Original Article

Systematic proteomics analysis revealed different expression of laminin interaction proteins in breast cancer: lower in luminal subtype and higher in claudin-low subtype

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Background: Breast cancer is a major public health concern. Proteomics enables identification of proteins with aberrant properties. Here, we identified proteins with abnormal expression levels in breast cancer tissues and systematically analyzed and validated the data to locate potential diagnostic and therapeutic targets.

Methods: Protein expression level in breast cancer tissues and para-carcinoma tissues were detected by Isobaric Tags for Relative and Absolute Quantification (iTRAQ) technology and further screened through Gene Expression Profiling Interactive Analysis (GEPIA) database. Cellular components, protein domain and Reactome pathway analysis were performed to screen functional targets. Abnormal expression levels of functional targets were validated by Oncomine database, quantitative real time polymerase chain reaction (qRT-PCR) and proteomics detection. Protein correlation analysis was performed to explain the abnormal expression levels of potential targets in breast cancer.

Results: Overall, 207 and 207 proteins were up- and down-regulated, respectively, in breast cancer tissues, and approximately 50% were also detected in the GEPIA database. The overlapping proteins were mainly extracellular proteins containing epidermal growth factor-like domain in leukocyte adhesion molecule (EGF-Lam) domain and enriched in laminin interaction pathway. Moreover, the downregulated laminin interaction proteins could be functional targets, which were also validated through Oncomine-Richardson and Oncomine-Curtis database. However, the lower expression level of laminin interaction proteins only fit for luminal breast cancer cells with no or low metastasis ability because the proteins achieved higher expression level in more invasive claudin-low breast cancer cells. In addition, when compared with corresponding *in situ* carcinoma tissues, above-mentioned proteins also showed higher expression levels in invasive carcinoma tissues. Finally, we have revealed the negative correlation between the laminin interaction proteins and the claudins.

Conclusions: The laminin interaction protein, especially for laminins with β1 and γ1 subunits and their integrin receptors with α1 and α6 subunits, showed lower expression levels in luminal breast cancer with no or lower metastatic ability, but showed higher expression levels in claudin-low breast cancer with higher

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metastatic ability; and their higher expression could be related to the low claudin expression.

Keywords: Breast cancer; integrin receptors; laminins; proteomics

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Introduction

Breast cancer in women is a major concern across the world, and it is associated with high incidence rate and early onset age (1,2). In addition to BRCA1, BRCA2, and TP53 (3-5), many classical biomarkers exist (6,7); nevertheless, identifying new effective biomarkers and targets remains pivotal. With the emergence of proteomics, systematically investigating protein expression (8,9), modification (10-12), and classification (13,14) has become possible in breast cancer. Although more potential biomarkers and targets can be identified using this approach, such a complex method is bound to generate some inaccurate results. Thus, it is important to discriminate between accurate and inaccurate data. Several cancer-related databases are available, providing comprehensive information pertaining to potential targets. For example, Yuan *et al.* explored the expression pattern and prognostic value of FUNDC1 in pan-cancer across multiple databases, including Oncomine, PrognoScan, Gene Expression Profiling Interactive Analysis (GEPIA), and Kaplan-Meier Plotter (15). Further, Feng *et al.* identified significant genes with poor prognosis in ovarian cancer via bioinformatical analysis involving databases such as Gene Expression Omnibus, Database for Annotation, Visualization and Integration Discovery and GEPIA (16). Both proteomics and multi-database analysis can thus lead to the identification of potential cancer targets, but most previous studies involved only one of the two approaches; Thus, combining proteomics with multi-database analysis to identify valid cancer targets is accordingly desirable.

Breast cancer is a type of heterogeneous tumor (17,18), and depending on the expression level of the estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), and Ki-67, it can be classified into luminal A ($HER2^-$, ER^+ and PR^+), luminal B ($HER2^-$, ER^+ or $HER2^+$, ER^+), $HER2^+$ overexpression ($HER2^+$, ER^- and PR^-), and triple-negative types ($HER2^-$, ER^- and PR^-) (19,20). According to histological classification, breast cancer can be generally classified into ductal carcinoma and lobular carcinoma (21). Hence, precise investigation for different breast cancer subtype is important.

Herein we assessed protein expression levels in breast cancer using the isobaric tags for relative and absolute quantification (iTRAQ) method and validated our results with muti-database analysis and biological experiments. We revealed laminins and their receptors as repeated targets for breast cancer, which showed lower expression levels in luminal breast cancer with no or low metastatic ability but showed higher expression levels in claudin-low breast cancer. In addition, we revealed negative correlation between laminins, integrins and claudins. Overall, we observed that laminins and their integrin receptors showed dynamic alterations in expression levels during breast initiation and development, which can facilitate the identification of new, effective biological markers and targets for different breast cancer types. We present this article in accordance with the MDAR reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-2214/rc).

Highlight box

Key findings

- Laminins and their integrin receptors showed lower expression levels in luminal breast cancer with no or lower metastatic ability but showed higher expression levels in claudin-low breast cancer with higher metastatic ability, their higher expression could be related to the low claudin expression in breast cancer.

What is known and what is new?

- Breast cancer is a kind of highly heterogeneous cancer, so it is very important to find targets according to its classification.
- We revealed lower and higher expression levels of laminins and their integrin receptors in luminal and claudin-low breast cancer respectively, which could be potential targets during tumor initiation and development.

What is the implication, and what should change now?

- This study facilitates the identification of new, effective biological markers and targets for different breast cancer types.

Methods

Sample preparation

Three pairs of invasive ductal breast carcinoma and para-carcinoma tissues (hereafter referred to as "normal tissues") were obtained from patients at the Second Affiliated Hospital of Qiqihar Medical University (Qiqihar, China) from January 2018 to December of 2018. All patients were diagnosed with breast cancer but had not undergone chemotherapy nor radiotherapy previously. The samples were stored and transported in liquid nitrogen immediately after the operation. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The experimental protocol was approved by the Qiqihar Medical Ethics Committee (approval No. [2021]25), and informed consent was obtained from all patients.

Cell culture

MCF-10A (SCSP-575), MCF-7 (SCSP-531), ZR-751 (TCHu126), BT-549 (TCHu93), and MDA-MB-231 (TCHu227) cells were purchased from the Cell Bank of Chinese Academy of Science. All cell lines were authenticated by short tandem repeat analysis, and also tested for morphology and mycoplasma contamination. MCF-10A cells were cultured using the MEGM kit (Lonza/Clonetics) in the presence of 100 ng/mL cholera toxin (Sigma, Darmstadt, Germany). MCF7 cells were cultured in MEM (HyClone, Logan, Utah, USA) supplemented with 10% fetal bovine serum (FBS, HyClone). ZR-751 and BT-549 cells were cultured in RPMI 1640 (HyClone) with 10% and 20% FBS, respectively, and in the presence of 2.5 g/L glucose (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) and 0.11 g/L sodium pyruvate. BT-549 cells also required 0.023 IU/mL insulin (Sigma-Aldrich; Merck KGaA). All cells were cultured at 37 ℃ and in a humidified atmosphere of 5% CO_2. MDA-MB-231 cells were cultured in L15 (HyClone) supplemented with 10% FBS at 37 ℃ in a humidified atmosphere without CO_2. All the cells were cloned less than 30 generations.

Quantitative protein analysis

The protocols pertaining to protein extraction and digestion, tandem mass tag labeling, peptide separation, MS/MS analysis, database search, and proteomics data validation were as previously reported (22).

GEPIA database analysis

The proteins that were up- (fold change ≥1.5) and downregulated (fold change ≤1/1.5) in our quantification results and in GEPIA database (http://gepia.cancer-pku.cn/) (23) were analyzed using Venn analysis in FunRich v3.1.3 (24). Overlapping proteins with differential expression levels were chosen for further analyses. RGUI v3.4.2 was used to create a heat map of expression levels of these proteins in normal and breast cancer tissues (25). The correlation between laminins, integrins and claudins were also analyzed by GEPIA database.

Analyzing components, protein domains, and pathways

The components, protein domains, and pathways of target proteins were analyzed using FunRich v3.1.3 (24) based on the information retrieved from the UniProt database (https://www.uniprot.org/) (26). Entries with a corrected P value of <0.05 were considered to indicate a statistically significant difference.

Oncomine database analysis

The gene expression levels of laminin interaction proteins in normal and breast cancer tissues were analyzed using the Oncomine-Richardson (27) and Oncomine-Curtis database (28) and those of laminins and their integrin receptors in invasive ductal breast carcinoma and corresponding *in situ* tissues were analyzed using the Oncomine-Schuetz database (29).

