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Original Article

Overexpression of galectin2 (LGALS2) predicts a better prognosis in human breast cancer

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Abstract: Background: Galectins (LGALS) are a family of carbohydrate-binding proteins, and LGALS family members have shown prognostic roles in various types of cancers. However, the prognostic significance of some LGALS family members has not been studied in breast malignancy. Methods: The prognostic value of LGALS family mRNA expression in breast cancer patients was investigated according to distinct clinicopathological features (including lymph node, intrinsic subtype, pathological grade, HER2, and TP53 status) using the Kaplan-Meier plotter database. Quantitative real-time polymerase chain reaction and western blotting were used to detect the mRNA and protein expression of LGALS in breast cancer and normal breast cells. The aberrant expression of specific LGALS and its correlation with breast cancer outcomes remains elusive. In the present analysis, we comprehensively explored an immunohistochemistry-based map of protein expression profiles in normal tissues, cancer, and cell lines from the widely available Human Protein Atlas (HPA) database. Immunohistochemistry was applied to evaluate the expression of LGALS between cancer and normal tissues. Results: Our results showed that overexpression of LGALS2 mRNA were correlated with satisfactory overall survival among all breast cancer patients. Furthermore, LGALS2 and LGALS4 expression correlated with a better overall survival (OS) in grade III breast cancer patients; LGALS2 also predicted a better OS in basal-like subtype patients, luminal B patients, HER2-overexpressing patients, TP53 mutated and wild breast cancer patients. Notably, the mRNA and protein expression levels of LGALS2 were decreased in cancer cells compared with normal cells ($P<0.05$). Furthermore, LGALS2 expression in immunostaining score was lower in cancer tissues than in normal tissues ($P<0.005$). Conclusion: In conclusion, LGALS2 has potential as a valuable biomarker for envisaging a satisfactory prognosis in patients with breast tumours, particularly those with luminal and basal B types, all stages and grade III tumours.

Keywords: Prognosis, breast cancer, galectins (LGALS)

Introduction

Breast carcinoma is one of the most common malignancies and is the number one reason for cancer-related mortality in women throughout the world [1, 2]. Although the death rate is declining due to progress in screening, diagnostic and treatment modalities, the incidence of breast carcinoma is rising, and tumour recurrence and metastatic relapse are the main causes of death [3, 4]. Preventive agents and therapies targeting the progesterone receptor, oestrogen receptor, and human epidermal growth factor receptor 2 (HER2) have enhanced the medical results for numerous women with breast tumours, but tough challenges remain in managing cancers that do not express these molecular targets [5]. Thus, novel biomarkers that can be used to predict disease progression or identify new target molecules for more effective treatment modalities of breast cancer are being explored.

Galectins (LGALS) are a family of galactoside-binding glycoproteins with preserved carbohydrate recognition domains (CRDs) of approximately 130 amino acids [6]. Currently, 15 mammalian LGALS have been recognized (LGALS 1-15), which are widely expressed in normal and neoplastic cells. They have been associated with a wide range of biological processes, such as inflammatory responses, intracellular

signalling, tumour metastasis, cell adhesion, and differentiation [7, 8]. Numerous previous studies have shown that LGALS is aberrantly expressed and closely related to metastasis and invasion in certain types of tumours [9-12]. In addition, Thijssen et al. [13] reported that LGALS expression was associated with typical predictive indicators of cancers, such as clinical stage, lymph node status, and cancer grade. These family members have been well studied as prognostic markers in different types of cancer [14-28]. Nevertheless, the predictive significance of some family members of LGALS has rarely been studied in breast cancer. Hence, the analytical and predictive relevance of LGALS was highlighted, while opposing statistics concerning the type of LGALS and comparative malignancies have been published [15, 16]. However, the prognostic and functional roles of LGALS in women with breast carcinoma remain uncertain.

This study aimed to determine the prognostic value of LGALS mRNA expression in breast tumour patients according to distinct clinicopathological features (including lymph node, intrinsic subtype, pathological grade, HER2, and TP53 status) using Kaplan-Meier plotter (KM plotter). Furthermore, to identify prognostic biomarkers for patients with breast tumours, dissimilar LGALS expression among breast cancer cell lines and normal breast cell lines was observed. HPA using the Protein Atlas samples contains 20 unique cancer types, 48 unique normal human tissues, and 47 unique haematopoietic cell types and 12 human cell lines from patients [16, 17]. Expression analysis of cell lines in HPA is accomplished via programmed image examination of immunohistochemical (IHC) staining, and a similar scoring method has been used to describe the expression level. The databank hence keenly supports protein expression patterns representing whether a particular protein might be used as a biomarker.

Methods and materials

In the present study, the association between individual LGALS mRNA expression and overall survival (OS) of breast tumour patients was investigated using the Kaplan-Meier plotter database (www.kmplot.com.) [29]. Currently, this database is proficient for assessing the prognostic role of 54,675 genes in lung carcinoma [30], ovarian carcinoma [31], gastric carcinoma [32], and breast carcinoma [33]. Gene expression data and OS data of breast tumour patients from the Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/) [29] are included in this database. Additionally, they provide clinical data, such as intrinsic subtype, lymph node status, HER2 status, TP53, and differentiation grade status of breast cancer patients.

In simpler terms, fifteen LGALS members (LGALS1, LGALS2, LGALS3, LGALS4, LGALS5, LGALS6, LGALS7, LGALS8, LGALS9, LGALS10, LGALS11, LGALS12, LGALS13, LGALS14, and LGALS15) were introduced into the database (http://kmplot.com/analysis/index.php?p=service&cancer=breast) to obtain Kaplan-Meier survival plots. The selected breast malignancy patients were classified into “high” and “low” according to the LGALS mRNA level with the auto select best cut-off value. Then, the two groups of patients were matched with a Kaplan-Meier survival plot and analysed by setting different clinicopathological parameters. In addition, 95% confidence intervals (CIs), hazard ratios (HRs), and log-rank *P* values were taken from the website. A *P* value of <0.05 was found to be statistically significant; furthermore, if the HR>1 and the lower limit of the 95% CI of the HR>1, this implied that LGALS expression predicted a poor outcome of breast cancer patients; however, if the HR<1 and the upper limit of the 95% CI of the HR<1, this suggested that LGALS expression predicted a better outcome of breast cancer patients.

This study was subject to approval by the Ethics Committee Board of The First Affiliated Hospital of Shantou University, Guangdong, People’s Republic of China, SUMC-IRB-2021.

The HPA

HPA (www.proteinatlas.org) comprises IHC-based expression information for 20 cancer types, each of which is represented by 12 distinct tumours [34]. The database recognizes that tumour type-specific expression patterns can be classified and that proteins are expressed differently in distinct tumour types. Using the HPA database, it was discovered that more than half of the tumour tissue sections used a subjective variety of protein expression.

To identify the IHC image, successive sections of gastric cancer and normal human tissues were stained with two distinct antibodies: HPA and CAB. Different protein expression patterns were endorsed for direct comparison within tissue and subcellular slices. Finally, a database was used to examine the protein expression results of specific LGALS genes in normal tissues and breast cancer tissues.

Cell lines and cell culture

The human breast tumour cell lines T47D, MDA-MB-231, and MCF-7 and the human normal breast epithelial cell line MCF10A were obtained from Fuheng (Shanghai, China). All cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) (Biosharp, China) supplemented with 10% foetal bovine serum (FBS) (Biosharp, China) and 1% antibiotics (penicillin-streptomycin). All cells were incubated at 37°C in a humidified atmosphere comprising 5% CO_2. Furthermore, the cells were cultured to a confluence of 80% and passaged by using 1× trypsin with 0.2% ethylenediaminetetraacetic acid (EDTA).

RNA extraction and quantitative real-time PCR (qRT-PCR)

Total RNA was obtained using TRIzol reagent (Biosharp, China) as directed by the manufacturer, and cDNAs were created using reverse transcription. The subsequent reaction conditions were 94.0°C for 30 seconds for qRT-PCR, followed by 39 cycles of 94.0°C for 5 seconds and 60.0°C for 30 seconds with the PCR Kit (Biosharp, China). For gene quantification, the internal control of GAPDH was set. With qRT-PCR analysis, the number of technical and biological duplicates for each gene was at least three. The primers that were utilized in this study are listed below.

Human LGALS2: Forward: 5'-ATGACGGGGGAACTTGAGGT-3', Reverse: 5'-CAGGTTCAGCTTGTCTGTCC-3'. *Human GAPDH:* Forward: 5'-AAGAAGGTGGTGAAGCAGG-3', Reverse: 5'-GTCAAAGGTGGAGGAGTGG-3'.

