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BCL11A in Breast Cancer Progression

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Abstract

Background

This study aimed to investigate the expression and clinical significance of BCL11A, estrogen receptor (ER), and human epidermal growth factor receptor 2 (HER2) in breast cancer.

Methods

The study included two cohorts. Part 1 consisted of 119 surgical specimens from patients with invasive breast cancer (IBC). Part 2 included 44 surgical specimens from patients with both IBC and synchronous ductal carcinoma in situ (DCIS). BCL11A expression was assessed using quantitative polymerase chain reaction (qPCR) and immunohistochemistry (IHC). Statistical analyses were performed to evaluate associations among ER, HER2, BCL11A protein expression, and clinicopathological parameters.

Results

(1) In 44 IBC samples, BCL11A protein showed no or weak expression in peritumoral tissues, but high expression in IBC and synchronous DCIS tissues. (2) BCL11A expression differed significantly between breast cancer and non-cancerous tissues ($P < 0.01$), but not between IBC and synchronous DCIS ($P = 0.84$). (3) In IBC tissues, BCL11A and HER2 expression correlated with primary tumor T stage ($P < 0.01$), but not with age, histological grade, or N stage ($P > 0.05$). (4) Among eight IBC samples, the absolute BCL11A expression level was highest in one ER–/HER2 + case, while relative expression was higher in two ER–/HER2– cases than in other samples.

Conclusions

BCL11A and HER2 are expressed at the pre-invasive stage and may serve as early triggering factors in human breast cancer. BCL11A may drive the progression from DCIS to IBC and ipsilateral axillary lymph node metastasis.

Introduction

Breast cancer (BC) is the most common malignant tumor among women worldwide. Clinical management of invasive breast cancer (IBC) relies on multidisciplinary, individualized treatment plans based on histological type, disease stage, and biomarker expression. Among current strategies, molecular targeted therapy with high efficacy and safety has become a major focus of cancer research.

Ductal carcinoma in situ (DCIS) can progress to IBC, and most patients receive breast-conserving surgery and radiotherapy similar to that for early-stage IBC[1]. For advanced breast cancer, treatment decisions consider lymph node status, hormone receptor (HR) and HER2 expression, and menopausal status, often combining surgery with adjuvant systemic therapy[1]. Nevertheless, breast cancer remains a major threat to patient

survival, accounting for 23% of all cancer-related deaths in postmenopausal women[2]. Therefore, identifying effective diagnostic markers for breast cancer is urgently needed.

The BCL11A gene was originally identified in mice as CTIP1 (chicken ovalbumin upstream promoter transcription factor interacting protein1) and also named EVI9 (ecotropic virus integration site 9 homolog)[3,4]. Recent evidence indicates that BCL11A functions as an oncogene. High BCL11A expression in lymphoid malignancies is associated with tumor progression[5–7], making it a potential diagnostic and prognostic marker for certain hematopoietic malignancies. Elevated BCL11A expression has also been reported in triple-negative breast cancer[8], neuroblastoma, non-small cell lung cancer[9], endometrial cancer[10], cervical cancer, and other solid tumors, where it often correlates with tumor progression and poor prognosis. BCL11A is considered a novel breast cancer risk gene involved in signal transduction, cell proliferation, cell cycle regulation, apoptosis, and androgen receptor expression[8,11], but its exact mechanisms remain unclear. Therefore, investigating the roles and clinical significance of ER, HER2, and BCL11A in breast cancer development and progression is of particular importance.

Materials and Methods

Study subjects

All tissue samples were obtained from the pathology database of our department. Diagnoses were confirmed by two senior pathologists. The study protocol was approved by the Ethics Committee of the Second Affiliated Hospital of Chengdu Medical College, China National Nuclear Corporation 416 Hospital. Written informed consent was obtained from all participants.

Immunohistochemistry (IHC)

Tissue specimens were serially sectioned at 4 μm thickness and placed onto treated glass slides (at least five backup slides per specimen). Sections were deparaffinized, rehydrated, and rinsed with tap water. Antigen retrieval was performed according to the primary antibody requirements. After blocking endogenous peroxidase, sections were incubated with primary antibody (60 min at room temperature or overnight at 4°C), followed by biotinylated secondary antibody and streptavidin-alkaline phosphatase reagent. DAB or AEC was used for chromogenic detection, and sections were counterstained with hematoxylin.

