Original Research

Systemic analysis of the expression levels and prognosis of breast cancer-related cadherins

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Impact statement

This work investigated the expression of all the reported breast cancer-related cadherins and evaluated their potential as therapeutic targets and prognostic biomarkers. It includes distinct analyses, including genetic alteration, interaction, GO, and KEGG pathways, to elucidate underlying mechanisms of specific BC-related cadherins. We believe that these results would aid in the diagnosis and treatment of BC. On the other hand, they could provide theoretical support to the researchers interested in BC-related cadherins.

Abstract

Cadherins form connection between cells, facilitate communication, and serve as essential agents in the progression of multiple cancers. Over 100 cadherins have been identified and they are mainly divided into four groups: classical cadherins (CDHs), protocadherins (PCDHs), desmosomal (DSC), and cadherin-related proteins. Accumulating evidence has indicated that several members of the cadherins are involved in breast cancer development. Nevertheless, the expression profiles and corresponding prognostic outcomes of these breast cancer-related cadherins are yet to be analyzed. Here, we examined the expression levels and prognostic potential of these breast cancer-related cadherins from the specific databases viz. oncomine, gene expression profiling interactive analysis, human protein atlas, UALCAN, Kaplan–Meier Plotter, and cBioPortal. We found that the CDH2/11 levels were higher in breast cancer tissues, compared to healthy breast tissues, whereas with CDH3-5, PCDH8/10, and DSC3, the levels were lower in the former than in the latter. Additionally, for CDH1/6/13/17/23, PCDH7, and FAT4, trancript level alterations between breast cancer and healthy tissues varied across different databases. The CDH1 protein levels were elevated in breast cancer tissues versus healthy breast tissues, whereas the protein levels of CDH3/11 and PCDH8/10 were reduced in breast cancer, compared to healthy breast tissues. For CDH15 and CDH23, the expression levels paralleled tumor stage. Survival analysis, using the Kaplan–Meier Plotter database, demonstrated that elevated CDH1-3 levels correlated with diminished relapse-free survival in breast cancer patients. Alternately, enhanced CDH4-6/15/17/23, PCDH10, DSC3, and FAT4 levels estimated a rise in relapse-free survival of breast cancer patients. These data suggest CDH1-3 to be a promising target for breast cancer precision therapy and CDH4-6/15/17/23, PCDH10, DSC3, and FAT4 to be novel biomarkers for breast cancer prognosis.

Keywords: Breast cancer, expression, biomarkers, bioinformatics, cadherin

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Introduction

Breast cancer (BC) ranks among the top five cancers afflicting women across the world.[1] In 2020, 30% of cancer incidences involved BC, accounting for 15% of all cancer-associated female mortality within the United States of America.[1] The primary approach adopted to treat BC is surgery, chemotherapy, and radiation therapy, which have displayed improved therapeutic effects. Despite advancements in novel therapeutic targets and targeted drugs have enhanced the efficacy of BC treatment, ~20% of metastatic BC patients has a survival rate of <5 years.[2] Furthermore, owing to tumor heterogeneity, available biomarkers are limited in their prognosis of BC. Therefore, novel biomarkers are crucial for the enhancement of disease prognosis and develop precision medicine.

Cadherins, which have been known to act as tumor suppressors, regulate tissue development and differentiation. Therefore, dysregulated cadherins, brought on by genetic, epigenetic, and mutational factors could lead to tumor growth, invasion, and metastasis. Certain members of this superfamily are crucial to mammary cell development, such as CDH1/2/3/4/5/6/11/13/15/17, PCDH7/8/10, DSC3, FAT4, and CDH23. They have also been reported to contribute to tumor formation and BC metastases. Although the roles of known BC-associated cadherins have been characterized in prior studies, further analysis is yet to be performed to determine their prognostic potential.

Here, we performed an extensive bioinformatics analyses on the expression profile of BC-related cadherins and assessed their candidacy as therapeutic targets and/or prognostic biomarkers. We believe that our analyses would provide important help in the diagnosis and treatment of BC.

Materials and methods

Ethics statement

The Academic Committee of the North Sichuan Medical College agreed to our research protocols. All information for analysis was obtained from certified online sources, thus, confirming the procurement of written informed consents.

Expression pattern analysis for BC-related cadherins

The Oncomine database was employed for the comparison of the transcript levels of BC-associated cadherins in clinically diagnosed cancer specimens versus healthy controls. Significance was considered at a *P*-value of 0.01, fold change of 2, and gene rank within the leading 10%.

We analyzed the differential mRNA levels of the select genes in tumorous versus healthy tissues using the UALCAN database.[3]The cutoff *P* value was defined as 0.05.

After examining the mRNA profile of BC-related cadherins, we tried to analyze the protein expression patterns with the help of immunohistochemistry staining images from the HPA database, which details the mapping of all human proteins within cells, tissues, and organs, using a combination of multiple omics mechanics.[4]

Correlation between BC-related cadherins transcript levels and the clinicopathological variables

The relationship between BC-related cadherin expression and BC sub classes was analyzed with the help of the UALCAN database, while the relationship with the tumor stages was estimated using the GEPIA database. The GEPIA database offers customizable tools such as tumor/normal differential expression analysis, profiling based on specific cancer forms or pathological phases, correlation analysis,[5] with 0.05 as the significant threshold of *P*-value.

Prognostic potential for BC-related cadherins

Survival analyses including OS and RFS of the BC-related cadherins (median expression) were evaluated by using the Kaplan–Meier Plotter, which is usually used to evaluate the prognostic value.[6] Only the JeSet best probe set was selected.

Genetic alteration and co-expression in cBioPortal database

Genetic alteration frequency and co-expression of BC-related cadherins were analyzed using the cBioPortal database, which offers an open resource for interactive inspection of numerous cancer genome datasets.[7]

Analysis of gene interaction network of BC-related cadherins

Interactions among BC-related cadherins were analyzed with the help of STRING, a database that was used to predict protein–protein interactions.[8] The network of BC-related cadherins and its closely related neighbor genes was performed by Cytoscape, an open source platform for the visualization of complicated pathways and their interaction with any other available data.[9]

Function and pathway analysis for BC-related cadherins by DAVID

The Database for Annotation, Visualization, and Integrated (DAVID) offers an extensive array of functional interpretation tools that aid in the understanding of the function of a large set of genes.[10] Here, gene ontology (GO) enrichment analysis and Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analysis of the BC-related cadherins and intimately associated neighbor genes were obtained from DAVID and visualized with Excel. Biological processes (BP), cellular components (CC), and molecular function (MF) were incorporated into the GO enrichment analysis.

Results

All the BC-related cadherins show abnormal expression at transcriptional or/and protein level

To elucidate the distinct prognostic and possible therapeutic significance of BC-associated cadherins, mRNA expression and protein expression of the molecules were analyzed. As shown in Figure 1 and Table 1, transcript levels of CDH2/11 and PCDH7 in BC tissues were markedly elevated, while CDH3/4/5, PCDH8/10, and DSC3 were drastically reduced in BC tissues when compared to healthy breast tissues. The levels of CDH1/13/23 and FAT4 varied across different databases, and all of them displayed a lower expression in the BC tissues. In the cases of invasive BC (Figures 2 and 3), the mRNA levels of CDH1/2/11 were upregulated in tumor tissues, while CDH3/4/5/6/13/17/23, PCDH8/10, DSC3, and FAT4 were significantly downregulated. Protein expression patterns are shown in Figure 3. As evident, CDH1 staining was poor in normal breast

