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ORIGINAL RESEARCH

The Expression and Clinical Significance of ALDOA in Breast Cancer

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Background: Several malignant tumors have been shown to overexpress aldolase A (ALDOA), a crucial enzyme in the glycolytic cycle. Though, it is still unknown how ALDOA contributes to breast cancer (BC).

Methods: Using GEPIA, TIMER, UALCAN, BC-GenExMiner v5.1 database, and immunohistochemistry on 96 BC patients, the expression of ALDOA was investigated. The correlation between ALDOA expression and the prognosis was evaluated by employing the Kaplan-Meier (KM) plotter in breast cancer patients.

Results: The expression of ALDOA mRNA was higher in BC compared to the normal tissues. Certain subtypes of BC showed higher ALDOA expression, including micropapillary, luminal B, non-basal-like, non-triple negative breast cancer (TNBC), and luminal androgen receptor (LAR). Overexpression of ALDOA was related to the presence of lymph node metastasis (LNM), older age, high Ki67 expression, estrogen receptor (ER) and progesterone receptor (PR) positivity, and advanced Scarff-Bloom-Richardson (SBR) and Nottingham Prognostic Index (NPI) grades, while decreased ALDOA mRNA levels were observed in TNBC and basal-like BC. KM plotter showed that higher ALDOA mRNA levels predicted worse overall survival (OS), relapse-free survival (RFS), and distant metastasis-free survival (DMFS) overall. However, in BC patients with LNM, higher ALDOA levels correlated to better DMFS.

Conclusion: ALDOA was a crucial prognostic factor required for BC advancement, indicating a possible target for BC treatment.

Keywords: ALDOA, expression, prognostic value, breast cancer

Introduction

The most prevalent malignancy among women is breast cancer (BC).[1] Metabolic reprogramming and immune evasion are two main features of the malignant transformation of BC, which facilitate cancer cell proliferation.[2] The accumulation of reactive oxygen species (ROS) and oxidative stress are associated with many risk factors, including age, genetic susceptibility, exposure to ionizing radiation, and estrogen metabolism.[3]

Aldolase A (ALDOA) is a crucial enzyme within the glycolytic pathway. The process enables the reversible conversion of fructose-1,6-bisphosphate into glyceraldehyde 3-phosphate and dihydroxyacetone phosphate. Vertebrates possess three aldolase isozymes, ALDOA, ALDOB, and ALDOC, characterized by distinct electrophoretic and catalytic properties. ALDOA represents the primary aldolase isozyme in tumor tissues, and the systemic increase in aldolase activity due to ALDOA upregulation in these tissues is a distinctive characteristic of cancer.[4,5] ALDOA has been observed to be overexpressed in a variety of malignant tumors, including gastric cancer,[6] hepatocellular carcinoma,[7] colorectal cancer,[8] cervical cancer,[9] kidney cancer[10] and triple-negative BC.[11,12] Numerous studies indicate that ALDOA may be an independent prognostic factor.[13–15] Chang et al reported ALDOA was overexpressed in breast cancer tissues and a correlation between ALDOA expression levels and overall survival.[16] However, the potential correlation between ALDOA expression and clinicopathological features remains elusive and needs further evaluation.

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To ascertain the clinical implications of ALDOA in BC, this study examined any potential correlation between its expression and clinicopathological traits. Furthermore, we assessed the predictive significance of ALDOA in BC using the Kaplan-Meier plotter.

Methods

ALDOA Expression Analysis

The mRNA expression of ALDOA in pan-cancer data was analyzed by using the Tumor Immune Estimate Resource (TIMER) database (https://cistrome.shinyapps.io/timer/). The data of ALDOA expression in BC tissue and in normal tissue was analyzed using the Gene Expressing Profiling Interactive Analysis (GEPIA; http://gepia.cancer-pku.cn/) platform, the UALCAN web portal (http://ualcan.path.uab.edu/), and the BC Gene-expression miner (bc-GenExminer) v5.1 (http://bcgenex.ico.unicancer.fr).

Correlation Analysis of ALDOA and Clinicopathological Features

The Gene Expressing Profiling Interactive Analysis (GEPIA; http://gepia.cancer-pku.cn/) platform, the UALCAN web portal (http://ualcan.path.uab.edu/), and the BC Gene-expression miner (bc-GenExminer) v5.1 (http://bcgenex.ico.unicancer.fr) were used to analyze the correlation of ALDOA and clinicopathological features of BC patients. We used these databases based on the Pearson χ^2 test to analyze ALDOA mRNA expression among tumor and normal samples and P values < 0.05 to evaluate the statistical significance. The user-friendly web portal bc-GenExminer v5.1 includes BC patients' clinicopathological data based on microarray and RNA-seq (Jézéquel et al, 2012). The expression of GSTMs according to Scarff-Bloom-Richardson (SBR) grade and intrinsic molecular subtypes was identified by the Prediction Analysis of Microarray 50 (PAM50) test. The significant P value ($P < 0.05$) was determined using Dunnett-Tukey-Kramer test and Welch's t-test.

Survival Analysis

The association between ALDOA mRNA expression and BC patient survival, including overall survival (OS), recurrence-free survival (RFS) and distance metastasis-free survival (DMFS), was evaluated while using the Kaplan-Meier (K-M) Plotter (https://kmplot.com) database. $P < 0.05$ were considered as significant.

Human BC Tissues

The Department of General Surgery at Soochow University's First Affiliated Hospital collected 96 BC and 50 adjacent healthy samples from BC patients with histological diagnoses who had radical surgery. None of the participants had chemotherapy or radiotherapy before surgery. The ethics board of the relevant institution approved this study (IRB number is 2022-083), which was conducted in compliance with the Declaration of Helsinki. Written informed consent was obtained from all patients prior to study commencement.

Immunohistochemistry (IHC)

Following defined procedures, the paraffin-embedded tissues were sectioned to a thickness of 5 μm, incubated at 4°C overnight with a monoclonal human ALDOA antibody (dilution 1:100; #11305-1-AP, Proteintech), stained using a staining kit (Zhongshan Biotechnology, Bei-jing, China), followed by visualization. After this, the staining score was evaluated based on positive cell rate and color intensity.[17,18] The staining was divided by color intensity into not colored, light yellow, brown, and tan and is recorded as 0, 1, 2, and 3, respectively. Positive cell rate of <25% was a score of 1, positive cell rate of 25–50% was a score of 2, positive cell rate of 51–75% was a score of 3, positive cell rate of >75% was a score of 4. The final score was calculated by the multiple of the intensity and extent score. A final score of 0 was considered as −; 1–4 as +; 5–8 as ++; 9–12 as +++. In our study, ++ or +++ was considered as high expression, and – or + as no or low expression.

Statistical Analysis

The statistical significance was assessed by one-way analysis of variance or Student's t-test (paired, unpaired, or two-tailed). The difference in mRNA expression between groups was made by Welch's and Dunnett-Turkey-Kramer's tests.

The associations between ALDOA expression and clinicopathologic variables were examined using the Pearson χ^2 test. K-M analysis was used for survival analysis. It was determined that $P < 0.05$ was statistically significant.

Results

ALDOA Expression Levels are Higher in BC Tumor Tissues Compared to Normal Tissues

The RNA-seq data analysis from the TIMER database shows that most cancer tissues had ALDOA expression higher than in normal tissues, including 13 malignancies like BC (Figure 1A). According to the UALCAN database and the bc-GenExMiner v5.1 cohort, BC tissues had a considerably higher amount of ALDOA than normal tissues (Figure 1B and C, $P < 0.001$). ALDOA expression was higher in BC tumors than in normal breast tissues in the GEPIA dataset (Figure 1D; insignificant but marginal).

Furthermore, IHC was used to assess the expression of the ALDOA protein in BC tissues and normal breast tissues. In BC tissue, ALDOA protein-positive staining increased (Figure 1E). Importantly, human BC tissues had higher IHC scores than normal tissues (Figure 1F; $P < 0.001$).

Relationship Between ALDOA Expression and BC Patients' Clinicopathological Features

This study used the bc-GenExMiner v5.1 (Table 1) and UALCAN (Table 2) databases to examine the relationship between ALDOA expression and clinicopathological variables. Higher expression of ALDOA was linked to micropapillary BC, lymph node metastasis (LNM), older age, and high Ki67 expression. However, the correlations between ALDOA expressions with the staging of LNM patient's gender and TNM stage were not significant (Figure 2).

Afterward, this study examined the correlation between ALDOA expression and the pathological factors of BC. In the bc-GenExMiner v5.1 database, elevated expression of ALDOA was associated with ER+ (Figure 3A), PR+ (Figure 3B), and ER+/PR+ (Figure 3C). However, the correlations between ALDOA expression and HER2 status were insignificant (Figure 3D). ALDOA expression in BC tumors based on PAM50 molecular subtypes was also investigated, and results showed that non-basal-like (including HER2-enriched (HER2-E), Luminal A and Luminal B subgroups) BC possessed higher ALDOA expression than the basal-like subtype (Figure 3E and F). The ALDOA expression of non-triple negative breast cancer (TNBC) tissue was higher than that of TNBC (including LAR, MLIA, BLIA, and BLIS subgroups) tissue ($P = 0.0043$, Figure 3G and H). Among different subtypes of TNBC, the luminal androgen receptor (LAR) subgroup showed higher ALDOA expression than other subtypes ($P < 0.0001$, Figure 3H and I).

