

Overexpression of ECT2 is a strong poor prognostic factor in ER(+) breast cancer

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Abstract. Epithelial cell transforming sequence 2 (ECT2) is a guanine nucleotide exchange factor encoded by the ECT2 gene, which is located on the 3q26.31 chromosomal region and is directly associated with the occurrence of cancers. The aim of the present study was to examine the expression and prognostic importance of ECT2 in various breast cancer subtypes using the online tools, Gene Expression Profiling Interactive Analysis, Kaplan-Meier-plotter and bc-GenExMiner. ECT2 mRNA expression was significantly different in oestrogen receptor ER(+) breast cancer; overexpression of ECT2 was associated with poor prognosis in ER⁺ breast cancer. The mRNA expression levels of ECT2 were increased in basal-like breast cancer and triple negative breast cancer, but were not significant for prognostic prediction. We identified ECT2-correlated genes and their corresponding Gene Ontology (GO) enrichment terms. The results revealed that GO: 0005524 (protein binding) had the greatest number of correlated genes and also contained ECT2. This suggested that overexpression of ECT2 may be a significant prognostic factor for poor outcome in ER⁺ breast cancer; however, the precise role of ECT2 in breast cancer requires further investigation.

Introduction

Breast cancer is the second most common type of cancer worldwide, with the highest incidence and leading cause of mortality in females (1). According to data from Globocan, ~1.67 million novel cases of breast cancer were diagnosed in 2012 (2). The rates of incidence vary between less developed and developed countries, with rates ranging from 27 cases per 100,000 in Central Africa and Eastern Asia compared with 96

cases per 100,000 in Western Europe (2). In China, the largest developing and highly populated country worldwide, and has a low incidence of breast cancer; however, the burden of breast cancer in China is increasing annually (3). In 2008, 169,452 novel cases of breast cancer and 44,908 breast cancer-associated mortalities in China were reported, accounting for 12.2% of cases and 9.6% of associated mortalities globally (4). At present, the 5-year survival rate for primary breast cancer is relatively high, at 92% (5); however, the survival rate of patients with metastatic breast cancer remains <25% (2,5). Developments concerning the pathogenesis of breast cancer have been made, yet few drugs for the treatment of breast cancer exhibit satisfactory outcomes. Therefore, investigating the occurrence and development of breast cancer is a primary research objective.

Epithelial cell transforming sequence 2 (ECT2) is a guanine nucleotide exchange factor encoded by the ECT2 gene in humans and is directly associated with the occurrence of cancers (6). ECT2 is primarily upregulated in certain cancers and is considered a major oncogene involved in the onset or progression of cancer (7,8). In ovarian cancers, ECT2 mRNA is frequently amplified, and the protein is highly expressed in the nucleus but not in the cytoplasm in advanced disease; ECT2 acts as a Rho guanine nucleotide exchange factor specifically within the nucleus to drive ovarian cancer cell transformation (9). In breast cancer cells, ECT2 serves a significant role in the progression of breast cancer (10); however, the effects of ECT2 on the prognosis of different molecular types of breast cancer remains unclear. In the present study, Breast Cancer Gene-Expression Miner v4.1 (11), was employed to analyse the expression of ECT2 in various types of breast cancer. The aim of our study was to investigate whether ECT2 expression could predict the prognosis of different types of breast cancer.

Materials and methods

Gene expression levels of ECT2. We obtained the RNA sequence and expression profile of ECT2 in normal and tumour tissues from Gene Expression Profiling Interactive Analysis (GEPIA; <http://gepia.cancer-pku.cn/index.html>), using a standard system to analyse 9,736 tumour and 8,587 normal samples from the Cancer Genome Atlas (TCGA; <https://cancergenome.nih.gov/>) and Genotype-Tissue Expression projects (12). The differential expression of ECT2 across PAM50 subtypes, oestrogen

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Key words: epithelial cell transforming sequence 2, breast cancer, biomarker, prognosis