Western blot analysis

The cells were isolated and lysed in lysis buffer and the protein concentration was measured with the bicinchoninic acid (BCA) technique. Protein samples (30 μg) were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to PVDF membranes (Millipore). The membranes were incubated with primary antibodies against LGALS2 (1:4000, mouse anti-human, B Boster, China) using a 5% skim milk blocking agent, and anti-GAPDH (1:2000, mouse anti-human, Abbkine, China) at 4°C overnight. At room temperature, the membranes were incubated for 2 hours with a conjugated secondary antibody before being imaged with chemiluminescence reagent using gel imaging equipment. The amounts of protein were determined using the NIH ImageJ program and densitometry analysis (Rockville, MD, USA). The experiments were carried out three times.

Immunohistochemistry

We constructed 5 breast cancer tissue specimens obtained from staging surgery or cytoreductive surgery performed for patients with breast cancer and 5 normal breast tissue samples from patients receiving surgery for other gynaecological diseases after receiving appropriate approval from the ethics committee at The First Affiliated Hospital of Shantou University Medical College. Non-pregnant, non-breastfeeding women over the age of 18 are included in the study. Immunohistochemistry was done on tissue slices (4 m) from 10 formalin-fixed, paraffin-embedded breast tumor tissues and 5 pathologically verified normal breast tissues. The slides were dehydrated in xylene and graded ethanol and then treated with 0.3 percent hydrogen peroxide before being blocked with 10% normal goat serum. The sections were then incubated at 4°C overnight with primary antibodies antiLGALS2 (1:20, mouse antihuman, Santa Cruze). The sections were then treated with a secondary antibody before being stained with 3'3-diaminobenzidine tetrahydrochloride (DAB) (1:50 dilution, GIBCO) and counterstained with hematoxylin. For each experiment, positive and negative controls were established.

Two authors blindly viewed and rated the staining strength based on the positive cell percentage and positive cell staining density. The proportion of positively stained cells was scored as follows: 0 for no stained cells, 1 for 1%-25% stained cells, 2 for 26%-50% stained cells, 3 for 51%-75% stained cells, and 4 for 76%-

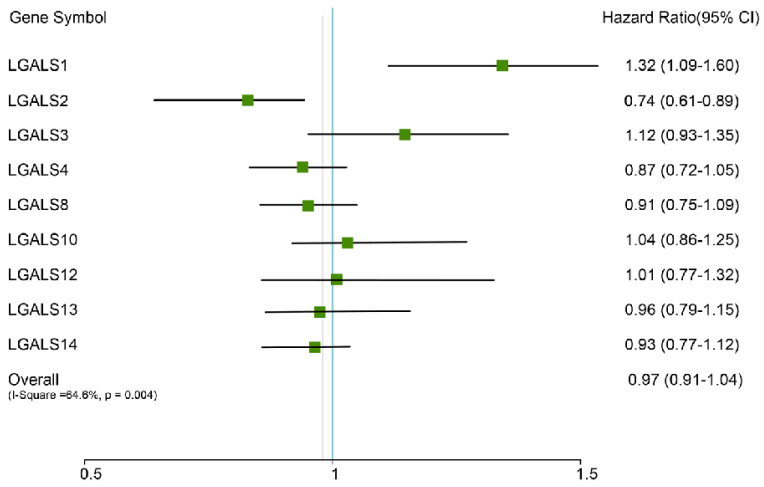

Figure 1. The prognostic HRs of individual LGALS members in all breast cancers in www.kmplot.com. Only 9 members (LGALS1, LGALS2, LGALS3, LGALS4, LGALS8, LGALS10, LGALS12, LGALS13, and LGALS14) were found on www.kmplot.com. Overexpression of LGALS2 mRNA was correlated with satisfactory overall survival in all breast cancer patients HR: Hazard ratio; CI: confidence intervals.

100% stained cells. The density of positive cell staining was scored as 0 (no staining), 1 (light yellow staining), 2 (yellow staining), and 3 (brown staining). Finally, by multiplying the proportion of positively stained cells by the staining intensity, the immunoreactivity score (IRS) was calculated (score ranged from 0 to 12). The final score was calculated using the average of the two referees' values.

Statistical analyses

For statistical analysis, SPSS 17.0 (SPSS, Chicago, USA) software was used. The mean and standard deviation (SD) are presented, and Student's t-test was performed to test the significance between groups. The Kaplan-Meier method was used to plot survival curves, and the log-rank test was used to associate them. The log-rank P, confidence intervals (95% CI), and hazard ratios (HRs) were analysed. Statistical significance was measured by a *P* value of <0.05.

Results

The prognostic values of LGALS members in all breast carcinoma patients

There are fifteen members in the LGALS family; however, only 9 members (LGALS1, LGALS2, LGALS3, LGALS4, LGALS8, LGALS10, LGALS12, LGALS13, and LGALS14) were found in www.kmplot.com. We show the prognostic values of the mRNA expression of these 9 LGALS family members in **Figure 1**. High mRNA expression of LGALS2 was suggestively correlated with favourable OS in all breast tumour patients (HR=0.74 (0.61-0.89), P=0.0018), whereas LGALS1 was correlated with a poor OS in all breast tumour patients HR=1.32 (1.09-1.6), P=0.004. However, high mRNA expression of LGALS3, LGALS4, LGALS8, LGALS10, LGALS12, LGALS13 and LGALS14 showed no correlation with OS in all breast cancer patients, HR=1.12 (0.93-1.35), P=0.23; HR=0.87 (0.72-1.05), P=0.15; HR=0.91 (0.75-1.09), P=0.3; HR=1.04 (0.86-1.25), P=0.72; HR=1.01 (0.77-1.32), P=0.95; HR=0.96 (0.79-1.15), P=0.65; HR=0.93 (0.77-1.12), P=0.43 (**Figure 2A-I**).

The prognostic values of LGALS members in different breast tumour subtypes

Then, the prognostic values of LGALS family members were assessed in different types of breast tumours, including basal-like tumours, different intrinsic subtypes, luminal A tumours, luminal B tumours, and HER2-high tumours (**Table 1**). In basal-like breast carcinoma, increased expression of LGALS2 mRNA was significantly linked with a better OS; however, LGALS1 and LGALS13 expression were associated with poor OS. In addition, other members of the LGALS family were not related to OS in basal-like breast carcinoma patients.

In luminal A-type breast malignancy, high levels of LGALS members showed no association with OS.

In luminal B-type breast malignancy, LGALS2 mRNA expression was significantly linked with better OS. However, LGALS1 and LGALS3 predicted a poor outcome in luminal B-type breast tumour patients. The rest of the LGALS members were not associated with OS in luminal B-type breast tumour patients.

