Identification of CLDN18 as a potential diagnostic biomarker and therapeutic target for pancreatic cancer based on multiomic analysis

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Research

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Abstract

Background Claudin 18 (CLDN18) is a transmembrane protein localized in the apical regions to form a tight-junction complex. The expression of CLDN18 was restricted to normal lung and stomach tissues but was aberrantly activated in some types of cancers, and this molecule can serve as a promising target for targeted treatment. Methods The mRNA expression, genetic variations and prognostic values of CLDN18 in pancreatic cancer (PC) were analyzed using public data from The Cancer Genome Atlas (TCGA), Gene Expression Omnibus (GEO), Human Protein Atlas (HPA) databases and multiple online tools. The role of cyclic AMP-responsive element binding protein (CREB) and DNA methylation in regulating the expression of CLDN18 was determined by correlation analysis. Results The expression of CLDN18 was restricted to the lung and stomach in normal tissues, but was ectopically overexpressed in PC. CLDN18 mRNA expression was higher in cancers with advanced local invasion, later clinical stage, infiltrating duct or mucinous carcinoma types. Genetic variations of CLDN18 are rarely observed in PC. Correlation analysis revealed that CREB3L1, CREB3L3, and CREB3L4 were significantly positively coexpressed with CLDN18, and CLDN18 expression increased as methylation gradually decreased. The expression of CLDN18 was not observed to plays a role in predicting the survival of PC patients. Conclusions This data-driven study summarizes the expression features of CLDN18 in PC and demonstrates that it may serve as an ideal biomarker and therapy target for PC.

Background

Pancreatic cancer (PC) is among the most common malignancies and one of the deadliest diseases and ranks fourth in the causes of cancer-related mortality worldwide [1]. Most patients present with locally advanced incurable or metastatic PC due to delayed diagnosis resulting from insidious onset and nonspecific clinical symptoms, and the 5-year survival rate is less than 5% [2, 3]. In addition to surgery, systemic treatment of PC is currently based primarily on highly toxic chemotherapy regimens. Recently, agents targeting antigens expressed on tumor cells or in the tumor microenvironment have been evaluated in patients with solid cancers with promising effects [5, 6]. However, there are few approved treatment options for PC, and studies found marginal benefit or most patients lacking antigen expression limited the usage of these agents in PC patients [6]. Therefore, there is an urgent medical need for the identification and characterization of novel molecules that can be exploited for targeted treatment.

A previous study identified the tight junction protein claudin 18 (CLDN18) as a promising target for the treatment of several solid tumors [7]. The expression of this transmembrane protein is strictly confined to gastric and lung tissues as two tissue-specific splice variants and is typically buried in the tight junction supramolecular complex. However, upon malignant transformation, it is aberrantly expressed in many types of cancer. Furthermore, perturbations in tight junctions cause epitopes of claudin 18 to be exposed on the surface of tumor cells [7]. These expression characteristics satisfy conditions of an ideal target for antibody-mediated cancer immunotherapy: first, there is no expression or inaccessibility to antibodies in most normal tissues to avoid adverse effects; second, the expression should be positive, and its epitopes should be exposed in corresponding malignant tissues, rendering them targetable by antibodies.

Therefore, a chimeric IgG1 monoclonal antibody, zolbetuximab (IMAB362), was developed as a specific claudin 18.2-targeting therapeutic mAb. This antibody was demonstrated to mediate cancer cell death through antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). In several early clinical trials for the treatment of advanced gastric cancer (GC), zolbetuximab alone or in combination with standard chemotherapy significantly prolonged survival with acceptable safety and tolerability [8, 9].

Aberrant expression of claudin 18.2 is especially obvious in PC, and the prevalence was reported to be 60–90% in pancreatic ductal adenocarcinoma [10, 11]. These previous studies showed that aberrant activation of CLDN18 transcription in PC may cause a fraction of patients to be targetable by zolbetuximab. Together with the evidence demonstrating the beneficial effects of zolbetuximab in GC, these findings prompted the exploration of claudin 18.2 as a potential therapeutic target for PC. Therefore, we conducted this study to explore the expression pattern of CLDN18 in PC patients and the potential mechanism of ectopic expression by applying different bioinformatic methods.

Methods

Analysis of *CLDN18* mRNA expression in different cancer types and corresponding normal tissues

To study the mRNA expression profile of *CLDN18* in different cancer and normal tissues, we first used the public database Oncomine (https://www.oncomine.org/resource/main.html) [12] to perform online analysis. The threshold for analysis was as follows: *P*-value: 0.05; FC: 2; gene ranking: all; analysis type: cancer vs. normal; data type: mRNA. Furthermore, the online database Gene Expression Profiling Interactive Analysis (GEPIA2) (http://gepia2.cancer-pku.cn/index.html) [13] was employed to confirm the expression of the *CLDN18* gene in different cancer tissues and normal tissues. The expression of transcript isoforms of *CLDN18* was also investigated using GEPIA2.

Analysis of the transcriptomic profile of *CLDN18* in pancreatic normal and cancer tissues

We extracted microarray data from the *Gene Expression Omnibus* (GEO) database (https://www.ncbi.nlm.nih.gov/geo/) to compare the transcriptomic profile of *CLDN18* between PC and normal tissue samples. The terms “pancreatic” and “cancer” or “tumor” or “carcinoma” or “neoplasm” were used as the search parameters. “*Homo sapiens*” and “sample count > 50 with at least 10 samples in the normal and cancer groups” were used to limit the search range. The differential expression of *CLDN18* was analyzed with GEO2R. We set a threshold of $\log_2$FC > 1 and adj. $P < 0.05$ was considered to be a significant overexpression when compared to the PC group versus the normal group. We also compared the expression difference between PC and normal tissues using GEPIA2, which included samples from TCGA-PAAD and the Genotype-Tissue Expression (GTEx) projects.

Protein expression of *CLDN18* detected by immunohistochemistry

The Human Protein Atlas (HPA) (https://www.proteinatlas.org) is an interactive open-access database containing the protein expression data generated within the framework of the HPA using a tissue microarray-based analysis of major cancer and different normal tissue types [14]. In this study, a comparison of claudin 18 protein expression among normal and cancer tissues was performed using the HPA database. The intensity of moderate and strong staining was considered positive.

Association with *CLDN18* expression and clinicopathological characteristics

The gene expression level of *CLDN18* and the currently available clinical information of corresponding patients were obtained from the TCGA data portal (https://portal.gdc.cancer.gov/; accessed January 15, 2020). Relevant search strategies were as follows: data category: transcriptome profiling; data type: gene expression quantification; experimental strategy: RNA-Seq; workflow type: HTSeq-counts; project: TCGA-PAAD (pancreatic adenocarcinoma). We used the $\log_2$(counts+1) transformation to convert the expression level of *CLDN18* for further analysis. Association with *CLDN18* expression and clinicopathological characteristics was analyzed.

