Overexpression of a novel oncogene Cadherin 6 correlates with tumor progression and poor prognosis in gastric cancer

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Abstract

Background: Gastric cancer (GC) is one of the most common and fatal cancers worldwide and effective biomarkers aids in GC management and prognosis. Hence, we explored the role and function of cadherin 6 (CDH6) in diagnosis and prognosis of gastric cancer.

Methods: The expression level of CDH6 in GC tissue and normal gastric tissue were analyzed using multiple public databases. Gene set enrichment analysis (GSEA) was performed using The Cancer Genome Atlas dataset (TCGA). The diagnostic efficiency of CDH6 expression in GC patients was determined through receiver operating characteristic (ROC) curve analysis. The associations between clinical variables and expression of CDH6 were evaluated statistically and the prognostic factors for overall survival were analyzed by univariate and multivariate Cox regression. Forty-four GC tissues, corresponding adjacent normal tissues (n=20), and detailed clinical information were collected from Tianjin Medical University General Hospital, CDH6 expression level was detected for further validation.

Results: CDH6 was upregulated in GC samples compared with normal gastric tissue, and GSEA identified the citrate cycle tricarboxylic (TCA) cycle, extracellular matrix (ECM) receptor interaction, glyoxylate and dicarboxylate metabolism oxidative phosphorylation, and pentose phosphate pathway as differentially enriched in GCs. According to the area under the ROC curve (AUC) (AUC=0.829 in TCGA and 0.966 in GSE54129), CDH6 had high diagnostic efficiency. Patients with high expression of CDH6 was associated with higher T classification and worse prognoses than those with low CDH6 expression in GC. Univariate and multivariate Cox regression analysis showed that CDH6 was an independent risk factor for

overall survival (univariate: HR = 1.305, P = 0.002, multivariate: HR = 1.481, P < 0.001).

Conclusion: CDH6 was upregulated in GC and high CDH6 expression indicated higher T classification and worse prognoses. CDH6 could be a potentially independent molecular biomarker for diagnosis and prognosis of GC.

Keywords:

cadherin 6, gastric cancer, tumor progression, prognosis, oncogene.

Background

Gastric cancer (GC) is one of the most common and lethal cancers worldwide. It is the third most-common cancer type and second common cause of cancer-related deaths in China[1]. In addition, the most common type of stomach cancer is adenocarcinoma. With improvements in surgical techniques, traditional radiotherapy, chemotherapy, and the implementation of neoadjuvant therapy, satisfactory therapeutic effects have been achieved for early GC[2]. Because of the nonspecific symptoms of GC, most patients with the disease are diagnosed only when the tumor has reached an advanced stage, at which point the disease has likely metastasized, leading to poor prognosis, low 5-year overall survival rates, and missed opportunity of radical operation[3, 4]. Therefore, further research is urgently needed to identify biomarkers with high sensitivity and specificity for early and accurate diagnosis of GC in order to increase long-term survival of patients.

Cadherins (CDHs) are a multigene family of proteins that mediate homophilic calcium-dependent cell adhesion and are considered to play critical roles in morphogenesis by mediating specific intercellular adhesion and organization of the cytoskeleton[5]. In addition, CDHs can also serve as sensors of the surrounding microenvironment and as signaling centers for cellular pathways[6]. Recently, several studies have found that CDHs can participate in the promotion of tumorigenesis, tumor growth and malignant progression, diagnosis, and survival prediction of cancer, and even be therapeutic targets[7-9]. For example, transcriptional silencing or mutation of E-cadherin is correlated with familial diffuse GC, which may serve as biomarkers for early diagnosis[10]. Besides, CDH2 was considered a potential prognostic and predictive biomarker for the grading and treatment of gliomas[11].

Cadherin-6 (CDH6) is a class II CDH, mainly involved in the morphogenesis of the central nervous system and kidney[12, 13]. Previous studies have declared that CDH6 can be abnormally upregulated and promotes epithelial mesenchymal transition (EMT) and cancer metastasis by restraining autophagy in papillary thyroid carcinomas[14, 15]. In addition, increased expression of CDH6 has been reported in several malignancies, including nasopharyngeal cancers, ovarian cancers, oral squamous cell cancers, and renal cancers, and is associated with lymph node metastasis and poor prognosis[16-18]. However, the clinical significance, diagnosis, and prognostic value of CDH6 in GC remains unclear, and further investigations are required to understand whether it can be used as a novel biomarker for GC diagnosis, prognosis, and as a therapeutic target in GC. In this report, we provide a comprehensive and systematic analysis of CDH6 expression level in GC tissues as compared to normal gastric tissues. To further study the function of CDH6, we used Gene Set Enrichment Analysis (GSEA) to evaluate the biological pathways involved in GC pathogenesis. Survival analyses (Cox regression analyses) were also performed to assess the prognostic value of CDH6 expression and other clinicopathological features.