In the bc-GenExMiner v5.1 database, the correlations between ALDOA expression in BC tumors with breast cancer susceptibility gene (BRCA) 1 (Figure 4A, $P = 0.9913$), BRCA2 (Figure 4B, $P = 0.3623$) or BRCA1/2 status (Figure 4C, $P = 0.3494$) were not significant. The GES analysis from bc-GenExMiner database revealed that the ALDOA expression level of p53 wild-type BC was higher than p53 mutated (Figure 4D, $P = 0.0002$). However, in the IHC analysis from bc-GenExMiner database and the UALCAN database, the status of P53 was not related to the ALDOA expression in BC (Figure 4E and F). Moreover, higher ALDOA levels were substantially correlated with higher Scarff-Bloom-Richardson (SBR) grade (Figure 4G, $P = 0.002$) and Nottingham Prognostic Index (NPI) (Figure 4H, $P = 0.0004$). Asian patients showed the highest ALDOA expression among all three races (Figure 4I, $P < 0.001$).

Moreover, the relationship between ALDOA expression and the clinical characteristics in 96 BC patients who underwent radical surgery was investigated using IHC (Table 3). Increased ALODA expression was shown to be significantly associated with higher histological grade (Table 3, $P < 0.001$) and lymph node metastasis (Table 3, $P = 0.043$). However, no significant correlation was found between ALDOA expression and TNM stage, vascular invasion, tumor location, tumor size, or age (Table 3, $P > 0.05$).

The OS of BC Patients is Correlated with ALDOA Expression in Various Subgroups

The correlation between ALDOA mRNA expression in various subgroups and OS of BC patients was determined using the K-M survival curve analysis from the K-M Plotter database. In BC patients, shorter OS was predicted by higher ALDOA expression (Figure 5A, HR = 1.34, $P = 0.0027$). The results of the subgroup analysis showed that in the ER-

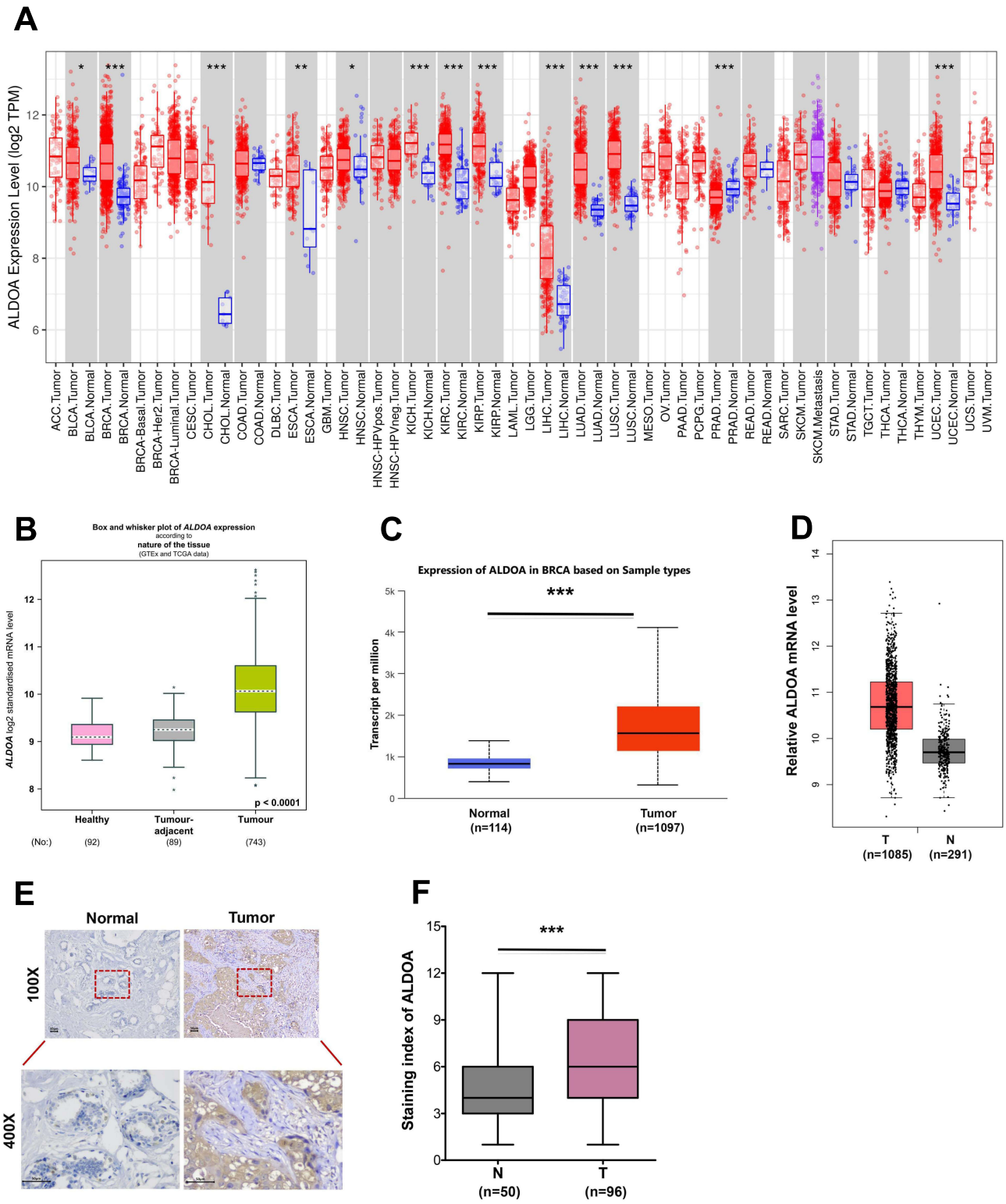

Figure 1 ALDOA expression in human BC tissues. (**A**) ALDOA expression in tumor and normal tissues in TIMER database. (**B**–**D**) ALDOA expression in BC tumor and normal tissues in bc-GenExMiner v5.1 (**B**), UALCAN (**C**) and GEPIA (**D**) databases. (**E**) Representative IHC staining of ALDOA in human BC tissues and normal tissues. (**F**) Analysis of ALDOA IHC scores in human BC tissues and normal tissues. ***$P < 0.001$.

positive (Figure 5B, HR = 1.42, $P = 0.003$), ER-negative (Figure 5C, HR = 1.6, $P = 0.0067$), PR-positive (Figure 5D, HR = 2.89, $P = 0.006$), and HER2-positive (Figure 5F, HR = 1.57, $P = 0.014$) subgroups, a shorter OS rate was associated with higher ALDOA expression. ALDOA expression did not, however, significantly correlate with the OS

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Table 1 The Correlation Between ALDOA Expression and Clinicopathological Features of BC Patients Using the bc-GenExMiner v5.1 Database

Variables		No.	*P* value
Histological Types	IDC	3694	< 0.0001
	ILC	375	
	IDC&ILC	125	
	Mucinous	61	
	Micropapillary	49	
Lymph node metastasis	N-	4337	< 0.0001
	N+	3649	
Age	21–40	846	< 0.0001
	40–70	5488	
	70–97	1396	
Ki67 Status	Ki67-low	7981	0.0043
	Ki67-high	1157	
Tumor Stage	IA	64	0.2507
	IB	0	
	IIA	257	
	IIB	179	
	IIIA	104	
	IIIB	18	
	IIIC	29	
	IV	0	
BRCA1 Status	Wild Type	2116	0.9913
	Mutated	72	
BRCA2 Status	Wild Type	2130	0.3623
	Mutated	56	
BRCA1/2 Status	Wild Type	12	> 0.05
	Mutated	1075	
P53 Status (GES)	Wild Type	2003	0.0002
	Mutated	725	
P53 Status (IHC)	Wild Type	643	0.8712
	Mutated	299	
SBR	SBR1	1024	0.02
	SBR2	3276	
	SBR3	3354	
NPI	NPI1	1230	0.0004
	NPI2	2201	
	NPI3	766	

of BC patients who were PR negative (Figure 5E, HR = 1.56, $P = 0.071$) or HER2 negative (Figure 5G, HR = 1.23, $P = 0.076$). Higher ALDOA level was significantly associated with shorter overall survival in lymph node-negative patients (Figure 5I, HR = 1.58, $P = 0.0086$), this correlation was not significant in the lymph node-positive cohort (Figure 5H, HR = 1.23, $P = 0.23$).