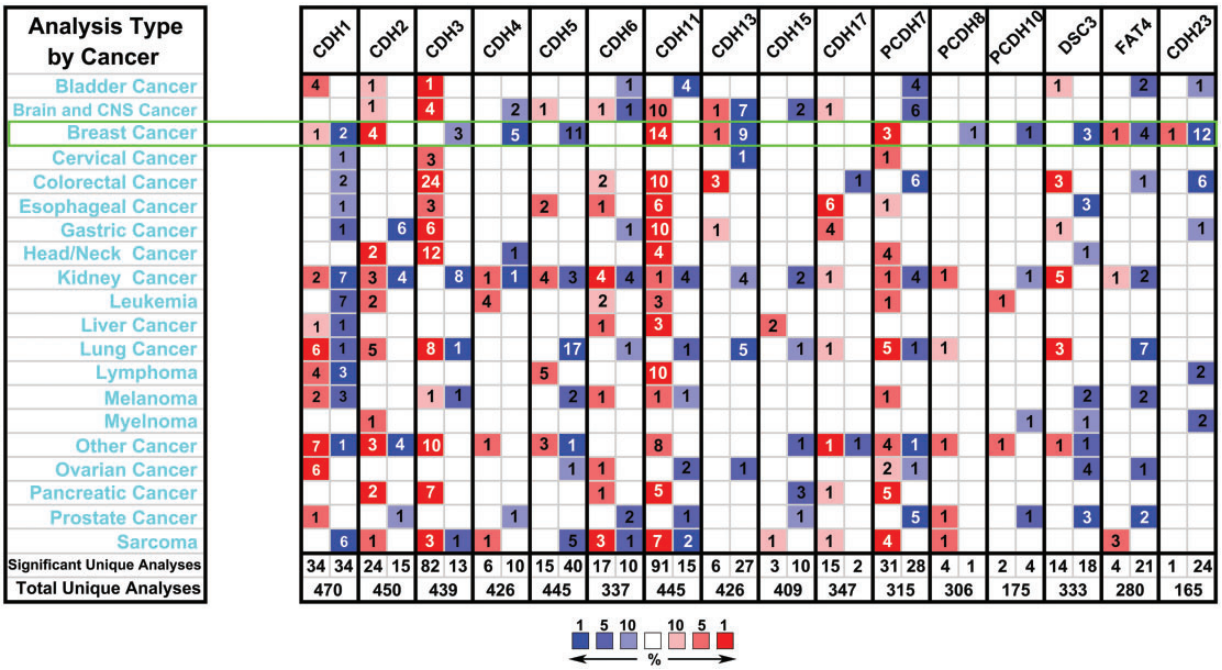


Figure 1. Transcript levels of the BC-related cadherins in various cancers (Oncomine). Number of datasets included in the current study with statistically significant BC-related cadherins upregulation (red) or downregulation (blue). The thresholds: *P*-value≤ 0.01; fold change≥ 2; gene rank ≤ 10%; data type: mRNA. (A color version of this figure is available in the online journal.)

tissues, while higher signal was observed in BC tissues. The staining patterns of normal tissues vs. BC tissues for CDH3, CDH11, PCDH8, and PCDH10 were medium vs. low, low vs. not detected, high vs. medium, and medium vs. not detected, respectively. Furthermore, distinct BC-related cadherins showed a similar staining profile in normal and BC tissues, such as CDH2/5/6/13/15/17, PCDH7, and DSC3. There was no available data for CDH4. In conclusion, we found that the CDH1 protein levels were enhanced in BC tissues, compared to healthy breast tissues, while the CDH3/11 and PCDH8/10 levels were lower.

Expression levels of CDH5/23, and other BC-related cadherins were related to tumor stages and subclasses

After the evaluation of the expression profiles, we investigated the correlation between BC-related cadherins mRNA levels and clinicopathological variables, such as, the specific stages of a patient's cancer and tumor subclasses. We demonstrated that the CDH5 and CDH23 levels correlated with specific tumor stages (Figure 4).

All BC-related cadherins except for CDH15 (Figure 5) displayed significant differences among the various tumor subclasses. CDH1 was markedly elevated in the $Her2^+$ BC, compared to the other two types of BC, while CDH17, PCDH7, and DSC3 displayed an opposite trend. Besides, CDH5/6/11 levels were remarkably high in luminal BC, relative to the $Her2^+$ and TNBC. In the caseofPCDH7, a significantly lower mRNA level was observed in TNBC than in the other two subclasses. Among the three subclasses of BC, the transcriptional levels of CDH3 and PCDH10 were significantly different.

Expression of CDH4-6/15/17/23, PCDH10, DSC3, and FAT4 was significantly associated with BC prognosis

There was ample evidence of an association between BC-related cadherins transcript levels and BC patient survival. In Figure 6, we show that the mRNA levels of CDH1/2/3/5 and FAT4 were strongly correlated with OS (overall survival) ($P < 0.05$), and BC patients with low CDH1-3 transcript levels and high CDH5 and FAT4 transcript levels were estimated to experience higher OS. The mRNA levels of CDH1-6/15/17/23, PCDH10, DSC3, and FAT4 were also strongly correlated with RFS ($P< 0.05$). The patients with reduced mRNA levels of CDH1-3 or high mRNA levels of CDH4-6/15/17/23, PCDH10, DSC3, and FAT4 were predicted to have high RFS (Figure 7). Based on these data, CDH4-6/15/17/23, PCDH10, DSC3, and FAT4 transcript levels were significantly associated with BC prognosis and might serve as effective biomarkers for BC patient prognosis.

Genetic changes, binding analyses, and neighbor gene network of the BC-related cadherins

We analyzed genetic alterations in BC-related cadherins for invasive BC. As a result, these cadherins showed marked differences in 680 samples of 1101 patients with invasive BC (61%). CDH1/2/3/4/5/6/11/13/15/17, PCDH7/8/10, DSC3, FAT4, and CDH23 were altered in 18%, 6%, 9%, 10%, 6%, 7%, 6%, 5%,4%, 17%, 4%, 3%, 3%, 4%, 4%, 6% of the queried BC samples, respectively (Figure 8(a)). Thereafter, correlations based on the mRNA expression of these cadherins in invasive BC were analyzed, and the data suggested marked positive associations in the following cadherins: CDH2 with CDH11; CDH3 with DSC3; CDH5 with CDH6/13 and FAT4; CDH6 with CDH11/13 and

Table 1. Significant changes of CDHs expression in transcription level between different types of breast cancer and normal breast tissues (oncomine).

		BC versus normal			
Gene name	**Type of breast cancer**	**Fold change**	***P* value**	***t* test**	**Source and/or reference**
CDH1	Lobular breast carcinoma	−5.088	1.37E-08	−8.564	Zhao breast statistics
	Mucinous breast carcinoma	4.661	0.008	3.213	TCGA
	Invasive lobular breast carcinoma	−4.415	2.74E-08	−5.962	
CDH2	Invasive breast carcinoma	2.217	5.33E-16	12.626	Gluck breast statistics
	Invasive ductal breast carcinoma	3.902	6.73E-04	3.726	Karnoub breast statistics
	Invasive ductal breast carcinoma stroma	2.192	9.60E-04	3.78	Ma breast 4 statistics
	Breast phyllodes tumor	3.661	0.004	4.852	Curtis breast statistics
CDH3	Lobular breast carcinoma	−2.43	0.002	−4.359	Zhao breast statistics
	Invasive ductal breast carcinoma	−2.62	0.001	−4.685	
	Ductal breast carcinoma	−3.039	8.97E-05	−4.45	Richardson breast 2 statistics
CDH4	Invasive ductal and lobular carcinoma	−3.214	5.00E-03	−6.37	TCGA
	Mixed lobular and ductal breast carcinoma	−2.831	4.63E-09	−9.691	
	Mucinous breast carcinoma	−2.643	4.96E-07	−9.472	
	Invasive lobular breast carcinoma	−2.659	4.88E-15	−9.339	
	Male breast carcinoma	−3.2	4.21E-06	−11.135	
CDH5	Fibroadenoma	−2.595	9.00E-03	−3.656	Sorlie breast 2 statistics
	Fibroadenoma	−2.85	9.00E-03	−3.485	Sorlie breast statistics
	Breast carcinoma	−3.043	3.10E-11	−11.746	Curtis breast statistics
	Tubular breast carcinoma	−2.785	6.34E-34	−15.231	
	Invasive ductal and invasive lobular breast carcinoma	−2.607	9.49E-38	−15.542	
	Invasive breast carcinoma	−2.87	3.86E-10	−9.091	
	Medullary breast carcinoma	−3.353	2.34E-16	−11.963	
	Invasive lobular breast carcinoma	−2.254	1.32E-33	−13.797	
	Mucinous breast carcinoma	−2.312	1.09E-19	−11.152	
	Invasive ductal breast carcinoma	−2.889	1.12E-51	−22.611	
	Ductal breast carcinoma	−4.05	3.18E-06	−6.972	Richardson breast 2 statistics
CDH6	NA	NA	NA	NA	NA
CDH11	Invasive breast carcinoma	14.244	1.23E-27	20.81	Finak breast statistics
	Mixed lobular and ductal breast carcinoma	3.753	1.91E-09	11.441	TCGA
	Invasive lobular breast carcinoma	3.277	2.74E-17	10.611	
	Invasive breast carcinoma	2.844	5.20E-18	9.945	
	Invasive ductal breast carcinoma	2.575	6.53E-20	11.479	
	Lobular breast carcinoma	4.111	7.00E-03	4.851	Perou breast statistics
	Breast phyllodes tumor	7.638	1.20E-04	10.995	Curtis breast statistics
	Tubular breast carcinoma	2.481	3.19E-21	11.665	
	Invasive mixed breast carcinoma	3.414	1.00E-03	4.008	Radvanyi breast statistics
	Invasive ductal breast carcinoma	2.854	3.00E-03	3.421	
	Invasive lobular breast carcinoma	2.604	7.00E-03	2.82	
	Invasive ductal breast carcinoma	3.889	1.00E-02	2.829	Turashvili breast statistics
	Invasive ductal breast carcinoma stroma	2.78	2.00E-03	3.465	Karnoub breast statistics
	Ductal breast carcinoma *in situ* stroma	2.332	5.00E-03	2.823	Ma breast 4 statistics

(continued)

Table 1. Continued.

