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Synergistic potential of CDH3 in targeting CRC metastasis and enhancing immunotherapy

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Abstract

Background Colorectal cancer (CRC) remains a leading cause of cancer-related mortality, particularly due to advanced-stage metastasis. P-cadherin (CDH3), a potential therapeutic target, is highly expressed in CRC tissues and associated with poor prognosis and metastasis. However, the mechanisms underlying its role in CRC progression and its translational potential remain poorly understood.

Materials and methods This study integrated multiple public databases (TCGA, HCMDB, UALCAN, HPA, UniProt, cBioPortal, and GEO) to evaluate CDH3 expression, construct a prognostic model, and perform functional and translational analyses. Immunohistochemistry was used to validate CDH3 protein expression in clinical samples. Additional analyses included correlations with clinicopathological parameters, immune infiltration (TIDE, TISIDB), functional enrichment (KEGG, GSEA), drug sensitivity (GSCA), and molecular docking (MOE). Single-cell sequencing (CancerSEA, HPA) was also conducted to explore CDH3's role at the single-cell level.

Results CDH3 expression was significantly elevated in CRC tissues and correlated with poor prognosis, recurrence, and metastasis. CDH3 expression was associated with the infiltration of resting immune cells, particularly dendritic cells, and enrichment analysis revealed its critical role in CRC metastasis through extracellular matrix (ECM) and local adhesion pathways. Notably, afatinib emerged as a promising candidate for targeting CDH3 via "drug repositioning," a process involving the repurposing of existing drugs for new therapeutic applications.

Conclusion This study provides novel insights into CDH3's role in CRC metastasis and its potential as a therapeutic target. The translational potential of CDH3, including its integration with immunotherapy and drug repositioning strategies, offers a promising avenue for the treatment of metastatic CRC.

Keywords CDH3, Colon cancer, Immune, Prognosis, EMT, Drug target

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Introduction

Colorectal cancer (CRC) remains a significant global health burden, ranking as the third most common cancer and the second leading cause of cancer-related deaths worldwide [1, 2]. Despite advancements in treatment, metastasis continues to be the primary cause of mortality in CRC patients, with liver metastases occurring in 25–30% of cases [3, 4]. Current therapeutic options for metastatic CRC are limited, highlighting the urgent need for novel biomarkers and therapeutic targets [5].

E-cadherin (CDH1) is a member of a family of homogeneous transmembrane glycoproteins in epithelial tissue and is responsible for calcium (Ca^{2+})-dependent cell adhesion [6]. The structural characteristics of CDH3 exhibit a significant resemblance to those of CDH1. CDH1 functions as a tumor suppressor gene and is expressed in nearly all epithelial tissues, with the exception of certain locations, such as the proximal tubules.

CDH3 and CDH1 are located in close proximity on chromosome 16q22.1, and their levels are inversely correlated in CRC. The knockout of CDH1 leads to increased CDH3 expression, which may restore adhesion junctions but promote cell migration and proliferation, features characteristic of invasive tumors [15]. This switch from CDH1 to CDH3 has been identified as a biomarker of EMT in various cancers, including CRC [7]. Furthermore, CDH3 expression is absent in normal intestinal epithelial cells but present in inflamed, hyperplastic, and dysplastic intestinal mucosa [8], suggesting its role in early neoplastic progression.

We found that *CDH3*, a gene encoding P-cadherin, is a major contributor to cell–cell adhesion in epithelial tissues, plays a key role in important morphogenesis and differentiation processes during development, and plays a critical role in maintaining tissue integrity and homeostasis [9–11]. Recent clinical studies have shown that abnormal expression of CDH3 is associated with poor prognosis in patients with squamous cell carcinoma of the tongue [12], gastric cancer [13], or cervical cancer [14]. The discovery that tumor-associated antigens that are highly expressed only in tumors can trigger an immune response has prompted research into the possible role of CDH3 in colon cancer invasion [15–19].

In this study, we identified 1913 differentially expressed genes (DEGs) between colon cancer samples and paracancerous samples by searching data from multiple public databases, analysed and compared 1913 DEGs between different stages of colon cancer tissues and paracancer tissues and obtained four DEGs by taking their intersection. The candidate gene CDH3 was obtained via prognostic analysis and weighted gene coexpression network analysis screening and was validated at the protein level. The value of CDH3 in colon cancer metastasis was assessed by analysing the relationships between CDH3 and metastasis-related clinicopathological factors. Risk models were constructed via univariate and multivariate Cox regression analyses [20]. The prognosis of elevated CDH3 in colon cancer is worse than that of normal intestinal tissue, while the prognosis of CDH3 in metastatic colorectal cancer is worse. The correlation between CDH3 expression and colon cancer metastasis was elucidated; immune correlation analysis was also performed to explore the relationships between CDH3 expression and immune infiltration, immune checkpoints, and immune regulatory genes. Further studies on drug sensitivity revealed that CDH3 could be a potential target for drug therapy and attempted to determine the role and potential molecular mechanisms of CDH3 in the biological function of colon cancer cells (Fig. 1).

Materials and methods

Summary of databases involved in CDH3 analysis

mRNA expressed: The Cancer Genome Atlas (TCGA) database (https://www.cancer.gov/) [21], protein expressed: University of Alabama at Birmingham (UALCAN) database (http://ualcan.path.uab.edu/index.html) [22], Human Protein Atlas(HPA)database (http://www.proteinatlas.org/) [23], Universal Protein (UniProt) database (https://www.uniprot.org) [24], Cancer meta stasis: Human Cancer Metastasis Database (HCMDB) database (https://hcmdb.i-sanger.com/) [25], Mutatio n analysis: cBio Cancer Genomics Portal (cBioPortal) database (http://cbioportal.org) [26], Protein modificat ion: Posttranslational modifications (qPTM) (http://qptm.omicsbio.info/) [27], Immune Cell-Related Analysis Database: tumor immune estimation resource, Version 2 (TIMER2.0) database (http://timer.cistrome.org/) [28], Cell Signaling Technology (https://www.cellsignal.com) and R&D Systems (https://www.rndsystems.com) [29], Gene Expression Profiling Interactive Analysis (GEPIA) database(http://gepia.cancer-pku.cn/) [30], Tumor Immune Dysfunction and Exclusion (TIDE) database (http://tide.dfci.harvard.edu/) [31] (Supplementary protoc ol 1), an integrated repository portal for tumor-immune system interactions (TISIDB) (http://cis.hku.hk/TISIDB/) [32], Gene function enrichment: The Database for Annotation, Visualization and Integrated Discovery (DAVID) (https://david.ncifcrf.gov) [33], Drug sensitivity analysis: Gene Set Cancer Analysis (GSCA) database (http://bioinfo.life.hust.edu.cn/GSCA/#/) [34], Single cell sequencing databases: CancerSEA database (http://biocc.hrbmu.edu.cn/CancerSEA/) [35], HPA database (https://www.proteinatlas.org/). This study employs the integration of various databases, each with distinct functions and areas of focus, to conduct a thorough and multifaceted examination of the mechanisms underlying the action and potential application of CDH3 in colorectal cancer (CRC). The investigation encompasses several dimensions, including

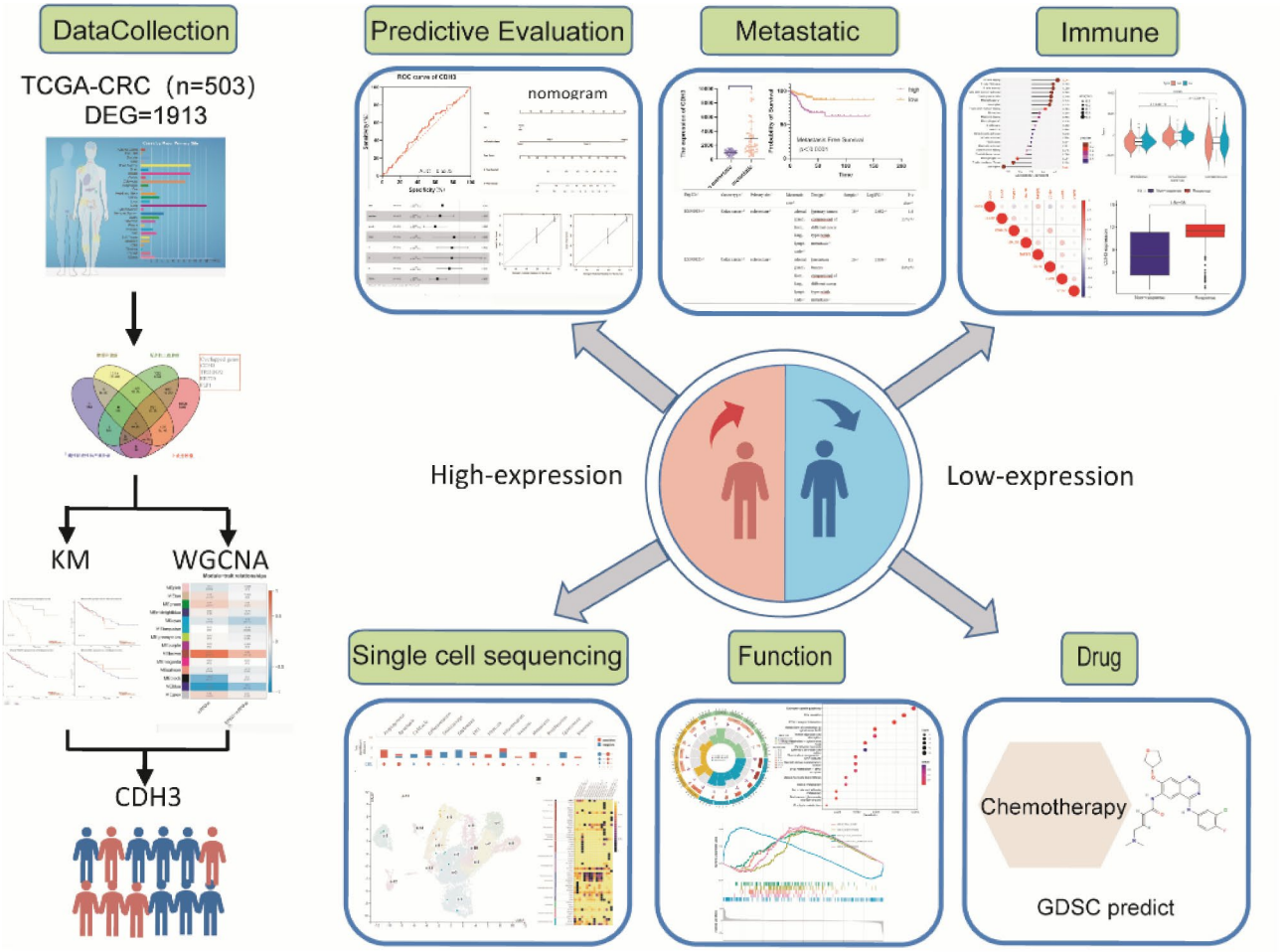

Fig. 1 Workflow for the screening and analysis of CDH3, a prognostic marker for targeted colon cancer metastases

gene expression, protein functionality, clinical prognosis, the immune microenvironment, and drug sensitivity. This comprehensive approach yields substantial information and theoretical support for the diagnosis, treatment, and prognostic evaluation of CRC.

