Original Article

Expression and prognostic impact of *VDAC3* in colorectal adenocarcinoma

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Background: Colorectal adenocarcinoma (COAD) is a malignant tumor with high mortality and low 5-year survival rate. Voltage-dependent anion channel 3 (VDAC3) is the least understood isoform of voltage-dependent anion-selective channels in the mitochondrial outer membrane. In this thesis, we aimed to investigate the prognostic value of *VDAC3* and provide new insights into colon adenocarcinoma.

Methods: We utilized The Cancer Genome Atlas (TCGA) database, Gene Expression Omnibus (GEO) database, Human Protein Atlas online database, and the University of ALabama at Birmingham CANcer data analysis Portal (UALCAN) database to analyze *VDAC3* expression in COAD and assess patient survival rates. Univariate and multivariate Cox regression analyses were employed to evaluate *VDAC3*'s prognostic significance for COAD. Gene set variation analysis (GSVA) was utilized to explore COAD-related signaling pathways associated with *VDAC3*. Additionally, we predicted the relationship between *VDAC3* expression and anticancer drug sensitivity using the CellMiner database.

Results: In the TCGA database, *VDAC3* demonstrated elevated expression levels in COAD, which was further validated by findings from the GEO database. Survival analysis conducted using Kaplan-Meier (K-M) curves highlighted that the patients with decreased *VDAC3* expression exhibited significantly shorter overall survival durations. *VDAC3* expression demonstrated correlation with COAD pathological stage. *VDAC3* gene mutation was linked to COAD outcomes. Cox regression analysis showed that *VDAC3* was an independent predictor. In addition, GSVA analysis showed that *VDAC3* was closely related to mitochondria-related biological processes and involved in the occurrence and development of mitochondria-related diseases. Finally, analysis of the CellMiner database predicted that *VDAC3* expression was positively correlated with chelerythrine and cladribine, but negatively correlated with Ergenyl.

Conclusions: Our study suggests that *VDAC3* may be a potential biomarker for early diagnosis, prognosis, and treatment of COAD.

Keywords: Colon adenocarcinoma; voltage-dependent anion channel 3 (*VDAC3*); biomarker; prognosis

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Introduction

Colorectal adenocarcinoma (COAD) is the most prevalent malignant gastrointestinal disease, ranking as one of the primary global causes of cancer-related mortality (1). In 2020, approximately 1.14 million individuals received diagnoses of COAD, resulting in 5.7 million deaths (2,3). Not only the mortality rate of COAD is on the rise, but also the trend of COAD patients is younger (4). Despite extensive research into treatment modalities, survival rates for advanced-stage COAD patients remain notably low, with only around 10% of individuals with distant metastases achieving a 5-year survival rate (5). Nevertheless, the mechanisms of COAD development remain obscure. Early diagnosis of colorectal cancer (CRC) continues to pose a significant challenge in the field of oncology (6). Therefore, the focus of current research is to explore the key molecules that affect the occurrence and metastasis of COAD, and to search for early diagnostic and prognostic biomarkers.

The voltage-dependent anion channels (VDACs) constitute a compact family of proteins primarily tasked with forming aqueous pores across the outer mitochondrial membrane (OMM). These channels facilitate the exchange of vital metabolites and molecules, playing a crucial role in cellular metabolism and homeostasis (7). In proliferating cells, VDACs facilitate the regulated transport of ATP/ADP and other respiratory substrates across the OMM. This process is essential for balancing the demands of oxidative phosphorylation and aerobic glycolysis, thereby ensuring the energy supply and the synthesis of biomass necessary for cell growth (8). By managing molecular exchange between cellular compartments, VDAC regulates various cellular processes such as apoptosis, metabolism, ion homeostasis, thereby affecting many diseases including cancer (9,10). VDACs family includes VDAC1, VDAC2 and VDAC3. Of the three subtypes, VDAC3 was found the latest and the least studied. However, recent studies have shown that the expression of *VDAC3* is closely related to the occurrence and development of certain cancers (11-13). The expression of *VDAC3* is up-regulated in human malignant tumors such as melanoma and thyroid tumors (14). VDAC3 is a binding site of anticancer drug erastin, and overexpression of VDAC3 can increase the sensitivity to erastin (15). Furthermore, ubiquitination of *VDAC3* has been shown to regulate ferroptosis in gastric cancer (16). In hepatocellular carcinoma cells, VDAC3 may play a pivotal role in mitochondrial autophagy. Studies have indicated that the knockout of VDAC3 renders breast cancer cells hypersensitive to the antiproliferative effects mediated by dankastatin B, suggesting that VDAC3 is involved in the anticancer efficacy of this natural product. This makes VDAC3 an attractive target for drug development (17,18).

