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Promising prognostic value of Cadherin-related family member 2(CDHR2) in human cervical cancer

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Abstract

Background: In 2018, the incidence (6.6%) and mortality (7.5%) of cervical cancer remained at a relatively high level. The National Cancer Institute and the National Human Genome Institute jointly initiated the Tumor Genome Atlas (TCGA) project, using large-scale sequencing-based genome analysis technology to explore changes in various cancer-promoting and tumor suppressor genes in the development of cancer. Accordingly, it may be possible to screen new biomarkers that reliably detect the prognosis of cervical cancer using this database.

Methods: The corresponding survival data and expression profiling of cervical cancer patients in the TCGA database were compared, and differentially expressed genes (DEG) between cervical cancer and para-carcinoma tissue were identified using a volcano diagram and Venn analysis. Survival analysis verification was performed in the database, and the selected candidate gene was analyzed with Gene Ontology annotation in order to study its functions. Fluorescent quantification PCR was then used to determine the gene expression in fresh cervical cancer tissues and adjacent tissues, after which its correlation with clinical pathological variables were ascertained. Western blot was used for the screening of experimental cells. The biological roles of CDHR2 in the metastatic activities of cervix carcinoma were investigated in vitro through the Cell migration test and Transwell assays

Results: Cadherin-associated family member 2 (CDHR2) was selected as a candidate factor closely associated with the prognostic results of CC patients via the TCGA database. The outcomes of Gene Ontology study revealed that CDHR2 participated in numerous activities biologically, like cell agglutination and differentiative activities of cells. The experimental results confirmed that CDHR2 is upregulated in cervix carcinoma samples, with their clinical stages, tissue and lymph node metastasis being closely related. CDHR2 was also found to be positively correlated with poor clinical prognosis. Additionally, CDHR2 can promote the migrative and invasive activities of oncocytes.

Conclusion: there is an upregulation of CDHR2 in cervix carcinoma, and its increased expression might be an underlying biological marker in predicting inferior prognostic results in cervix carcinoma.

Keywords: cervical cancer, CDHR2, biomarker, prognosis

Introduction

Cervix carcinoma (CC) is the 4th most commonly seen tumor among females across the world and the 2nd most commonly seen in the non-developed world. In 2018, approximately 570,000 patients with CC were diagnosed along with 311,000 fatalities (Bray F. et al. 2018). Cervical cancer is mainly induced by HR-HPVgenotype infection (Celewicz, A. et al. 2018). Currently, treatment methods mainly include surgery, radiotherapy, and platinum-based chemotherapy (Bhatla N. et al. 2018). Moreover, up to one-third of diagnosed patients develop progressive or recurrent tumors (Munro A. et al. 2017). In contrast to 91.5% of local cervix carcinoma, the survival ratio within five years of metastasized cervix carcinoma stands at 16.5%; its mortality rate is very high due to late diagnosis

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Running title: CDHR2 overexpression serves as a biomarker in the poor prognosis of advanced cervical cancer

and poor prognosis (Ferlay J. et al. 2013), with reasons for poor efficacy being lack of sufficient tumor specificity and drug resistance (Yi Zhu. et al. 2017). Routine clinical diagnosis and pathological variables (such as FIGO staging) as well as certain biomarkers (such as serum squamous cell carcinoma antigen) are still widely used to predict prognosis (Kim S-W. et al. 2017). However, the sensitivity and specificity of these parameters are insufficient, thus limiting their clinical application. Ideal anti-cancer drugs should act on specific molecular abnormalities that drive malignant progression in order to be more effective. Hence, it's essential to develop promising biomarkers for prognosis and novel treatment targets in order to identify the prognosis of CC while being able to offer individualized treatment options.

In recent years, high-throughput technologies have been used as a very significant tool for many novel biomarkers, therapeutic targets, cancer stage, cancer early diagnosis, and prognosis prediction (Yang, L. et al. 2018). The combination of gene expression profiling and bioinformatics enables the comprehensive detection of mRNA expression changes in cervical cancer, identification of differentially expressed genes (DEG) related to prognosis, and research of related pathways. Previous studies have developed promising prognostic biomarkers for cervical cancer. In this study, we directly screened the mRNAs of different groups in the TCGA database according to the prognosis, and identified CDHR2 as a candidate factor.

CDHR2 (also known as PCDH24) belongs to the proto-cadherin (PCDH) family and is vital for calcium-dependent cell agglutination (Takeichi M. 1990) (Morishita H. et al. 2007). CDHR2 could be a cancer inhibitor by inducing contact suppression of tumor formation and is vital for contact suppression at the facies lateralis of epitheliums (Okazaki N. et al. 2002). It is a group of transmembrane proteins, composed of 1310 amino acids, including 7 cadherin repeat domains, 1 transmembrane region and PDZ binding domain in structure, which is mainly expressed in the human liver, kidney, colorectal and other organs (Ziyuan Xia. et al. 2019) (Brasch J. et al. 2012). Researches have revealed that CDHR2 is tightly associated with tumors and has different tumor suppressors or tumor promoting effects according to tumor types. However, no studies concerning whether the highly expressed CDHR2 is associated with the prognosis of cervical carcinoma exists. Therefore, our aim lies at observing the expression of CDHR2 in cervix carcinoma and analyzing its clinical significance and prognostic value in cervix carcinoma. Accordingly, the outcomes of this research might offer a theoretical and experimental foundation for the prognostic assessment and targeted therapy of cervical cancer.

