Clinical and Experimental Obstetrics & Gynecology

Clin. Exp. Obstet. Gynecol. **2023**; 50(8): 169
https://doi.org/10.31083/j.ceog5008169

Original Research

Expression Value of Rab10 in Breast Cancer

Jian Zhuo[1,†], Yanchun Zhao[2,†], Jianjun Han[3,†], He Li[1], Ruiying Hao[1], Yan Yang[1], Luxian Dai[4], Ankang Sheng[4], Xiaohong Yang[4], Weiguang Liu[3,*]

[1]School of Clinical Medicine, Hebei University of Engineering, 056000 Handan, Hebei, China
[2]Department of Outpatient, Affiliated Hospital of Hebei University of Engineering, 056000 Handan, Hebei, China
[3]Department of Breast Surgery, Affiliated Hospital of Hebei University of Engineering, 056000 Handan, Hebei, China
[4]Department of Breast Surgery, Yangzhou Maternal and Child Health Hospital Affiliated to Yangzhou University Medica College, 225007 Yangzhou, Jiangsu, China
*Correspondence: lwg1943@163.com (Weiguang Liu)
[†]These authors contributed equally.
Academic Editor: Ambrogio Pietro Londero
Submitted: 21 February 2023 Revised: 15 April 2023 Accepted: 20 April 2023 Published: 14 August 2023

Abstract

Background: Rab10 is a small GTPase protein belonging to the Ras superfamily. It is expressed and plays a role in a variety of malignant tumours. However, the expression of Rab10 and its role in breast cancer (BC) prognosis remains unclear. The aim of this study was to analyse the differential expression and prognostic value of Rab10 in BC using bioinformatics techniques and immunohistochemistry in a clinical cohort. **Methods**: The TIMER2, GEPIA2, and UALCAN databases were used to analyse the correlation between the differential expression of Rab10 and BC. Rab10 and BC prognosis were correlated using the Kaplan–Meier Plotter and UALCAN databases. The expression of Rab10 in BC tissues was detected using immunohistochemistry, and its correlation with the BC clinical cohort was analysed using Chi-squared tests and logistic regression analysis. **Results**: The expression of Rab10 mRNA identified in BC patients using TIMER2, GEPIA2, and UALCAN databases was higher than that in para-cancerous tissues. Kaplan–Meier plotter and the UALCAN database revealed that increased Rab10 expression was associated with poor prognosis in BC patients. Immunohistochemistry showed that Rab10 was expressed on cell membranes and in cytoplasm of BC tissues. In a clinical cohort, Rab10 expression correlated with histological grade, (human epidermal growth factor receptor 2) HER2 status, and molecular typing. **Conclusions**: Rab10 can be used as an effective clinical prognostic biomarker for BC.

Keywords: breast cancer; Rab10; prognosis; HER2

1. Introduction

Breast cancer (BC) is the most common malignant tumour in women worldwide, with the highest rates of disability and death [1]. The global burden of BC is rapidly increasing, especially in China, where BC incidence and mortality rates are rapidly rising [2]. According to international consensus guidelines, the current treatment for BC mainly includes chemotherapy, radiotherapy, targeted therapy, immunotherapy, and endocrine therapy before and after surgery [3]. Traditional treatment modalities, such as surgery, chemotherapy, and radiotherapy, have improved the prognosis of patients with BC. However, tumour progression and metastasis in BC patients remains a great clinical challenge. Therefore, it is crucial to elucidate the pathogenesis of BC and identify new therapeutic targets.

The GTP-binding protein Rab10 belongs to the Ras superfamily of small GTPases, which are mainly distributed in cytosolic compartments and are used to regulate intracellular vesicle transport [4]. Rab10 resides on glucose transporter 4 (GLUT4) vesicles and coordinates with myosin Va to store GLUT4 in vesicles and deliver GLUT4 to the plasma membrane for glucose uptake in adipocytes and muscle tissue [5]. Rab10 is a substrate of leucine-rich repertoire kinase 2 (LRRK2) and is highly expressed in phagocytes in the immune system. Reducing Rab10 expression inhibits AKT (protein kinase B, PKB) activation and chemotaxis, and Rab10 phosphorylation mediated by LRRK2 impedes rapid vesicle cycling and promotes PI3K-Akt immune responses [6].