Detection of mRNA expression levels by quantitative real time polymerase chain reaction (qRT-PCR)

Approximately 1×10^6 cells were collected and treated with 1 mL TRIzol, followed by total RNA isolation. The extracted RNA was then converted into cDNA. mRNA expression was determined using TaKaRa TB Green Premix Ex Taq, as per manufacturer instructions. The reaction mixture comprised 0.25 U DNA polymerase, 150 μg cDNA, 0.2 μM forward and reverse primer each, and 1× PCR buffer; ddH_2O was used to adjust the final volume to 10 μL. The cycling conditions were as follows: 95 ℃ for 30 s, 40 cycles of 95 ℃ for 5 s and 60 ℃ for 30 s, and finally 95 ℃ for 30 s. The temperature was then increased from 65 ℃ to 95 ℃ at a rate of 0.5 ℃/s for melting curve analysis. The experiments were replicated

Table 1 Forwards and reverse primer sequences used in quantitative real time polymerase chain reaction experiments

Primer	Sequence (5'-3')
ITGA1	
Forward	CTCTGCTACTGCTTCTTCTG
Reverse	CTGTTCTCCACTGAGCGTCT
ITGA6	
Forward	TGGGCTATCCTCAAGAGTTCAGTT
Reverse	GTTATGGGAATGGGACGCAG
LAMA2	
Forward	CTTGAATCCGCTGTCTCCTAT
Reverse	CACAGATGCCACCAAAAATAGT
LAMA4	
Forward	GCGCTCGGTTCTGCCTCTGT
Reverse	TCGTCTCAGGCGGGTCTTGC
LAMB1	
Forward	GAGTGCCTGAAGGGGCTTATTTG
Reverse	TTGTCATCATCGGGGATGGTATTA
LAMB2	
Forward	CTGGGATGATGTAGTCTGTGAG
Reverse	ACAGGTTCTGAATCCGTGAG
LAMC1	
Forward	CTCTTAATCGCCTGAACACT
Reverse	TCGTTCTTCATACACTCGC

three times. *Table 1* lists the primers used for qRT-PCR.

Statistical analysis

Enrichment analysis was performed by Bonferroni Method and Benjamini-Hochberg method (BH method). The statistical difference between two groups was performed using student's *t* test, the data were replicated through three times. The significance level was set at *P≤0.05, **P≤0.01 and ***P≤0.001. Error bars denoted the standard deviation.

Results

Screening proteins with aberrant expression levels in breast cancer tissues

Overall, 3,792 proteins were identified, of which 3,753 were quantified from human breast cancer and corresponding normal tissues. When the quantification ratio was set at 1.5, 207 proteins were found to be upregulated and 207 proteins were downregulated (22). For further validation, we compared our results with those from the GEPIA database. When the fold-change cut-off was set at ≥1.5 or ≤1/1.5, 44% (92/207) of up- and 58% (120/207) of downregulated proteins were also detected in the database (*Figure 1A*,*1B*). The indicated common proteins and their differential expression levels in invasive ductal breast carcinoma and normal tissues are shown in Tables S1,S2 and *Figure 1C*,*1D*, they were selected for further analyses.

Proteins with aberrant expression levels were enriched in the laminin interaction pathway

Our cellular component analyses revealed that the overlapping proteins were significantly enriched in extracellular components, such as extracellular exosome, extracellular matrix, focal adhesion, extracellular region, and extracellular space (*Figure 2A*,*2B*). To investigate the function of these extracellular proteins, we further analyzed them. Protein domain analysis indicated that most of them contained the epidermal growth factor-like domain in leukocyte adhesion molecule (EGF-Lam) domain (*Figure 2C*), and pathway analysis showed that they were significantly enriched in the laminin interaction pathway (*Figure 2D*). In addition, all proteins with the EGF-Lam) domain were observed to participate in the laminin interaction pathway (*Figure 2E*). These findings indicated that proteins involved in laminin interaction may be reliable potential targets.

Lower expression levels of laminin interaction-related proteins in breast cancer tissues

Laminin interaction usually involves the combination between heterotrimeric laminin (also referred to laminins) and their receptors on cell membrane and often affects the migration, invasion, and self-renewal of tumor cells (30-34). Heterotrimeric laminin contains one α subunit, one β subunit and one γ subunit, which are encoded by the genes named *laminin A*, *laminin B* and *laminin C* respectively (35). Thus, they are usually named according to their subunit make-up. For example, laminin-211 is just composed of subunit α2, β1 and γ1, which is encoded by gene named laminin subunit α2 (*LAMA2*), laminin subunit β1 (*LAMB1*) and laminin subunit γ1 (*LAMC1*) respectively (36). In our

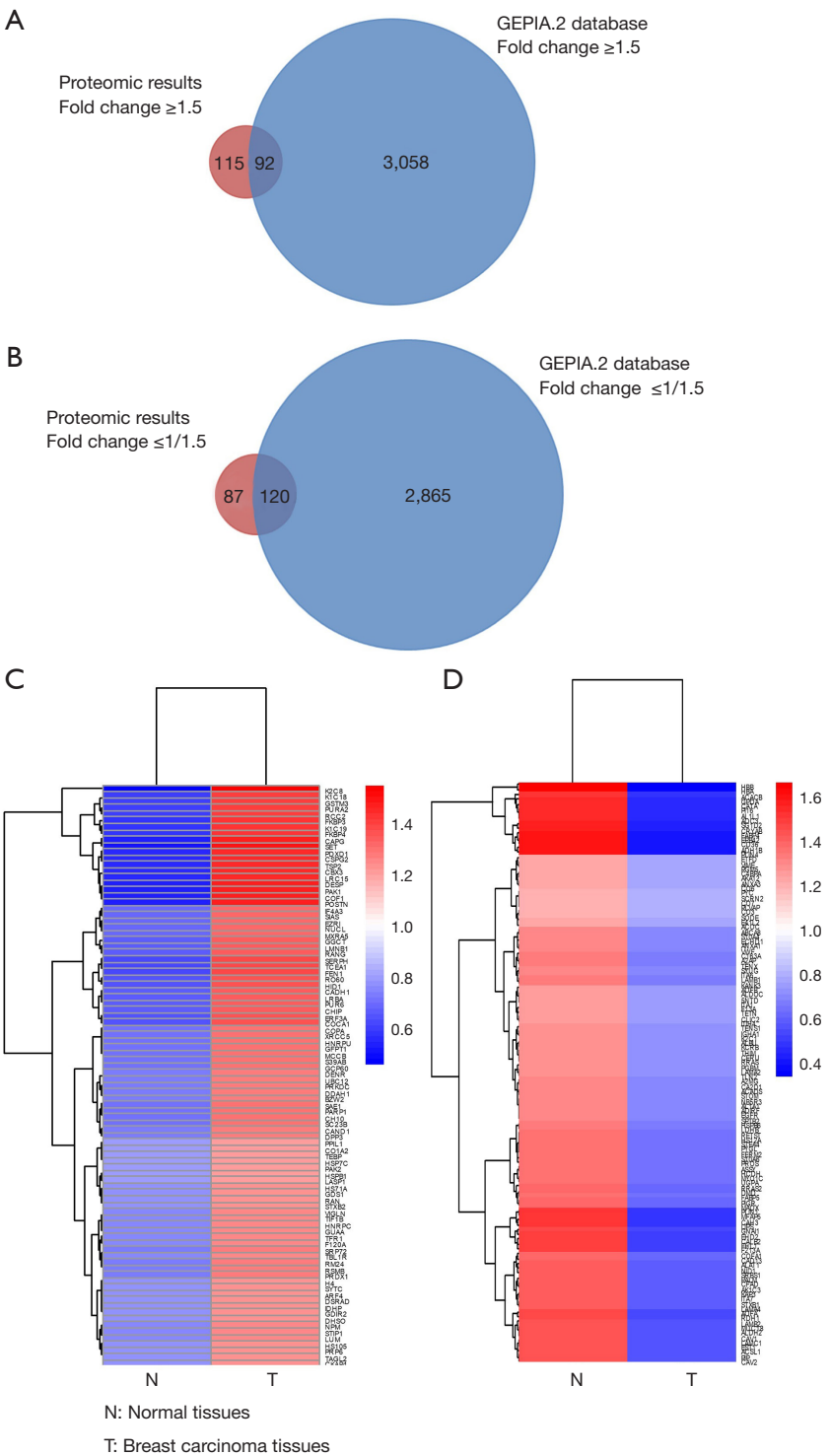

Figure 1 Proteomics and GEPIA database analysis for differentially expressed proteins between breast cancer and normal tissues. (A) Venn diagrams for upregulated proteins (fold change ≥1.5) in proteomics and GEPIA database; (B) Venn diagrams for downregulated proteins (fold change ≤1/1.5) in proteomics and GEPIA database; (C) expression information of upregulated proteins in breast cancer and normal tissues; (D) expression information of downregulated proteins in breast cancer and normal tissues. Specific proteins in (C,D) are listed in Tables S1,S2. GEPIA, Gene Expression Profiling Interactive Analysis.

