The Expression of *CLDN11* in Invasive Ductal and Invasive Lobular Breast Cancer and Multivariate Prognostic Analysis

Yu Cui
Affiliated Hospital of Qinghai University
Qiupei Du
Affiliated Hospital of Qinghai University
Zhanhong Zhang
Affiliated Hospital of Qinghai University
Haojie Feng
Affiliated Hospital of Qinghai University
Wang Hu
Affiliated Hospital of Qinghai University
Guoquan Jin
Affiliated Hospital of Qinghai University
Haining Fan (fanhaining@medmail.com)
Affiliated Hospital of Qinghai University

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Abstract

Background: Breast cancer (BRCA) remains the most common malignancy among women worldwide. Invasive ductal (IDC) and invasive lobular breast cancer (ILC) are the most frequent histological subtypes. Many genetic heterogeneities are related to the carcinogenesis of breast cancer, but exact reasons are still unclear. The study aimed to perform a clinical multivariate analysis of *CLDN11* in ILC and IDC.

Methods: Datasets analyzed *CLDN11* expression in IDC and ILC from The Cancer Genome Atlas (TCGA) database, and the multiple analysis of stratification in terms of clinical pathologic features and *CLDN11* was using the Chi-square test.

Results: *CLDN11* expression was significantly decreased in IDC and ILC data of GEO and TCGA compared to the normal breast tissues. Besides, *CLDN11* is negatively correlated with pro-oncogene *IKBKE* expression and positively correlated with tumor suppressor *ETS1* expression in IDC and ILC samples. Multiple factors, including age, clinical stage, subtype, and mutation status, indicated that the expression of *CLDN11* is positively associated with the progression of IDC and ILC.

Conclusions: *CLDN11* acted as a tumor suppressor in IDC and ILC patients and serves as a drug-gable target against IDC and ILC.

Introduction

Breast cancer (BRCA) is the most commonly diagnosed cancer in women worldwide, with an estimated 268,600 newly diagnosed women in 2019 alone, accounting for approximately 15.2%-30% of all new cancer cases in women[1]. Incidence rates are higher in high-income areas and lower in low-income areas. The natural history of breast cancer includes a progression from an in situ proliferative lesions to invasive cancer, leading to metastatic disease[2]. Environmental factors, lifestyle, and genetic diseases are the main risk factors for breast cancer[3,4]. As important subtypes of breast cancer, invasive neoplasms are broadly divided into two major types, invasive ductal breast cancer (IDC) and invasive lobular breast cancer (ILC). Based on the molecular properties, breast cancer can be subdivided into luminal A, luminal B, HER2 enriched, basal-like, and normal five groups[5].

The Claudin (CLDN) gene encodes a tetra-transmembrane family of proteins that play an essential role in tight junctions' formation and function. They prevent the free movement of lipids and proteins across the membrane by separating the membrane's apical and basolateral compartments. They also act as a regulator of cell proliferation and differentiation[6–8]. *CLDN11* is the core component of tight junctions and cell adhesion, and belongs to claudin family[9]. At the same time, it is also an essential part of the myelin sheath in the central nervous system, which plays a dual regulatory role in enhancing bone formation and inhibiting the occurrence of osteoclasts. Recently, *CLDN11* has been reported to be down-regulated in some cancers, such as hepatocellular carcinoma, malignant melanoma, gastric cancer, or cutaneous squamous cell carcinoma[10]. Thus, *CLDN11* has emerged as a hot area of cancer research.

High throughput screening (HTS) has become a convenient tool in molecular and biological studies that allows automated testing of large numbers of genes or proteins associated with disease progression. At present, relevant studies on *CLDN11* in IDC and ILC have not been carried out yet. To investigate whether it can be used as a specific marker for diagnosis and prognosis of IDC and ILC, we conducted a comprehensive and objective analysis to evaluate the expression, overall survival (OS), and disease-free survival (DFS) in IDC and ILC based on data obtained from Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/) dataset and The Cancer Genome Atlas (TCGA, https://cancergenome.nih.gov/). The details are as follows:

Material And Methods

CLDN11 expression analysis

The GEO of the National Center for Biotechnology Information (NCBI) has become the leading complete public repository of gene expression data. TCGA database, which is a collaboration between the National Cancer Institute (NCI) and National Human Genome Research Institute (NHGRI). Microarray datasets used for expression analysis of *CLDN11* in IDC and ILC were downloaded from The TCGA data was downloaded from cBioPortal for Cancer Genomics (http://www.cbioportal.org/). The dataset contains mRNA expression counts, gene mutations, and survival data with clinical information. Raw data were processed log2 transformation before conducting the expression analysis.

Microarray correlation analysis

Pearson correlation analysis of *CLDN11* with the inhibitor of nuclear factor kappa-B kinase subunit epsilon (*IKBKE*) and erythroblastosis virus transcription factor-1 (*ETS1*) was applied to evaluate microarray data from a set of human invasive breast cancer samples representing different stages of development. The data for overall survival in IDC and ILC and the correlation with *CLDN11* were obtained from the TCGA database.

Clinical significance of CLDN 11 in IDC and ILC patients

The overall survival (OS) and disease-free survival (DFS) of *CLDN11* with multivariate in IDC and ILC patients were accessed using the TCGA database. Clinical characteristics data including age, TNM stage, subtype, and mutation status were extracted. Log2-transformation was performed with the expression of *CLDN11* for further analyses.

Statistical analysis

For expression analysis, gene change was considered dramatically if the fold changes ≥ 1.5 and p-value $\leq 5\%$. Chi-square tests assessed the putative associations between conventional clinical pathology parameters (age at diagnosis, gender, TNM stage, and mutational status of *TP53* and *PIK3CA*) and survival outcomes. For survival analysis, the overall survival and the disease-free survival rate were

performed using a log-rank test and considered different if the p-value was less than 5% based on the one-way ANOVA with Tukey's post-test.

Results

mRNA level of CLDN11 is lowly expressed in IDC and ILC patients

Compared to *CLDN11* expression in IDC and ILC cancer patient samples, the original Series Matrix.txt File(s) were downloaded from GEO and TCGA databases. Original intensity files were performed MAS 5.0 normalization before further analysis. mRNA level of *CLDN11* was decreased in IDC and ILC samples compared to the standard group. Data were shown in Figs. 1A, C, and Figs. 1B, D, with a total of 4.496, 1.684, 6.340, and 3.099 fold changes, respectively ($P < 0.0001$).