Materials and methods

Microarray data

Microarray data were acquired via the TCGA (http://cancergenome.nih.gov/). This database contains the gene expression data of various cancers (Tomczak K. et al. 2015). In order to determine potential genes related to poor survival of cervical cancer patients, GEO2R analysis tools were employed so as to sift the DEGs in each data set of the OS microarray data (Table 1). In addition, the expression level of these DEGs was verified in the cervical cancer-related transcription profile. The P value below 0.05 was deemed as significance on statistics.

Investigating differentially expressed genes (DEGs)

The TCGA cervical cancer data set was standardized and functionally interpreted prior to carrying out the comparison. The normal cervical tissue group random was used as the control group and variance model (RVM) t-test. A volcano map was then drawn in RStudio (version 4.3.0). Then, hierarchical cluster analysis was applied in order to divide the data into two groups based on overall survival and screen out the DEG associated with the prognosis between cervical cancer and normal cervical epithelial cells. The differential expressions of the genes in all samples were illustrated using the Venn diagrams (http://bioinformatics.psb.ugent.be/webtools/Venn). The survival curve of the expression level of the two groups was then drawn from the collected overall survival rate information of all patients. The specific genes associated with the prognosis of cervical cancer patients were then identified.

GO annotation analysis

The Gene Ontology (GO) method (http://www.geneontology.org), which is broadly adopted to facilitate the vital annotation of different genes and their products in various organic matters, was employed to carry out a functional analysis of differentially expressed genes. The DAVID (The Database for Annotation, Visualization and Integrated Discovery) analysis method on the Internet was used to carry out function annotation cluster classification (Gene ontology, GO) and pathway analysis (KEGG pathway) on the screened DEGs to determine the main functions and signal pathways involved in the selected CDHR2 gene.

Clinical specimens

A total of 102 cervix carcinoma samples and adjacent healthy cervix tissue were analyzed from sufferers who were diagnosed and received surgical treatment in the Department of Gynecologic tumor ward of the Third Affiliated Hospital of Zhengzhou University (Zhengzhou, China) between 2015 and 2017. Our research was accepted by the Institutional Ethics Committee of the Third Affiliated Hospital of Zhengzhou University (ethics number: (2019) Medical Ethics Committee Review Approval No. 102). All specimens were acquired from the operations and reserved in liquid nitrogen (-80℃) immediately. Patients' clinical features and parameters are given in Table 2. All subjects offered informed consent and their follow-up data were accessible.

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

Accordingly, 102 cases of tumor and normal tissue samples from CC patients were used in this study. The entire RNA was obtained from tissues via Trizol solution (Invitrogen, America). Then, PrimeScript RT reagent kit (Takara, Shiga, Japan) was employed to convert RNA into cDNA special primers and SYBR Green qPCR Master Mix (Takara) to perform qRT-PCR study as per the specification from the supplier. The following primers were adopted: 5'-GGAGCCAAAAGGGTCATCATCTC-3' sense primer and 5'-GAGGGGCCATCCACAGTCTTCT-3' antisense primer for GAPDH, 5'-GTCTACCCGAATCCCCCACT-3' sense primer and 5'-CACATCCGCAGGGACTGCAT-3' antisense primer for CDHR2. GAPDH was taken as an interior control, and the comparative expression of the target gene as well as GAPDH were calculated using the $2^{\Delta Ct}$ method (Δ Ct = Δ Ct target gene-Δ Ct reference gene) (Yue C. et al. 2019).

Cell lines and transfection

Cell cultivation and transfection

The cervix carcinoma lineage cells of mankind (SiHa, HeLa) and normal cervical cells of mankind (H8) were purchased from the American Type Culture Collection (ATCC). All cell lines were placed in DMEM intermediate with 10% FBS and cultivated at 37°C, 5% carbon dioxide. SiHa cells were selected for transfection after detecting the expression of CDHR2 in each cell line. Small interfering RNA (siRNA) which targeted CDHR2 gene, and negative control siRNA (NC-siRNA) were prepared by Huzhou Hippo Biotechnology Co., Ltd. (Zhejiang, China). The SiHa cells were transfected and assigned into 2 groups: the negative control (NC) group (NC-siRNA transfection) and the si-CDHR2 group (CDHR2 siRNA transfection). The SiHa cells were suspended at 10 × 105 cells/ml after the treatment of 0.25% trypsin. The SiHa cells were placed into plates with six wells and treated with transfection when cell growth reached 50% confluence. Lipofectamine 3000 transfection reagent (thermo fisher, USA) was purchased for cell transfection.