Scoring followed the Fromowitz semi-quantitative method, evaluating staining intensity (0 = no staining, 1 = light yellow, 2 = brown-yellow, 3 = brown-black) and positive cell proportion (0 = < 10%, 1 = 10–25%, 2 = 26–50%, 3 = 51–80%, 4 = > 80%). The sum of the two scores was interpreted as: 0–1 = negative, 2–7 = positive.

RNA isolation and cDNA synthesis

Total RNA was extracted from breast cancer tissues, breast lobular hyperplasia samples, and cell lines (MCF-7, SK-BR-3, MDA-MB-231, MDA-MB-468, MCF-10A) using the RNeasy Mini Kit (QIAGEN, Germany). RNA concentration and purity were measured using a NanoDrop1000 spectrophotometer (Thermo Fisher Scientific,

USA). Reverse transcription was performed using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, USA) in a SimpliAmp™ Thermal Cycler (Applied Biosystems). Conditions: 10 min at 25°C, 120 min at 37°C, and 5 min at 85°C.

Quantitative real-time PCR (qPCR)

BCL11A mRNA levels were evaluated using a 7500 real-time PCR system (Applied Biosystems). GAPDH was used as the endogenous control. TaqMan gene expression assays with specific primers and probes were used. Reactions were run in triplicate under the following conditions: 50°C for 2 min, 95°C for 10 min, followed by 45 cycles of 95°C for 15 s and 60°C for 1 min. Relative BCL11A expression was calculated using the ΔΔCt method.

Statistical analysis

Statistical analyses were performed using SPSS software (version not specified). Group comparisons were made using chi-square tests or Fisher's exact test as appropriate. Spearman correlation was used to assess relationships between variables. A P value < 0.05 was considered statistically significant.

Results

Expression of ER and HER2 in 119 IBC tissues, DCIS, and peritumoral tissues

Among 119 IBC samples, 24 contained peritumoral tissues and 22 contained DCIS components based on HE and p63 IHC staining. ER and HER2 expression patterns in DCIS were consistent with those in IBC (Fig. 1). ER expression within and across tissue types varied: some cases showed consistent (both positive or both negative) expression, while others exhibited differential expression. Significant differences in ER and HER2 expression were observed among IBC, synchronous DCIS, and peritumoral normal tissues (Tables 1, 2).

Table 1
Expression of ER and HER2 in peritumoral normal tissues, DCIS and IBC

	n	ER		χ^2	*P*	HER2		χ^2	*P*
		-	+			-	+		
				36.605	< 0.01			17.711	< 0.01
Peritumor	24	1	23			24	0		
DCIS	22	7	15			10	12		
IBC	119	39	80			75	44		
Age(years)				1.083	0.298			0.005	0.944
<51	60	17	43			38	22		
≥ 51	59	22	37			37	22		
Grades				1.575	0.209			0.062	0.803
G1-2	63	18	45			39	24		
G3-4	53	21	32			34	19		
Other	3								
T				1.065	0.302			6.881	< 0.01
T1-2	92	28	64			63	29		
T3-4	12	6	6			3	9		
Unknown	15								
N				0.662	0.416			0.017	0.895
N0-1	93	32	61			59	34		
N2-3	20	5	15			13	7		
Unknown	6								

Table 2
Co-expression of ER/HER2 in pericancerous tissue, DCIS and IBC and its clinical significance in IBC

	n	ER/HER2				χ^2	*P*
		+/-	+/+	-/+	-/-		
						24.460	< 0.01
Peritumor	24	23	0	0	1		
DCIS	22	8	7	5	2		
IBC	119	48	32	18	21		
Age(years)						1.287	0.732
<51	60	29	14	8	9		
≥ 51	59	24	13	9	13		
Grades						3.859	0.277
G1-2	63	31	14	10	8		
G3-4	53	20	12	7	14		
Other	3						
T						9.298	0.026
T1-2	92	46	18	11	17		
T3-4	12	2	4	5	1		
Unknown	15						
N						1.680	0.641
N0-1	93	40	21	13	19		
N2-3	20	11	4	3	2		
Unknown	6						

BCL11A expression in IBC with synchronous DCIS

BCL11A protein expression was assessed by IHC in 44 IBC specimens containing synchronous DCIS (Fig. 2). Weak BCL11A expression was observed in normal breast ductal epithelium, moderate nuclear expression in DCIS, and variable (weak to strong) cytoplasmic/nuclear expression in IBC.