In recent years, an increasing number of studies have discovered that Rab10 is aberrantly expressed in a variety of malignancies, including liver cancer, glioma, and cervical cancer, and is associated with poor patient prognosis [7–9]. In addition, Rab10 plays an important role in tumour cell proliferation, apoptosis, invasion, and metastasis, as well as in tumour autophagy [7–11]. Studies have found that FAM49B promotes proliferation, metastasis, and chemoresistance in BC by regulating the downstream Rab10/TLR4 pathway [12]. However, the expression profile of Rab10 in BC and its association with clinical prognosis remain unclear. We analysed the differential expression of Rab10 in tumours and adjacent tissues using the GEPIA2 and TIMER2 databases and found that Rab10 expression was significantly higher in BC tissues than in normal breast tissues. In this study, we further investigated the expression of Rab10 in BC tissues, analysed its biological functions, and correlated it with prognosis of BC patients.

2. Materials and Methods

2.1 Online Data Acquisition and Analyses

Differential expression of Rab10 mRNA in BC and paraneoplastic tissues was analysed using the TIMER2 (http://timer.cistrome.org/), GEPIA2 (http://gepia2.cancer-pku.cn/), and UALCAN (http://ualcan.path.uab.edu/) databases. Correlation between Rab10 and BC overall survival (OS) and recurrence-free survival (RFS) was explored using the Kaplan–Meier Plotter website (https://kmplot.com/analysis/) and the UALCAN databases.

2.2 Human Participants

This study included 106 patients with invasive BC who underwent breast surgery at the Affiliated Hospital of Hebei Engineering University between January 2014 and December 2015. Patient inclusion criteria were as follows: (1) none of the patients had neoadjuvant treatment, such as radiotherapy or chemotherapy before surgery; (2) specimens were all analysed pathologically for diagnosis; (3) all patients were treated with standard adjuvant therapy following radical surgery. Exclusion criteria were as follows: (1) distant metastasis of the tumour; (2) combination of other malignant tumours; (3) combination of immune system-related diseases; (4) presence of severe liver, kidney or cardiac defects; (5) mental disorders; (6) not meeting the indications for breast cancer surgery; (7) age <18 years.

Our study used the 2013 St. Gallen International Expert Consensus Report to identify four subtypes of BC: luminal A, luminal B, human epidermal growth factor receptor 2 (HER2) overexpression, and triple-negative.

Our study and all experimental methods were approved by the Ethics Committee of the Affiliated Hospital of Hebei Engineering University.

2.3 Immunohistochemistry (IHC)

Immunohistochemistry (IHC) was performed as follows. Paraffin-embedded mammary sections were first placed in fresh xylene solution for 15 min, and then in 100%, 95%, 90%, 80%, and 70% ethanol for 5 min each, washed three times (3 min each) in phosphate-buffered saline (PBS), after which the sections were placed in a 3% H_2O_2 solution for immersion (10 min) and rinsed with PBS. After the above treatment, the slices were heated in sodium citrate buffer (10 mM trisodium citrate, 0.05% Tween-20, pH 6.0) for 15 min in a microwave oven and then removed and cooled naturally to room temperature. After three rinses with PBS, 1% bovine serum albumin (BSA) was added and incubated at 37 °C for 20 min, followed by dropwise addition of primary antibody (1:100) at 4 °C overnight. The slices were rinsed again with PBS three times, followed by addition of enhanced enzyme-labelled goat anti-rabbit IgG polymer, and incubated at 37 °C for 20 min. Afterwards, PBS was used again for washing three times. The staining of the sections was then observed under a microscope using diaminobenzidine (DAB) working solution, and colour development was terminated by immersion of the sections in double-distilled water after completion of colour development. Next, the sections were stained in hematoxylin working solution for 3 min, rinsed with tap water, and then divided using 1% hydrochloric acid alcohol. Finally, the film was sealed with ethanol solution rehydration, xylene transparency, and neutral resin.

Rab10 expression in breast tissue was analysed separately and semi-quantitatively by two senior pathologists.

2.4 Statistical Methods

Clinical data were analysed using SPSS v.26.0 (IBM Corp., Armonk, NY, USA), and the χ^2 test was used to assess correlations between Rab10 expression and tumour clinicopathological data. Statistical significance was set at $p < 0.05$.