Methods

Data collection

The gene expression profiles and associated clinicopathological data of patients with gastric adenocarcinoma were downloaded from the TCGA Genomic Data Commons data portal (https://portal.gdc.cancer.gov/repository) on 25 May, 2020. RNA-Seq gene expression HTSeq-FPKM data for 343 cancer tissue samples and 30 normal, adjacent tissue samples were

collected for further analysis. To ensure the accuracy of the TCGA results, we systematically retrieved the GEO (Gene Expression Omnibus) microarray, and five datasets (GSE50710, GSE70880, GES109476, GSE118916, GSE54129) were obtained. Oncomine database (http://www.oncomine.org), a web-based microarray database, was used to analyze the expression level of CDH6 in gastric cancer tissues and normal control samples.

Gene set enrichment analysis

The GSEA is a computational method to detect whether *a priori* defined gene sets have statistically significant and consistent differences between two biological states[19]. Datasets and phenotype label files from TCGA were generated and uploaded into the GSEA software. The phenotype labels were CDH6-high expression and CDH6-low expression. Gene set permutations were conducted 1000 times for each analysis. Gene sets with |ES| > 0.6, FWER P values < 0.05 were considered as enriched.

Validation using cell culture and clinical samples

GC cell lines NCI-N87 was purchased from National Experimental Cell Resource Sharing Platform (Beijing, China). Two GC cell lines HGC-27, MGC-803 and one normal gastric epithelial cell line GES-1 were collected form Laboratory of General surgery, Tianjin Medical University General Hospital (Tianjin, China). Cells were cultured in 1640 medium (Thermo Fisher Scientific, Waltham, MA, USA) containing 10% FBS (Thermo Fisher Scientific), 80 $U \cdot mL^{-1}$ penicillin and 0.08 $mg \cdot mL^{-1}$ streptomycin under a humidified atmosphere with 5% CO2 at 37°C. The culture medium was replaced every 48 h. The cells were screened periodically for mycoplasma contamination using One-step Quickcolor Mycoplasma Detection Kit (Shanghai,

China). Forty-four gastric cancer tissues and 20 normal adjacent tissue samples were collected from the Tianjin Medical University General Hospital. Corresponding clinical characteristics (age, stage, grade, distant metastasis status, lymph node status, survival time, and survival status) were also collected and analyzed. Written informed consent was obtained from all patients; the hospital ethics review committees approved this study.

Quantitative real-time-polymerase chain reaction (qRT-PCR)

Total RNA was extracted from cells using RNAprep Pure Tissue kit (Tiangen, Beijing, China). Then, complementary DNA (cDNA) was reverse synthesized using FastKing gDNA Dispelling RT SuperMix for qPCR (Tiangen, Beijing, China). Real-time PCR was performed using the 2×SYBR Green qPCR Master Mix (Tiangen, Beijing, China). The relative expression level of mRNA was calculated using the $2^{-\Delta\Delta Ct}$ method. The primer sequences were as follows: CHD6, forward (5'-TAT CAG ACC CCG ACC ATA TT-3') and reverse (5'-GAC CAT AAA CTT CCG GCT T-3'); β-actin, forward (5'-CTC CTC CAC CTT TGA CGC TG-3') and reverse (5'-TCC TCT TGT GCT CTT GCT GG-3'). All gene primers were obtained from Aoke Dingsheng Biotechnology (Beijing, China). The thermocycling conditions comprised an initial denaturation at 95℃ for 15 s, followed by 40 cycles of 53℃ for 30 s and 72℃ for 30 s (40 cycles).

Survival analysis and COX analysis

We divided the TCGA samples into two groups by the median value of *CDH6* gene expression to construct the survival curve. Univariate and multivariate Cox analysis were used to

investigate the role of CDH6 expression and other clinical characteristics (age, stage, grade, distant metastasis status, and lymph node status) in overall survival. In addition, survival analysis was directly verified using the Kaplan–Meier Plotter (http://kmplot.com/) online.

Statistical analysis

R3.5.2, Bioconductor (https://www.bioconductor.org/) and GraphPad Prism 8 were used for statistical analysis. Survival curves were plotted using the Kaplan–Meier method and were compared using the log-rank test. Cox regression analyses were completed by using the 'survival' R package. The relationship of clinical pathologic features and CDH6 expression were completed using Wilcox test and Kruskal–Wallis test. $P<0.05$ was considered to indicate statistical significance.

Results

CDH6 is over expression in GC

According to TCGA, CDH6 was over expressed in 343 GC tissues as compared to the 30 normal tissues (P=2.16e-09, Fig. 1a) and in 25 GC tissues compared to the matching paired normal tissues (P=1.069e−05, Fig. 1b). Meanwhile, we analyzed the differential expression of CDH6 in 111 GC tissues compared with 21 normal volunteer gastric tissues from GSE54129 (P=1.413e-11, Fig. 1c) and in 50 GC tissues compared with paired gastric tissues from the four datasets (GSE50710, GSE70880, GES109476, GSE118916) (P=0.035, Fig. 1d). According to the Oncomine database, CDH6 was upregulated in GC tissues including Cho Gastric, Cui Gastric, DErrico Gastric, and Wang Gastric (P=0.034, Fig. 1e). To further verify CDH6 expression, we measured the expression level of CDH6 in normal gastric epithelial cell lines

(GES-1), three GC cell lines (HGC-27, MGC-803, NCI-N87) ($P<0.05$, Fig. 1f) and 20 paired gastric cancer tissues by qRT-PCR ($P<0.05$, Fig. 1g); all were statistically significant.