Target gene screening and identification

Differential expression analysis

Using the TCGA database, mRNA expression datasets from colon cancer and paracancerous samples were obtained. Differential expression analysis was performed using the limma package (version 3.38.3) in R (version 4.0.1). Significant differential expression was determined with a fold change cutoff of ≥ 2 or ≤ 0.5 and a p-value < 0.05 after Benjamini-Hochberg correction for multiple comparisons [36]. Heatmap and volcano map of differentially expressed genes. Candidate genes were screened by Venn diagrams and survival curves (K–M) (Supplementary protocol 2).

Weighted gene coexpression network analysis (WGCNA)

WGCNA was conducted using the WGCNA package in R. Data cleaning involved removing low-quality data and genes with low variance. Coexpression networks were constructed, and modules associated with colon cancer progression were identified. Block module functions were used to construct mRNA coexpression networks in colon cancer and complete target gene screening. Data cleaning was performed to further screen for genes related to colon carcinogenesis and metastasis [37, 38].

Validation of CDH3

CDH3 mRNA expression was validated using the HPA database. Protein expression was confirmed via IHC, with scoring based on staining intensity and percentage of positive cells. To validate the mRNA expression levels of the screened target genes in colon cancer vs. paracancerous tissue, colon cancer tissue vs. paracancerous tissue were analysed to visualize the mRNA expression of *CDH3* in colon cancer and paracancerous samples. The HPA database was used to validate the protein expression of CDH3 in colon tissues (as shown by IHC) [39, 40].

Relationship between CDH3 expression and recurrent metastasis in colon cancer

The relationship between *CDH3* expression and recurrent colon cancer metastasis was analysed on the basis of clinical data from the TCGA, and K–M curves were plotted. The relationship between CDH3 expression and recurrent metastasis in patients with colon cancer was further validated with a GEO database dataset (GSE38174). The HCMDB database was used to explore the metastatic potential of CDH3 in the TCGA-COAD cohort [41].

Prognostic analysis of CDH3

Cox proportional risk regression model based on CDH3

To evaluate the effect of CDH3 on survival time in colon cancer patients, we performed univariate Cox proportional risk regression analysis via the SURVIVAL package of R software (version 4.0.1) [42]. A Cox proportional hazards regression model was built using the survival package in R. Variables included age, sex, tumor stage, and treatment history. A hazard ratio (HR) with a 95% confidence interval (CI) and $P<0.05$ indicated statistical significance. A multivariate Cox proportional risk regression analysis was conducted via stepwise regression methods and mathematical models to identify factors associated with the prognosis of patients with colon cancer, and a Cox proportional risk regression model was constructed [43].

CDH3 prognostic analysis

CDH3 expression was dichotomized at the median. ROC curves were used to assess the correlation between *CDH3* expression and patient survival. *CDH3* expression values were divided into high and low-expression groups according to the median for colon cancer patients, and subject operating characteristic (ROC) curves were plotted to analyse and evaluate the correlation between *CDH3* expression and prognosis in colon cancer patients [44] (Supplementary protocol 3).

Survival model analysis

The final column line plot was created via the "rms" package in R to estimate the probability of survival for individual colon cancer patients and to visualize the results of multiple Cox regression [45]. Prognosis-related clinical factors and genetic signature models were used to construct column line graphs. Each factor in the column line graph is scored according to its weight, and the predictive model accuracy is assessed and compared via decision curve analysis (DCA) [46]. Based on the distribution of CDH3 expression in the discovery cohort, a median value was used to categorize patients into high and low expression groups. The Kaplan-Meier method was used to estimate survival curves, and the log-rank test was applied to compare survival distributions between the high and low CDH3 expression groups. To adjust for potential confounders, a multivariate Cox proportional hazards regression model was used. Variables included in the model were age, sex, tumor stage, and treatment history. A hazard ratio (HR) with a 95% confidence interval (CI) not overlapping 1.0 and a $P<0.05$ were considered indicative of a statistically significant association between CDH3 expression and survival outcomes.

Clinical tissue experiments

Patients and tissue specimens

Tissue samples were obtained from individuals diagnosed with COAD at the Gastrointestinal Surgery Ward of the Fourth Hospital of China Medical University between 2008 and 2012. Samples were collected from CRC patients at the Fourth Hospital of China Medical University. Inclusion criteria included histologically confirmed CRC, availability of follow-up data, and no prior anticancer therapy. Exclusion criteria were metastatic disease at diagnosis and incomplete clinical data. The samples comprised 50 instances of COAD tissue, 50 instances of normal tissue, and 20 instances of metastatic COAD tissue. This study was executed in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of China Medical University. Informed consent was obtained from all participants. To validate the findings from our initial discovery cohort, we employed an independent validation cohorts. The following characteristics were collected for each patient in the validation cohorts: age, sex, tumor stage, histological grade, and treatment history.

Tissue microarray (TMA) and immunohistochemistry (IHC)

The tissue microarray chip was deparaffinized with xylene, followed by debenzene and hydration through a decreasing gradient of ethanol. The chip was subsequently immersed in citrate buffer (pH 6.0) and subjected to high pressure of 80 kPa for 10 min for repair. Following treatment with 3% hydrogen peroxide/methanol and 10% nonimmune normal goat serum, the chip was incubated overnight at 4 °C with a mouse anti-human CDH3 protein monoclonal antibody (Aviva Systems Biology Cat# ARP45170_T100, RRID: AB_937861) added dropwise. The following day, a biotin-labelled secondary antibody (UltraSensitive SP Mouse/Rabbit IHC Kit, China) was applied to the chip, followed by the dropwise addition of freshly prepared 3,3'-diaminobenzidine (DAB). Finally, the sections were counterstained with hematoxylin, dehydrated, made transparent with xylene, and mounted with neutral gum for observation.

Immunohistochemistry evaluation

Two proficient clinicians assessed the degree of staining on the tissue microarray chip, noting the expression of the CDH3 protein primarily in the cytomembrane. The staining level was determined by multiplying the percentage of stained cells (ranging from 0 to 100%) by the staining intensity (categorized as 0 for no staining, 1 for weak positive, 2 for medium positive, and 3 for strong positive). This calculation resulted in a total score range of 0–300%. Survival status was assessed via receiver operating characteristic (ROC) curve analysis, with the total score serving as the threshold value to differentiate between negative and positive protein expression.

Immune cell correlation and immune infiltration analysis

Immune cell correlations

Using the cibersort package, immune cell subtypes were analyzed for correlation with CDH3 expression. The immune cell subtypes (B cells naive, B cells memory, plasma cells, T cells CD8, T cells CD4 naive, T cells CD4 memory quiescent, T cells CD4 memory activated, T cells follicular helper, T cells regulatory (Tregs), T cells gamma delta, NK cells quiescent, NK cells activated, monocytes, M0 macrophages, M1 macrophages, M2 macrophages, quiescent dendritic cells, and activated dendritic cells). activation, mast cell quiescence, mast cell activation, eosinophils, and neutrophils) expression matrix explore the correlation between the CDH3 gene and immune cells [47].

Immunological score

In the examination of the mechanisms through which CDH3 affects immune cell functionality, it is essential to recognize the intricate interactions among diverse cell types and signaling pathways present within the tumor microenvironment. Beyond the direct interactions between CDH3 and the surface molecules of immune cells, it is imperative to account for the regulatory influences exerted by additional factors within the tumor microenvironment, as well as the stroma score, on immune cell activity. These elements may serve as confounding variables, thereby impacting the relationship between CDH3 and immune cells. The ESTIMATE package in R provides tumor purity, viewing, and downloading of stromal, immune, and estimated scores for each sample of colon cancer on all TCGA platforms to infer the proportion of stromal and immune cells in tumor samples [48]. Generation of a violin plot of CDH3 gene expression and immune score in colon cancer.

Immune cell infiltration analysis

We assessed cancer stage in colon cancer patients on the basis of cancer stage in TCGA clinical data for each patient's immune cell subtype (B-cell naive, B-cell memory, plasma cells, T-cell CD8 + T cell, T-cell CD4 + T-cell naïve, T-cell CD4 + memory resting, T-cell CD4 + memory activated, T-cell regulatory (Tregs), T-cell gamma delta, NK-cell resting, NK-cell activated, monocyte, M0 macrophage, M1 macrophage, M2 macrophage, resting dendritic, activated dendritic, resting mast-cell, activated mast-cell activated, eosinophils, and neutrophils) and assessed the impact of infiltration scores [49]. The impact of CDH3 on immune cell prognosis was assessed via the TIMER 2.0 database. When analyzing the relationship between CDH3 expression and immune cell infiltration, it is necessary to consider the potential impact of factors such as the patient's age, gender, tumor stage, and treatment history on immune cell infiltration. These factors may influence the distribution and function of immune cells within the tumor microenvironment independently of CDH3.

Relationships between CDH3 expression and immune cell markers and immune checkpoint-related genes

In addition to analysing immune cell infiltration, we investigated the relationship between CDH3 expression and many immune cell markers to identify the immune cells associated with their expression. CDH3 expression was correlated with immune checkpoint-related genes using the GEPIA database. Tumors in different patients may have distinct molecular subtypes and biological characteristics, which may affect the expression of immune checkpoint-related genes and their relationship with CDH3. We propose to categorize tumor samples based on the median expression level of CDH3, and then analyze the correlation between CDH3 expression and immune checkpoint-related genes to reduce the confounding effects brought by disease heterogeneity. Immunogenic markers were selected from Cell Signaling Technology and R&D Systems. These include B cells, CD8 + T cells, CD4 + T cells, Tfhs, Th1 cells, Th2 cells, Th9 cells, Th17 cells, Th22 cells, Tregs, Tex cells, M1 macrophages, M2 macrophages, tumor-associated macrophages (TAMs), monocytes, NK cells, neutrophils, dendritic cells (DCs) and Bregs [50, 51]. We explored the relationship between CDH3 and immune cell marker expression via the GEPIA database.

Analysis of immunotherapy efficacy

On the basis of normalized transcriptomic data from the TCGA colon cancer dataset, TIDE scores were retrieved from the TIDE database, and T-cell dysfunction scores were retrieved to synthesize the response of patients with colon cancer to immune dysfunction and rejection to effectively predict the efficacy of immune checkpoint suppression therapy and assess the correlation between *CDH3* expression and the response to immunotherapy [52]. The calculation of the TIDE score involves three

main steps: first, select gene sets related to immune cell dysfunction and rejection based on literature and bioinformatics analysis. Then, analyze the expression levels of these genes in tumor samples. Finally, calculate the tumor immune dysfunction and rejection score based on the gene expression levels. The TIDE score includes an immune dysfunction score, which assesses the degree of immune cell dysfunction in the tumor microenvironment; and an immune rejection score, which assesses the degree of immune cell rejection in the tumor microenvironment, such as the presence of immunosuppressive cytokines and immunosuppressive cells.

Functional enrichment analysis

After verifying differential expression, gene function analysis, including Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses, was performed via the DAVID enrichment database. The above steps were also used to screen the biological functions of the DEGs, including enrichment in biological processes, molecular functions, and cellular components, and to draw circle and bubble maps. Gene set enrichment analysis (GSEA) is a method for analysing and interpreting microarrays and such data via biological knowledge [53, 54]. GO and KEGG analyses were performed using DAVID, with significant terms determined by $P < 0.05$.

