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Original Article

Cadherin family genes in non-small cell lung cancer: implications for diagnosis, prognosis, and targeted therapy

Yirui Wang[1*], Xuan Qin[2*], Wei Dong[2*], Changjiang Lei[1], Su Zheng[3], Mohamed M Salem[4], Mounir M Bekhit[5], Nihal Almuraikhi[6]

[1]Department of Oncology, The Fifth Hospital of Wuhan, Wuhan 430050, Hubei, China; [2]Department of Radiology, The Fifth Hospital of Wuhan, Wuhan 430050, Hubei, China; [3]Department of Rehabilitation, Taihe Hospital (Affiliated Hospital of Hubei University of Medical), Shiyan 442000, Hubei, China; [4]College of Medicine, Huazhong University of Science and Technology, Wuhan 430030, Hubei, China; [5]Department of Pharmaceutics, College of Pharmacy, King Saud University, PO Box 2457, Riyadh 11451, Saudi Arabia; [6]Stem Cell Unit, Department of Anatomy, College of Medicine, King Saud University, Riyadh 11461, Saudi Arabia. [*]Equal contributors.

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Abstract: Objectives: This study aimed to explore the diagnostic, prognostic, and therapeutic values of cadherin family genes (CDH1, CDH2, and CDH3) in non-small cell lung cancer (NSCLC) subtypes: lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC). Methodology: We analyzed the expression of CDH1, CDH2, and CDH3 in LUAD and LUSC using TCGA and TIMER2 data, and evaluated protein levels through immunostaining data from the HPA database. Gene expression across LUAD and LUSC stages was examined using GEPIA2. Methylation and mutation analyses were conducted vby OncoDB and cBioPortal, respectively. Prognostic significance was assessed through survival analyses using the KM Plotter tool. Gene enrichment and immune infiltration correlations were investigated using DAVID and GSCA databases. Knockdown experiments in PC9 cells were performed to assess the effects of CDH1 and CDH2 on cell proliferation, colony formation, and wound healing. Results: The expression of CDH1, CDH2, and CDH3 was significantly elevated in both LUAD and LUSC. Methylation analysis revealed reduced promoter methylation of cadherin genes in tumor samples compared to normal tissues. Mutational analysis showed that CDH2 exhibited the highest mutation frequency (63%), followed by CDH3 (23%) and CDH1 (19%). Survival analysis indicated that higher expression of CDH1, CDH2, and CDH3 was associated with poor prognosis in both LUAD and LUSC. Knockdown of CDH1 and CDH2 in PC9 cells resulted in reduced cell proliferation, colony formation, and impaired wound healing, with CDH2 knockdown showing more pronounced effects. Conclusion: CDH1, CDH2, and CDH3 were upregulated in LUAD and LUSC, contributing to tumor progression and poor prognosis. Knockdown of CDH1 and CDH2 in PC9 cells impaired proliferation, colony formation, and wound healing, highlighting their potential as therapeutic targets.

Keywords: NSCLC, LUAD, LUSC, prognosis: immune cells

Introduction

Non-small cell lung cancer (NSCLC) is the most common and deadly type of lung cancer [1, 2], accounting for approximately 85% of all cases worldwide [3, 4]. It is a major public health challenge with high morbidity and mortality, despite advances in diagnostic and therapeutic interventions [5, 6]. NSCLC includes several subtypes, including adenocarcinoma, squamous cell carcinoma, and large cell carcinoma [4, 7]. Early detection of NSCLC remains difficult, and by the time the disease is diagnosed, many patients present with metastatic disease, leading to a poor prognosis [8, 9]. The five-year survival rate for NSCLC remains low, especially for patients with advanced stages of the disease [10, 11].

Current diagnostic strategies primarily rely on imaging techniques such as chest X-rays, CT scans, and PET scans, as well as biopsy-based histopathologic examination for confirmation [12]. However, these methods often lack sensi-

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tivity, especially in early-stage disease or small lesions, which contributes to a delay in diagnosis [13]. Additionally, molecular profiling through liquid biopsy and genetic testing is still in the early stages of adoption, with limited availability and high cost being significant barriers in clinical practice [14]. On the therapeutic front, treatment options for NSCLC include surgery, chemotherapy, radiation therapy, targeted therapy, and immunotherapy [15]. While targeted therapies and immune checkpoint inhibitors have shown promise, they are only effective in specific patient subgroups defined by molecular markers such as EGFR mutations, ALK rearrangements, and PD-L1 expression [16]. However, the efficacy of these treatments is limited by the development of resistance and the heterogeneous nature of the disease. Furthermore, the complexity of NSCLC subtypes poses a significant challenge in developing universally effective therapies. Therefore, there is a critical need for better diagnostic biomarkers, prognostic indicators, and therapeutic targets to improve patient outcome and guide personalized treatment strategy [17].

The progression of NSCLC, like many other cancers, is driven by a series of genetic mutations and molecular alterations that promote uncontrolled cell growth, resistance to cell death, and metastasis [18-20]. One such group of molecules that have been implicated in cancer progression are cadherins, a family of transmembrane glycoproteins responsible for mediating cell-cell adhesion [19]. Cadherins play essential roles in maintaining tissue architecture and cellular integrity [21, 22]. The cadherin family is composed of several isoforms, including E-cadherin (CDH1), N-cadherin (CDH2), and P-cadherin (CDH3), each of which has distinct biologic functions and relevance to cancer biology [23, 24].

E-cadherin (CDH1) is a key molecule in epithelial cells that promotes cell-cell adhesion and inhibits cellular motility, playing a crucial role in maintaining tissue integrity [25]. Loss of E-cadherin expression is a hallmark of epithelial-to-mesenchymal transition (EMT), a process that contributes to cancer metastasis [26]. Reduced expression of E-cadherin has been associated with poor prognosis and increased metastatic potential in a variety of cancers [27, 28]. N-cadherin (CDH2), which is primarily expressed in mesenchymal cells, is upregulated in many cancers and is involved in promoting EMT [29]. The upregulation of N-cadherin facilitates cellular migration and invasion, contributing to cancer progression and metastasis [30]. Similarly, P-cadherin (CDH3) has been implicated in several types of cancer, including breast and lung cancers, and has been shown to play a role in tumor cell migration, invasion, and survival [23, 31].

Although significant progress has been made in understanding the role of cadherins in cancer biology, their full diagnostic and therapeutic potential, particularly in NSCLC, remains to be fully realized. Restoration of E-cadherin expression or inhibition of N-cadherin and P-cadherin signaling offers promise as a therapeutic strategy to reverse EMT and prevent metastasis [22, 32]. However, challenges remain in translating these findings into effective clinical intervention.

This study aimed to analyze the diagnostic, prognostic, and therapeutic values of cadherin family genes (CDH1, CDH2, and CDH3) in NSCLC through both in silico [33, 34] and in vitro experiments [35, 36]. By investigating the expression patterns, functional roles, and therapeutic implications of these genes, we seek to identify novel biomarkers for early detection and progression of NSCLC. Additionally, we aimed to explore the use of targeting cadherin-mediated signaling pathways as a therapeutic approach for improving the outcomes of NSCLC patients. Ultimately, our research endeavors to contribute to the growing body of knowledge surrounding cadherin family genes in cancer biology and their application for clinical management.

Materials and methods

Expression landscape of cadherin genes

The expression analysis of cadherin genes (CDH1, CDH2, and CDH3) in LUAD and LUSC was conducted using publicly available databases to assess both mRNA and protein expression levels. For mRNA expression data, the TIMER2 database (https://cistrome.shinyapps.io/timer/) [37] was used, which provides immune-related gene expression profiling and tumor immune infiltration analysis based on data from The Cancer Genome Atlas (TCGA). This database was used in the study to analyze

the mRNA expression levels of CDH1, CDH2, and CDH3 across LUAD and LUSC samples. For protein expression data, the Human Protein Atlas (https://www.proteinatlas.org/) [38] was used, which offers comprehensive tissue and cell expression data derived from immunohistochemistry (IHC) analysis. This database was instrumental in the work in confirming the protein expression levels of CDH1, CDH2, and CDH3 in LUAD and LUSC samples.

Expression analysis of cadherin gene expression across different stages of LUAD and LUSC

The GEPIA2 database (http://gepia2.cancer-pku.cn/) [39] was used to examine the expression of these cadherin genes across different stages (I-IV) of LUAD and LUSC. GEPIA2 provides gene expression analysis based on data from TCGA and GTEx. To further investigate the molecular involvement of cadherin genes in LUAD and LUSC, we used the GSCA database (http://bioinfo.life.hust.edu.cn/GSCA/#/) [40] for Gene Set Enrichment Analysis (GSEA). GSCA is a web tool that provides comprehensive analysis of gene set enrichment, offering insight into the molecular mechanisms driving cancer.

Methylation analysis of cadherin genes in LUAD and LUSC

The OncoDB database (https://oncodb.org/) [41] was used for methylation analysis of CDH1, CDH2, and CDH3 in LUAD and LUSC tumors. OncoDB provides comprehensive cancer-related genomic data, including methylation profiles of genes in various cancer types. In our study, the OncoDB database was employed to compare the methylation levels of cadherin genes in tumor samples and normal tissues.