Western blot

After the cells in all groups were collected and cultured, the entire protein of harvested cells was extracted using RIPA lysis buffer for half an hour through ice. Next, the protein abundance was determined using Bicinchoninic Acid Assay Kit (Thermo Fisher Scientific, America). SDS-PAGE was applied for the separation of the proteins, which afterwards were moved to PVDF films. Then, 5% skimmed milk in Tris-treated NS (normal saline) with 0.05% Tween-20 was utilized to block the films, which was then separately incubated with primary antibodies against FZD3, β-catenin, c-myc, and GAPDH, all of which came from CST in America. Afterward, the membranes with corresponding anti-rabbit secondary antibody (Boster, China) were incubated for 60 min at ambient temperature. The binds were measured via a strengthened chemical luminescence detection kit (Beyotime; Beijing; China).

Cell invasion test

The invasion ability of cells was observed through the Transwell test. The diluted matrigel (BD) was applied in the Transwell chamber. Then, the treated samples (1 × 105 cells/well) in the intermediate without sera were placed in the upper chamber with a complete intermedium containing serum in the lower chamber. After 36 h, the chamber was immersed in 4% paraformaldehyde and fixed for twenty-five minutes, dyed with 0.1% gentian violet and protected from light. It was then observed under an inverted microscope, and Image J software (version 1.51) was employed to calculate the cellular invasion.

Cell migration test

The migrative ability of cells was evaluated through the scratch test. The treated SiHa cells were put in plates with six wells (3×10^5 cells/well) and cultivated for 24h. Next, a sterilized 200 µl pipette tip was used to draw linesscore evenly on the bottom of the plate. posterior to three times of cleaning via PBS, the cells were continued to be cultured for 48 h, which was recorded via photos.

Statistical analysis

The entire bioinformation studies were carried

out in R software (Version 3.4, Austria). The χ2 test was applied to compare clinicopathologic factors, while the student t test or one-way analysis of variance approach was adopted to compare continuous variates. Kaplan-Meier curves and the log-rank test were adopted to study the survival status. The outcomes would be deemed as important on statistics if the P value was below 0.05.

Results

Gene expression analysis

RNA sequencing data of CC samples from the TCGA were used. The original data and clinical information of the CC patients were downloaded for further analysis. In order to determine potential genes associated with the poor survival of CC patients, patients were initially divided in the TCGA database into two groups according to OS: short OS (<543 days) and long OS (>543 days). Compared to para-carcinoma tissues, a total of 1382 differentially expressed DEGs (fold change> 2) in tumor tissues were observed via Volcano diagrams, including 936 upregulated genes and 446 downregulated genes (Figure 1A-C). Incorporating two groups of long and short overall survival into the analysis, the hierarchical cluster analysis depicted the expression of 162 DEGs related to survival (fold change> 2), which included 96 upregulated genes and 66 downregulated genes (P <0.05, FDR <0.05, FC> 2) (Figure 1D-F). The Venn diagram shows 3 crossed DEGs based on two screening methods, namely, cadherin-related family member 2 (CDHR2), γ-glutamyltransferase light chain 1 (GGTLC1) and cytokine dependent hematopoietic cell adaptor protein (CLNK) (Figure 1G). The expression of these 3 DEGs was analyzed through TCGA databases (Table 3).

High expression and poor prognosis of DEGs verified in database

To screen the prognostic implication of the recognized DEGs, the DEG expression was verified in the database. The Kaplan-Meier survival study unveiled that greater expressed CDHR2 or GGTLC1 was closely related to poorer survival (Figure 2A-B), and the expression of CLNK had no prognostic significance in CC patients (Figure 2C). Accordingly, CDHR2 and GGTLC1 were identified as two candidate genes for the poor prognosis of CC patients. A further analysis only on CDHR2 was then conducted, which has not previously been studied for the prognosis of cervical cancer.

Significant GOs and pathways

In order to forecast the role of CDHR2 in CC development, GO enrichment study and KEGG pathway study were carried out. GO analysis outcomes show that CDHR2 was involved in various biological processes, including brush border assembly, protein localization to microvillus, regulation of microvillus length, spanning component of plasma membrane, microvillus membrane, cell adhesion, epithelial cell differentiation, and so forth (Figure 1H).

Validation of clinical patients

To evaluate the clinical implication in the expression of CDHR2, samples from 51 CC patients were analyzed, in which the relationship between the mRNA expression and clinical pathologic parameters were ascertained (Table 4). The results showed that CDHR2 was significantly increased in CC tumor tissues (P <0.001) (Figure 3A). Subsequently, the associations between the CDHR2 expression and clinical pathologic parameters were studied. The results showed that the expression of CDHR2 was found to be related to FIGO staging (P=0.001). The FIGO advanced cervical cancer patients were found to have a higher expression of CDHR2 (Figure 3B), which was also found to be related to lymphatic metastasis (P=0.007), in which the expression of lymph node metastasis was relatively high (Figure 3C). Moreover, it was observed to be related to the level of tissue differentiative status (P=0.002), where the expression of low differentiation was relatively high (Figure 3D). Additionally, the higher expression of CDHR2 in cancer samples was related to worse prognostic results (P=0.005) (Figure 3E). The expression of CDHR2 was not significantly different between epidermoid carcinoma and adenocarcinoma (P>0.05). Overall, CDHR2 expression was vital for the prediction of prognosis for CC patients, as shown by the above findings.