Identification of potential therapeutic drugs that target CDH3

We used the GSCA database to analyse relevant gene–drug interaction networks to predict the effects of various drugs on CDH3 protein expression.

Molecular docking

The DrugBank database was utilized to obtain the 2D structure of afatinib, which was subsequently converted from SDF format to PDB format via Open Babel version 2.3.2 [55]. An analysis of the crystal structure of the CDH3 protein was conducted (PDB: 4oy9), during which the receptor protein underwent desolvation and ligand removal through the application of PyMOL version 2.3.4. Additional modifications, including the addition of hydrogen atoms, were executed via AutoDock Tools, facilitating the conversion of both the receptor protein and the ligand small molecules into pdbqt format [56]. Molecular docking was performed with AutoDock Vina version 1.1.2, with the conformation exhibiting the lowest binding energy selected as the docking outcome. Typically, a binding energy threshold of < -5.0 kcal/mol is indicative of favourable binding potential. The results of the molecular docking were visualized via PyMOL software.

Analytical methods

We investigated the prognostic or predictive accuracy of each feature and protein classifier via time-dependent receiver operating characteristic (ROC) analysis. We used the area under curve (AUC) at different cutoff times to measure prognostic or predictive accuracy. A fold change cutoff of ≥ 2 or ≤ 0.5 was used to define significant upregulation or downregulation of CDH3 expression, respectively. A $P < 0.05$, following adjustment for multiple comparisons using the [Bonferroni/FDR/etc.] method, was considered statistically significant. Employing multivariate linear regression and Cox proportional hazards regression models, this study incorporates potential confounding variables into the analysis to more precisely evaluate the independent relationship between CDH3 expression and immune cell infiltration. In this study, Cox regression and survival analysis were conducted using R software (version 4.0.1). We compared two groups via the t test for continuous variables and the chi-square test for IHC. Specifically, the SURVIVAL package was used to perform univariate Cox proportional hazards regression analysis, where the CDH3 expression values were incorporated into the model to evaluate their independent impact on the survival time of colon cancer patients. Factors such as age, gender, and tumor stage, which may affect survival, were also included. The relative risk (HR) and 95% confidence interval (CI) for each factor were calculated, and the statistical significance was determined by the *P*-value, thereby identifying potential factors associated with survival. Variables were gradually included or excluded to determine the final factors to be included in the model, constructing an optimized Cox proportional hazards regression model. Additionally, the "rms" package was used to construct nomograms, providing each patient with an intuitive prediction of survival probability. This is based on the results of multivariate Cox regression, considering the weights of multiple factors, and assessing the consistency between the predicted values and the actual observations through calibration curves to ensure the reliability of the model.

Decision curve analysis (DCA) calculates the net benefit at different thresholds to comprehensively evaluate the clinical application value of the model, helping to determine whether the model can bring actual benefits to patients. In survival analysis, the Kaplan-Meier method was used to draw survival curves, visually displaying the survival status of different patient groups (such as high and low CDH3 expression groups). The log-rank test was used to compare the differences in survival curves between groups, determining whether they are statistically significant, providing a strong basis for judging the relationship between CDH3 expression and patient prognosis.

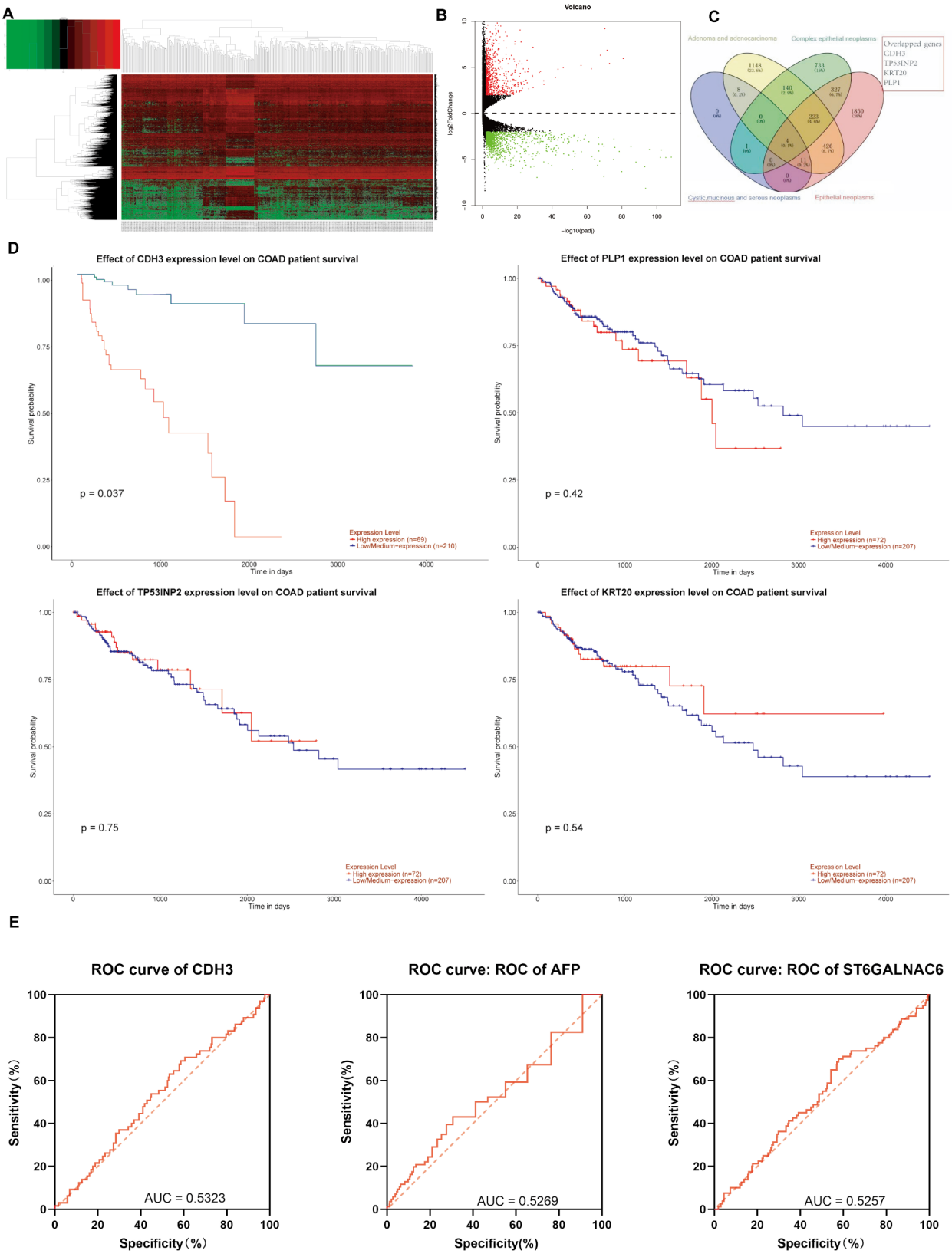

Fig. 2 (See legend on next page.)

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Fig. 2 Identification of DEGs in colon cancer: (**A**, **B**) Heatmap of DEGs in colon cancer and normal samples in the TCGA dataset; volcano plot. The vertical axis represents the -log (adjusted P value [adj. P]), and the horizontal axis represents log2 (FC). Red dots represent upregulated genes, and green dots represent downregulated genes ($P < 0.01$ and|log2(FC)| > 1). (**C**) Venn diagram showing the 4 DEGs. (**D**) K–M survival analysis of DEGs. (**E**) It illustrates the receiver operating characteristic (ROC) curves for CDH3, AFP, and CA19-9. The ROC curve was used to evaluate the diagnostic performance of biomarkers. The larger the area under the curve (AUC), the better the diagnostic performance of biomarkers. The figure shows that the AUC of CDH3 is 0.5323, the AUC of AFP is 0.5269, and the AUC of CA19-9 is 0.5257, indicating that CDH3 has some potential as a tumor biomarker

Results

Screening and expression of target genes for colorectal cancer

In this subsection, differential gene expression analysis and WGCNA were conducted to screen for target genes in colorectal cancer. CDH3 was identified as a candidate gene, and its expression was found to be significantly elevated in CRC tissues compared to normal tissues. Additionally, CDH3 expression was associated with recurrent metastasis and poor prognosis in colon cancer patients.

Differential gene expression analysis and WGCNA screening for colon cancer

We downloaded 461 samples of colon cancer and 42 samples of paracancerous tissue from the TCGA - Colon Cancer Project. Differential expression analysis of each type of colon cancer yielded 1913 DEGs (658 upregulated and 1255 downregulated). Heatmaps and volcano plots were drawn based on these DEGs (Fig. 2A, B). The intersecting genes in the Venn diagram of different types of colon cancer DEGs were CDH3, TP53INP2, KRT20, and PLP1, defined as candidate DEGs (Fig. 2C). K - M survival curves were generated for these four genes, and only CDH3 was significantly associated with the survival of patients with colon cancer ($P < 0.05$). The overall survival (OS) curve of the high CDH3 expression group was significantly lower than that of the low expression group, indicating a higher risk of recurrence or death for patients with high CDH3 expression (Fig. 2D). To further explore the potential of CDH3 as a tumor biomarker, we collated its expression data with tumor biomarkers (AFP, CA19–9) through the TCGA database and plotted ROC curves. CDH3 had the highest AUC (AUC = 0.5323 for CDH3, AUC = 0.5269 for AFP, and AUC = 0.5257 for CA19–9), suggesting it has some potential as a tumor biomarker(Fig. 2E) [57, 58]. Analysis of CDH3 expression in colon cancer and paracancerous samples from the TCGA database demonstrated that CDH3 mRNA expression was significantly upregulated in both paired and unpaired clinical colon cancer tissues relative to normal tissues. According to the UniProt database, the CDH3 protein is a single - channel type I membrane protein expressed on cell membranes. Immunohistochemical images from the HPA database showed that CDH3 staining levels were low in normal tissues but high in colon cancer tissues (Supplementary Fig. 1A-C).

WGCNA screening

A weighted gene coexpression network was constructed via the WGCNA package for 1913 DEGs between the CC group and the paracancer group. The optimal soft threshold $\beta = 4$ was selected by scale - free topology (Fig. 3A), and all selected genes were clustered into 16 modules (Fig. 3B, C). *CDH3* was found to belong to the BLUE module(Fig. 3D). Overall, *CDH3* was selected as a prognostic marker for CRC.

CDH3 expression in metastatic and nonmetastatic colon cancer

Clinical data on colon cancer in the TCGA cohort showed a significant positive correlation between CDH3 expression and recurrent metastasis in patients with colon cancer ($P < 0.001$). High CDH3 expression was observed in patients with metastatic colon cancer, and there were significant differences in metastasis - free survival (MFS) outcomes (Fig. 3E, F). Comparison of CDH3 expression levels in metastatic and non - metastatic patients with primary tumor sites in colon cancer via the HCMDB database also revealed significant differences (Table 1), validating that CDH3 is a good predictor of distant tumor metastasis. The prognostic expression of CDH3 was notably different between metastatic and non - metastatic colon cancer patients ($P < 0.001$), with higher CDH3 expression in metastatic patients correlating with a poorer prognosis.