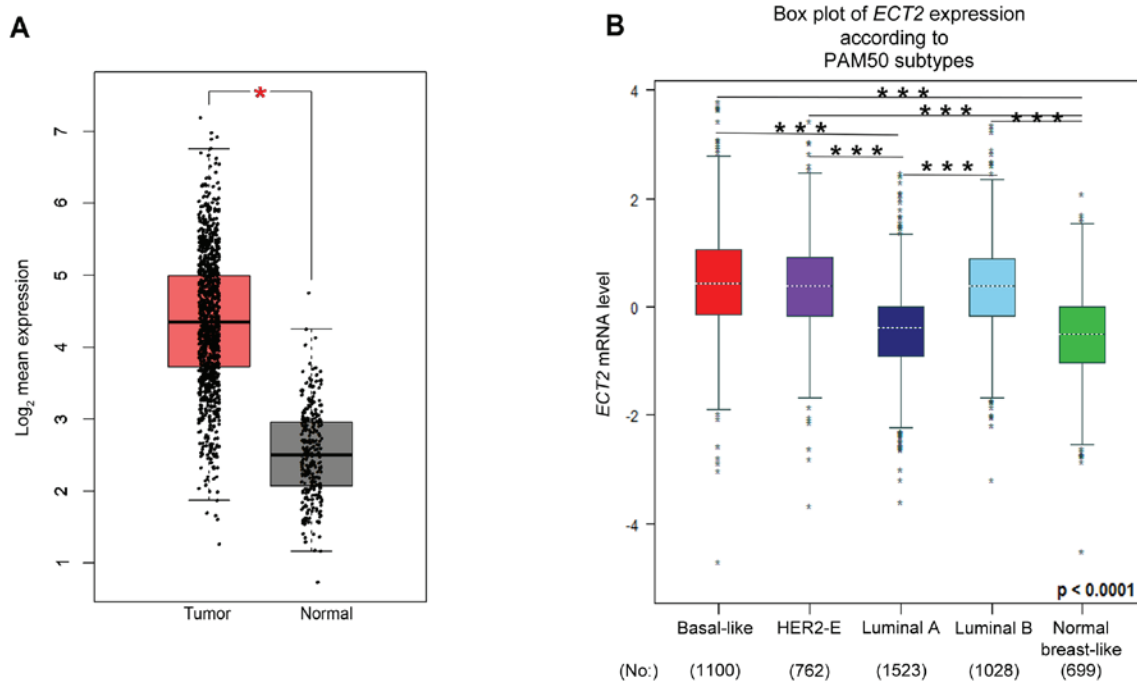


Figure 1. ECT2 expression in various breast cancer tissue types and normal breast tissues. (A) ECT2 expression in breast cancer and normal tissues, and (B) PAM50 subtypes of breast cancer. *P<0.01, ***P<0.0001. ECT2, epithelial cell transforming sequence 2.

receptor (ER, -/+) and basal-like and/or triple negative breast cancers (TNBCs), were analysed using bc-GenExMiner v4.1 (<http://bcgenex.centregauducheau.fr/BC-GEM/GEM-Accueil.php?js=1>), a web-based tool with a MySQL relational database. Subsequently, boxplots were created to visualize the results. There were 32 datasets with containing ECT2 expression data of 5,277 patients for analysis; gene correlation analysis was conducted using data from 36 datasets.

Survival analysis of patients with breast cancer associated with ECT2. The association between the expression of ECT2, and the overall survival (OS) and progression-free survival (PFS) in patients with breast cancer was determined using Kaplan-Meier-plotter (KM plotter, <http://kmplot.com/analysis/>), which can analyse 54,675 genes with respect to survival using 10,461 cancer samples (13). The longest follow up was conducted for 300 months. OS data for patients with ER (-/+), different grades and basal-like and/or TNBCs were obtained from KM plotter and bc-GenExMiner v4.1, with a MySQL relational database (11,13). A total of 5,206 patients from 32 datasets were used for prognostic analysis with respect to ECT2 with any nodal status, ER status and any event (AE).

Univariate Cox analysis of ECT2. Univariate cox analysis was conducted with data of ECT2 for the survival of patients with breast cancer and basal-like and/or TNBC using bc-GenExMiner v4.1, with a MySQL relational database (11).

Correlation between ECT2 and Gene Ontology (GO) 'biological process' enrichment. Using bc-GenExMiner v4.1 with a MySQL relational database, we determined genes that were correlated with ECT2 and performed corresponding GO 'biological process' enrichment analysis (12).

Statistical analysis. Data are presented as the mean \pm standard deviation. The survival plot was generated by GEPIA and the Log-rank test was used for comparison of survival curves. Kaplan-Meier survival curves and univariate Cox analysis were conducted via bc-GenEx-Miner v4.1 to analyse the association between ECT2 expression and ER. Box and whiskers plots were used to compare ECT2 expression among the different population groups by bc-GenEx-Miner v4.1 and a Welch's test. In addition, when there were at least three different groups, Dunnett-Tukey-Kramer's test was used for two-by-two comparisons. P<0.05 was considered to indicate a statistically significant difference.

Results

ECT2 expression in different types of breast cancer tissue and normal breast tissues. Using GEPIA expression analysis, it was revealed that the expression of ECT2 was significantly higher in breast cancer tissues compared with normal breast tissues (P<0.0001; Fig. 1A). We used bc-GenExMiner to demonstrate that ECT2 expression significantly varied in PAM50 subtypes (P<0.0001; Fig. 1B), and basal-like breast cancer exhibited higher ECT2 expression than all other subtypes.

OS and PFS of patients with breast cancer exhibiting high and low expression levels of ECT2. Survival analysis from the Kaplan-Meier-plotter was employed to confirm whether high ECT2 expression was associated with significantly worse OS (P=3.7 $\times 10^{-7}$; Fig. 2A) and PFS (P<1 $\times 10^{-16}$; Fig. 2B) in patients with breast cancer. The results indicates that ECT2 expression was associated with significantly worse PFS and OS. As presented in Fig. 2B that the patient with the longest follow-up succumbed to mortality. All observed subjects

Table I. Univariate Cox analysis of ECT2 for the survival of the patients with breast cancer.

No.	Nodal status	ER status	Event status	P-value	Hazard ratio	95% CI	No. patients	No. events
1	Nm	ER+	AE	<0.0001	1.32	1.24-1.41	3,722	1,177
2	Nm	ER+	MR	<0.0001	1.48	1.36-1.61	2,711	652
3	Nm	ERm	AE	<0.0001	1.24	1.18-1.30	5,206	1,750
4	Nm	ERm	MR	<0.0001	1.32	1.23-1.41	3,751	979
5	N-	ER+	MR	<0.0001	1.62	1.43-1.84	1,358	310
6	N-	ER+	AE	<0.0001	1.45	1.31-1.60	1,740	502
7	N-	ERm	AE	<0.0001	1.31	1.21-1.42	2,385	717
8	N-	ERm	MR	<0.0001	1.40	1.27-1.54	1,841	448
9	N+	ER+	MR	<0.0001	1.42	1.20-1.67	662	198
10	N+	ER+	AE	0.0002	1.26	1.12-1.43	1,033	397
11	N+	ERm	MR	0.0010	1.23	1.09-1.39	951	314
12	N+	ERm	AE	0.0046	1.15	1.04-1.26	1,466	606
13	N+	ER-	AE	0.2871	0.92	0.79-1.07	426	208
14	N+	ER-	MR	0.3561	0.92	0.76-1.10	283	115
15	N-	ER-	AE	0.7096	1.03	0.89-1.18	622	211
16	N-	ER-	MR	0.8359	1.02	0.86-1.21	466	137
17	Nm	ER-	AE	0.9181	1.00	0.92-1.10	1,439	563
18	Nm	ER-	MR	0.9462	1.00	0.89-1.11	1,014	323

Bold font indicates significant findings. ECT2, epithelial cell transforming sequence 2; CI, confidence interval; AE, any event; MR, metastatic relapse; ER, oestrogen receptor status; N, nodal status; +, positive; -, negative; m, mixed.

