). As shown in Table 1, positive ATG5 staining was observed in 81 (40.5%) of the 200 cases according to the scoring criteria described in Materials and methods section. Moreover,

Table 1
Clinicopathological variables of and ATG5 and FIP200 expression in the study cohort.

Variable	Number of patients	ATG5 expression		P^a Value	FIP200 expression		P^a Value
		Negative *N* (%)	Positive *N* (%)		Negative *N* (%)	Positive *N* (%)	
Total	200	119 (59.5)	81 (40.5)		128 (64.0)	72 (36.0)	
Age				0.716			0.795
≤50 years	92 (46.0)	56 (28.0)	36 (18.0)		58 (29.0)	34 (17.0)	
>50 years	108 (54.0)	63 (31.5)	45 (22.5)		70 (35.0)	38 (19.0)	
Menopausal status				0.460			0.286
Premenopause	90 (45.0)	51 (25.5)	39 (19.5)		54 (27.0)	36 (18.0)	
Postmenopause	110 (55.0)	68 (34.0)	42 (21.0)		74 (37.0)	36 (18.0)	
Tumor size				0.024			0.160
≤2 cm	90 (45.0)	52 (26.0)	38 (19.0)		53 (26.5)	37 (18.5)	
>2, ≤5 cm	98 (49.0)	56 (28.0)	42 (21.0)		64 (32.0)	34 (17.0)	
>5 cm	10 (5.0)	10 (5.0)	0 (0.0)		9 (4.5)	1 (0.5)	
Not measurable	2 (1.0)	1 (0.5)	1 (0.5)		2 (1.0)	0 (0.0)	
Lymph node status				0.099			0.744
Negative	122 (61.0)	67 (33.5)	55 (27.5)		77 (38.5)	45 (22.5)	
Positive	78 (39.0)	52 (26.0)	26 (13.0)		51 (25.5)	27 (13.5)	
TNM stage				0.644			0.653
I	65 (32.5)	36 (18.0)	29 (14.5)		40 (20.0)	25 (12.5)	
II	101 (50.5)	60 (30.0)	41 (20.5)		64 (32.0)	37 (18.5)	
III	32 (16.0)	22 (11.0)	10 (5.0)		22 (11.0)	10 (5.0)	
Unknown	2 (1.0)	1 (0.5)	1 (0.5)		2 (1.0)	0 (0.0)	
Grade				0.532			0.954
1	4 (2.0)	2 (1.0)	2 (1.0)		3 (1.5)	1 (0.5)	
2	113 (56.5)	66 (33.0)	47 (23.5)		73 (36.5)	40 (20.0)	
3	52 (26.0)	29 (14.5)	23 (11.5)		33 (16.5)	19 (9.5)	
Unknown	31 (15.5)	22 (11.0)	9 (4.5)		19 (9.5)	12 (6.0)	
ER status				0.318			0.170
Negative	115 (57.5)	65 (32.5)	50 (25.0)		69 (34.5)	46 (23.0)	
Positive	85 (42.5)	54 (27.0)	31 (15.5)		59 (29.5)	26 (13.0)	
PR status				0.633			0.304
Negative	147 (73.5)	86 (43.0)	61 (30.5)		91 (45.5)	56 (28.0)	
Positive	53 (26.5)	33 (16.5)	20 (10.0)		37 (18.5)	16 (8.0)	
HER-2/neu status				0.405			0.260
Negative	123 (61.5)	76 (38.0)	47 (23.5)		75 (37.5)	48 (24.0)	
Positive	77 (38.5)	43 (21.5)	34 (17.0)		53 (26.5)	24 (12.0)	
Subtype[b]				0.397			0.068
Luminal	85 (42.5)	54 (27.0)	31 (15.5)		59 (29.5)	26 (13.0)	
HER-2$^+$	34 (17.0)	17 (8.5)	17 (8.5)		16 (8.0)	18 (9.0)	
Triple-negative	81 (40.5)	48 (24.0)	33 (16.5)		53 (26.5)	28 (14.0)	

Abbreviations: *ATG5*, autophagy-related gene 5; *FIP200*, FAK family kinase-interacting protein of 200 kDa; *ER*, estrogen receptor; *PR*, progesterone receptor; *HER-2*, human epidermal growth factor receptor 2.