Mutational analysis of cadherin genes in LUAD and LUSC samples

The cBioPortal database (https://www.cbioportal.org/) [42] was used in this study to perform mutational analysis of CDH1, CDH2, and CDH3 in LUAD and LUSC samples. cBioPortal is an online platform that provides access to a wide range of cancer genomic data, including mutation frequencies, variant types, and copy number variations (CNVs) in various cancer types.

Prognostic analysis of cadherin genes in LUAD and LUSC samples

The KM Plotter tool (https://kmplot.com/analysis/) [43] was used to analyze the correlation between the expression levels of cadherin genes and overall survival in LUAD and LUSC samples. KM Plotter is a widely used online tool for assessing the prognostic value of gene expression in cancer patients, providing survival curves by the Kaplan-Meier method.

Correlation of cadherin gene expression with immune inhibitors in LUAD and LUSC

The TISIDB database (http://cis.hku.hk/TISIDB/) [44] was used to analyze the correlation between the expression of cadherin genes (CDH1, CDH2, and CDH3) and various immune inhibitors in LUAD and LUSC. TISIDB is an integrated resource for studying tumor-immune interactions, including the relationship between gene expression and immune checkpoint inhibitors across various cancer types.

Gene enrichment analysis of cadherin genes

The DAVID tool (https://david.ncifcrf.gov/) [45] was employed to perform gene enrichment analysis for cadherin genes (CDH1, CDH2, and CDH3) in LUAD and LUSC. DAVID is a comprehensive online resource for functional annotation and pathway analysis of gene lists. The DAVID tool was used to examine the cellular component, molecular function, biological process, and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways associated with cadherin genes.

Correlations of cadherin gene expression with immune infiltrates and drug sensitivity

The GSCA database (http://bioinfo.life.hust.edu.cn/GSCA/) [40] was used to assess the correlation between cadherin gene expression and immune infiltrates in LUAD and LUSC, as well as to analyze the potential drug sensitivity associated with these genes. The GSCA database provides comprehensive cancer-related datasets and tools for exploring gene expression, immune infiltrates, and drug responses.

Cell culture, transfection, and expression analysis

PC9 cells (purchased from ATCC, USA) were cultured in RPMI-1640 medium (Thermo Fisher Scientific, Catalog No. 11875093) supplemented with 10% fetal bovine serum (FBS) (Thermo Fisher Scientific, Catalog No. 16000044) and 1% penicillin-streptomycin (Thermo Fisher Scientific, Catalog No. 15140122) at 37°C in a 5% CO_2 incubator.

The cells were transfected with small interfering RNAs (siRNAs) targeting CDH1 and CDH2 (Thermo Fisher Scientific, Catalog Nos. AM-16708 and AM16702) using Lipofectamine™ RNAiMAX Transfection Reagent (Thermo Fisher Scientific, Catalog No. 13778150) according to the manufacturer's protocol. A non-targeting control siRNA was used as a negative control. The final siRNA concentration used was 20 nM for each gene.

Total RNA was isolated from PC9 cells using the RNAqueous™ Total RNA Isolation Kit (Thermo Fisher Scientific, Catalog No. AM1912) following the manufacturer's instructions. The isolated RNA was reverse transcribed into complementary DNA (cDNA) using the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, Catalog No. 4368814) as per the manufacturer's instructions. Briefly, 1 µg of RNA was used in a 20 µL reaction mixture, which was incubated at 25°C for 10 minutes, 37°C for 120 minutes, and then 85°C for 5 minutes to inactivate the reverse transcriptase.

mRNA expression of CDH1 and CDH2 was quantified using the PowerUp™ SYBR™ Green Master Mix (Thermo Fisher Scientific, Catalog No. A25742). Reactions were performed using a 20 µL reaction volume consisting of 10 µL PowerUp™ SYBR™ Green Master Mix, 1 µL cDNA, and 0.2 µM primers for CDH1, CDH2, and GAPDH (Thermo Fisher Scientific, Catalog Nos. Hs01023894_m1, Hs00983056_m1, and Hs02786624_g1, respectively). Data were normalized to GAPDH expression, and relative gene expression was determined using the $2^{-\Delta\Delta Ct}$ method.

Total protein was extracted from the transfected PC9 cells using the RIPA Lysis and Extraction Buffer (Thermo Fisher Scientific, Catalog No. 89900) supplemented with a protease inhibitor cocktail (Thermo Fisher Scientific, Catalog No. 78443). The protein concentration was determined using the Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific, Catalog No. 23225). Equal amounts of protein (30 µg) were separated by SDS-PAGE on 10% gels and transferred onto a PVDF membrane (Thermo Fisher Scientific, Catalog No. 88518). The membranes were blocked with 5% non-fat dry milk in TBS with 0.1% Tween-20 for 1 hour at room temperature. After blocking, the membranes were incubated with primary antibodies against CDH1 (Thermo Fisher Scientific, Catalog No. 32-1700) and CDH2 (Thermo Fisher Scientific, Catalog No. TA503933) at 1:1000 dilution overnight at 4°C. After washing, the membranes were incubated with secondary antibody (Thermo Fisher Scientific, Catalog No. 31430) at a 1:5000 dilution for 1 hour at room temperature. Protein bands were detected using the Pierce™ ECL Western Blotting Substrate (Thermo Fisher Scientific, Catalog No. 32209).

Cell proliferation assay

Cell proliferation was assessed by measuring the levels of CDK-8 expression in PC9 cells using a CDK-8 ELISA kit (Thermo Fisher Scientific, Catalog No. PV4402). PC9 cells were seeded at 1×10^4 cells/well in a 96-well plate and transfected with CDH1 and CDH2 siRNAs. After 24-, 48-, and 72-hour post-transfection, the cell culture supernatant was collected for analysis. The levels of CDK-8 were determined according to the manufacturer's protocol, which involves binding of the CDK-8-specific antibody to the immobilized antigen. The reaction was developed using a substrate solution, and the absorbance was measured at 450 nm using a microplate reader. The relative CDK-8 levels were normalized to the control group to assess the effect of the gene knockdown on cell proliferation.

Colony formation assay

PC9 cells were seeded at 500 cells per well in a 6-well plate and allowed to form colonies for 10-14 days. Colonies were fixed with 4% paraformaldehyde (Thermo Fisher Scientific, Catalog No. 28908) and stained with 0.1% crystal violet (Thermo Fisher Scientific, Catalog No. C3886). Colonies were counted using ImageJ software (NIH) to quantify the number of colonies formed.

Wound healing assay

PC9 cells were grown in a 6-well plate to 90% confluency. A linear scratch was created using a sterile 200 µL pipette tip, and the wells were washed with PBS (Thermo Fisher Scientific, Catalog No. 10010023) to remove any detached cells. Cells were incubated in RPMI-1640 medium supplemented with 2% FBS. Images of the wound area were captured at 0

and 24 hours using a light microscope (Thermo Fisher Scientific, Catalog No. ECLIPSE TS100). The percentage of wound closure was calculated by comparing the initial wound area to the remaining open area at each time point.

Statistical analysis

Statistical analysis was performed using GraphPad Prism software (Version 9.5.1). Data were expressed as mean ± standard deviation (SD) from at least three independent biological replicates. Comparisons between two groups were performed using an unpaired Student's t-test, and multiple group comparisons were analyzed using one-way or two-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Correlations between gene expression and clinical data were evaluated using Pearson's correlation coefficient. Survival analysis was conducted using the Kaplan-Meier method, and differences in overall survival were assessed using the log-rank test. Statistical significance was set at *P < 0.05, **P < 0.01, and ***P < 0.001.

Results

Expression landscape of cadherin genes

The expression levels of cadherin genes (CDH1, CDH2, and CDH3) were analyzed across NSCLC subtypes (LUAD and LUSC), using data from TCGA datasets by the TIMER2 database. Results showed that CDH1, CDH2, and CDH3 exhibited significantly (*p*-value < 0.05) elevated expression levels in LUAD and LUSC samples (**Figure 1A**), indicating a potential involvement of these genes in the tumorigenesis and progression of NSCLC. Furthermore, protein expression analysis using IHC data from the HPA database revealed that the protein levels of CDH1, CDH2, and CDH3 were also high in both LUAD and LUSC samples (**Figure 1B**). These results are consistent with the mRNA expression data and suggest that the cadherin family genes are upregulated in NSCLC, and contribute to the malignant characteristics of these tumors.