Gene name	Type of breast cancer	BC versus normal			Source and/or reference
		Fold change	*P* value	*t* test	
CDH13	Ductal breast carcinoma *in situ* stroma	2.183	4.08E-05	5.087	Ma breast 4 statistics
	Fibroadenoma	−2.771	2.00E-03	−8.568	Sorlie breast 2 statistics
	Ductal breast carcinoma	−2.007	1.32E-06	−10.181	
	Fibroadenoma	−3.043	6.00E-03	−6.354	Sorlie breast statistics
	Invasive ductal breast carcinoma	−2.891	1.08E-24	−14.806	TCGA
	Invasive ductal and lobular carcinoma	−3.13	9.38E-04	−8.446	
	Invasive lobular breast carcinoma	−2.694	2.83E-16	−10.002	
	Mixed lobular and ductal breast carcinoma	−2.798	1.94E-07	−9.035	
	Invasive breast carcinoma	−2.398	5.99E-17	−9.52	
	Ductal breast carcinoma	−2.046	1.00E-03	−4.72	Perou breast statistics
CDH15	NA	NA	NA	NA	NA
PCDH7	Invasive mixed breast carcinoma	2.174	6.61E-04	4.653	Radvanyi breast statistics
	Invasive ductal breast carcinoma stroma	2.778	7.14E-04	4.168	Ma breast 4 statistics
	Ductal breast carcinoma *in situ* stroma	2.144	8.50E-04	3.738	
PCDH8	Intraductal cribriform breast adenocarcinoma	−2.059	7.00E-03	−3.78	TCGA
PCDH10	Invasive ductal breast carcinoma	−2.911	3.00E-03	−3.502	Turashvili breast statistics
DSC3	Invasive ductal breast carcinoma	−11.356	2.99E-06	−7.299	Turashvili breast statistics
	Lobular breast carcinoma	−2.224	4.00E-03	−4.893	Zhao breast statistics
	Mucinous breast carcinoma	−20.788	3.00E-03	−5.348	TCGA
FAT4	Invasive mixed breast carcinoma	3.638	8.00E-03	3.387	Radvanyi breast statistics
	Invasive ductal breast carcinoma stroma	−2.917	7.54E-04	−3.68	Ma breast 4 statistics
	Invasive ductal breast carcinoma	−3.235	2.39E-30	−17.04	TCGA
	Invasive breast carcinoma	−2.361	2.12E-16	−9.257	
	Invasive breast carcinoma	−2.555	1.76E-04	−9.995	Gluck breast statistics
CDH23	Invasive lobular breast carcinoma	2.626	7.00E-03	2.725	Turashvili breast statistics
	Invasive breast carcinoma	−2.3	7.46E-20	−15.774	Curtis breast statistics
	Tubular breast carcinoma	−2.133	2.57E-40	−17.355	
	Medullary breast carcinoma	−2.517	2.86E-24	−16.488	
	Mucinous breast carcinoma	−3.621	3.41E-29	−16.233	
	Invasive ductal and invasive Lobular breast carcinoma	−2.148	2.37E-40	−16.683	
	Invasive ductal breast carcinoma	−2.237	2.54E-59	−25.648	
	Breast carcinoma	−2.365	1.31E-05	−6.077	
	Invasive ductal and lobular carcinoma	−2.684	4.11E-06	−8.304	TCGA
	Intraductal cribriform breast adenocarcinoma	−3.841	8.91E-04	−7.873	
	Invasive ductal breast carcinoma	−5.043	4.01E-25	−14.992	
	Invasive lobular breast carcinoma	−3.009	3.94E-10	−6.997	
	Invasive breast carcinoma	−3.137	8.92E-14	−8.209	

NA: not available; TCGA: The Cancer Genome Atlas.

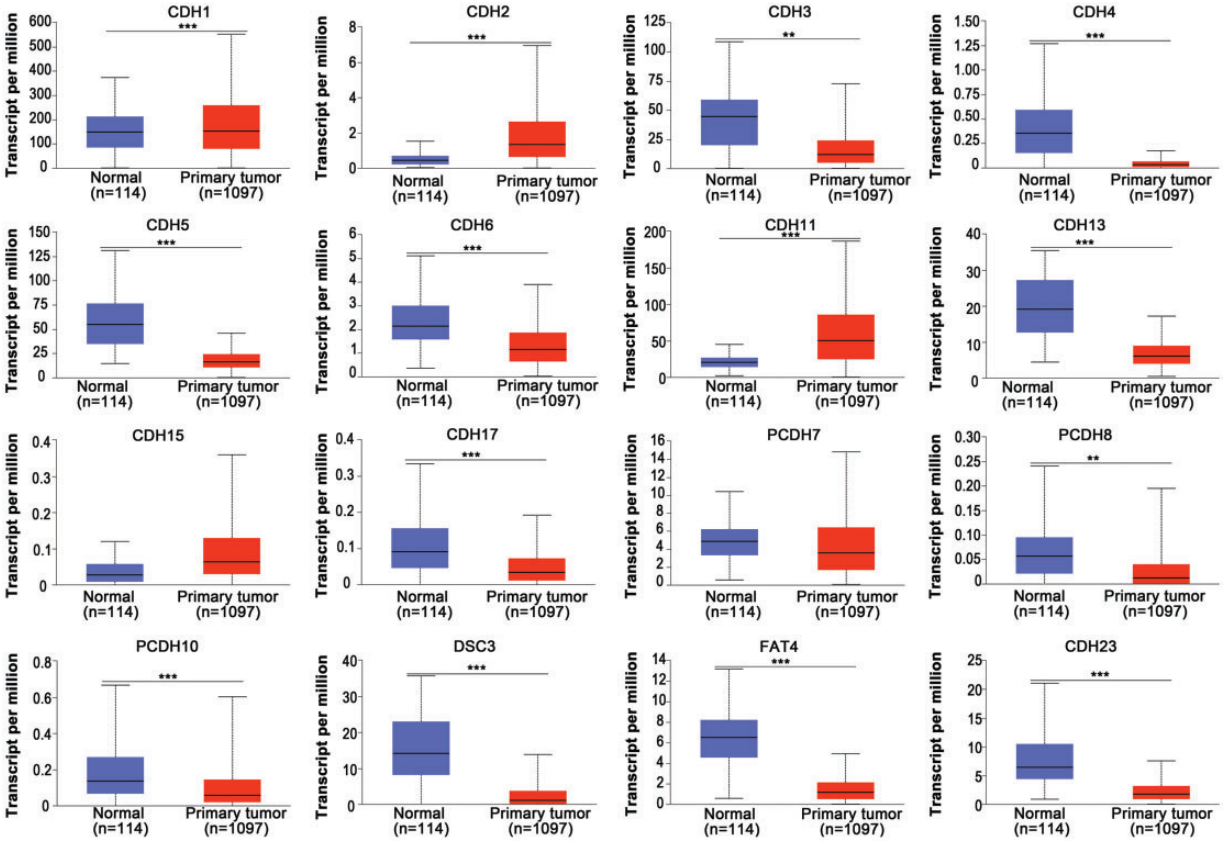


Figure 2. mRNA expression levels of the BC-related cadherins (UALCAN). (*$P<0.05$; **$P<0.01$; ***$P<0.001$) (A color version of this figure is available in the online journal.)