Correlation among BCL11A, ER, and HER2

Spearman correlation analysis showed that BCL11A expression was positively correlated with breast cancer occurrence (r_s=0.434, P < 0.01). Significant differences in BCL11A expression were found among the three

tissue types, consistent with ER and HER2 differences (Table 3).

Table 3
Expression of BCL11A in IBC, synchronous DCIS, and pericancerous normal tissue

n	Peritumor	DCIS	IBC	χ^2	*P*
	34	**28**	**44**		
BCL11A				23.095	<0.01
0	23	9	10		
1+	11	10	17		
2+	0	7	13		
3+	0	2	4		
Standard a				17.066	<0.01
negative	23	9	10		
positive	11	19	34		
Standard b				16.657	<0.01
negative	34	19	27		
positive	0	9	17		
ER				18.384	0.005
0	1	7	19		
1+	7	3	4		
2+	26	18	21		
HER2				32.617	<0.01
0	14	4	13		
1+	19	9	6		
2+	1	1	1		
3+	0	14	24		

Pairwise comparisons of BCL11A among tissue types

BCL11A expression was significantly higher in IBC and DCIS than in peritumoral tissues ($P < 0.01$), but did not differ significantly between IBC and DCIS ($P = 0.84$, Table 4).

Table 4
Pairwise comparison of BCL11A expression in three tissue types

n	Peri	DCIS	χ^2	*P*	Peri	IBC	χ^2	*P*	DCIS	IBC	χ^2	*P*
	34	**28**			**34**	**44**			**28**	**44**		
BCL11A			14.72	<0.01			22.49	<0.01			0.81	0.84
0	23	9			23	10			9	10		
1+	11	10			11	17			10	17		
2+	0	7			0	13			7	13		
3+	0	2			0	4			2	4		
Standard a			7.75	<0.01			15.85	<0.01			0.78	0.37
negative	23	9			23	10			9	10		
positive	11	19			11	34			19	34		
Standard b			10.32	<0.01			16.79	<0.01			0.31	0.57
negative	34	19			34	27			19	27		
positive	0	9			0	17			9	17		

Clinical significance of BCL11A expression

BCL11A expression correlated significantly with tumor diameter and T stage ($P < 0.05$), but not with age, lymph node metastasis, or grade (Table 5).

Table 5
Clinical significance of BCL11A expression in invasive breast cancer

	n	BCL11A				χ^2	*P*
		0	1+	2+	3+		
Age(years)						1.193	0.75
<51	20	5	6	7	2		
≥ 51	24	5	11	6	2		
Grades						9.506	0.147
G1-2	29	6	10	12	1		
G3	15	4	7	1	3		
diameter							0.033*
≤ 5cm	32	2	14	12	4		
>5cm	7	4	2	1	0		
Unknown	5						
LN						1.837	0.606
No	25	4	10	8	3		
Yes	19	6	7	5	1		
T							0.041*
T1-2	31	2	14	11	4		
T3-4	8	4	2	2	0		
Unknown	5						
N						1.819	0.994
N0-1	37	7	15	11	4		
N2-3	6	2	2	2	0		
Unknown	1						

qPCR analysis of BCL11A in eight IBC patients

BCL11A mRNA expression varied by ER/HER2 subgroup. Lower expression was observed in ER-positive cases. One ER–/HER2 + case showed the highest absolute BCL11A expression, while two ER–/HER2–cases showed the highest relative expression compared to peritumoral tissues (Figs. 3, 4).