Expression of cadherin genes across different stages of LUAD and LUSC

The expression of cadherin genes (CDH1, CDH2, and CDH3) was analyzed across different stages (I-IV) of both LUAD and LUSC using the GEPIA2 database. The analysis revealed that there were no significant stage-dependent variations in the expression of these genes in either LUAD or LUSC. For LUAD, the F-values for CDH1, CDH2, and CDH3 were 0.567 (Pr(>F) = 0.637), 0.789 (Pr(>F) = 0.501), and 1.81 (Pr(>F) = 0.145), respectively, indicating no significant differences in expression across stages (**Figure 2A**). Similarly, for LUSC, the F-values for CDH1, CDH2, and CDH3 were 1.3 (Pr(>F) = 0.273), 0.181 (Pr(>F) = 0.91), and 2.42 (Pr(>F) = 0.0657), showing no stage-specific expression patterns (**Figure 2A**). GSEA analysis via the GSCA database revealed significant enrichment of CDH1, CDH2, and CDH3 in LUAD, with enrichment scores above 0.25 for each gene (**Figure 2B**). This indicates that cadherin genes are involved in the molecular processes driving LUAD, and may contribute to the pathogenesis of the disease.

Methylation analysis of cadherin genes in LUAD and LUSC

The methylation analysis (promoter and gene body) of cadherin genes (CDH1, CDH2, and CDH3) in LUAD and LUSC tumors by the OncoDB database revealed lower methylation levels in tumor samples compared to normal tissues. In both LUAD and LUSC, methylation levels were significantly (*p*-value < 0.05) reduced in the promoter regions of these genes, indicating hypomethylation in the tumor samples (**Figure 3**). This suggests that the reduced promoter methylation of cadherin genes in both LUAD and LUSC may be an important factor in their altered expression.

Mutational landscape of cadherin genes in LUAD and LUSC

Mutational analysis of cadherin family genes (CDH1, CDH2, and CDH3) was performed in LUAD and LUSC samples using the cBioPortal database. In the analyzed samples, CDH1, CDH2, and CDH3 were found to have varying mutation frequencies. CDH2 exhibited the highest mutation frequency, with 63% of the samples showing alterations, followed by CDH3 with 23% and CDH1 with 19% (**Figure 4A**). Among the observed mutations, missense mutations were the most prevalent type, while nonsense mutations and frameshift deletions were less common (**Figure 4B**). In terms of vari-

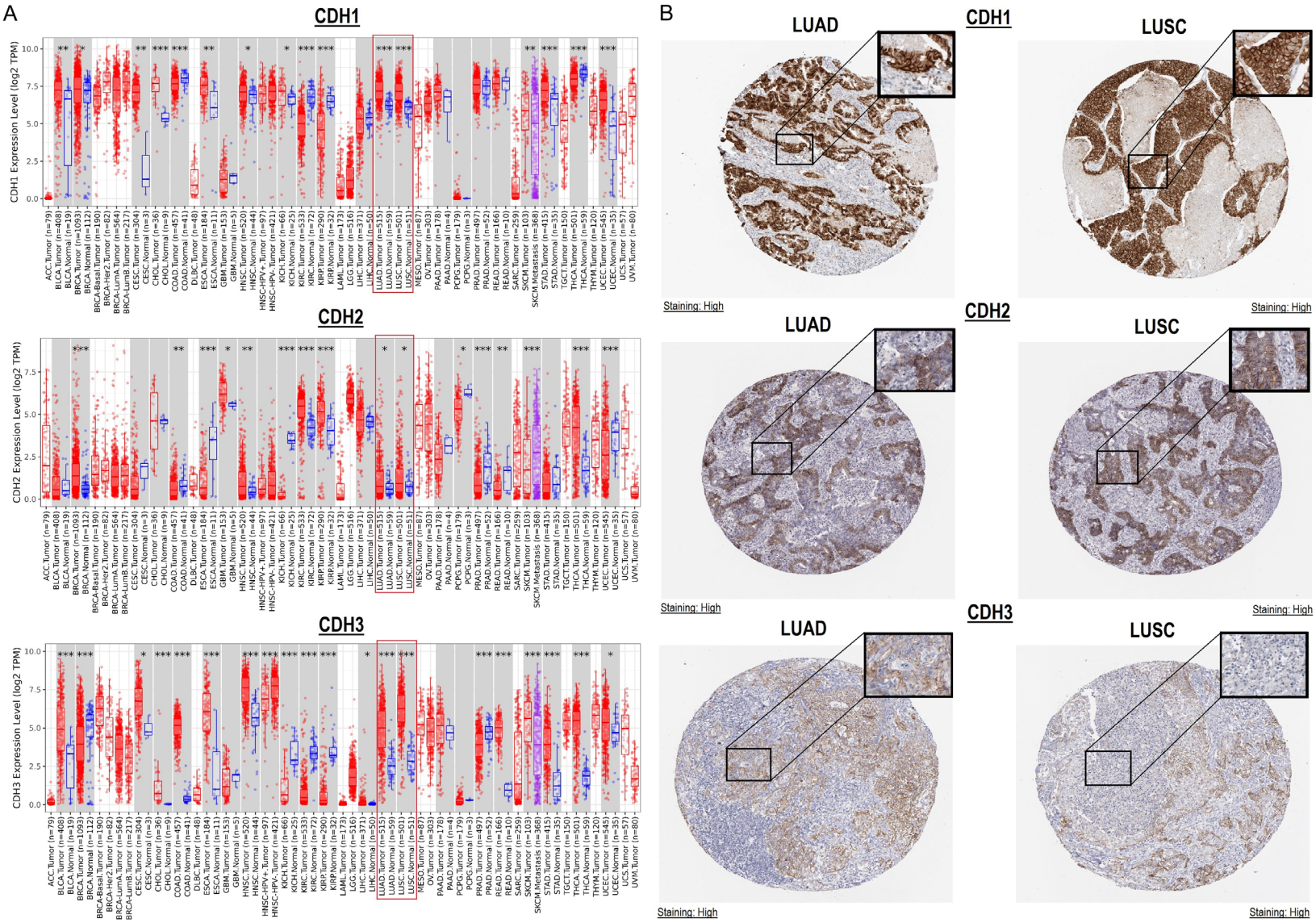

Figure 1. Expression landscape of cadherin family genes in lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC). A. The expression levels of CDH1, CDH2, and CDH3 were analyzed across LUAD and LUSC samples using The Cancer Genome Atlas (TCGA) datasets. B. Protein expression analysis by immunohistochemistry (IHC) data from the Human Protein Atlas (HPA) database revealed high protein levels of CDH1, CDH2, and CDH3 in LUAD and LUSC samples. **P*-value < 0.05, ***P*-value < 0.01, and ***P*-value < 0.001.