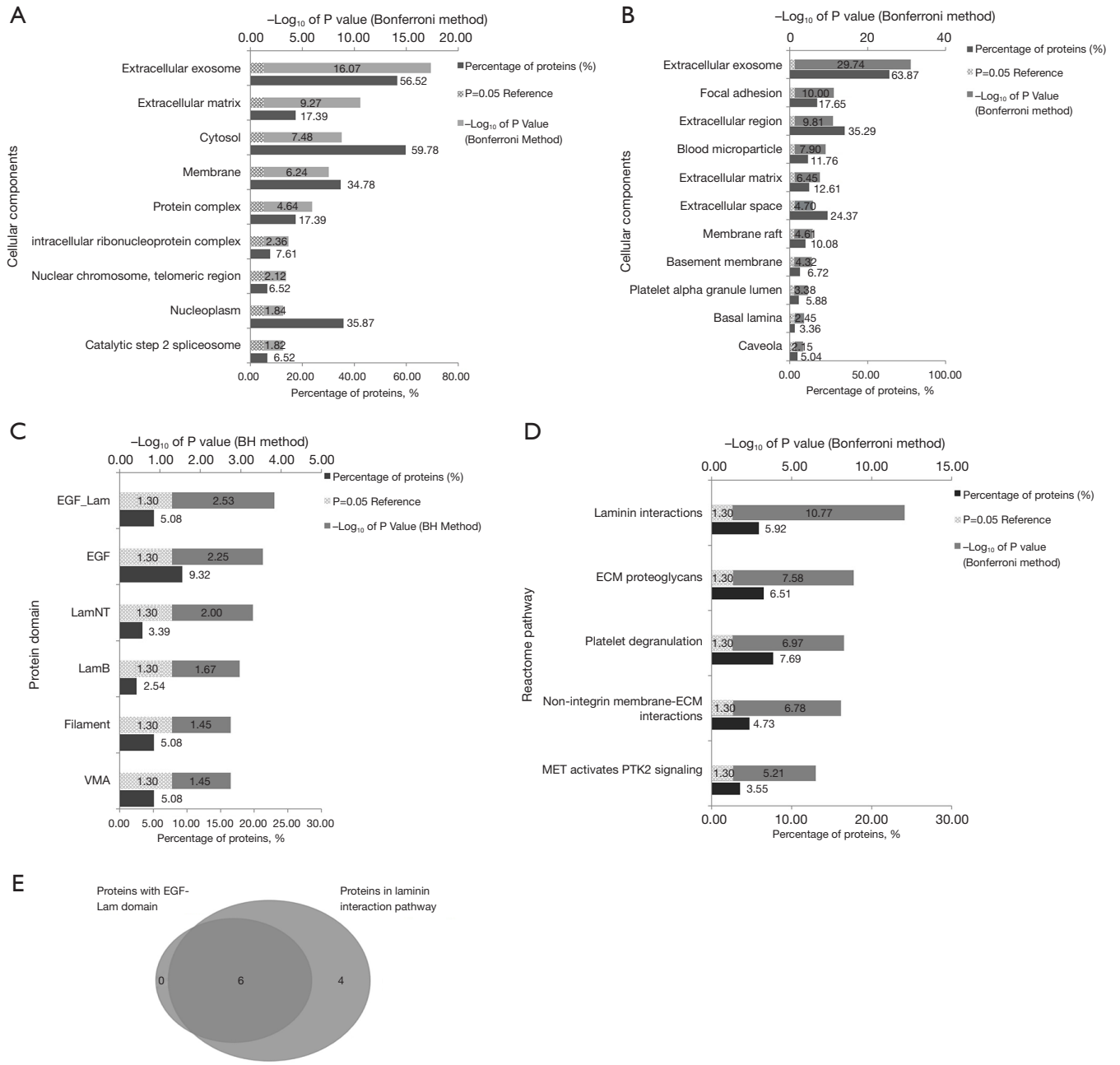

Figure 2 Bioinformatics analysis of proteins with differential expression level in breast cancer and normal tissues. (A) Cellular component analysis of upregulated targets, P≤0.05 entries are listed; (B) cellular component analysis of downregulated targets, P≤0.05 entries are listed; (C) protein domain analysis of extracellular proteins in (A,B), P≤0.05 entries are listed; (D) Reactome pathway analysis of extracellular proteins in (A,B), P≤0.05 entries are listed; (E) Venn diagram of proteins with the EGF-Lam domain and in the laminin interaction pathway. BH method, Benjamini-Hochberg method; EGF-Lam, epidermal growth factor-like domain in leukocyte adhesion molecule; EGF, epidermal growth factor; LamNT, N-terminal domain of laminins; LamB, B domain of laminins; VWA, von Willebrand factor A domain; ECM, extracellular matrix; MET, metastasis-associated protein; PTK2, protein tyrosine kinase 2.

proteomics results, the abnormal expressed proteins in laminin interaction pathway involved laminin subunit β2 (LAMB2), LAMC1, laminin subunit α4 (LAMA4), LAMB1, LAMA2 and their receptors including integrin α7 (ITGA7), integrin α6 (ITGA6), integrin α1 (ITGA1), nidogen-1 (NID1) and basement membrane-specific heparan sulfate proteoglycan core protein (PGBM). In addition, all the above proteins were downregulated in breast cancer tissues when compared with normal tissues (*Figure 3A*,*3B*). To validate our results, we analyzed their gene expression levels using the Oncomine database. In Oncomine-Curtis Breast database (27), which includes data more than 2,000 samples, the results showed that genes encoding above 10 proteins were downregulated in ductal breast cancer tissues (*Figure 3C*). Moreover, Oncomine-Richardson database (28) also suggested that besides *ITGA1*, all the other nine genes showed significant lower expression level in invasive ductal breast cancer tissues (*Figure 3D*). Above results revealed lower expression levels of laminin interaction-related proteins in breast cancer tissues.

Lower expression levels of laminin interaction-related proteins occurred in luminal but not claudin-low breast cancer cells

To further validate above results acquired from tissue samples, we detected the mRNA expression levels of above laminin interaction-related proteins in different breast cancer cell types. Because laminin subunits and integrin subunits occupied with most of the above ten proteins, thus we chose the proteins above for further validation. Except for *LAMA2* and *ITGA7*, of which the cycle threshold value were more than 35 cycles, we got the data of the other six genes including *LAMB2*, *LAMA4*, *LAMC1*, *LAMB1*, *TIGA1* and *ITGA6*. The results indicated that when compared with MCF-10A cells, the six genes showed significant lower expression levels in non-metastatic luminal MCF-7 cells; five of them (*LAMB2*, *LAMC1*, *LAMB1*, *TIGA1* and *ITGA6*) showed significant lower expression levels in lower-metastatic luminal ZR-751 cells; only two of them (*ITGA1* and *ITGA6*) showed significant low expression levels in the more invasive claudin-low BT-549 cells (*Figure 4A*). The results suggested that the lower expression level of laminins and their integrin receptors seems fit to the luminal breast cancer cells with no or lower invasive potential. To validate our hypotheses, we detected the mRNA expression level of above-mentioned genes in MDA-MB-231 cells, which is also claudin-low type and shows strong metastatic ability. As expected, all the six genes showed conversely higher expression levels in MDA-MB-231 cells (*Figure 4B*). In addition to mRNA expression level, we detected by proteomics techniques the significantly lowered protein expression levels of laminins and their integrin receptors in luminal less metastatic breast cancer cell lines including T47D and ZR-75-1, and their apparently higher expression levels in the more invasive claudin-low MDA-MB-231, MDA-MB-436 and BT-549 cells (*Figure 4C*,*4D*). Moreover, due to the Oncomine-Schuetz database (21), the expression level of above proteins in invasive ductal tissues was higher than that of corresponding *in situ* tissues (*Figure 4E*). All the above results indicated that the laminins and their integrin receptors may show lower expression during the initiation of breast cancer and achieve higher expression during tumor development.