Discussion

BCL11A is a newly recognized breast cancer risk gene. Previous studies have largely focused on triple-negative breast cancer and investigated cell proliferation, androgen receptor expression, and cell viability[8,11]. In this study, we included all molecular subtypes of IBC and, for the first time, reported BCL11A expression in synchronous DCIS. We also evaluated BCL11A expression in adjacent ductal-lobular tissues to explore its potential role in breast cancer initiation and progression.

We found that BCL11A protein was localized in both the cytoplasm and nucleus. A previous study showed that the BCL11A-S isoform is confined to the cytoplasm, whereas two longer isoforms are nuclear[12]. BCL11A is normally expressed in the brain and most hematopoietic cells, including hematopoietic stem cells, common lymphoid progenitors, and early T-cell progenitors, but is only weakly expressed in T lymphocytes[13,14]. Different isoforms exhibit specific expression patterns: BCL11A-XL is expressed in normal B cells, while BCL11A-S is found in malignant B cells[15]. In brain tissue from humans and rats, BCL11A-S is widely distributed, but BCL11A-L is mainly cortical[16,17]. The functional implications of these different expression patterns remain unclear.

The normal expression of BCL11A is tightly regulated. Imbalances or genetic variations in regulatory factors can lead to BCL11A dysregulation. Our findings suggest that BCL11A may act in the pre-invasive stage of breast cancer. Previous reports have shown that BCL11A is regulated by miRNAs, transcription factors, and genetic variations[18,19]; however, its role and regulation in solid tumors, especially breast cancer, remain poorly understood.

We observed higher BCL11A protein expression in IBC and synchronous DCIS than in adjacent ductal-lobular tissues, suggesting that BCL11A dysregulation may be an early event in breast cancer development. A recent study using multiplexed ion beam imaging (MIBI-TOF) and machine learning revealed coordinated transitions in the tumor microenvironment (TME) among normal breast, DCIS, and IBC[20]. That study highlighted the disruption of myoepithelial cells during the transition from DCIS to IBC. Whether BCL11A contributes to such myoepithelial disruption warrants further investigation.

Currently, there is a lack of specific biomarkers to distinguish low-risk DCIS from DCIS with higher progression potential. As a result, many patients receive surgery or adjuvant therapy, which may be overtreatment in low-risk cases. Better risk stratification could improve clinical decision-making. Genomic studies comparing DCIS and IBC from the same patient have revealed clonal evolution processes[21,22], but consistent explanations for the DCIS–IBC transition are still lacking. Our results suggest that BCL11A may be a driver gene in this process, though this requires validation with larger cohorts and ideally with subtype stratification[23].

This study has several limitations: the sample size is relatively small, the analyses are descriptive, there is no external validation cohort, and no survival analysis was performed. Additionally, we did not stratify DCIS by histological subtype. Future studies with larger, multicenter cohorts and molecular subtyping are needed to confirm our findings.

Advances in molecular technologies (FISH,qRT-PCR, gene chips,NGS,and ctDNA detection) have significantly improved our understanding of breast cancer genetics. However, standardization of detection methods

across institutions remains a challenge in China and elsewhere.

In conclusion, this study provides preliminary evidence that BCL11A is expressed in the pre-invasive stage of breast cancer and may drive breast cancer progression. Future work should explore how BCL11A recruits epigenetic modifiers to regulate downstream target genes, thereby elucidating its mechanism in breast cancer and potentially offering targeted treatment strategies for BCL11A-positive patients.

Declarations

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Author contributions

Jinrong Chen and Yulan Liu: concept and design.Bingyin Su and Lingmiao Kong: immunohistochemistry.Shuaijun Zhang and Shigao Chen: collect specimens and corresponding clinical information.Shurong Li: clinical data analysis.Xuemin Xie: draft the manuscript.All authors have read and agreed to the published version of the manuscript.

Data availability The raw sequence data reported in this paper have been deposited in the Genome Sequence Archive (Genomics, Proteomics & Bioinformatics 2025) in National Genomics Data Center (Nucleic Acids Res 2025), China National Center for Bioinformation/Beijing Institute of Genomics, Chinese Academy of Sciences (GSA-Human: HRA018699) that are publicly accessible at https://ngdc.cncb.ac.cn/gsa-human.