OS was significantly shortened in high ALDOA expressing basal (Figure 6A, HR = 1.94, $P = 0.0011$), luminal A (Figure 6B, HR = 1.42, $P = 0.033$), and luminal B (Figure 6C, HR = 1.56, $P = 0.017$) BC patients when taking StGallen molecular subtypes into account. However, there was no significant correlation between the OS of BC patients with HER2 and ALDOA expression (Figure 6D, HR = 1.64, $P = 0.086$). Patients with p53 wild-type tumors exhibiting high ALDOA expression demonstrated significantly shorter overall survival (Figure 6F, HR = 1.95, $P = 0.032$). This association was not significant in the p53-mutated subgroup (Figure 6E, HR = 2.2, $P = 0.094$). Furthermore, high ALDOA expression is related to shorter OS in histological grade 1 (Figure 6G, HR = 2.29, $P =$

Table 2 The Correlation Between ALDOA Expression and Clinicopathological Features of BC Patients Using the UALCAN Database

Variables		No.	*P* value
Nodal Metastasis Status	N0	516	> 0.05
	N1	362	
	N2	120	
	N3	77	
Age	21–40	97	< 0.001
	41–60	505	
	61–80	431	
	80–100	54	
Gender	Male	12	0.18
	Female	1075	
Cancer Stage	I	183	> 0.05
	II	615	
	III	247	
	IV	20	
BRCA1 Status	Wild Type	2116	0.9913
	Mutated	72	
BRCA2 Status	Wild Type	2130	0.3623
	Mutated	56	
BRCA1/2 Status	Wild Type	12	> 0.05
	Mutated	1075	
P53 Status (GES)	Wild Type	2003	0.0002
	Mutated	725	
P53 Status (IHC)	Wild Type	643	0.8712
	Mutated	299	
SBR	SBR1	1024	0.02
	SBR2	3276	
	SBR3	3354	
NPI	NPI1	1230	0.0004
	NPI2	2201	
	NPI3	766	
Race	Caucasian	748	< 0.001
	African-American	179	
	Asian	61	

0.053, not significant, but marginal) and grade 2 (Figure 6H, HR = 1.67, $P = 0.011$) subgroups. Still, there was no discernible correlation between the OS of the histological grade 3 subgroup and the expression of ALDOA mRNA (Figure 6I, HR = 1.23, $P = 0.26$).

The RFS of BC Patients is Correlated with ALDOA Expression in Various Subgroups

K-M survival curve analysis employing the K-M Plotter database showed that ALDOA mRNA expression was linked to relapse-free survival (RFS) of BC patients in several categories. BC patients with high ALDOA expression generally had significantly shorter RFS than those with low ALDOA expression (Figure 7A, HR = 1.15, $P = 0.0085$). Considering different pathological subgroups, shortened RFS was related to higher ALDOA expression regardless of the ER and PR status (Figure 7B–E), and also in HER2 positive BC patients (Figure 7F, HR = 1.42, $P = 0.0017$). However, among HER2-negative patients, there was no significant correlation between ALDOA expression and RFS (Figure 7G, HR = 1.09, $P = 0.12$). Elevated ALDOA expression corresponded to reduced RFS in LNM negative breast cancer cases (Figure 7I, HR = 1.22, $P = 0.018$). Conversely, nodal metastasis-positive patients exhibited no statistically

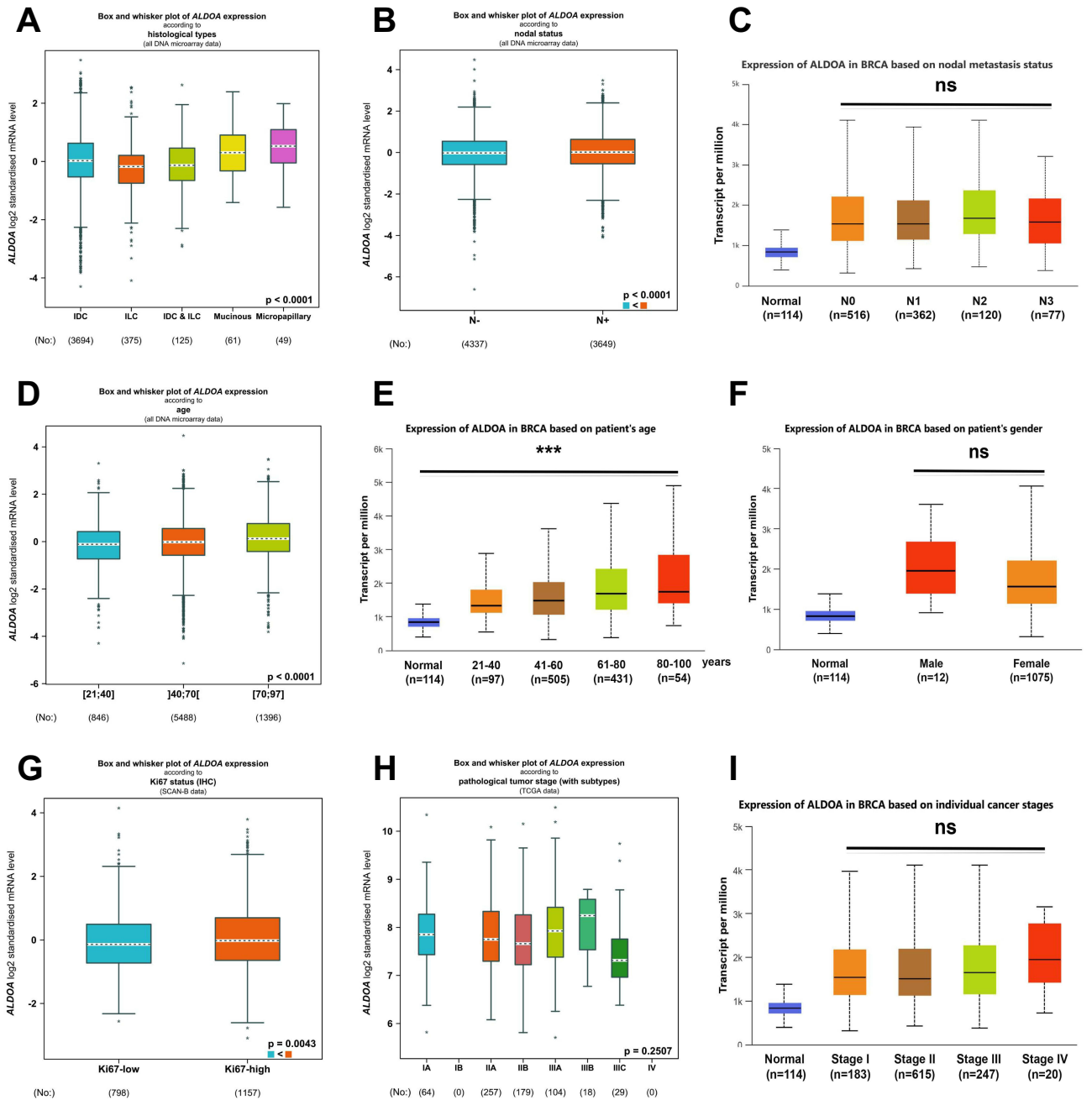

Figure 2 ALDOA expression in different subgroups of human BC tumor tissues in bc-GenExMiner v5.1 and UALCAN databases. (**A**) ALDOA expression in BC tumors of different histological types in bc-GenExMiner v5.1 database. (**B** and **C**) ALDOA expression in BC tumors with or without LNM in bc-GenExMiner v5.1 (**B**) and UALCAN (**C**) databases. (**D** and **E**) ALDOA expression in BC tumors based on patient's age in bc-GenExMiner v5.1 (**D**) and UALCAN (**E**) databases. (**F**) ALDOA expression in BC tumors based on patient's gender in UALCAN database. (**G**) ALDOA expression in BC tumors based on ki-67 status in bc-GenExMiner v5.1 database. (**H** and **I**) ALDOA expression in BC tumors based on tumor stages in bc-GenExMiner v5.1 (**H**) and UALCAN (**I**) databases. ***$P < 0.001$.
Abbreviation: ns, nonsignificant.

association between ALDOA levels and RFS outcomes (Figure 7H, HR = 1.17, $P = 0.13$). Considering LNM status, shortened RFS was related to higher ALDOA expression in BC patients without LNM (Figure 7I, HR = 1.22, $P = 0.018$). In contrast, in patients with LNM, the correlation was not significant (Figure 7H, HR = 1.17, $P = 0.13$).

In the luminal A (Figure 8B, HR = 1.28, $P = 0.0044$), basal (Figure 8A, HR = 1.32, $P = 0.014$), and luminal B (Figure 8C, HR = 1.21, $P = 0.033$) cohorts, pronounced RFS reduction is significantly related to high ALDOA expression. In contrast, the association between ALDOA expression and the RFS of HER2-positive BC patients (Figure 8D, HR = 1.39, $P = 0.067$) was not significant. RFS was remarkably shortened in high ALDOA expressing