CDH3 prognostic correlation analysis

This subsection focuses on the prognostic correlation analysis of CDH3. The Cox proportional risk regression model showed that CDH3 expression is an independent prognostic factor for CRC patients. A nomogram model was constructed, which has good predictive performance in estimating the survival probability of colon cancer patients.

Cox proportional risk regression model

Univariate analysis revealed that age, clinical stage, and T, N, and M stages influenced the OS of patients with colon cancer ($P < 0.05$). Cox multifactor regression analysis suggested that age was an independent factor affecting the prognosis of colon cancer patients ($P < 0.05$) (HR = 0.882, 95% Cl = 0.643–1.209; $P = 0.43$; Fig. 3G)). CDH3 expression was also found to be an independent prognostic factor for CRC patients. Patients with high CDH3

expression had significantly worse survival outcomes compared to those with low expression, emphasizing the importance of CDH3 in predicting patient prognosis.

Nomogram model construction

Based on the above analysis, a column line graph model was constructed using prognostic correlates, including patients' clinical T classification and age (Fig. 3H). The 3 - and 5 - year survival probabilities predicted by the DCA curves were highly consistent with the analysis results(Fig. 3I). The nomogram, by inputting parameters such as the patient's age, tumor stage, and CDH3 expression level, can identify corresponding survival probability points on the curve. The prognostic ROC curves showed good predictive performance of the CDH3 - based prognostic index (3 - year AUC = 0.868 and 5 - year AUC = 0.817), indicating the clinical applicability of the model in predicting the prognosis of patients with colon cancer (Fig. 3J).

Experimental verification of CDH3 in clinical samples

Immunohistochemistry was used to analyze CDH3 expression in clinical samples. The results confirmed that CDH3 expression was highest in metastatic CRC tissues, followed by in - situ CRC tissues, and lowest in normal tissues. High CDH3 expression was associated with poor prognosis in colorectal cancer patients.

We utilized immunohistochemistry(IHC) techniques to analyze the expression of CDH3 in clinical sample chips from normal individuals, patients with in - situ CRC, and patients with metastatic CRC (Fig. 4A). The clinical statistical data are presented in Table 2. Comparative immunohistochemical microscopic imaging demonstrated that patients with metastatic colorectal cancer presented the highest CDH3 expression, followed by patients with in - situ colorectal cancer, and the lowest CDH3 expression was observed in the normal population (Fig. 4B). Combined with the analysis of clinical prognosis data, high CDH3 expression was associated with poor prognosis (Fig. 4C), leading to a significant decrease in survival rates among patients with metastatic colorectal cancer (Fig. 4D).

Immunological correlation analysis

This section explores the immunological correlations of CDH3. CDH3 expression was found to be inversely associated with immune infiltration, especially in resting NK cells. It also has significant relationships with immune checkpoint - related genes, and high CDH3 expression may predict a better response to immunotherapy in colon cancer patients.

Correlation between the CDH3 gene and immune cell infiltration in colon cancer

We assessed the relationship between CDH3 expression levels and immune cell subpopulation infiltration in colon cancer. A positive correlation was detected between CDH3 expression and resting NK cells ($R = 0.45$, $P = 0.041$) (Fig. 5A). Analysis of colon cancer samples from the TCGA database and calculation of stromal and immune scores via ESTIMATE found a significant negative correlation between CDH3 expression and the level of immune infiltration (Fig. 5B). Clinical data from colon cancer patients showed that the M stage, T stage, and immune infiltration of dendritic cells were significantly correlated (Fig. 5C, D), with higher levels of infiltration in the M0 stage and in the T3 and T4 stages. These results suggest that CDH3 upregulation inhibits immune infiltration and promotes the occurrence of colon cancer metastasis.

Relationships between CDH3 expression and immune checkpoint-related genes

We analysed the associations between *CDH3* and immune cell markers to further investigate the potential link between *CDH3* and invading immune cells (Supplementary Table 1). B cells, CD8 + T cells, CD4 + T cells, M1/M2 macrophages, tumor-associated macrophages, monocytes, NK cells, neutrophils, DC cells, and regulatory B cells were identified via these markers. We also analysed various subtypes of T cells, including follicular helper T, Th1, Th2, Th9, Th17, Th22, Tregs, and exhausted T cells. *CDH3* expression was associated with 22 of 40 immune cell markers in colon cancer, and *CDH3* expression was significantly correlated with B cell, CD4 + T cell, DC, Breg, monocyte, NK, Th22, and M2 markers ($P < 0.05$). Among them, a significant negative correlation was found with markers of DC cells. The TIDE score can be used as a more accurate predictor of immune checkpoint blockade (ICB) therapy [59]. A low TIDE score indicates a lower likelihood of tumor immune escape and a greater likelihood of benefiting from anti-PD-1/CTLA4 therapy. We found that colon cancer patients in the high-CDH3 expression group had lower TIDE and dysfunction scores than patients in the low-CDH3 expression group did (Fig. 5E), suggesting that patients in the high-CDH3 expression group are candidates for ICB therapy. We analysed the relationships between *CDH3* expression and immune checkpoint-related genes (Fig. 5F) and found that *CDH3* expression was positively correlated with HHLA2, TNFSF9, CD276, and VTCN1 expression and negatively correlated with CD40LG, LGALS9, and CD160 expression. Patient response to anti-PD-L1 therapy was predicted (Fig. 5G). We further confirmed that the CDH3 expression had a significant relationship immunological checkpoint-related genes

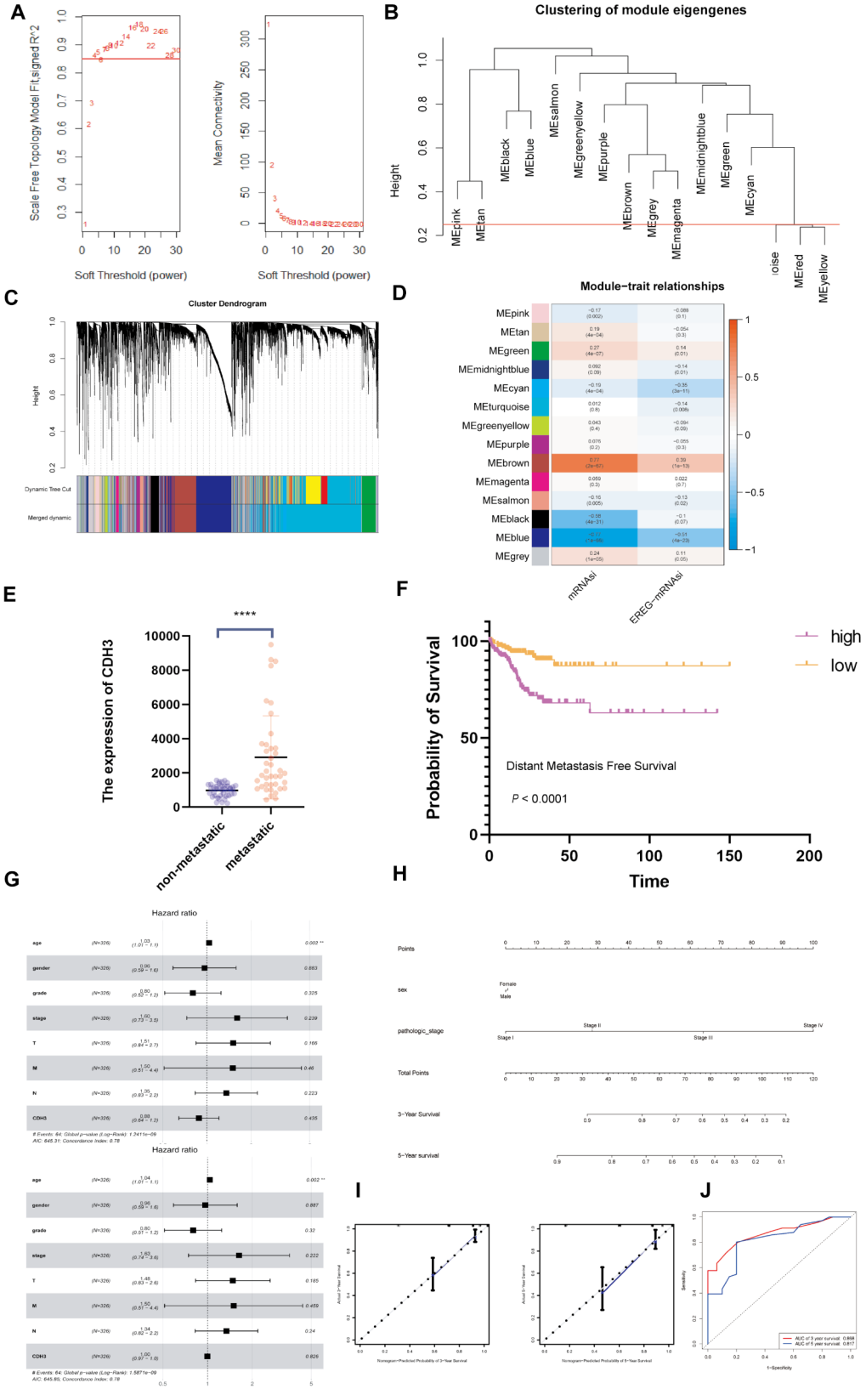

Fig. 3 (See legend on next page.)

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Fig. 3 WGCNA-based construction of coexpression modules for colon cancer RNA-seq data: (**A**) Network topology analysis of various soft-threshold powers. To examine the unscaled topology, the adjacency matrix was defined via soft thresholds with β = 4. (**B**) Line plots showing gene expression trends for each module. (**C**) Clustering of gene dendrograms with variability on the basis of topological overlap and the specified module colors. (**D**) Heatmap visualizing gene networks. (**E**) Expression of *CDH3* in metastatic, nonmetastatic colon cancer. (**F**) K–M curves of *CDH3* expression and metastasis-free survival in patients with colon cancer. (**G**) Univariate and multivariate Cox regression analyses of genes. **P* < 0.05, ***P* < 0.01 and ****P* < 0.001. (**H**) Columnar line plots for predicting the 3- or 5-year OS of patients. (**I**) Calibration curves for column line plots showing the agreement between the predicted and observed 3- and 5-year outcomes in the training cohort. (**J**) The ROC curve of CDH3-based prognostic index for detection of risk factors for colon cancer is shown. The AUC at 3 and 5 years was 0.868 and 0.817, respectively. The high AUC values indicated that the CDH3-based prognostic index had good performance in predicting the prognosis of colon cancer patients

(Fig. 6A), suggesting that patients with metastatic colon cancer are better candidates for immunotherapy. Moreover, we transformed each expression value using the log2(x + 0.001) transformation. Next, we calculated the Pearson correlation coefficients for the CDH3 gene and the marker genes of the five immune pathways. CDH3 is correlated with the majority of immune regulatory genes (Fig. 6B).

Furthermore, we investigated the relationship between CDH3 expression and immune checkpoint-related genes. CDH3 expression was negatively correlated with the expression of CD40LG, a gene associated with dendritic cell maturation and antigen presentation. This finding implies that CDH3 may interfere with the immune response by affecting dendritic cell function.