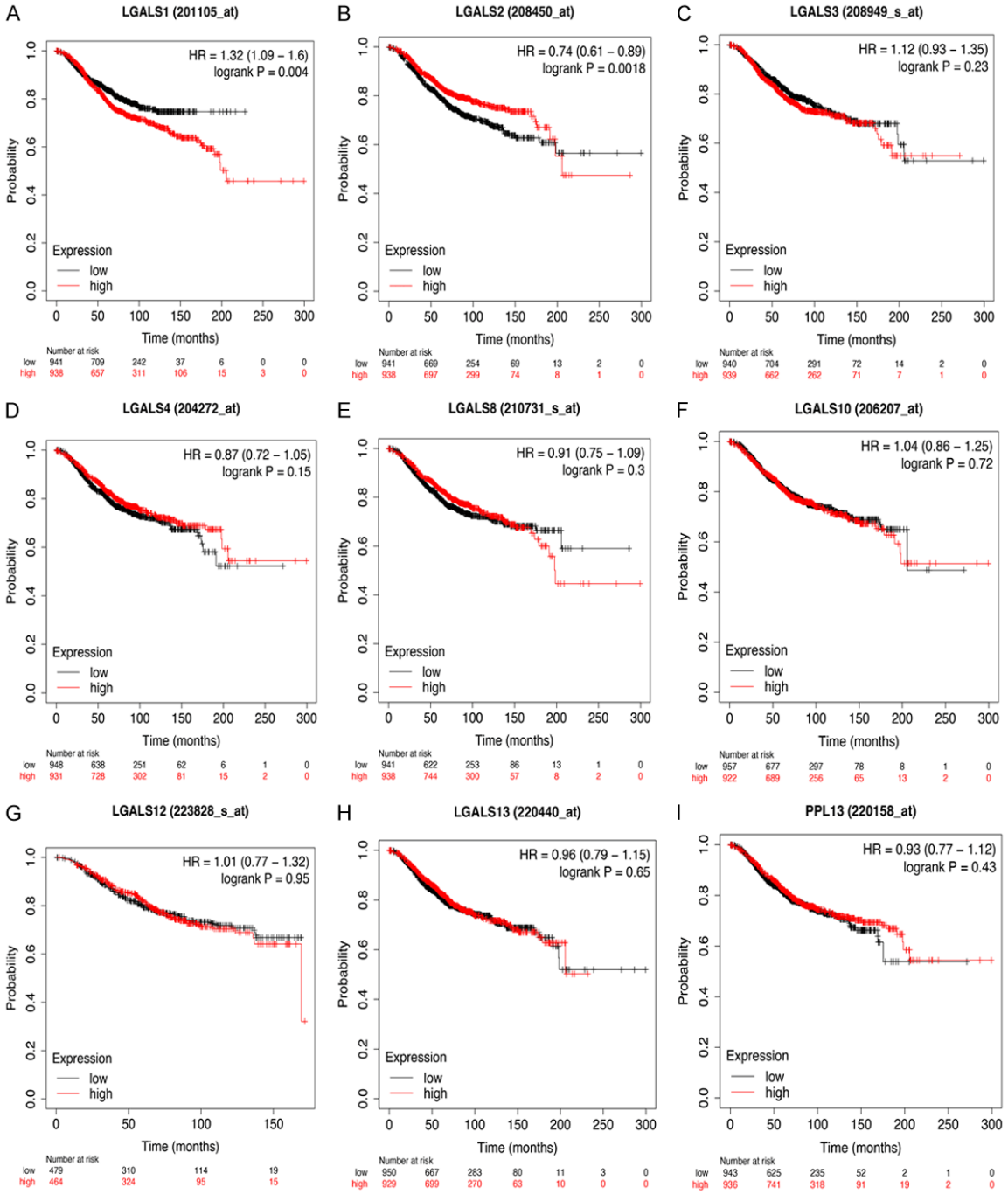

Figure 2. The prognostic value of LGALS member expression in breast cancer with Affymetrix IDs. The prognostic survival curves of LGALS1 (A) 201105_at, LGALS2 (B) 208450_at, LGALS3 (C) 208949_s_at, LGALS4 (D) 204272_at, LGALS8 (E) 210731_s_at, LGALS10 (F) 206207_at, LGALS12 (G) 223828_s_at, LGALS13 (H) 220440_at, and LGALS14 (I) 220158_at were plotted for all breast cancer patients (n=943 for LGALS12 and n=1879 for the rest of all LGALS members).

In HER2-overexpressing breast tumour patients, high mRNA expression of LGALS2 was correlated with a better prognosis. Nevertheless, the remaining LGALS members were not linked with the prognosis in HER2-overexpressing breast carcinoma patients.

The prognostic value of LGALS members in breast tumour patients with different clinicopathological features

In our current research, we observed that high mRNA expression of LGALS10, LGALS12, and

Table 1. Correlation of LGALS expression level with OS in different pathological grades in breast cancer patients

LGALS	Intrinsic Subtypes	Overall Survival HR (95% CI)	Log rank P	CASES	
LGALS1	all types	1.32 (1.09-1.6)	0.004	1879	201105_at
	basal type	1.9 (1.29-2.82)	0.0011	404	
	luminal A	1.07 (0.78-1.47)	0.69	794	
	luminal B	1.83 (1.27-2.63)	0.00093	515	
	HER2+	0.61 (0.34-1.07)	0.083	166	
LGALS2	all types	0.74 (0.61-0.89)	0.0018*	1879	208450_at
	basal type	0.54 (0.37-0.8)	0.0019*	404	
	luminal A	0.84 (0.61-1.15)	0.27	794	
	luminal B	0.7 (0.49-1)	0.046*	515	
	HER2+	0.54 (0.3-0.97)	0.035*	166	
LGALS3	all types	1.12 (0.93-1.35)	0.23	1879	208949_s_at
	basal type	1.33 (0.91-1.94)	0.15	404	
	luminal A	0.76 (0.56-1.05)	0.095	794	
	luminal B	1.47 (1.03-2.08)	0.032	515	
	HER2+	1.16 (0.66-2.05)	0.6	166	
LGALS4	all types	0.87 (0.72-1.05)	0.15	1879	204272_at
	basal type	1.02 (0.7-1.5)	0.9	404	
	luminal A	0.76 (0.55-1.04)	0.087	794	
	luminal B	1.02 (0.72-1.45)	0.91	515	
	HER2+	1.15 (0.65-2.02)	0.64	166	
LGALS8	all types	0.91 (0.75-1.09)	0.3	1879	210731_s_at
	basal type	1.37 (0.93-2.01)	0.11	404	
	luminal A	0.76 (0.55-1.04)	0.088	794	
	luminal B	1.01 (0.71-1.43)	0.97	515	
	HER2+	1.18 (0.67-2.09)	0.57	166	
LGALS10	all types	1.04 (0.86-1.25)	0.72	1879	206207_at
	basal type	1.21 (0.82-1.76)	0.33	404	
	luminal A	0.9 (0.66-1.24)	0.53	794	
	luminal B	1.22 (0.86-1.74)	0.26	515	
	HER2+	1.34 (0.75-2.36)	0.32	166	
LGALS12	all types	1.01 (0.77-1.32)	0.95	943	223828_s_at
	basal type	1.16 (0.73-1.84)	0.53	278	
	luminal A	0.99 (0.62-1.59)	0.96	377	
	luminal B	0.76 (0.41-1.41)	0.38	177	
	HER2+	0.98 (0.5-1.95)	0.96	111	
LGALS13	all types	0.96 (0.79-1.15)	0.65	1879	220440_at
	basal type	1.39 (0.95-2.05)	0.088	404	
	luminal A	0.74 (0.54-1.02)	0.068	794	
	luminal B	1.05 (0.74-1.49)	0.77	515	
	HER2+	1.31 (0.74-2.32)	0.35	166	
LGALS14	all types	0.93 (0.77-1.12)	0.43	1879	220158_at
	basal type	1.18 (0.81-1.73)	0.39	404	
	luminal A	0.85 (0.62-1.16)	0.3	794	
	luminal B	1.03 (0.72-1.47)	0.87	515	
	HER2+	0.79 (0.45-1.41)	0.43	166	

*P<0.05.

Table 2. Correlation of LGALS expression level with OS in different clinical stages in breast cancer patients

LGALS	Grades	Overall Survival HR (95% CI)	Log rank P	Cases
LGALS1	I	1.22 (0.49-3.07)	0.67	175
	II	1.11 (0.74-1.66)	0.62	443
	III	1.2 (0.89-1.63)	0.23	586
LGALS2	I	0.78 (0.32-1.89)	0.58	175
	II	0.9 (0.6-1.34)	0.6	443
	III	0.63 (0.46-0.85)	0.0027*	586
LGALS3	I	0.36 (0.13-0.98)	0.037*	175
	II	1.13 (0.76-1.68)	0.55	443
	III	1.27 (0.94-1.72)	0.12	586
LGALS4	I	0.69 (0.28-1.68)	0.41	175
	II	1.27 (0.85-1.9)	0.25	443
	III	0.72 (0.53-0.97)	0.029*	586
LGALS8	I	1.44 (0.59-3.55)	0.42	175
	II	0.98 (0.65-1.46)	0.91	443
	III	1.14 (0.84-1.53)	0.41	586
LGALS10	I	1.36 (0.56-3.3)	0.5	175
	II	1.2 (0.8-1.78)	0.38	443
	III	1.23 (0.91-1.67)	0.17	586
LGALS12	I	4.64 (0.36-60.4)	0.21	26
	II	1.51 (0.48-4.77)	0.48	64
	III	1.09 (0.66-1.82)	0.73	204
LGALS13	I	0.76 (0.29-1.95)	0.56	175
	II	1.07 (0.72-1.6)	0.74	443
	III	0.93 (0.69-1.25)	0.64	586
LGALS14	I	0.65 (0.25-1.67)	0.36	175
	II	0.93 (0.62-1.4)	0.75	443
	III	0.9 (0.67-1.22)	0.5	586

*P<0.05.

LGALS14 exhibited no association with the prognosis of all breast tumour patients, basal-like breast tumour patients, luminal A and B type breast carcinoma patients, and HER2-overexpression breast tumour patients. Hence, we further assessed the correlation of the LGALS members with other clinicopathological features, such as pathological grade (**Table 2**), HER2 status (**Table 3**), lymph node status (**Table 4**), and TP53 status (**Table 5**), of breast tumour patients. As mentioned in **Table 2**, we discovered that high mRNA expression of LGALS2 and LGALS4 was correlated with a better prognosis in grade III breast tumour patients. Furthermore, LGALS3 was associated with a favourable prognosis in grade I breast tumour patients. However, overexpression of LGALS1, LGALS8, LGALS10, LGALS12, LGALS13, and LGALS14 mRNA showed no correlation with all pathological grades of breast tumours.