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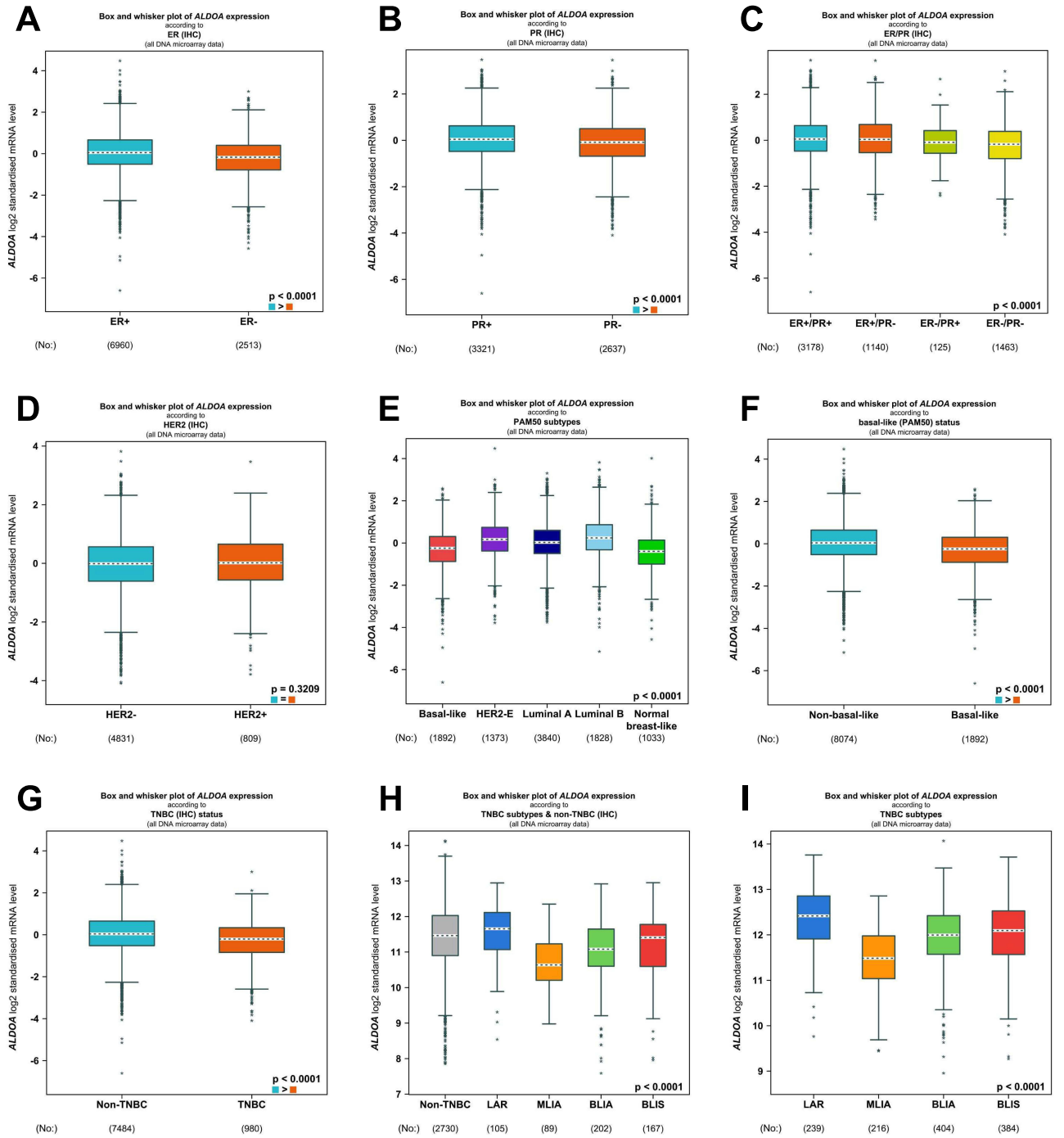

Figure 3 ALDOA expression in different subgroups of human BC tumor tissues in bc-GenExMiner v5.1 database. (**A**–**D**) ALDOA expression in BC tumors based on ER (**A**), PR (**B**), ER/PR (**C**) and HER2 (**D**) status. (**E**) ALDOA expression in BC tumors based on PAM50 molecular subtypes. (**F** and **G**) ALDOA expression in BC tumors based on basal-like (PAM50 (**F**)) and TNBC (**G**) and status. (**H** and **I**) ALDOA expression in BC tumors based on non-TNBC and TNBC subtypes.

p53 mutated BC patients (Figure 8E, HR = 3.61, P = 3.1e-05) and in the p53 wild type individuals (Figure 8F, HR = 1.39, P = 0.17), the association was not significant. Considering histological grade status, shortened RFS was related to higher ALDOA expression in BC patients with grade 1 subgroup (Figure 8G, HR = 1.65, P = 0.054, not significant, but marginal). The ALDOA mRNA expression and RFS of the histological grade 2 (Figure 8H, HR = 1.2, P = 0.1) and grade 3 (Figure 8I, HR = 1.19, P = 0.12) subgroups showed no discernible correlation.

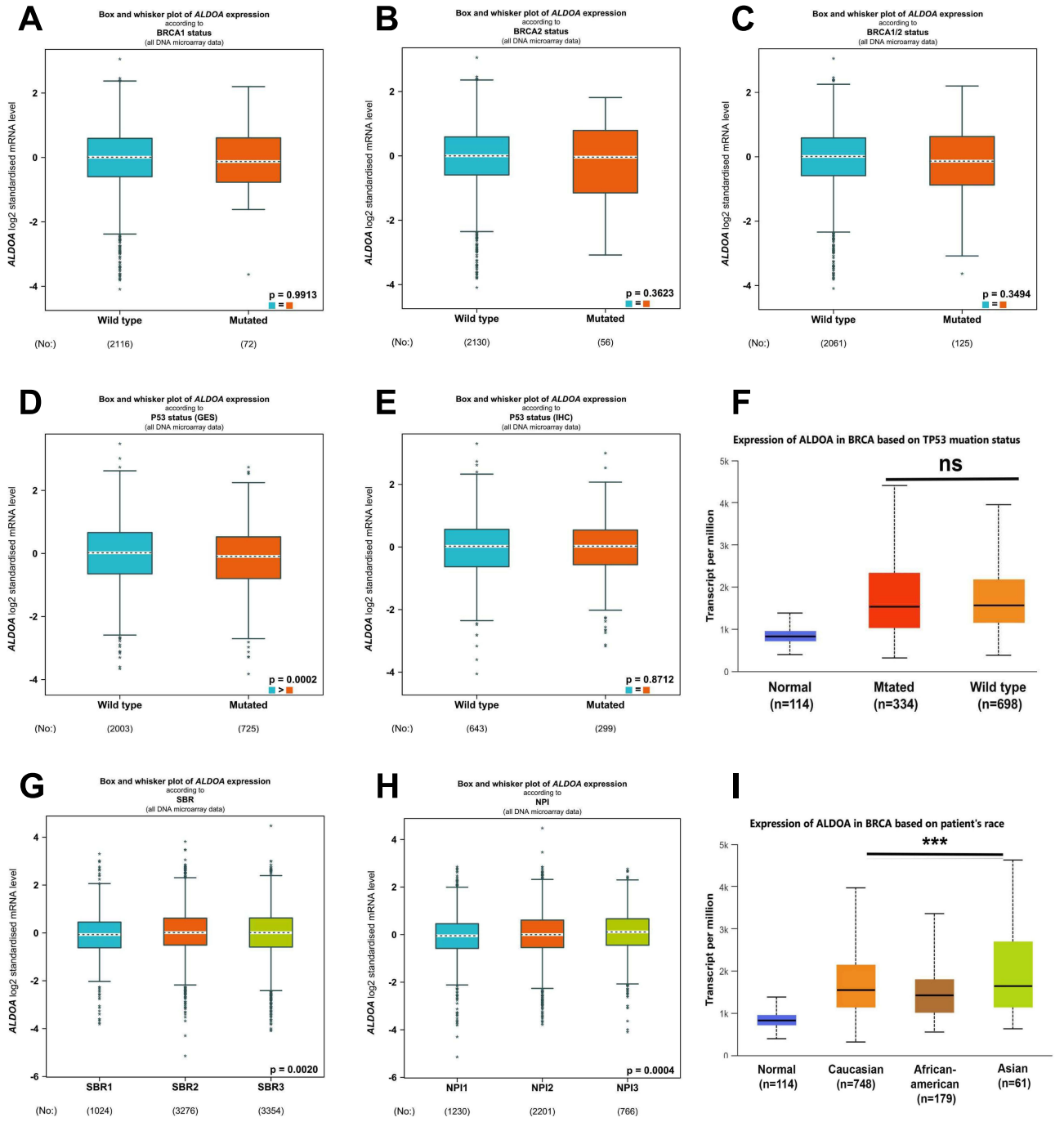

Figure 4 ALDOA expression in different subgroups of human BC tumor tissues in bc-GenExMiner v5.1 and UALCAN databases. (**A**–**C**) ALDOA expression in BC tumors based on BRCA1 (**A**), BRCA2 (**B**) AND BRCA1/2 status in bc-GenExMiner v5.1 database. (**D** and **E**) ALDOA expression in BC tumors based on p53 (GES (**D**)), p53 (IHC (**E**)) status in bc-GenExMiner v5.1 database. (**F**) ALDOA expression in BC tumors based on p53 status in UALCAN database. (**G** and **H**) ALDOA expression in BC tumors based on Scarff-Bloom-Richardson (SBR (**G**)) grade and NPI (Nottingham prognostic index (**H**)) status in bc-GenExMiner v5.1 database. (**I**) ALDOA expression in BC tumors based on patient's race in UALCAN database. ***$P < 0.001$.
Abbreviation: ns, nonsignificant.