Functional enrichment analysis

Functional enrichment analysis was performed to understand the biological pathways involved in colon cancer metastasis related to CDH3. The results showed that CDH3 is associated with processes such as EMT and is enriched in pathways like ECM - receptor interaction, cell cycle, endocytosis, and focal adhesion. To further understand the biological pathways involved in the mechanism of colon cancer metastasis, biological enrichment analyses (KEGG pathway and GO analyses) were performed on the TCGA dataset (Fig. 7A, B). The biological processes associated with CDH3 include epidermis development, epidermal cell differentiation, keratinocyte differentiation, skin development, and intermediate filament organization. The above processes suggest that CDH3 has some relevance to EMT; the KEGG pathway is enriched mainly in the extracellular matrix (ECM)-receptor interaction pathway. The ECM is a highly active part of the TME and affects the metastatic ability of colon tumor cells [60]. We performed gene set enrichment analysis (GSEA) on the gene sets (Fig. 7C) and found associations with the cell cycle, endocytosis, and focal adhesion.

CDH3 protein and drug sensitivity analysis

This part focuses on the relationship between CDH3 protein and drug sensitivity. Afatinib was found to have the strongest correlation with CDH3 among the drugs analyzed. It has a high binding affinity to CDH3, suggesting it could be a potential drug for targeting CDH3 in CRC treatment.

There are currently no drugs in the FDA that directly target CDH3. We performed Spearman correlation analysis of CDH3 expression with small molecule/drug sensitivity, and the top 30 small molecule/drug sensitivities are shown in Fig. 8A and Table 3. The table indicates that the impact of CDH3 expression on drug sensitivity is not uniform across different drugs. CDH3 can either enhance or reduce the sensitivity of cancer cells to various treatments, suggesting a complex role for CDH3 in cancer biology and treatment response.

Afatinib, an epidermal growth factor receptor (EGFR) inhibitor, was found to be the most strongly correlated with CDH3. The IC50 of afatinib in the group with high CDH3 expression was significantly lower than that in the group with low CDH3 expression (Fig. 8B), and the docking score of afatinib with the CDH3 protein was − 7.359, indicating strong binding ability (Fig. 8C and D).

We performed drug sensitivity analysis showed the strongest correlation with CDH3 expression and exhibited high binding affinity to CDH3. This suggests that Afatinib could be a viable option for targeting CDH3 in CRC treatment.

CDH3 single-cell sequencing analysis

Single-cell sequencing analysis of *CDH3* further supported its role in CRC metastasis and immunotherapy. *CDH3* was positively correlated with EMT, metastasis, and proliferation, and was associated with immune cell populations, especially DC cells.

We analysed the correlation between the 14 functional states of the *CDH3* gene and colon cancer (Fig. 9A). *CDH3* was found to be most significantly positively correlated with inflammatory immunity and apoptosis and most significantly negatively correlated with the cell cycle. *CDH3* was positively correlated with EMT, metastasis, and proliferation in colon cancer and was correlated with colon carcinogenesis development and invasive metastasis, which is consistent with the results of the previous analysis. Colon cancer cells were analysed via the HPA database, and 16 different cell populations were analysed by descending and clustering analysis via a uniform manifold approximation and projection (UMAP) plot. The results revealed that *CDH3* was most strongly

Table 1 Comparison of CDH3 expression levels in primary tumors from patients with metastatic and nonmetastatic disease in the HCMDB

Exp ID	Cancer type	Primary site	Metastasis site	Design	Sample	Log_2FC	*P* value
EXP00124	Colon cancer	colorectum	adrenal gland, liver, lung, lymph node	[primary tumors comparison] of different cancer types with metastasis	18	2.682	$1.625◊10^{-4}$
EXP00125	Colon cancer	colorectum	adrenal gland, liver, lung, lymph node	[metastasis tumors comparison] of different cancer types with metastasis	26	2.809	$8.584◊10^{-4}$

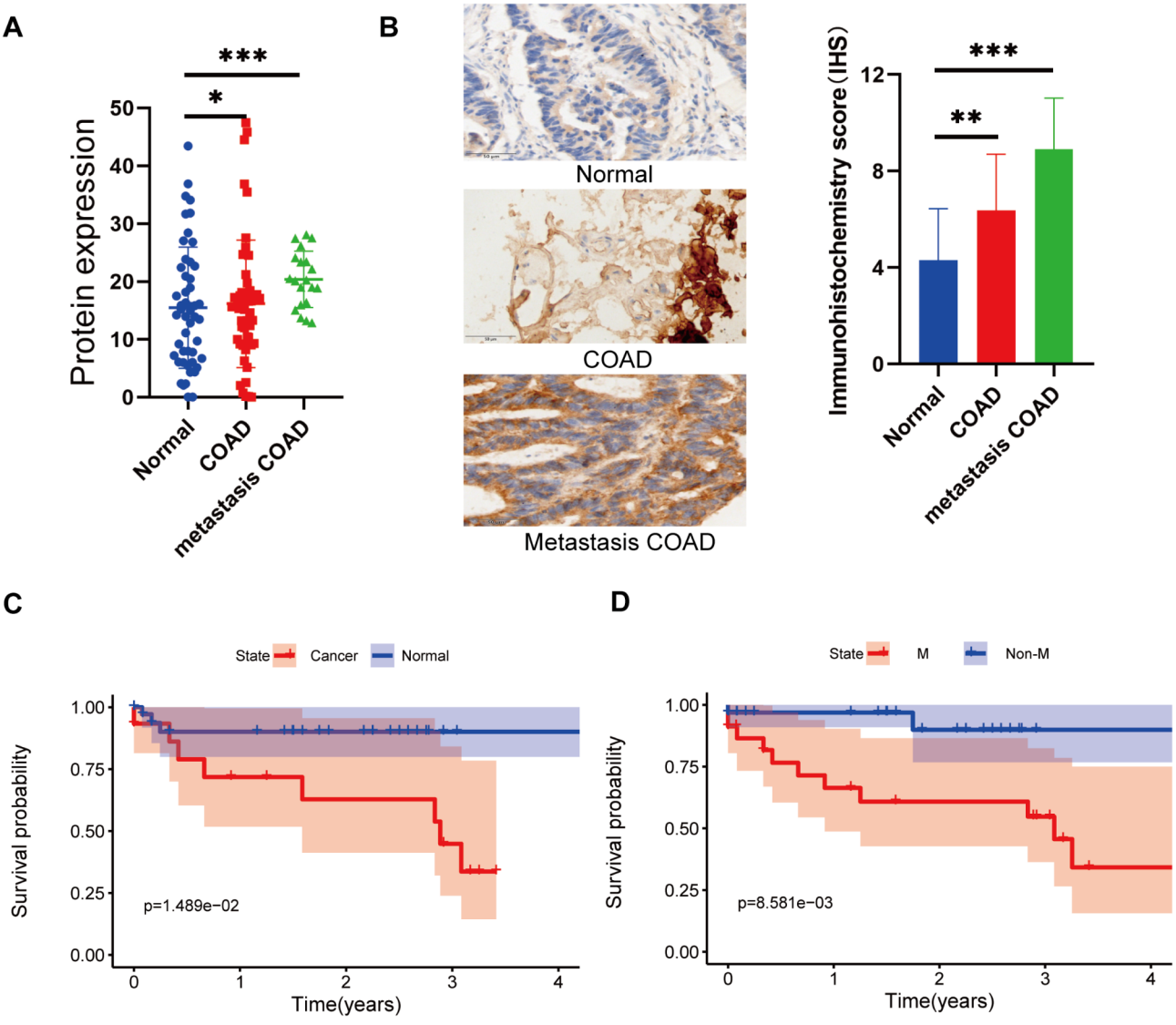

Fig. 4 Experimental verification of CDH3 (**A**) protein expression in normal, colon cancer, and metastatic colon cancer tissues from clinical samples. (**B**) Immunohistochemical results and statistics of normal, colon cancer, and metastatic colon cancer tissues. (**C**) Comparison of survival curves between clinical colorectal cancer patients and normal patients. (**D**) Comparison of survival curves between clinical colorectal cancer patients and metastatic colorectal cancer patients

associated with cluster 3 (T cells) (Fig. 9B) and correlated with DC cells in immune cells (Fig. 9C). The single-cell sequencing results validated the function of *CDH3* in colon carcinogenesis, metastasis and immunotherapy, as previously described. Tumor samples were obtained from patients with colon cancer, and the tumor microenvironment was carefully dissected to include both cancer cells and immune cells. Single cells were isolated using fluorescence-activated cell sorting (FACS) based on cell surface markers specific to cancer cells and dendritic

Table 2 Clinicopathological characteristics of patients with colon cancer

Features	Categories	Frequency	Percent
Sex	Male	54	45.0
	Female	66	55.0
Age(years)	≤ 60	49	40.8
	>60	71	59.2
Family history	No	54	81.8
	Yes	12	18.2
	Low + Medium	62	51.7
Invasion depth	Inside muscular	16	13.3
	Outside muscular	42	35.0
Lymphatic metastasis	Yes	20	28.6
	No	50	71.4
CEA	Negative	57	64.8
	Positive	31	35.2
CA-125	Negative	58	76.3
	Positive	18	23.7
CA19-9	Negative	67	80.7
	Positive	16	19.3

cells. Isolated single cells were subjected to scRNA-seq, which captures the transcriptomic profile of each cell. Sequencing data were processed using bioinformatics tools designed for scRNA-seq data, including cell clustering, dimensionality reduction, and differential expression analysis. We specifically focused on identifying dendritic cell subsets and characterizing their gene expression profiles in relation to neighboring cancer cells.

Single-cell RNA sequencing analysis further supported the role of CDH3 in CRC metastasis and immunotherapy. CDH3 expression was positively correlated with EMT, metastasis, and proliferation, confirming its role in CRC progression. Additionally, CDH3 was found to be associated with immune cell populations, particularly DC cells, further supporting its involvement in immune regulation.

Discussion

CDH3 is a cell adhesion molecule associated with the binding of cells to the extracellular matrix (ECM) [61]. CDH3 can influence intracellular signaling pathways that affect the expression of immune checkpoints and other molecules involved in immune regulation. This remodeling can create a more permissive environment for immune cell infiltration. For example, a decrease in CDH3 expression might lead to a more disorganized ECM, allowing immune cells to penetrate the tumor more effectively [87]. Immune infiltration is closely related to cancer development and progression, and infiltration of immune cells in the tumor microenvironment is associated with the response to immunotherapy [62]. Studies have shown that immunotherapy is a first-line treatment for metastatic colorectal cancer; however, only a small proportion of patients benefit from immune checkpoint inhibitors [63]. Exploring potential biomarkers is therefore a clinical priority. Immune cell infiltration can help identify the key pathways driving tumor formation. In our study, *CDH3* expression was found to be significantly correlated with the infiltration of resting NK cells. As immune infiltration progresses, it promotes tumor progression and inhibits immune cell-mediated cytotoxicity. DCs play a key role in the coordination of innate and adaptive antitumour immunity [64]. One study reported a high rate of DC infiltration in colon cancer, promoting tumor cell migration, tumor stem cell stemness, and EMT by increasing the number of Treg cells and decreasing CD8 + T-cell cytotoxicity [65, 66]. In our study, M stage, T stage, and immune infiltration of dendritic cells were significantly correlated, further validating the relationship between DC infiltration and colon cancer metastasis. NK cells recognize and kill tumor cells through a balance of activating and inhibitory signals received from the interaction with tumor cell surface molecules. CDH3 expression might affect the presentation of ligands for NK cell receptors, such as NKG2D ligands, which are often upregulated in stressed or transformed cells. A decrease in CDH3 expression could lead to the upregulation of these ligands, thereby enhancing NK cell activation and tumor cell lysis. Dendritic cells are key antigen-presenting cells that can initiate and modulate immune responses. CDH3 might influence the interaction between tumor cells and DCs, affecting the capture and presentation of tumor antigens to T cells. A altered CDH3 expression could impact the efficiency of this process, potentially leading to a more robust antitumor immune response.