Next, we evaluated the prognostic significance among the nine LGALS mRNA expression levels and HER2 status in breast tumour patients (**Table 3**). Our results indicated that high mRNA expression of LGALS2 (HER-2 positive) and LGALS8 (HER-2 negative) was related to a longer survival of HER2-negative breast tumour patients. However, LGALS3 in HER2-positive breast tumour patients and LGALS1 in HER2-negative breast tumour patients predicted poor survival rates. The *P* values of LGALS2 (HER2-positive and negative) and LGALS8 (HER2-negative) were less than 0.05 and the HR<1; however, the upper limit of the 95% CI of the HR>1, implying that these correlations were not statistically significant. Thus, high mRNA expression of LGALS4, LGALS10, LGALS12, LGALS13, and LGALS14 showed no association with outcomes in HER2 positive breast tumour patients.

Additionally, from **Table 4**, we observed that high mRNA expression of LGALS2, LGALS8, and LGALS14 presented favourable survival in lymph node-negative breast tumour patients. In addition, a high level of LGALS1 was associated with lymph node-negative breast tumour patients. However, LGALS3, LGALS4, LGALS12, LGALS13, and LGALS14 had no correlation with outcomes for lymph node-negative and positive breast tumour patients.

Finally, we examined the connection among the nine LGALS members and the prognosis according to TP53 status in breast tumours (**Table 5**). Elevated LGALS2 mRNA expression was associated with satisfactory OS in TP53 wild-type breast cancer patients. However, LGALS1, LGALS3, LGALS4, LGALS8, LGALS10, LGALS12, LGALS13, and LGALS14 showed no association with OS in either TP53-mutated or wild-type breast tumour patients.

HPA

From the HPA database, fifteen LGALS gene members in normal breast tissues and breast cancer tissues were chosen. We went through all of the accessible immunohistochemistry

Table 3. Correlation of LGALS expression level with OS in breast cancer patients with different HER2 statuses

LGALS	HER2 status	Overall Survival HR (95% CI)	Log rank P	Cases
LGALS1	Positive	1.11 (0.77-1.59)	0.59	420
	Negative	1.41 (1.12-1.76)	0.0028	1459
LGALS2	Positive	0.5 (0.34-0.73)	0.00022*	420
	Negative	0.8 (0.64-1)	0.051	130
LGALS3	Positive	1.5 (1.04-2.16)	0.028	420
	Negative	0.97 (0.78-1.21)	0.8	1459
LGALS4	Positive	1.1 (0.77-1.58)	0.61	420
	Negative	0.85 (0.68-1.05)	0.14	1459
LGALS8	Positive	1.1 (0.76-1.58)	0.61	420
	Negative	0.76 (0.61-0.95)	0.014*	1459
LGALS10	Positive	0.9 (0.63-1.29)	0.57	420
	Negative	0.98 (0.79-1.23)	0.89	1459
LGALS12	Positive	1.04 (0.63-1.71)	0.89	223
	Negative	1.01 (0.74-1.39)	0.93	720
LGALS13	Positive	1.02 (0.71-1.47)	0.9	420
	Negative	0.94 (0.76-1.18)	0.61	1459
LGALS14	Positive	1.16 (0.81-1.67)	0.41	420
	Negative	0.86 (0.69-1.08)	0.19	1459

*P<0.05.

Table 4. Correlation of LGALS expression level with OS in breast cancer patients with different lymph node status

LGALS	Lymph node status	Overall Survival HR (95% CI)	Log rank P	Cases
LGALS1	Positive	0.97 (0.7-1.34)	0.84	452
	Negative	1.55 (1.08-2.21)	0.016	726
LGALS2	Positive	0.87 (0.63-1.22)	0.43	452
	Negative	0.69 (0.49-0.98)	0.035*	726
LGALS3	Positive	0.97 (0.7-1.34)	0.84	452
	Negative	0.97 (0.69-1.36)	0.84	726
LGALS4	Positive	1.05 (0.75-1.45)	0.79	452
	Negative	0.76 (0.54-1.06)	0.11	726
LGALS8	Positive	1.34 (0.95-1.87)	0.09	452
	Negative	0.65 (0.46-0.92)	0.014*	726
LGALS10	Positive	1.32 (0.95-1.84)	0.096	452
	Negative	0.98 (0.7-1.37)	0.89	726
LGALS12	Positive	1.18 (0.73-1.91)	0.5	230
	Negative	0.93 (0.42-2.05)	0.86	180
LGALS13	Positive	1.23 (0.88-1.71)	0.23	452
	Negative	0.99 (0.7-1.39)	0.95	726
LGALS14	Positive	1.25 (0.89-1.74)	0.19	452
	Negative	0.65 (0.46-0.92)	0.015*	726

*P<0.05.

photos for all of the proteins in the database one by one. Although image quality disparities were found, numerous proteins with fascinating forms were identified with differential expression in normal breast tissue relative to breast cancer tissue (**Figure 3**). We discovered that the LGALS4 was not expressed in either normal or breast cancer tissues in this study. Whereas, LGALS1 and LGALS8 showed higher expression in cancer tissues compared to the normal breast cancer tissues. Nevertheless, LGALS2 expression was shown to be higher in normal breast tissues, while mild expression was found in the cytoplasmic and membranous portions of breast cancer tissues, respectively. However, other LGALS were not associated with the expression in both normal and cancer breast tissues. Information about the LGALS2 IHC slides is provided in the **Table 7**.

The expression of LGALS2 mRNA in breast cancer cells and normal breast cells and the protein expression of LGALS2 in breast cancer cells and normal breast cells

As presented in **Figure 4**, LGALS2 mRNA expression was considerably downregulated in the human breast tumour cell lines compared to the normal breast cell lines (P<0.05). On the other hand, the protein level of LGALS2 in breast cancer cell lines was decreased compared to that in normal breast cells (*P<0.05; **P<0.01; ***P<0.001).

The expression of LGALS2 protein between breast cancer tissues and normal breast tissues

Table 6 demonstrated the clinical features of the patients LGALS2

Table 5. Correlation of AQP gene expression with OS in breast cancer patients with TP53 mutation status

LGALS	TP53 mutation	Overall Survival HR (95% CI)	Log rank P	Cases
LGALS1	Mutated	1.4 (0.72-2.74)	0.32	130
	Wild	0.95 (0.51-1.75)	0.86	197
LGALS2	Mutated	0.52 (0.26-1.04)	0.059	130
	Wild	0.48 (0.25-0.91)	0.022*	197
LGALS3	Mutated	1.21 (0.62-2.35)	0.58	130
	Wild	1.19 (0.64-2.21)	0.57	197
LGALS4	Mutated	1.46 (0.71-3.03)	0.3	130
	Wild	0.86 (0.47-1.6)	0.64	197
LGALS8	Mutated	1.55 (0.76-3.15)	0.23	130
	Wild	0.93 (0.5-1.72)	0.81	197
LGALS10	Mutated	1.63 (0.82-3.24)	0.16	130
	Wild	1.68 (0.9-3.15)	0.1	197
LGALS12	Mutated	0.65 (0.17-2.47)	0.53	56
	Wild	N/A		6
LGALS13	Mutated	1.64 (0.81-3.33)	0.16	130
	Wild	1.32 (0.71-2.45)	0.38	197
LGALS14	Mutated	1.09 (0.53-2.21)	0.82	130
	Wild	1.14 (0.62-2.11)	0.67	197

*P<0.05.

immunostaining was found in the cytoplasm of positive cells (**Figure 5**). In breast cancer tissues, the staining scores of LGALS2 were 1.32±0.58, which was lower than those in normal breast tissues (6.14±1.80) (P<0.005).

Discussion

In a recent report, we carefully explored the prognostic importance of LGALS family members in breast tumour patients by means of an online KM plotter database. Our data showed that high LGALS2 expression was related to a prolonged survival rate in all breast tumour patients. Although LGALS3, LGLAS4, and LGALS8 showed no association with the prognosis of all breast tumour patients, they showed a significant prognostic relationship with other clinicopathological features (intrinsic subtype, pathological grade, HER2 status, and lymph node status). LGALS1 was associated with a poor prognosis in all breast tumour patients and had a significant correlation with prognosis and other clinicopathological features. Meanwhile, we determined that LGALS10, LGALS12, LGALS13, and LGALS14 mRNA were not correlated with the prognosis of all breast tumour patients or any intrinsic subtype breast tumour patients; thus, more investigations are needed to confirm their role in breast cancer.