Researchers have observed an upregulation of mitochondrial pyruvate kinase M2 (PKM2) and VDAC3 in human CRC tissues, and have identified a positive correlation between the two. It has been suggested that mitochondrial PKM2 and VDAC3 contribute to the metabolic reprogramming of tumor cells, thereby counteracting the environmental stress encountered during tumorigenesis (8). Unfortunately, the role of *VDAC3* in COAD remains largely unexplored. To address this knowledge gap, we conducted a comprehensive bioinformatics analysis to investigate the biological function of *VDAC3* in COAD. Our analysis revealed that *VDAC3* expression was significantly upregulated in COAD patients and correlated with a worse clinical outcome. Furthermore, through rigorous univariate and multivariate Cox regression analyses, we identified *VDAC3* as an independent

Highlight box

Key findings

- Voltage-dependent anion channel 3 (*VDAC3*) is associated with the prognosis of colorectal adenocarcinoma (COAD) and is a potential biomarker for early diagnosis, prognosis and treatment.
- Kaplan-Meier survival curve analysis showed that patients with low *VDAC3* expression had a significantly shorter overall survival. The expression of *VDAC3* was correlated with the pathological stage of COAD. *VDAC3* gene mutation was associated with the prognosis of COAD.
- Univariate and multivariate Cox regression analyses showed that *VDAC3* was an independent predictor.
- *VDAC3* was closely related to mitochondria-related biological processes and involved in the occurrence and development of mitochondria-related diseases.
- The expression of *VDAC3* was positively correlated with chelerythrine and cladribine, but negatively correlated with Ergenyl.

What is known and what is new?

- *VDAC3* was known to play a crucial role in cell biology and was involved in the regulation of molecular exchange and ion homeostasis between cells.
- Our study clarified the significance of *VDAC3* in the prognosis and diagnosis of colorectal adenocarcinoma.

What is the implication, and what should change now?

- *VDAC3* can be used as diagnostic, prognostic, and therapeutic biomarker for COAD.

predictor of COAD prognosis. To elucidate the underlying mechanisms, we employed gene set variation analysis (GSVA) to confirm the pathways regulated by *VDAC3* in COAD. In addition, we explored the relationship between *VDAC3* expression and genomic heterogeneity. Lastly, we leveraged the CellMiner database to predict a link between *VDAC3* expression and anticancer drug sensitivity. This approach offers valuable insights for the development of novel therapeutic strategies in the context of COAD. We present this article in accordance with the REMARK reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-402/rc).

Methods

Data collection

The expression data and pertinent clinical information for this analysis were obtained from 41 non-cancerous colon tissues and 483 COAD tissues within The Cancer Genome Atlas (TCGA) dataset, which can be accessed at https://tcga-data.nci.nih.gov/tcga/. Calibrated fragments per kilobase million (FPKM) values were used for the analysis. Publicly available data include RNA-seq profiles of CRC lymph node metastatic tissues (N1, N2) and non-metastatic tissues (N0). Additionally, RNA-seq profiles of CRC primary tumors with diameters less than or equal to 5 cm (T1, T2) and greater than 5 cm (T3, T4) were included. The dataset encompasses groups with and without metastatic COAD. Patients were stratified into elderly and young groups based on the age of 65 years. COAD was classified into stage I, II, III, and IV according to the TNM staging system. Furthermore, gene expression data from the GSE44861 dataset is available on the Gene Expression Omnibus (GEO) database website (https://www.ncbi.nlm.nih.gov/geo/). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Biological functional enrichment scores

GSVA was conducted on tumor transcriptome sequencing data using default parameters with the R package 'gsva' (19). To identify relevant genes associated with *VDAC3* or characteristic genes within cell clusters, the gene lists were uploaded to the Database for Annotation, Visualization, and Integrated Discovery (DAVID, v6.8). Official gene symbols were utilized as identifiers, and Homo sapiens was selected as the species. Subsequently, enrichment results were obtained through Gene Ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis.

Analysis of VDAC3 in HPA database and UALCAN database

VDAC3 protein expression in COAD was verified through immunohistochemical staining in the Human Protein Atlas (HPA, http://www.proteinatlas.org/) online database (20). Subsequently, we examined VDAC3 total protein expression in both COAD and normal tissues using the University of ALabama at Birmingham CANcer data analysis Portal (UALCAN) database. Additionally, we investigated the association between VDAC3 total protein levels and the histological or pathological stages of COAD.

Univariate and multifactor Cox risk regression analyses of VDAC3

Univariate and multivariate Cox regression analyses were conducted to determine whether *VDAC3* and clinicopathological parameters were independent factors associated with COAD. Based on the FPKM value, expressions >41 were designated as high, while expressions ≤41 were classified as low.

The construction of protein-protein interaction (PPI) network

The PPI network was also constructed using the STRING database (https://string-db.org/) to investigate genes with potential functional interactions with VDAC3 (21).