The DMFS of BC Patients is Correlated with ALDOA Expression in Various Subgroups

We then investigated the connection between ALDOA expression in different subgroups and the DMFS of BC patients using the K-M survival curve analysis from the K-M Plotter database. In general, the association between ALDOA expression and the distant metastasis-free survival (DMFS) of BC patients was insignificant (Figure 9A, HR = 0.93, $P = 0.37$). Shorter DMFS was linked to increased ALDOA expression in BC patients with both ER-positive (Figure 9B, HR = 1.2, $P = 0.066$,

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Table 3 The Relationships Between ALDOA and Clinicopathological Factors in 96 Patients with Breast Cancer

Clinic Parameters	Total	ALDOA Expression		χ^2	*P* value
		Low	High		
Total	96	32 (33.3%)	64 (66.7%)		
Age (years)					
<=45	12	3 (25.0%)	9 (75.0%)	0.104	0.743
>45	84	29 (34.5%)	55 (65.5%)		
Tumor size					
<=2cm	19	5 (26.3%)	14 (73.7%)	0.525	0.469
>2cm	77	27 (35.1%)	50 (64.9%)		
Tumor location					
Left	53	19 (35.8%)	34 (64.2%)	0.337	0.562
Right	43	13 (30.2%)	30 (69.8%)		
Vascular invasion					
No	84	31 (36.9%)	53 (63.1%)	2.679	0.102
Yes	12	1 (8.3%)	11 (91.7%)		
Histological grade					
Grade 2	42	19 (45.2%)	23 (54.8%)	19.477	0.000***
Grade 3	43	5 (11.6%)	38 (88.4%)		
Others	11	8 (72.7%)	3 (27.3%)		
Lymph node metastasis					
No	52	22 (42.3%)	30 (57.7%)	4.112	0.043*
Yes	44	10 (22.7%)	34 (77.3%)		
TNM stage					
I/II	79	27 (31.2%)	52 (65.8%)	0.143	0.705
III	17	5 (29.4%)	12 (70.6%)		

Notes: *$P < 0.05$; ***$P < 0.001$.

insignificant, but marginal) and ER-negative (Figure 9C, HR = 1.34, $P = 0.039$) subgroups. Regardless of PR and HER2 status, there was no discernible correlation between DMFS and ALDOA mRNA expression in BC patients (Figure 9D–G). Unexpected, prolonged DMFS was related to higher ALDOA expression in BC patients with LNM (Figure 9H, HR = 0.72, $P = 0.01$), while in patients without LNM, the correlation was not significant (Figure 9I, HR = 1.19, $P = 0.18$).

Regarding the StGallen molecular subtypes, DMFS was significantly shortened in high ALDOA expressing basal (Figure 10A, HR = 1.64, $P = 0.014$) and luminal A (Figure 10B, HR = 1.4, $P = 0.017$) BC patients. The correlation between ALDOA expression and DMFS in the luminal B (Figure 10C, HR = 1.14, $P = 0.36$) and HER2 positive subgroups (Figure 10D, HR = 0.75, $P = 0.32$) was not statistically significant. Shortened DMFS was associated with high ALDOA expression in both p53 mutated (Figure 10E, HR = 3.29, $P = 0.019$) and p53 wild type (Figure 10F, HR = 2.19, $P = 0.029$) subgroups. Additionally, high ALDOA expression was related to shorter DMFS in histological grade 1 BC patients (Figure 10G, HR = 3.91, $P = 0.00039$). Still, the ALDOA mRNA expression and DMFS of histological grade 2 (Figure 10H, HR = 0.85, P = 0.28) and grade 3 (Figure 10I, HR = 0.85, $P = 0.21$) BC patients showed no significant correlation.

Discussion

Despite extensive research on molecular targeted drugs, chemotherapy and hormone therapy are still the first line of BC treatment.[1] However, an improved comprehension of the mechanisms by which malignant cells evade the immune system and the advancement of specific immune checkpoint antagonists has introduced novel therapeutic options.[19] It is necessary to identify new molecular targets. This study offers insight into ALDOA's potential function as a BC biomarker.

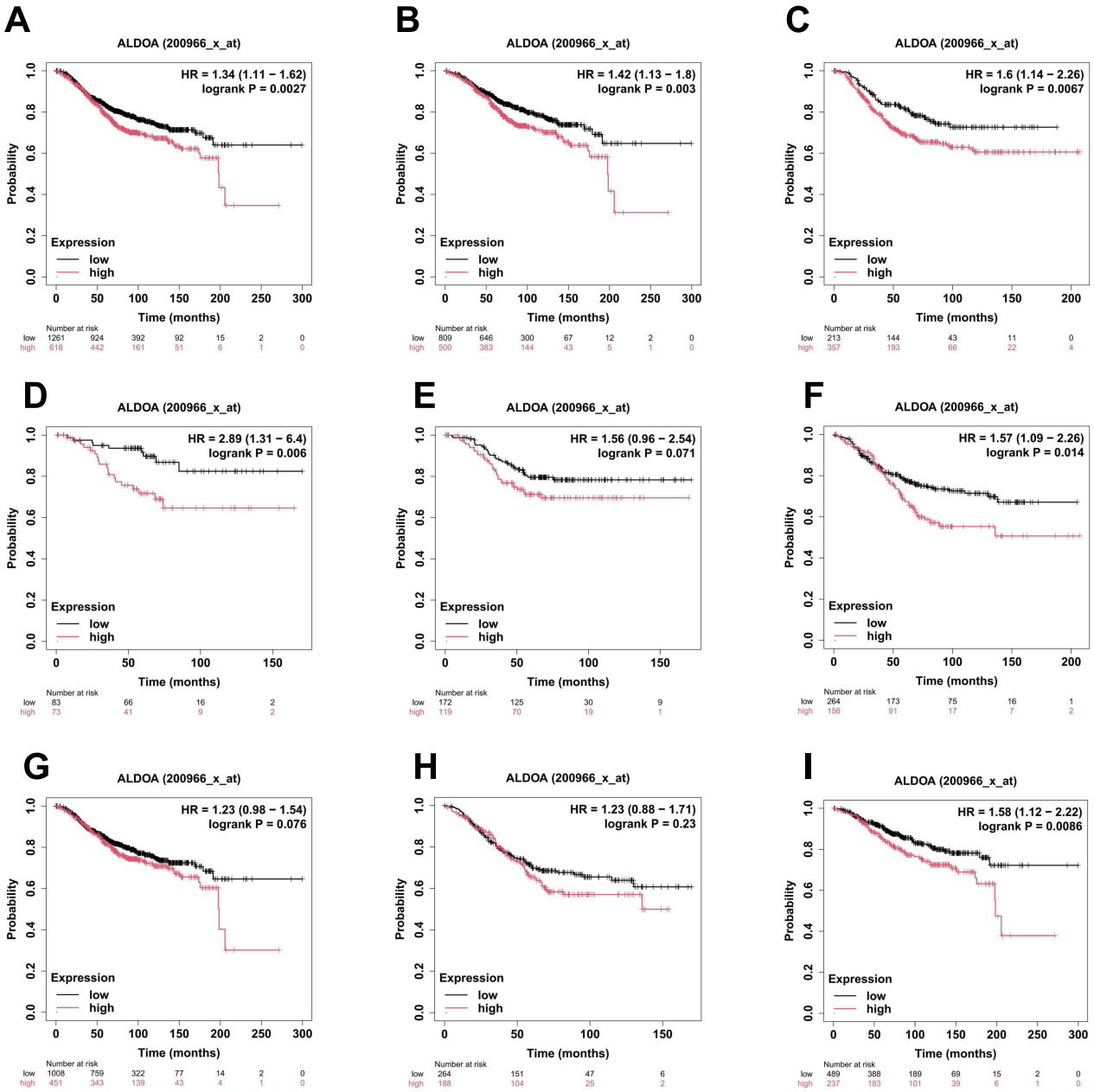

Figure 5 ALDOA expression in different subgroups correlates with overall survival (OS) of patients with BC from Kaplan-Meier plotter database. (**A**) Kaplan-Meier survival curve analysis shows OS of BC patients. (**B** and **C**) Kaplan-Meier survival curve analysis shows OS of BC patients based on ER status ((**B**) ER positive; (**C**) ER negative). (**D** and **E**) Kaplan-Meier survival curve analysis shows OS of BC patients based on PR status ((**D**) PR positive; (**E**) PR negative). (**F** and **G**) Kaplan-Meier survival curve analysis shows OS of BC patients based on HER2 status ((**F**) HER2 positive; (**G**) HER2 negative). (**H** and **I**) Kaplan-Meier survival curve analysis shows OS of BC patients with LNM (**H**) or without LNM (**I**).
Abbreviation: HR, hazard ratio.

Initially, data from internet datasets were analyzed to compare ALDOA expression. ALDOA mRNA levels were observed to be elevated in breast tumors relative to normal and tumor-adjacent tissues, corroborating prior studies that demonstrated a significant increase in ALDOA transcript expression across all breast tumor subtypes compared to normal tissues, suggesting that ALDOA may represent a novel oncogene.[12] The above conclusion was verified by IHC. Comparative analysis of various breast cancer subtypes revealed elevated ALDOA expression in specific subtypes, including micropapillary, luminal B, non-basal-like, non-TNBC, and LAR, influencing therapeutic strategies and prognostic outcomes.