Higher laminins and integrin receptors expression could be correlated with low-claudin expression in breast cancer

Basing on above results, we observed higher expression level of laminins and their integrin receptors in the more invasive breast cancer cells including BT-549, MDA-MB-436 and MDA-MB-231, which belong to triple-negative and claudin-low (claudin-3, claudin-4 and claudin-7 low) breast cancer cells (37-39). To investigate whether laminins and their integrin receptors have something to do with these factors involved in classification, including ER, PR, HER2, claudin-3, claudin-4 and claudin-7, we analyzed the gene expression correlation among them. In our results, neither laminins nor integrins showed the regular relationship with *ER*, *PR* and *HER2* (data not shown). However, except for the higher P value between genes encoding ITGA6 and claudin-4, all the genes encoding laminin subunits (LAMA2, LAMA4, LAMB1 and LAMC1) and integrin subunits (ITGA1 and ITGA6) showed significant negative correlation with that encoding claudin-3, claudin-4 and claudin-7 (*Figure 5A-5F*). These results indicate that higher laminins and integrins expression could be correlated with low claudin expression.

Discussion

At present, proteomics is being widely used for elucidating protein expression, protein modification (22,40) and tumor classification (41,42) for breast cancer. Although proteomics-based methods are well established, their reproducibility is evidently a major challenge for the differences among

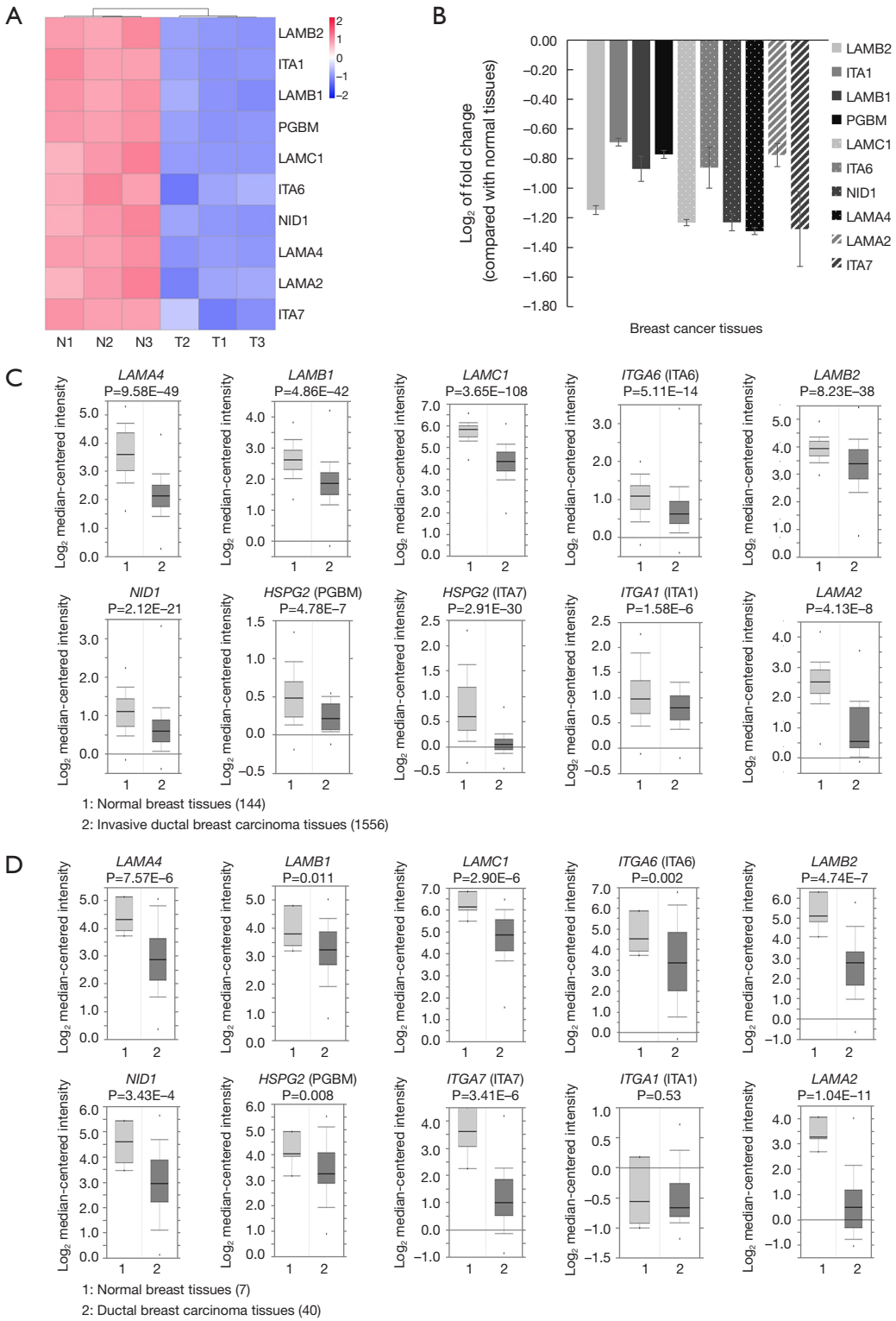

Figure 3 Expression levels of laminin interaction proteins in breast cancer tissues. (A) Protein expression levels of laminin subunits and their receptors in normal (N) and breast cancer tissues (T) assayed by proteomics detection; (B) quantification of (A); (C) expression levels of laminin subunits and their receptors in normal and breast cancer tissues using the Oncomine-Curtis database; (D) expression levels of laminin subunits and their receptors in normal and breast cancer tissues using the Oncomine-Richardson database.