Analysis of genetic alterations of *CLDN18* in PC

The cBioPortal (https://www.cbioportal.org/) is a website for exploring, analyzing and visualizing multidimensional cancer genomics datasets [15]. Mutations and copy number alterations (CNAs) of *CLDN18* in PC were analyzed using the cBioPortal tool. The OncoPrint subtool was utilized to display an overview of the integrated status of genetic alterations for *CLDN18*. The Cancer Types Summary subtool showed the details of genetic alterations in different datasets and in different histological types.

Coexpression relationship between *CLDN18* and the cyclic AMP-responsive element binding protein (CREB) family

Studies have found that the activation of *CLDN18* depends on the transcription factor CREB [7]. Therefore, we also downloaded the gene expression level of the *CREB* family in PC from the TCGA data portal and used $\log_2$(counts+1) transformation to convert the expression value for further analysis. The coexpression relationship between *CLDN18* and the *CREB* gene family was measured by the Pearson coefficient, and $P < 0.05$ represents a significant coexpression relationship between the two genes.

Correlation between *CLDN18* mRNA expression and methylation

The methylation of CpGs in promoter regions has been reported as a mechanism for ectopic activation of genes in cancer [16]. Therefore, the MEXPRESS tool was used to analyze the expression and methylation data of *CLDN18* available in TCGA (http://mexpress.be/) [17]. This website executed the Pearson correlation to evaluate the difference between mRNA expression and methylation for one single gene in the specific cancer type.

Prognostic analysis based on *CLDN18* mRNA expression

The prognostic value of *CLDN18* expression was explored by the TCGA-PAAD cohort downloaded by this study and series from GEO, which has *CLDN18* expression and survival data. Patients with follow-up or survival time less than 1 month were excluded. The patients were divided into high and low expression groups according to the median level of *CLDN18*. Hazard ratios (HRs) with 95% confidence intervals (CIs) and log-rank *P* values were calculated. A *P*-value < 0.05 was considered to indicate a significant difference.

Statistical analysis

Online analyses were conducted following the statistical methods used by individual bioinformatic websites, and the corresponding parameters were described above. For data analyzed in this study, significant differences in all variables among groups were determined using the Kruskal-Wallis H or Mann-Whitney U test. The statistical analyses were performed using IBM SPSS Statistics ver. 22.0 (IBM Co., Armonk, NY, USA). The graphs, survival curves, Pearson correlation analysis and log-rank tests were completed by GraphPad Prism 8.0 (GraphPad Software, San Diego, CA, USA). For all performed statistical analyses, a two-sided significance level of 0.05 was assumed.

Results

Expression of the *CLDN18* gene in different cancer types and corresponding normal tissues

In the Oncomine database, 312 unique analyses compared the *CLDN18* mRNA expression difference between cancer and normal tissues; among these analyses, 49 showed significance($P < 0.05$). The results revealed that *CLDN18* expression was downregulated in GC, lung cancer, breast cancer and sarcoma, whereas *CLDN18* expression was upregulated significantly in esophageal and pancreatic cancer compared to their normal counterparts (Figure 1A). The results in GEPIA2 showed that in normal tissues, the mRNA expression of *CLDN18* was restricted to the lung and stomach, while the expression nearly disappeared in lung cancer tissues and was significantly downregulated in GC tissues. In contrast, obvious ectopic expression of *CLDN18* was observed in PC (Figure 1B).

mRNA expression analysis of *CLDN18* in pancreatic normal and tumor tissues

In the Oncomine database, 5 of 8 analyses from 7 datasets reported upregulated *CLDN18* mRNA expression in PC tissues, while no analysis reported downregulated *CLDN18* mRNA expression (Figure 1A). Because the sample sizes of analyses were small in studies included in Oncomine, we further compared the expression difference between PC and normal tissues using series from GEO. Seven series were obtained from GEO based on our predefined criterion, including 381 cancer samples and 209 normal samples. A total of 15 probes reported the expression level of *CLDN18*. All probes reported an upregulated *CLDN18* mRNA expression difference with significance ($\log_2 FC > 1$, $P < 0.05$) (Table 1). In addition, the results from GEPIA2 showed that the expression of *CLDN18* was clearly higher in PC tissues than in normal tissues (Figure 3A).

Expression of alternatively spliced transcript variants of *CLDN18*

Analysis using GEPIA2 showed that *CLDN18-001*, which encodes isoform 2, was mostly expressed in normal gastric and cancer tissues. When compared with *CLDN18-001* expression in normal tissues, the expression was downregulated in GC tissues (Figure 2A). The expression of *CLDN18-002*, which encodes isoform 1, was restricted to pulmonary normal tissues and was obviously downregulated in lung cancer tissues (Figure 2B). The ectopic expression in PC tissues was mainly *CLDN18-001* (Figure 2A), and the transcript level of *CLDN18-001* was obviously higher than that of *CLDN18-002* and *CLDN18-003*; the latter is an instance of nonsense-mediated decay (Figure 2C).

Correlation between *CLDN18* expression and clinicopathological characteristics

Gene expression and currently available clinical data of 177 patients were extracted from the TCGA database based on our search strategies. We compared the expression differences among groups with different clinicopathological characteristics. As shown in Figure 3, there were no relationships between *CLDN18* expression and age, sex, race, node metastasis, distant metastasis, alcohol history or tumor location. However, increased expression of *CLDN18* correlated significantly with the infiltration depth, tumor stage and histology type, and the expression was higher in tumor tissues with advanced local invasion (T3, T4), later clinical stage (TNM III, IV), infiltrating duct or mucinous carcinoma types (Figure 3B-K).

Protein expression of *CLDN18* in normal and various cancer tissues

Three antibodies against claudin 18 were available in the HPA database, of which antibody HPA018446 was used to detect both isoforms of claudin 18, while the detected isoforms of antibodies CAB013010 and CAB013243 were not exactly known. A high staining score was only found in gastric glandular cells among normal tissues, but a high positive rate was found in pancreatic, gastric, lung and ovarian cancer tissues (Figure 4). In PC tissues, claudin 18 was detected in the cytoplasm and on the membrane, and the positive rates of claudin 18 were 58.3% (7/12), 81.8% (9/11), and 30% (3/10) for antibodies HPA018446, CAB013010 and CAB013243, respectively.

Analysis of *CLDN18* genetic alterations in PC

The TCGA PanCan Atlas studies were used to analyze genetic alterations of *CLDN18* in different cancers. The results showed that *CLDN18* alterations mainly occurred in lung squamous, cervical, esophageal, head and neck, and ovarian cancers with the main type being amplification, uterine cancer, with the main type being mutation, and stomach cancer, with the main type being fusion (Figure 5A). cBioPortal has 10 archived datasets of *CLDN18* alterations in human PC, and two datasets including PC patients from different cohorts (Queensland Centre for Medical Genomics and TCGA) were used for analysis. Finally, *CLDN18* was altered in only 0.4% (2/640) of patients with pancreatic adenocarcinoma. These alterations were truncating mutations with unknown significance in 1 case and amplification in 1 case (Figure 5B).