There are numerous studies on immune infiltration in CRC, however, a few more recent references on immune checkpoint inhibitors and their correlation with metastasis, Treatment options for patients with metastatic colorectal cancer (mCRC) are limited, and the prognosis is poor. Although immunotherapy with checkpoint inhibitors (ICI) holds promise, some patients do not respond. There is a discussion in the literature about the role of the chemokine receptor CCR5 and its ligand CCL5 in mCRC, including their effects on the tumor microenvironment, immune response, and treatment efficacy [67–69].

Cancer growth and progression are linked to immunosuppression, and the use of monoclonal antibodies targeting immune checkpoints represents a breakthrough in cancer treatment [70]. Programmed cell death receptor 1 (PD-1) and/or cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) checkpoint inhibition is highly effective in the treatment of patients with advanced mismatch-repair-deficient (dMMR) CRC [71]. The Food and Drug Administration (FDA) approved the first use of immunotherapy drugs for the treatment of metastatic colon cancer in 2017 [72, 73]. Immune checkpoint inhibitors

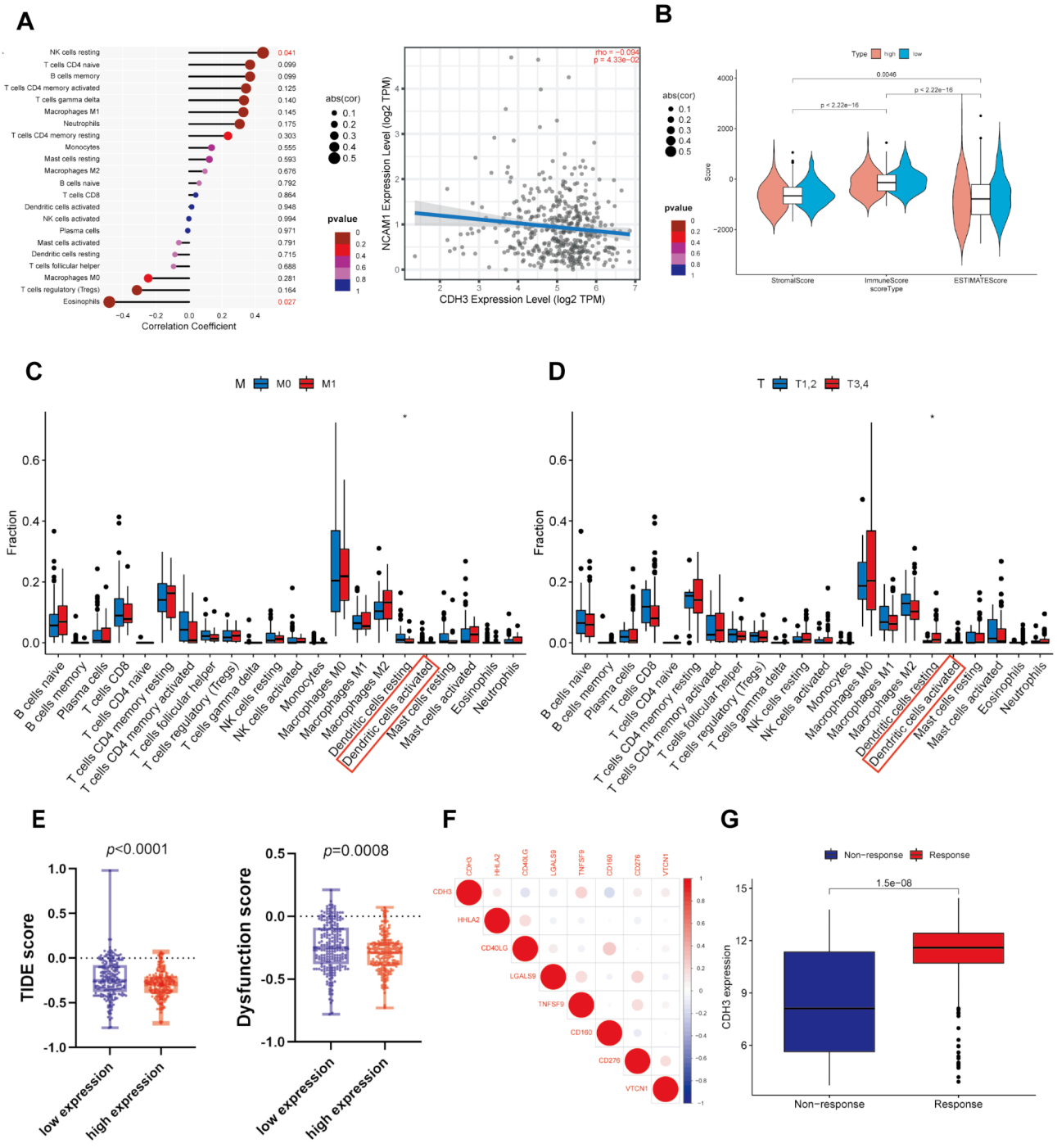

Fig. 5 Predicting immunotherapy in patients with colon cancer. (**A**) An analysis of differences in *CDH3* expression and immune cells in colon cancer. Each dot in the figure represents an immune cell subtype, and the color and position of the dot reflect its association with *CDH3* expression and its *P* value. (**B**) *CDH3* expression in the TCGA database versus immune infiltration score, immune score, and stromal score immune infiltration versus clinical outcome in the TCGA-COAD dataset. (**C**, **D**) Clinical stage (M0, M1) with immune infiltration assessment. (**E**) TIDE scores and T cell dysfunction scores for colon cancer patients with low CDH3 expression and those with high *CDH3* expression. TIDE score can be used to predict the response to immune checkpoint blockade (ICB) therapy. Low TIDE score indicates that the possibility of tumor immune escape is low and patients are more likely to benefit from anti-PD-1 / CTLA4 treatment. The figure shows that the TIDE score and dysfunction score were lower in the high *CDH3* expression group, suggesting that patients in this group may be better candidates for ICB treatment. (**F**) The results of the correlation analysis between *CDH3* gene expression and immune checkpoint related gene expression. Each point in the figure represents an immune checkpoint-related gene, and the direction and slope of the line reflect its association with *CDH3* expression. (**G**) The association between *CDH3* expression and immune response by the TIDE algorithm. Groups of *CDH3* expression levels are represented on the patients' response to immunotherapy (as non-response or response) is represented on the ordinate. This graph is used to show the relationship between *CDH3* expression and immunotherapy response to aid in determining the role of *CDH3* in immunotherapy

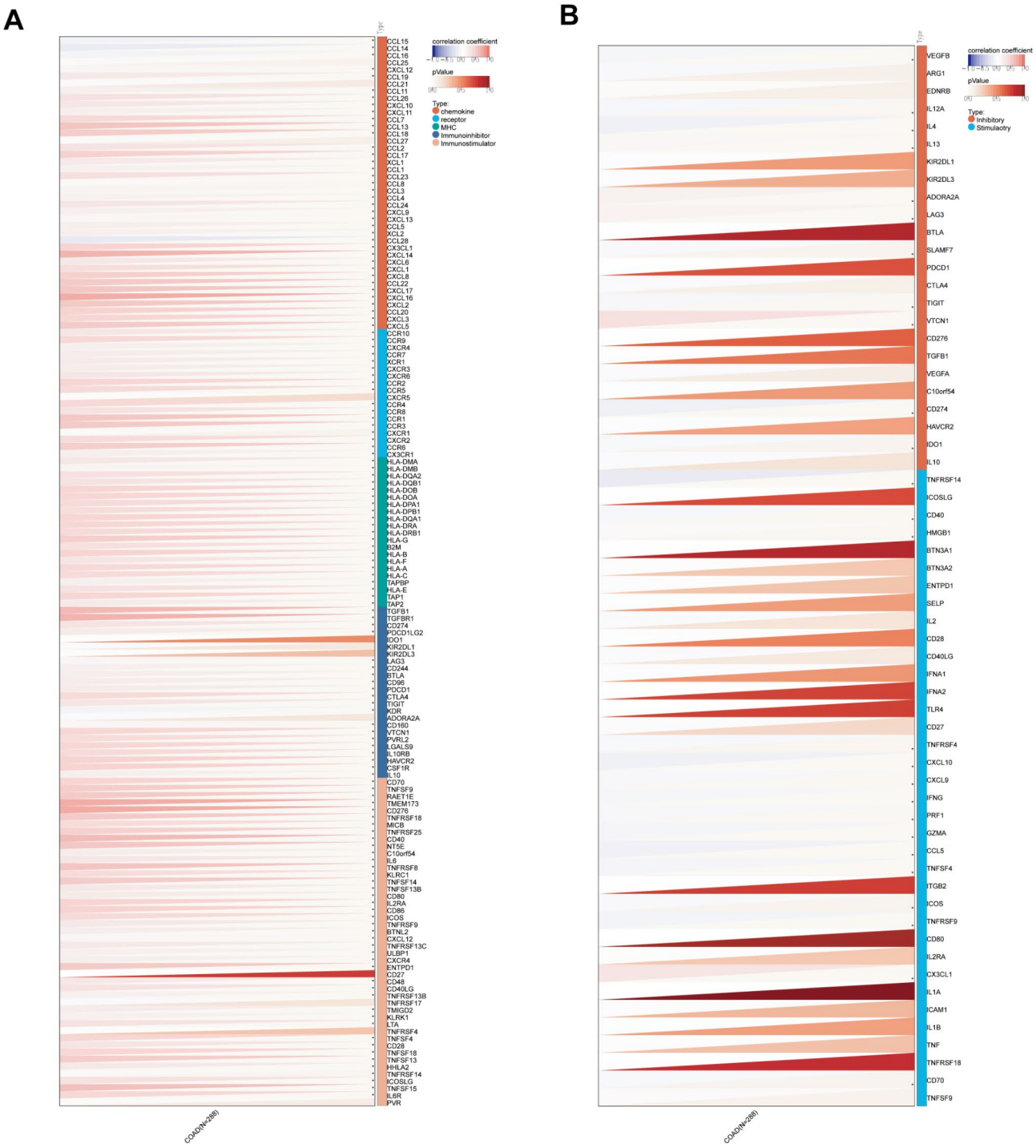

Fig. 6 The relationship of CDH3 and immune checkpoint modulation and immune regulatory genes. (**A**) The correlation between *CDH3* gene expression and immune checkpoint related gene expression. This figure further validates the significant relationship between CDH3 expression and immune checkpoint related genes, providing a basis for investigating the role of CDH3 in immune regulation. (**B**) The correlation between CDH3 gene expression and immune checkpoint regulated gene expression. The figure shows that CDH3 is correlated with most immunomodulatory genes, which will help us to understand the mechanism of CDH3 in the tumor immune microenvironment

stand as pivotal tools in immunotherapy, capable of reinvigorating the immune system, bolstering therapeutic efficacy, and prognosticating immune therapy responses. It is imperative to recognize that immune checkpoint inhibitors do not universally apply to all patients; their efficacy is influenced by factors such as tumor types and individual patient variabilities. We found a negative correlation between the expression of CDH3 and CD40LG, a