CLDN11 is negatively correlated with pro-oncogene IKBKE expression in IDC and ILC samples

To identify the role of *CLDN11* in IDC and ILC progression, correlated analysis of *CLDN11* and *IKBKE*/*ETS-1* was conducted using the TCGA database. Studies have shown that *IKBKE* was highly expressed in breast cancer, which was first identified *IKBKE* as a new oncogene in breast cancer[11]. Guan et al. demonstrated that *IKBKE* had elevated mRNA and protein levels in glioma cell lines and human primary glioma tissues[12]. Kang and colleagues found that *IKBKE* was upregulated in esophageal squamous cell carcinoma by immunohistochemical staining[13]. As shown in Fig. 2A, expression of *CLDN11* was found to be consistently negatively correlated with *IKBKE* expression. Thus, *CLDN11* is a tumor suppressor gene in IDC and ILC.

CLDN11 has positively correlated with tumor suppressor ETS-1 expression in IDC and ILC samples

The carcinogenic and tumor-suppressive activities shown by *ETS-1* depend on the analysis conditions. High expression of *ETS-1* is closely related to higher metastatic potential and poor prognosis in ovarian cancer, lung adenocarcinoma, malignant melanoma, colorectal cancer, and other cancers[14–17]. However, a recent study found that the poor prognosis of BRCA patients was negatively correlated with the expression of *ETS-1*, suggesting that *ETS-1* is a tumor suppressor gene in BRCA cells[18]. Therefore, we believe that *ETS-1* is a tumor suppressor gene in breast cancer. At the same time, we discovered that *CLDN11* was positively correlated with the expression of *ETS-1* (Fig. 2B). Thus, these findings demonstrate that *CLDN11* served as a cancer suppressor gene in IDC and ILC patients.

Association of clinicopathological variables to survival outcome

The associations between clinical variables and survival outcomes in this study are summarized in Table 1. According to the literature, we divide the age as 60 years old, the stage into Ⅰ-Ⅳ, and the subtype into Luminal A, Luminal B, HER2 enriched, and basal-like.

Table 1
Association between the clinicopathological characteristics and survival outcome

	Overall survival			Disease-free survival		
	Living	Deceased	*P* value[a]	Living	Deceased	*P* value[a]
Age						
≤ 60	415 (56.6%)	45 (43.7%)	0.009	370 (54.7%)	36 (63.2%)	0.138
> 60	318 (43.4%)	58 (56.3%)		306 (45.3%)	21 (36.8%)	
AJCC TNM stage						
I and II	578 (78.9%)	60 (58.3%)	<0.001	543 (80.3%)	33 (58.0%)	<0.001
III and IV	155 (21.1%)	43 (41.7%)		133 (19.7%)	24 (42.0%)	
Subtype						
LumA	395 (53.9%)	43 (41.7%)	0.031	358 (52.9%)	26 (45.7%)	0.334
LumB	149 (20.3%)	27 (26.2%)		144 (21.3%)	10 (17.5%)	
Her2	57 (7.8%)	15 (14.6%)		54 (8.0%)	6 (10.5%)	
Basal	132 (18.0%)	18 (17.5%)		120 (17.8%)	15 (26.3%)	
*TP53*status						
WT	469 (64.0%)	61 (59.2%)	0.203	428 (63.3%)	33 (58.0%)	0.250
Mut	264 (36.0%)	42 (40.8%)		248 (36.7%)	24 (42.0%)	
*PIK3CA*status						
WT	434 (59.2%)	58 (56.3%)	0.324	396 (58.6%)	37 (65.0%)	0.215
Mut	299 (40.8%)	45 (43.7%)		280 (41.4%)	20 (35.0%)	

[a]Calculated using the Chi-square test

Age > 60 (43.4%) was associated with worse OS compared to age ≤ 60 (46.6%) (P = 0.009), and the DFS has the same trend without significance. The OS of early-stage (Ⅰ-Ⅱ) was higher than the advanced stage (Ⅲ-Ⅳ) (P < 0.001), so did the DFS (P < 0.001). Luminal A has the highest OS (P = 0.031) and DFS in all subtypes of BRCA, although the DFS was not statistically significant. The wild type of *TP53* and *PIK3CA*

were associated with better OS and DFS compared to the mutant type, while they had no statistical significance.

CLDN11 high expression is associated with better OS and DFS in IDC and ILC samples

According to the average value, patients were divided into two groups with high and low expression of *CLDN11*. Figure 3 showed that high expression of *CLDN11* was associated with better OS in IDC and ILC patients, while the DFS had the same trend with no significance.

Age, TNM stage, and gene mutation status are the stratification factors of CLDN11. The subtype is not a stratification factor of CLDN11 in IDC and ILC

Studies have shown that women's incidence in their 40 s and 50 s has remained stable, but women's incidence in their 60 s and over has increased[19]. To verify whether age is a stratification factor of CLDN11, we found that patients ≥ 60 years old and < 60 years old have a better OS and DFS with high expression of CLDN11 than low expression in both IDC and ILC(Figure 6A, B and Fig. 6C, D), but only the OS of over 60 years old is statistically significant.

As for the TNM stage, one of the variates in breast cancer indicated an effect on survival analysis. High expression of *CLDN11* in all disease stages may predict a good OS and DFS, although without statistical significance (Fig. 7).

The subtype is also an essential factor in breast cancer, but in Fig. 8 showed, there was no apparent correlation between the subtypes and prognosis.

Tumor protein p53 (*TP53*) and phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α (*PIK3CA*) were revealed as mutant genes in breast cancer[20,21]. As shown in Fig. 4, *TP53* and *PIK3CA* are two of the most frequently mutated gene in breast cancer. Therefore, we analyze the association between mutation and survival rate in IDC and ILC. In Fig. 5, we found that the high expression of the wild type of *TP53* and the mutant type of *PIK3CA* had better OS with statistically significant. Nevertheless, none have a noticeable effect on DFS.

Discussion

Precision medicine (PM) is a disease treatment and prevention strategy that considers individual differences in genes, environment, and lifestyle[22]. Compared with stratified medicine (SM), which includes a population-indicating drug changed according to a specific molecule, PM is designed to indicate treatment individually[23]. However, current opinions and technical limitations are not entirely

correct or precise. PM is still an indispensable way to reduce cancer treatment's toxicity and increase its benefit to patients[24].