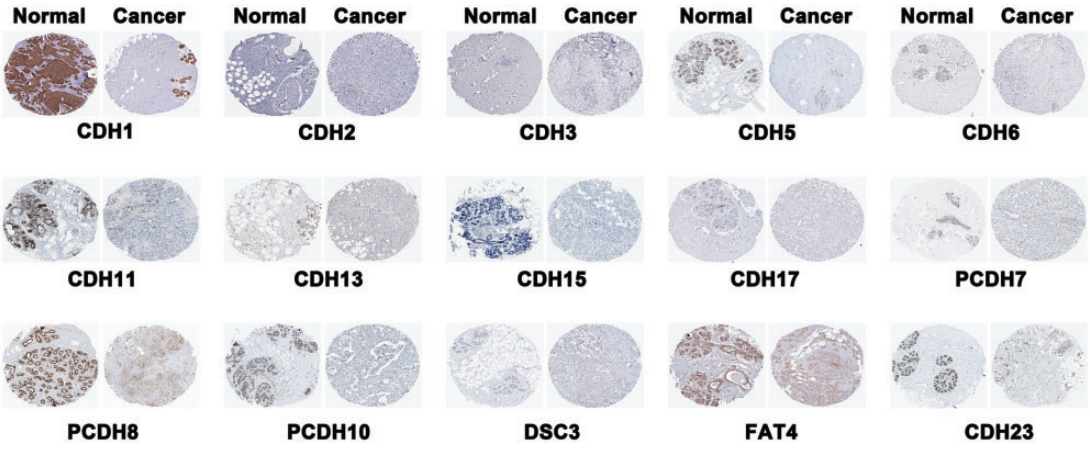
FAT4; CDH11 with PCDH7 and FAT4; CDH13 with FAT4; and PCDH7 with FAT4. Furthermore, we observed a close connection between CDH1/2/3/4/5/6/11/13/15/17 with the help of STRING (Figure 8(b)). We also constructed a network for the BC-related cadherins and the 50 highly altered neighboring genes. Based on our results, TP53, SYNE1, TTN, PTEN, ANK2, KMT2C, FAT3, PCLO, USH2A, and DMD were closely associated with alterations in BC-related cadherins (Figure 8(c)).

The functions of the BC-related cadherins and the 50 highly altered neighboring genes were predicted, and Figure 9(a) to (c) shows the top 10 highly enriched GO items. Within the BP category, homophilic cell adhesion via plasma membrane adhesion molecules, adherens junction organization, cell adhesion, calcium-dependent cell–cell adhesion via plasma membrane cell adhesion molecules, cellular response to caffeine, sensory perception of light stimulus, heterophilic cell–cell adhesion via plasma membrane cell adhesion molecules, cerebral cortex development, lipoprotein transport, and O-glycan processing were among the ten leading highly enriched factors. The Z disc, membrane, integral part of membrane, plasma membrane, M band, sarcoplasmic reticulum membrane, sarcoplasmic reticulum, sarcolemma, extracellular exosome, and dynein complex were among the 10 leading highly enriched factors within the CC group. In the MF category, the BC-related cadherins and their neighboring genes were primarily enriched in calcium ion binding, ATPase activity, calcium-induced calcium release activity, ryanodine-sensitive calcium-release channel activity, structural constituent of muscle, calcium-release channel activity, gamma-catenin binding, actin binding, calmodulin binding, and protease binding.

KEGG analysis was employed to define the signaling networks associated with the alterations of BC-related cadherins and their highly altered neighboring genes. Nine networks were associated with the BC-related cadherin alterations. As depicted in Figure 9(d), they were cell adhesion molecules (CAMs), endometrial cancer, arrhythmogenic right ventricular cardiomyopathy (ARVC), melanoma, hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy, circadian entrainment, thyroid hormone signaling pathway, vitamin digestion, and absorption.

Discussion

Specific associations between cancerous cells, neighboring cells, and extracellular matrix, mediated by CAMs, modulate the development of cancer. As important components of CAMs, the cadherin protein family is involved in the development of multiple cancers, including BC. Albeit the confirmatory role of specific cadherins in BC development has been underscored, there is a dearth of information on the prognostic potential of discrete cadherins. Here, the level, mutation, and prognostic potential of BC-related cadherins were analyzed.



Protein	Antibody	Normal staining	Cancer staining
CDH1	CAB000087	Low	High
CDH2	CAB000141	Not detected	Not detected
CDH3	CAB001767	Medium	Low
CDH4	NA	NA	NA
CDH5	CAB028366	Medium	Medium
CDH6	CAB025274	Not detected	Not detected
CDH11	CAB013072	Low	Not detected
CDH13	CAB025863	Not detected	Not detected
CDH15	HPA009139	Not detected	Not detected
CDH17	HPA026556	Not detected	Not detected
PCDH7	HPA011866	Low	Low
PCDH8	HPA010509	High	Medium
PCDH10	HPA011220	Medium	Not detected
DSC3	CAB037328	Not detected	Not detected
FAT4	HPA052819	Medium	Medium
CDH23	HPA017232	Medium	Medium

Figure 3. Immunohistochemistry staining images of BC-related cadherins proteins (HPA). (NA: no available data). (A color version of this figure is available in the online journal.)

Classical cadherins

CDH1, known as epithelial cadherin (E-cadherin), is known to regulate development of BC.[11] The loss of CDH1 is a known characteristic of lobular neoplasia and invasive lobular carcinomas,[12] and the depletion is also related to enhanced tumor size, advanced histological grade, and occurrence of distant metastases. Moreover, patients with lower CDH1 expression perform poorly when assessed for the disease-free survival (DFS)[13] and OS[14] parameters. In our results, CDH1 showed a lower expression level in lobular breast carcinoma and invasive lobular breast carcinoma than in healthy samples in the Oncomine database. However, in the UALCAN (invasive BRCA) and HPA databases, CDH1 levels were elevated in BC, compared to healthy normal tissues, which was strongly correlated with worse OS and RFS in all BC patients, with follow-ups lasting 200 months. These variations might be attributed to different numbers and types of samples in the studied subjects. Moreover, when the association between CDH1 transcript levels and disease state of BC patients was analyzed, no significant difference among the stages of BC was observed. A significant difference was observed between the normal and BC subclasses of luminal/Her2$^+$, luminal and Her2$^+$, and Her2$^+$ and TNBC cases.

CDH2, known as neuronal cadherin (N-cadherin), works synergistically with CDH1 to stimulate breast tumor growth.[15] High CDH2 levels were observed in triple-negative breast cancer (TNBC) cases with lymphatic invasion, and in node metastasis-positive cases,[16] which was identical to our results based on the Oncomine database. High CDH2 levels were strongly associated with poor OS and RFS in all BC patients. Moreover, we demonstrated a marked difference between the healthy and the three types of BC (including luminal, Her2$^+$, and TNBC), while its levels were not related to tumor phase.