Coexpression relationship between *CLDN18* and the *CREB* gene family

The expression levels of 9 *CREB* genes were detected by TCGA-PAAD project. Of these genes, 3 were negatively coexpressed, and 6 were positively coexpressed. In total, 4 genes reported significant coexpression associated with *CLDN18*. Only the *CREBL2* gene reported significant negative coexpression associated with *CLDN18* with a Pearson r of 0.2165 (P=0.0037), while *CREB3L1*, *CREB3L3,* and *CREB3L4* were reported to have significant positive coexpression associated with *CLDN18* with Pearson r values of 0.7236 (P < 0.0001), 0.5425 (P < 0.0001), and 0.3506 (P < 0.0001), respectively (Figure 6).

CLDN18 expression is downregulated through methylation in PC

As shown in Figure 7, the methylation of *CLDN18* was tested by 21 probes distributed in different regions of the gene (the localization of each probe is represented in the figure, and those localized in the promoter region are highlighted in dark blue). Nineteen regions including all the promoter regions analyzed presented a negative correlation with respect to *CLDN18* gene expression (Pearson's correlation coefficients are indicated on the right), and only one region at the end of the gene analyzed presented a positive correlation with respect to *CLDN18* gene expression (Figure 7). The results showed that *CLDN18* expression increased as methylation gradually decreased.

Prognostic value of *CLDN18*

There were 171 patients with eligible survival data in the TCGA-PAAD cohort. Through searching the GEO database, 5 datasets with a total of 359 patients (GSE21501: N=102; GSE57495: N=63; GSE62452: N=66; GSE79229: N=49; GSE85916: N=79) were obtained together for survival analysis. Two and three probes were used to test the mRNA expression of *CLDN18* in GSE57495 and GSE85916, respectively. All analyses showed that the expression of *CLDN18* is not related to the overall survival of PC patients (Figure 8).

Discussion

Although claudin 18.2 was a pan-cancer target suitable for therapeutic antibody development and the beneficial effects of zolbetuximab in GC were reported many years ago, few studies have investigated this molecule in PC to date. In this study, we provided a more systemic analysis of CLDN18 gene expression to evaluate the potential roles and clinical significance of CLDN18 in PC.

Claudin 18 possesses common claudin family structures, including four transmembrane domains and an N-terminus and a C-terminus in the cytoplasm. These proteins are mainly localized in the apical regions to form tight-junction complexes in various types of epithelial cell sheets, playing a critical role in cell-cell adhesion, maintenance of cell polarity and selective paracellular permeability [18–20]. Consistent with previous studies [7, 21], multiomics analysis in this study found that CLDN18 has two isoforms. In normal tissues, CLDN18 mRNA expression was restricted to the lung, in which it was spliced as CLDN18-002 (claudin 18.1), and the stomach, in which it was spliced as CLDN18-001 (claudin 18.2). The expression features were contradictory among different cancer type tissues. CLDN18 mRNA expression

nearly disappeared in lung cancer tissues and was significantly downregulated in GC tissues, while CLDN18-001 was ectopically upregulated in PC tissues.

In line with our finding that CLDN18 was highly overexpressed in tissues compared with normal pancreatic tissues, ectopic activation of CLDN18 is an early effect and occurs in premalignant lesions reported by other studies [22, 23], suggesting a biological role for this gene in PC but is not entirely understood. Contradictory expression patterns among a variety of cancer types indicate dichotomous roles of this gene. As a tumor suppressor, loss of claudin 18 may create an inflammation setting for carcinogenesis and may lead to activation or translocation of some kinases among a number of pro-oncogenic pathways [24]. As a tumor promoter, claudin 18 may be expressed in the cytoplasm and nucleus, where it can function as other tight junction proteins, such as ZO-1, which is involved in the induction of invasion through epidermal growth factor receptor (EGFR) activation [25]. On the other hand, the activation of CLDN18 expression may be merely a result of malignant transformation, rather than participating in the development of PC. These hypotheses lead to important questions on the role of CLDN18 in PC that warrant further research.

Genetic alterations were a rare event in PC, indicating that CLDN18 was not activated by mutation or CNAs but through other mechanisms. The methylation of CpGs in promoter regions has been reported as a mechanism for both the lineage-specific expression of differentiation genes, as well as the ectopic activation of genes in cancer, and the activation of CLDN18 depends on the binding of the transcription factor CREB to its unmethylated consensus site [7]. We further confirmed this conclusion using bioinformatic analysis. Correlation analysis revealed that CREB3L1, CREB3L3, and CREB3L4 were significantly positively coexpressed with CLDN18, and CLDN18 expression increased as methylation gradually diminished. However, some CREBs showed a negative expression relationship with CLDN18, and a study found that phorbol-12-myristate 13-acetate enhances CLDN18 expression via activator protein-1 motifs [26]. Future studies need to verify these findings and to explore other factors in regulating CLDN18 expression.

Although the biological role of CLDN18 in PC is ambiguous, ectopic expression can serve as an exceptionally useful early stage or molecular typing marker and as a promising treatment target. However, there has been little research on the relationship between CLDN18 expression and clinical characteristics, which may be important for deciding which patients would in principle be eligible for a targeting approach. In two previous immunohistochemistry studies, the authors found that the positive rate was higher in well-differentiated cancers, and one study reported a higher positive rate in pN1 stage cancers [11, 22]. In contrast, in this study using the TCGA-PAAD cohort in which the expression of CLDN18 was detected by RNA sequencing, we found that the expression level was higher in patients with advanced local invasion, later clinical stage, infiltrating duct or mucinous carcinoma types. Differences were also observed in survival predicting roles. One study showed that patients whose carcinomas were strongly and diffusely labeled with the antibody to claudin 18 had a significantly better survival than did patients whose carcinomas were weakly labeled or not labeled [10]. However, analysis using TCGA-PAAD and GEO series in this study showed that the expression of CLDN18 plays no role in predicting the

survival of GC patients. These contradictory results can be explained by the heterogeneity of the patients (the majority included had with stage II cancer) and small samples, as well as the difference between mRNA and protein detection for expression of CLDN18.

Conclusions

The expression of CLDN18 was restricted to normal lung and stomach tissues but was aberrantly activated in PC. Therefore, claudin 18 may be a candidate biomarker and therapeutic target for PC. In addition, the regulatory mechanisms of CLDN18 may highlight key pathways in normal phenotypic maintenance and in pancreatic carcinogenesis. In-depth experiments and well-defined detection approaches are needed to investigate molecular mechanism, to develop targeted agents and to screen for patients suitable for treatment.