Lung cancer, colon cancer, and breast cancer are the three most common cancers in the world[25,26]. Women diagnosed with breast cancer have a 5-year survival rate of 89%, a 10-year survival rate of 83%, and a 15-year survival rate of 78%. Although improvements have been made in early diagnosis, new targets against BRCA still cannot satisfy clinical requirements. Studies have shown that each type of breast cancer has a high level of heterogeneity. It is composed of several molecular subgroups driven by different molecular subgroups, indicating that it can be treated according to the individual molecular conditions of the tumor[27].

A large amount of data shows that claudins are abnormally expressed in a variety of cancers, so the role of claudins in carcinogenesis and progression to metastasis has become a hot area[28]. The *CLDN* expression changes observed in normal and cancerous tissues are cancer-specific. For example, *CLDN3* is up-regulated in prostate cancer, ovarian cancer, and breast cancer[29–31]. *CLDN7* is low expressed in head and neck tumors and breast cancer, but highly expressed in cervical cancer and ovarian cancer[32–34]. *CLDN1* is lost in glioblastoma multiforme and Melanoma, while it is overexpressed in colon cancer and cervical cancer[33,35–37]. *CLDN10* is increased in both thyroid cancer and hepatocellular carcinoma[38,39]. *CLDN4* is sometimes increased and sometimes decreased in breast and gastric cancer, while it is always highly expressed in prostate cancer and ovarian cancer[29–31,40–42]. Although the functional role of *CLDN11* in cancer is still unclear, the differential expression between tumor and normal cells, and the location of the membrane makes it a significant candidate for cancer treatment[43].

To further study the role of *CLDN11* in IDC and ILC patients, we performed the expression, correlation, and survival analysis of *CLDN11* in IDC and ILC patients. Microarray expression analysis revealed that the mRNA level of *CLDN11* down-regulated in patients with IDC and ILC. *CLDN11* is negatively correlated with pro-oncogene *IKBKE* expression and positively correlated with tumor suppressor *EST1* expression in IDC and ILC samples. Meanwhile, higher expression of *CLDN11* indicated a more prolonged overall survival and disease-free survival time than the lower groups, which suggested *CLDN11* as a tumor suppressor gene in this disease.

Clinical variables, including age, stage, subtype, and gene mutation status, may predict cancers' development. To better understand how these factors influenced breast cancer patient progression, the *CLDN11* level was classified into high and low expression groups according to the average value for OS and DFS analysis. Increasing the *CLDN11* level has a better prognosis for patients both over and under 60-year-old, and those over 60-year-old are statistically significant. As for the stage, higher expression of *CLDN11* level has a higher overall survival in the early stage(□-□) and the advanced stage(□-□). There is no statistically significant, but the trend is evident. We also find that the wild type status of *TP53* and the mutation status of *PIK3CA* showed better OS when the *CLDN11* expression level was high. Also, regardless of the high or low expression of *CLDN11*, there is no significant effect on the OS and DFS of

different subtypes of IDC and ILC. In general, age, TNM stage, and gene mutation are all related factors for IDC and ILC, except to subtypes. These suggest that the targeted therapy of *CLDN11* may have good significance for age, TNM staging, wild-type *TP53*, and mutant *PIK3CA*.

IKBKE, a nonclassical IKK family member, plays an essential role in regulating inflammation, activating and increasing immune cells, and metabolic diseases[44]. Studies have shown that *IKBKE* plays a carcinogenic effect in pancreatic cancer induced by KRAS[45]. Besides, compared with normal hematopoietic cells, the expression level of *IKBKE* in myeloid leukemia cells is higher. Inhibition of *IKBKE* can reduce the viability of AML cells[46]. Jie Lu et al. found that *IKBKE* increased the two crucial downstream factors of the hippo pathway and induce epithelial-mesenchymal transition (EMT), ultimately leading to tumor invasion and metastasis[47]. Boehm and colleagues indicate that *IKBKE* is amplified and overexpressed in a considerable number of breast cancer cell lines and primary breast tumors, so *IKBKE* is described as an oncogene of breast cancer[11]. *ETS1* is defined as an oncogene because it contributes to tumor angiogenesis and invasiveness in cancer strongly expressed in vascular endothelial cells and the adjacent interstitial cells[48,49]. *ETS1* promotes the metastasis of non-small cell lung cancer (NSCLC) and is associated with poor prognosis[50]. *ETS-1* regulates intracellular glutathione levels and promoting the development of ovarian cancer[51]. In melanoma, the upregulation of *ETS1* contributes to the malignant phenotype[16]. Besides, studies shown that *ETS1* is involved in the invasion and metastasis of colon cancer. In human breast cancer, overexpression of *ETS-1* has been found to a strong independent relationship with the poor prognosis of breast cancer, which may be related to epithelial-to-mesenchymal transition (EMT)[52,53].

There are several genes known to be associated with the prognosis of invasive breast cancer. The *TP53* gene, which is the most frequently mutated gene in human cancer, called 'the guardian of the genome'[54]. It has been found playing an essential role in inhibiting the progression of bone and soft tissue sarcoma[55]. Mutations of *TP53* in the direct DNA contact area lead to accelerated tumor progression of head and neck squamous cell carcinoma and reduced response to treatment[56,57]. In multiple bone marrow, changes in the tumor suppressor gene *TP53* are associated with poor prognosis[58]. *TP53* is the most frequently mutated gene in invasive breast cancer, the survival rate of patients with *TP53* mutations is significantly reduced[59]. *TP53* has different roles in different subtypes, among the most prevalent breast cancer subtypes, the frequency of *TP53* mutation is the lowest in luminal A, while the basal-like subtype is the highest[60]. Besides, *TP53* is also mutated in most TN breast cancers[20].

Phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) are crucial lipid kinases in neoplasia[61]. *PIK3CA*, which encodes the p110αcatalytic subunit of PI3K, has been found to be mutated in a variety of tumors, and may act as an oncogene in human cancers[62,63]. Pearson and colleagues showed that *PIK3CA* mutation correlates with low-grade prostate cancer patient survival[64]. In gastric cancer, the prevalence of somatic mutations in *PIK3CA* is relatively high, and its mutations cluster within the MSI (microsatellite unstable tumors) subset of gastric tumors, supporting *PIK3CA* as the main oncogene in gastric

cancer[65,66]. Studies have also indicated that *PIK3CA* mutation is related to the poor survival rate in resectable stage I to III colon cancer, providing a new research direction for the treatment of colon cancer[67]. In addition, it has found that *PIK3CA* mutations occur with high frequency in breast cancer[68]. And targeted inhibitors of PI3K/Akt/mTOR pathway can inhibit brain metastasis of *PIK3CA*-mutant breast cancer[69]. In our study, we found that mutate *PIK3CA* and the wild type of *TP53* are significantly correlated with the OS of invasive breast cancer, and the high expression of *CLDN11* is associated with a better survival time, which may provide new ideas for targeted drug research.

In conclusion, *CLDN11* acted as a tumor suppressor gene in IDC and ILC patients, and downregulation of the *CLDN11* gene is related to the poor prognosis in IDC and ILC patients. Besides, age, TNM stage, and gene mutation are stratification factors of *CLDN11* in IDC and ILC. Therefore, the determination of *CLDN11* gene expression may be useful for predicting prognosis and provide significant implications for novel treatment strategy based on precision medicine in IDC and ILC.

Abbreviations

GEO, Gene Expression Omnibus; TCGA, The Cancer Genome Atlas; OS, overall survival; DFS, disease-free survival; BRCA, breast cancer; IDC, invasive ductal breast cancer; ILC, invasive lobular breast cancer; CLDN, claudin; TJ, tight junction; HTS, high throughput screening; *IKBKE*, an inhibitor of nuclear factor kappa-B kinase subunit epsilon; *ETS1*, ETS proto-oncogene 1, transcription factor; *TP53*, tumor protein p53; PI3K, Phosphatidylinositol-4,5-bisphosphate 3-kinase; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α; MSI, microsatellite unstable tumors; PM, Precision medicine; SM, stratified medicine.

Declarations

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Authors' contributions

Yu Cui initiated the research, Qiupei Du developed the concept of the paper; Zhanhong Zhang, Haojie Feng, and Wang Hu analyzed and interpreted the data; Haining Fan provided valuable support and input of the project; All the authors approved the manuscript.

Consent for publication

No patient personal information was directly involved in this study.

Disclosure

The authors declare no competing conflicts of interest.

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Figures

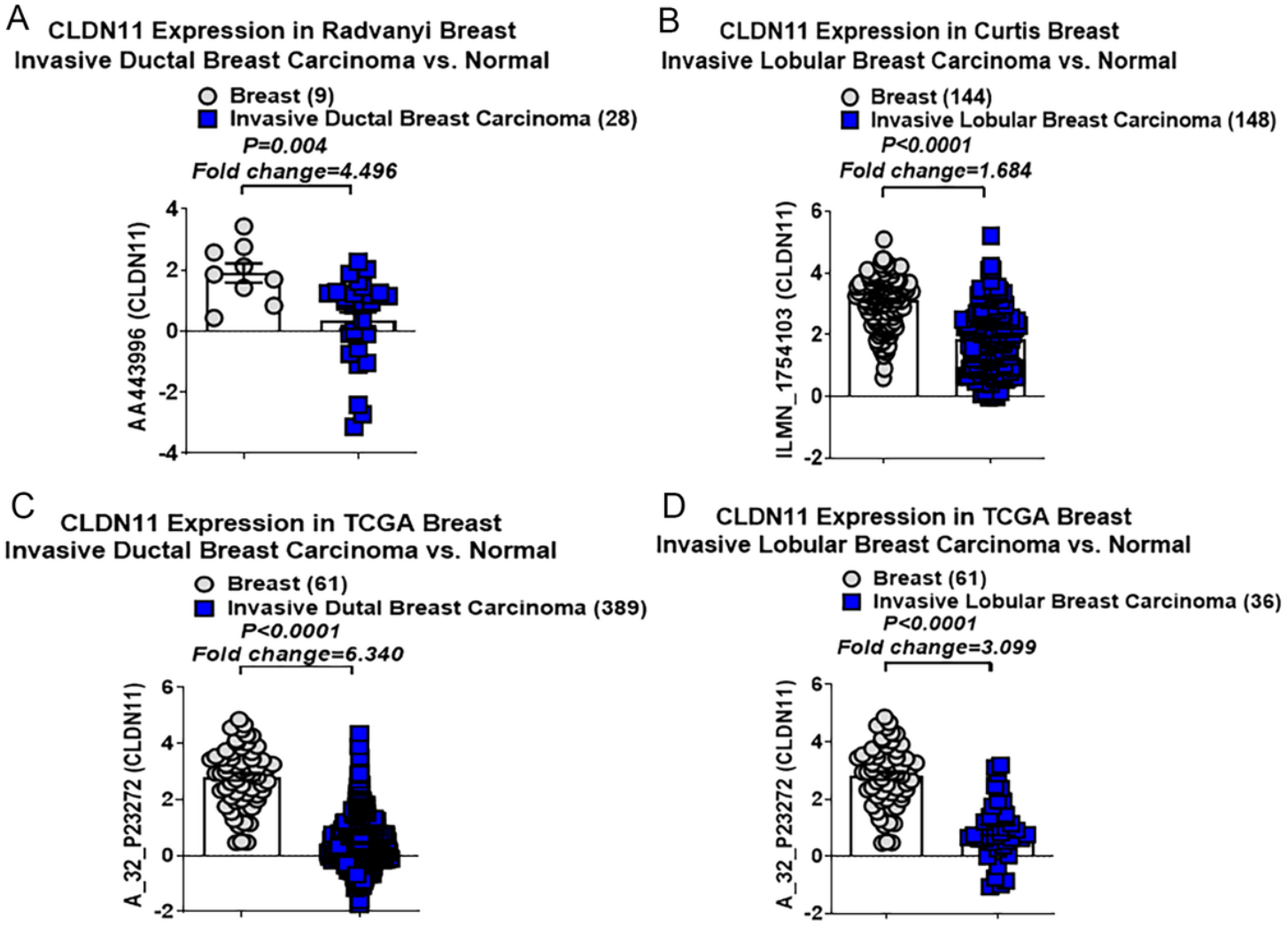

Figure 1

mRNA level of CLDN11 in GEO dataset of IDC(A) and ILC(B). mRNA level of CLDN11 in TCGA database of IDC (C) and ILC(D). Expression analysis for CLDN11 was performed using student t-test method. P value and fold change were extracted directly without any manual modification.