GSEA identifies functions and signaling pathways

To analyze biologic characteristics shared by the different CDH6 expression levels and predict the functions and pathways in which CDH6 may be involved, we performed the GSEA assay. GO analysis indicated that ATP metabolic process, cellular respiration, inner mitochondrial membrane protein complex, intrinsic component of the mitochondrial inner membrane, mitochondrial matrix, mitochondrial protein complex, mitochondrial respiratory chain complex assembly, and mitochondrial transmembrane transport, oxidoreductase complex, ribosome biogenesis were mainly enrichment items (Fig. 2a). In addition, KEGG analysis found that the TCA cycle, glyoxylate and dicarboxylate metabolism, oxidative phosphorylation, and pentose phosphate pathway were significant enrichment items in the CDH6 high-expression phenotype. On the other hand, ECM receptor interaction was significant in the CDH6 low-expression phenotype (Fig. 2b).

CDH6 has high diagnostic value in GC

To evaluate the diagnostic value of CDH6, the receiver operating characteristic (ROC) curve was constructed using the expression data from 343 GC tissues and 30 normal tissues from TCGA. The area under the ROC curve (AUC) was 0.829 [95% confidence interval (CI): 76.9–89.0%], the sensitivity was 61.8% (95%CI: 56.6–66.8%), and the specificity was 93.3% (95%CI: 78.7%–98.8%) (Fig. 3a). For further verification, we generated another ROC curve using the expression data from 111 GC patients and 21 healthy individuals from GSE54129. The AUC

was 0.966 (95%CI: 0.938–0.994), sensitivity was 89.2% (95%CI: 0.820–0.937), and the specificity was 95.2% (95%CI: 77.3%–99.8%) (Fig. 3b). Collectively, both ROCs indicated the potential diagnostic value of CDH6 in GC. To evaluate the diagnostic value of CDH6 in early detection of GC, the ROC curve was constructed using the expression data from 50 stage I tissues and 30 normal tissues from TCGA (Fig. 3c [AUC=0.747, 95%CI: 0.641–0.853, p=0.0002]).

High CDH6 expression is associated with tumor progression

We analyzed the clinical pathologic data of 343 patients with GC from TGCA, including the patients' age, sex, clinical stage, histological grade, and tumor-lymph node-metastasis (TNM) classification. As shown in Figure 4a, expression of CDH6 was only significantly associated with T stage (p=0.046). And CDH6 was unrelated to age, sex, clinical stage, histological grade, lymph node metastasis, and distant metastasis. The same analysis outcomes were observed in the 44 patients with GC from Tianjin Medical University General Hospital (T1-2 VS T3-4, p=0.031) (Fig. 4b).

Survival analysis and Cox analysis

As shown in Figure 5a, high expression of CDH6 was closely associated with poor overall survival (P<0.01). This relationship was further validated by the online Kaplan–Meier Plotter (http://kmplot.com/) (Fig. 5b, p<0.01). The above results were re-verified in the 44 patients with GC (Fig. 5c, p<0.05). The univariate Cox analysis revealed that high CDH6 expression was significantly associated with poor overall survival (hazard ratio [HR]: 1.305, 95%CI: 1.102–

1.544, P=0.002); as well as age (HR: 1.023, 95%CI: 1.004–1.044, P=0.020); stage (HR=1.451, 95%CI: 1.144–1.841, P=0.002); and N stage (HR=1.305, 95%CI: 1.102–1.544, P=0.002) among GC patients (Fig. 6a). Besides, multivariate Cox analysis indicated that high CDH6 expression remained an independent risk factor for overall survival with an HR of 1.481 (95%CI: 1.206–1.819, P<0.001), as well as age (HR=1.040, 95%CI: 1.018–1.063, P<0.001) among GC patients (Fig. 6b).

Discussion

The primary function of CDH family is considered to determine the cell-cell and cell-matrix adhesion, define the structure and organization of the cellular interactions with the surrounding microenvironment[6]. In cancer, any disfunction in the cell-cell and cell-matrix adhesion are related to tumor progression, lymph node infiltration, and distant metastases[20]. Tumor growth, malignant progression, and distant metastasis were associated with cellular adhesion molecules such as CDHs, integrins, and immunoglobulins[21, 22]. With the deepening of the research on cadherins, several studies have indicated that cadherins not only have structural function but also can regulate complex biological signals and participate in the promotion of tumorigenesis, tumor growth, and malignant progression. For example, CDH1 gene is associated with familial diffuse gastric cancer and the process of EMT[10, 23]. In glioma, low CDH2 expression had an improved prognosis and benefited from temozolomide therapy[11]. In thyroid cancer cell line, downregulated CDH3 inhibited proliferation, migration, and invasion[24].Cadherin-6 (CDH6) is a transmembrane glycoprotein and a member of the CDH family. Recent studies have shown that CDH6 can be aberrantly over expressed in cancer. In thyroid cancer, CDH6 expression is

strongly associated with EMT, metastatic behavior, and worse outcome[14]. In other malignancies, CDH6 was reportedly associated with tumor growth and poor prognosis [16, 18]. However, studies regarding the function of CDH6 in GC are inadequate.