Abbreviations

CLDN: claudin; PC: pancreatic cancer; ADCC: antibody-dependent cellular cytotoxicity; CDC: complement-dependent cytotoxicity; GC: gastric cancer; TCGA: the Cancer Genome Atlas; GEO: Gene Expression Omnibus; HPA: Human Protein Atlas; CREB: cyclic AMP-responsive element binding protein; GEPIA: Gene Expression Profiling Interactive Analysis; GTEx: Genotype-Tissue Expression; PAAD: pancreatic adenocarcinoma; CNAs: copy number alterations; HRs: hazard ratios; CIs: confidence intervals; EGFR: epidermal growth factor receptor.

Declarations

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Author contributions

The study was conceived by Jian Li. Data analysis and figure preparation were conducted by Yao Zhang and Jian Li. The statistical analysis was performed by Jian Li. Jian Li and Dengmin Hu wrote the paper and all authors provided critical contributions to the write-up and approved submission.

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Availability of data and materials

Data supporting the results reported in this article can be obtained from individual public database.

Ethics approval and consent to participate

This study was conducted according to the principles expressed in the Declaration of Helsinki. All information in this study was retrieved from public datasets; therefore, written informed consent was not necessary. This study meets the publication guidelines provided by the individual public datasets.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1. *CLDN18* expression difference between PC and normal tissues using series from GEO

Series	Country	Year	Platform	Cancer (N)	Normal (N)	Probe ID	logFC	*P* value
GSE15471	**Romania**	2009	GPL570	39	39	221133_s_at	1.61	2.91e-04
						221132_at	1.99	7.49e-05
						214135_at	2.09	2.62e-05
						232578_at	3.00	2.45e-06
GSE16515	USA	2009	GPL570	36	16	221133_s_at	4.41	4.95e-06
						221132_at	4.04	2.73e-05
						214135_at	5.15	7.21e-07
						232578_at	5.26	1.19e-06
GSE28735	USA	2012	GPL6244	45	45	8082928	2.13	2.41e-08
GSE60979	Norway	2015	GPL14550	49	12	A_33_P3240752	4.48	2.02e-04
GSE62165	Belgium	2016	GPL13667	118	13	11751677_a_at	2.00	7.18e-03
						11752402_x_at	2.26	4.75e-03
						11722312_a_at	2.88	2.16e-03
GSE62452	USA	2016	GPL6244	69	61	8082928	1.72	1.16e-08
GSE91035	USA	2016	GPL22763	25	23	A_33_P3240752	2.57	1.51e-02

PC: pancreatic cancer; FC: fold change

Figures

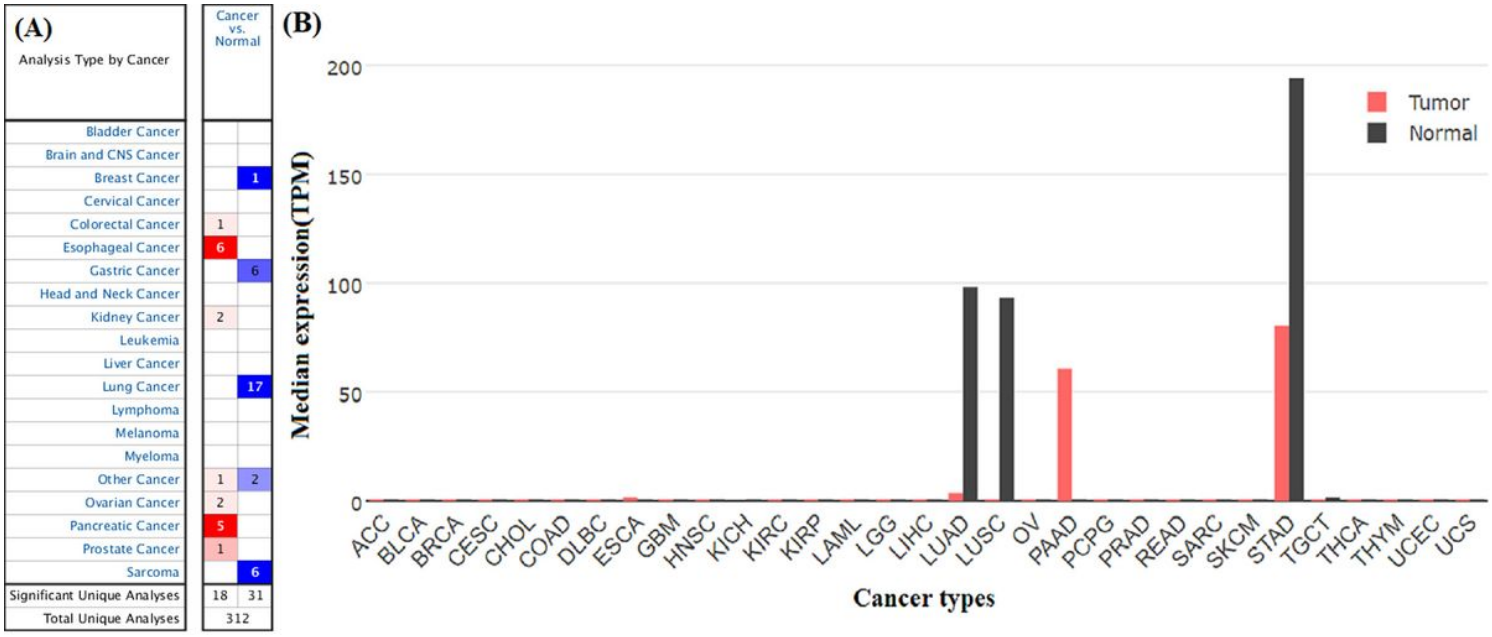

Figure 1

Gene expression profiles of CLDN18 across all tumor samples. (A) Analysis using the Oncomine database. The cell number represents the number of datasets that meet the thresholds. The color intensity is directly proportional to the significance level of dysregulation. (B) Analysis using the GEPIA2 database. The height of the bar represents the median expression level. TPM, transcripts per kilobase of exon model per million mapped reads.