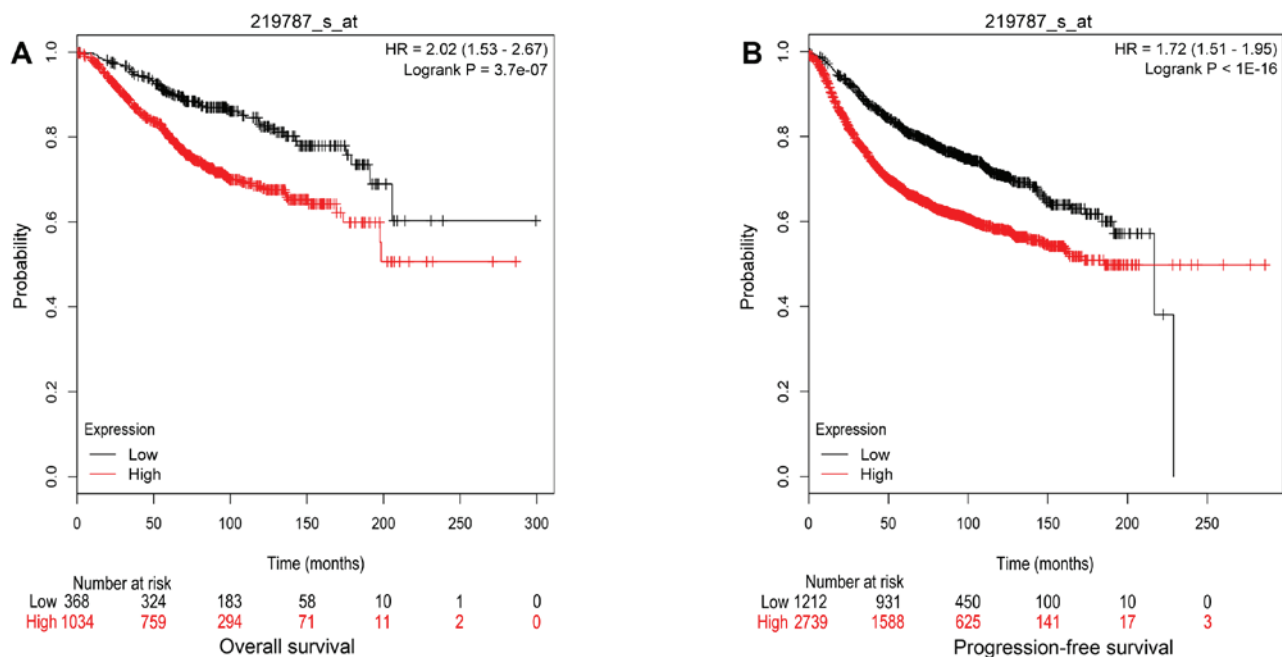


Figure 2. Kaplan-Meier survival curves of breast cancer patients exhibiting high or low ECT2 expression. (A) Overall survival and (B) progression-free survival. ECT2, epithelial cell transforming sequence 2; HR, hazard ratio.

succumbed by the last time point in the low ECT2 expression group; however, high ECT2 expression was associated with significantly worse PFS.

ECT2 expression and OS in patients with ER⁺ and ER⁻ breast cancer. There were 12 significant results (P<0.05) among

the 18 total findings from the Univariate Cox analysis from bc-GenExMiner v4.1 data (Table I). ECT2 mRNA expression was significantly different between patients with ER⁺ and ER⁻ breast cancer (Fig. 3A); ECT2 expression was upregulated in ER⁻ breast cancer than in ER⁺ breast cancer. On the contrary, the present study reported that higher expression levels of ECT2

Table II. Univariate Cox analysis of ECT2 for the survival of the patients with basal-like and/or triple negative breast cancer.

Population	P-value	Hazard ratio	95% CI	No. patients	No. events
Basal-like	0.2609	1.07	0.95-1.19	1,091	384
TNBC	0.3510	0.91	0.76-1.10	371	120
Basal-like and TNBC	0.6229	1.07	0.81-1.42	242	66

ECT2, epithelial cell transforming sequence 2; CI, confidence interval.