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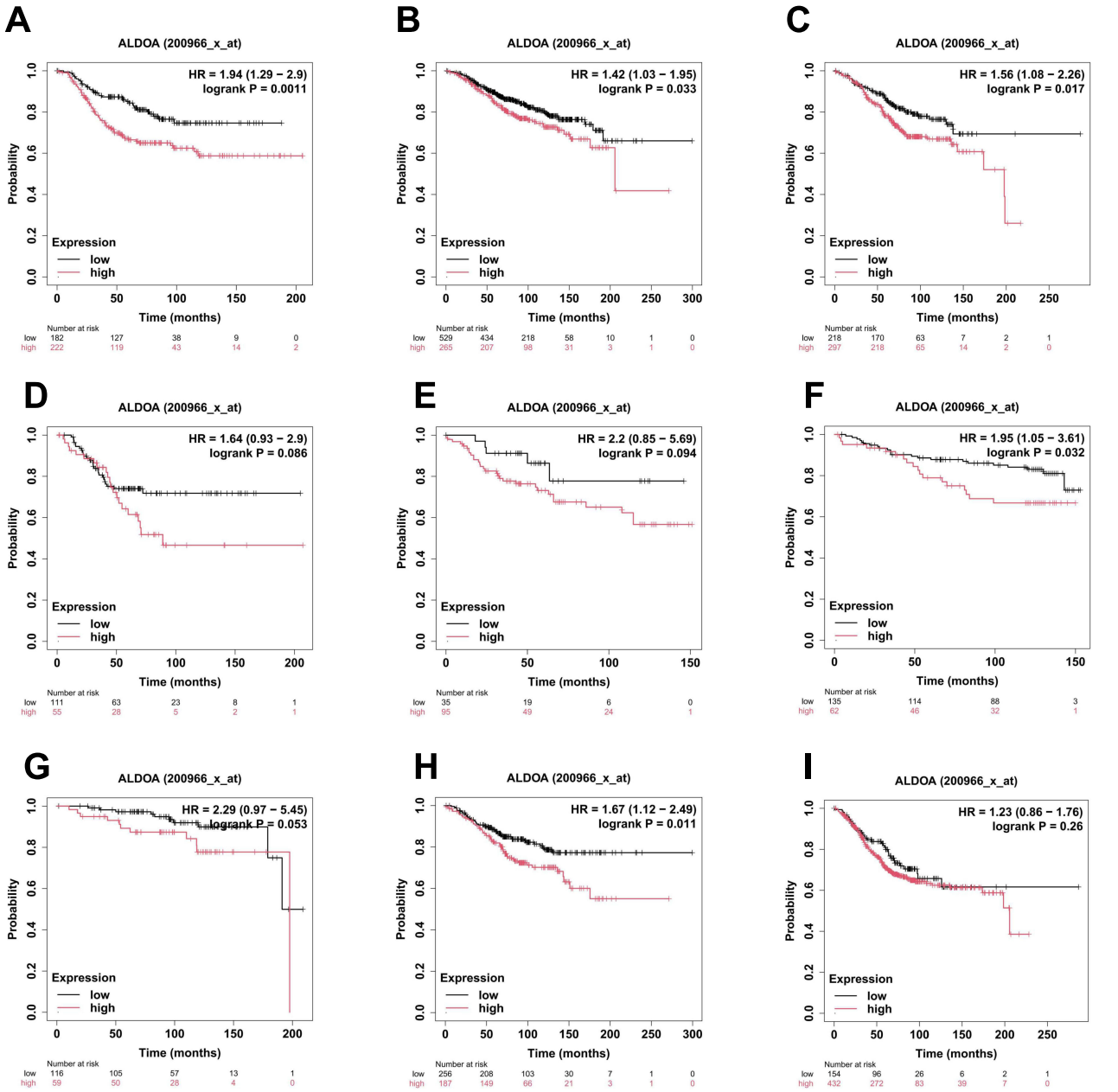

Figure 6 ALDOA expression in different subgroups correlates with OS of patients with BC from Kaplan-Meier plotter database. (**A**–**D**) Kaplan-Meier survival curve analysis shows OS of BC patients based on StGallen molecular subtypes ((**A**) basal; (**B**) luminal A; (**C**) luminal B; (**D**) HER2 positive). (**E** and **F**) Kaplan-Meier survival curve analysis shows OS of BC patients based on p53 status ((**E**) mutated; (**F**) wild type). (**G**–**I**) Kaplan-Meier survival curve analysis shows OS of BC patients based on histological grade ((**G**) grade 1; (**H**) grade 2; (**I**) grade 3).

Following this, the bc-GenExMiner v5.1 and UALCAN databases were employed to systematically analyze the correlation between ALDOA expression and several clinicopathological variables. Overexpression of ALDOA was linked to LNM, older age, and high Ki67 expression. There was no statistical significance in gender, cancer stage, or HER2 status in the analysis. Notably, although ALDOA expression was correlated with LNM, the stage of LNM did not affect it. Aside from that, high ALDOA expression was found to follow ER and PR positivity, while decreased ALDOA mRNA levels were associated with TNBC and basal-like BC, which might indicate that steroids increase the levels of ALDOA in breast epithelial cells. Tubule formation, nuclear characteristics of pleiomorphism, and the mitotic index are all assessed by the SBR grade, a histological grade.[20] NPI grade is a clinicopathological grading system based on tumor size, histopathological grade, and lymph node stage.[21] Our study showed that ALDOA expression was higher with BC

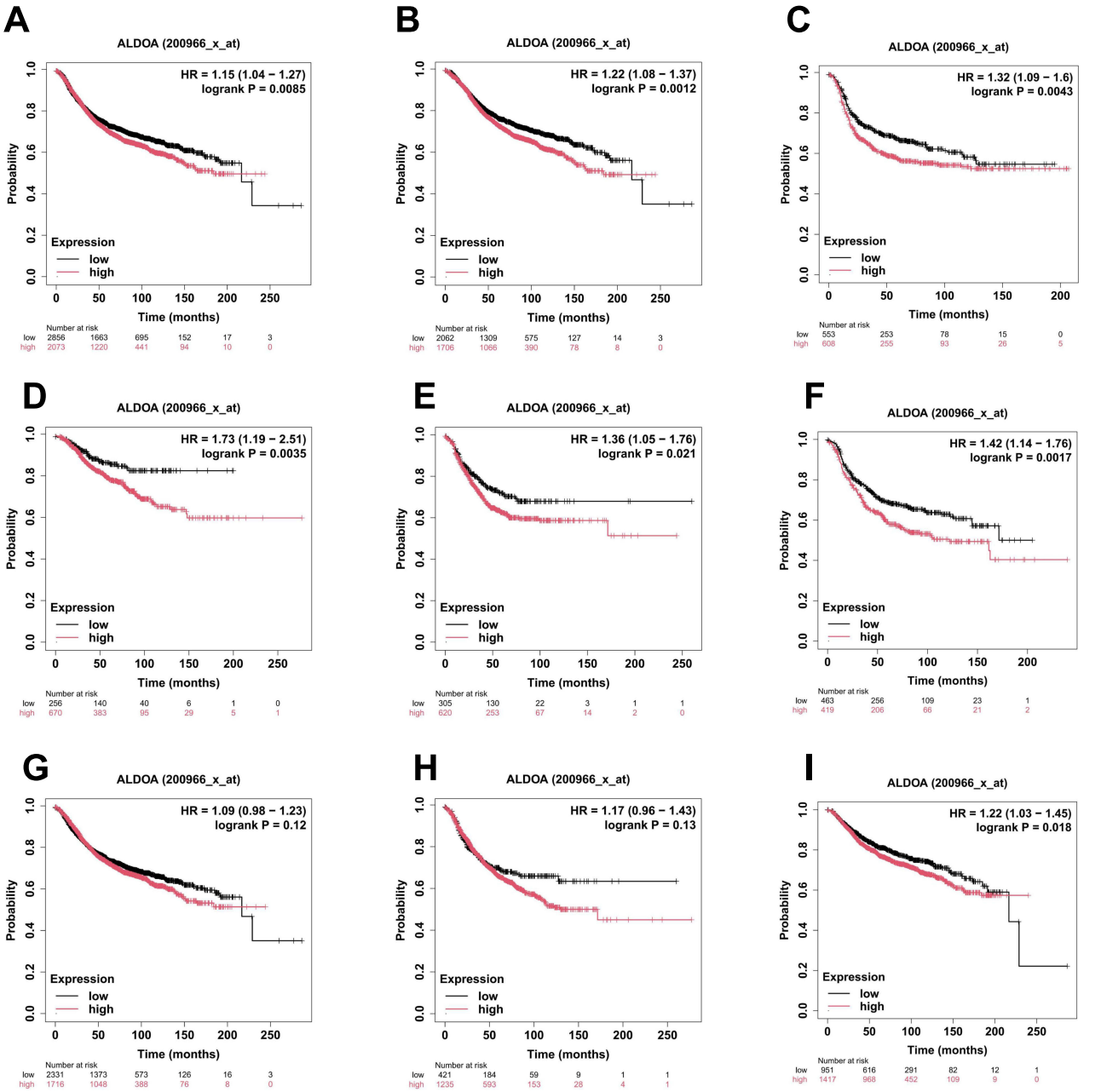

Figure 7 ALDOA expression in different subgroups correlates with relapse-free survival (RFS) of patients with BC from Kaplan-Meier plotter database. (**A**) Kaplan-Meier survival curve analysis shows RFS of BC patients. (**B** and **C**) Kaplan-Meier survival curve analysis shows RFS of BC patients based on ER status ((**B**) ER positive; (**C**) ER negative). (**D** and **E**) Kaplan-Meier survival curve analysis shows RFS of BC patients based on PR status ((**D**) PR positive; (**E**) PR negative). (**F** and **G**) Kaplan-Meier survival curve analysis shows RFS of BC patients based on HER2 status ((**F**) HER2 positive; (**G**) HER2 negative). (**H** and **I**) Kaplan-Meier survival curve analysis shows RFS of BC patients with LNM (**H**) or without LNM (**I**).