[a] Based on the Pearson χ^2 test (Fisher's exact test was used when needed).

[b] Definitions of subtypes: luminal (ER- and/or PR-positive), HER-2$^+$ (ER- and PR-negative, HER-2-positive), and triple-negative (ER-negative, PR-negative, and HER-2-negative).

positive FIP200 staining was detected in 72 (36.0%) of the 200 cases. We found that higher ATG5 expression levels were associated with a smaller tumor size ($P = 0.024$; Table 1). There was no significant correlation between the expression levels of ATG5 and FIP200 ($P = 0.801$).

3.2. ATG5 and FIP200 expression redefines patients with different risks of relapse

To evaluate the clinical significance of ATG5 and FIP200 expression in breast cancer, we investigated the relationship between the status of these two autophagy-related proteins and DFS. As shown by the survival curves in Fig. 2A and B, patients with high ATG5 expression generally exhibited better DFS ($P = 0.015$), and positive staining for FIP200 correlated with a favorable clinical outcome ($P = 0.039$). By evaluating the combination of ATG5 expression and FIP200 expression, all patients were classified into one of four subgroups: ATG5+/FIP200+ ($n = 30$), ATG5+/FIP200- ($n = 51$), ATG5-/FIP200+ ($n = 42$), and ATG5-/FIP200- ($n = 77$). Differences in DFS were significant among the four groups ($P = 0.013$; Fig. 2C). More specifically, patients that were negative for the expression of both ATG5 and FIP200 had a high risk of relapse, with 5-year DFS of 71.6%, whereas the 5-year DFS of patients who were positive for both ATG5 and FIP200 was 86.3%. In the univariate analysis, elevated ATG5 expression was associated with a better clinical outcome in terms of DFS ($P = 0.017$; Table 2), and cases with positive FIP200 staining also exhibited a lower likelihood of DFS events ($P = 0.043$; Table 2).

3.3. Univariate and multivariate analyses and risk prediction modeling

As shown in Table 2, when the correlation between DFS and each clinicopathological variable was examined in the univariate analysis, several factors demonstrated a significant association. Positive lymph node status, tumor size >5 cm, stage III cancer were associated with a higher risk of recurrence (all $P < 0.05$). In contrast, PR status was related to longer DFS ($P = 0.018$). To select a small set of traditional clinical variables that could adequately predict survival, a stepwise multivariate Cox model was used to examine the clinical parameters that were prognostic factors for DFS (Table 3). As a result, age, menopausal status, PR status, and lymph node status were identified as significant prognostic factors for DFS (all $P < 0.05$). Thus, the traditional model ($M_{traditional}$) was as follows:

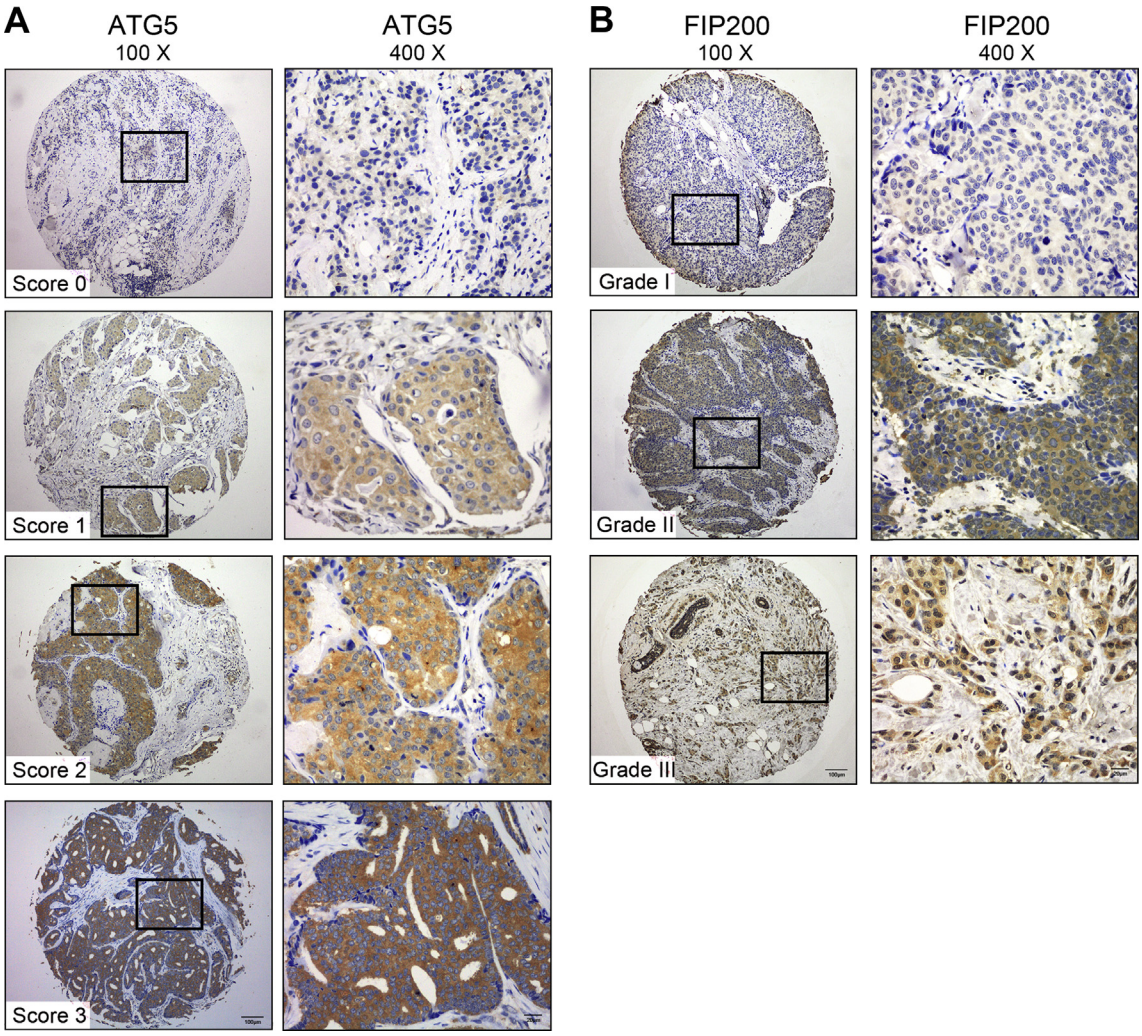

Fig. 1. Representative ATG5 and FIP200 immunohistochemical staining in malignant cells from breast cancer tissue specimens in low-magnification (100 ×) and high-magnification (400 ×) images. (**A**) The ATG5 staining intensities were classified as negative (score of 0), weak (score of 1), moderate (score of 2), or strong (score of 3). (**B**) FIP200 expression was classified into 3 grades: cytoplasm-/nuclei- (Grade I), cytoplasm+/nuclei- (Grade II), or cytoplasm + or -/nuclei+ (Grade III).

$$M_{traditional} = -0.74 \times \text{Age} + 0.75 \times \text{Menopausal Status} - 1.06 \times \text{PR} + 0.84 \times \text{LN}$$

After adjustment for age, menopausal status, PR status and lymph node status, the expression levels of ATG5 ($P = 0.022$) and FIP200 ($P = 0.023$) retained significant prognostic power (Table 4). The combined model ($M_{combined}$) was as follows:

$$M_{combined} = -0.69 \times \text{Age} + 0.62 \times \text{Menopausal Status} - 1.24 \times \text{PR} + 0.85 \times \text{LN} - 0.74 \times \text{ATG5} - 0.74 \times \text{FIP200}$$