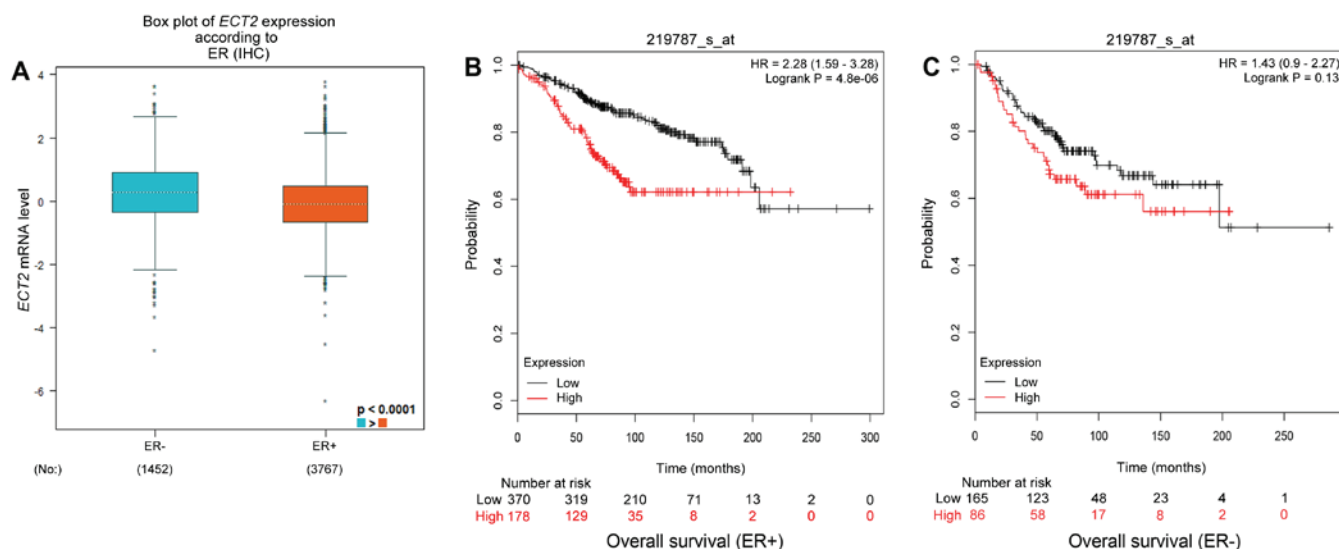


Figure 3. ECT2 expression and overall survival in patients with ER⁺ and ER⁻ breast cancer. (A) Differences in ECT2 expression according to ER (-/+) breast cancer. The overall survival curves of (B) ER (+) breast cancer patients and (C) ER (-) breast cancer patients. ECT2, epithelial cell transforming sequence 2; ER, oestrogen receptor; HR, hazard ratio.

were associated with poor prognosis in breast cancer in the mixed nodal status (Nm)/ER⁺/AE, Nm/ER⁺/MR, Nm/mixed ER status (ERm)/AE, Nm/ERm/MR, negative nodal status (N⁻)/ER⁺/MR, N⁻/ER⁺/AE, N⁻/ERm/AE, N⁻/ERm/MR, positive nodal status (N⁺)/ER⁺/MR and N⁺/ER⁺/AE subtypes, particularly in ER⁺ breast cancer, high expression of ECT2 is associated with poor prognosis in all ER⁺ breast cancers. In addition, we observed that high ECT2 expression was associated with significantly worse OS in patients with ER⁺ breast cancer (Fig. 3B), while the expression of ECT2 in patients with ER⁻ breast cancer was not significantly associated with survival (Fig. 3C).

ECT2 expression and OS in patients with basal-like breast cancer and/or TNBC. There were no significant differences ($P > 0.05$) among the three total results from univariate Cox analysis of ECT2 for the survival of patients with basal-like and/or TNBC from bc-GenExMiner v4.1 data (Table II). Significant differences in the expression of ECT2 mRNA between patients with basal-like breast cancer and/or TNBC were observed. The expression levels of ECT2 mRNA in patients with basal-like breast cancer and/or TNBC were upregulated than in patients without these subtypes ($P < 0.05$; Fig. 4A). In addition, the expression of ECT2 was not associated with OS in basal-like breast cancer and/or TNBC with concomitant AE via KM analysis ($P > 0.05$; Fig. 4B).

Genes correlated with ECT2. The top 10 closest positive/negative correlations with ECT2 were identified via extensive gene correlation analysis of breast cancer (Table III). Then, GO enrichment analysis was performed for the genes correlated with ECT2 via bc-GenExMiner (Table IV). Among them, GO:0005524 (protein binding) had the greatest number of associated genes and also contained ECT2 itself.

Discussion

The ECT2 gene was initially identified as a proto-oncogene located in the 3q26.31 chromosomal region that can transform NIH/3T3 fibroblasts into cancer cells (14). As a guanine nucleotide exchange factor of the Rho guanosine triphosphate (GTP)ase family, ECT2 regulates cell division and the cell cycle (15,16), which affects various biological functions, including the proliferation, invasion and migration of tumour cells, associated with numerous signalling pathways (17-19). ECT2 catalyses the dissociation of bound guanosine diphosphate (GDP) and promotes the replacement of GDP with GTP, thereby activating Ras GTP enzymes in cell signalling pathways (20,21). A study indicated that the formation of lung cancer depends on rRNA synthesis regulated by ECT2 (22). Numerous studies have reported that ECT2 expression correlated with the prognosis of patients with prostate cancer and gastrointestinal tumours,

Table III. The top 10 positive and/or negative genes correlated with ECT2.