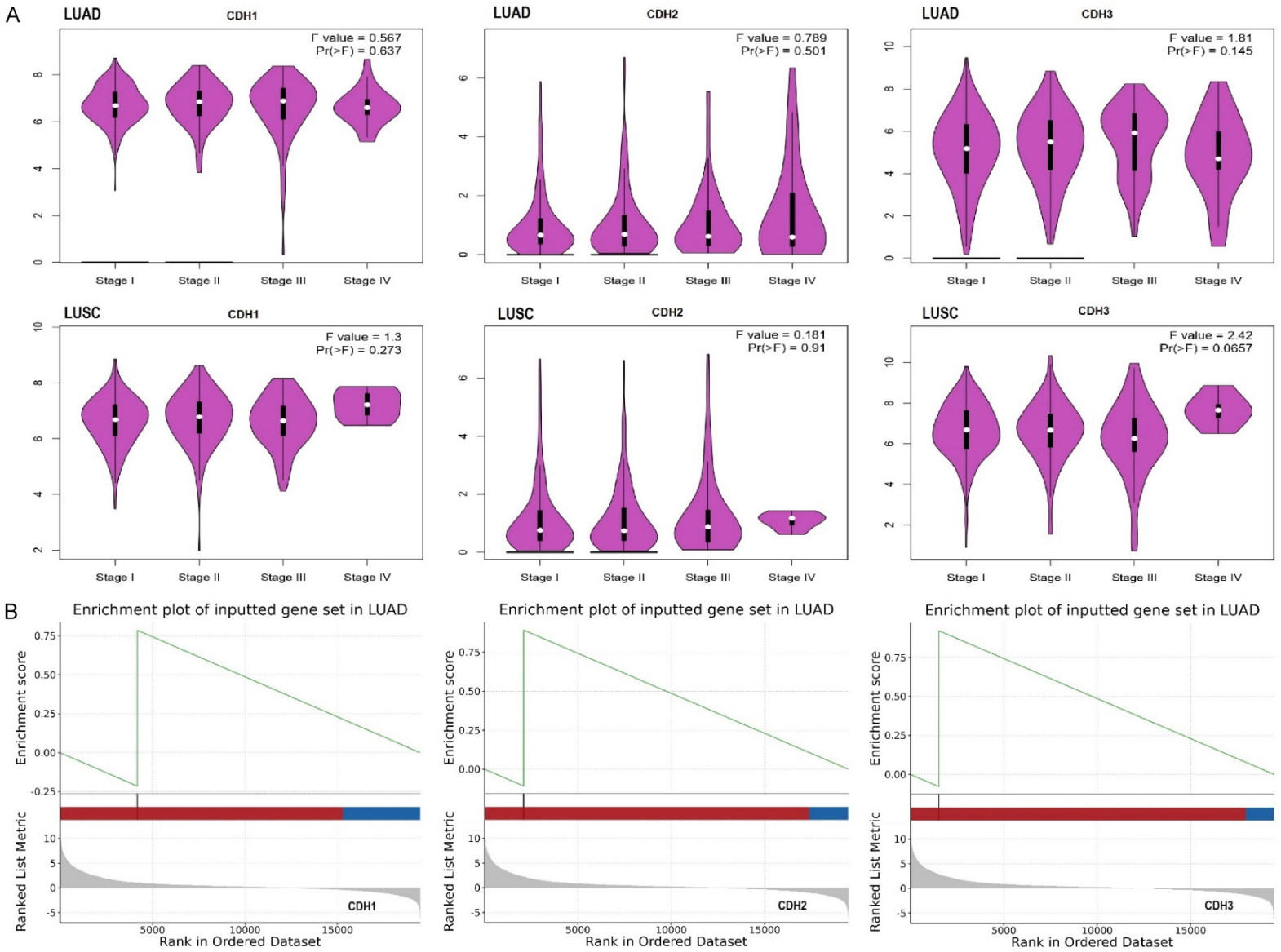

Figure 2. Expression of cadherin genes across different stages of in lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC). A. The expression of CDH1, CDH2, and CDH3 was analyzed across different stages (I-IV) of LUAD and LUSC using the Gene Expression Profiling Interactive Analysis (GEPIA2) database. B. Gene set enrichment analysis (GSEA) via the Gene Set Cancer Analysis (GSCA) database revealed significant enrichment of CDH1, CDH2, and CDH3 in LUAD and LUSC. *P*-value < 0.05.

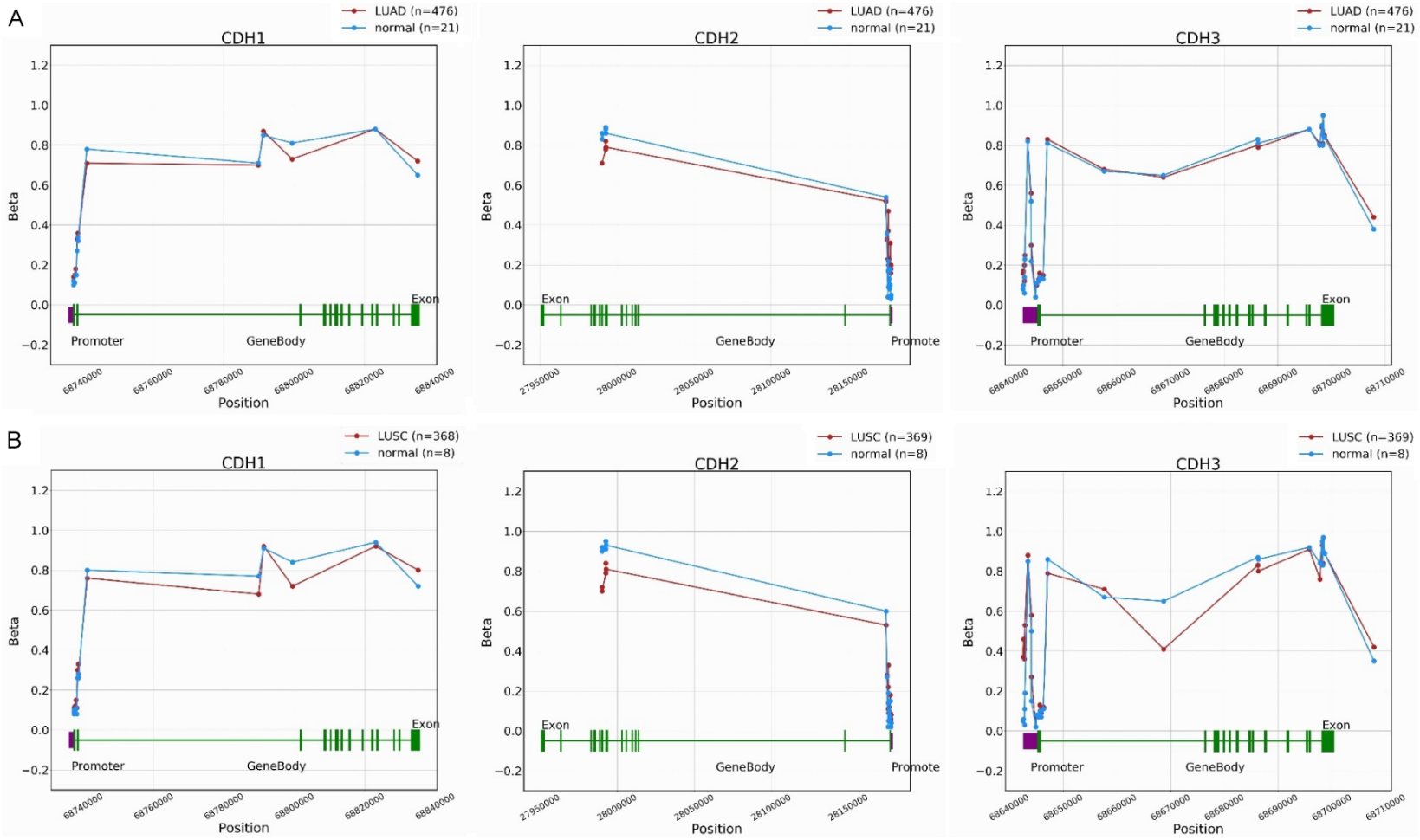

Figure 3. Methylation analysis of cadherin genes in lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC). Methylation analysis of CDH1, CDH2, and CDH3 in LUAD and LUSC tumors was performed using the OncoDB database. *P*-value < 0.05.

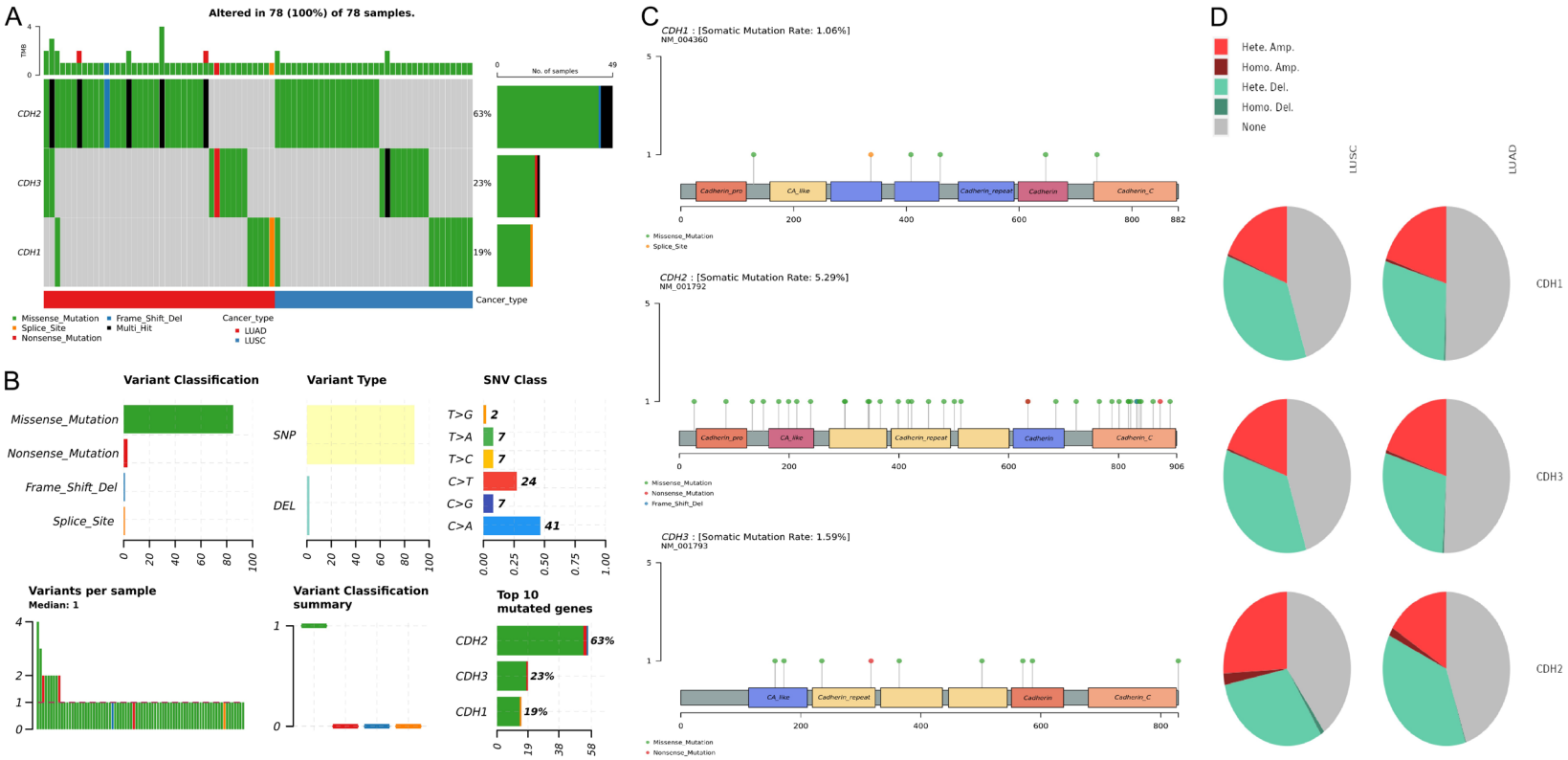

Figure 4. Mutational landscape of cadherin family genes in lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC). A. Mutational frequencies of CDH1, CDH2, and CDH3 in LUAD and LUSC samples were analyzed using the cBioPortal database. B. Types of mutations observed in cadherin genes. C. Somatic mutation rates in cadherin genes. D. Copy number variations (CNVs) of cadherin genes.