Ethics approval and consent to participate This study protocol related to human use has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration, and has been approved by the Ethics Committee of the Second Affiliated Hospital of Chengdu Medical College, China National Nuclear Corporation 416 Hospital.Consent for publication Informed consent was obtained for experimentation with human subjects.

Competing interests The authors declare that they have no competing interests.

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Figures

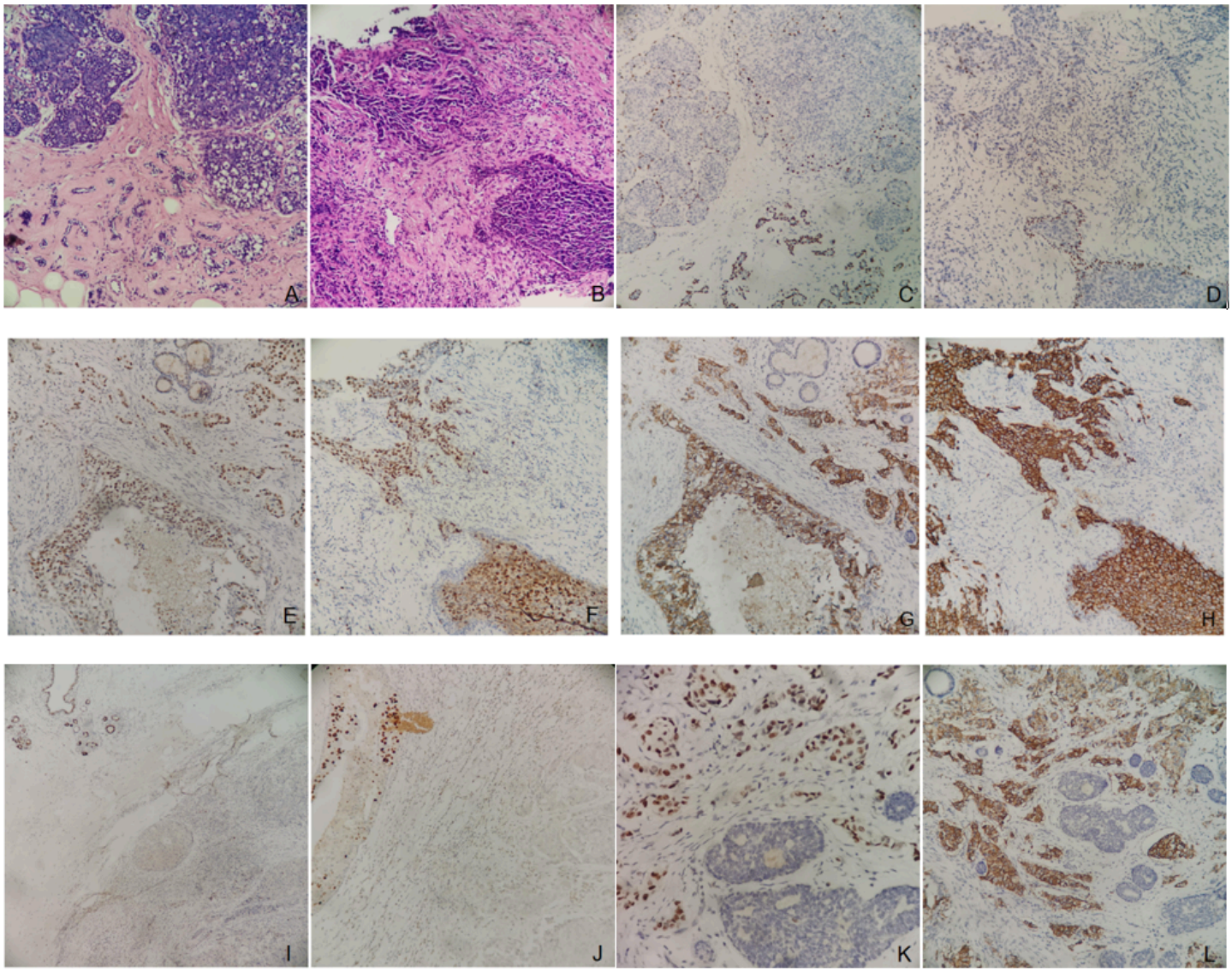


Figure 1

Histological characteristics and immunohistochemical expression of invasive breast cancer, synchronous DCIS and pericancerous normal tissue