Positive correlations			Negative correlations		
Gene	P-value	No. patients	Gene symbol	P-value	No. patients
PRINS	<0.0001 ^a	155	LOC100507412	<0.0001	155
CDK1	<0.0001 ^a	5,277	LINC01660	<0.0001	155
SMC4	<0.0001 ^a	5,277	LOC286161	<0.0001	214
CENPW	<0.0001 ^a	2,607	IGHV5-78	<0.0001	206
CLPSL1	<0.0001 ^a	139	TARP	0.0003	51
CEP55	<0.0001 ^a	5,277	TRBV19	0.0011	50
RPL39P5	<0.0001 ^a	139	MIR600HG	<0.0001	400
MELK	<0.0001 ^a	5,277	USP17L6P	<0.0001	135
RFC4	<0.0001 ^a	5,277	BMS1P21	<0.0001	326
RRM2	<0.0001 ^a	5,106	AARSD1	0.0013	59

^aP<0.05. AARSD1, alanyl-tRNA synthetase domain containing 1; BMS1P21, BMS1, ribosome biogenesis factor pseudogene 21; CDK1, cyclin-dependent kinase 1; CEP55, centrosomal protein 55; CENPW, centromere protein W; CLPSL1, colipase like 1; ECT2, epithelial cell transforming sequence 2; IGHV5-78, immunoglobulin heavy variable 5-78; LINC01660, long intergenic non-protein coding RNA 1660; PRINS, psoriasis associated non-protein coding RNA induced by stress; MELK, maternal embryonic leucine zipper kinase; RFC4, replication factor C subunit 4; RPL39P5, ribosomal protein L39 pseudogene 5; RRM2, ribonucleotide reductase regulatory subunit 2; SMC4, structural maintenance of chromosomes 4; TARP, TCR γ alternate reading frame protein; TRBV19, T cell receptor β variable 19; USP17L6P, ubiquitin carboxyl-terminal hydrolase 17-like protein 6.

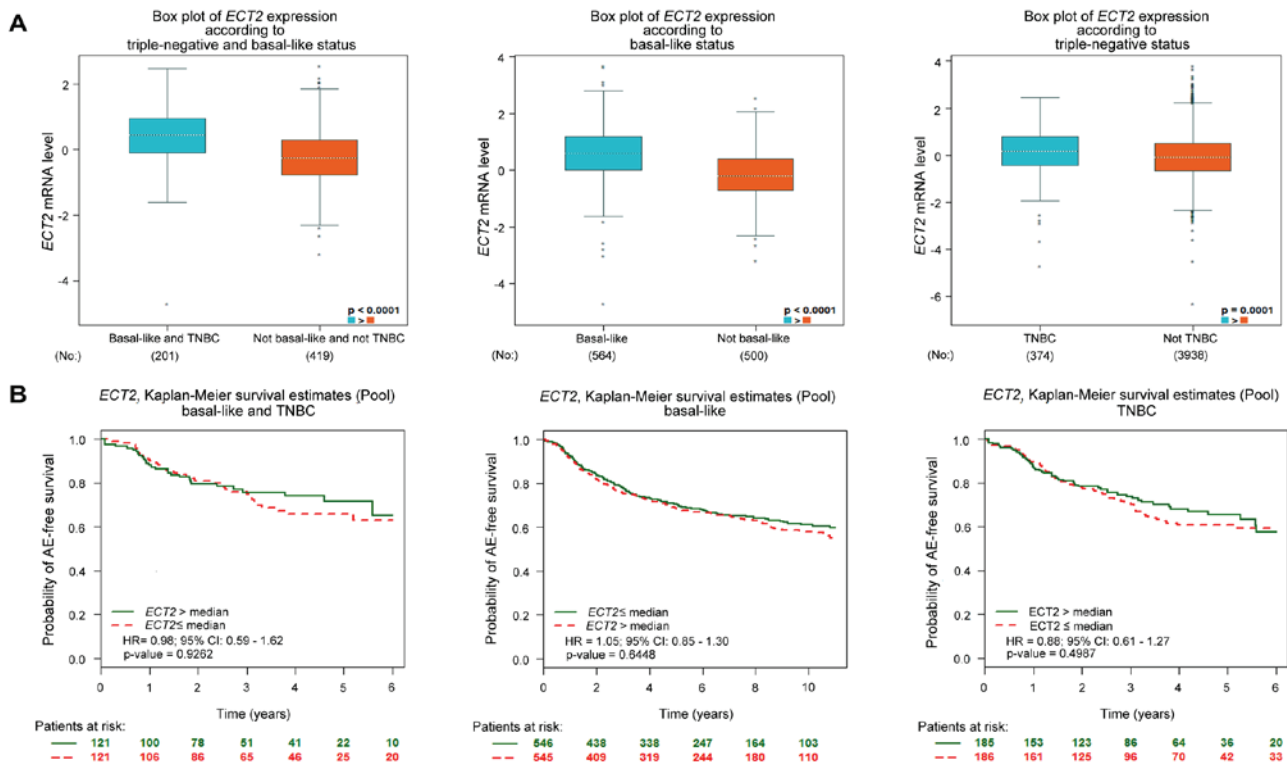


Figure 4. ECT2 expression and overall survival in patients with basal-like breast cancer and/or TNBC. (A) Differences in ECT2 expression according to basal-like and/or triple negative breast cancers. (B) overall survival curves of basal-like and/or triple negative breast cancers patients. AE, any event; ECT2, epithelial cell transforming sequence 2; HR, hazard ratio; CI, confidence interval.

including gastric cancer, colorectal cancer and oesophageal cancer (15,21,23,24).

The present study demonstrated that the expression of ECT2 was significantly higher in breast cancer tissues than in

normal breast tissues using data from GEPIA. Therefore, ECT2 may serve an important role in the occurrence and development of breast cancer. Furthermore, the expression of ECT2 in PAM50 subtypes was significantly different as determined

Table IV. GO enrichment of genes correlated with ECT2.