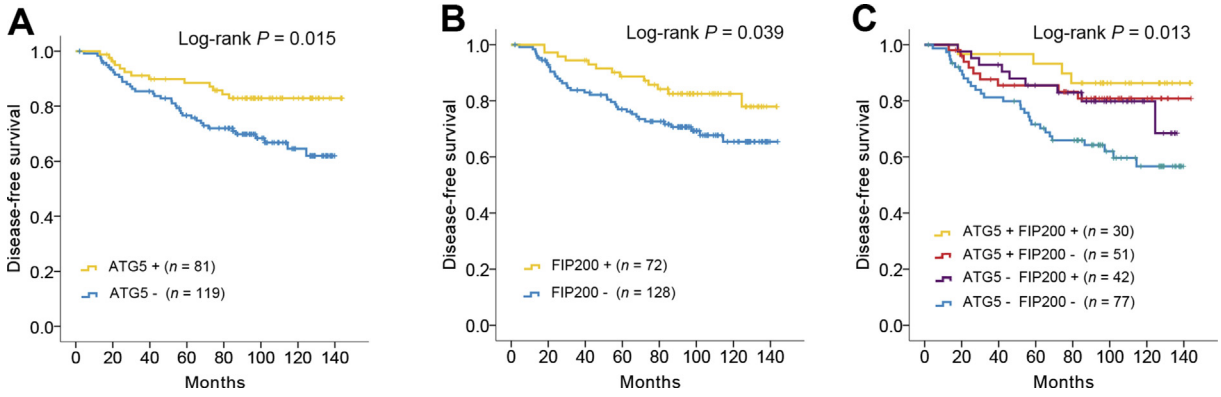

Fig. 2. High expression of ATG5 or FIP200 indicates favorable DFS in patients with breast cancer. Cumulative DFS curves for breast cancer patients classified according to (**A**) ATG5+ ($n = 81$) and ATG5- ($n = 119$); (**B**) FIP200+ ($n = 72$) and FIP200- ($n = 128$); or (**C**) ATG5+/FIP200+ ($n = 30$), ATG5+/FIP200- ($n = 51$), ATG5-/FIP200+ ($n = 42$) and ATG5-/FIP200- ($n = 77$).

Table 2
Univariate survival analysis of factors associated with disease-free survival.

	HR (95% CI)	*P* value
Age		
≤50 years	1	
>50 years	0.839 (0.484–1.453)	0.531
Menopausal status		
Premenopause	1	
Postmenopause	1.523 (0.858–2.705)	0.151
Tumor size		
≤2 cm	1	
>2, ≤5 cm	1.133 (0.627–2.047)	0.680
>5 cm	4.481 (1.793–11.195)	**0.001**
Not measurable	3.908 (0.517–29.511)	0.186
Lymph node status		
Negative	1	
Positive	2.322 (1.336–4.034)	**0.003**
TNM stage		
I	1	
II	1.453 (0.730–2.894)	0.287
III	3.155 (1.437–6.928)	**0.004**
Unknown	4.924 (0.631–38.451)	0.128
Grade		
1 or 2	1	
3	1.791 (0.991–3.239)	0.054
Unknown	0.843 (0.347–2.049)	0.706
ER status		
Negative	1	
Positive	0.754 (0.429–1.324)	0.325
PR status		
Negative	1	
Positive	0.381 (0.172–0.847)	**0.018**
HER-2/neu status		
Negative	1	
Positive	0.951 (0.542–1.669)	0.861
ATG5		
Negative	1	
Positive	0.465 (0.247–0.872)	**0.017**
FIP200		
Negative	1	
Positive	0.521 (0.278–0.979)	**0.043**
Subtype		
Luminal	1	
HER-2$^+$	1.273 (0.595–2.712)	0.534
TNBC	1.354 (0.733–2.501)	0.333

Abbreviations: *ATG5*, autophagy-related gene 5; *FIP200*, FAK family kinase-interacting protein of 200 kDa; *ER*, estrogen receptor; *PR*, progesterone receptor; *HER-2*, human epidermal growth factor receptor 2; *TNBC*, triple-negative breast cancer.

In the combined model, the regression coefficients for traditional clinical parameters changed slightly. The multivariate Cox analysis identified four independent prognostic factors for DFS: PR status was the strongest predictor, followed by lymph node status, ATG5 expression and FIP200 expression.