Our research showed that CDH6 was highly expressed in GC compared with normal gastric tissues based on multiple public databases, which was verified in cell lines and 20 paired gastric tissues by qRT-PCR. To analyze biologic functions of CDH6 in GC, GSEA was completed. As shown in GSEA (Fig. 2a, 2b). ECM receptor interaction was enriched significantly in CDH6 low expression groups, which was consistent with several previous bioinformatic studies of patients with GC[25-27]. This result might be related to the decrease of intercellular adhesion and instability of cellular interactions. In addition, CDH6 was closely related to energy metabolism such as citrate cycle TCA cycle, glyoxylate and dicarboxylate metabolism, oxidative phosphorylation, and pentose phosphate pathway. In addition, GO analyses suggested that CDH6 might participate in the formation of mitochondrial membrane structure including the intrinsic component of mitochondrial inner membrane, mitochondrial matrix, mitochondrial protein complex, and mitochondrial respiratory chain complex assembly.

To evaluate the diagnostic value of CDH6, the AUC from TCGA was 0.829, and the AUC from GSE54129 was 0.966, which indicated that the diagnostic efficacy of CDH6 was credible. Some conventional biomarkers such as CEA, CA199, and CA72-4 that have shown limited diagnostic efficacy for early detection of GC [28]. However, CDH6 played a good diagnostic value for the early stage (stage I, AUC=0.747). In particular, CDHs have been found not only between the tumor cells but also in body fluids (mainly in blood), which indicated that CDH6 has high clinical diagnostic significance[29]. According to the associations of CDH6 and

clinical pathologic features and survival outcome, higher expression level of CDH6 was found more frequently in advanced tumors with advanced T stage and poor prognosis. Our univariate and multivariate Cox analyses indicated that the CDH6 expression level was a potential independent marker for poor prognosis in GC. Besides, survival analyses of the 44 patients with GC from Tianjin Medical University General Hospital and Kaplan–Meier plots all supported the same conclusion.

In this work, we mainly focused on the gene expression of CDH6 and clinical pathologic features and survival outcome. Although the Human Protein Atlas (HPA) showed that gene expression of CDH6 was consistent with protein expression results, further protein evidences and functional experiments also need to be completed.

Noticeably, a previous study reported that expression of CDH6 in the oral squamous cell carcinoma was relative to lymph node metastasis and poor prognosis[18]. *Gugnoni M, Sancisi V et al* reported that CDH6 was associated with the structure and function of mitochondria and promotes EMT and cancer metastasis in papillary thyroid carcinomas[14]. On the contrary, *Benjamin Goeppert* reported that CDH6 was a putative tumor suppressor and downregulation of CDH6 and association with poor patient outcome in cholangiocarcinoma[30]. These results alert a different role of CDH6 in specific tumors that needs to be further elucidated.

Conclusion

CDH6 was over-expressed in gastric cancer, and CDH6 high expression may be a potential diagnostic and prognostic molecular marker in GC. In addition, high expression CDH6 was

significantly associated with more advanced T stage and poor survival outcome.

Declarations

Availability of data and materials

The datasets generated during the current study are not publicly available since they will contain patient data and the informed consent agreement does not include sharing data publicly. An anonymized form of the data could be made available from the corresponding author upon reasonable request.

Abbreviations

CDH6: Cadherin 6

GC: Gastric cancer

ROC: Receiver operating characteristic curve

GSEA: Gene set enrichment analysis

TCGA: The Cancer Genome Atlas dataset

AUC: Area under the ROC curve

TCA: Tricarboxylic acid cycle

ECM: Extracellular matrix

GEO: Gene Expression Omnibus

EMT: Epithelial mesenchymal transition

qRT-PCR: Quantitative real-time-polymerase chain reaction

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Contributions

Zongxian Zhao and Shuliang Li performed the data analysis and assisted in writing the manuscript. Jun Wang and Hai Lin designed the study and assisted in writing the manuscript. Shilong Li assisted in language polishing. Weihua Fu read and approved the final manuscript.

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Ethics Declarations

Ethics approval and consent to participate

All patients were from Tianjin Medical University General Hospital, and approval was obtained from the hospital's ethics review committees.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Figure legends

Figure 1: a, CDH6 expression in 343 GC tissues and 30 normal tissues from TCGA; b, CDH6 expression in 25 GC tissues and matched normal tissues from TCGA; c, CDH6 expression in 111 GC tissues and 21 normal volunteer tissues from GEO (GSE54129); d, CDH6 expression in 50 GC tissues and matched normal tissues from GEO (GSE50710, GSE70880, GES109476, GSE118916); e, meta-analysis of CDH6 expression using Oncomine online analysis tools; f, CDH6 expression in normal gastric epithelial cell lines (GES-1) and three GC cell lines (HGC-27, MGC-803, NCI-N87) by qRT-PCR; F, CDH6 expression in 20 patients' GC tissues and matched normal tissues from Tianjin Medical University General Hospital.

Figure 2: Enrichment plots from gene set enrichment analysis (GSEA), a, GO analysis; b, KEGG analysis.

Figure 3: ROC curve for CDH6 expression in normal gastric tissue and GC, a, TCGA GC vs. normal tissue; b, GSE54129 GC vs. normal tissue; c, TCGA GC stage I vs. normal tissue.