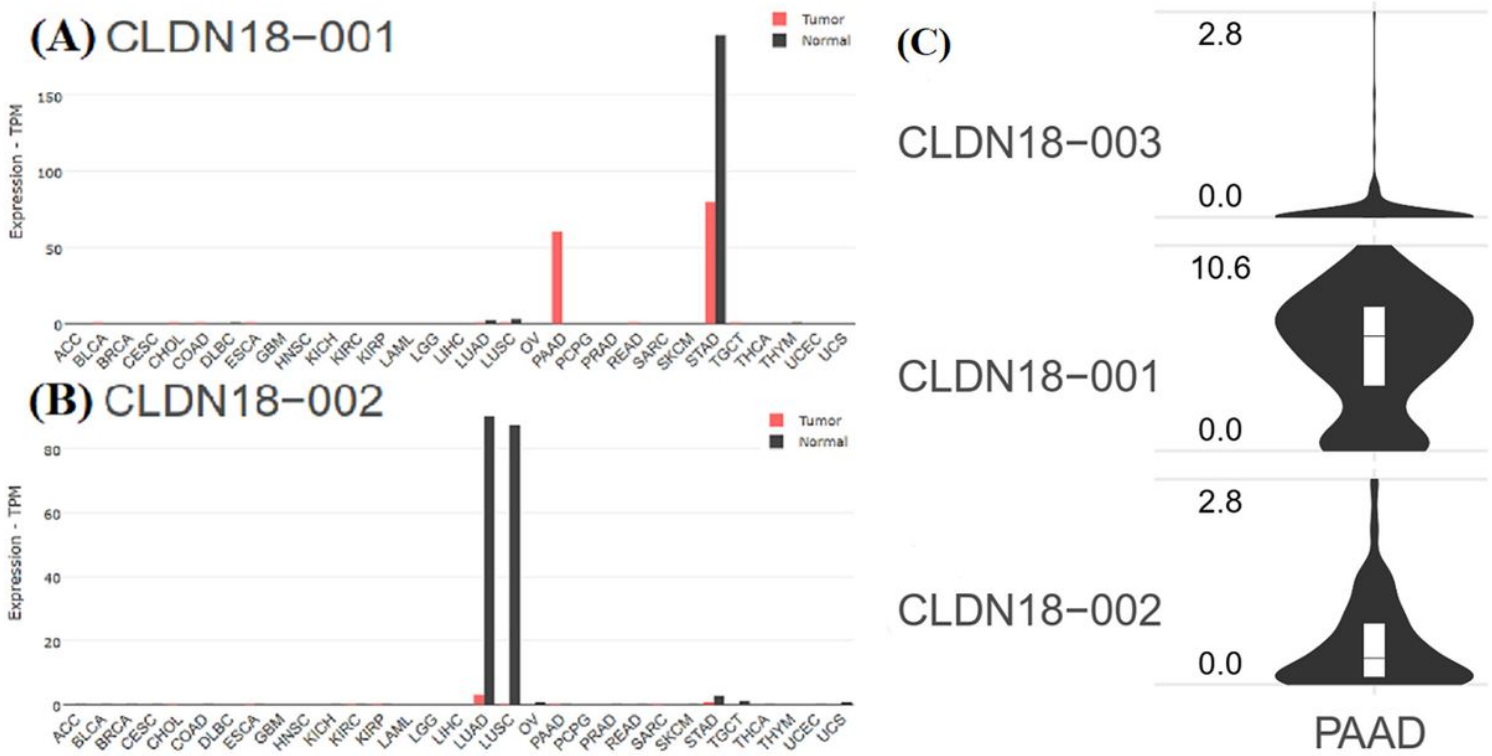

Figure 2

Transcription levels of isoforms of CLDN18 in the GEPIA2 database. (A) The transcription levels of CLDN18-001 across all tumor samples and paired normal tissues. (B) The transcription levels of CLDN18-002 across all tumor samples and paired normal tissues. (C) The transcription levels of three isoforms of CLDN18 in PC tissues. The height of the bars in (A) and (B) represents the median expression levels transformed by TPM. The Y axis of (C) represents the expression level transformed by log2(counts+1). TPM, transcripts per kilobase of exon model per million mapped reads.

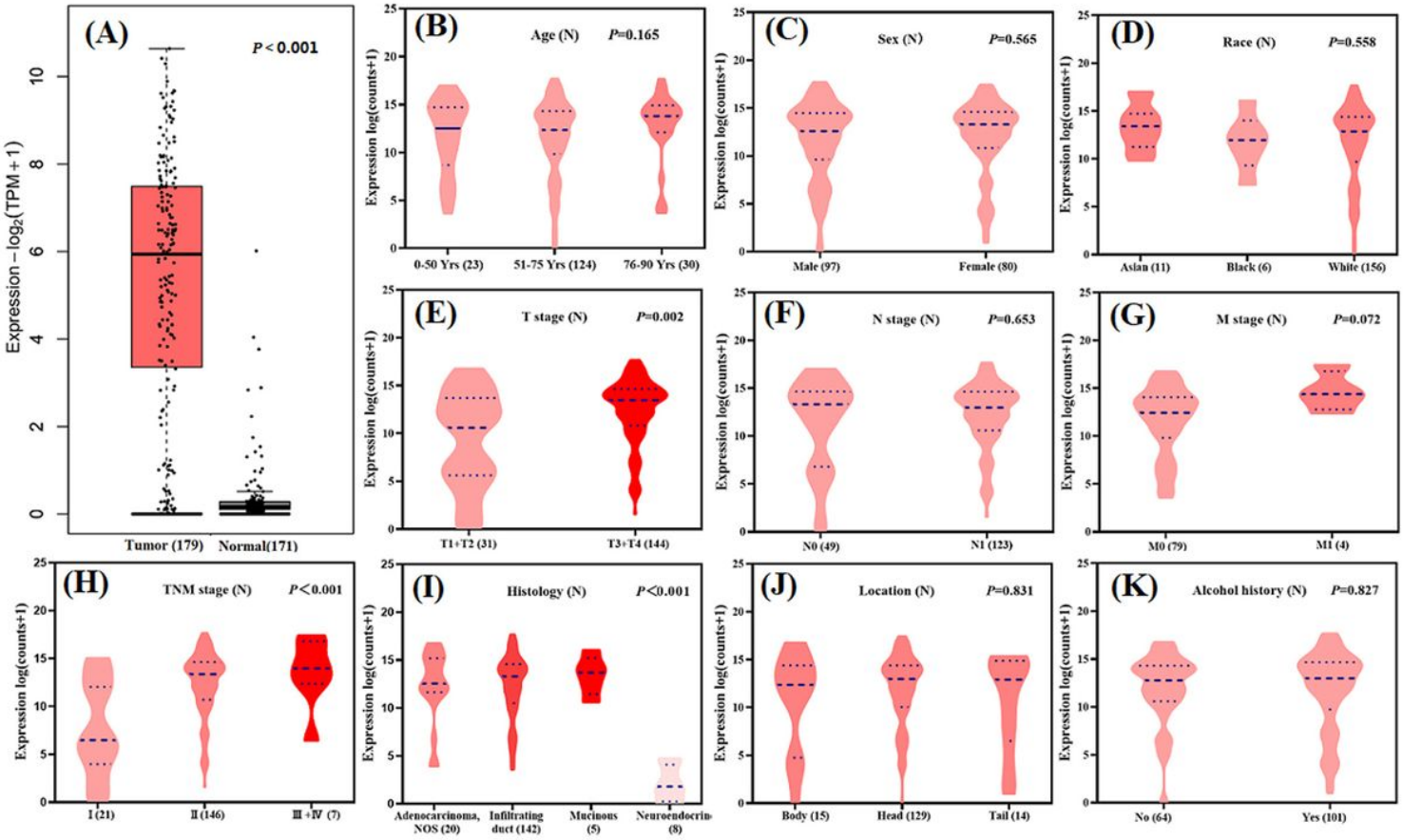

Figure 3

. (A) CLDN18 expression difference between PC and normal tissues using GEPIA2. The solid line in the box represents the median. (B-K) Correlation analysis of CLDN18 expression and clinicopathological characteristics (TCGA-PAAD). The dotted lines represent the median and quartiles. TPM, transcripts per kilobase of exon model per million mapped reads.