In vitro cell experiment

The Western Blot study showed that the CDHR2 protein expression in the NC group was greater versus that in the si-CDHR2 group (Figure 4A). In the cell invasion test, the number of penetrating cells in the si-CDHR2 group was less than that within the NC group after 36 hours (Figure 4B), indicating that CDHR2 has the function of promoting wound healing and cervical cancer cell invasion. In the cell invasion test, the 48h migration rates of the NC group and the si-CDHR2 group were calculated to be 45.19% ± 0.03% and 27.54 ± 0.04% (Figure 4C). The experimental group was remarkably lower versus the NC group (P<0.05), showing that CDHR2 may facilitate the migrative ability of cervical oncocytes.

Discussion

In this study, mRNA study was carried out via the TCGA microarray analysis database to identify new genes associated with the poor prognosis of cervix carcinoma. The results indicated that three DEGs (CDHR2, GGTLC1, and CLNK) in CC patients were significantly upregulated. However, CLNK was not found to have prognostic value in cervix carcinoma as per the Kaplan-Meier curves. In this case, CDHR2 was screened out for clinical sample verification. The consistent results of the present clinical data along with the TCGA database support high CDHR2 expression as a novel candidate biomarker gene of poor prognosis in cervix carcinoma. Nevertheless, the specific process of CDHR2's carcinoma-promoting effect in cervical cancer remains unclear. We selected CDHR2 for GO analysis, which was found to be mainly related to brush border assembly, protein localization to microvillus, regulation of microvillus length, spanning component of plasma membrane, microvillus membrane, cell adhesion, epithelial cell differentiation. The first few biological functions are more manifested in the colorectum. Cervical cancer is an epithelial cell lesion, and the function of CDHR2 may be more towards cell adhesion and epithelial cell differentiation.

In the past 20 years, the dysregulation of cadherin was noted to lead to diverse dimensions of tumor development, such as medicine-resistant ability, angiogenic process, and invasive and metastatic activities of oncocytes. Previous studies have shown that CDHR2 can affect cell-cell adhesion and contact suppression in epitheliums by interacting with β-catenin in the cytoplasmic tail; hence, it is considered to be a potential tumor suppressor (Okazaki N. et al. 2002) (Rui Ose. et al. 2009). PCDH10, which is in the same PCDH family, has a poor prognosis in gastric cancer due to frequent promoter methylation (JunYu. et al. 2009). Its low expression in liver cancer facilitates the proliferative activities of liver oncocytes and promotes the progression of the disease, resulting in a worse prognosis (Yuntao Bing. et al. 2018). Even though the specific mechanism of CDHR2's cancer-promoting effect in cervical cancer is still unclear, it still serves as a very promising candidate in clinical diagnosis and prognosis, as well as a potential therapeutic target.

Some scholars also proposed that CDHR2 was found to closely be related to tumors, having different tumor suppressors or cancer-promoting effects according to tumor types. For example, it has a tumor suppressor effect in liver cancer and colon cancer but a cancer-promoting effect in cholangiocarcinoma (Ziyuan Xia. et al. 2019) (Rui Ose. et al. 2009) (Duangkumpha K. et al. 2019). In liver cancer, AKT inactivation and downregulation of COX-2 expression are used to achieve protection (Ziyuan Xia. et al. 2019). CDHR2 can inhibit the growth and proliferative activities of HCC cells in vitro and reduce tumorigenesis and cancer development in vivo. Elevated CDHR2 expression in HCT116 colon oncocytes causes contact inhibition, eliminating tumor formation (Rui Ose. et al. 2009). In contrast, CDHR2's expression is significantly upregulated in patients with cholangiocarcinoma (CCA), which is highly positive in cancer tissues and urine (Duangkumpha K. et al. 2019). It has been identified as a candidate biomarker for CCA diagnosis. In addition, the dysregulation of CDHR2 mRNA expression has been shown to be related to the development and prognosis of gastric cancer (Yang Chao. et al. 2020).

According to other studies, cervical cancer can promote epithelial to mesenchymal transition (EMT) via the Wnt/β-catenin pathway (Han Li. et al. 2019) (Qiqi Wang. et al. 2018). EMT is an activity where polarized epithelial cells stop maintaining cell-to-cell contact, lose the expression of characteristic epithelial cell markers and acquire the characteristics of mesenchymal cell markers (Deborah P. et al. 2020). During the occurrence and development of tumor cells, EMT can result in the reduction of certain features of epitheliums for oncocytes to gain certain features of mesenchymal cells to obtain stronger invasion and detachment capabilities. However, epithelial cells lose their polarity, and their interaction with neighboring cells and matrix cells are decreased, while the mutual effects among cells are decreased, enabling them to obtain stronger migration and movement capabilities. In fact, CDHR2 was noted to have a promoting effect on the invasive and metastatic activities of oncocytes via in vitro cell function experiments. Accordingly, we speculate that CDHR2 may promote cancer in cervical cancer via EMT and cancer stem cell (CSC) features. Therefore, further experimental verification is required to determine the precise mechanism of CDHR2 in the progression of cervical cancer to establish therapeutic targets that can improve the prognosis of cervical cancer patients.