3.4. Risk model combining ATG5 and FIP200 shows superior prognostic power

Next, we sought to evaluate the additive ability of ATG5 and FIP200 to identify patients with breast cancer who had a higher risk of experiencing DFS events over 3 and 5 years. In the absence of ATG5 and FIP200, the traditional model exhibited modest prognostic accuracy, with bootstrap-corrected AUC values of 0.680 (95% CI 0.569–0.790) for 3-year DFS and 0.699 (95% CI 0.606–0.792) for 5-year DFS (Fig. 3A and B). The addition of ATG5 and FIP200 expression to the traditional model resulted in a significant improvement in both bootstrap-corrected AUC values, with 0.748 (95% CI 0.634–0.861) for 3-year DFS and 0.756 (95% CI 0.665–0.847) for 5-year DFS ($P < 0.001$ for all; Fig. 3A and B). Both of the ROC curves for 3-year and 5-year DFS had the same optimal cutoff value of −0.397. The entire patient cohort was subsequently reclassified as high risk (risk score > −0.397, $n = 84$) or low-risk (risk score ≤ −0.397, $n = 116$). The survival curves showed a significant difference in survival between the two groups ($P < 0.001$), with 5-year DFS rates of 66.3% for the high-risk group and 91.9% for the low-risk group (Fig. 3C). After being stratified by subtypes, high risk was associated with worse DFS in patients with luminal, Her-2-enriched or triple-negative breast cancer (TNBC) ($P < 0.05$ for all; Fig. S1).

Table 3
Multivariate Cox regression model for disease-free survival (traditional model).

	HR (95% CI)	*P* Value
Age (>50 years vs ≤ 50 years)	0.478 (0.245–0.935)	**0.031**
Menopausal status (postmenopause vs premenopause)	2.117 (1.054–4.253)	**0.035**
Lymph node status (positive vs negative)	2.326 (1.337–4.047)	**0.010**
PR status (positive vs negative)	0.346 (0.155–0.775)	**0.003**

Table 4
Multivariate Cox regression model for disease-free survival including ATG5 and FIP200 expression (combined model).

	HR (95% CI)	*P* Value
Age (>50 years vs ≤ 50 years)	0.500 (0.254–0.985)	**0.045**
Menopausal status (postmenopause vs premenopause)	1.868 (0.922–3.782)	0.083
Lymph node status (positive vs negative)	2.330 (1.336–4.063)	**0.003**
PR status (positive vs negative)	0.288 (0.128–0.650)	**0.003**
ATG5 (positive vs negative)	0.476 (0.252–0.899)	**0.022**
FIP200 (positive vs negative)	0.479 (0.253–0.905)	**0.023**

4. Discussion

Increasing recognition of the active role of autophagy in tumorigenesis has led to the identification of novel autophagy markers for prognosis prediction [6,7]. In the present study, we sought to evaluate the relationship of the autophagy-related proteins ATG5 and FIP200 with the risk of recurrence and metastasis in invasive ductal breast cancer. Our results supported the conclusion that ATG5 and FIP200 are independent prognostic factors, and a risk model incorporating these proteins was able to classify breast cancer patients into two risk recurrence categories. Our risk model incorporates both biomarkers and clinical factors and includes all subtypes of breast cancer. To the best of our knowledge, this is the first study supporting the prognostic value of these two autophagy-related proteins in breast cancer.

In this study, high ATG5 expression was associated with longer DFS, which is consistent with the emerging role of ATG5 as a molecular link in the interplay between autophagy and apoptosis that accelerates cancer cell death [10]. The switch from autophagy to apoptosis in response to ATG5 cleavage has been observed in doxorubicin-treated cells *in vitro* [10]. In addition, in the current study, we found that nuclear expression of FIP200, which we defined as a positive result, correlated with favorable DFS, which supported the tumor-suppressive role of FIP200 in breast cancer [17]. All patients who were classified based on a combination of their ATG5 and FIP200 expression statuses presented different DFS, which suggested that combining these two markers provided additional prognostic information.