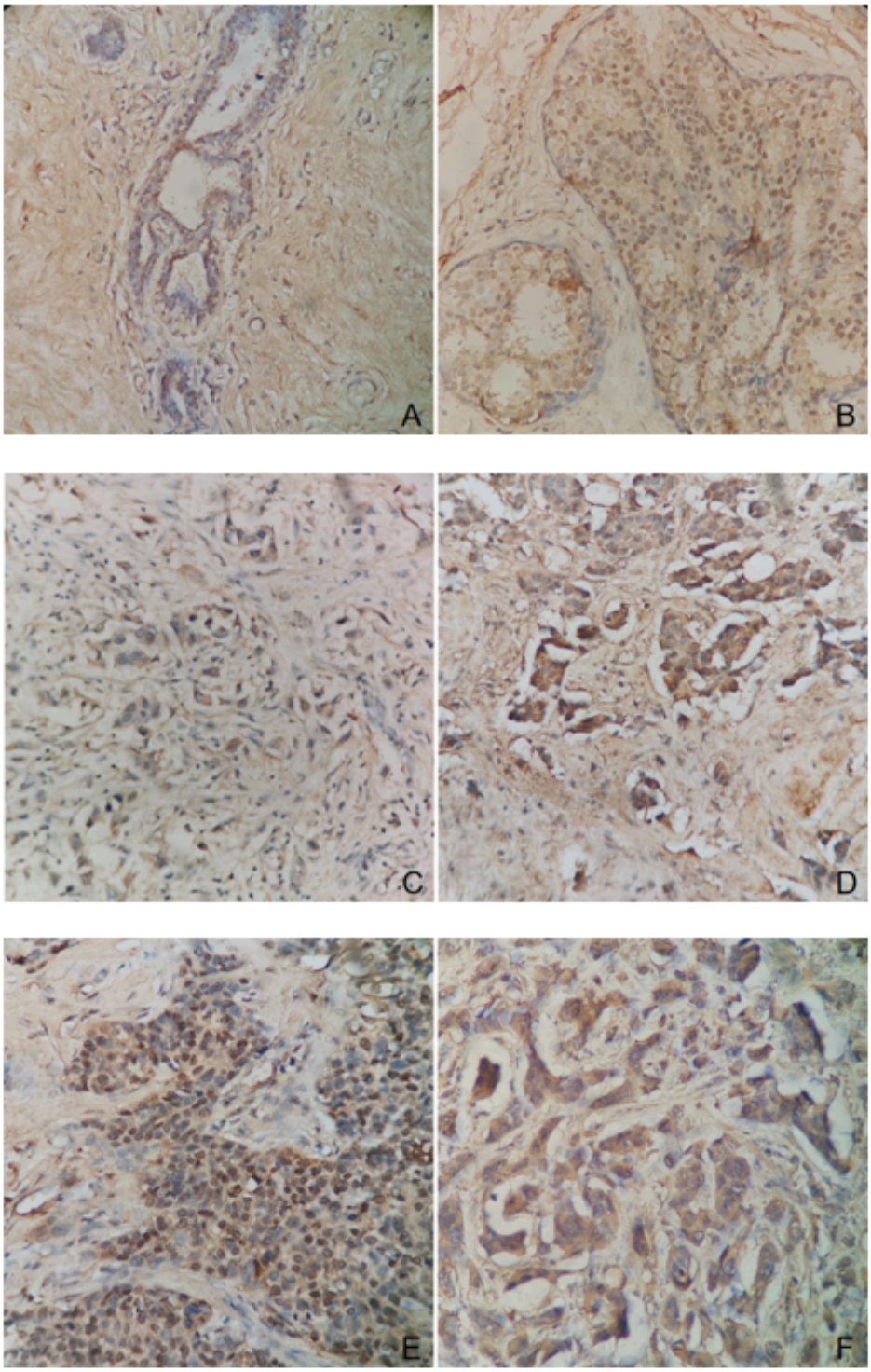


Figure 2

Protein expression of BCL11A in breast cancer tissues, synchronous DCIS and pericancerous tissues