3. Results

3.1 RAB10 is Highly Expressed in BC

Analysis of the TIMER2 database revealed that Rab10 mRNA was expressed in a variety of tumour tissues, and the expression levels of Rab10 mRNA in BC and paraneoplastic tissues were statistically different, as shown in Fig. 1A. Analysis of the GEPIA2 database revealed differential expression of Rab10 in a variety of tumours, and BC included a total of 1376 samples (tumour = 1085; normal = 291) with much higher Rab10 expression than in paraneoplastic tissues (Fig. 1B).

Similarly, analysis of 1211 BC samples from the UALCAN database revealed that Rab10 mRNA was significantly upregulated in BC tissues (Fig. 1C), consistent with the GEPIA2 data.

3.2 High Rab10 Expression is Associated with Poor BC Prognosis

Analysis of the UALCAN database showed that BC patients with high Rab10 mRNA expression had worse OS, and the difference was statistically significant ($p < 0.05$) (Fig. 1D). Kaplan–Meier Plotter website analysis showed that BC patients with high Rab10 expression at 5 years had worse RFS [hazard ratio (HR) = 1.44 (1.21–1.7), $p = 2.3 \times 10^{-5}$] (Fig. 1E) and OS [HR = 1.43 (1.03–1.9), $p = 0.033$] (Fig. 1F), and the results were statistically significant.

3.3 Correlation Analysis of Rab10 Expression with Clinical Samples

A total of 106 female patients with BC with a mean age of 54.93 years (range, 26–82 years) were included in this study.

IHC results showed that Rab10 was mainly present in BC glandular cells and myoepithelial cells, localised in the cell membrane and cytoplasm, and the cytoplasmic staining was stronger than the cell membrane staining (Fig. 2).

A clinical cohort analysis revealed that Rab10 positivity in BC tissue was 27.4% (29/106), and Rab10 positivity in HER2+ BC was as high as 48.15% (13/27). Addition-