GO terms	Description	P-value	Associated genes
Biological process GO: 0051301	Cell division	<0.0001 ^a	CDK1, SMC4, CENPW, CCNB1, MAD2L1, NEK2, BUB1B, NCAPG, KIF18B, FBXO5, KIF11, FAM83D, CKS2, CCNA2, CDCA8, UBE2C, KIF14, ZWINT, CCNE2, PTTG1, TPX2, CENPF, OIP5, SMC2, NDC80, KNSTRN, AURKA, CDC20, ERCC6L, NCAPH, CDCA2, KIF2C, NUF2, GPSM2, CKS1B, CDCA3, SPAG5, BIRC5, MASTL, SGO2, CCNB2, CENPE, NCAPG2, BUB1, SKA3, CDCA5, KIFC1
GO: 00007062	Sister chromatid cohesion	<0.0001 ^a	MAD2L1, BUB1B, CDCA8, ZWINT, CENPA, CENPF, NDC80, CDC20, ERCC6L, KIF2C, NUF2, CENPK, CENPL, BIRC5, CENPU, SGO2, KIF18A, CENPE, CENPM, CENPN, BUB1, CDCA5
GO: 0000278	Mitotic cell cycle	<0.0001 ^a	CENPW, NEK2, BUB1B, PBK, KIF18B, KIF11, TPX2, CENPF, NDC80, AURKA, KIF2C, NUF2, KIF15, MASTL, RAB6C, CENPE, SKA3, CDCA5
GO: 0000070	Mitotic sister chromatid segregation	<0.0001 ^a	SMC4, MAD2L1, NEK2, NUSAP1, KIF18B, ZWINT, NDC80, KNSTRN, SPAG5, ESPL1, KIFC1
Cellular component GO: 0005654	Nucleoplasm	<0.0001 ^a	CDK1, SMC4, CENPW, RFC4, ANLN, FANCI, KPNA2, CCNB1, MAD2L1, NEK2, PRC1, UBE2T, RACGAP1, FBXO5, DTL, RAD51API, TOP2A, CCNA2, KIF4A, CDCA8, UBE2C, KIF23, ZWINT, CCNE2, KIF20A, CENPA, TPX2, CENPF, EZH2, OIP5, SMC2, NDC80, FOXM1, MCM6, PCNA, ACTL6A, MCM2, ATAD2, AURKA, CDC20, CDCA2, KIF2C, CENPK, CKS1B, ASF1B, HJURP, PCLAF, CENPL, FEN1, RMI2, ZNF367, BIRC5, CENPU, DBF4, MCM10, MASTL, SGO2, EXO1, CHEK1, GMNN, RAD51, NCAPG2, MCM4, CSE1L, DEK, CENPM, TOPBP1, RNASEH2A, CENPN, BUB1, SNRPD1, CDCA5, NUDCD1
GO: 0000777	Condensed chromosome kinetochore	<0.0001 ^a	CENPW, MAD2L1, NEK2, BUB1B, ZWINT, NDC80, KNSTRN, ERCC6L, KIF2C, NUF2, CENPK, HJURP, SPAG5, BIRC5, CENPU, SGO2, CENPM, CENPN, BUB1
GO: 0000775	Cytosol	<0.0001 ^a	ECT2, CDK1, SMC4, RRM2, FANCI, KPNA2, CCNB1, DLGAP5, MAD2L1, NEK2, PRC1, RACGAP1, BUB1B, NCAPG, KIF18B, FBXO5, DTL, KIF11, CDKN3, CCNA2, DEPDC1B, KIF4A, CDCA8, UBE2C, KIF23, KIF14, ZWINT, CCNE2, PTTG1, CENPA, TPX2, CENPF, OIP5, SMC2, NDC80, CKAP2L, HMMR, MCM2, MTHFD2, KNSTRN, AURKA, CDC20, ERCC6L, NCAPH, CDCA2, KIF2C, NUF2, GPSM2, CENPK, HJURP, CENPL, CDCA3, RMI2, KIF15, BIRC5, CENPU, SGO2, KIF18A, STIL, CHEK1, GMNN, ESPL1, TYMS, GMPs, RAD51, CCNB2, RAB6C, CENPE, PDCD10, CSE1L, CENPM, RNASEH2A, CENPN, BUB1, SNRPD1, PLK4, CDCA5, NUDCD1
GO: 0030496	Nucleus	<0.0001 ^a	PRC1, UBE2T, RACGAP1, NCAPG, PBK, KIF18B, FBXO5, DTL, CDKN3, RAD51API, TOP2A, CCNA2, CDCA8, KIF23, KIF14, ZWINT, H2AFZ, PTTG1, CENPA, TPX2, CENPF, EZH2, OIP5, SMC2, NDC80, FOXM1, MCM6, PCNA, ACTL6A, MCM2, ATAD2, KNSTRN, AURKA, TRIP13, DONSON, NCAPH, NUF2, HJURP, PCLAF, FEN1, DEPDC1, ZNF367, BIRC5, CENPU, DBF4, MCM10, MASTL, TCF19, KIF18A, EXO1, CHEK1, GMNN, ESPL1, UHRF1, TYMS, RAD51, CCNB2, RAB6C, CENPE, MCM4, CSE1L, DEK, TOPBP1, CENPN, SNRPD1, CDCA5, TMPO, KIFC1

Table IV. Continued.