ant types, single nucleotide polymorphisms (SNPs), especially T>C substitutions, were the most frequent, contributing significantly to the overall mutational landscape (**Figure 4B**). The somatic mutation rate was highest in CDH2, with 5.29% of the samples showing somatic mutations (**Figure 4C**). Missense mutations were primarily observed in this gene, spread across various regions. On the other hand, CDH1 and CDH3 had lower somatic mutation rates (1.06% and 1.59%, respectively), with missense mutations again being the dominant type in both genes (**Figure 4C**). Regarding CNVs, both LUAD and LUSC samples showed significant amplification and deletion events in cadherin family genes. CDH2 was notably amplified in both LUAD and LUSC, with a higher frequency of heterozygous amplifications observed, particularly in LUAD (**Figure 4D**). CDH1 showed a moderate frequency of amplifications in LUAD, while CDH3 displayed a combination of homozygous deletions and heterozygous amplifications in LUSC (**Figure 4D**).

Prognostic values of cadherin genes in LUAD and LUSC

The analysis of the prognostic value of cadherin family genes in NSCLC (LUAD and LUSC) was performed using the KM plotter tool. In LUAD samples, high expression of CDH1 was associated with a significantly worse overall survival (HR = 1.33, logrank P = 2.9e-06) (**Figure 5A**), indicating that higher CDH1 expression may act as a negative prognostic factor. Similarly, CDH2 showed that higher expression was associated with poor prognosis (HR = 1.26, logrank P = 0.00014) (**Figure 5A**), while CDH3 expression also significantly correlated with reduced survival (HR = 1.22, logrank P = 0.00092) (**Figure 5A**). For LUSC, CDH1 expression showed an even stronger association with poor prognosis (HR = 1.48, logrank P = 7.3e-06) (**Figure 5B**), suggesting it may be a more critical prognostic marker in LUSC. The expression of CDH2 (HR = 1.19, logrank P = 0.041) and CDH3 (HR = 1.19, logrank P = 0.042) were also correlated with worse overall survival in LUSC (**Figure 5B**).

Correlation of cadherin gene expression with immune inhibitors in LUAD and LUSC

The correlations between cadherin genes and various immune inhibitors in LUAD and LUSC were analyzed using TISIDB database. The correlation results suggested positive correlations between the expression of cadherin genes and immune inhibitors in both LUAD and LUSC (**Figure 6A**). For example, in LUAD, CDH1 expression showed a moderate positive correlation with VTCN1 (rho = 0.163, P = 0.000193) (**Figure 6B**), indicating that higher expression of CDH1 was associated with increased expression of VTCN1. Similarly, CDH2 expression correlated positively with VTCN1 (rho = 0.214, P = 9.34e-07), and CDH3 expression showed a slightly stronger positive correlation with VTCN1 (rho = 0.22, P = 4.7e-07) (**Figure 6B**). In LUSC, the same trends were observed, with CDH1 positively correlating with VTCN1 (rho = 0.32, P = 3.06e-13), CDH2 showing a positive correlation with VTCN1 (rho = 0.214, P = 9.34e-07), and CDH3 showing a slightly weaker correlation with VTCN1 (rho = 0.199, P = 7.35e-06) (**Figure 6B**).

Gene enrichment analysis of cadherin genes

The gene enrichment analysis by the DAVID tool revealed several key findings across different categories. The cellular component terms highlighted a strong involvement of cell junctions, with terms such as “zonula adherens”, “focal adhesion”, “membrane microdomain”, and “adhesion junction” showing significant enrichment (**Figure 7A**). Molecular function terms demonstrated enrichment in categories like “cadherin binding”, “integrin binding”, “tyrosine kinase binding”, and “protein kinase binding (**Figure 7B**)”. Biological process terms showed enrichment in processes like “cell junction assembly”, “canonical Wnt signaling pathway”, and “cell adhesion (**Figure 7C**)”. These enriched terms suggested that key cellular processes involving adhesion and Wnt signaling were significantly impacted in the samples. Finally, KEGG pathway analysis revealed the enrichment of pathways such as “adhesions junction”, “gastric cancer”, and “prostate cancer (**Figure 7D**)”.

Correlations of cadherin gene expression with immune infiltrates and drug sensitivity

A correlation between cadherin gene expression and immune infiltrates was assessed in LUAD and LUSC using the GSCA database. In LUAD, several negative correlations were observed between the expression of CDH1, CDH2, and CDH3 and specific immune cell

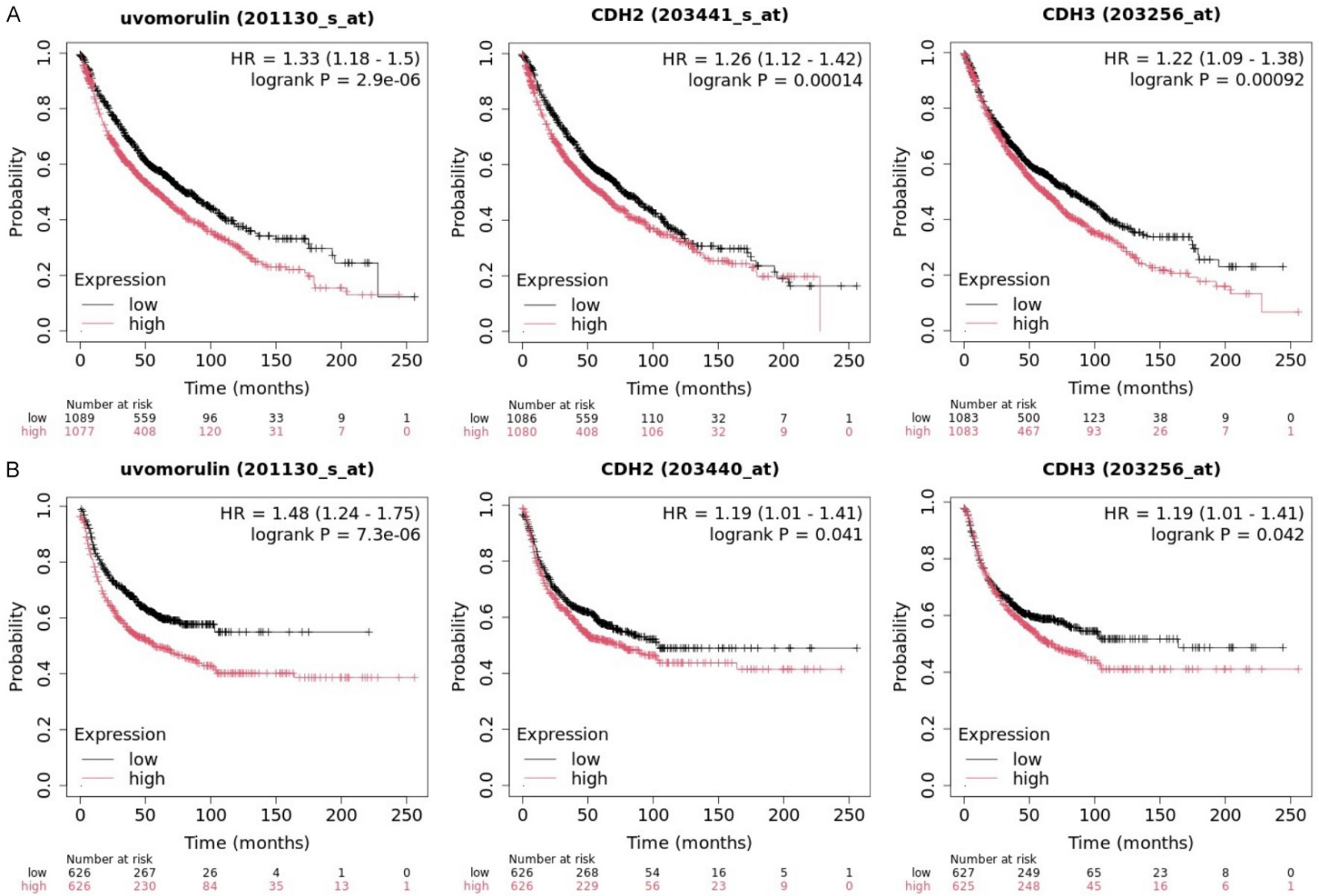

Figure 5. Prognostic values of cadherin genes in lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC). A. Kaplan-Meier survival analysis of CDH1, CDH2, and CDH3 expression in LUAD samples. B. Kaplan-Meier survival analysis of CDH1, CDH2, and CDH3 expression in LUSC samples. *P*-value < 0.05.