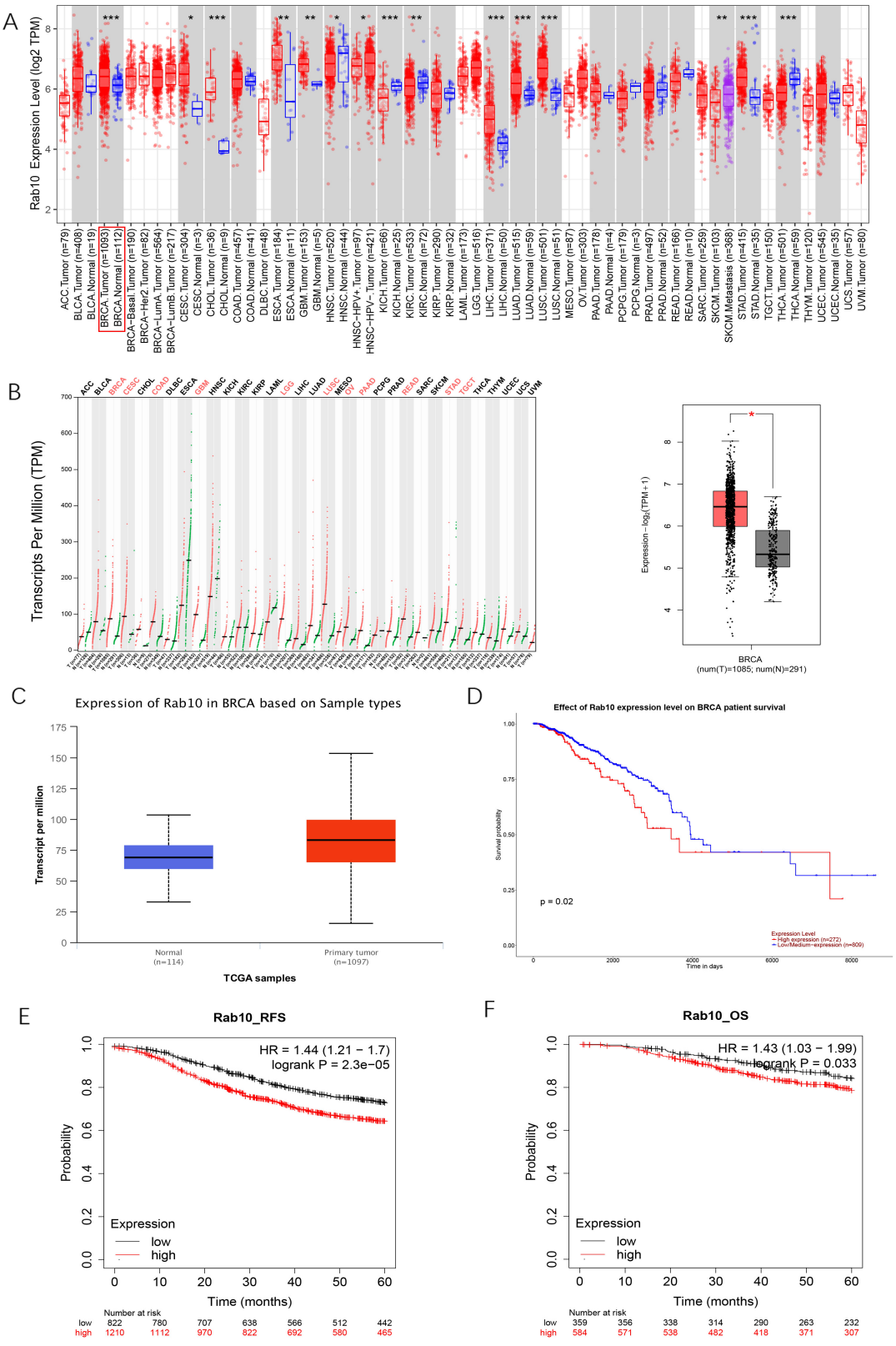

Fig. 1. Differential expression of Rab10 in BC cells and BC prognosis. (A) Determination of Rab10 expression levels in different tumour types using TIMER (*$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). (B) Rab10 is expressed in various cancers and is increased in BC by GEPIA. (C) The UALCAN database analysed Rab10 for high expression in BC. (D) The UALCAN database analysed Rab10 expression in association with poor BC prognosis ($p < 0.05$). (E,F) In the Kaplan–Meier drawing platform, Rab10 was associated with OS and RFS, and prognosis of the high expression group was poor ($p < 0.05$). BC, breast cancer; OS, overall survival; RFS, recurrence-free survival.