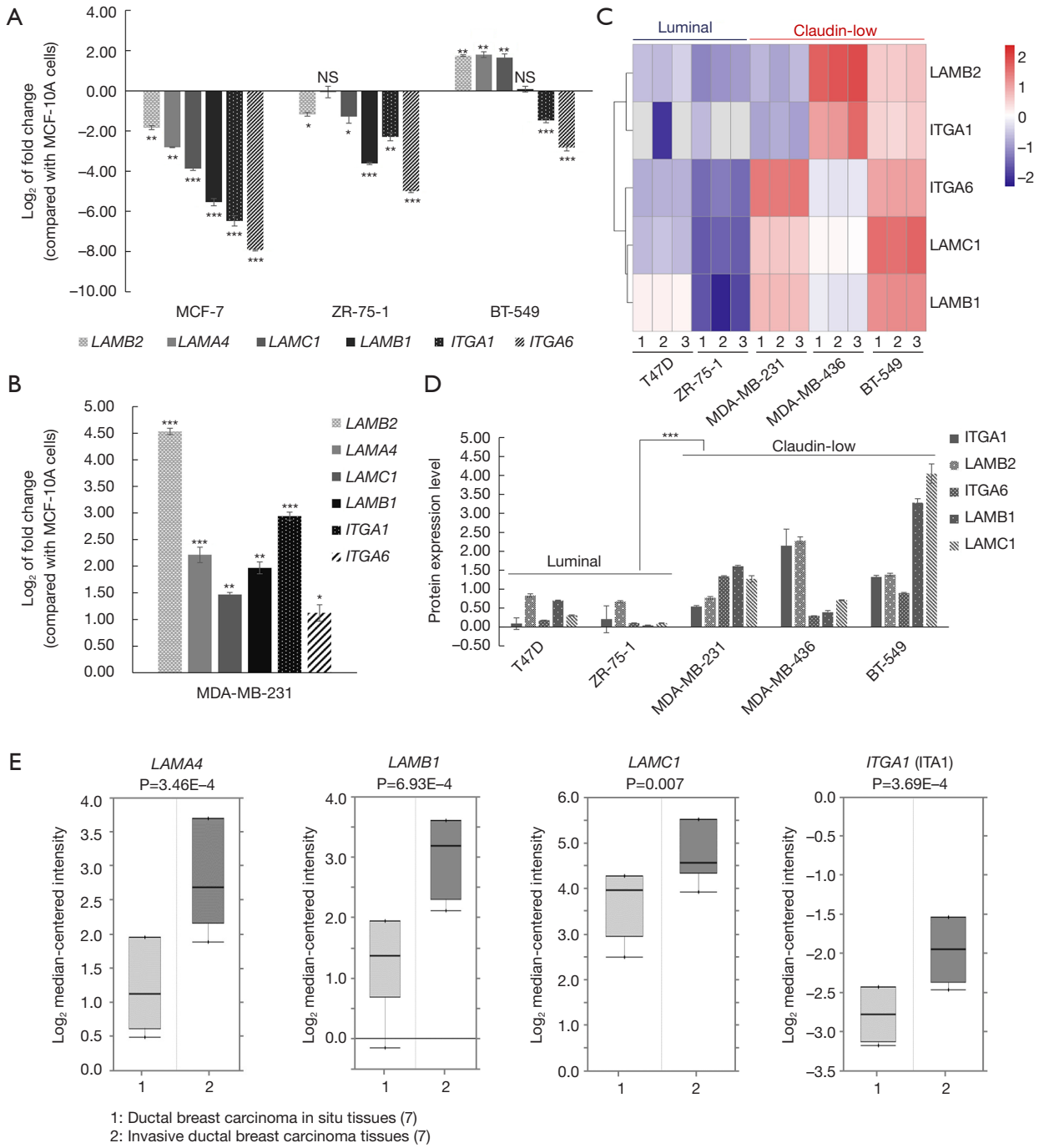

Figure 4 Expression levels of laminin interaction proteins in breast cancer cells. (A) Expression levels of genes encoding laminins and their integrin receptors in breast cancer cells (MCF-7, ZR-75-1 and BT-549) assessed by qRT-PCR. MCF-10A cells were used as the control group, β-actin served as the reference gene. The experiment was repeated three times. (B) Expression levels of genes encoding laminins and their integrin receptors in MDA-MB-231 cells assessed by qRT-PCR. MCF-10A cells were used as the control group, β-actin served as the reference gene. The experiment was repeated for three times. (C) Expression clusters of laminins and integrin receptors in MCF-10A, BT-549, MDA-MB-436, T47D and ZR-75-1 cells, which were detected by proteomics detection. (D) Quantification of protein expression levels in (C). (E) Expression levels of target laminins in invasive breast cancer tissues and corresponding *in situ* tissues using the Oncomine-Schuetz database. *, P≤0.05; **, P≤0.01; ***, P≤0.001; NS, no significance; qRT-PCR, quantitative real time polymerase chain reaction.

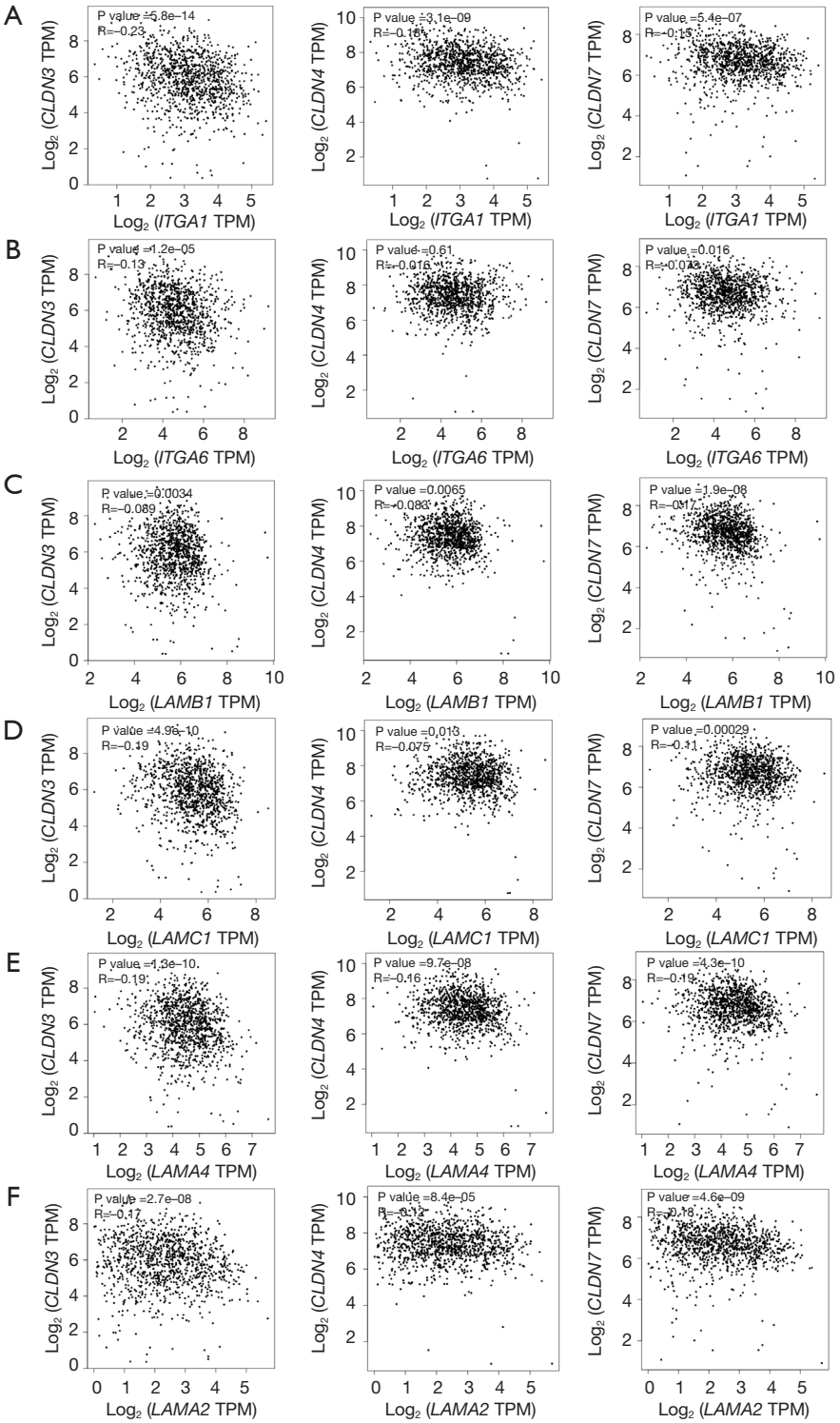

Figure 5 Gene correlation analysis between laminin interaction proteins and claudins. (A) Correlation between *ITGA1* and *CLDN3*, *CLDN4* and *CLDN*7; (B) correlation between *ITGA6* and *CLDN3*, *CLDN4* and *CLDN*7; (C) correlation between *LAMB1* and *CLDN3*, *CLDN4* and *CLDN*7; (D) correlation between *LAMC1* and *CLDN3*, *CLDN4* and *CLDN*7; (E) correlation between *LAMA4* and *CLDN3*, *CLDN4* and *CLDN*7; (F) correlation between *LAMA2* and *CLDN3*, *CLDN4* and *CLDN*7. *ITGA1*, integrin α1; *CLDN3*, gene encoding claudin-3; *LAMA2*, laminin subunit α2; *LAMB1*, laminin subunit β1; *LAMC1*, laminin subunit γ1; TPM, transcripts per million.

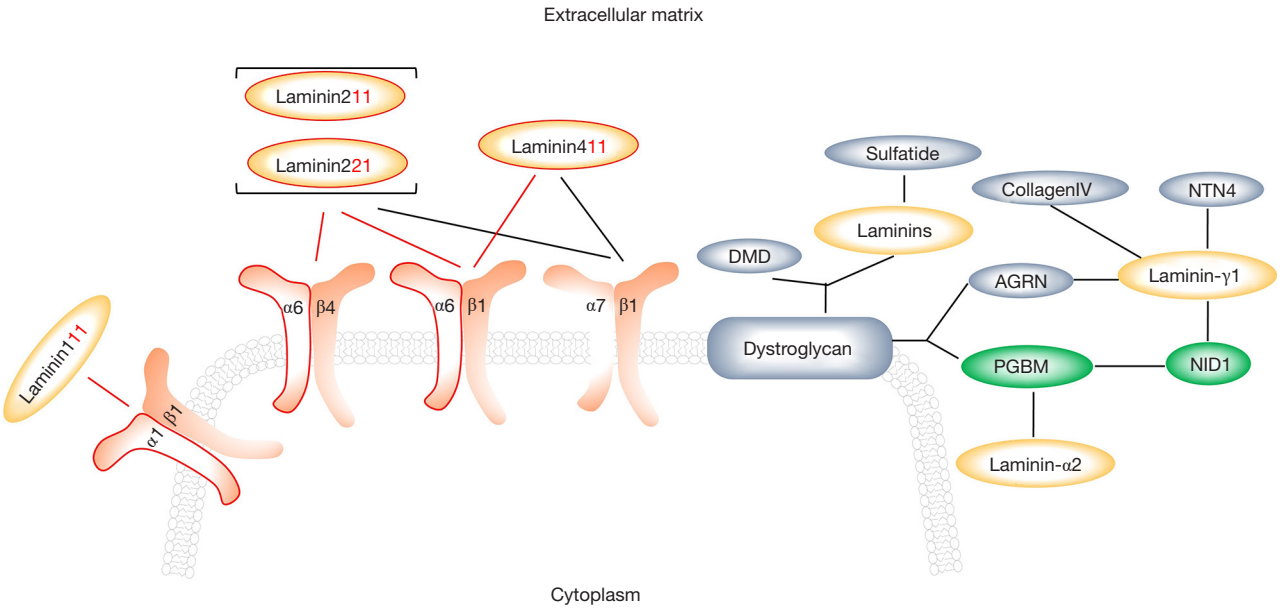

Figure 6 Simplified laminin interaction pathway involved proteins with abnormal expression level in breast cancer. Red fonts and lines represent the core targets and their combination revealed in our results.