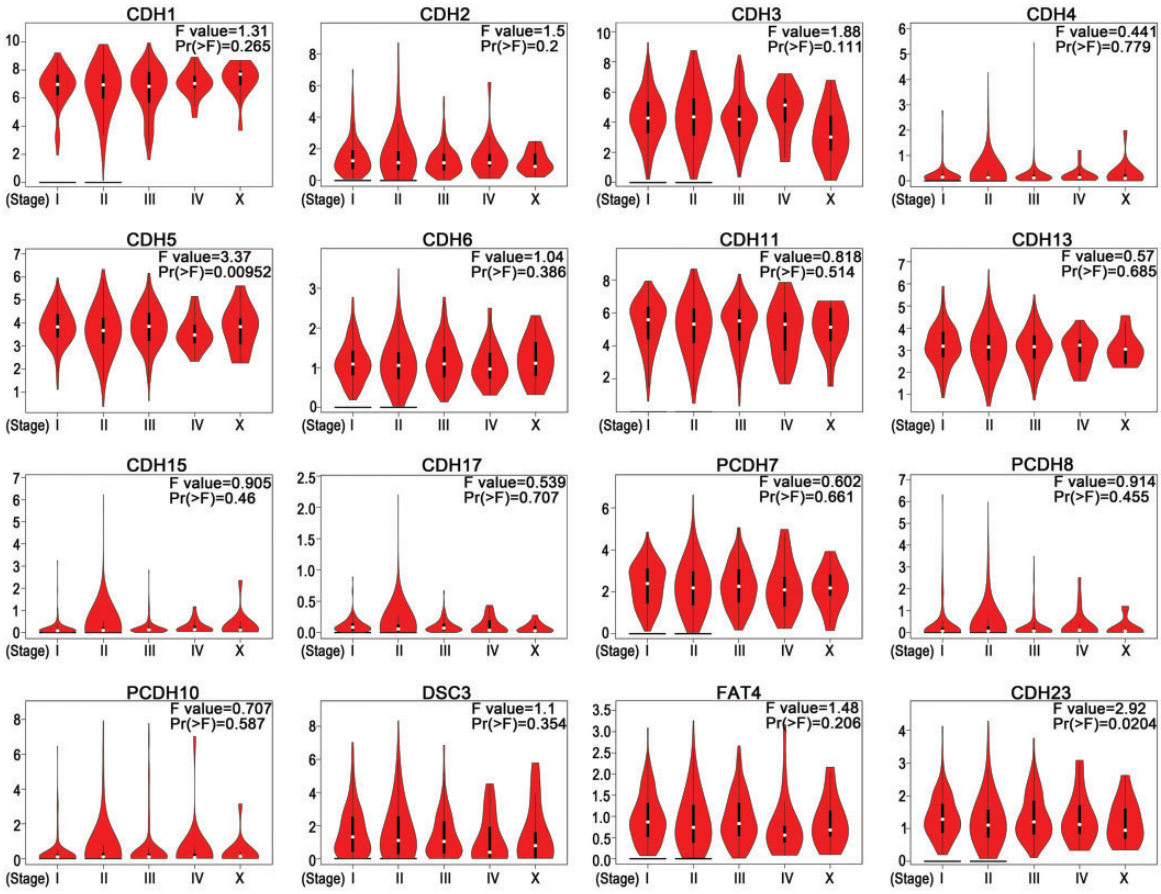


Figure 4. Relationship between the BC-related cadherins mRNA levels and tumor stages (GEPIA). (A color version of this figure is available in the online journal.)

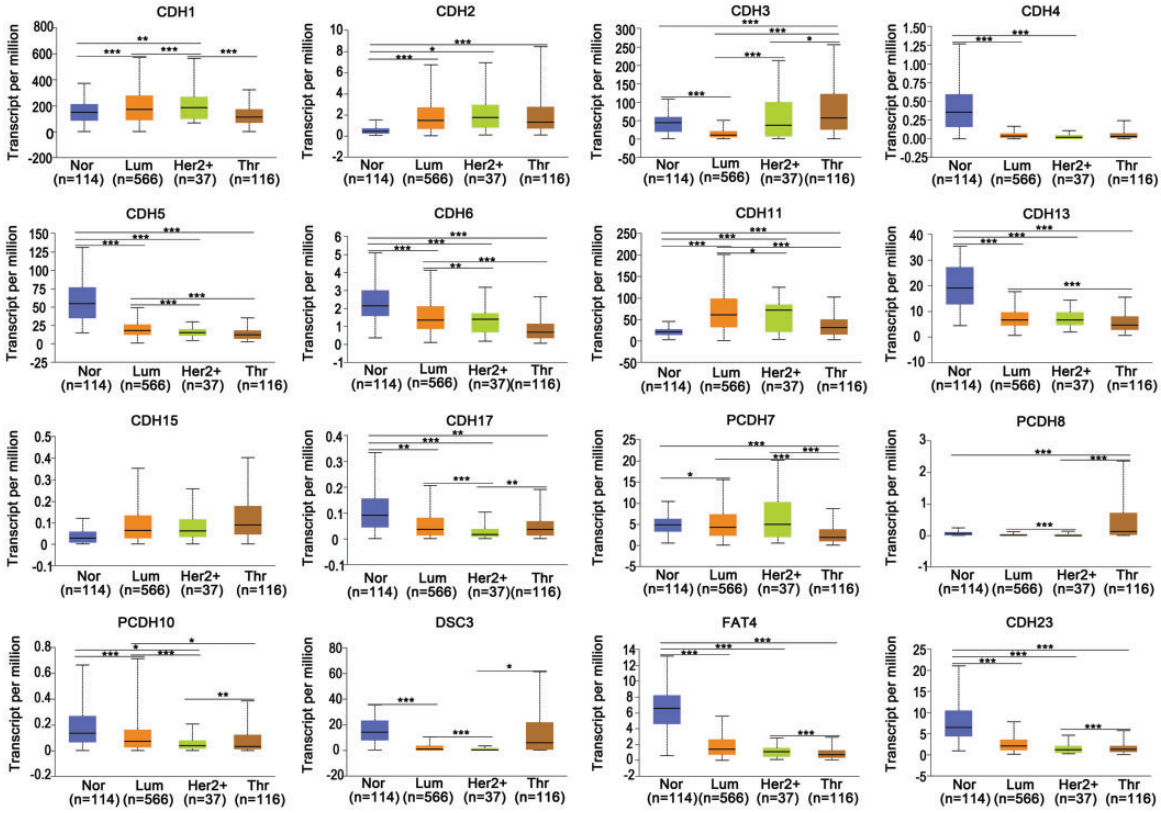


Figure 5. Relationship between BC-associated cadherins mRNA levels and tumor subclasses (UALCAN). (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$). (A color version of this figure is available in the online journal.)

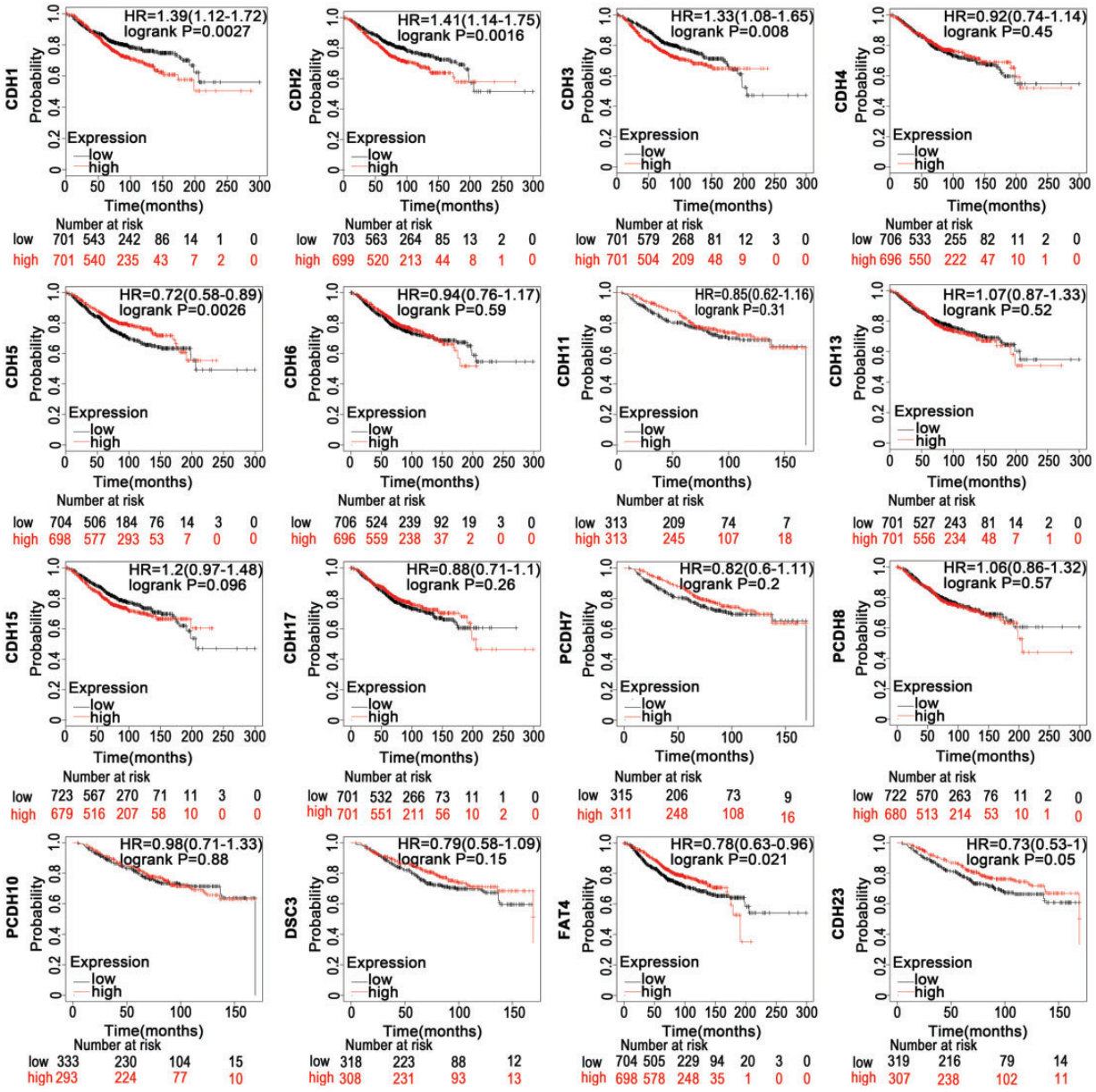


Figure 6. OS (overall survival) of the BC-related cadherins (KM-Plotter). (A color version of this figure is available in the online journal.)