The present study found that CDHR2 is regulated upward in cervix carcinoma samples and lineage cells, and its obvious expression is closely related to FIGO staging, histological differentiation and lymph node metastasis. We also found that the upregulation of CDHR2 in CC is closely related to poor prognosis. Therefore, CDHR2 may be a treatment target and prognostic biomarker for CC. A further comprehension of the function of CDHR2

can offer precious hints for the progression and prognosis of cervical cancer and establish new therapeutic targets that can improve the prognosis in these patients.

This study has some limitations that are worthy of further discussion. The survival rate of cervical cancer has been increasing in recent years. A larger number of clinical cases should be analyzed, which will result in a more accurate survival curve. Additionally, the current findings lack verification of in vivo experiments. Accordingly, animal model tests should be conducted in subsequent experiments. In our next study, it would be necessary to unveil the function of CDHR2 in cervix carcinoma or study its specific mechanisms and detailed roles in cancer progression.

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Interest clash disclosure

None.

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Table 1. **The TCGA data set is grouped according to the OS.**

OS	sampleID
Short (<543 days)	TCGA-C5-A3HF-01 TCGA-C5-A7UC-01 TCGA-VS-A94X-01 TCGA-VS-A9V5-01 TCGA-DS-A7WF-01 TCGA-VS-A953-01 TCGA-C5-A7CL-01 TCGA-VS-AA62-01 TCGA-VS-A9UL-01 TCGA-C5-A7X5-01 TCGA-UC-A7PG-06 TCGA-UC-A7PG-01 TCGA-UC-A7PD-01 TCGA-VS-A8QC-01 TCGA-C5-A0TN-01 TCGA-VS-A8EB-01 TCGA-C5-A7X3-01 TCGA-Q1-A6DT-01 TCGA-DS-A1O9-01 TCGA-EX-A69M-01 TCGA-DS-A7WI-01 TCGA-JW-A852-01 TCGA-EA-A50E-01 TCGA-C5-A1BN-01 TCGA-VS-A9V1-01 TCGA-VS-A94Y-01 TCGA-VS-A9V4-01 TCGA-VS-A9UV-01 TCGA-JW-A5VH-01 TCGA-C5-A1MK-01 TCGA-VS-A9UJ-01 TCGA-C5-A1MJ-01
Long (>543 days)	TCGA-C5-A7CG-01 TCGA-JX-A3Q0-01 TCGA-C5-A1BK-01 TCGA-C5-A1BL-01 TCGA-C5-A905-01 TCGA-C5-A7UE-01 TCGA-C5-A7CH-01 TCGA-IR-A3L7-01 TCGA-C5-A7CO-01 TCGA-C5-A8XJ-01 TCGA-C5-A1BJ-01 TCGA-IR-A3LA-01 TCGA-C5-A7UH-01 TCGA-DS-A1OD-01 TCGA-DS-A0VN-01 TCGA-DS-A0VM-01 TCGA-IR-A3LC-01 TCGA-EK-A2IR-01 TCGA-C5-A8XK-01 TCGA-IR-A3LF-01 TCGA-DR-A0ZL-01 TCGA-C5-A2LX-01 TCGA-IR-A3LI-01 TCGA-IR-A3LH-01 TCGA-C5-A2LY-01 TCGA-C5-A2LV-01 TCGA-C5-A2LT-01 TCGA-ZJ-A8QQ-01 TCGA-VS-A9UZ-01 TCGA-UC-A7PI-01 TCGA-VS-A8EL-01 TCGA-DG-A2KJ-01 TCGA-DR-A0ZM-01 TCGA-C5-A1ME-01 TCGA-BI-A0VS-01 TCGA-MY-A5BD-01 TCGA-C5-A1MF-01 TCGA-VS-A8QF-01 TCGA-C5-A3HD-01 TCGA-C5-A7XC-01 TCGA-VS-A952-01 TCGA-DG-A2KK-01 TCGA-VS-A954-01 TCGA-VS-A958-01 TCGA-BI-A0VR-01 TCGA-VS-A957-01 TCGA-VS-A9UH-01 TCGA-C5-A1M7-01 TCGA-VS-A8Q9-01 TCGA-EK-A2H0-01 TCGA-VS-A959-01 TCGA-DG-A2KL-01 TCGA-VS-A9U5-01 TCGA-C5-A2LS-01 TCGA-VS-A9U6-01 TCGA-VS-A9UP-01 TCGA-VS-A9U7-01 TCGA-VS-A9UO-01 TCGA-VS-A9UI-01 TCGA-VS-A8EC-01 TCGA-DG-A2KM-01 TCGA-VS-A950-01 TCGA-C5-A2M1-01 TCGA-VS-A8EG-01 TCGA-C5-A1BI-01 TCGA-FU-A3HZ-01 TCGA-VS-A94W-01 TCGA-VS-A8QA-01 TCGA-VS-A9UQ-01 TCGA-MY-A5BE-01 TCGA-MU-A5YI-01 TCGA-C5-A1MQ-01 TCGA-EA-A3HU-01 TCGA-IR-A3LL-01 TCGA-FU-A3HY-01 TCGA-EA-A3HR-01 TCGA-JX-A3Q8-0 TCGA-C5-A1M8-01 TCGA-JW-A69B-01 TCGA-JW-A5VG-01 TCGA-VS-A8EH-01 TCGA-EK-A2H1-01 TCGA-EA-A43B-01 TCGA-WL-A834-01 TCGA-EA-A411-01 TCGA-JW-A5VI-01 TCGA-VS-A94Z-01 TCGA-FU-A23L-01 TCGA-DS-A3LQ-01 TCGA-VS-A9UC-01 TCGA-MA-AA3W-01 TCGA-VS-A9UA-01 TCGA-VS-A9UB-01 TCGA-C5-A8ZZ-01 TCGA-MY-A5BF-01 TCGA-JW-A5VK-01 TCGA-C5-A3HL-01 TCGA-C5-A7CM-01 TCGA-EX-A1H5-01 TCGA-VS-A9UR-01 TCGA-EX-A69L-01 TCGA-VS-A9UD-01 TCGA-R2-A69V-01 TCGA-MA-AA3X-01 TCGA-MA-AA3Z-01 TCGA-JW-A5VJ-01 TCGA-VS-A9V0-01 TCGA-VS-A8EI-01 TCGA-C5-A3HE-01