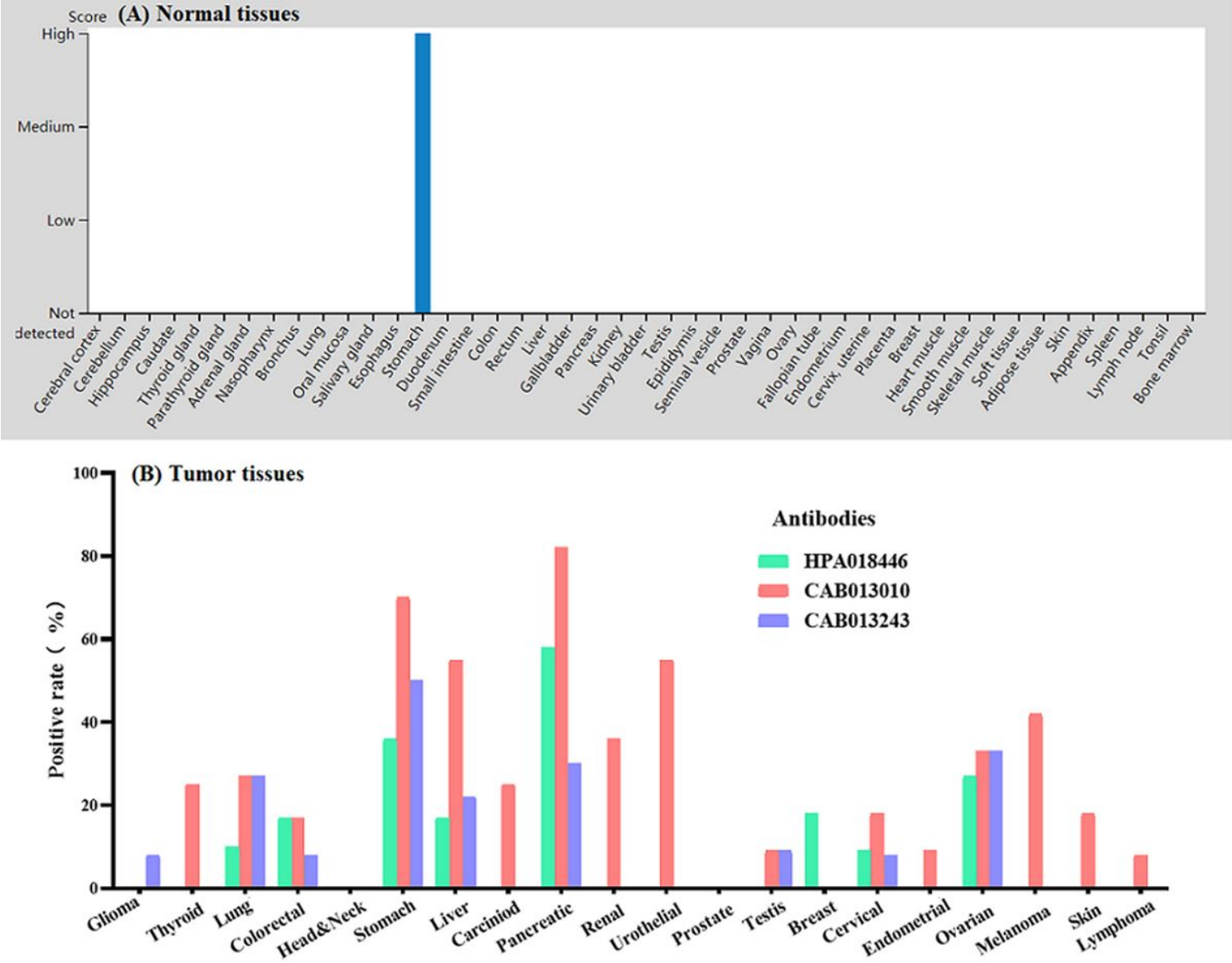

Figure 4

Protein expression of CLDN18 in normal and tumor tissues. (A) In normal tissues. (B) In tumor tissues.