Among all fifteen LGALS family members, LGALS10, LGALS12, LGALS13, and LGALS14 have been less studied. To our knowledge, only one study using quantitative proteomics methods showed that the LGALS10 protein expression level was progressively upregulated in the multistage carcinogenesis of colorectal cancer [34]. Laderach et al. [35] found that LGALS12 expression was downregulated in malignant prostate tissue compared with normal (benign hyperplasia) prostate tissue. Subsequently, Katzenmaier et al. [36] also demonstrated that LGALS12 mRNA expression was decreased in colorectal cancer tissue compared to adjacent normal tissue; therefore, they concluded that LGALS12 might play a tumour-suppressive role in colorectal cancer. In addition, Leithy et al. [14] demonstrated that lower LGALS12 gene expression was significantly related to worse overall survival in acute myeloid leukaemia patients. LGALS13, also called human placental tissue protein 13 (PP-13), is highly expressed in syncytiotrophoblasts, trophoblast cells, and nuclei of syncytiotrophoblasts as well as in extravillous trophoblast cells of the placenta and it plays a vital role in the regulation of the maternal immune system with its anti-inflammatory function in the placenta during pregnancy [37, 38]. However, to date, there have been no studies on the prognostic roles of these four LGALS members in solid tumours, including breast carcinoma. In the current study, we discovered that increased levels of LGALS10, LGALS12, LGALS13, and LGALS14 were not associated with the prognosis of breast tumour patients. Further investigations of these LGALS are needed to verify these findings.

Several studies have reported that LGALS1 and LGALS3, the most studied members of the LGALS family, play prognostic roles in cancer and are linked to patient outcomes. Increased expression of LGALS1 was related to a poor prognosis in patients with prostate carcinoma

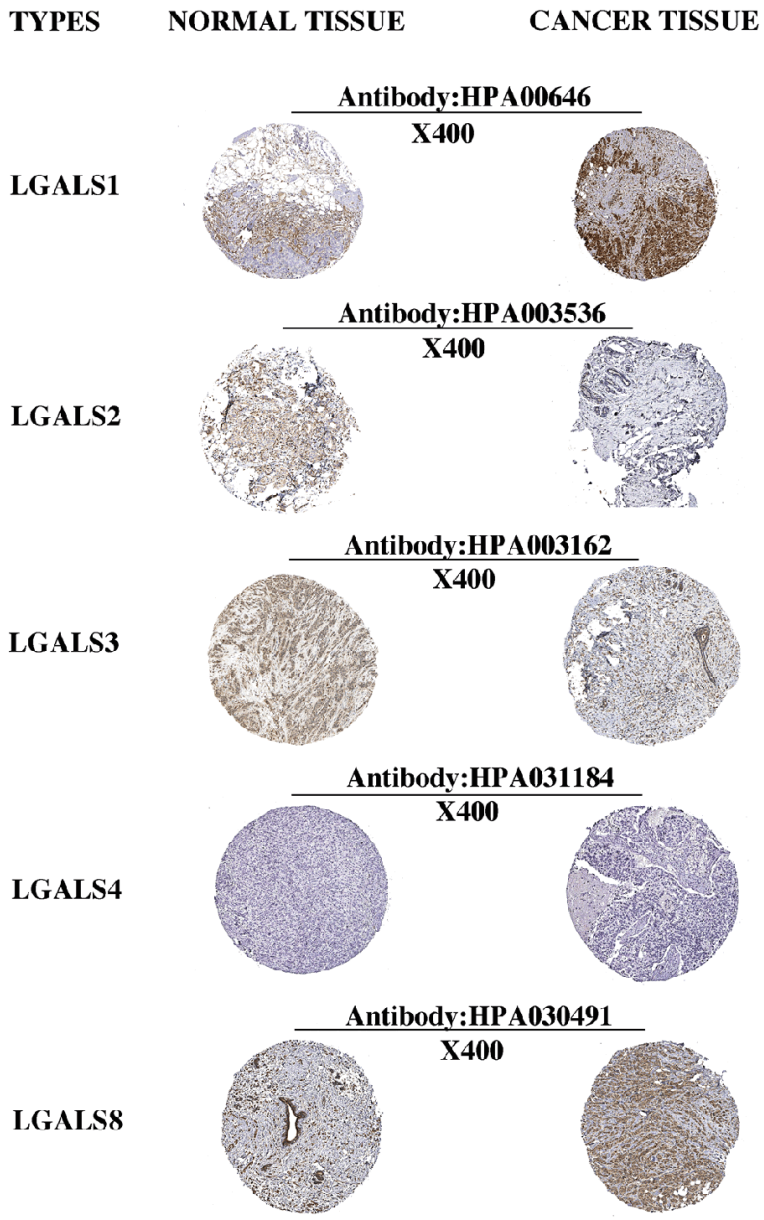

Figure 3. Immunohistochemistry images of magnification ×400 were obtained from HPA. The selected image of LGALS member proteins detected in the HPA database showed trends towards differential expression in normal and breast cancer tissues.

[15], ovarian carcinoma [16], glioblastoma multiforme [17], gingival squamous cell carcinoma [18], nasopharyngeal carcinoma [19], pancreatic ductal adenocarcinoma [20], etc. Furthermore, Jung et al. [39] revealed that LGALS1 expression was upregulated in breast cancer tissues, and they showed that LGALS1 expression in cancer-associated stromal cells was associated with tumour progression and tumour invasiveness in breast carcinoma. Numerous studies have focused on the prognostic value of LGALS3; however, the results were inconsistent and dependent on the type of cancer. Okada et al. [21] demonstrated that high LGALS3 expression was significantly related to a satisfactory prognosis in gastric carcinoma. However, high levels of LGALS3 predicted poor outcomes in patients with colorectal cancer [22, 23], brain cancer [24], cervical cancer [40], distal cholangiocarcinoma [25], and so on. In addition, Honjo et al. [41] reported that blockage of LGALS3 expression in MDA-MB-435 human breast carcinoma cells led to partial reversion of the transformed phenotype *in vitro* and to significant suppression of tumour growth *in vivo*, suggesting that LGALS3 plays a crucial role in breast cancer. Moisa et al. [26] found that the presence of LGALS3 in the stroma, but not the nucleus or cytoplasm in tumour cells, was related to more aggressive tumours and predicted a poor outcome of breast cancer. Consistent with previous findings, we found that high LGALS1 mRNA expression was significantly linked with poor survival rates in basal-like and luminal B subtype breast tumour patients; LGALS3 was strongly related to poor survival rates in luminal B subtype breast tumour patients. Moreover, LGALS1 expression was correlated with significantly decreased survival in patients with negative lymph node breast carcinoma. LGALS3 mRNA expression showed a null prognosis in grade II and III breast cancer patients. Taken together, high LGALS1 and LGALS3 mRNA expression predicted a worse outcome in patients with breast carcinoma.

Jung et al. [42] found that low expression of LGALS2 was markedly associated with lymph node metastasis and advanced clinical stage in

Table 6. Clinical types of breast cancer patients and control patients

Variables	Patients with breast cancer (N=5)	Patients with normal breast cancer (N=5)
Median age (years)	45 (range 30-55)	50 (range 35-60)
Marital status, n (%)		
Married	5 (100%)	5 (100%)
Unmarried	0	0
Histology, n (%)		
Basal	5 (90%)	-
Luminal	0	-
TNM stages, n (%)		
I	1 (30%)	-
II	1 (30%)	-
III	3 (60%)	-

Table 7. The table shows the basic information of the IHC

Tissue Type	ID	AGE	Gender
Normal (LGALS1)	319	55 years	Female
Cancer (LGALS1)	108	91 years	Female
Normal (LGALS2)	2259	23 years	Female
Cancer (LGALS2)	1838	40 years	Female
Normal (LGALS3)	2252	47 years	Female
Cancer (LGALS3)	2091	40 years	Female
Normal (LGALS4)	2160	83 years	Female
Cancer (LGALS4)	4193	43 years	Female
Normal (LGALS8)	2565	51 years	Female
Cancer (LGALS8)	2252	47 years	Female

gastric carcinoma, suggesting that loss of LGALS2 may promote the aggressiveness of gastric carcinoma. Reports on the role of LGALS2 in human cancers, however, are very few. LGALS2 expression correlated with a better overall survival (OS) in grade III breast cancer patients; LGALS2 also predicted a better OS in basal-like subtype patients, luminal B patients, HER2-overexpressing patients, TP53 mutated and wild breast cancer patients. It implies that LGALS2 possibly exerts its predictive role in breast cancer. Specially, our study further identified the downregulation of LGALS2 in breast cancer cells compared with those in normal breast cell at mRNA and protein levels, and also showed the similar decreased expression in normal breast tissues compared with those in breast tumor tissues. It was speculated that LGALS2 might be a tumor suppressor in ovarian cancer, which needs more biological research. Overall, it suggests that LGALS2 may be an important marker in predicting a better prognosis for breast cancer patients. The study of Cai et al. [27] showed that low expression of LGALS4 was closely associated with hepatocellular carcinoma progression (microvascular invasion, larger tumour size, more advanced stage, and malignant differentiation) and might serve as a prognostic biomarker to classify patients with poor clinical results. Wu et al. [28] found that low LGALS8 expression was related to disease-free survival and poor overall survival in patients with gastric carcinoma after surgical resection.