Table 2. **Clinical characteristics of 51 patients with cervical adenocarcinoma.**

Characteristics	group	N	percentage (%)
Patient age(year)	<50	26	60.0
	≥50	25	49.0
FIGO stage	I-II	36	70.6
	III~IV	15	29.4
Myometrial invasion	<2/3	33	64.7
	≥2/3	17	33.3
	no data	1	2.0
LNM	Yes	12	23.5
	No	36	70.6
	no data	3	5.9
Histological type	Squamous cell carcinoma	39	76.5
	adenocarcinoma	9	17.6
	no data	3	5.9
Differentiation	L	10	19.6
	M-H	41	80.4
Scc	<1.5	40	78.4
	≥1.5	11	21.6

Table 3. **Expression of 3 genes associated with a poor cervical cancer prognosis in TCGA.**

Gene symbol	Gene ID	Description	Style
CDHR2	54825	cadherin-related family member 2	up
GGTLC1	92086	gamma-glutamyltransferase light chain 1	up
CLNK	116449	Cytokine-dependent hematopoietic cell linker	up

Table 4. **Correlation between the expression of CDHR2 and clinic-pathological characteristics of cervical cancer cases (n=51).**

Characteristics	group	N	CDHR2 expression		χ^2	P
			H	L		
Patient age(year)	<50	26	12	14	0.321	0.571
	≥50	25	13	11		
FIGO stage	I-II	36	14	22	7.161	0.007
	III~IV	15	12	3		
Myometrial invasion	<2/3	33	14	19	2.228	0.136
	≥2/3	17	11	6		
LNM	No	36	14	22	4.703	0.030
	Yes	12	9	3		
Histological type	Squamous cell carcinoma	39	20	19	0.697	0.643
	adenocarcinoma	9	6	3		
Differentiation	L	10	8	2	4.192	0.041
	M-H	41	18	23		
Scc	<1.5	40	19	21	0.899	0.343
	≥1.5	11	7	4		