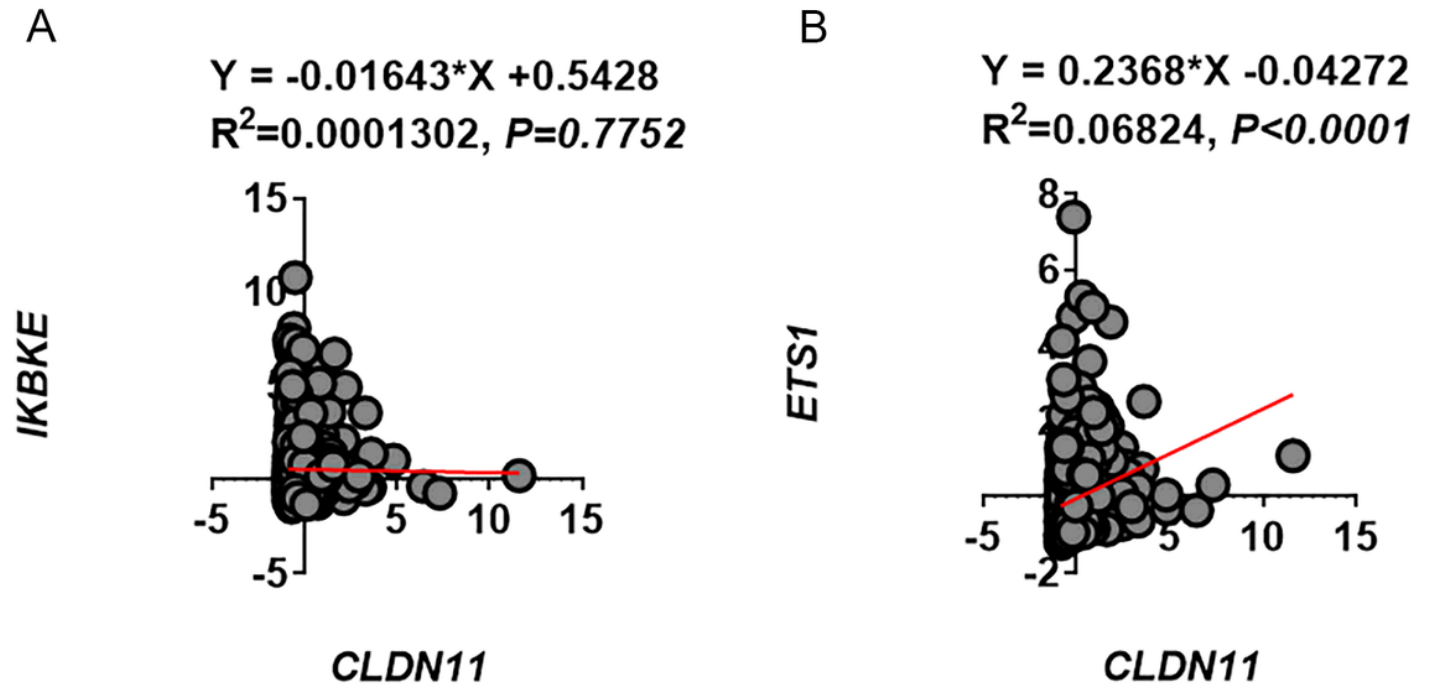

Figure 2

Correlation analysis of CLDN11 and IKBKE expression with IDC and ILC patients in TCGA database(A). Correlation analysis of CLDN11 and ETS1 expression with IDC and ILC patients in TCGA database(B).

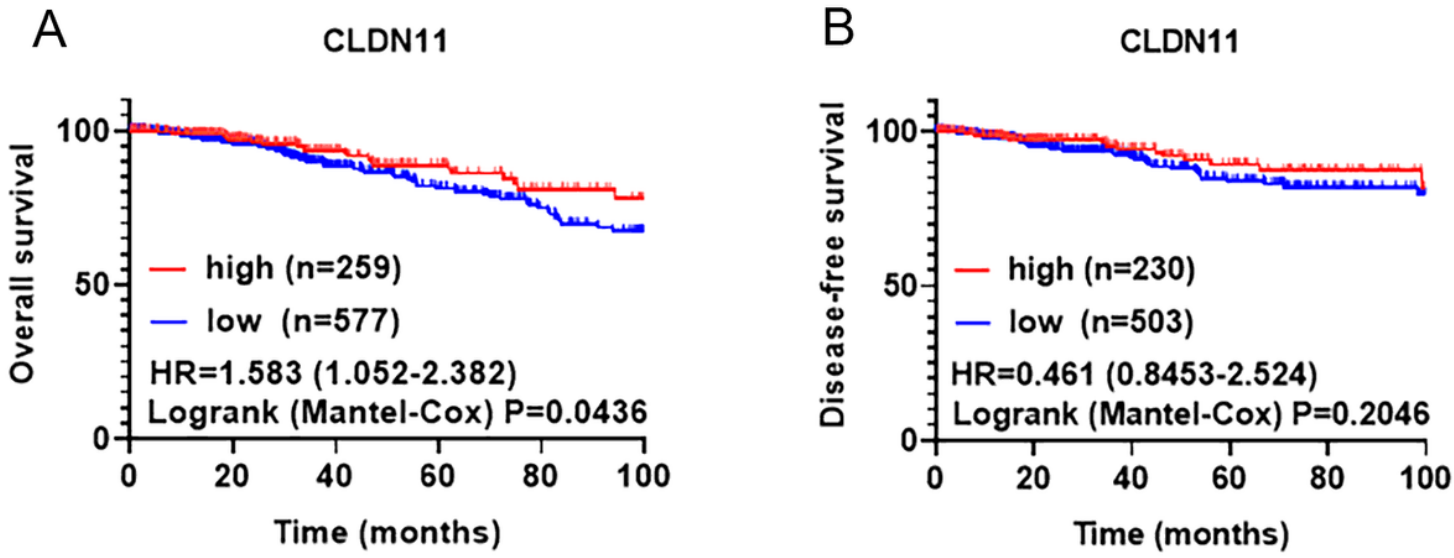

Figure 3

Patients were categorized into high and low CLDN11 expression groups according to the average value. OS (A) and DFS (B) analysis of CLDN11 in TCGA database. Survival analysis was performed using a log-rank test. *p<0.05, **p<0.01 and ***p<0.001 (one-way ANOVA with Tukey's post-test).