Regarding the new LGALS14 member little is known about its function in malignancies. Furthermore, there are almost no studies about the prognostic value of these LGALS family members in breast carcinoma. We reported for the first time that increased LGALS2 mRNA expression was linked with a favourable prognosis in all breast tumour patients. Additionally, the database results showed that high levels of LGALS3, LGALS4, LGLAS8, and LGALS13 were significantly related to a favourable prognosis in luminal A breast cancer patients. In addition, high levels of LGALS2, LGALS8, and LGALS14 expression predicted a better outcome in lymph node-negative breast tumour patients; LGALS1 was linked with poor survival in lymph node-negative breast tumour patients. Likewise, high LGALS2 and LGALS4 mRNA expression were correlated with a better prognosis in grade III breast tumour patients. Additionally, LGALS2 also predicted a better prognosis in basal-like subtype patients, luminal B patients, and HER2-overexpressing breast cancer patients. Based on our study, high levels of LGALS2 and LGALS4 predicted a favourable outcome in breast tumour patients. This implies that LGALS2 may play a vital role in breast cancer progression. Our research found downregulation of LGALS2 at the mRNA and protein levels in normal breast cells relative to cancer cells, as well as lower expression in normal breast tissues compared to breast tumour tissues. LGALS2 is thought to be a tumour suppressor in breast carcinoma, yet more biological research is needed. LGALS2 may be a crucial marker for improving outcomes of breast tumour patients, according to the findings.

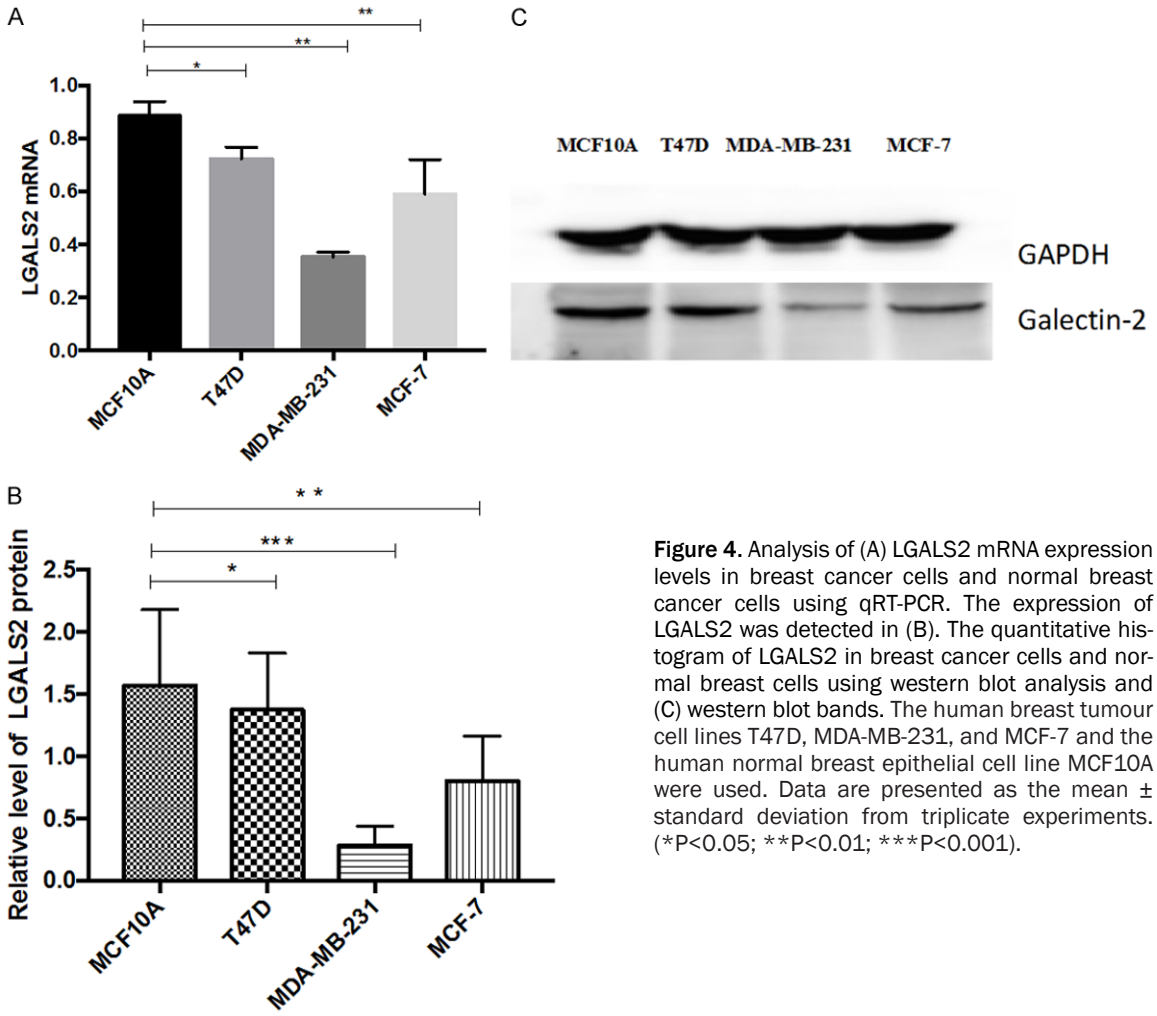

Figure 4. Analysis of (A) LGALS2 mRNA expression levels in breast cancer cells and normal breast cancer cells using qRT-PCR. The expression of LGALS2 was detected in (B). The quantitative histogram of LGALS2 in breast cancer cells and normal breast cells using western blot analysis and (C) western blot bands. The human breast tumour cell lines T47D, MDA-MB-231, and MCF-7 and the human normal breast epithelial cell line MCF10A were used. Data are presented as the mean ± standard deviation from triplicate experiments. (*P<0.05; **P<0.01; ***P<0.001).

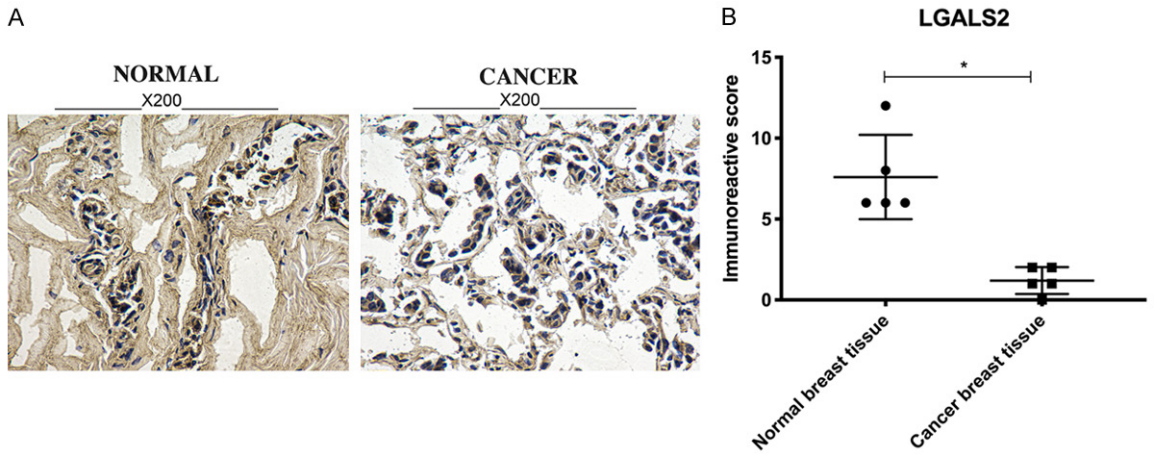

Figure 5. The associated protein expression of LGALS2 in human breast tissues was detected by immunohistochemistry (SP staining, ×200). A and B. Representative images and scores of IHC for LGALS2 in human breast cancer tissue and matched normal breast tissue. Values were expressed as the mean +/- SD. *P<0.05.