Figure legends

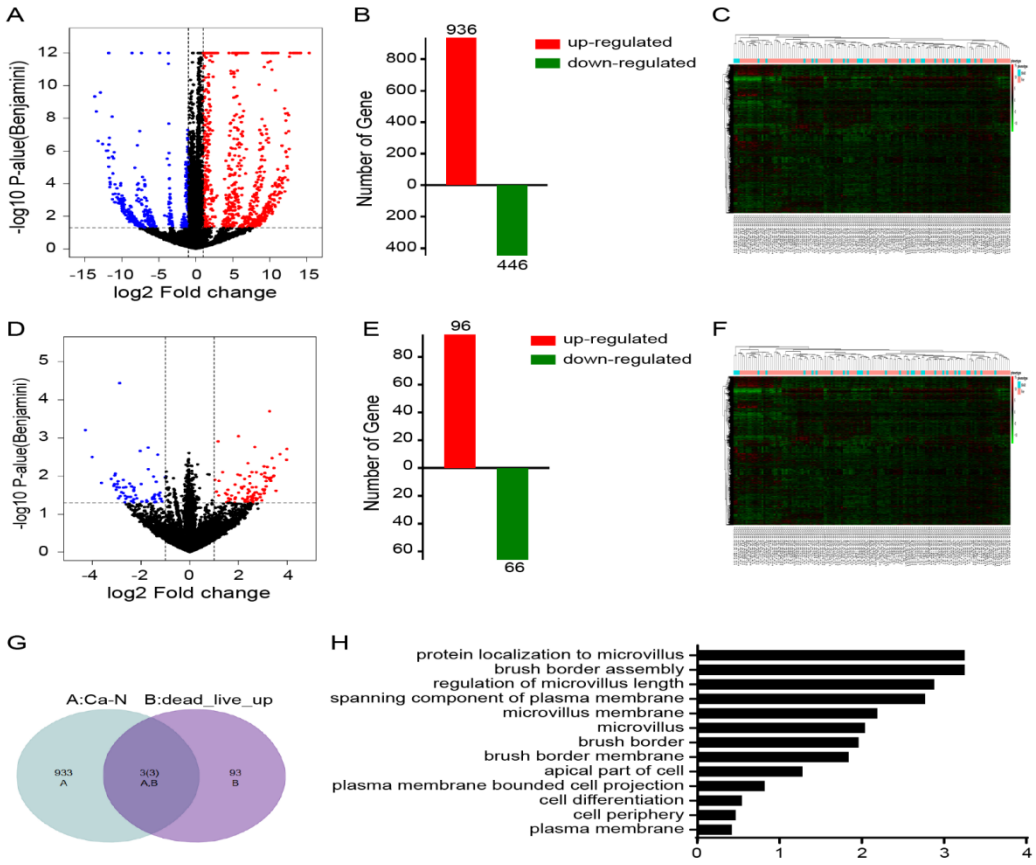

Figure 1. **Screening of differentially expressed genes related to the prognosis of cervical cancer.**

A volcano map of differentially expressed genes based on the results of RNA-seq analysis of cervix carcinoma and healthy cervix samples from the TCGA database. The x-axis denotes the log2 conversion of the fold change ratio, and the y-axis is the p score with adjustment after log10 conversion. (the threshold of p<0.05, false discovery rate (FDR) p<0.05 and the red colored dots denote the DEGs as per fold change> 2)

A. The plot of 936 up-regulated and 446 down-regulated genes based on volcano analysis.

B. Hierarchical cluster study of the RNA-seq information of diverse genes in cervical cancer and normal cervical tissues from the TCGA data center.

C. The volcano plot exhibited the different genes when comparing the long OS patient group with the short OS patient group. (a threshold of P<0.05, FDR <0.05 and fold change >2)

D. The plot showed 96 genes with upregulation and 66 genes with downregulation based on the above-mentioned volcano analysis.

E. Hierarchical cluster study of the candidate genes related to the survival data obtained from TCGA database.

F. Venn diagram reflecting the dispersion of DEGs in diverse groups. 3 There was expression of DEGs in CA and short survival sufferers.

G. GO analysis showed CDHR2 was closely related to brush border assembly, protein localization to microvillus, regulation of microvillus length, spanning component of plasma membrane, microvillus membrane, cell adhesion, epithelial cell differentiation.

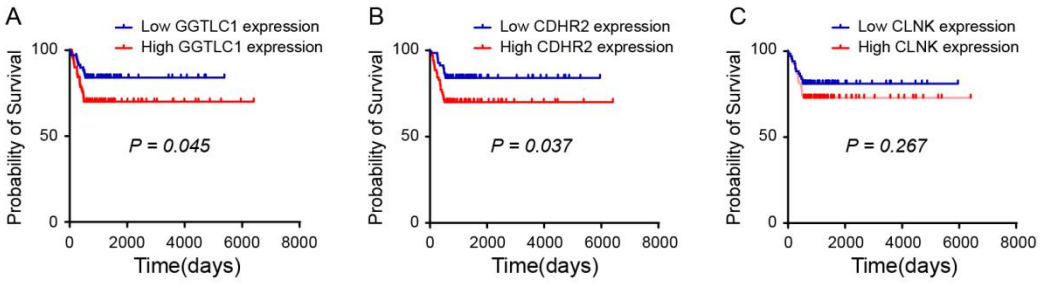

Figure 2. **Kaplan-Meier curve of these 3 genes (GGTLC1, CDHR2 and CLNK) were provided by patients from TCGA data.**

A. Highly expressed GGTLC1 is related to poorer survival of cervical carcinoma cases (P=0.045).
B. Highly expressed CDHR2 is related to poorer survival of cervical carcinoma cases (P=0.0379).
C. No important association on statistics exists between highly expressed GGTLC1 and poorer survival in cervical cancer patients (P>0.05).