Abbreviation: HR, hazard ratio.

patients' advanced SBR grade and NPI. Consistent with previous findings,[22] ALDOA may predict a poorer prognosis in BC. Although a strong correlation between age and ALDOA expression was demonstrated in both databases, no such correlation was observed in the patient samples collected from individuals who underwent surgery. This is probably due to selection bias and a low sample size, because the clinical information for all 96 patients who underwent surgery was obtained from the same center. The clinicopathological information of BC patients from multiple centers need to be obtained in future subsequent studies.

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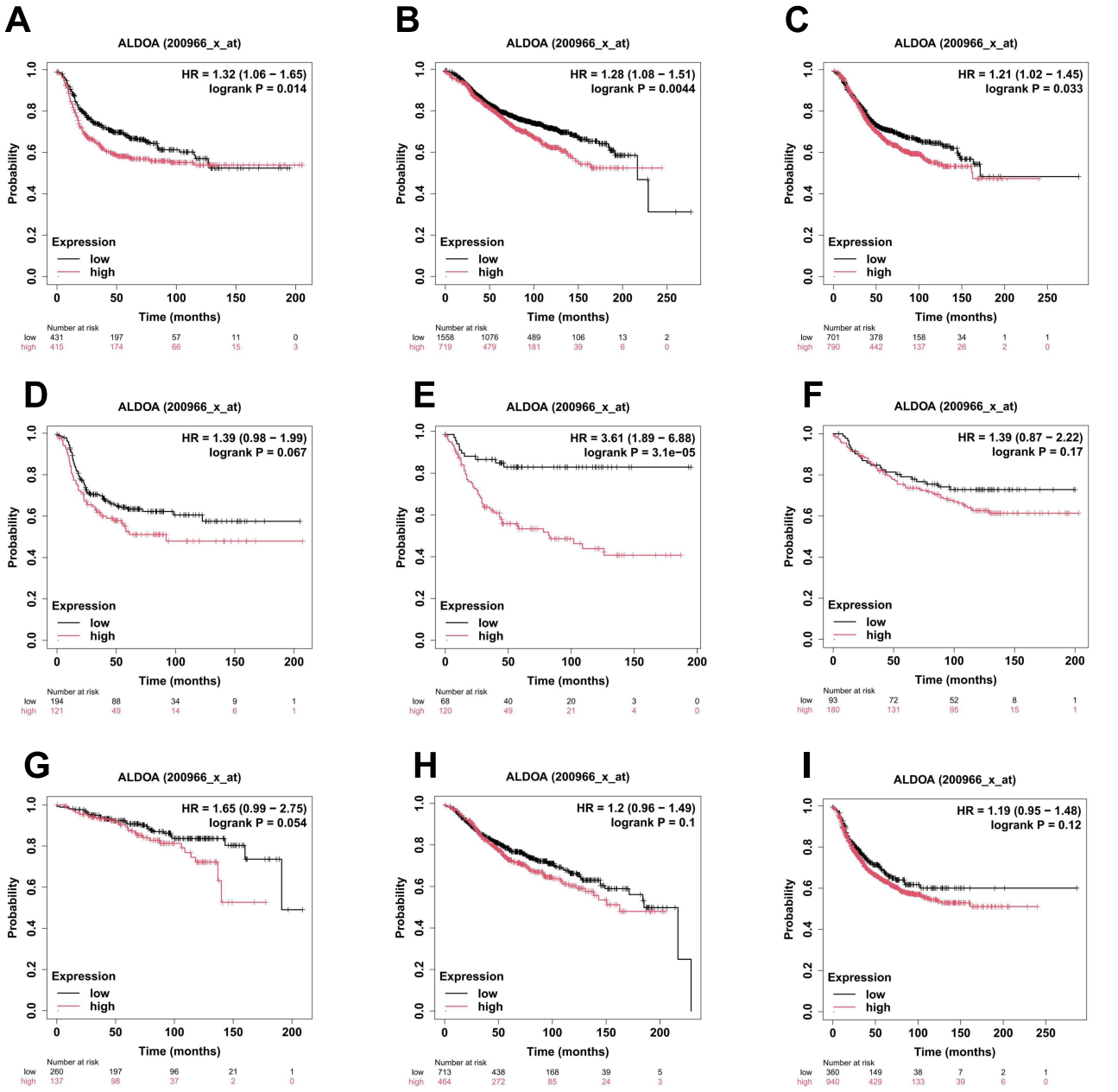

Figure 8 ALDOA expression in different subgroups correlates with RFS of patients with BC from Kaplan-Meier plotter database. (**A**–**D**) Kaplan-Meier survival curve analysis shows RFS of BC patients based on StGallen molecular subtypes ((**A**) basal; (**B**) luminal A; (**C**) luminal B; (**D**) HER2 positive). (**E** and **F**) Kaplan-Meier survival curve analysis shows RFS of BC patients based on p53 status ((**E**) mutated; (**F**) wild type). (**G**–**I**) Kaplan-Meier survival curve analysis shows RFS of BC patients based on histological grade ((**G**) grade 1; (**H**) grade 2; (**I**) grade 3).

The KM plotter was used in this study to assess the relationship between BC patients' survival and ALDOA expression. The survival curves showed higher ALDOA mRNA levels were generally linked to worse OS, worse RFS, and worse DMFS. This is in line with earlier findings that suggested high ALDOA levels are linked to poor patient survival in a variety of solid tumors, including BC.[23] However, ALDOA expression was correlated to better DMFS of BC patients with LNM, which disagreed with the overall tendency and previous study.[22]

Tu et al found that ALDOA can control tumor progression through the ALDOA-adenosine-50-monophosphate (AMP) activated protein kinase (AMPK) pathway, a nutrient sensor linked to aberrant activation of metabolic pathways, mitochondrial dynamics and functions, and epigenetic regulation. Depending on the specific cellular context, AMPK can be an oncogene or a tumor suppressor. Studies have shown the significance of ALDOA in cancers, but the underlying