operators, equipment, and samples. Thus, it is crucial to obtain solid results and to validate them. Here, through our proteomics detection (both for tissue and cell samples), investigation with different databases (GEPIA, Oncomine-Richardson, Oncomine-Curtis and Oncomine-Schuetz), and qRT-PCR detection, we revealed dynamic expression of laminins and their integrin receptors in breast cancer, as well as their correlation with claudins.

As compared with their normal counterpart, breast cancer tissues showed lower expression levels of laminins and their integrin receptors (*Figure 3*). It has been reported that laminins are necessary for a "soft" extracellular matrix (43) and the loss of the laminin signal can result in a "stiff" extracellular environment and abnormal tissue function, which are hallmarks of solid tumors (44). Thus, the lower expression levels of laminins and their integrin receptors could be one of the reasons for the tumorigenesis of mammary epithelial cells, which is consistent with our results. However, the lower expression level seems to be characteristic for luminal breast cancer cells with no- or lower-invasive ability (such as MCF-7, T47D and ZR-75-1), for we found higher expression level of laminin and their integrins in the more invasive claudin-low breast cancer cells, such as MDA-MB-436, BT-549 and MDA-MB-231 (*Figure 4A-4D*). In addition, we also found that some laminins and integrins showed higher expression levels in invasive ductal tissues than their corresponding *in situ* tissues (*Figure 4E*). That is, the laminins and integrins showed a rebounded expression during cancer progression. As the important components of extracellular matrix, laminins can interact with their receptors and several active intracellular signaling pathway including phosphatidylinositol-3-kinase/protein kinase B (PI3K/AKT), mitogen activated protein kinase/extracellular signal regulated kinase (MAPK/ERK), and Rho GTPases, which involve in the development of some cancer types, including bladder cancer, colorectal cancer, lung cancer, and head and neck squamous carcinomas (45-47). In addition, overexpression of laminins has been reported in breast cancer for facilitating cell metastasis (48). Thus, we speculated that the rebounded expression of laminins and integrins could be one of the main reasons for breast cancer progression, especially for claudin-low expression breast cancer. Furthermore, our continuous validated results suggested that above-mentioned dynamic expression is mainly characteristic for laminins with β1 and γ1 subunits and their integrin receptors with α1 and α6 subunits (*Figure 6*).

The claudin-low breast cancer is characterized by decreased expression of claudin-3, claudinin-4 and claudin-7, enrichment for markers involved in epithelial-mesenchymal transition and possessing features involved in mammary cancer stem cells (39), and usually shows poor

prognosis (37,49). We also found the negative correlation between laminins, integrins and claudin3, claudin4, and claudin7. Moreover, it has been reported that targeting integrin-β1 can sensitize claudin-low cells to mitogen-activated protein kinase kinase (MEK) inhibition (50), indicating its oncogenic role in claudin-low breast cancer. However, there is no reported proof about the correlation and regulation between other laminins, integrins and claudins in breast cancer, which needs our further investigation.

Conclusions

Here, through proteomics detection and experiment validation, we found contrary expression levels of laminins and their integrin receptors according to different breast cancer: they exhibited lower expression levels in luminal breast cancer with no or lower metastatic ability but achieved higher expression levels in claudin-low breast cancer with higher metastatic ability and their higher expression could be related with the low claudin expression.

Acknowledgments

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-2214/rc

Data Sharing Statement: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-2214/dss

Peer Review File: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-2214/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-2214/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Experiments involving human breast cancer and para-carcinoma tissues were approved by the Qiqihar Medical Ethics Committee (approval No. [2021]25). Informed consent to participate in this study was provided by all patients.

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Table S1 Screened down-regulated proteins by proteomic detection and GEPIA.2 database