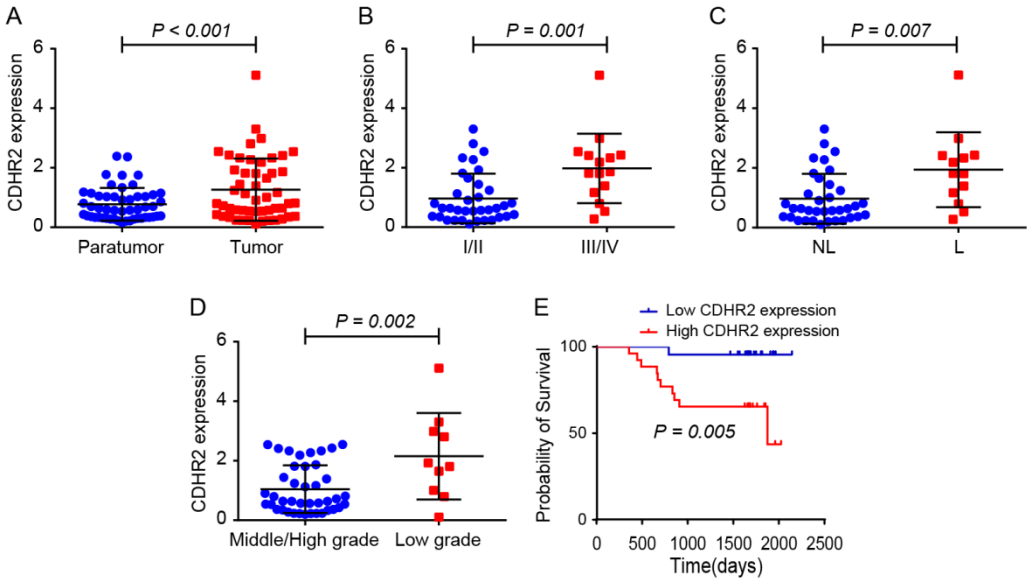

Figure 3. **CDHR2 is a predictor of poor prognosis.**

A. The mRNA expression level of CDHR2 in different groups were displayed.
B. The mRNA expression level of CDHR2 were shown in correlation to FIGO stage.
C. The mRNA level of CDHR2 were compared in terms of histological differentiation.
D. The mRNA level of CDHR 2 were compared in terms of lymph node metastasis.
E. Impact of CDHR2 expression on overall survival in clinical patients. (n=51)

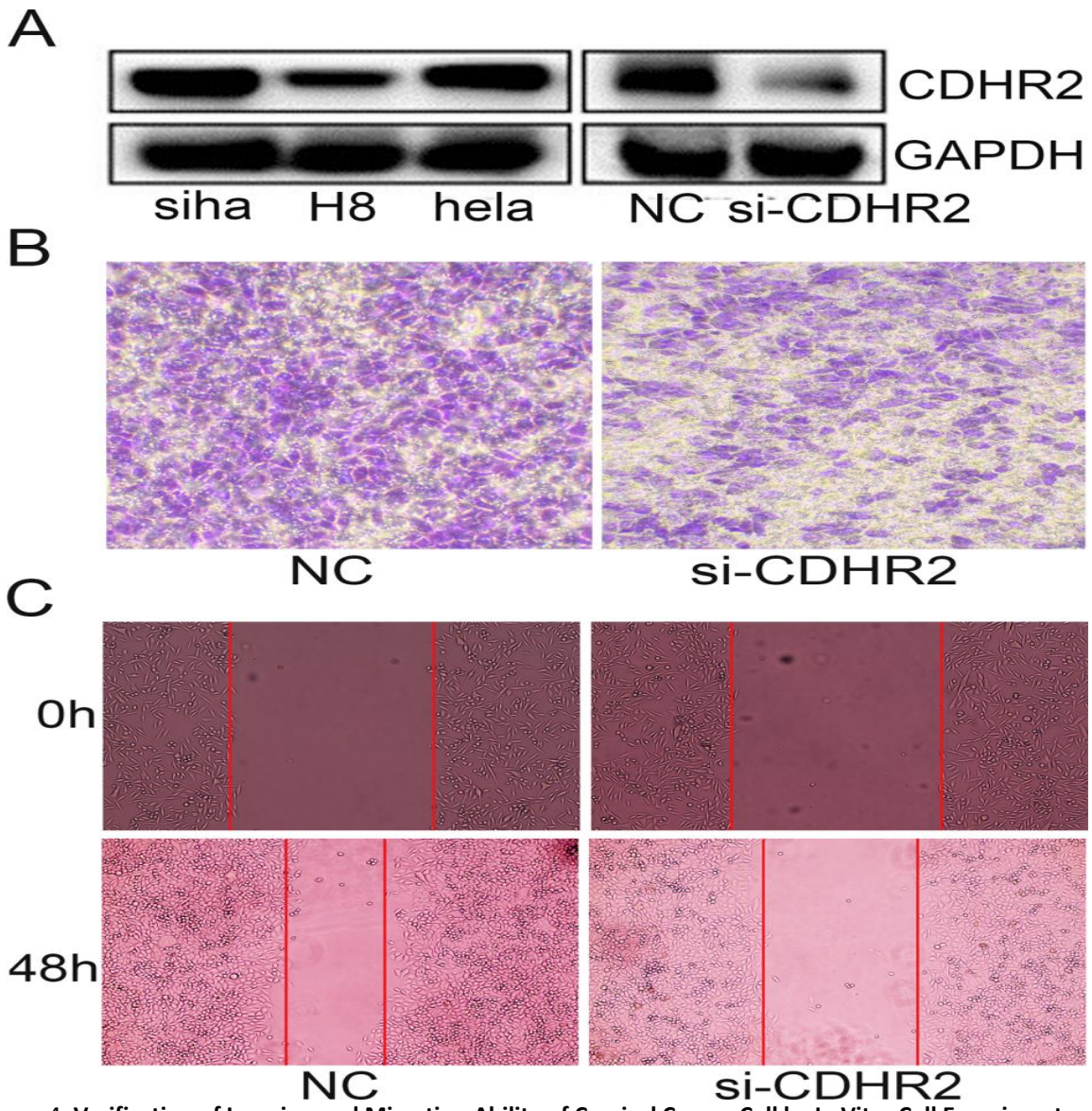

Figure 4. **Verification of Invasion and Migration Ability of Cervical Cancer Cell by In Vitro Cell Experiment.**
A. The expressed CDHR2 in different lineage cells and different groups. (SiHa cells on the right)
B. Contrast of the invasive capability of cells between experimental group and NC group at 36h.
C. Contrast of the migrative capability of cells between experimental group and NC group at 0h and 48h.