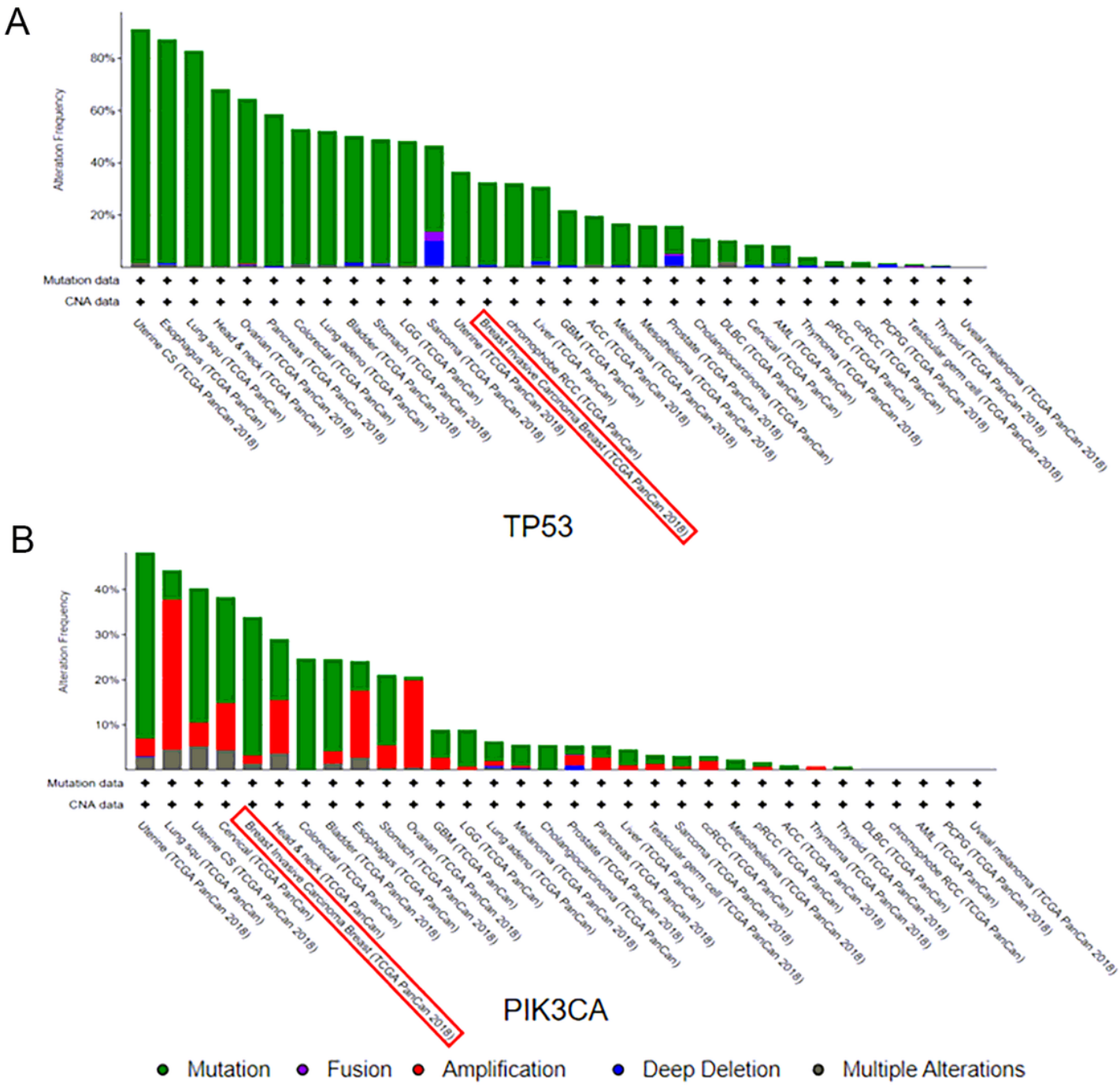

Figure 4

Mutations of TP53 (A) and PIK3CA (B) in invasive breast cancer.