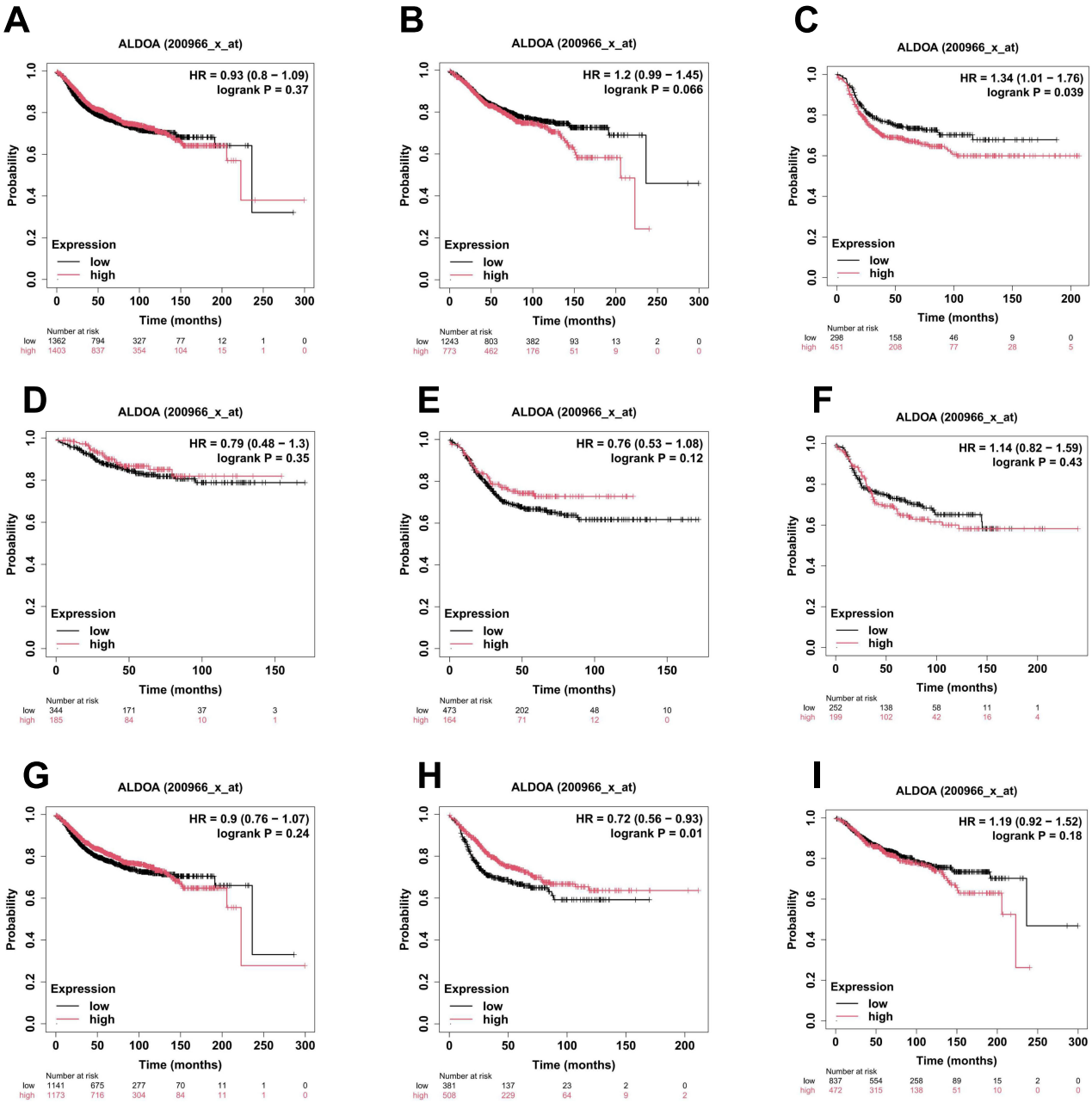

Figure 9 ALDOA expression in different subgroups correlates with distance metastasis-free survival (DMFS) of patients with BC from Kaplan-Meier plotter database. (**A**) Kaplan-Meier survival curve analysis shows DMFS of BC patients. (**B** and **C**) Kaplan-Meier survival curve analysis shows DMFS of BC patients based on ER status ((**B**) ER positive; (**C**) ER negative). (**D** and **E**) Kaplan-Meier survival curve analysis shows DMFS of BC patients based on PR status ((**D**) PR positive; (**E**) PR negative). (**F** and **G**) Kaplan-Meier survival curve analysis shows DMFS of BC patients based on HER2 status ((**F**) HER2 positive; (**G**) HER2 negative). (**H** and **I**) Kaplan-Meier survival curve analysis shows DMFS of BC patients with LNM (**H**) or without LNM (**I**).
Abbreviation: HR, hazard ratio.

mechanisms are still unclear.[22,24] In intrahepatic cholangiocarcinoma and radioresistance cervical cancer, ALDOA promotes tumor proliferation and migration by enhancing tumor cell glycolysis.[9,25] According to Gu et al, ADOLA controlled the activity of the EGFR receptor and its downstream targets, ERK1/2 and AKT. Through the EGFR pathway, overexpression of ALDOA increased gastric cancer cells' proliferation and cisplatin resistance.[26] Research conducted by Lu et al has shown that ALDOA promoted the proliferation and metastasis of colorectal cancer through its interaction with and regulation of the protein COPS6, thereby activating the mitogen-activated protein kinase (MAPK) signaling

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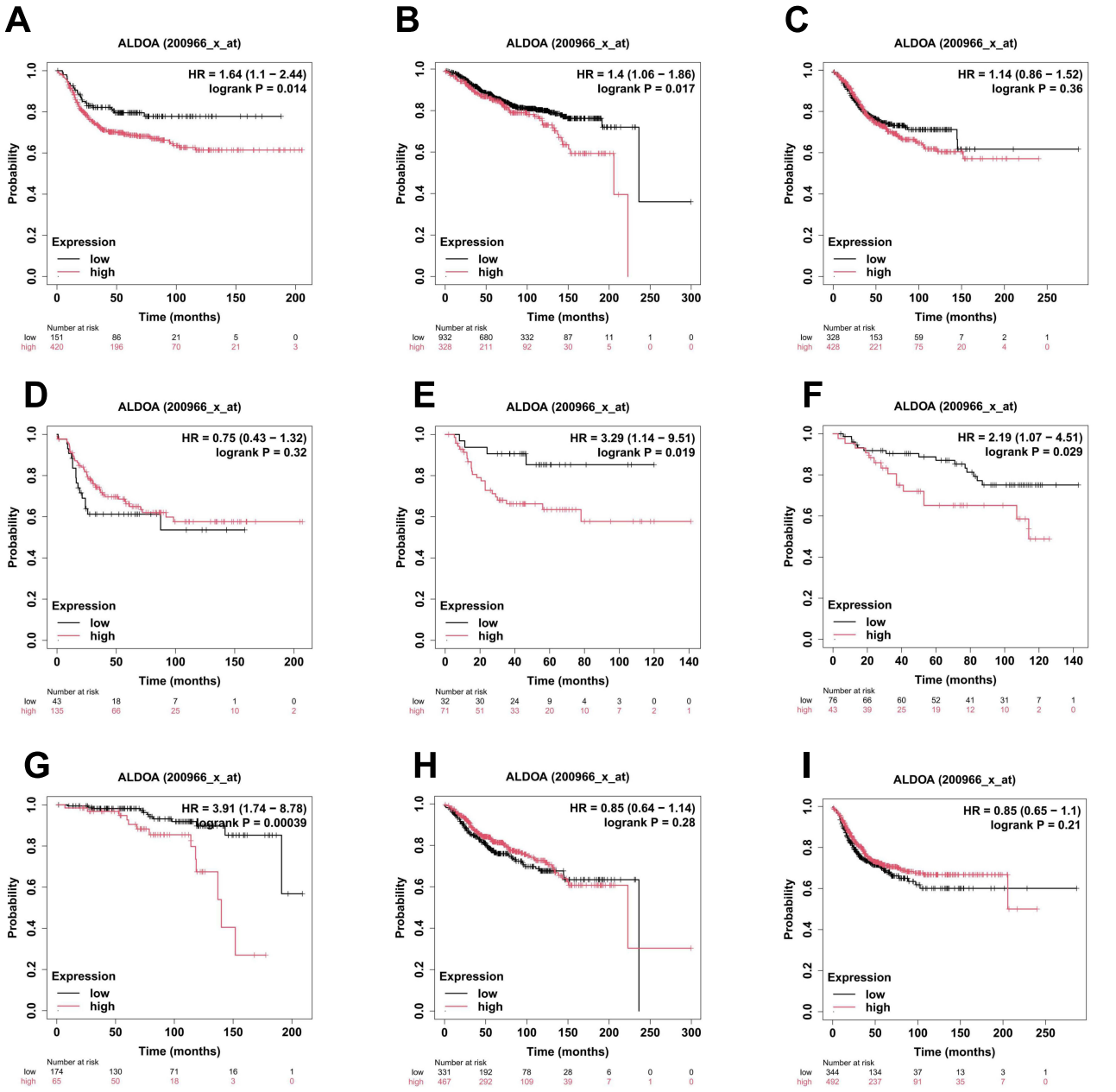

Figure 10 ALDOA expression in different subgroups correlates with DMFS of patients with BC from Kaplan-Meier plotter database. (**A**–**D**) Kaplan-Meier survival curve analysis shows DMFS of BC patients based on StGallen molecular subtypes ((**A**) basal; (**B**) luminal A; (**C**) luminal B; (**D**) HER2 positive). (**E** and **F**) Kaplan-Meier survival curve analysis shows DMFS of BC patients based on p53 status ((**E**) mutated; (**F**) wild type). (**G**–**I**) Kaplan-Meier survival curve analysis shows DMFS of BC patients based on histological grade ((**G**) grade 1; (**H**) grade 2; (**I**) grade 3).

pathway and initiating EMT.[27] By suppressing miR-145 expression and activating the Oct4/DUSP4/TRAF4 pathway, ALDOA enhances the stemness of lung cancer.[28]

There are some limitations in our study. Firstly, more research is necessary to understand the fundamental molecular process of ALDOA in BC cells. Secondly, more BC tumor tissues should be collected to further explore the expression and prognostic value of ALDOA in BC.

These findings indicated that ALDOA expression was much higher in BC tissues and closely correlated with clinical characteristics. For BC patients, a higher ALDOA indicated a lower chance of survival. The current research suggests that ALDOA may be a significant prognostic factor and a potential target for BC treatment.

Abbreviations

ALODA, Aldolase A; BC, breast cancer; BLIA, basal-like immune-activated; BLIS, basal-like immune-suppressed; BRCA, breast cancer susceptibility gene; DMFS, distant metastasis-free survival; ER, estrogen receptor; FP, first progression; GES, gene expression signature; HER2, human epidermal growth factor receptor 2; IDC, invasive ductal carcinoma; IHC, immunohistochemistry staining; ILC, invasive lobular carcinoma; KM, Kaplan-Meier; LAR, luminal androgen receptor; LNM, lymph node metastasis; MLIA, mesenchymal-like immune-altered; NPI, Nottingham Prognostic Index; OS, overall survival; PAM50, intrinsic molecular subtypes from Parker's SSP; PPS, post-progression survival; PR, progesterone receptor; RFS, relapse-free survival; SBR, Scarff-Bloom-Richardson; TNBC, triple negative breast cancer.

Data Sharing Statement

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

Ethics Statement

The study was approved by the Ethics Committee of the First Affiliated Hospital of Soochow University.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no competing interests in this work.

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