Figure 4: a, Association of CDH6 expression with clinical variables from TCGA; b, CDH6 expression and clinical variables from Tianjin Medical University General Hospital

Figure 5: CDH6 expression and overall survival in GC patients in TCGA cohort (a), Kaplan–Meier Plotter (http://kmplot.com/) online (b), and Tianjin Medical University General Hospital (c).

Figure 6: a: The univariate Cox analysis of CDH6 and clinical data; b: multivariate Cox analysis of CDH6 and clinical data.

Figures

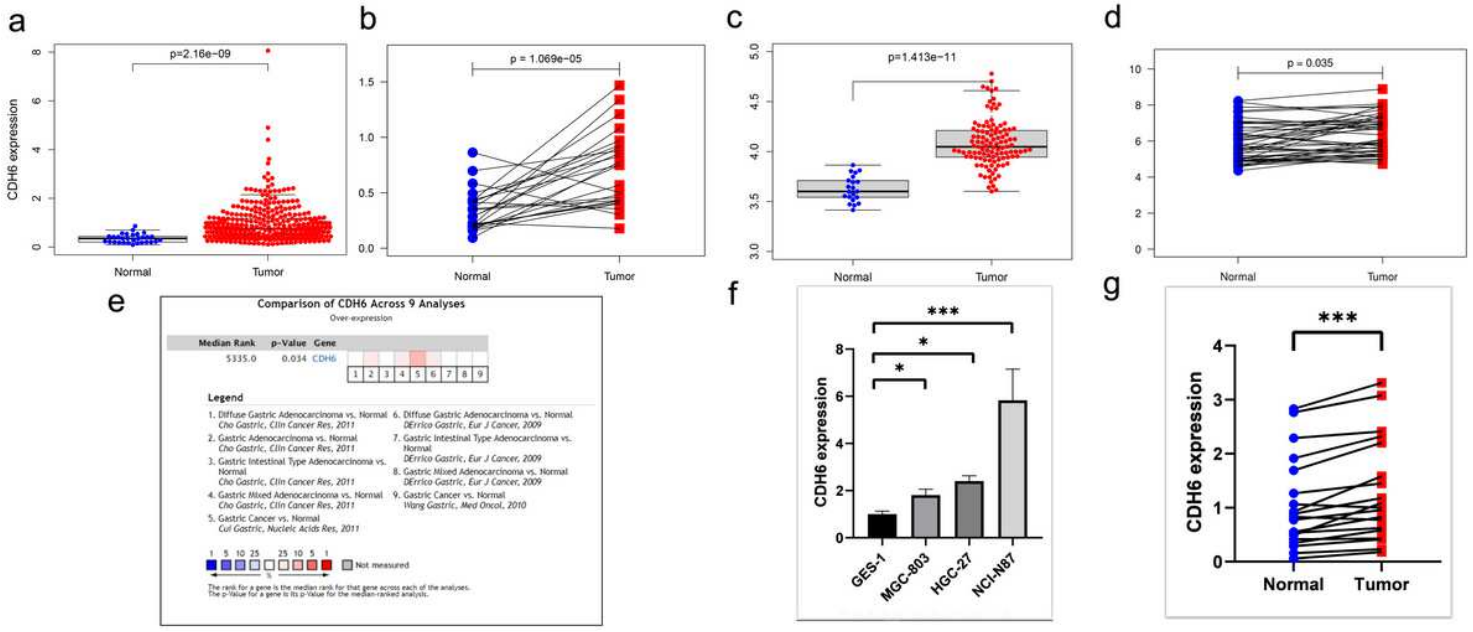

Figure 1

a, CDH6 expression in 343 GC tissues and 30 normal tissues from TCGA; b, CDH6 expression in 25 GC tissues and matched normal tissues from TCGA; c, CDH6 expression in 111 GC tissues and 21 normal volunteer tissues from GEO (GSE54129); d, CDH6 expression in 50 GC tissues and matched normal tissues from GEO (GSE50710, GSE70880, GES109476, GSE118916); e, meta-analysis of CDH6 expression using Oncomine online analysis tools; f, CDH6 expression in normal gastric epithelial cell lines (GES-1) and three GC cell lines (HGC-27, MGC-803, NCI-N87) by qRT-PCR; F, CDH6 expression in 20 patients' GC tissues and matched normal tissues from Tianjin Medical University General Hospital.