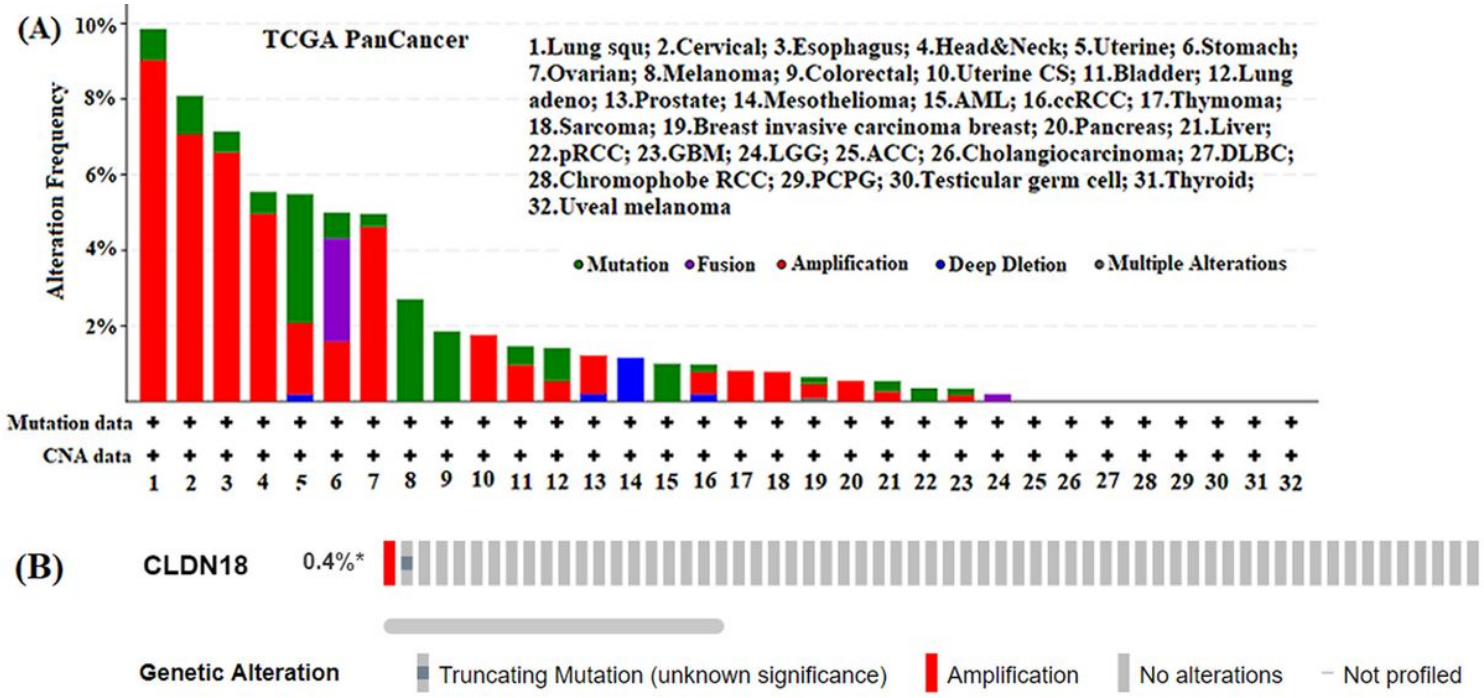

Figure 5

Genetic alterations of CLDN18 in cancers. (A) Frequency of genetic alterations in various types of cancer derived from TCGA PanCan datasets. (B) OncoPrint visual summary of variations in PC on a query of CLDN18. CNA, copy number alteration.

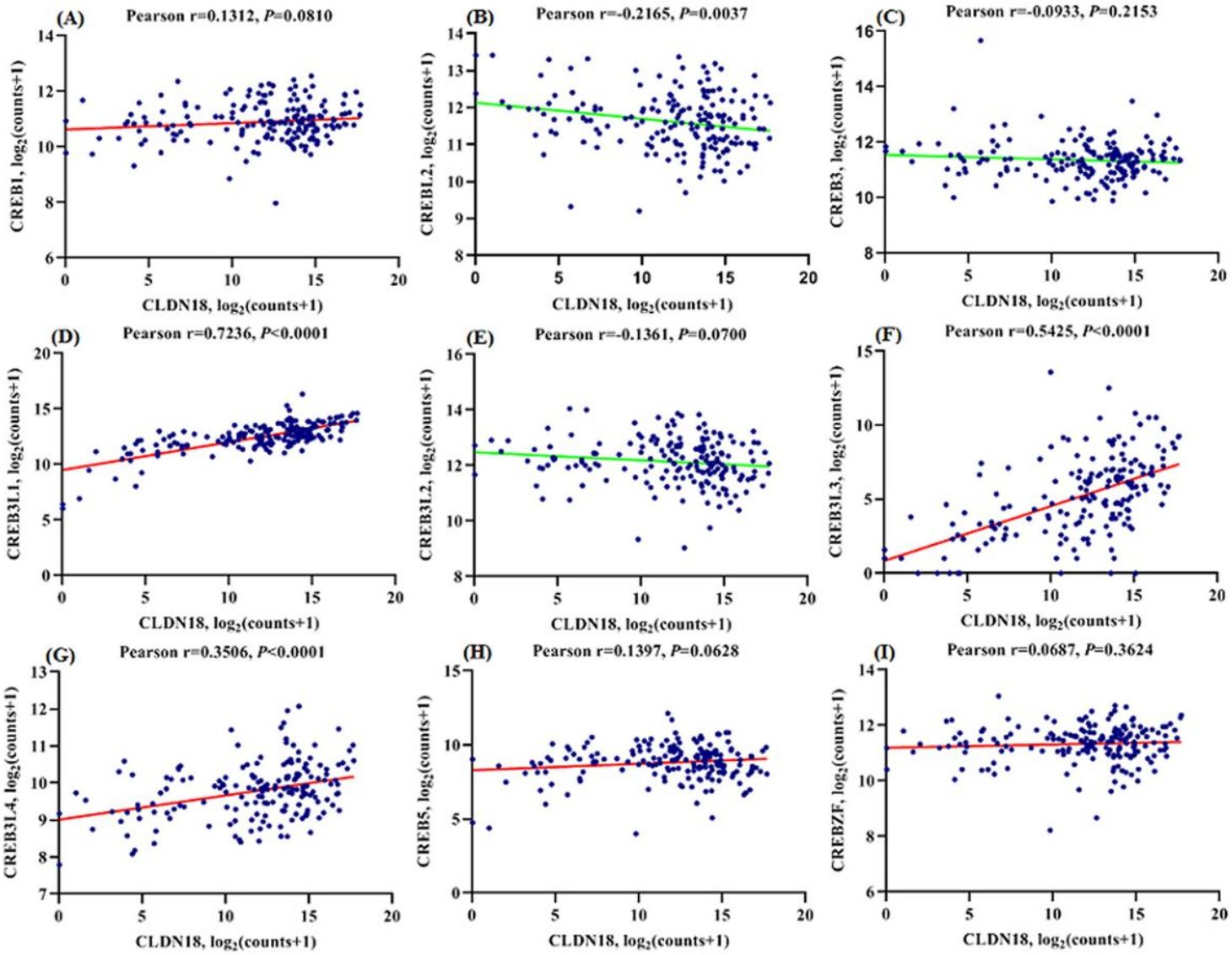

Figure 6

Coexpression analysis between CLDN18 and the CREB family.