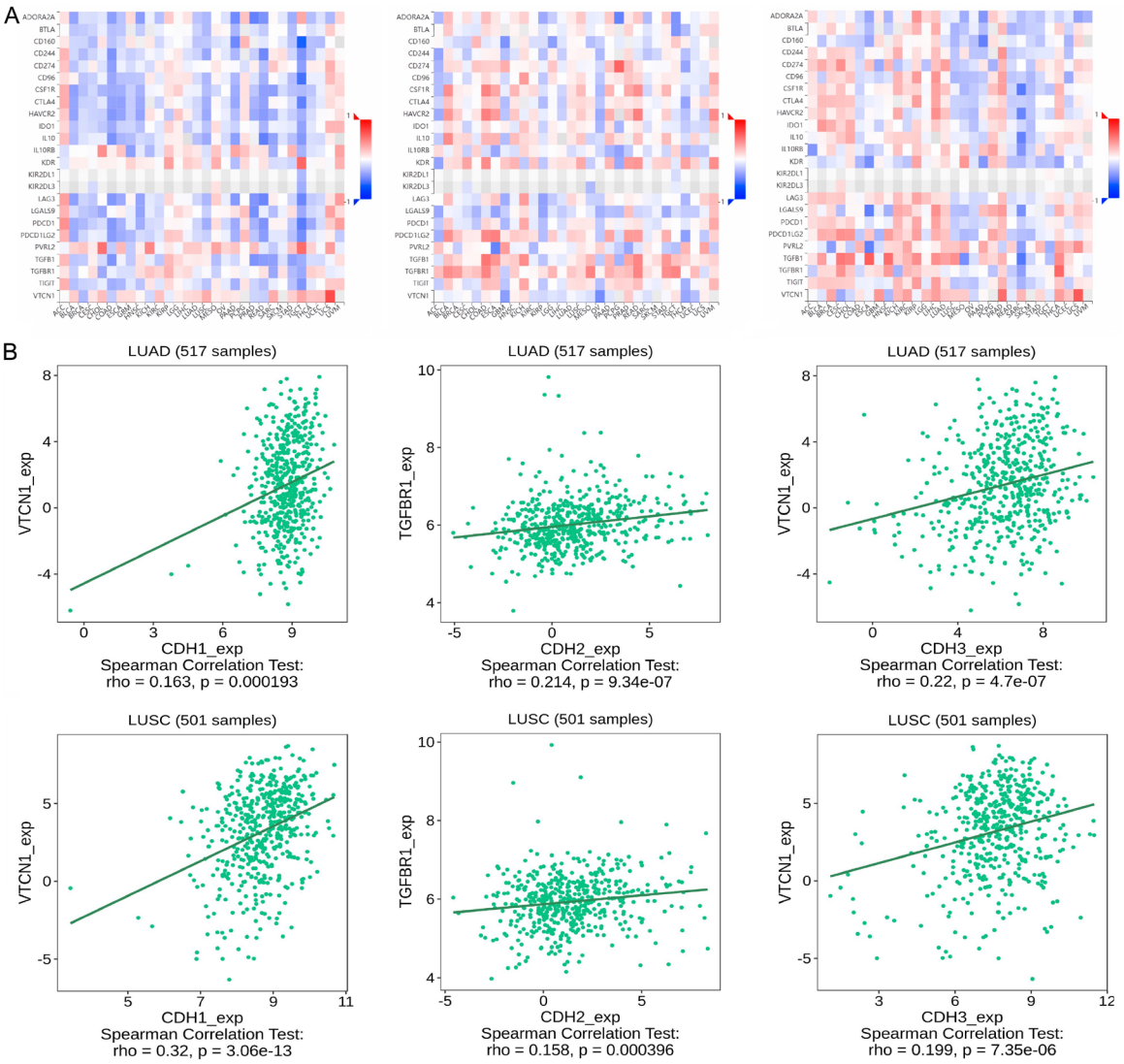

Figure 6. Correlation of cadherin gene expression with immune inhibitors in lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC). A. Correlation analysis between cadherin genes and immune inhibitors in LUAD and LUSC using the TISIDB database. B. Correlations between cadherin genes and selective immune inhibitors in LUAD and LUSC. *P*-value < 0.05.

types, including NK and MAIT cells (**Figure 8A**). In LUSC, the negative correlations between cadherin genes and immune infiltrates were more pronounced for CDH1, CDH2, and CDH3. These genes showed significant negative relationships with various immune cells such as gamma_delta cells and Th1 cells (**Figure 8B**). In the analysis of cadherin gene expression and drug sensitivity, significant positive correlations were found for all three cadherin genes (CDH1, CDH2, and CDH3) with various drugs, including 8-oxo-dG, IWR-1, and AZD-0530 (**Figure 8C**). These results indicated that elevated expression of cadherin genes may contribute to resistance against these therapeutic agents, suggesting they have a role in influencing drug response.

Knockdown effects of CDH1 and CDH2 on, cell proliferation, colony formation, and wound healing in PC9 cells

In the final part of the study, the effects of CDH1 and CDH2 knockdown on various cellular processes in PC9 cells were investigated. After knockdown experiments, we observed a significant (*p*-value < 0.001) reduction in mRNA expression of CDH1 and CDH2 (**Figure 9A**). This was confirmed by western blot analysis, which showed a corresponding decrease in CDH1 and

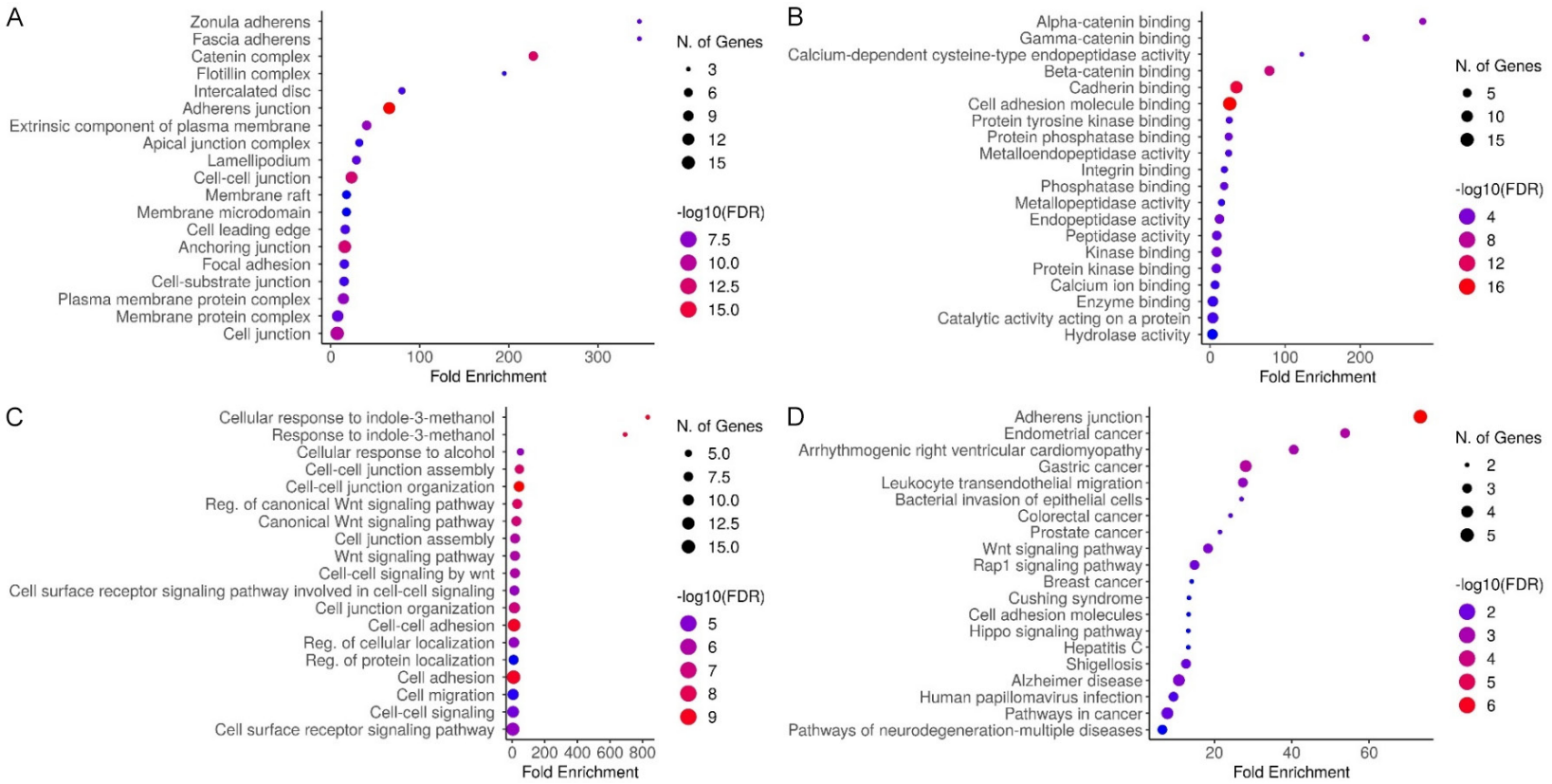

Figure 7. Gene enrichment analysis of cadherin genes. A. Cellular component enrichment terms. B. Molecular function enrichment terms. C. Biological process enrichment terms. D. KEGG pathway enrichment analysis. *P*-value < 0.05.