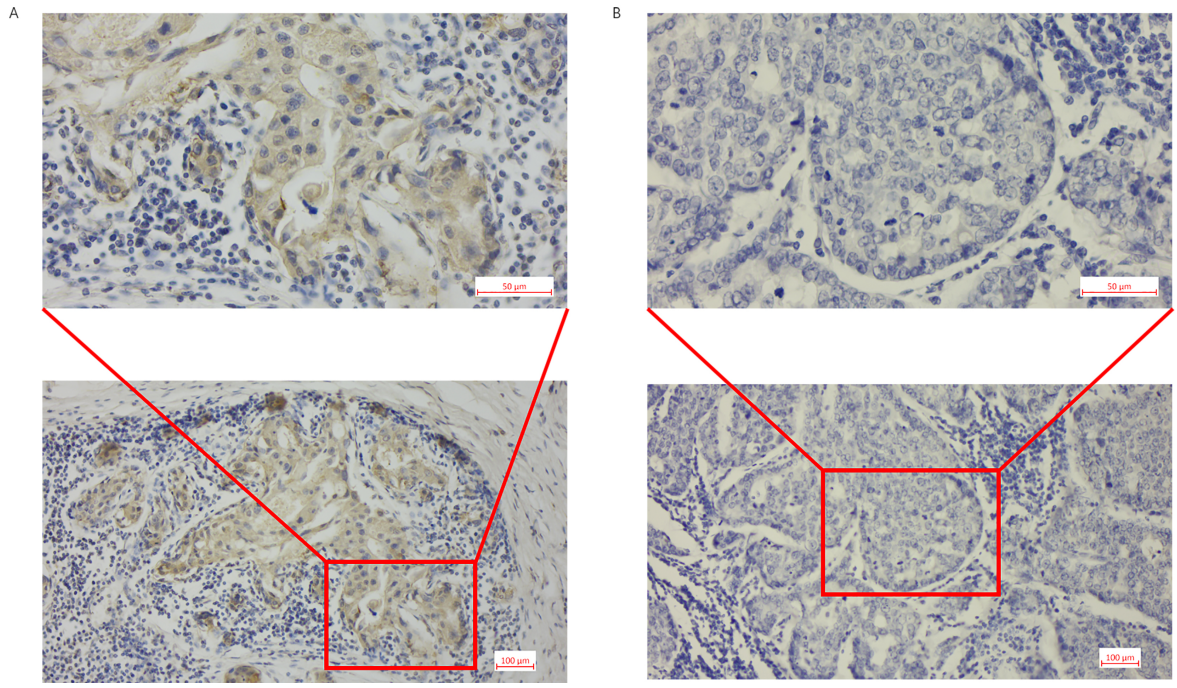

Fig. 2. Immunohistochemical analysis of Rab10 expression in BC tissues. (A) Brown indicates Rab10 positively expressed in BC tissue (magnification 400×). (B) BC tissue in which Rab10 is not expressed (magnification 400×).

ally, Rab10 expression was associated with the histological grade (χ^2 = 8.39, p = 0.02), molecular typing (χ^2 = 8.45, p = 0.04), and HER2 status (χ^2 = 4.54, p = 0.03) of BC (Table 1).

To exclude the influence of confounding factors, we conducted a logistic analysis of the above factors. Multifactorial analysis showed that Rab10 expression was positively correlated with HER2 status (Exp (B) = 3.31, p = 0.03) and histological grade (Exp (B) = 2.66, p = 0.01). Unfortunately, Rab10 expression was not associated with BC molecular typing (Exp (B) = 0.54, p = 0.22) (Table 2), which could be related to the number of clinical samples.

4. Discussion

This is the first report of a study involving Rab10 and a BC clinical cohort. Rab10 has been reported in recent years to be found in a variety of tumours. Our previous study showed that Rab10 can promote BC progression by regulating the TLR4 pathway [12]. However, a correlation between Rab10 and BC has not yet been extensively studied. Therefore, in the present study, we found that Rab10 expression was much higher in BC than in paraneoplastic tissues using bioinformatics analysis, which suggests that Rab10 may be involved in BC development and progression.

To demonstrate whether Rab10 offers potential as a clinical prognostic biomarker, we used the UALCAN database and Kaplan–Meier Plotter website to analyse the correlation between Rab10 and BC patient prognosis. The results showed that Rab10 was associated with RFS and OS in BC cases at 5 years. Unfortunately, the relationship between Rab10 and survival in clinical cohorts was not confirmed due to the lack of clinical data.

In a clinical cohort study, we found that Rab10 was expressed in human BC tissues and was associated with histological grade, molecular typing, and HER2 status. After excluding confounding factors, Rab10 expression was positively correlated with clinicopathological variables, including histological grade and HER2 status. Higher Rab10 expression levels implied higher tissue grading and HER2 expression. Histological grading can reveal the degree of differentiation of tumour cells. HER2 is amplified or overexpressed in approximately 20% of BCs and indicates a poor prognosis in the absence of systemic therapy [13]. This evidence further suggests that Rab10 may be a potential prognostic biomarker for BC or a molecular target for BC.

Since this was a retrospective study with a small sample size, there may have been potential selection bias. In addition, given the small tissue volume of BC samples from surgical punctures in neoadjuvant patients and the altered pathological characteristics of the samples after neoadjuvant therapy, neoadjuvant BC patients were not included in the study, so any correlation between Rab10 and BC molecular typing still needs to be validated in a larger clinical cohort for confirmation.

Due to the lack of tissue specimens and limitations inherent to IHC, only the location of Rab10 expression in BC tissues was confirmed in this study, and it was difficult to assess the differential expression of Rab10 protein in BC and paracancerous tissues. In addition, the results may be more convincing if the survival status analysis of Rab10 is performed using the BC clinical cohort.

Table 1. The association with Rab10 and clinicopathological factors in invasive BC (N = 106).

Clinicopathologic characteristics	N	Rab10(+)	Rab10(–)	χ^2	p
Nationality					
Han	106	29	77		
Year					
>60	35	7	28	1.42	0.23
<60	71	22	49		
Grading					
1	22	3	19	8.39	**0.02**
2	35	6	29		
3	49	20	29		
pTNM stage					
I	31	10	21	2.02	0.36
II	52	11	41		
III	23	8	15		
T stage					
T1	38	12	26	1.39	0.71
T2	50	11	39		
T3	15	5	10		
T4	3	1	2		
N stage					
N0	57	15	42	0.91	0.82
N1	34	9	25		
N2	14	5	9		
N3	1	0	1		
Subtype					
LA	30	5	25	8.45	**0.04**
LB	32	8	24		
HER2+	27	13	14		
TN	17	3	14		
ER status					
Positive	60	13	47	2.25	0.13
Negative	46	16	30		
PR status					
Positive	55	12	43	1.77	0.18
Negative	51	17	34		
HER2 status					
Positive	59	21	38	4.54	**0.03**
Negative	47	8	39		