The HER2 oncogene is amplified in 25%-30% of human breast carcinomas, and this modification is related to poor prognosis in human breast carcinoma [43-47]. Several previous

studies showed that LGALS7 expression was upregulated in HER2-positive breast tumour patients [48-50]. Demers et al. [48] demonstrated that high LGALS7 expression is linked with lymph node metastasis in HER2-positive breast tumour patients. In the present analysis, we discovered that high LGALS3 mRNA expression was related to a poor prognosis in HER2-positive breast malignancy patients.

Various studies have explored the connection between p53 protein expression and the prognosis of breast tumour patients; however, the results were conflicting. Therefore, Pharoah et al. [51] conducted a meta-analysis to produce a more precise estimate of the prognostic value of p53 mutations. They confirmed that p53 mutations predicted poor overall survival and disease-free survival in patients with breast malignancy. However, to date, no published study has mentioned the prognostic value of LGALS family members in different TP53 statuses of breast tumour patients. In the present analysis, we discovered that mRNA expression of LGALS2 was linked with favourable OS in both TP53 mutant and wild breast tumour patients.

Our study was the first to look at the LGALS family's predictive function in patients with breast cancer. However, there were several limitations to our research that should be considered. First, we did not investigate the predictive roles of 6 LGALS members (LGALS5, LGALS6, LGALS7, LGALS9, LGALS11 and LGALS15) in breast cancer since there was no data accessible in the K-M plotter database among these fifteen LGALS members, despite earlier study into their functions. Second, the mechanism by which LGALS2 was linked to a higher survival result and expressed differentially in breast cancer and normal breast cells/tissues remained unknown, which would be the focus of our future research. Finally, because no fresh breast cancer or normal tissues were gathered, our research only looked at the distinct mRNA and protein expression of LGALS members in cell lines, not tissue samples. Instead, we used immunohistochemistry on paraffin slices of tissues to detect the various expressions of LGALS members, which will be further investigated using RT-PCR and western blot to investigate LGALS expression in breast cancer patients.

Conclusion

Our results showed that LGALS2 expression was linked with improved outcomes in all breast tumour patients by applying the Kaplan-Meier database. Additionally, increased mRNA expression of LGALS2 was associated with discrete clinicopathological parameters, such as pathological grade, TP53 status, HER2, and lymph node status. Although LGALS3, LGALS4, and LGLAS8 revealed no association with the prognosis of all breast tumour patients, they showed a significant prognostic relationship with other clinicopathological features (intrinsic subtype, metastatic lymph node and pathological grade). However, LGALS10, LGALS12, and LGALS13 expression showed no involvement in all breast cancer patients or any intrinsic subtype breast cancer patients. Taken together, the database outcomes suggest that LGALS2 expression predicts a favourable outcome. In brief, our results indicated that LGALS2 is associated with an improved OS and reduced OS in breast cancer cells and tissues, suggesting that LGALS2 might be a promising prognostic marker for patients with breast carcinoma, specifically in patients with luminal carcinoma, all clinical stages and grade III carcinoma. Nevertheless, investigations of the biological mechanism are necessary to authenticate the role of LGALS2.

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Disclosure of conflict of interest

None.

Abbreviations

OS, Overall Survival; HER2, Human Epidermal Growth Factor Receptor 2; CRDs, Carbohydrate Recognition Domains; KM plotter, Kaplan-Meier plotter; HR, Hazard Ratio; CI, Confidence Intervals.

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References

[1] Harbeck N and Gnant M. Breast cancer. Lancet 2017; 389: 1134-1150.

[2] Rugo HS, Olopade OI, DeMichele A, Yau C, van 't Veer LJ, Buxton MB, Hogarth M, Hylton NM, Paoloni M, Perlmutter J, Symmans WF, Yee D, Chien AJ, Wallace AM, Kaplan HG, Boughey JC, Haddad TC, Albain KS, Liu MC, Isaacs C, Khan QJ, Lang JE, Viscusi RK, Pusztai L, Moulder SL, Chui SY, Kemmer KA, Elias AD, Edmiston KK, Euhus DM, Haley BB, Nanda R, Northfelt DW, Tripathy D, Wood WC, Ewing C, Schwab R, Lyandres J, Davis SE, Hirst GL, Sanil A, Berry DA and Esserman LJ; I-SPY 2 Investigators. Adaptive randomization of veliparib-carboplatin treatment in breast cancer. N Engl J Med 2016; 375: 23-34.

[3] Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ and He J. Cancer statistics in China, 2015. CA Cancer J Clin 2016; 66: 115-132.

[4] Berry DA, Cronin KA, Plevritis SK, Fryback DG, Clarke L, Zelen M, Mandelblatt JS, Yakovlev AY, Habbema JD and Feuer EJ. Effect of screening and adjuvant therapy on mortality from breast cancer. N Engl J Med 2005; 353: 1784-1792.

[5] Schneider BP, Winer EP, Foulkes WD, Garber J, Perou CM, Richardson A, Sledge GW and Carey LA. Triple-negative breast cancer: risk factors to potential targets. Clin Cancer Res 2008; 14: 8010-8018.

[6] Ito K, Stannard K, Gabutero E, Clark AM, Neo SY, Onturk S, Blanchard H and Ralph SJ. Galectin-1 as a potent target for cancer therapy: role in the tumor microenvironment. Cancer Metastasis Rev 2012; 31: 763-778.

[7] Liu FT and Rabinovich GA. Galectins as modulators of tumour progression. Nat Rev Cancer 2005; 5: 29-41.

[8] Rumilla KM, Erickson LA, Erickson AK and Lloyd RV. Galectin-4 expression in carcinoid tumors. Endocr Pathol 2006; 17: 243-249.

[9] Tsuboi K, Shimura T, Masuda N, Ide M, Tsutsumi S, Yamaguchi S, Asao T and Kuwano H. Galectin-3 expression in colorectal cancer: relation to invasion and metastasis. Anticancer Res 2007; 27: 2289-2296.

[10] Wu MH, Hong TM, Cheng HW, Pan SH, Liang YR, Hong HC, Chiang WF, Wong TY, Shieh DB, Shiau AL, Jin YT and Chen YL. Galectin-1-mediated tumor invasion and metastasis, up-regulated matrix metalloproteinase expression, and reorganized actin cytoskeletons. Mol Cancer Res 2009; 7: 311-318.

[11] Kim HJ, Do IG, Jeon HK, Cho YJ, Park YA, Choi JJ, Sung CO, Lee YY, Choi CH, Kim TJ, Kim BG, Lee JW and Bae DS. Galectin 1 expression is associated with tumor invasion and metastasis in stage IB to IIA cervical cancer. Hum Pathol 2013; 44: 62-68.

[12] Zhang D, Chen ZG, Liu SH, Dong ZQ, Dalin M, Bao SS, Hu YW and Wei FC. Galectin-3 gene silencing inhibits migration and invasion of human tongue cancer cells in vitro via downregulating beta-catenin. Acta Pharmacol Sin 2013; 34: 176-184.

[13] Thijssen VL, Heusschen R, Caers J and Griffioen AW. Galectin expression in cancer diagnosis and prognosis: a systematic review. Biochim Biophys Acta 2015; 1855: 235-247.

[14] El Leithy AA, Helwa R, Assem MM and Hassan NH. Expression profiling of cancer-related galectins in acute myeloid leukemia. Tumour Biol 2015; 36: 7929-7939.

[15] van den Brule FA, Waltregny D and Castronovo V. Increased expression of galectin-1 in carcinoma-associated stroma predicts poor outcome in prostate carcinoma patients. J Pathol 2001; 193: 80-87.

[16] Chen L, Yao Y, Sun L and Tang J. Galectin-1 promotes tumor progression via NF-kappaB signaling pathway in epithelial ovarian cancer. J Cancer 2017; 8: 3733-3741.

[17] Chou SY, Yen SL, Huang CC and Huang EY. Galectin-1 is a poor prognostic factor in patients with glioblastoma multiforme after radiotherapy. BMC Cancer 2018; 18: 105.

[18] Noda Y, Kishino M, Sato S, Hirose K, Sakai M, Fukuda Y, Murakami S and Toyosawa S. Galectin-1 expression is associated with tumour immunity and prognosis in gingival squamous cell carcinoma. J Clin Pathol 2017; 70: 126-133.

[19] Chang SL, Li CF, Lin C and Lin YS. Galectin-1 overexpression in nasopharyngeal carcinoma: effect on survival. Acta Otolaryngol 2014; 134: 536-542.