CDH3, known as placental cadherin (P-cadherin), has been shown to augment invasion and aggression of tumor cells. Some studies have shown that CDH3 is overexpressed in TNBC to an extent of over 50%, which is markedly correlated with poor OS,[17,18] and associated with the promotion of key cancer features like augmented cell migration, invasion, and tumorigenic and metastatic ability in BC models.[19,20] A recent study revealed a strong association between high-expressing CDH3 and BC, and an inverse correlation between high-expressing CDH3 and poor BC subtype prognosis.[21] In our study, there was lower expression of CDH3 mRNA and protein in BC tissues when compared to healthy tissues. Moreover, elevated CDH3 levels were significantly related to poor OS and RFS in BC patients. Additionally, we found marked difference between healthy and luminal/TNBC, luminal and $Her2^+$/TNBC, $Her2^+$ and TNBC cases. However, its expression was not correlated with tumor stage.

CDH4 or retinal cadherin can be found in myoblast–derived rhabdomyosarcomas but not in healthy myoblasts. It might have a metastasis-related function in BC.[22] Overexpression and knockdown studies in mammary gland cell lines validated that CDH4 inhibits malignancy. Moreover, CDH4 levels were mostly reduced in carcinomas, as opposed to healthy breast tissues,[23] which concurs with our analysis using the Oncomine database. Moreover, low CDH4 levels were strongly associated with poor RFS in BC patients; a significant difference between the normal and BC subclasses of luminal/$Her2^+$cases. However, the expression of CDH4 was not correlated with specific tumor stages.

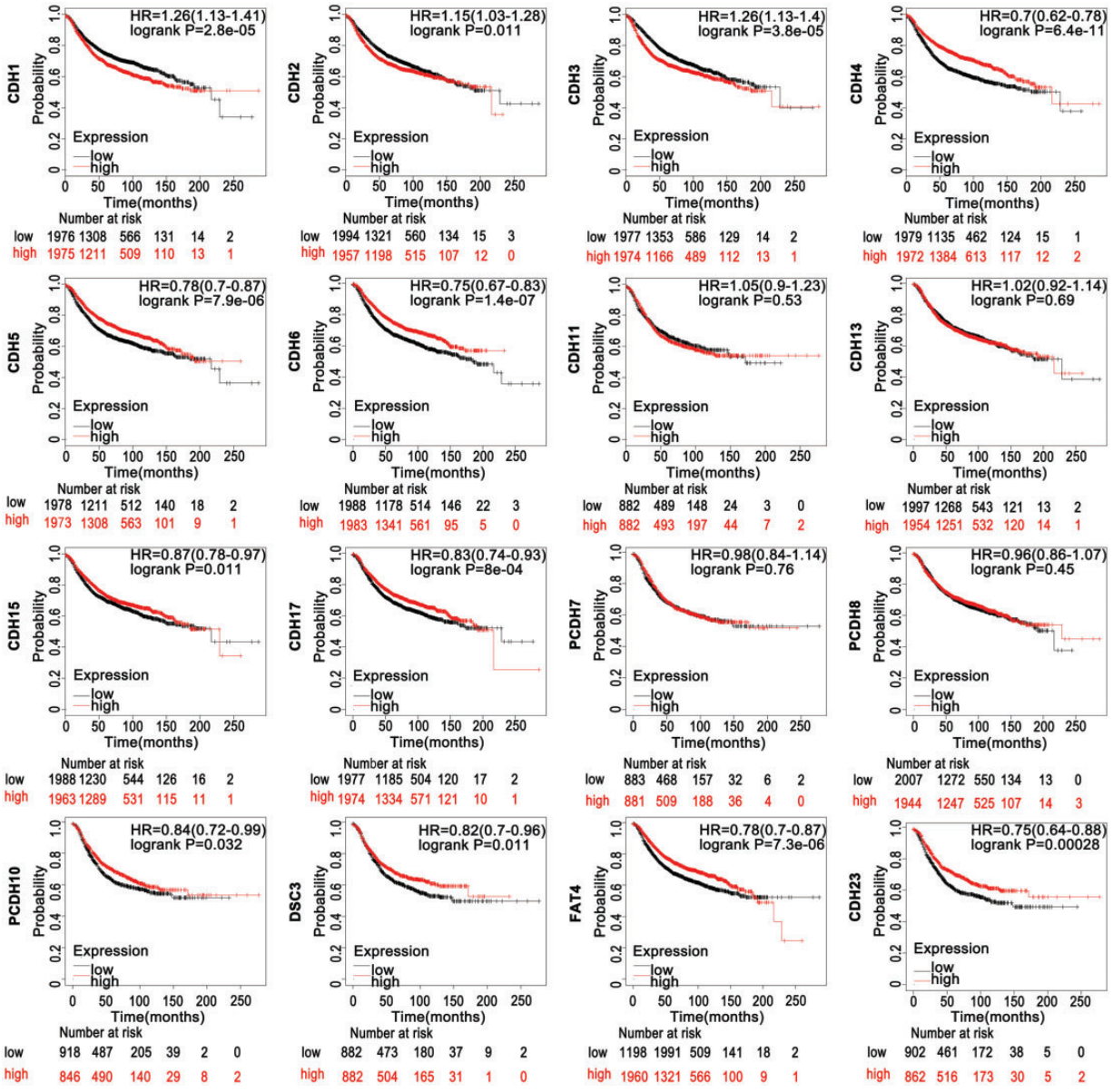
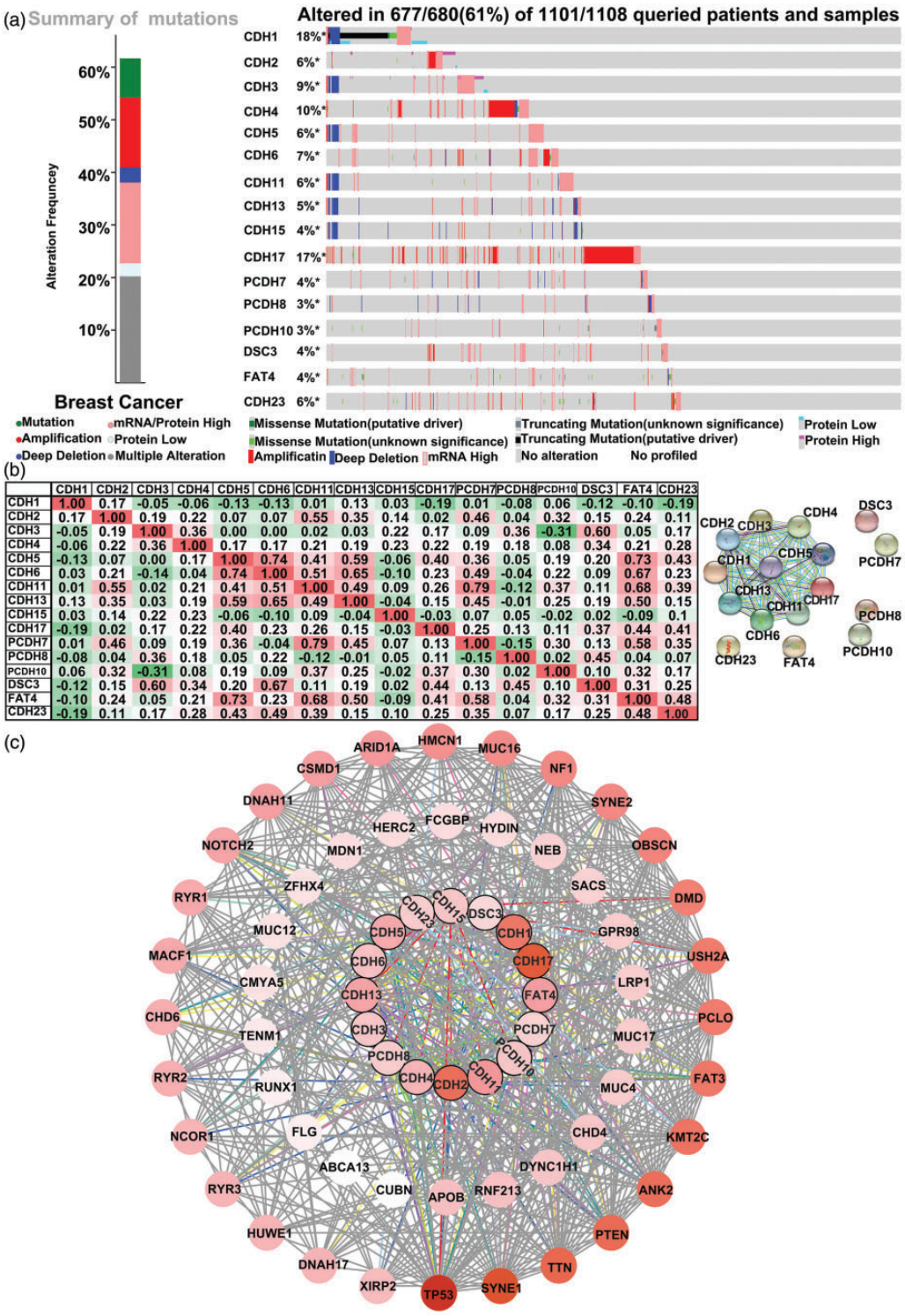


Figure 7. RFS (relapse-free survival) of the BC-related cadherins (KM-Plotter). (A color version of this figure is available in the online journal.)