pTNM stage, refer to AJCC 8th edition; T, tumer size; N, lymph node involvement; ER, estrogen receptor; PR, progesterone receptor; HER2, Human epidermal growth factor receptor 2; LA, Luminal A; LB, Luminal B; HER2+, HER2 over expression; TN, Triple-negative.

The bold: $p < 0.05$ is statistically significant.

Table 2. Multivariate analysis of the factors related to Rab10 expression (N = 106).

Characteristic	B	Exp (B)	95% CI for Exp (B)	p
Year	–0.87	0.42	0.13–1.32	0.14
Grading	0.98	2.66	1.28–5.55	**0.01**
pTNM stage	–0.76	0.47	0.10–2.26	0.35
T stage	0.00	1.00	0.35–2.90	1.00
N stage	0.29	1.34	0.44–4.05	0.60
Subtype	–0.63	0.54	0.20–1.47	0.22
ER status	–1.71	0.18	0.02–1.86	0.15
PR status	0.42	1.52	0.24–9.62	0.66
HER2 status	1.20	3.31	1.14–9.66	**0.03**

ER, estrogen receptor; PR, progesterone receptor; HER2, Human epidermal growth factor receptor 2; CI, Confidence interval.

The bold: $p < 0.05$ is statistically significant.

HER2 is a type I transmembrane growth factor receptor localised to the cell membrane, and its biological activity is influenced by such cell membrane localisation [14]. In addition, activation of HER2 can cause cascade activation of AKT and MAPK signalling pathways, and the termination of these signalling cascades is mainly dependent on epidermal growth factor receptor (EGFR) endocytosis and its corresponding ligand-forming complexes recycled to the cell surface or degraded by various enzymes [15]. We note that Rab10, as a small GTPase, is a key regulator in eukaryotic membrane exocytosis and endocytosis pathways, involved in a wide range of membrane transport events [16]. Furthermore, dysfunction of Rab10 is able to activate AKT and MAPK signalling pathways [17]. We therefore hypothesised that Rab10 may influence HER2 expression and signal transduction at the cell membrane by participating in HER2 transport and localisation and may also participate in the co-regulation of AKT or MAPK signalling pathways for interactive functions. In addition, upregulation of Rab10 protein was observed in extracellular vesicles released from HER2+ BC cells resistant to trastuzumab, suggesting a possible association of Rab10 with HER2+ BC resistance [18]. Knockdown of Rab10 in hepatocellular carcinoma inhibits the proliferative capacity of hepatocellular carcinoma cells, induces G0/G1 phase arrest, and increases the level of apoptosis in hepatocellular carcinoma cells [7]. HER2 gene amplification in BC is associated with increased tumour cell proliferation, cell cycle progression, and decreased apoptosis [19]. The mechanism of action of Rab10 in BC, especially in HER2-positive BC, deserves further in-depth study.

5. Conclusions

In conclusion, this study indicates that Rab10 mRNA and protein are highly expressed in BC tissues, which is related to histological grading, molecular subtypes, and HER2 status. High expression of Rab10 reflects poor prognosis in BC patients and can be used as a potential clinical biomarker.

Availability of Data and Materials

The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

Author Contributions

JZ took part in the data curation, formal analysis, investigation and drafting the article. YZ and JH were involved in clinical resources. HL, RH, YY, LD and AS analyzed the data. XY was responsible for methodology and project administration. WL made conceptualization, funding acquisition and final review of the manuscript. All authors contributed to editorial changes in the manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki and the Ethics Committee of the Affiliated Hospital of Hebei Engineering University approved study number 2021[K]019 for approval.

Acknowledgment

Not applicable.

Funding

This work was supported by the Natural Science Foundation of Hebei Province (H2021402015) and the Medical Science Research Project of Hebei Province (20231527).

Conflict of Interest

The authors declare no conflict of interest.

References

[1] Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, *et al*. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA: A Cancer Journal for Clinicians. 2021; 71: 209–249.