In previous studies, high-intensity expression of the autophagy marker LC3B was correlated with a poor prognosis in breast cancer [7]. In addition, we have shown that LC3B expression in the residual tumor predicts a worse outcome in locally advanced breast cancer

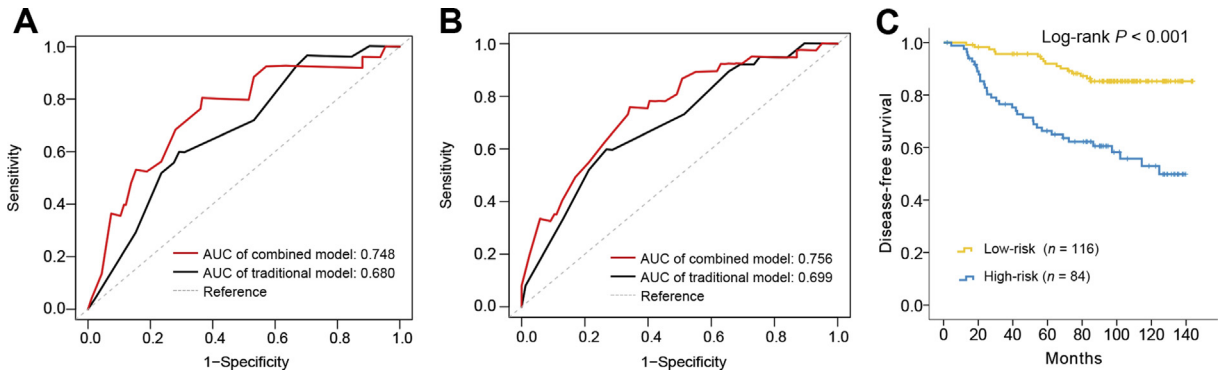

Fig. 3. Time-dependent ROC curves showing that a combination of ATG5 and FIP200 expression levels (red) improves the prognostic accuracy of the traditional model (black) regarding 3-year (**A**) and 5-year (**B**) DFS. All $P < 0.001$ for AUC comparison. (**C**) Kaplan–Meier estimates of DFS according to low risk ($n = 116$) or high risk ($n = 84$), classified according to the combined model (log-rank, $P < 0.001$).

(LABC) after NCT [6]. These two studies indicate a cytoprotective role for autophagy as a response of cancer cells to both chemotherapy and radiotherapy, which is the basis of multiple clinical trials combining an autophagy inhibitor (chloroquine or hydroxychloroquine) with conventional treatment modalities [19]. Thus, augment the efficacy of various cancer therapies by a blockade of treatment-induced autophagy appears to be promising. However, recent studies have revealed that cytostatic and cytotoxic forms of autophagy, which act as a precursor to apoptosis, mediate cell growth inhibition or promote cell death following their induction and that inhibition of this phenomenon may lead to the protection of cancer cells [20]. Our results provide additional evidence that certain essential components of autophagy are tumor suppressive. Because ATG5 and FIP200 also participate in the apoptosis pathway [4], they may act as a molecular link between autophagy and apoptosis that results in cytotoxic effects under specific conditions. However, this topic needs to be further studied.

The results of our study are slightly limited by the sample size. Because the number of patients with stage III breast cancer in our cohort was too small ($n = 32$), the risk model should be applied to this subgroup with caution. Although the stratification analysis revealed good prognostic power of the model among all cancer subtypes, the composition of the study cohort was not exactly representative of the general breast cancer patient population. Further studies using larger and consistent cohorts of breast cancer patients are thus required before this model can be recommended for clinical application.

In conclusion, our study highlights the prognostic value of ATG5 and FIP200 in breast cancer. The addition of these two autophagy-related proteins to conventional prognostic models improves the accuracy of predicting DFS events. Our findings may therefore promote the further clinical application of ATG5 and FIP200, providing additional prognostic information to oncologists with regard to assessing risk and applying personalized treatment strategies following breast cancer resection.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.02.037.

Transparency document

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