GO terms	Description	P-value	Associated genes
Molecular function			
GO: 0008017	Microtubule binding	<0.0001 ^a	PRC1, NUSAP1, RACGAP1, KIF18B, KIF11, FAM83D, KIF4A, KIF23, KIF14, KIF20A, SPAG5, KIF15, BIRC5, KIF18A, CENPE, KIFC1
GO: 0005524	Protein binding	<0.0001 ^a	ECT2, CDK1, SMC4, CENPW, CEP55, MELK, RFC4, RRM2, FANCI, KPNA2, CCNB1, DLGAP5, MAD2L1, NEK2, PRC1, NUSAP1, UBE2T, RACGAP1, BUB1B, NCAPG, PBK, TTK, KIF18B, FBXO5, DTL, CDKN3, RAD51AP1, TOP2A, FAM83D, CKS2, CCNA2, KIF4A, CDCA8, UBE2C, KIF23, KIF14, ZWINT, H2AFZ, CCNE2, PTTG1, KIF20A, CENPA, TPX2, CENPE, EZH2, OIP5, SMC2, NDC80, FOXM1, MCM6, HMMR, PCNA, ACTL6A, MCM2, MTRF2, ATAD2, KNSTRN, AURKA, CDC20, TRIP13, ERCC6L, DONSON, NCAPH, KIF2C, NUF2, GPSM2, CENPK, CKS1B, ASF1B, HJURP, PCLAF, CENPL, CDCA3, FEN1, SPAG5, DEPD1, KIF15, BIRC5, CENPU, DBF4, MCM10, SGO2, TCF19, KIF18A, EXO1, STIL, CHEK1, GMNN, ESPL1, UHRF1, RAD51, CCNB2, FBXO45, CENPE, PDCD10, MCM4, CSE1L, SHCBP1, TOPBP1, FAM111B, BUB1, SKA3, SNRNP1, PLK4, CDCA5, NUDCD1
GO: 0003777	Microtubule motor activity	<0.0001 ^a	KIF18B, KIF11, KIF4A, KIF23, KIF14, KIF20A, KIF2C, KIF15, CENPE, KIFC1
GO: 0005515	ATP binding	<0.0001 ^a	CDK1, SMC4, MELK, RFC4, NEK2, UBE2T, BUB1B, PBK, TTK, KIF18B, KIF11, TOP2A, KIF4A, UBE2C, KIF23, KIF14, KIF20A, TPX2, SMC2, MCM6, MCM2, ATAD2, AURKA, TRIP13, ERCC6L, KIF2C, KIF15, MASTL, KIF18A, CHEK1, GMPS, RAD51, CENPE, MCM4, BUB1, PLK4, KIFC1

^aP<0.05; GO, Gene Ontology.

via bc-GenExMiner analysis, indicating that the expression of ECT2 in luminal A and normal breast-like breast cancers were significantly lower than in other types. Several studies have proposed the overexpression of ECT2 to be an independent prognostic index in patients with various malignant disorders. For example, Luo *et al* (21) reported that patients with colorectal cancer and high ECT2 expression had notably shorter overall survival; Cox regression analysis revealed that ECT2 expression may be a significant independent prognostic factor for the OS rate of patients with colorectal cancer. Guo *et al* (23) used a dataset of TCGA to investigate the prognostic value of ECT2 in prostate cancer. In addition, lower levels of ECT2 mRNA expression predicted increased OS and biochemical recurrence-free survival in all patients with prostate cancer or non-metastatic prostate cancer (23). Hirata *et al* (24) screened for genes that were overexpressed in tumors via gene expression profile analyses of 101 lung cancer and 19 esophageal squamous cell carcinoma (ESCC) tissues via a cDNA microarray comprising 27,648 genes or expressed sequence tags. It was reported that high levels of ECT2 expression were associated with the poor prognosis of patients with NSCLC and ESCC (24). In the present study, it was observed that high ECT2 expression was associated with significantly lower OS and PFS in patients with breast cancer using KM-plotter. In addition, we determined that ECT2 expression was lower in ER⁺ breast cancer; however, increased expression of ECT2 was associated with the poor prognosis of ER⁺ breast cancer, as demonstrated by ECT2 univariate Cox and KM curve analyses of bc-GenExMiner v4.1 data. Although, the expression of ECT2 in ER⁻ breast cancer was higher than in ER⁺ breast cancer, our results indicated that the high expression of ECT2 in ER⁺ breast cancer may be associated with poor prognosis. These results suggest the potential of ECT2 as a biomarker and a therapeutic target for ER⁺ breast cancer. Recently, TNBC and ECT2 were reported to be independent prognostic factors for patients with breast cancer (25). On the contrary, the results of the present study indicated that ECT2 expression is higher in basal-like breast cancer and TNBC; however, ECT2 was not an independent prognostic factor for both of these subtypes. In addition, we conducted GO enrichment for genes correlated with ECT2 via GO analysis of bc-GenExMiner v4.1 data. The results revealed that GO: 0005524 (protein binding) had the greatest number of genes correlated with and contained ECT2, which may provide insight into the mechanism of ECT2 in the progression of breast cancer; however, further investigation is required. The mechanism of ECT2 action in breast cancer must be confirmed by prospective experiments *in vitro* and *in vivo*.

In conclusion, ECT2 may represent a novel therapeutic target for the treatment of cancer based on its role in the occurrence and development of breast cancer, as well as its independent prognostic significance.

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Availability of data and materials

The data that support the findings of this study are available from GEPIA (<http://gepia.cancer-pku.cn/index.html>) and bc-GenExMiner v4.1 (<http://bcgenex.centregauducheau.fr/BC-GEM/GEM-Accueil.php?js=1>).