VDAC3 alteration analysis

The cBioPortal for Cancer Genomics (http://cbioportal.org) was employed to analyze the frequency, mutation type, and copy number alterations (CNAs) of *VDAC3* across all TCGA tumors. Additionally, differences in overall survival (OS), disease-free survival (DFS), progression-free survival (PFS), and disease-specific survival (DSS) between COAD cases with and without *VDAC3* gene alterations were explored using Kaplan-Meier (K-M) survival curves.

Relationship between VDAC3 and HRD, MSI, MATH, TMB, ploidy, LOH

The correlation between *VDAC3* gene expression and various genomic factors, including homologous

recombination deficiency (HRD), microsatellite instability (MSI), mutant-allele tumor heterogeneity (MATH), tumor mutational burden (TMB), ploidy, and loss of heterozygosity (LOH), was explored using the Spearman's rank correlation method. This analysis was performed utilizing the Sangerbox tool, an online platform (http://www.sangerbox.com/tool) (22), and the results were visualized via radar plots generated with the R package.

Relationship between VDAC3 expression and drug sensitivity

We conducted an analysis of the correlation between *VDAC3* expression and drug sensitivity utilizing the CellMiner database (https://discover.nci.nih.gov/cellminer/ home.do) and subsequently visualized the results using R (23).

Correlation analysis of VDAC3 expression with prognostic gene markers in COAD

The relation of *VDAC3* expression with various prognostic markers associated with COAD was investigated via the Gene Expression Profiling Interactive Analysis (GEPIA) (http://gepia.cancer-pku.cn/index.html). Furthermore, we conducted an analysis of the differential expression, pathological staging, and survival outcomes for *VDAC3* utilizing the aforementioned online platform (24).

Statistical analysis

Data were visualized with the above-mentioned packages in R version 4.2.2. Clinical characteristics related to patients' overall survival in TCGA were analyzed using Cox regression in SPSS (version 26.0). These results were considered as statistically significant at $P<0.05$.

Results

Selection of differentially expressed genes through GSVA analysis in COAD

Initially, we employed GSVA enrichment analysis to discern 20 distinct biological functions that exhibited significant disparities among CRC patients (*Figure 1A*). From this pool, we meticulously selected the five most significantly positively correlated and five negatively correlated biological functions (*Figure 1B*). Subsequently, we conducted batch COX regression analysis utilizing the included genes. This comprehensive analysis yielded a total of 64 genes that were intricately linked to the prognosis of CRC. Given the limited existing research on the impact of *VDAC3* in CRC, we undertook a comprehensive examination of *VDAC3* expression in both CRC and normal tissues, drawing from the TCGA database. Our findings revealed an upregulation of *VDAC3* expression in CRC tissues when contrasted with normal tissues (*Figure 1C*,*1D*, Figure S1A). To bolster our observations, we further validated these findings by acquiring Gene Expression Omnibus Series (GSE) data from the GEO database, where the results consistently demonstrated heightened expression of the *VDAC3* gene in tumor tissues (*Figure 1E*). In summary, these findings corroborate the association between *VDAC3* expression and the occurrence and progression of CRC.

Total protein expression levels of VDAC3 in COAD

In the HPA database, immunohistochemical microarrays indicated a significant aberrant increase in VDAC3 expression in CRC tissues (*Figure 2A*,*2B*). We further analyzed VDAC3 expression across ten different cancer types using the TCGA database, revealing notably elevated levels in specific cancers such as BRCA, UCEC, and LIHC (*Figure 2C*). Our investigation further unveiled a remarkable overexpression of VDAC3 in COAD tissues when compared to non-cancerous colon tissues, as evidenced by data from the UALCAN database ($P<0.001$) (*Figure 2D*). Additionally, we observed strong correlations between VDAC3 and patient race, with significant associations for Caucasians ($P<0.001$) (*Figure 2E*) and African Americans ($P<0.05$) (*Figure 2E*).

Furthermore, VDAC3 protein expression levels exhibited significant increases among CRC patients aged 41 to 80 years ($P<0.001$) (*Figure 2F*). Additionally, VDAC3 correlated with tumor stage, demonstrating significant associations with stage I ($P<0.05$) (*Figure 2G*), stage II ($P<0.001$) (*Figure 2G*), and stage III ($P<0.001$) (*Figure 2G*).