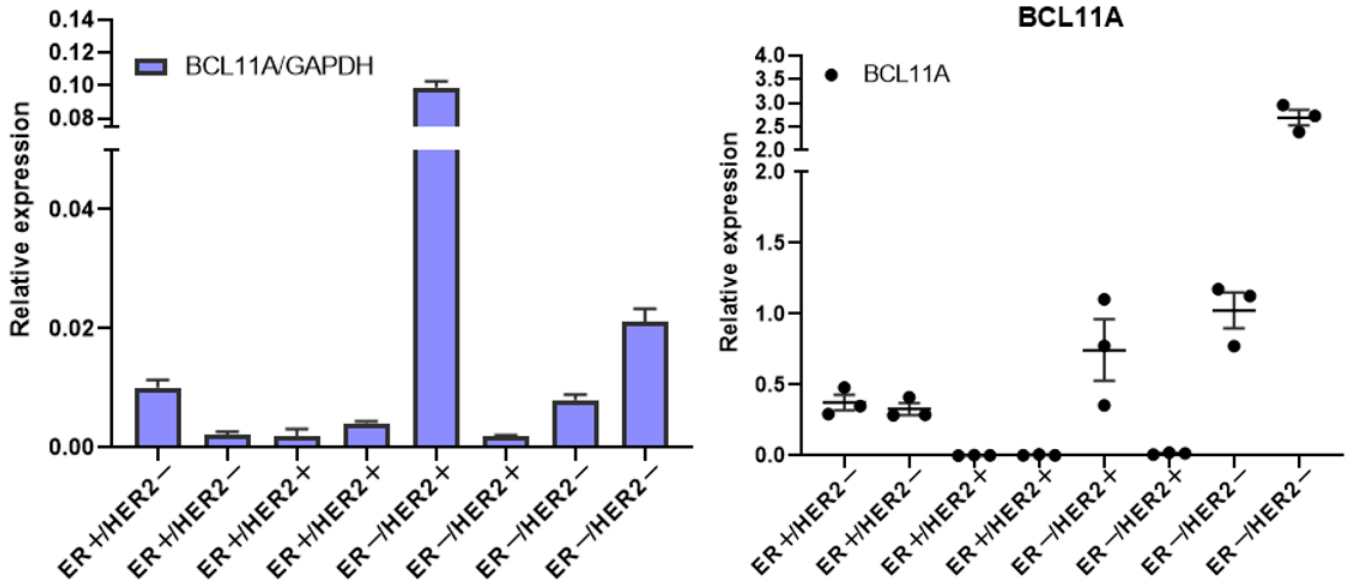


Figure 3

Bar plot (right) of absolute expression of BCL11A relative to internal reference gene and scatter plot (left) of relative expression to pericancerous tissue in 8 breast cancer tissue samples

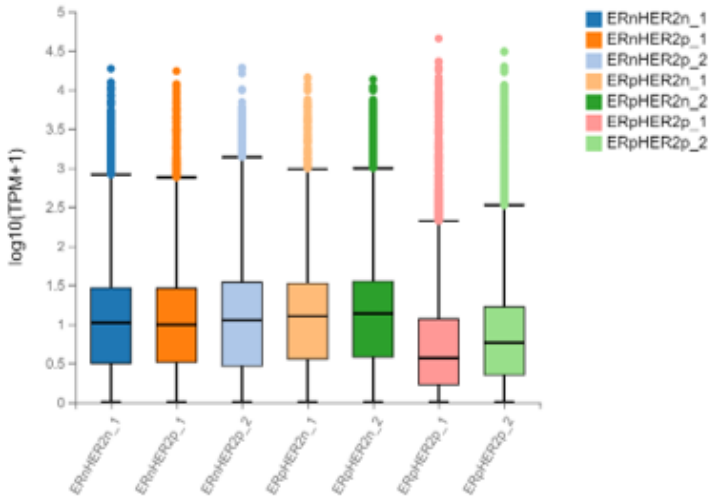


Figure 4

Boxplot of whole-genome transcriptional expression levels in 7 breast cancer tissue samples