CDH5 or vascular endothelial cadherin is ubiquitously expressed in endothelial cells but not found in normal epithelium. It has been identified as a biomarker of metastatic BC.[24,25] Invasive human breast tumors and a breast cancer mouse model[26] showed a rise in CDH5 levels, while in our results, CDH5 levels were substantially diminished in BC tissues, relative to healthy tissues in the Oncomine and UALCAN databases. Furthermore, we found that low CDH5 levels were strongly associated with poor OS and RFS in the BC patients with its expression being correlated with specific tumor stages. Besides, a marked difference in the expression of CDH5 was noted between the normal and BC subclasses of luminal/Her2$^+$/TNBC cases, and the luminal, and Her2$^+$/TNBC.

CDH11, colloquially known as osteoblast cadherin (OB-cadherin), is expressed exclusively in invasive BC cells and aggressive BC and facilitates cell proliferation and migration.[27–29] In our study, CDH11 showed high mRNA expression levels in the Oncomine and UALCAN databases, while in HPA, it displayed a lower protein expression level. This difference might be due to the translation, post-transcriptional and post-translational modification of CDH11. Furthermore, we found a substantial difference in CDH11 expression between the normal and BC subclasses of luminal/Her2$^+$/TNBC, and the luminal and Her2$^+$/TNBC cases. The expression of CDH11 was not correlated with any tumor stage.

CDH13, also known as truncated cadherin (T/H-cadherin), is present in numerous cell types within the breast tissue, including myoepithelial, epithelial, and endothelial cells. CDH13 expression is increased in endothelial cells during breast cancer, and the CDH13 levels are diminished in all but endothelial cells in mouse mammary tumor models.[30] CDH13 showed low mRNA expression levels



	CDH1	CDH2	CDH3	CDH4	CDH5	CDH6	CDH11	CDH13	CDH15	CDH17	PCDH7	PCDH8	PCDH10	DSC3	FAT4	CDH23
CDH1	1.00	0.17	-0.05	-0.06	-0.13	-0.13	0.01	0.13	0.03	-0.19	0.01	-0.08	0.06	-0.12	-0.10	-0.19
CDH2	0.17	1.00	0.19	0.22	0.07	0.07	0.55	0.35	0.14	0.02	0.46	0.04	0.32	0.15	0.24	0.11
CDH3	-0.05	0.19	1.00	0.36	0.00	0.00	0.02	0.03	0.22	0.17	0.09	0.36	-0.31	0.60	0.05	0.17
CDH4	-0.06	0.22	0.36	1.00	0.17	0.17	0.21	0.19	0.23	0.22	0.19	0.18	0.08	0.34	0.21	0.28
CDH5	-0.13	0.07	0.00	0.17	1.00	0.74	0.41	0.59	-0.06	0.40	0.36	0.05	0.19	0.20	0.73	0.43
CDH6	0.03	0.21	-0.14	0.04	0.74	1.00	0.51	0.65	-0.10	0.23	0.49	-0.04	0.22	0.09	0.67	0.23
CDH11	0.01	0.55	0.02	0.21	0.41	0.51	1.00	0.49	0.09	0.26	0.79	-0.12	0.37	0.11	0.68	0.39
CDH13	0.13	0.35	0.03	0.19	0.59	0.65	0.49	1.00	-0.04	0.15	0.45	-0.01	0.25	0.19	0.50	0.15
CDH15	0.03	0.14	0.22	0.23	-0.06	-0.10	0.09	-0.04	1.00	-0.03	0.07	0.05	-0.02	0.02	-0.09	0.1
CDH17	-0.19	0.02	0.17	0.22	0.40	0.23	0.26	0.15	-0.03	1.00	0.25	0.13	0.11	0.37	0.44	0.41
PCDH7	0.01	0.46	0.09	0.19	0.36	-0.04	0.79	0.45	0.07	0.13	1.00	-0.15	0.30	0.13	0.58	0.35
PCDH8	-0.08	0.04	0.36	0.18	0.05	0.22	-0.12	-0.01	0.05	0.11	-0.15	1.00	0.02	0.45	0.04	0.07
PCDH10	0.06	0.32	-0.31	0.08	0.19	0.09	0.37	0.25	-0.02	0.37	0.30	0.02	1.00	0.10	0.32	0.17
DSC3	-0.12	0.15	0.60	0.34	0.20	0.67	0.11	0.19	0.02	0.44	0.13	0.45	0.10	1.00	0.31	0.25
FAT4	-0.10	0.24	0.05	0.21	0.73	0.23	0.68	0.50	-0.09	0.41	0.58	0.04	0.32	0.31	1.00	0.48
CDH23	-0.19	0.11	0.17	0.28	0.43	0.49	0.39	0.15	0.10	0.25	0.35	0.07	0.17	0.25	0.48	1.00

Figure 8. Genetic changes, binding analyses, and neighboring gene network of the BC-related cadherins (cBioPortal). (a) Mutation analysis of CDHs in BC. (b) Protein–protein interaction network of CDHs. (c) Gene–gene interaction network of CDHs and 50 most frequently altered neighboring genes. (A color version of this figure is available in the online journal.)

in BC in the Oncomine and UALCAN databases. Moreover, CDH13 expression did not have an important relationship with the tumor phase and survival prognosis of BC patients, while a marked difference in the expression levels was seen between the normal and BC subclasses of luminal/Her2^{+}/TNBC, and the luminal and TNBC cases.

There are no signs of CDH15, often referred to as muscle cadherin (M–cadherin), in healthy breast tissue physiology, and its location on chromosome 8 is often missing in mouse breast tumors.[31] Here, we revealed that low CDH15 levels were significantly related to poor RFS in BC patients.

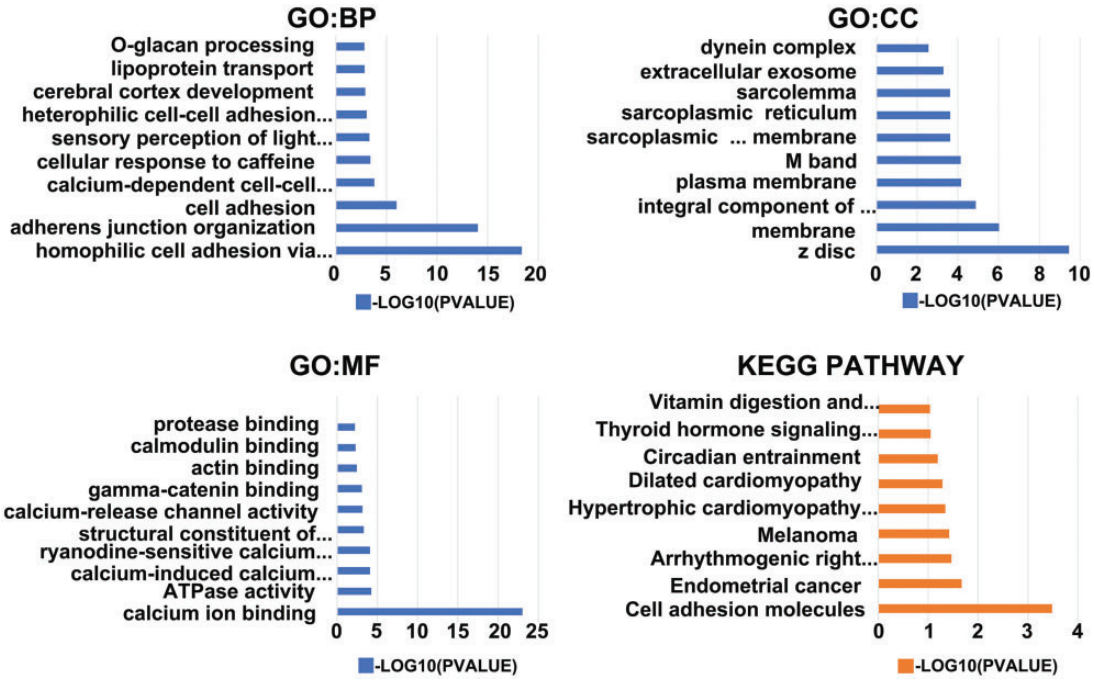


Figure 9. Enrichment analysis of the BC-related cadherins and 50 most commonly altered neighboring genes in BC. (a, b, and c) bar-plot of GO enrichment in cellular component terms, biological process terms, and molecular function terms. (d) Bar-plot of KEGG pathway. (A color version of this figure is available in the online journal.)