[2] Lei S, Zheng R, Zhang S, Wang S, Chen R, Sun K, *et al*. Global patterns of breast cancer incidence and mortality: A population-based cancer registry data analysis from 2000 to 2020. Cancer Communications. 2021; 41: 1183–1194.

[3] Cardoso F, Paluch-Shimon S, Senkus E, Curigliano G, Aapro MS, André F, *et al*. 5th ESO-ESMO international consensus guidelines for advanced breast cancer (ABC 5). Annals of Oncology. 2020; 31: 1623–1649.

[4] Vieira OV. Rab3a and Rab10 are regulators of lysosome exocytosis and plasma membrane repair. Small GTPases. 2018; 9: 349–351.

[5] Chen Y, Wang Y, Zhang J, Deng Y, Jiang L, Song E, *et al*. Rab10 and myosin-Va mediate insulin-stimulated GLUT4 storage vesicle translocation in adipocytes. The Journal of Cell Biology. 2012; 198: 545–560.

[6] Liu Z, Xu E, Zhao HT, Cole T, West AB. LRRK2 and Rab10 coordinate macropinocytosis to mediate immunological responses in phagocytes. The EMBO Journal. 2020; 39: e104862.

[7] Wang W, Jia WD, Hu B, Pan YY. RAB10 overexpression promotes tumor growth and indicates poor prognosis of hepatocellular carcinoma. Oncotarget. 2017; 8: 26434–26447.

[8] Zhang X, Wang S, Lin G, Wang D. Down-regulation of circ-PTN suppresses cell proliferation, invasion and glycolysis in glioma by regulating miR-432-5p/RAB10 axis. Neuroscience Letters. 2020; 735: 135153.

[9] Han H, Shao Q, Liu X. LINC00441 promotes cervical cancer progression by modulating miR-450b-5p/RAB10 axis. Cancer Cell International. 2020; 20: 368.

[10] Zhang YJ, Pan Q, Yu Y, Zhong XP. microRNA-519d Induces Autophagy and Apoptosis of Human Hepatocellular Carcinoma Cells Through Activation of the AMPK Signaling Pathway via Rab10. Cancer Management and Research. 2020; 12: 2589–2602.

[11] Mizushima N, Yoshimori T, Ohsumi Y. The role of Atg proteins in autophagosome formation. Annual Review of Cell and Developmental Biology. 2011; 27: 107–132.

[12] Li Y, Xiong Y, Wang Z, Han J, Shi S, He J, *et al*. FAM49B promotes breast cancer proliferation, metastasis, and chemoresistance by stabilizing ELAVL1 protein and regulating downstream Rab10/TLR4 pathway. Cancer Cell International. 2021; 21: 534.

[13] Waks AG, Winer EP. Breast Cancer Treatment: A Review. The Journal of the American Medical Association. 2019; 321: 288–300.

[14] Moasser MM. The oncogene HER2: its signaling and transforming functions and its role in human cancer pathogenesis. Oncogene. 2007; 26: 6469–6487.

[15] Lv Q, Meng Z, Yu Y, Jiang F, Guan D, Liang C, *et al*. Molecular Mechanisms and Translational Therapies for Human Epidermal Receptor 2 Positive Breast Cancer. International Journal of Molecular Sciences. 2016; 17: 2095.

[16] Chua CEL, Tang BL. Rab 10-a traffic controller in multiple cellular pathways and locations. Journal of Cellular Physiology. 2018; 233: 6483–6494.

[17] Actis Dato V, Chiabrando GA. Activated Alpha-2 Macroglobulin Improves Insulin Response via LRP1 in Lipid-Loaded HL-1 Cardiomyocytes. International Journal of Molecular Sciences. 2021; 22: 6915.

[18] Drucker A, Yoo BH, Khan IA, Choi D, Montermini L, Liu X, *et al*. Trastuzumab-induced upregulation of a protein set in extracellular vesicles emitted by ErbB2-positive breast cancer cells correlates with their trastuzumab sensitivity. Breast Cancer Research. 2020; 22: 105.

[19] Li JW, Zhang K, Shi ZT, Zhang X, Xie J, Liu JY, *et al*. Triple-negative invasive breast carcinoma: the association between the sonographic appearances with clinicopathological feature. Scientific Reports. 2018; 8: 9040.