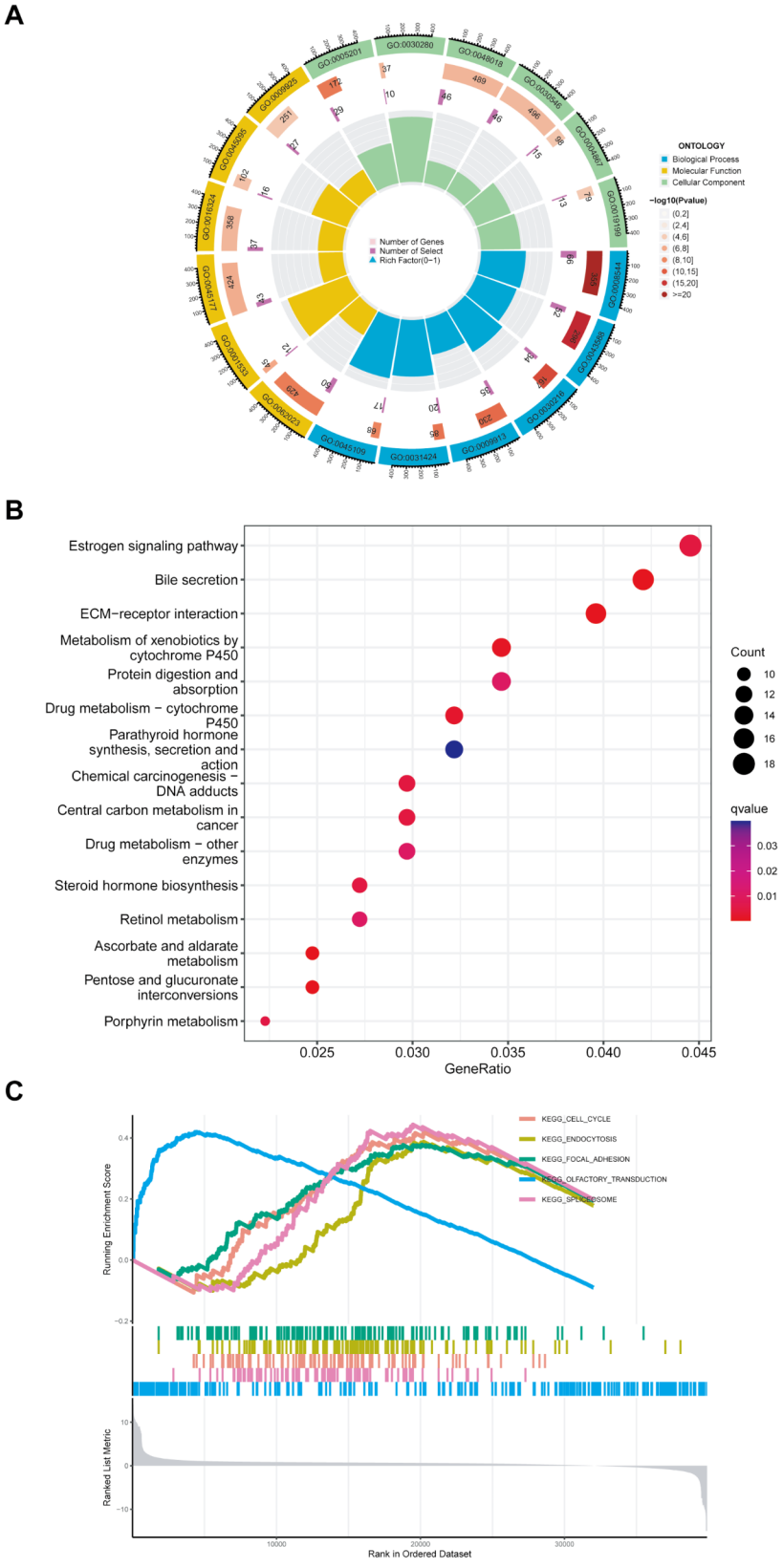

Fig. 7 (See legend on next page.)

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Fig. 7 Functional enrichment analysis of CDH3. (**A**) The results of GO pathway correlation analysis for CDH3. The ordinate is -log10 (Pvalue), and a higher value indicates a more significant correlation between this pathway and CDH3. (**B**) The results of KEGG correlation analysis of CDH3. The abscissa is GeneRatio, indicating the enrichment ratio of genes in this pathway. The ordinate is qvalue, which measures the false discovery rate. The figure shows the KEGG pathways that CDH3 is significantly enriched in, such as the ECM-receptor interaction pathway, revealing important biological pathways in which CDH3 is involved. (**C**) The GSEA results for CDH3. The abscissa is the ranking of genes in the ordered dataset, and the ordinate is the enrichment score (ES). The figure demonstrates the association of CDH3 with cell cycle, endocytosis, and focal adhesion pathways, suggesting that CDH3 has important functions in these pathways. $P < 0.05$* and FDR q < 0.25 (q value: a measure of the false discovery rate)

core prognostic gene associated with the tumor microenvironment belonging to the tumor necrosis factor (TNF) gene superfamily, which plays a key role in dendritic cell maturation, and the stimulation of CD40L/CD40 by the endogenous expression of CD40L has been shown to increase the immunomodulatory capacity of DCs in colorectal tumor cells [74]. The above results provide further evidence that CDH3 may play a role in the immune regulation of colon cancer metastasis through DCs and thus in immunotherapy.

Studies have indicated a correlation between TIDE scores and the efficacy of immune therapy, with tumor patients presenting lower TIDE scores being more inclined towards a response to immunotherapy. The TIDE score emerges as a valuable bioinformatics instrument, aiding in the anticipation of tumor immunotherapy responses and offering leads for the exploration of tumor immunotherapeutic interventions. Immature DCs in the tumor microenvironment are unable to effectively uptake, process, and present tumor antigens, leading to insufficient T cell activation and impaired immune surveillance function. This state of immune cell dysfunction is reflected in the TIDE score, causing it to increase, indicating an increased possibility of tumor immune escape and suggesting that patients may have a poor response to treatment with the PD-1 inhibitor Pembrolizumab [75]. The TIDE score and PD-1 immunotherapy prediction results suggest that CDH3 may provide new ideas for the treatment of colon cancer metastasis. This is crucial for the development of new therapeutic approaches for colon cancer metastasis, but further studies are needed to explore the exact mechanisms involved.

To identify the mechanism of the role of CDH3 in colon cancer metastasis, public databases such as TCGA and methods such as GSEA were used to locate CDH3-related pathways. CDH3 functional enrichment analysis revealed that CDH3 is associated with key pathways, such as cell adhesion and EMT, which are potential targets for the development of cancer therapeutic strategies [76]. Cell adhesion is a key mediator of cancer progression, with adhesion and interactions mediated by cell adhesion molecules (CAMs), which alter the ability of tumor cells to interact with other cells and extracellular matrix proteins and promote hallmarks of cancer, including immune escape and metastatic spread [77, 78]. In colon cancer, tumor cells become mesenchymal-like and have an increased ability to enter the circulation after the occurrence of EMT [79]. Enabling cells to stratify and locally invade from the primary tumor [80]. The EMT process confers migratory and invasive properties on tumor cells, and changes in the expression of cell adhesion molecules play important roles in EMT [81], suggesting that CDH3 may induce colon cancer progression and metastasis by promoting cell invasion and EMT.

As there is no small-molecule drug directly targeting CDH3 in the FDA, we have predicted a small-molecule drug targeting CDH3 through the drug-related website GDSC: afatinib, an irreversible blocker of EGFR, a receptor tyrosine kinase (RTK) associated with cell survival, growth, proliferation, and differentiation, which is a therapeutic target for human malignancies, and anti-EGFR therapy. Afatinib could be used in combination with standard chemotherapy regimens, such as FOLFOX or FOLFIRI, especially in patients who have become resistant to these treatments. The sequential or concurrent use of afatinib with these regimens could potentially overcome resistance mechanisms and improve overall survival. Additionally, the side effect profile of afatinib, including rash and diarrhea, should be carefully managed to ensure patient compliance and quality of life. The emerging role of immunotherapy in CRC has led to the approval of immune checkpoint inhibitors, such as pembrolizumab and nivolumab. The combination of afatinib with immunotherapy could exploit complementary mechanisms of action, potentially leading to synergistic effects. Afatinib's ability to downregulate EGFR, which can contribute to immune evasion, may enhance the efficacy of immunotherapy by making tumor cells more susceptible to immune attack [82, 83].

EGFR inhibitors have been shown to modulate the tumor microenvironment by reducing the expression of immune checkpoint ligands such as PD-L1, which can enhance the response to immunotherapy. In some cases, EGFR inhibitors can also increase the infiltration of immune cells, such as T cells, into the tumor. Some studies suggest that targeting CDH3 could lead to a more inflamed tumor microenvironment, which is often associated with a better response to immunotherapy [84]. These studies often demonstrate improved tumor control, increased survival rates, and a more robust immune response when both therapies are used together. Tumors with high levels of CDH3 expression may have specific genomic alterations that could make them more sensitive to immunotherapy when combined with EGFR