Protein	UniProt ID	P	T	Ratio	P value
O00159	MYO1C_HUMAN	1.36054422	0.6394558	0.47	6.1736E-19
O00763	ACACB_HUMAN	1.52788388	0.4721161	0.309	0.00054677
O14558	HSPB6_HUMAN	1.33958473	0.6604153	0.493	0.00023161
O15247	CLIC2_HUMAN	1.25234815	0.7476518	0.597	0.0042528
O43301	HS12A_HUMAN	1.35501355	0.6449864	0.476	1.0591E-06
O43491	E41L2_HUMAN	1.21359223	0.7864078	0.648	3.4257E-05
O60240	PLIN1_HUMAN	1.50432493	0.4956751	0.3295	1.597E-16
O75781	PALM_HUMAN	1.41242938	0.5875706	0.416	0.00049725
O75891	AL1L1_HUMAN	1.54202005	0.45798	0.297	1.0724E-08
O76070	SYUG_HUMAN	1.31752306	0.6824769	0.518	8.5772E-06
O94911	ABCA8_HUMAN	1.31233596	0.687664	0.524	0.00142807
O95969	SG1D2_HUMAN	1.57480315	0.4251969	0.27	0.00423645
P00325	ADH1B_HUMAN	1.60064026	0.3993597	0.2495	5.6319E-55
P00352	AL1A1_HUMAN	1.29282482	0.7071752	0.547	3.3168E-07
P00387	NB5R3_HUMAN	1.29366106	0.7063389	0.546	2.2404E-14
P00450	CERU_HUMAN	1.27795527	0.7220447	0.565	7.4447E-18
P00488	F13A_HUMAN	1.25549278	0.7445072	0.593	8.1569E-10
P00533	EGFR_HUMAN	1.29240711	0.7075929	0.5475	0.00492403
P00746	CFAD_HUMAN	1.40894681	0.5910532	0.4195	0.00066217
P00966	ASSY_HUMAN	1.36425648	0.6357435	0.466	5.4212E-08
P01023	A2MG_HUMAN	1.2987013	0.7012987	0.54	1.2797E-35
P01024	CO3_HUMAN	1.20772947	0.7922705	0.656	1.6639E-52
P01833	PIGR_HUMAN	1.37646249	0.6235375	0.453	8.4387E-05
P01876	IGHA1_HUMAN	1.26984127	0.7301587	0.575	4.7753E-14
P02511	CRYAB_HUMAN	1.56862745	0.4313725	0.275	1.6823E-08
P02768	ALBU_HUMAN	1.27632419	0.7236758	0.567	0
P04003	C4BPA_HUMAN	1.23533045	0.7646695	0.619	1.4031E-16
P04040	CATA_HUMAN	1.53905348	0.4609465	0.2995	1.3856E-18
P04083	ANXA1_HUMAN	1.30804447	0.6919555	0.529	5.7324E-18
P04264	K2C1_HUMAN	1.26984127	0.7301587	0.575	8.9272E-05
P04275	VWF_HUMAN	1.30633573	0.6936643	0.531	2.9529E-20
P05091	ALDH2_HUMAN	1.43369176	0.5663082	0.395	7.354E-06
P05452	TETN_HUMAN	1.24882922	0.7511708	0.6015	0.00963534
P06703	S10A6_HUMAN	1.34952767	0.6504723	0.482	0.0004362
P06737	PYGL_HUMAN	1.35455469	0.6454453	0.4765	3.2968E-10
P07195	LDHB_HUMAN	1.33958473	0.6604153	0.493	1.6062E-11
P07225	PROS_HUMAN	1.34725497	0.652745	0.4845	0.00087605
P07451	CAH3_HUMAN	1.51630023	0.4836998	0.319	3.3464E-05
P07942	LAMB1_HUMAN	1.32187707	0.6781229	0.513	2.2633E-13
P08294	SODE_HUMAN	1.20992136	0.7900786	0.653	0.00063633
P08670	VIME_HUMAN	1.22737036	0.7726296	0.6295	4.8199E-30
P08697	A2AP_HUMAN	1.32450331	0.6754967	0.51	3.5501E-06
P09972	ALDOC_HUMAN	1.25944584	0.7405542	0.588	0.0057946
P10301	RRAS_HUMAN	1.27795527	0.7220447	0.565	0.00180024
P10643	CO7_HUMAN	1.20156203	0.798438	0.6645	0.00110747
P11047	LAMC1_HUMAN	1.43833154	0.5616685	0.3905	2.5688E-23
P11498	PYC_HUMAN	1.20264582	0.7973542	0.663	8.9208E-06
P11532	DMD_HUMAN	1.37221269	0.6277873	0.4575	1.8884E-05
P12273	PIP_HUMAN	1.44300144	0.5569986	0.386	3.5211E-31
P12277	KCRB_HUMAN	1.2755102	0.7244898	0.568	4.2801E-09
P12429	ANXA3_HUMAN	1.23190638	0.7680936	0.6235	3.0465E-11
P13671	CO6_HUMAN	1.23304562	0.7669544	0.622	1.5572E-07
P14543	NID1_HUMAN	1.39958013	0.6004199	0.429	1.3424E-13
P15090	FABP4_HUMAN	1.58478605	0.4152139	0.262	7.8745E-53
P16219	ACADS_HUMAN	1.29659643	0.7034036	0.5425	8.4567E-05
P16452	EPB42_HUMAN	1.58667196	0.413328	0.2605	8.993E-06
P16671	CD36_HUMAN	1.58730159	0.4126984	0.26	7.484E-14
P21397	AOFA_HUMAN	1.46466496	0.535335	0.3655	1.6154E-06
P21399	ACOC_HUMAN	1.21580547	0.7841945	0.645	6.2279E-07
P21589	5NTD_HUMAN	1.26103405	0.738966	0.586	0.00759825
P21695	GPDA_HUMAN	1.54798762	0.4520124	0.292	1.7054E-18
P22105	TENX_HUMAN	1.32625995	0.6737401	0.508	8.6956E-30
P22676	CALB2_HUMAN	1.48533234	0.5146677	0.3465	1.0483E-06
P23141	EST1_HUMAN	1.43988481	0.5601152	0.389	2.8992E-07
P23229	ITA6_HUMAN	1.31752306	0.6824769	0.518	0.00049692
P24043	LAMA2_HUMAN	1.28328521	0.7167148	0.5585	0.00325765
P24298	ALAT1_HUMAN	1.40498771	0.5950123	0.4235	0.00031137
P26447	S10A4_HUMAN	1.31061599	0.689384	0.526	0.00021987
P27105	STOM_HUMAN	1.29533679	0.7046632	0.544	3.3943E-14
P27338	AOFB_HUMAN	1.2642225	0.7357775	0.582	9.6285E-05
P31323	KAP3_HUMAN	1.41643059	0.5835694	0.412	0.00013019
P33121	ACSL1_HUMAN	1.4461316	0.5538684	0.383	1.1233E-16
P39059	COFA1_HUMAN	1.39372822	0.6062718	0.435	7.2838E-06
P42330	AK1C3_HUMAN	1.41043724	0.5895628	0.418	6.5073E-06
P42765	THIM_HUMAN	1.27959053	0.7204095	0.563	5.0852E-07
P43121	MUC18_HUMAN	1.43575018	0.5642498	0.393	3.6785E-08
P48163	MAOX_HUMAN	1.37457045	0.6254296	0.455	0.00381169
P51636	CAV2_HUMAN	1.44196107	0.5580389	0.387	0.00412808
P54289	CA2D1_HUMAN	1.30039012	0.6996099	0.538	0.00216406
P55268	LAMB2_HUMAN	1.43061516	0.5693848	0.398	2.1333E-28
P55290	CAD13_HUMAN	1.39664804	0.603352	0.432	0.007346
P56199	ITA1_HUMAN	1.26143173	0.7385683	0.5855	0.00159665
P61764	STXB1_HUMAN	1.41944642	0.5805536	0.409	0.00276067
P62070	RRAS2_HUMAN	1.38504155	0.6149584	0.444	0.00111997
P63096	GNAI1_HUMAN	1.47383935	0.5261606	0.357	0.00017787
P68871	HBB_HUMAN	1.66251039	0.3374896	0.203	6.937E-166
P69905	HBA_HUMAN	1.65837479	0.3416252	0.206	4.4276E-75
P98160	PGBM_HUMAN	1.28824477	0.7117552	0.5525	8.1264E-32
Q01082	SPTB2_HUMAN	1.29198966	0.7080103	0.548	2.2168E-31
Q01469	FABP5_HUMAN	1.37033231	0.6296677	0.4595	6.4835E-11
Q02952	AKA12_HUMAN	1.23609394	0.7639061	0.618	1.6035E-05
Q03135	CAV1_HUMAN	1.43369176	0.5663082	0.395	1.2786E-11
Q05469	LIPS_HUMAN	1.5117158	0.4882842	0.323	1.2207E-10
Q13361	MFAP5_HUMAN	1.5060241	0.4939759	0.328	0.00186517
Q13642	FHL1_HUMAN	1.49031297	0.509687	0.342	2.7213E-08
Q13683	ITA7_HUMAN	1.41743444	0.5825656	0.411	1.8227E-06
Q14624	ITIH4_HUMAN	1.25078174	0.7492183	0.599	1.1423E-10
Q15124	PGM5_HUMAN	1.22774708	0.7722529	0.629	7.0245E-06
Q15847	ADIRF_HUMAN	1.29282482	0.7071752	0.547	0.00936148
Q16134	ETFD_HUMAN	1.22399021	0.7760098	0.634	1.9834E-05
Q16363	LAMA4_HUMAN	1.42045455	0.5795455	0.408	6.8847E-13
Q16836	HCDH_HUMAN	1.36379134	0.6362087	0.4665	2.3735E-05
Q16851	UGPA_HUMAN	1.36147039	0.6385296	0.469	2.5241E-18
Q16853	AOC3_HUMAN	1.54202005	0.45798	0.297	2.4608E-20
Q687X5	STEA4_HUMAN	1.35501355	0.6449864	0.476	0.00340973
Q6NUM9	RETST_HUMAN	1.35501355	0.6449864	0.476	0.00017121
Q6NY19	KANK3_HUMAN	1.32144037	0.6785596	0.5135	0.0058884
Q6UXB8	PI16_HUMAN	1.54202005	0.45798	0.297	0.00181115
Q86VB7	C163A_HUMAN	1.32802125	0.6719788	0.506	1.5536E-10
Q92781	RDH1_HUMAN	1.45666424	0.5433358	0.373	0.00273196
Q96AC1	FERM2_HUMAN	1.35409614	0.6459039	0.477	1.0905E-10
Q96FV2	SCRN2_HUMAN	1.20048019	0.7995198	0.666	0.00108376
Q96Q06	PLIN4_HUMAN	1.59616919	0.4038308	0.253	1.1336E-48
Q9BRX8	F213A_HUMAN	1.49253731	0.5074627	0.34	4.4825E-09
Q9BX66	SRBS1_HUMAN	1.40203295	0.5979671	0.4265	2.6473E-07
Q9BX97	PLVAP_HUMAN	1.2012012	0.7987988	0.665	0.00658363
Q9HBL0	TENS1_HUMAN	1.27226463	0.7277354	0.572	1.9054E-07
Q9NTX5	ECHD1_HUMAN	1.30420606	0.6957939	0.5335	2.0504E-05
Q9NZN4	EHD2_HUMAN	1.47928994	0.5207101	0.352	3.2169E-21
Q9Y4G6	TLN2_HUMAN	1.28534704	0.714653	0.556	4.4061E-20

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Table S2 Screened up-regulated proteins by proteomic detection and GEPIA.2 database