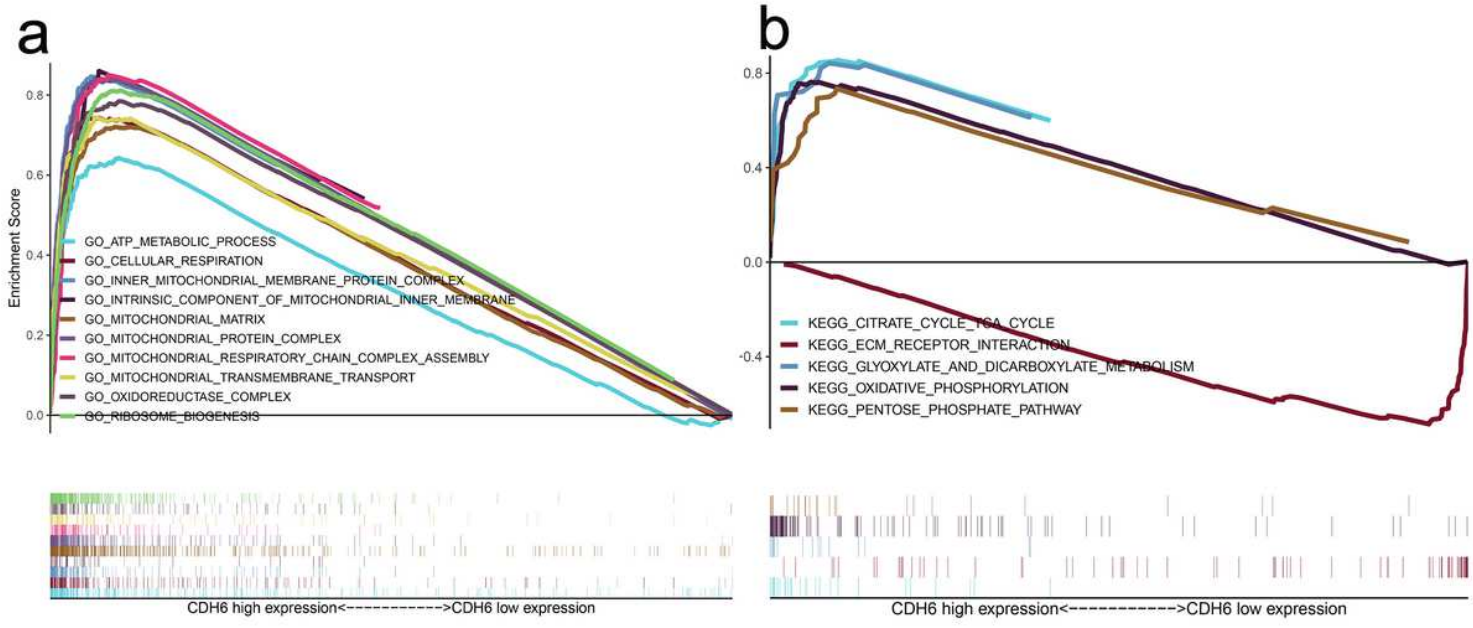

Figure 2

Enrichment plots from gene set enrichment analysis (GSEA), a, GO analysis; b, KEGG analysis.