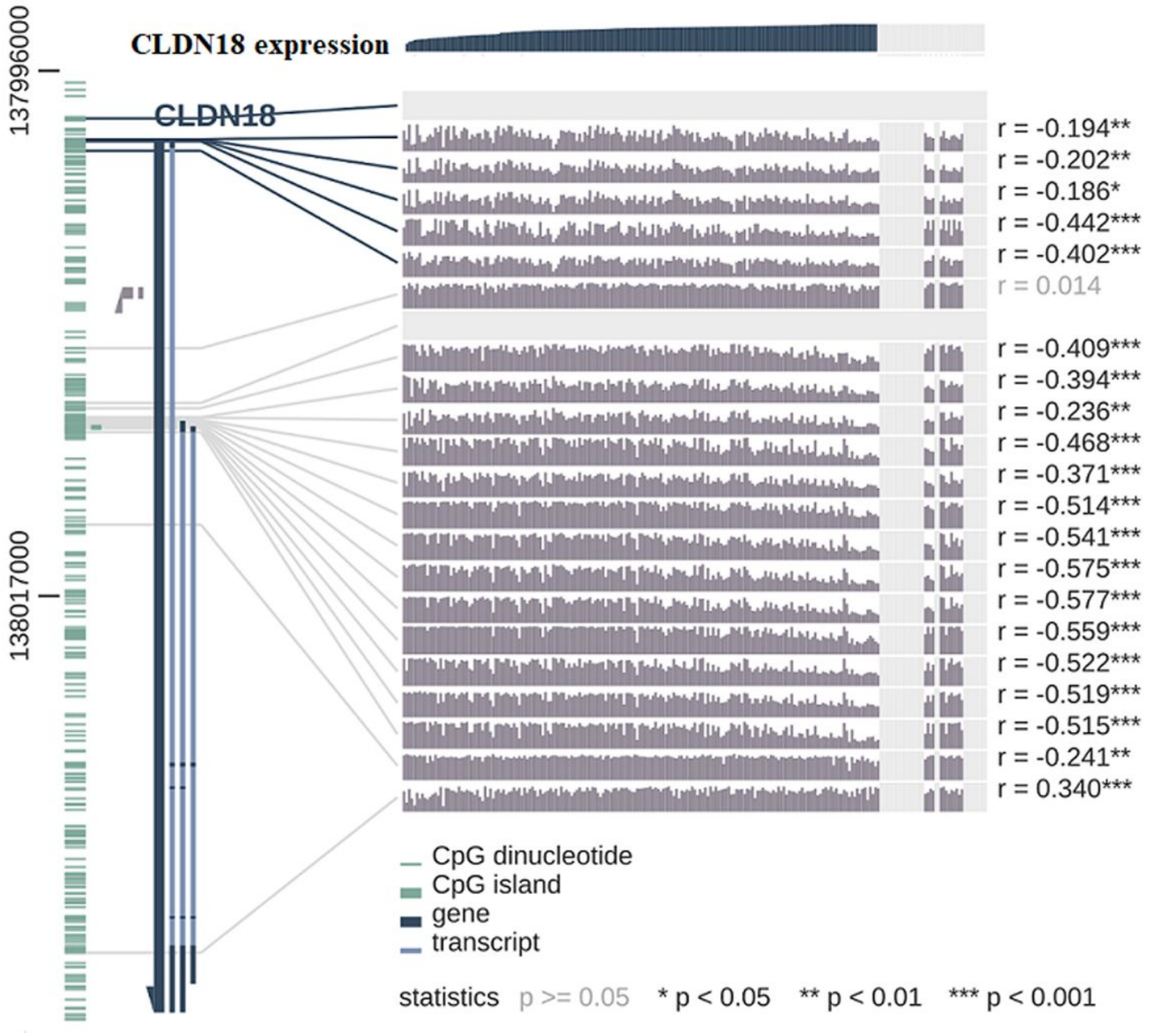

Figure 7

CLDN18 expression and methylation status in PC using MEXPRESS. On the right-hand side, the Pearson correlation coefficient and the P value are shown. The samples are ordered according to CLDN18 expression, with the highest expression on the right side and the lowest on the left. The height of the gray lines indicates the beta value for the probe. The probes localized in the promoter region of the gene are highlighted in dark blue.

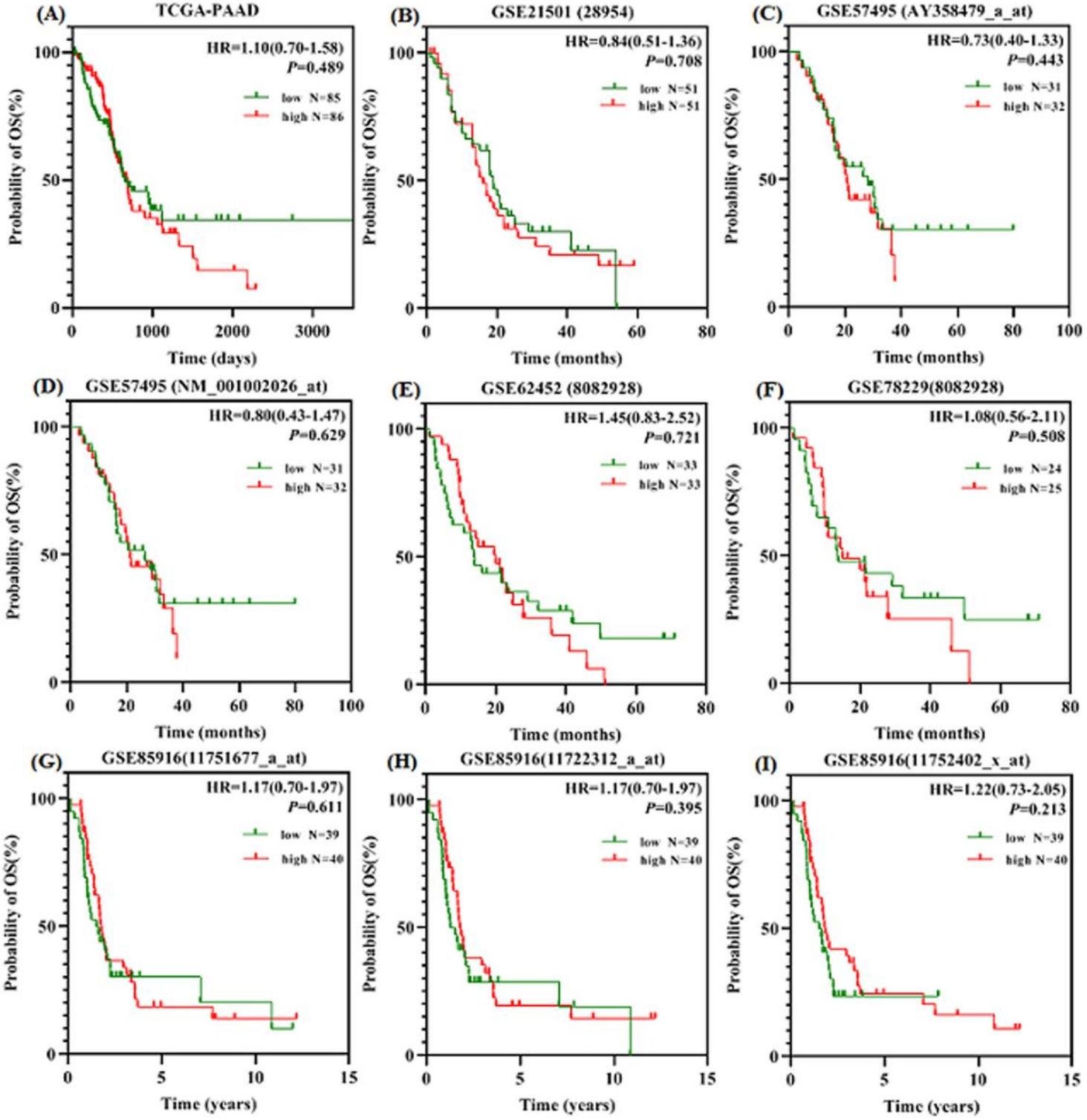

Figure 8

Overall survival of PC patients grouped by CLDN18 median cutoff.