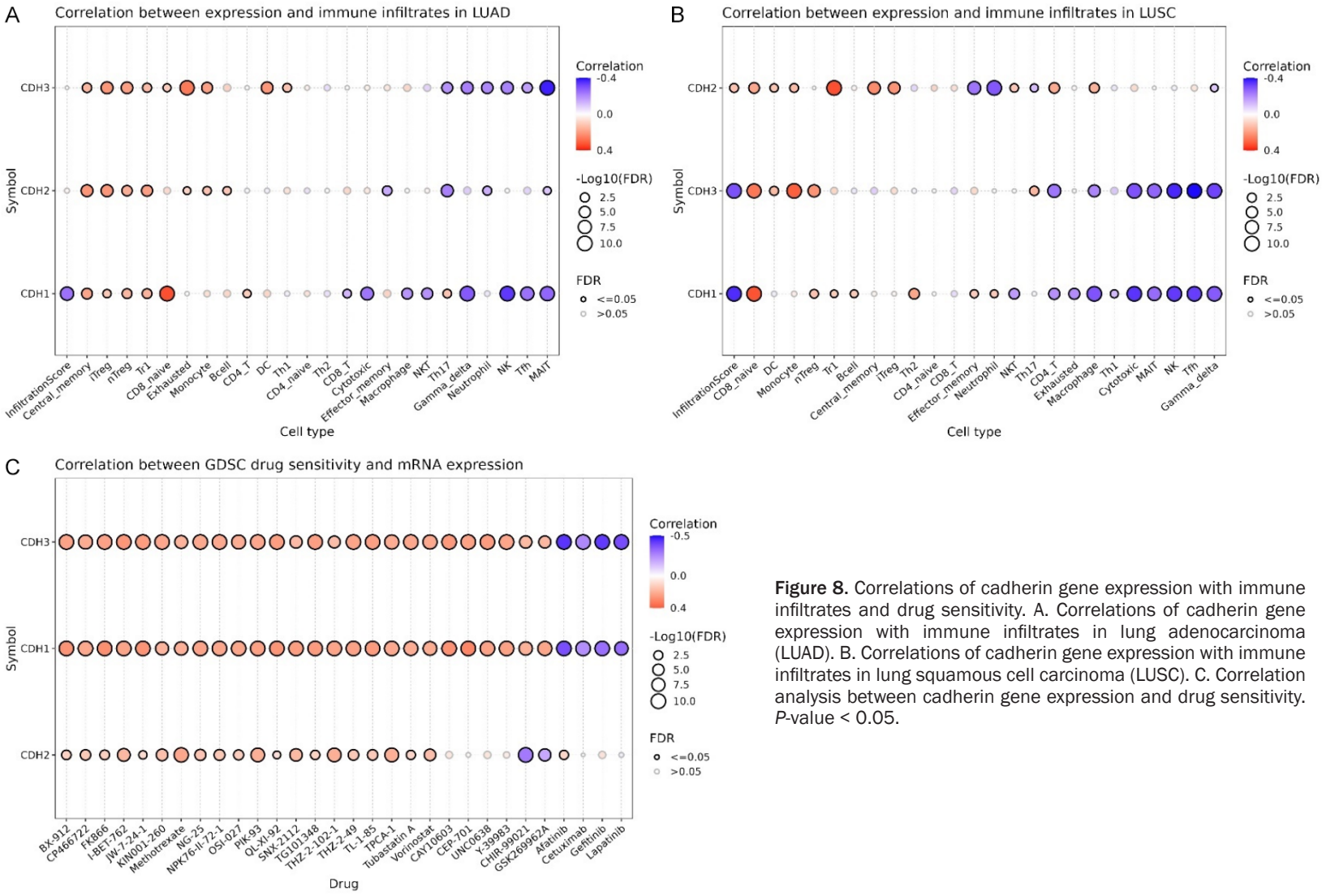

Figure 8. Correlations of cadherin gene expression with immune infiltrates and drug sensitivity. A. Correlations of cadherin gene expression with immune infiltrates in lung adenocarcinoma (LUAD). B. Correlations of cadherin gene expression with immune infiltrates in lung squamous cell carcinoma (LUSC). C. Correlation analysis between cadherin gene expression and drug sensitivity. *P*-value < 0.05.

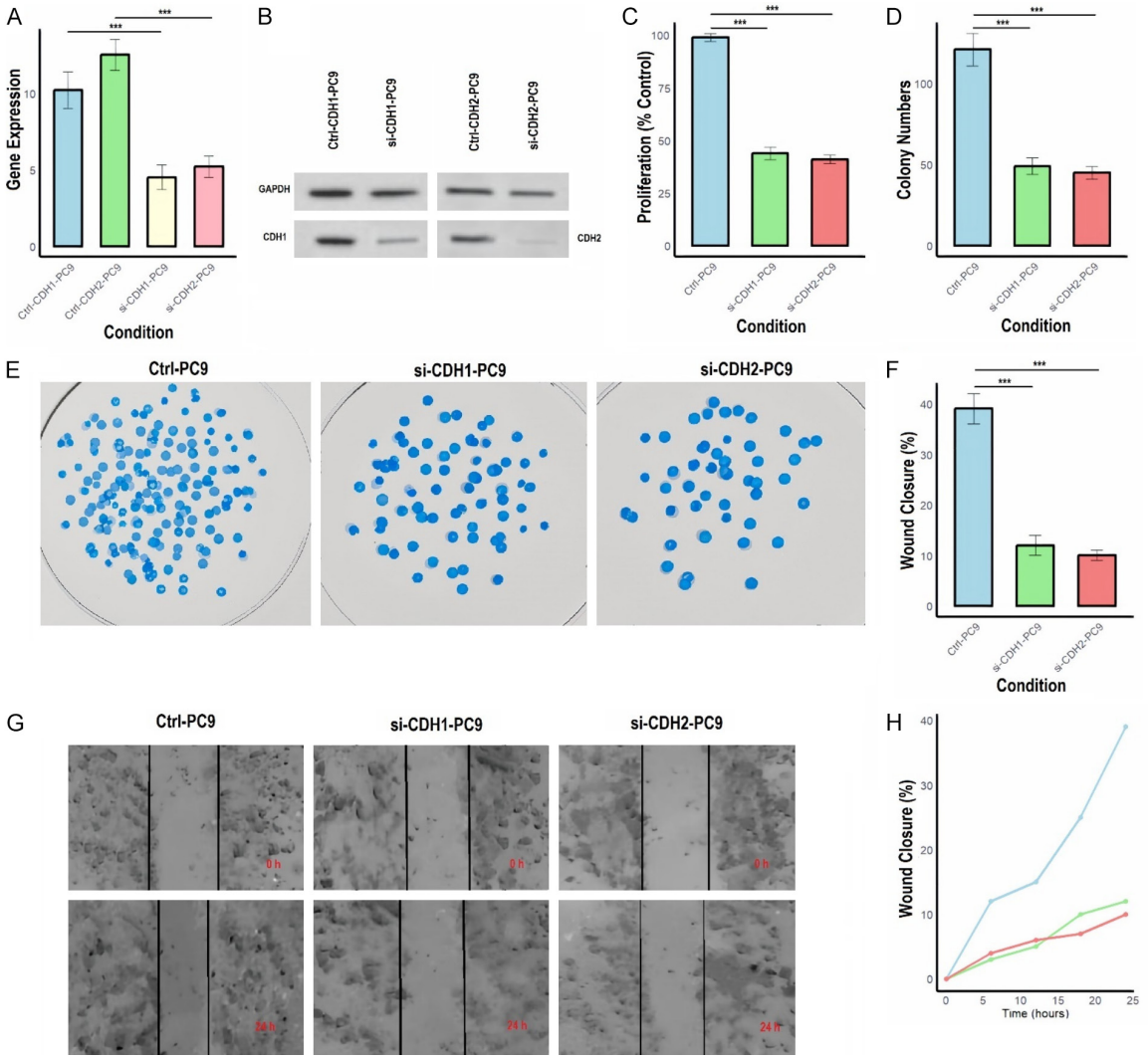

Figure 9. Knockdown effects of CDH1 and CDH2 on cell proliferation, colony formation, and wound healing in PC9 cells. A. mRNA expression levels of CDH1 and CDH2 after knockdown. B. Western blot analysis confirming the corresponding decrease in CDH1 and CDH2 protein levels in knockdown cells. C. Cell proliferation assays showing significant impairment in cell growth following CDH1 and CDH2 knockdown. D, E. Colony formation assays demonstrating that both knockdowns reduced colony numbers. F-H. Wound healing assays showing significant delays in wound closure in both CDH1 and CDH2 knockdown cells. ***P-value < 0.001.