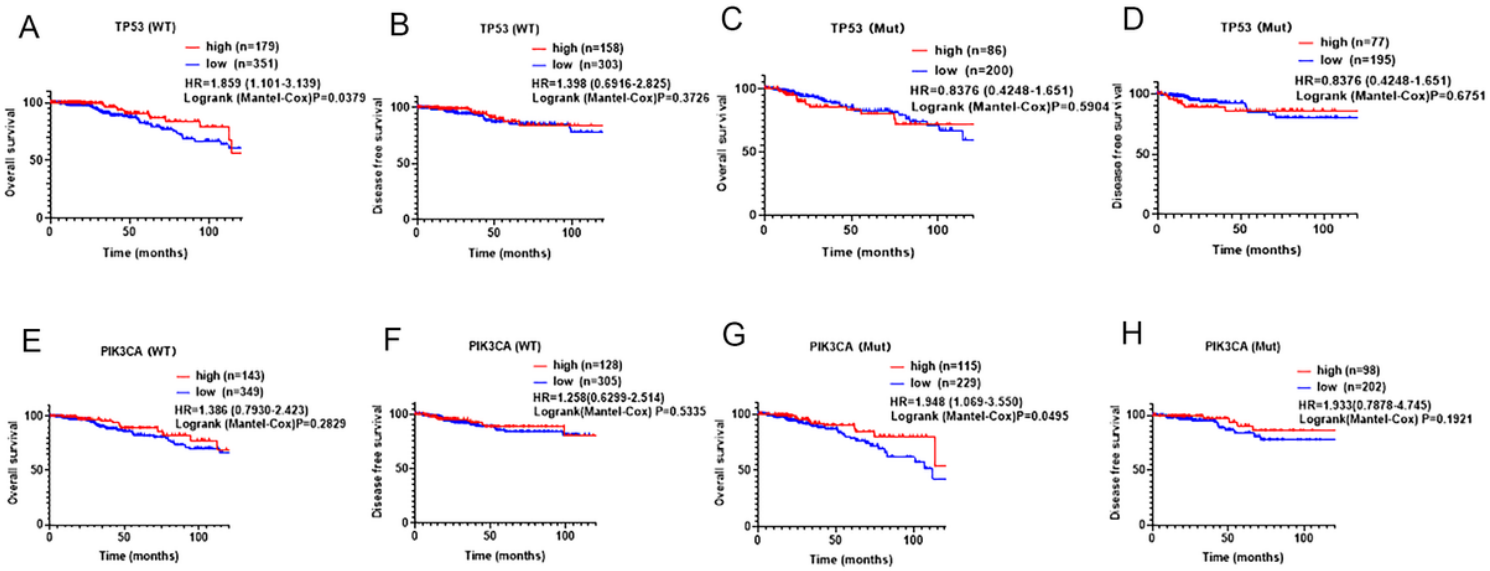

Figure 5

Patients were categorized into high and low CLDN11 expression groups according to the average value. Effect of CLDN11 expression on OS in IDC and ILC patients with TP53 wild type (A) and mutant type (C) in TCGA database. Effect of CLDN11 expression on DFS in IDC and ILC patients with TP53 mutant type (B) and wild type (D) in TCGA database. Effect of CLDN11 expression on OS in IDC and ILC patients with PIK3CA wild type (E) and mutant type (G) in TCGA database. Effect of CLDN11 expression on DFS in IDC and ILC patients with PIK3CA wild type (F) and mutant type (H) in TCGA database. Survival analysis was performed using a log-rank test. *p<0.05, **p<0.01 and ***p<0.001 (one-way ANOVA with Tukey's post-test)

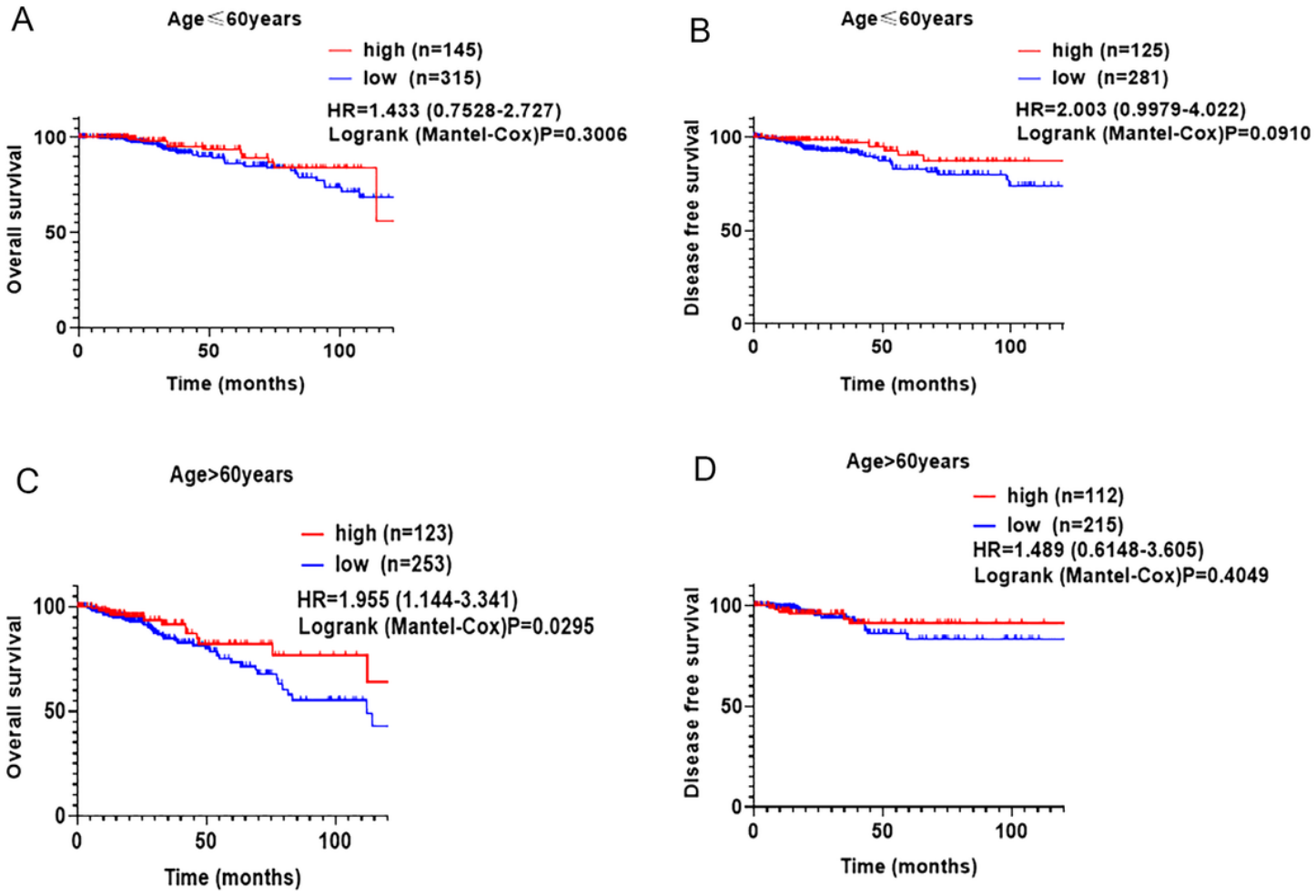

Figure 6

Effect of CLDN11 expression on OS in IDC and ILC patients with age≤60 (A), age60 (C). Effect of CLDN11 expression on DFS in IDC and ILC patients with age≤60 (B), age60 (D). Survival analysis was performed using a log-rank test. *p<0.05, **p<0.01 and ***p<0.001 (one-way ANOVA with Tukey's post-test)