Authors' contributions

XYX is the major contributor to the writing of the manuscript; XYX and HMW made substantial contributions to the design of the present study. LW, WTY and LY supervised the study and critically revised the manuscript for intellectually important content. All the authors have read and approved the final version of this manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Global Burden of Disease Cancer Collaboration, Fitzmaurice C, Dicker D, Pain A, Hamavid H, Moradi-Lakeh M, MacIntyre MF, Allen C, Hansen G, Woodbrook R, *et al*: The global burden of cancer 2013. *JAMA Oncol* 1: 505-527, 2015.
2. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D and Bray F: Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 136: E359-E386, 2015.
3. Chen WQ and Zheng RS: Incidence, mortality and survival analysis of breast cancer in China. *Chin J Clin Oncol* 42: 668-674, 2015.
4. Fan L, Strasser-Weippl K, Li JJ, St Louis J, Finkelstein DM, Yu KD, Chen WQ, Shao ZM and Goss PE: Breast cancer in China. *Lancet Oncol* 15: e279-e289, 2014.
5. Siegel RL, Miller KD and Jemal A: Cancer statistics, 2015. *CA Cancer J Clin* 65: 5-29, 2015.
6. Tatsumoto T, Xie X, Blumenthal R, Okamoto I and Miki T: Human ECT2 is an exchange factor for Rho GTPases, phosphorylated in G2/M phases, and involved in cytokinesis. *J Cell Biol* 147: 921-928, 1999.
7. Fields AP and Justilien V: The guanine nucleotide exchange factor (GEF) Ect2 is an oncogene in human cancer. *Adv Enzyme Regul* 50: 190-200, 2010.
8. Justilien V and Fields AP: Ect2 links the PKC α -Par6 α complex to Rac1 activation and cellular transformation. *Oncogene* 28: 3597-3607, 2009.
9. Huff LP, Decristo MJ, Trembath D, Kuan PF, Yim M, Liu J, Cook DR, Miller CR, Der CJ and Cox AD: The role of Ect2 nuclear RhoGEF activity in ovarian cancer cell transformation. *Genes Cancer* 4: 460-475, 2013.
10. Mansour M, Haupt S, Chan AL, Godde N, Rizzitelli A, Loi S, Caramia F, Deb S, Takano EA, Bishton M, *et al*: The E3-ligase E6AP represses breast cancer metastasis via regulation of ECT2-Rho signaling. *Cancer Res* 76: 4236-4248, 2016.
11. Jézéquel P, Frénel JS, Campion L, Guérin-Charbonnel C, Gouraud W, Ricolleau G and Campone M: bc-GenExMiner 3.0: New mining module computes breast cancer gene expression correlation analyses. *Database (Oxford)* 2013: bas060, 2013.
12. Tang Z, Li C, Kang B, Gao G, Li C and Zhang Z: GEPIA: A web server for cancer and normal gene expression profiling and interactive analyses. *Nucleic Acids Res* 45: W98-W102, 2017.

13. Szász AM, Lánckzy A, Nagy Á, Förster S, Hark K, Green JE, Boussioutas A, Busuttil R, Szabó A and Györfy B: Cross-validation of survival associated biomarkers in gastric cancer using transcriptomic data of 1,065 patients. *Oncotarget* 7: 49322-49333, 2016.
14. Miki T, Smith CL, Long JE, Eva A and Fleming TP: Oncogene *ect2* is related to regulators of small GTP-binding proteins. *Nature* 362: 462-465, 1993.
15. Wang HB, Yan HC and Liu Y: Clinical significance of ECT2 expression in tissue and serum of gastric cancer patients. *Clin Transl Oncol* 18: 735-742, 2016.
16. Scoumanne A and Chen X: The epithelial cell transforming sequence 2, a guanine nucleotide exchange factor for Rho GTPases, is repressed by p53 via protein methyltransferases and is required for G1-S transition. *Cancer Res* 66: 6271-6279, 2006.
17. Duquette PM and Lamarche-Vane N: Rho GTPases in embryonic development. *Small GTPases* 5: e972857, 2014.
18. Matsuoka T and Yashiro M: Rho/ROCK signaling in motility and metastasis of gastric cancer. *World J Gastroenterol* 20: 13756-13766, 2014.
19. Tanabe K, Shinsato Y, Furukawa T, Kita Y, Hatanaka K, Minami K, Kawahara K, Yamamoto M, Baba K, Mori S, *et al*: Filamin C promotes lymphatic invasion and lymphatic metastasis and increases cell motility by regulating Rho GTPase in esophageal squamous cell carcinoma. *Oncotarget* 8: 6353-6363, 2017.
20. Cook DR, Rossman KL and Der CJ: Rho guanine nucleotide exchange factors: Regulators of Rho GTPase activity in development and disease. *Oncogene* 33: 4021-4035, 2014.
21. Luo Y, Qin SL, Mu YF, Wang ZS, Zhong M and Bian ZQ: Elevated expression of ECT2 predicts unfavorable prognosis in patients with colorectal cancer. *Biomed Pharmacother* 73: 135-139, 2015.
22. Justilien V, Ali SA, Jamieson L, Yin N, Cox AD, Der CJ, Murray NR and Fields AP: Ect2-dependent rRNA synthesis is required for KRAS-TRP53-driven lung adenocarcinoma. *Cancer Cell* 31: 256-269, 2017.
23. Guo Z, Chen X, Du T, Zhu D, Lai Y, Dong W, Wu W, Lin C, Liu L and Huang H: Elevated levels of epithelial cell transforming sequence 2 predicts poor prognosis for prostate cancer. *Med Oncol* 34: 13, 2017.
24. Hirata D, Yamabuki T, Miki D, Ito T, Tsuchiya E, Fujita M, Hosokawa M, Chayama K, Nakamura Y and Daigo Y: Involvement of epithelial cell transforming sequence-2 oncoantigen in lung and esophageal cancer progression. *Clin Cancer Res* 15: 256-266, 2009.
25. Wang HK, Liang JF, Zheng HX and Xiao H: Expression and prognostic significance of ECT2 in invasive breast cancer. *J Clin Pathol* 71: 442-445, 2018.



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