CDH2 protein levels in the knockdown cells (**Figure 9B** and Supplementary Figure 1). Furthermore, cell proliferation was significantly impaired in both CDH1 and CDH2 knockdown cells, with the CDH2 knockdown leading to a greater reduction in proliferation (**Figure 9C**). Colony formation assays demonstrated that both knockdowns reduced colony numbers, especially in CDH2-silenced cells, which formed fewer colonies (**Figure 9D**, **9E**). Additionally, wound healing assays revealed that both CDH1 and CDH2 knockdowns significantly hindered wound closure, with the CDH2 knockdown showing the most severe delay in closure (**Figure 9F-H**).

Discussion

Non-small cell lung cancer (NSCLC) is the most prevalent form of lung cancer, accounting for approximately 85% of all lung cancer cases [46-49]. It is classified into two major subtypes: lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC) [50]. Despite advancements in treatment, the prognosis for NSCLC patients remains poor, primarily due to late-stage diagnosis, resistance to therapy, and metastatic spread [51]. Given the complexity of the molecular mechanisms driving tumorigenesis and progression in NSCLC, a deeper understanding of the genetic and epigenetic

alterations associated with the disease is essential for identifying therapeutic targets [52-54].

Cadherin family proteins, including CDH1 (E-cadherin), CDH2 (N-cadherin), and CDH3 (P-cadherin), play pivotal roles in cell-cell adhesion and tissue architecture [22]. Their dysregulation and roles in cell proliferation and migration has been rarely implicated in different malignancies, including NSCLC [24, 55]. The proliferation, migration, and invasion of NSCLC cells mediated by CDH1, CDH2, and CDH3 are likely influenced by several key signaling pathways, including the Wnt/β-catenin pathway, epithelial-mesenchymal transition (EMT), and other related pathways [22, 32]. Recent studies have highlighted the involvement of cadherin genes in regulating the Wnt/β-catenin pathway, where CDH1 has been shown to inhibit β-catenin-mediated transcriptional activation, thereby controlling cell proliferation and survival in cancer cells [22, 32]. Additionally, cadherins, particularly CDH2 (N-cadherin), play a pivotal role in promoting EMT, a process that enhances cell migration and invasion by altering cell-cell adhesion [22, 32]. Furthermore, cadherins are also implicated in TGF-β and Notch signaling pathways, both of which are involved in the regulation of cellular differentiation, migration, and metastasis, further supporting their use as therapeutic targets in NSCLC [21, 23].

This study aimed to investigate the expression, methylation, mutational landscape, and prognostic value of cadherin genes in LUAD and LUSC [56]. By understanding the role of cadherins in NSCLC, we hope to uncover novel molecular pathways that could be targeted for therapeutic intervention. The study also explored the effects of CDH1 and CDH2 knockdown on cellular processes such as proliferation, colony formation, and wound healing in PC9 cells, a commonly used NSCLC cell line.

Our results revealed that CDH1, CDH2, and CDH3 were significantly upregulated in both LUAD and LUSC samples. These findings were in line with previous studies showing that cadherins, particularly CDH1 and CDH2, are often upregulated in various cancers. For example, a study demonstrated increased CDH1 expression in breast cancer, particularly in advanced-stage tumors [57]. Similarly, another study found that CDH2 overexpression in breast was associated with worse prognosis, aligning with our finding of a possible role for CDH2 in driving the malignancy of LUAD and LUSC [58]. However, one novel aspect of our study is the inclusion of CDH3 in our analysis, which, to our knowledge, has not been as extensively studied in the context of NSCLC.

Our analysis of promoter methylation in LUAD and LUSC revealed hypomethylation of cadherin genes, which is consistent with earlier studies. For example, a previous study showed that hypomethylation of CDH1 was associated with increased expression and cancer progression in various tumor types, including lung cancer [59]. Similarly, another study found the reduced methylation levels in the promoter regions of cadherin genes, correlating with tumorigenesis in NSCLC [60]. Our results not only confirm these previous findings but also extend them by including CDH2 and CDH3, which have not been studied as extensively in terms of methylation status in NSCLC.

Our study highlights that cadherin gene expression may be a prognostic biomarker in NSCLC. We found that higher expression of CDH1, CDH2, and CDH3 was significantly associated with worse survival outcomes in both LUAD and LUSC. Previous studies have also linked cadherin expression to poor prognosis in various cancers. For instance, a study demonstrated that increased CDH1 expression was associated with poor survival in NSCLC [61]. Similarly, another study found that overexpression of CDH2 was correlated with reduced survival in breast patients [62]. Our study reinforced these findings but extended them by systematically analyzing the three cadherin genes (CDH1, CDH2, and CDH3) in both LUAD and LUSC and linking their expression to prognosis across both subtypes of NSCLC. A unique aspect of our study was the use of comprehensive survival analysis for all three cadherin genes across both LUAD and LUSC, offering a more nuanced view of their prognostic value. Additionally, we observed that CDH1 showed an even stronger association with poor prognosis in LUSC compared to LUAD, which contrasts with the findings of a previous study [63], that reported a similar trend but in a different cohort of lung cancer patients. Our study provides more robust evidence for CDH1 as a prognostic marker specifically for LUSC, suggesting

that subtype-specific differences in cadherin expression should be further explored.

Our findings on the correlation between cadherin gene expression and immune inhibitors add a novel layer to the understanding of cadherin-mediated immune regulation in NSCLC. Previous studies have shown that cadherins can influence immune cell recruitment and adhesion [64, 65]. In our study, we found positive correlations between cadherin expression and immune inhibitors like VTCN1 in both LUAD and LUSC, which may suggest a role for cadherins in modulating immune responses in NSCLC. This correlation may have implications for immune checkpoint therapy, although more research is needed to elucidate the functional significance of this relationship. Our analysis of cadherin gene expression and drug sensitivity also presents a novel finding. We observed that elevated expression of all three cadherin genes correlated with resistance to several therapeutic agents. This highlights the potential of cadherins as biomarkers for predicting drug response in NSCLC, suggesting that targeting cadherin pathways may enhance the efficacy of certain therapies, an underappreciated concept in lung cancer treatment.

Despite the comprehensive nature of our study, several limitations should be acknowledged. First, our analysis primarily relied on publicly available datasets, which may have inherent biases due to sample composition or data preprocessing methods, limiting the generalizability of our findings. Additionally, the cross-sectional nature of our data does not allow for causal inference, and the absence of functional validation experiments (e.g., RNA interference or CRISPR-based knockout) to confirm the role of cadherin genes in NSCLC progression limits the interpretation of their precise mechanisms. Moreover, while we have provided evidence for the prognostic significance of cadherin expression in both LUAD and LUSC, the influence of confounding factors, such as patient treatment history, comorbidities, and tumor microenvironment, was not accounted for in our analysis. Moreover, future work involving larger clinical cohorts will be crucial for the robustness of our results. Future prospective studies with larger cohorts and experimental validation are necessary to confirm the clinical relevance of cadherins and to explore their therapeutic use in NSCLC.

Conclusion

Our study provided compelling evidence that cadherin family genes (CDH1, CDH2, and CDH3) are upregulated in NSCLC and are associated with poor prognosis, particularly in LUAD and LUSC. Our findings not only corroborated previous studies but also contributed new insight into the role of CDH3 as a candidate therapeutic target. The correlation with immune inhibitors and drug sensitivity further highlighted the clinical relevance of these genes, offering new avenues for the development of personalized treatment in NSCLC.

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Disclosure of conflict of interest

None.

Address correspondence to: Changjiang Lei, Department of Oncology, The Fifth Hospital of Wuhan, Wuhan 430050, Hubei, China. E-mail: 305069071@qq.com; Su Zheng, Department of Rehabilitation, Taihe Hospital (Affiliated Hospital of Hubei University of Medical), Shiyan 442000, Hubei, China. E-mail: zhengsu0413@126.com

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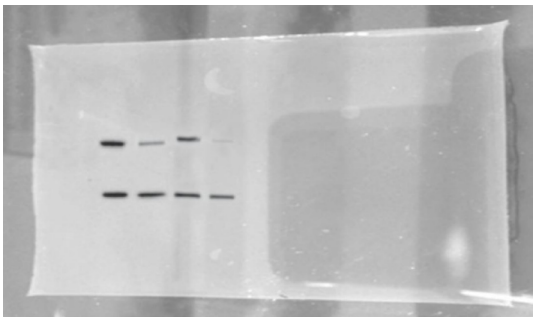

Supplementary Figure 1. Uncut western blot bands of CDH1, CDH2, and GAPDH.