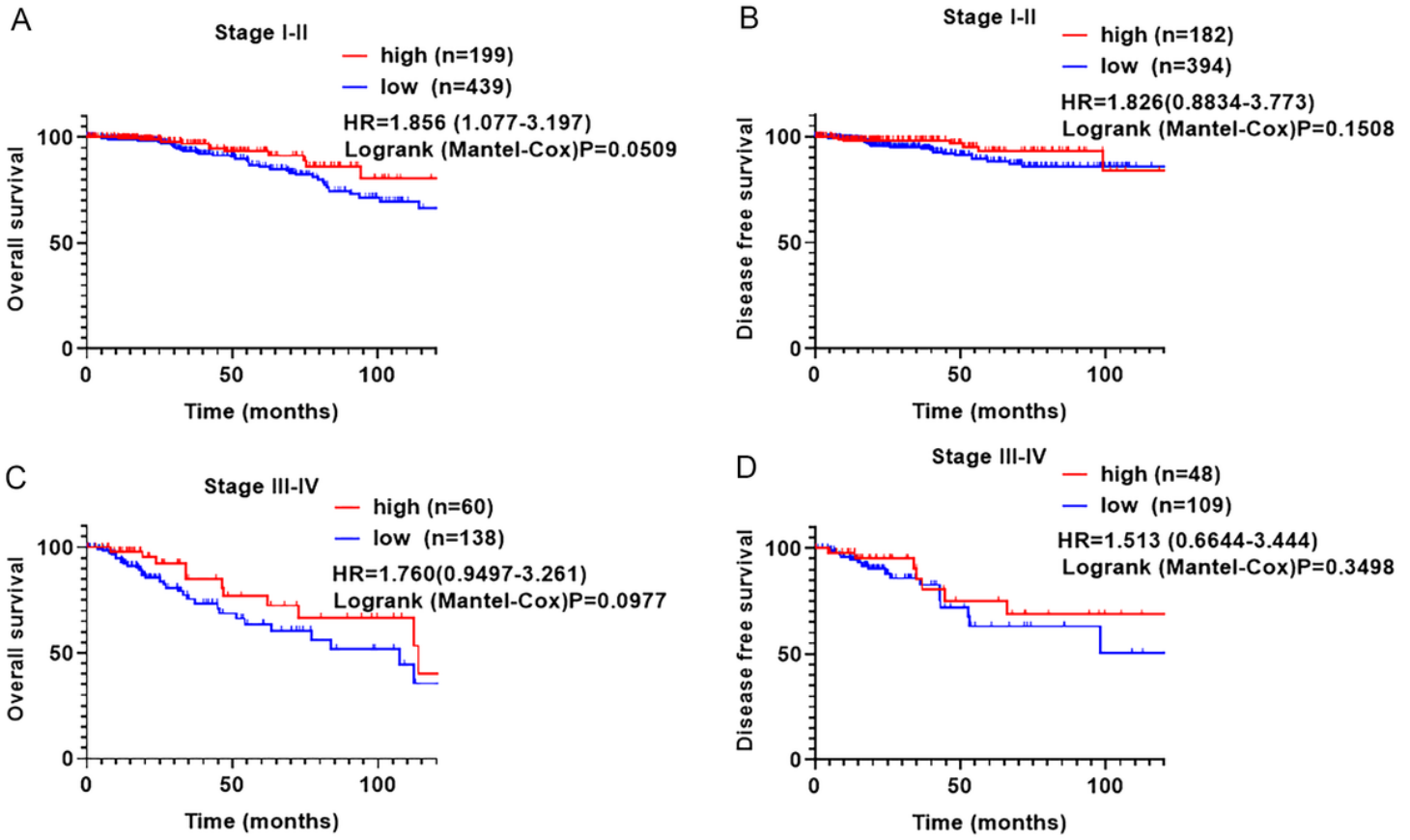

Figure 7

Patients were categorized into high and low CLDN11 expression groups according to the average value. Effect of CLDN11 expression on OS in IDC and ILC patients with stage Ⅰ-Ⅱ(A), stage Ⅲ-Ⅳ (C). Effect of CLDN11 expression on DFS in IDC and ILC patients with stage Ⅰ-Ⅱ(B), stage Ⅲ-Ⅳ (D). Survival analysis was performed using a log-rank test. *p<0.05, **p<0.01 and ***p<0.001 (one-way ANOVA with Tukey's post-test)

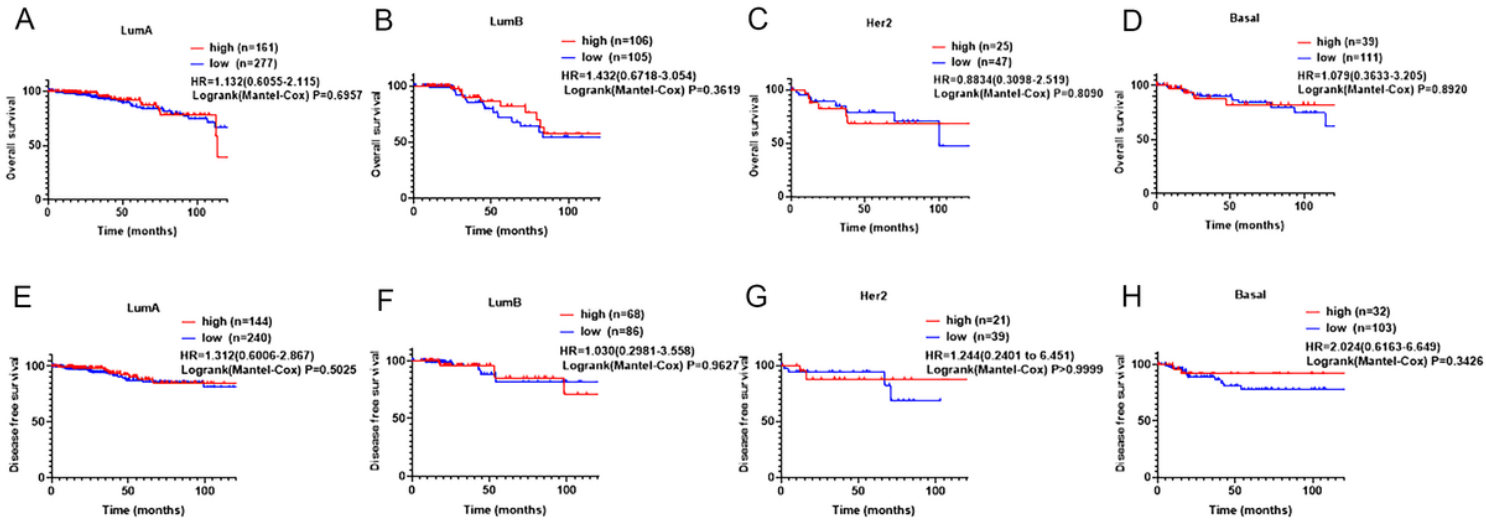

Figure 8

Effect of CLDN11 expression on OS in IDC and ILC patients with LumA, LumB, Her2, and basal in TCGA database (A-D). Effect of CLDN11 expression on OS in IDC and ILC patients with LumA, LumB, Her2, and basal in TCGA database (E-H). Survival analysis was performed using a log-rank test. *$p<0.05$, **$p<0.01$ and ***$p<0.001$ (one-way ANOVA with Tukey's post-test)