Summary Results of the BC-related Cadherins

Symbles	Expression			Expression & Survival		Expression & Stages	Expression & Subclasses						Potetial for therapeutic target	Prognosis biomarker
	Oncomine	ULCAN	HPA	OS	RFS		No:Lu	No:Her2	No:Thr	Lu:Her2	Lu:Thr	Her2:Thr		
CDH1	↑/↓	↑	↑	**	***	-	***	**	-	***	-	***	Yes	No
CDH2	↑	↑	-	**	*	-	***	*	***	-	-	-	Yes	No
CDH3	↓	↓	↓	**	***	-	***	-	***	***	***	*	Yes	No
CDH4	↓	↓	-		***	-	***	***	-	-	-	-	No	Yes
CDH5	↓	↓	-	**	***	**	***	***	***	***	***	-	No	Yes
CDH6	-	↓	-	-	***	-	***	***	***	**	***	-	No	Yes
CDH11	↑	↑	↓	-	-	-	***	***	***	*	***	-	No	No
CDH13	↑/↓	↓	-	-	-	-	***	***	***	-	***	-	No	No
CDH15	-	-	-	-	*	-	-	-	-	-	-	-	No	Yes
CDH17	-	↓	-	-	***	-	***	***	***	***	-	**	No	Yes
PCDH7	↑	-	-	-	-	-	*	-	***	-	***	**	No	No
PCDH8	↓	↓	↓	-	-	-	-	-	***	***	-	***	No	No
PCDH10	↓	↓	↓	-	*	-	***	*	-	***	*	**	No	Yes
DSC3	↓	↓	-	-	*	-	***	-	-	***	-	*	No	Yes
FAT4	↑/↓	↓	-	*	***	-	***	***	***	-	-	***	No	Yes
CDH23	↑/↓	↓	-	-	***	*	***	***	***	-	-	***	No	Yes

-,No significant difference; *,P<0.05; **,P<0.01; ***,P<0.001

No,Normal; Her2, Her2 positive; Lu, Luminal; Thr, Three negative

Figure 10. Summary of the relationship between BC-related cadherins and BC. (A color version of this figure is available in the online journal.)

Recent reports have shown that CDH6 or K-cadherin and CDH17 or liver-intestine cadherin (LI-cadherin) regulate cancer stem cells (CSCs) maintenance in TNBC.[32] In the UALCAN database, both were shown to have a lower expression in BC.

Proto-cadherins

PCDH7 levels are significantly elevated in BC models.[33] Moreover, its mRNA and protein levels were reported to be exceptionally high in primary BC patients with bone metastases, relative to BC patients without bone metastases.[34] Our study indicates that PCDH7 is strongly upregulated in BC tissues, compared to healthy controls as per the Oncomine database. Also, a marked difference in the expression profile of PCDH7 was seen between the normal and BC subclasses of luminal/TNBC, luminal/Her2$^+$, and TNBC.

PCDH8 mediates BC-originated brain metastasis. When PCDH8 was suppressed *in vitro*, BC cell growth, migration, and invasion were inhibited, whereas its overexpression produced bone metastasis *in vivo*.[35] PCDH8 showed low transcript and protein levels in BC, and there was a significant difference between the normal and the TNBC, Her2$^+$, and the luminal/TNBC cases.

For PCDH10, the expression reduced to 75% in BC cells owing to promoter methylation.[36] Similar to PCDH8, PCDH10 also showed low mRNA expression levels and protein expression levels in BC. Reduced PCDH10 levels were markedly associated with poor RFS and a significant difference in the expression between the normal and the BC subclasses of luminal/Her2$^+$/TBNC, Her2$^+$ and luminal/TNBC was observed.

Desmosomal cadherins

DSC3 is a desmosomal cadherin can be found in healthy breast cells but is strongly upregulated in tumors and tumor cell lines, with 72% and 79% repression, respectively.[37] A lower transcript level was seen for DSC3 in BC cases. A higher expression of DSC3 was strongly correlated with worse RFS in all BC patients who underwent follow-up lasting 200 months, and there was marked difference between healthy and luminal BC,TNBC, and luminal/Her2$^+$ cases.

Cadherin-related proteins

FAT4, Atypical cadherin 4, has been identified as a candidate tumor suppressor gene in BC, wherein its expression is lost.[38,39] Our results show that low mRNA expression levels in BC and a low expression level of FAT4 were significantly associated with worse OS and RFS in all patients with BC who were followed up for 200 months. There was a significant difference between the normal and three main BC subclasses, TNBC, and Her2$^+$ cases.

CDH23, also called CDHR23 (cadherin−related 23), is significantly elevated in both cancer and stromal cells, suggesting a strong role in breast cancer.[40] We demonstrated downregulation of CDH23 in BC, which was strongly correlated with worse RFS in BC. There was a significant difference between the normal and three main BC subclasses, TNBC, and Her2$^+$ cases.

It is generally known that clinical drug therapy for BC is mainly divided into chemical drug therapy, endocrine therapy (hormone therapy), and targeted therapy. There are distinct differences in the employment of therapeutic drugs for specific types of BC. While endocrine therapy has been found to be suitable for patients with hormone receptor (PR/ER) positive BC, targeted therapy is often deemed suitable for patients with Her2$^+$BC. In Figure 5, we show the expression of CDH3 in luminal and Her2$^+$ BC tissue was significantly low and none respectively when compared with normal breast tissues. However, its expression was significantly high in the luminal, Her2$^+$, and TNBC cases. The expression of multiple cadherins in different types of BC was similar, relative to healthy breast tissues. Moreover, no differential expression of CDH1/4 and PCDH10 was observed in the TNBC, no differential expression of PCDH7 in Her2$^+$, significantly high levels of PCDH8 in TNBC, significantly low expression of DSC3 in luminal, and the unchanged expression in the two unmentioned types. Therefore, the combination of these cadherins' expression conditions could predict the type of BC and help the diagnosis and treatment of BC.

Our work had certain limitations. Firstly, despite high mRNA levels of CDH1/2/3 and low mRNA levels of CDH5/FAT4 were stand-alone prognostic bio-markers for shorter OS in BC patients, future investigations encompassing larger sample sizes warranted to confirm the conclusions from this study and to explore potential of BC-related cadherins clinical application in treating BC. Secondly, the diagnostic and therapeutic outcomes of BC-associated cadherins were not examined in this paper. Thus, future studies are necessary to explore if BC-related cadherins can serve as diagnostic bio-markers or therapeutic targets. Finally, the underlying mechanisms of BC-related cadherins were not examined here. This investigation should be performed in detail to delineate the relationship between cadherins and BC.

In summary, we systematically analyzed the expression and prognostic potential of BC-related cadherins (Figure 10). Based on our data, elevated levels of CDH1 in BC tissues may contribute to BC oncogenesis. The mRNA expression levels of CDH5/23 could serve as molecular markers of BC patients in different stages, and CDH1/3/5/6/11/17, PCDH7/8/10, and DSC3 could improve the differential diagnosis of BC subclasses. Our work suggest that CDH1/2/3 can serve as a possible therapeutic target for BC, and transcriptional levels of CDH4-6/15/17/23, PCDH10, DSC3, and FAT4 can be prospective prognostic bio-markers for the detection and prognosis of BC.

AUTHORS' CONTRIBUTIONS

All authors participated in the planning and execution of experiments, analysis of the data, and preparation of the manuscript. MFX, conceptualized, planned, and conducted experiments; CYL, compiled the manuscript; SSD, was involved in critical manuscript revision; LLP, JRL, JJL, QWN and XL analyzed the data.

DECLARATION OF CONFLICTING INTERESTS

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