Protein	UniProt ID	P	T	Ratio	P value
O43583	DENR_HUMAN	0.728199527	1.271800473	1.7465	0.006164239
O75223	GGCT_HUMAN	0.665335995	1.334664005	2.006	0.003326817
O76094	SRP72_HUMAN	0.7390983	1.2609017	1.706	0.000612669
O94760	DDAH1_HUMAN	0.735023888	1.264976112	1.721	0.000901056
O94906	PRP6_HUMAN	0.767606985	1.232393015	1.6055	0.002135071
P02786	TFR1_HUMAN	0.745156483	1.254843517	1.684	0.002903714
P04792	HSPB1_HUMAN	0.789265983	1.210734017	1.534	7.77769E-05
P05783	K1C18_HUMAN	0.596569724	1.403430276	2.3525	1.38377E-08
P05787	K2C8_HUMAN	0.456777435	1.543222565	3.3785	3.49006E-13
P06748	NPM_HUMAN	0.758437619	1.241562381	1.637	0.005875224
P07910	HNRPC_HUMAN	0.747104968	1.252895032	1.677	0.004032246
P08123	CO1A2_HUMAN	0.795228628	1.204771372	1.515	6.11323E-10
P08727	K1C19_HUMAN	0.572573719	1.427426281	2.493	1.00891E-10
P09874	PARP1_HUMAN	0.716460684	1.283539316	1.7915	0.007477093
P0DMV8	HS71A_HUMAN	0.779423227	1.220576773	1.566	2.8258E-06
P10155	RO60_HUMAN	0.631313131	1.368686869	2.168	0.000968119
P11142	HSP7C_HUMAN	0.797289217	1.202710783	1.5085	2.50214E-13
P12830	CADH1_HUMAN	0.653915318	1.346084682	2.0585	1.42929E-05
P13010	XRCC5_HUMAN	0.70323488	1.29676512	1.844	3.97037E-06
P13611	CSPG2_HUMAN	0.552944429	1.447055571	2.617	4.99453E-06
P14678	RSMB_HUMAN	0.738279808	1.261720192	1.709	0.003967077
P15170	ERF3A_HUMAN	0.643293664	1.356706336	2.109	0.006605383
P15311	EZRI_HUMAN	0.680966973	1.319033027	1.937	0.000111799
P15924	DESP_HUMAN	0.538575468	1.461424532	2.7135	3.48866E-14
P18085	ARF4_HUMAN	0.768935025	1.231064975	1.601	0.007548843
P19338	NUCL_HUMAN	0.682943486	1.317056514	1.9285	4.88773E-05
P20700	LMNB1_HUMAN	0.670690812	1.329309188	1.982	6.80059E-05
P21266	GSTM3_HUMAN	0.597371565	1.402628435	2.348	2.26976E-05
P22234	PUR6_HUMAN	0.657894737	1.342105263	2.04	0.005674788
P23193	TCEA1_HUMAN	0.624414611	1.375585389	2.203	0.004442461
P23528	COF1_HUMAN	0.540248514	1.459751486	2.702	1.40437E-08
P26639	SYTC_HUMAN	0.769822941	1.230177059	1.598	0.002425976
P30520	PURA2_HUMAN	0.58979652	1.41020348	2.391	0.003515081
P31948	STIP1_HUMAN	0.759589821	1.240410179	1.633	3.67843E-10
P35442	TSP2_HUMAN	0.55233361	1.44766639	2.621	7.3699E-05
P37802	TAGL2_HUMAN	0.766283525	1.233716475	1.61	4.79149E-05
P38919	IF4A3_HUMAN	0.687285223	1.312714777	1.91	0.008213099
P39748	FEN1_HUMAN	0.625586487	1.374413513	2.197	0.005466157
P40121	CAPG_HUMAN	0.525210084	1.474789916	2.808	0.000100328
P43487	RANG_HUMAN	0.67351406	1.32648594	1.9695	0.007456127
P48735	IDHP_HUMAN	0.771010023	1.228989977	1.594	8.23933E-07
P49915	GUAA_HUMAN	0.748783227	1.251216773	1.671	0.000175359
P50454	SERPH_HUMAN	0.622858922	1.377141078	2.211	6.01505E-06
P50851	LRBA_HUMAN	0.658653055	1.341346945	2.0365	0.000609108
P51884	LUM_HUMAN	0.760600875	1.239399125	1.6295	6.53663E-13
P52306	GDS1_HUMAN	0.780031201	1.219968799	1.564	0.008913827
P52566	GDIR2_HUMAN	0.771753811	1.228246189	1.5915	0.005656912
P53621	COPA_HUMAN	0.709471444	1.290528556	1.819	3.57151E-08
P55265	DSRAD_HUMAN	0.769230769	1.230769231	1.6	0.002871291
P61081	UBC12_HUMAN	0.729128691	1.270871309	1.743	0.003600053
P61604	CH10_HUMAN	0.715563506	1.284436494	1.795	0.00066449
P62805	H4_HUMAN	0.774893452	1.225106548	1.581	1.61525E-08
P62826	RAN_HUMAN	0.781097442	1.218902558	1.5605	0.003167895
P78527	PRKDC_HUMAN	0.731796561	1.268203439	1.733	1.08588E-10
Q00341	VIGLN_HUMAN	0.750469043	1.249530957	1.665	1.07035E-05
Q00688	FKBP3_HUMAN	0.566732785	1.433267215	2.529	0.00651434
Q00796	DHSO_HUMAN	0.772200772	1.227799228	1.59	0.005067755
Q00839	HNRPU_HUMAN	0.703111267	1.296888733	1.8445	0.000159278
Q01105	SET_HUMAN	0.520020801	1.479979199	2.846	0.000293636
Q02790	FKBP4_HUMAN	0.577200577	1.422799423	2.465	8.29321E-05
Q06210	GFPT1_HUMAN	0.706214689	1.293785311	1.832	6.28233E-05
Q06830	PRDX1_HUMAN	0.738552437	1.261447563	1.708	5.45718E-08
Q07065	CKAP4_HUMAN	0.766430351	1.233569649	1.6095	5.17643E-06
Q13153	PAK1_HUMAN	0.538647994	1.461352006	2.713	0.00354872
Q13177	PAK2_HUMAN	0.796971508	1.203028492	1.5095	0.009001749
Q13185	CBX3_HUMAN	0.545033383	1.454966617	2.6695	0.00137969
Q13263	TIF1B_HUMAN	0.751597144	1.248402856	1.661	0.000230832
Q14847	LASP1_HUMAN	0.786936848	1.213063152	1.5415	0.008203029
Q15063	POSTN_HUMAN	0.541345243	1.458654757	2.6945	1.36919E-12
Q15185	TEBP_HUMAN	0.79633685	1.20366315	1.5115	0.000382492
Q15437	SC23B_HUMAN	0.715563506	1.284436494	1.795	0.002945394
Q15833	STXB2_HUMAN	0.781860829	1.218139171	1.558	0.005127278
Q6P996	PDXD1_HUMAN	0.550206327	1.449793673	2.635	1.08723E-05
Q86VP6	CAND1_HUMAN	0.723589001	1.276410999	1.764	2.33593E-10
Q8IV36	HID1_HUMAN	0.636841267	1.363158733	2.1405	0.002397717
Q8N1S5	S39AB_HUMAN	0.705467372	1.294532628	1.835	0.007754981
Q8TF66	LRC15_HUMAN	0.546298825	1.453701175	2.661	0.00294708
Q92598	HS105_HUMAN	0.76394194	1.23605806	1.618	0.002144996
Q96A35	RM24_HUMAN	0.737871241	1.262128759	1.7105	0.004032021
Q99715	COCA1_HUMAN	0.648508431	1.351491569	2.084	1.36339E-11
Q9BZK7	TBL1R_HUMAN	0.73964497	1.26035503	1.704	0.003795054
Q9H3P7	GCP60_HUMAN	0.705218618	1.294781382	1.836	0.000972587
Q9HCC0	MCCB_HUMAN	0.705716302	1.294283698	1.834	0.000144853
Q9NR45	SIAS_HUMAN	0.689061154	1.310938846	1.9025	5.07267E-05
Q9NR99	MXRA5_HUMAN	0.683176772	1.316823228	1.9275	0.004448784
Q9NY33	DPP3_HUMAN	0.720980534	1.279019466	1.774	0.000322739
Q9NZB2	F120A_HUMAN	0.743494424	1.256505576	1.69	2.25452E-05
Q9P258	RCC2_HUMAN	0.587975893	1.412024107	2.4015	0.009155968
Q9UBE0	SAE1_HUMAN	0.717360115	1.282639885	1.788	0.00053816
Q9UNE7	CHIP_HUMAN	0.657246139	1.342753861	2.043	0.000281691
Q9Y3C6	PPIL1_HUMAN	0.799360512	1.200639488	1.502	0.00120908
Q9Y6E2	BZW2_HUMAN	0.735158978	1.264841022	1.7205	0.005659967

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