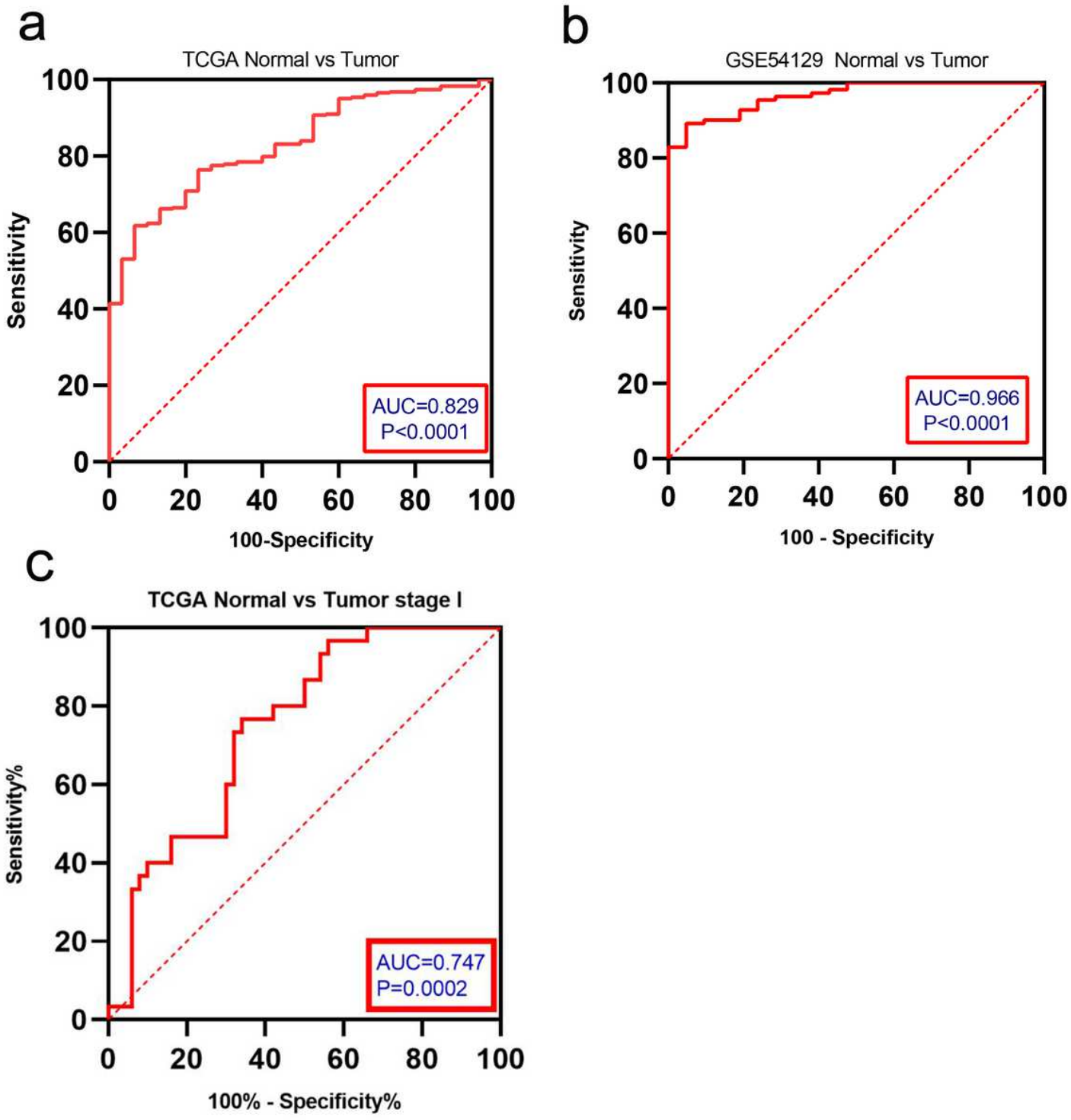

Figure 3

ROC curve for CDH6 expression in normal gastric tissue and GC, a, TCGA GC vs. normal tissue; b, GSE54129 GC vs. normal tissue; c, TCGA GC stage I vs. normal tissue.

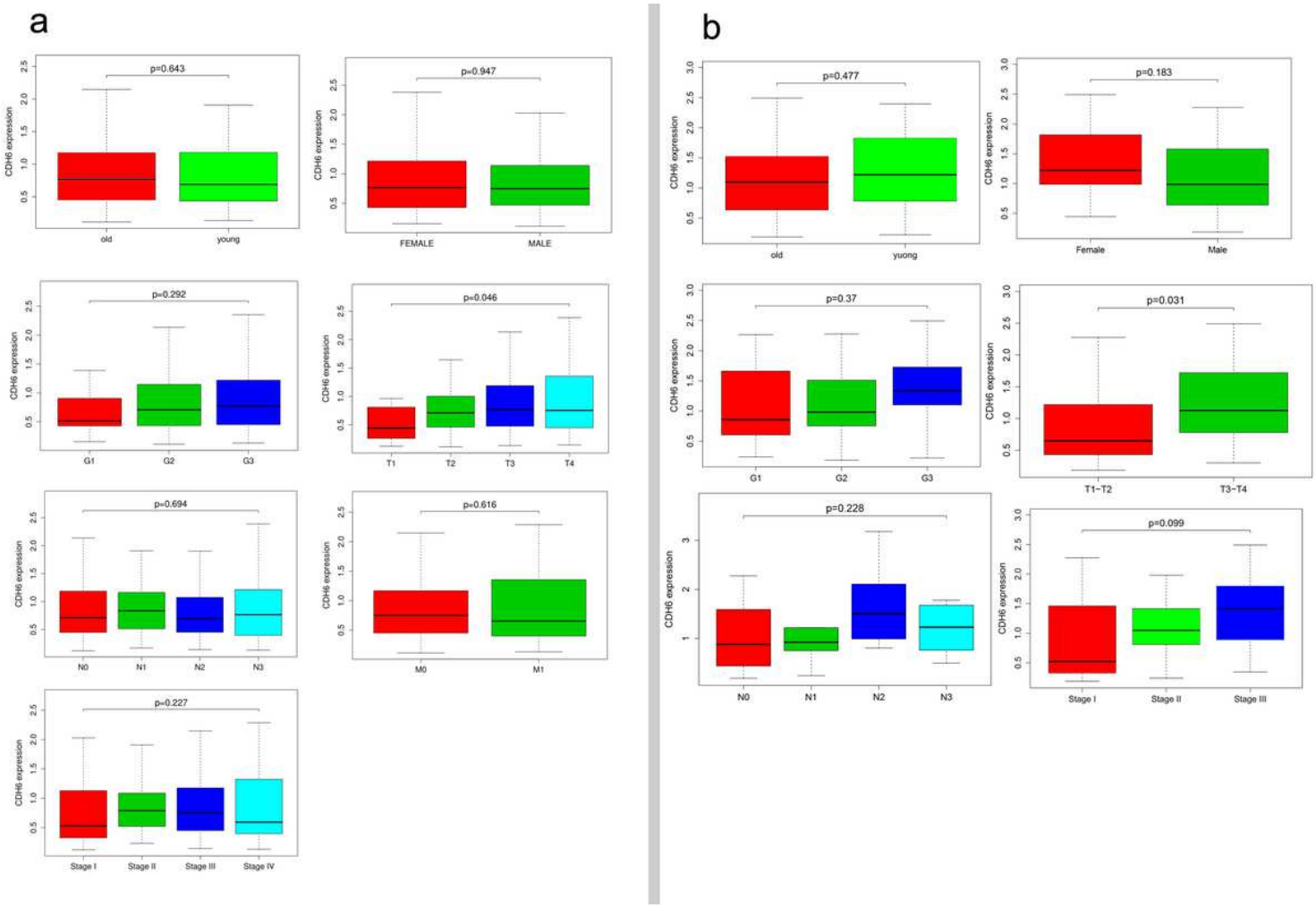

Figure 4

a, Association of CDH6 expression with clinical variables from TCGA; b, CDH6 expression and clinical variables from Tianjin Medical University General Hospital