[20] Tang D, Zhang J, Yuan Z, Gao J, Wang S, Ye N, Li P, Gao S, Miao Y, Wang D and Jiang K. Pancreatic satellite cells derived galectin-1 increase the progression and less survival of pancreatic ductal adenocarcinoma. PLoS One 2014; 9: e90476.

[21] Okada K, Shimura T, Suehiro T, Mochiki E and Kuwano H. Reduced galectin-3 expression is an indicator of unfavorable prognosis in gastric cancer. Anticancer Res 2006; 26: 1369-1376.

[22] Endo K, Kohnoe S, Tsujita E, Watanabe A, Nakashima H, Baba H and Maehara Y. Galectin-3 expression is a potent prognostic marker in colorectal cancer. Anticancer Res 2005; 25: 3117-3121.

[23] Tao L, Jin L, Dechun L, Hongqiang Y, Changhua K and Guijun L. Galectin-3 expression in

colorectal cancer and its correlation with clinical pathological characteristics and prognosis. Open Med (Wars) 2017; 12: 226-230.
[24] Camby I, Belot N, Rorive S, Lefranc F, Maurage CA, Lahm H, Kaltner H, Hadari Y, Ruchoux MM, Brotchi J, Zick Y, Salmon I, Gabius HJ and Kiss R. Galectins are differentially expressed in supratentorial pilocytic astrocytomas, astrocytomas, anaplastic astrocytomas and glioblastomas, and significantly modulate tumor astrocyte migration. Brain Pathol 2001; 11: 12-26.
[25] Shimura T, Kofunato Y, Okada R, Yashima R, Koyama Y, Araki K, Kuwano H and Takenoshita S. Intranuclear accumulation of galectin-3 is an independent prognostic factor for patients with distal cholangiocarcinoma. Oncol Lett 2017; 14: 819-829.
[26] Moisa A, Fritz P, Eck A, Wehner HD, Murdter T, Simon W and Gabius HJ. Growth/adhesion-regulatory tissue lectin galectin-3: stromal presence but not cytoplasmic/nuclear expression in tumor cells as a negative prognostic factor in breast cancer. Anticancer Res 2007; 27: 2131-2139.
[27] Cai Z, Zeng Y, Xu B, Gao Y, Wang S, Zeng J, Chen L, Huang A, Liu X and Liu J. Galectin-4 serves as a prognostic biomarker for the early recurrence/metastasis of hepatocellular carcinoma. Cancer Sci 2014; 105: 1510-1517.
[28] Wu S, Liu H, Zhang H, Lin C, Li R, Cao Y, He H, Li H, Shen Z, Qin J and Xu J. Galectin-8 is associated with recurrence and survival of patients with non-metastatic gastric cancer after surgery. Tumour Biol 2016; 37: 12635-12642.
[29] Gyorffy B, Lanczky A, Eklund AC, Denkert C, Budczies J, Li Q and Szallasi Z. An online survival analysis tool to rapidly assess the effect of 22,277 genes on breast cancer prognosis using microarray data of 1,809 patients. Breast Cancer Res Treat 2010; 123: 725-731.
[30] Hou GX, Liu P, Yang J and Wen S. Mining expression and prognosis of topoisomerase isoforms in non-small-cell lung cancer by using oncomine and kaplan-meier plotter. PLoS One 2017; 12: e0174515.
[31] Li S, Sheng B, Zhao M, Shen Q, Zhu H and Zhu X. The prognostic values of signal transducers activators of transcription family in ovarian cancer. Biosci Rep 2017; 37: BSR20170650.
[32] Cai XP, Chen LD, Song HB, Zhang CX, Yuan ZW and Xiang ZX. PLK1 promotes epithelial-mesenchymal transition and metastasis of gastric carcinoma cells. Am J Transl Res 2016; 8: 4172-4183.
[33] Zhang S, Wang Z, Liu W, Lei R, Shan J, Li L and Wang X. Distinct prognostic values of S100 mRNA expression in breast cancer. Sci Rep 2017; 7: 39786.
[34] Peng F, Huang Y, Li MY, Li GQ, Huang HC, Guan R, Chen ZC, Liang SP and Chen YH. Dissecting characteristics and dynamics of differentially expressed proteins during multistage carcinogenesis of human colorectal cancer. World J Gastroenterol 2016; 22: 4515-4528.
[35] Laderach DJ, Gentilini LD, Giribaldi L, Delgado VC, Nugnes L, Croci DO, Al Nakouzi N, Sacca P, Casas G, Mazza O, Shipp MA, Vazquez E, Chauchereau A, Kutok JL, Rodig SJ, Elola MT, Compagno D and Rabinovich GA. A unique galectin signature in human prostate cancer progression suggests galectin-1 as a key target for treatment of advanced disease. Cancer Res 2013; 73: 86-96.
[36] Katzenmaier EM, Kloor M, Gabius HJ, Gebert J and Kopitz J. Analyzing epigenetic control of galectin expression indicates silencing of galectin-12 by promoter methylation in colorectal cancer. IUBMB Life 2017; 69: 962-970.
[37] Unverdorben L, Huttenbrenner R, Knabl J, Jeschke U and Hutter S. Galectin-13/PP-13 expression in term placentas of gestational diabetes mellitus pregnancies. Placenta 2015; 36: 191-198.
[38] Visegrady B, Than NG, Kilar F, Sumegi B, Than GN and Bohn H. Homology modelling and molecular dynamics studies of human placental tissue protein 13 (galectin-13). Protein Eng 2001; 14: 875-880.
[39] Jung EJ, Moon HG, Cho BI, Jeong CY, Joo YT, Lee YJ, Hong SC, Choi SK, Ha WS, Kim JW, Lee CW, Lee JS and Park ST. Galectin-1 expression in cancer-associated stromal cells correlates tumor invasiveness and tumor progression in breast cancer. Int J Cancer 2007; 120: 2331-2338.
[40] Li M, Feng YM and Fang SQ. Overexpression of ezrin and galectin-3 as predictors of poor prognosis of cervical cancer. Braz J Med Biol Res 2017; 50: e5356.
[41] Honjo Y, Nangia-Makker P, Inohara H and Raz A. Down-regulation of galectin-3 suppresses tumorigenicity of human breast carcinoma cells. Clin Cancer Res 2001; 7: 661-668.
[42] Jung JH, Kim HJ, Yeom J, Yoo C, Shin J, Yoo J, Kang CS and Lee C. Lowered expression of galectin-2 is associated with lymph node metastasis in gastric cancer. J Gastroenterol 2012; 47: 37-48.
[43] Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SG, Keith DE, Levin WJ, Stuart SG, Udove J, Ullrich A, et al. Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. Science 1989; 244: 707-712.
[44] Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A and McGuire WL. Human breast cancer: correlation of relapse and survival with amplifi-

cation of the HER-2/neu oncogene. Science 1987; 235: 177-182.
[45] Paik S, Hazan R, Fisher ER, Sass RE, Fisher B, Redmond C, Schlessinger J, Lippman ME and King CR. Pathologic findings from the national surgical adjuvant breast and bowel project: prognostic significance of erbB-2 protein overexpression in primary breast cancer. J Clin Oncol 1990; 8: 103-112.
[46] Kallioniemi OP, Holli K, Visakorpi T, Koivula T, Helin HH and Isola JJ. Association of c-erbB-2 protein over-expression with high rate of cell proliferation, increased risk of visceral metastasis and poor long-term survival in breast cancer. Int J Cancer 1991; 49: 650-655.
[47] Toikkanen S, Helin H, Isola J and Joensuu H. Prognostic significance of HER-2 oncoprotein expression in breast cancer: a 30-year follow-up. J Clin Oncol 1992; 10: 1044-1048.
[48] Demers M, Rose AA, Grosset AA, Biron-Pain K, Gaboury L, Siegel PM and St-Pierre Y. Overexpression of galectin-7, a myoepithelial cell marker, enhances spontaneous metastasis of breast cancer cells. Am J Pathol 2010; 176: 3023-3031.
[49] Grosset AA, Poirier F, Gaboury L and St-Pierre Y. Galectin-7 expression potentiates HER-2-Positive phenotype in breast cancer. PLoS One 2016; 11: e0166731.
[50] Miwa HE, Koba WR, Fine EJ, Giricz O, Kenny PA and Stanley P. Bisected, complex N-glycans and galectins in mouse mammary tumor progression and human breast cancer. Glycobiology 2013; 23: 1477-1490.
[51] Pharoah PD, Day NE and Caldas C. Somatic mutations in the p53 gene and prognosis in breast cancer: a meta-analysis. Br J Cancer 1999; 80: 1968-1973.