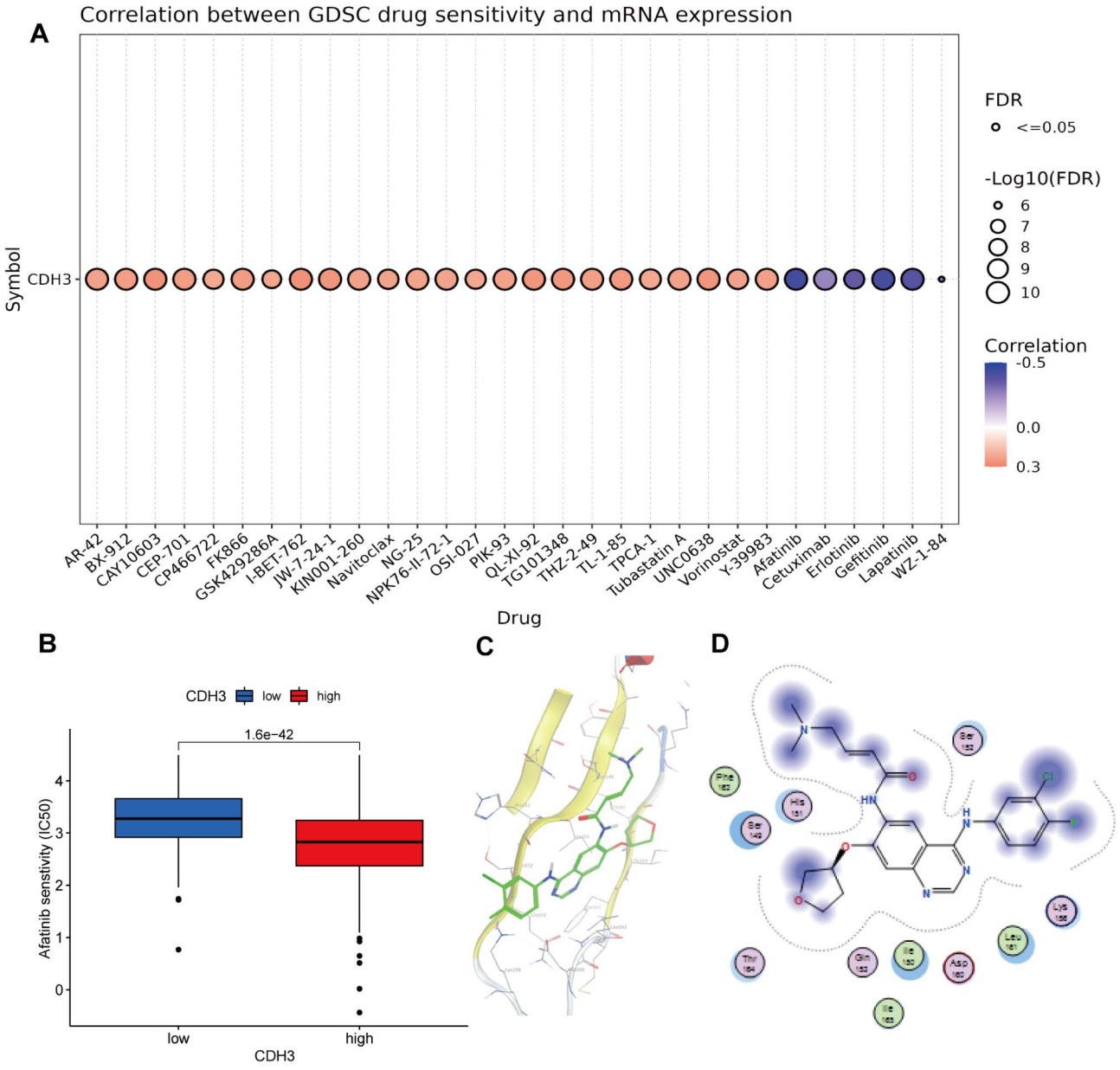

Fig. 8 CDH3 protein and drug correlation: (**A**) the results of the analysis of the predicted CDH3 protein with susceptibility to 30 drugs. The horizontal axis is the drug name, and the vertical axis is -Log10 (FDR). FDR was used to correct the P value after multiple comparisons, and the higher the value indicates the more significant the correlation between CDH3 expression and drug sensitivity. This figure was used for screening with CDH3 (**B**) Afatinib sensitivity in the CDH3 high and low groups, (**C**) Afatinib and CDH3 docking results, (**D**) Afatinib and CDH3 docking results 2D graphic

inhibitors. Researchers are investigating biomarkers, including CDH3 expression, that could predict which patients are most likely to benefit from the combination of EGFR inhibitors and immunotherapy.

As discussed, CDH3-targeted therapies could be combined with immunotherapies, such as checkpoint inhibitors, to modulate the tumor microenvironment and enhance immune responses. A high TMB is often associated with a better response to immunotherapy, as it can lead to the production of more neoantigens that can be recognized by the immune system. While PD-L1 expression is not always a reliable biomarker for response to immunotherapy, it can still be used in combination with other markers to predict responsiveness. The presence of certain immune cells, such as tumor-infiltrating lymphocytes (TILs), can be a marker of a more immunologically active tumor microenvironment and potentially a better response to immunotherapy. The expression of other cell adhesion molecules or the integrity of the epithelial-to-mesenchymal transition (EMT) pathway could also serve as biomarkers for response to CDH3-targeted therapies.

Table 3 Correlation between CDH3 expression and drug sensitivity

symbol	drug	correlation	FDR
CDH3	Afatinib	-0.433009802	1.59175E-42
CDH3	Gefitinib	-0.423051364	3.08454E-35
CDH3	Lapatinib	-0.384174618	6.7966E-13
CDH3	Erlotinib	-0.354267328	9.39737E-10
CDH3	WZ-1-84	-0.296077215	1.10929E-06
CDH3	I-BET-762	0.265982546	1.89529E-15
CDH3	CAY10603	0.259501778	1.99245E-14
CDH3	UNC0638	0.255066144	1.10165E-14
CDH3	Cetuximab	-0.254563214	3.46088E-12
CDH3	JW-7-24-1	0.252319155	9.49013E-14
CDH3	QL-XI-92	0.249700578	2.30795E-13
CDH3	TG101348	0.2468639	3.26258E-13
CDH3	TL-1-85	0.245228778	5.92687E-13
CDH3	CEP-701	0.240245129	4.98804E-11
CDH3	FK866	0.239090273	3.67029E-12
CDH3	BX-912	0.235503997	3.60883E-12
CDH3	THZ-2-49	0.232899603	2.13678E-11
CDH3	Y-39,983	0.229165486	4.23549E-11
CDH3	AR-42	0.228503845	2.71926E-11
CDH3	PIK-93	0.225767597	2.79669E-11
CDH3	Tubastatin A	0.224910368	4.46462E-11
CDH3	KIN001-260	0.223911482	7.51514E-11
CDH3	NG-25	0.223622644	5.93088E-11
CDH3	Nutlin-3a (-)	0.222378533	1.28988E-08
CDH3	Vorinostat	0.221326615	4.23034E-10
CDH3	NPK76-II-72-1	0.220386701	7.09517E-11
CDH3	Navitoclax	0.22016195	5.82113E-10
CDH3	GSK429286A	0.214582621	2.92726E-09
CDH3	TPCA-1	0.213691724	3.38968E-10
CDH3	OSI-027	0.212509268	7.64225E-10

While these findings align with prior studies linking cell adhesion molecules to immune modulation, several challenges and limitations warrant discussion. CDH3 downregulation may disrupt ECM organization, facilitating immune cell infiltration (e.g., resting NK cells) while paradoxically promoting DC-mediated immunosuppression through Treg recruitment. This dichotomy mirrors recent findings on CCR5/CCL5 axis dysregulation in metastatic CRC, where chemokine-driven immune cell recruitment paradoxically fosters a protumor microenvironment. Notably, our observation of CDH3-CD40LG inverse correlation echoes studies demonstrating TNF superfamily members' roles in DC maturation, highlighting CDH3 as a potential immunotherapeutic target.

Despite promising bioinformatic predictions (e.g., afatinib as a CDH3 modulator via EGFR inhibition), clinical translation faces hurdles: No FDA-approved CDH3 inhibitors exist, and afatinib's predicted effects require validation in CDH3-specific models. Off-target EGFR inhibition may exacerbate adverse effects (e.g., rash, diarrhea) without guaranteed efficacy. Tumor Microenvironment Complexity: While CDH3 correlates with TIDE scores, its interaction with PD-L1/PD-1 remains uncharacterized. Contrasting studies show EGFR inhibitors may either suppress PD-L1 or induce compensatory immune checkpoints, necessitating mechanistic studies. Although CDH3 expression associates with metastasis, its diagnostic utility lags behind established markers like CD44 or c-myc. Heterogeneity in TCGA data and retrospective study design limit clinical generalizability.

Our findings diverge from non-metastatic CRC studies where CDH3 primarily drives EMT, underscoring metastasis-specific immune roles. However, key limitations persist: Database-derived results may conflate primary/metastatic lesions. Single-cell sequencing (e.g., CancerSEA) could resolve CDH3's cell-type-specific roles. Predicted afatinib-CDH3 interactions require in vitro/in vivo functional assays. Current conclusions rely on bioinformatics; prospective cohorts are needed to assess CDH3's predictive value for immunotherapy response.

Current tumor markers for the clinical detection of colorectal cancer metastases are still limited [85]. Studies have been conducted to analyse the prognostic role of CDH3 in nonmetastatic colon cancer [17, 86, 87]. To our knowledge, our study is the first analysis of metastasis in colon cancer by CDH3 and provides new insights into gene expression, immune cell infiltration, functional enrichment, and colon cancer prognosis, all of which provide a solid foundation for further research into the diagnosis and treatment of metastatic colon cancer. Although multiplex analyses based on different databases can provide a wealth of data for research, differences in results may be reflected in the method of data collection, the fact that the data are to some extent heterogeneous, and the fact that the underlying mechanisms associated with different biological properties are unclear. Integrating CDH3 into multimodal biomarker panels (e.g., CDH3/PD-L1/TMB) may improve patient stratification. Preclinical studies should explore CDH3 knockout models to dissect its immune versus adhesion functions, while clinical trials could test afatinib-immunotherapy combinations in CDH3-high metastatic CRC subgroups.

Conclusion

In summary, this study investigated the role of CDH3 in colon cancer metastasis and explored its expression, clinical prognosis, immune infiltration, and potential as a drug target. Our findings provide a more comprehensive understanding of CDH3's role in colon cancer metastasis. The results show that CDH3 is upregulated in colon cancer metastasis, negatively impacting prognosis, and suggest its potential as a prognostic marker, therapeutic target, and immunotherapy candidate for colon cancer. The drugs screened in this study not only inhibit CDH3

Fig. 9 *CDH3* single-cell sequencing analysis. (**A**) CancerSEA single-cell RNA sequencing (scRNA-seq) results: provides a functional state map of single cancer cells, involving 14 functional states from colon cancer. (**B**) UMAP map showing 16 cell types in colon cancer, patient origin, tumor, or adjacent normal tissue origin. (**C**) Heatmap showing the relative enrichment of each cell type in colon cancer and adjacent normal tissues

expression but also compensate for the limitations of immunotherapy through the synergistic effects of CDH3 targeting and immune modulation. Despite these contributions, the study has limitations, such as the need for further experimental validation of CDH3-targeted therapy. Future research should focus on conducting in vitro and in vivo experiments with varying CDH3 expression levels, performing retrospective analyses of patient samples to explore relationships between CDH3 expression and clinical outcomes, and investigating its interactions with immune cells. Additionally, exploring the potential of combining CDH3-targeted therapy with existing treatments could provide innovative strategies for colon cancer management, offering new hope for improving patient outcomes. These findings may enable the identification of patients who could benefit from anti-tumor immunotherapy and provide novel insights into immunotherapeutic approaches for colon cancer. The clinical translation of these results could lead to the development of precision medicine strategies, improving prognosis and treatment responses for patients with metastatic colon cancer. This study underscores the importance of continued research into CDH3's role in CRC, with the potential to transform current clinical practices and treatment paradigms.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12885-025-13845-2.

Supplementary Material 1

Acknowledgements

Not applicable.

Author contributions

Chen Fu and Zhaojin Yu conceived the project. Jia Fu, Chaoyue Liu, and Chen Fu conducted the research. Jia Fu, Chaoyue Liu, and Chen Fu analysed and interpreted the data. Jia Fu, Chaoyue Liu, and Chen Fu were responsible for providing the materials. Zhaojin Yu provided research guidance. Jia Fu, Chaoyue Liu, Chen Fu, and Zhaojin Yu were major contributors to the writing of the manuscript. All the authors read and approved the final manuscript.

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Data availability

All the data generated or analysed during this study are included in this published article and its supplementary information files.

Declarations

Ethics approval and consent to participate

The study was approved by the Medical Ethics Committee of China Medical University. Patients consented to have tissue collected at the time of admission for surgery via protocols approved by the Regional Human Ethics Committee of China Medical University. The patients did not receive radiotherapy or chemotherapy before surgery, and the histological determination was performed according to WHO (World Health Organization) standards. Informed consent was obtained from all participants. All procedures performed in studies involving human participants followed the

principles of the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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