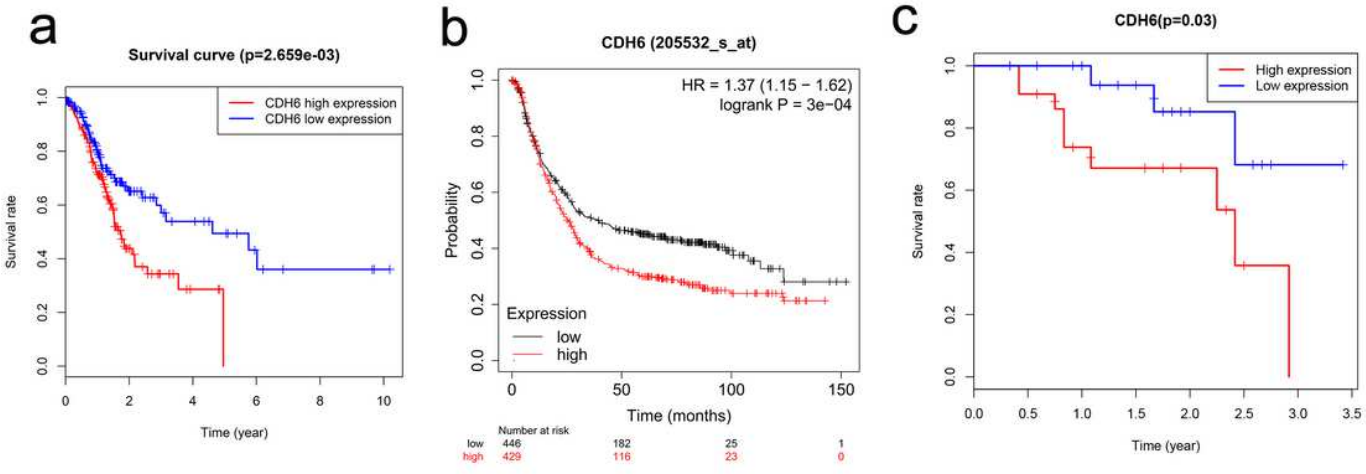

Figure 5

CDH6 expression and overall survival in GC patients in TCGA cohort (a), Kaplan–Meier Plotter (http://kmplot.com/) online (b), and Tianjin Medical University General Hospital (c).

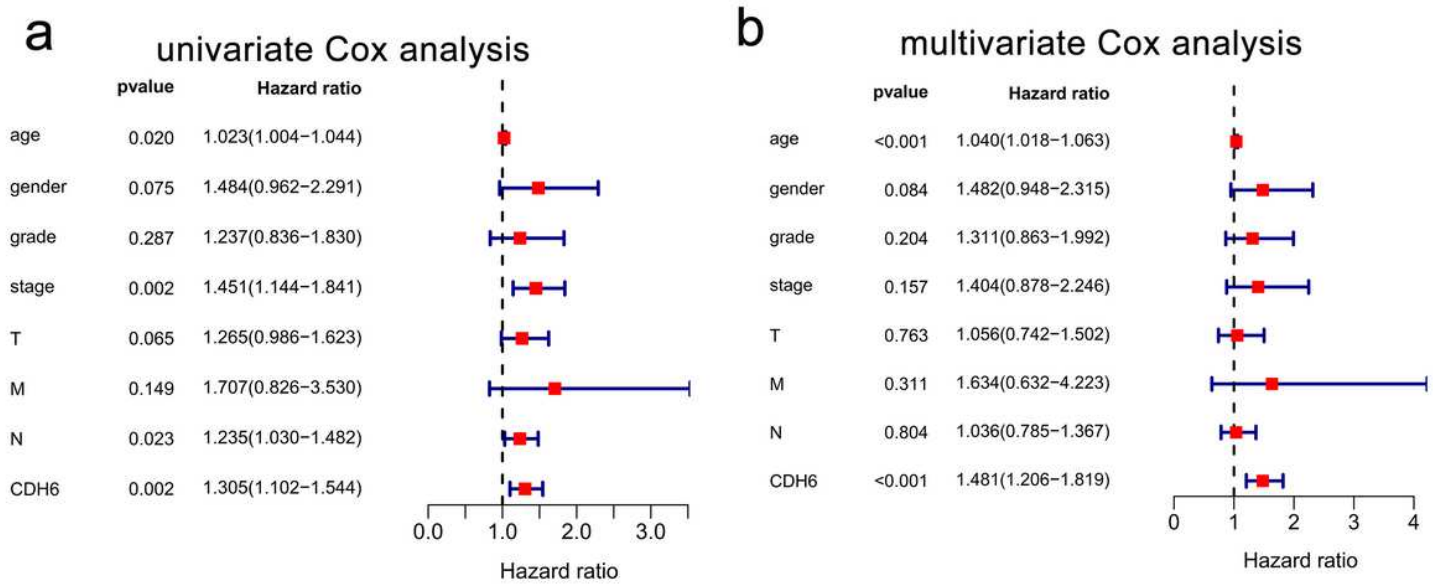

Figure 6

a: The univariate Cox analysis of CDH6 and clinical data; b: multivariate Cox analysis of CDH6 and clinical data.