Relationship between VDAC3 expression and clinicopathological parameters

Differential expression of *VDAC3* in patients revealed distinctive clinical and pathological patterns. The analysis of TCGA datasets displayed asymmetric distributions of *VDAC3* levels, N-stage, T-stage, and World Health Organization (WHO) grade (*Figure 3A*). We conducted a comparative analysis across various sample groups. Because

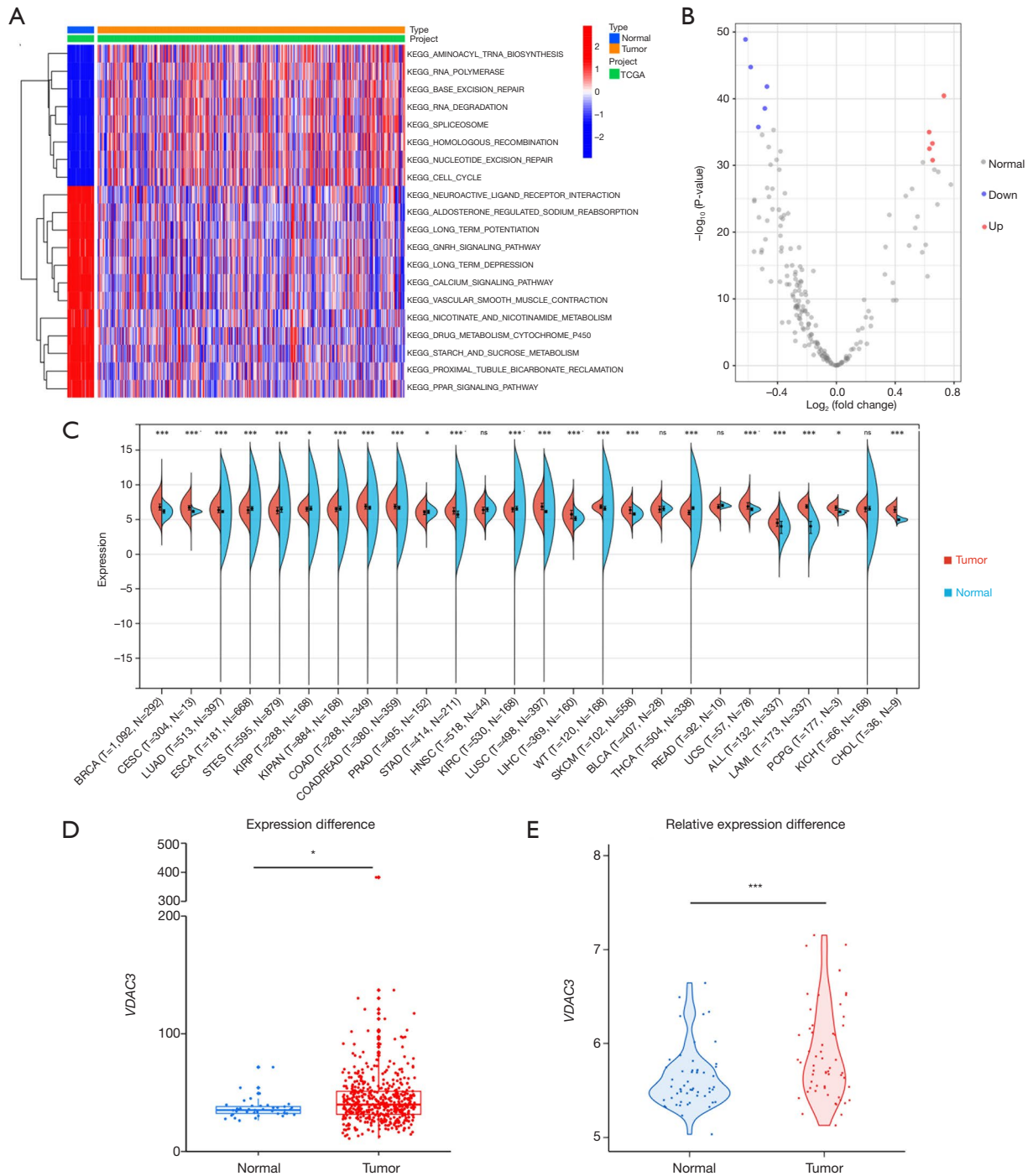

Figure 1 GSVA analysis in COAD and expression analysis of *VDAC3*. (A) Identification of 20 pathways exhibiting significant differential expression in COAD. (B) Select the top 5 positively correlated pathways and the top 5 negatively correlated pathways with the highest differences for further analysis. (C) Pan-cancer analysis of *VDAC3* expression. *, P<0.05; ***, P<0.001; ns, not statistically significant. (D) Evaluate the relative expression of *VDAC3* in colorectal adenocarcinoma in comparison to non-cancerous colon tissues utilizing the TCGA database. *, P<0.05. (E) Evaluate the relative expression of *VDAC3* in colorectal adenocarcinoma in comparison to non-cancerous colon tissues utilizing the GSE database. ***, P<0.001. KEGG, Kyoto Encyclopedia of Genes and Genomes; TCGA, The Cancer Genome Atlas; GSVA, gene set variation analysis; COAD, colorectal adenocarcinoma; *VDAC3*, voltage-dependent anion channel 3; GSE, Gene Expression Omnibus.

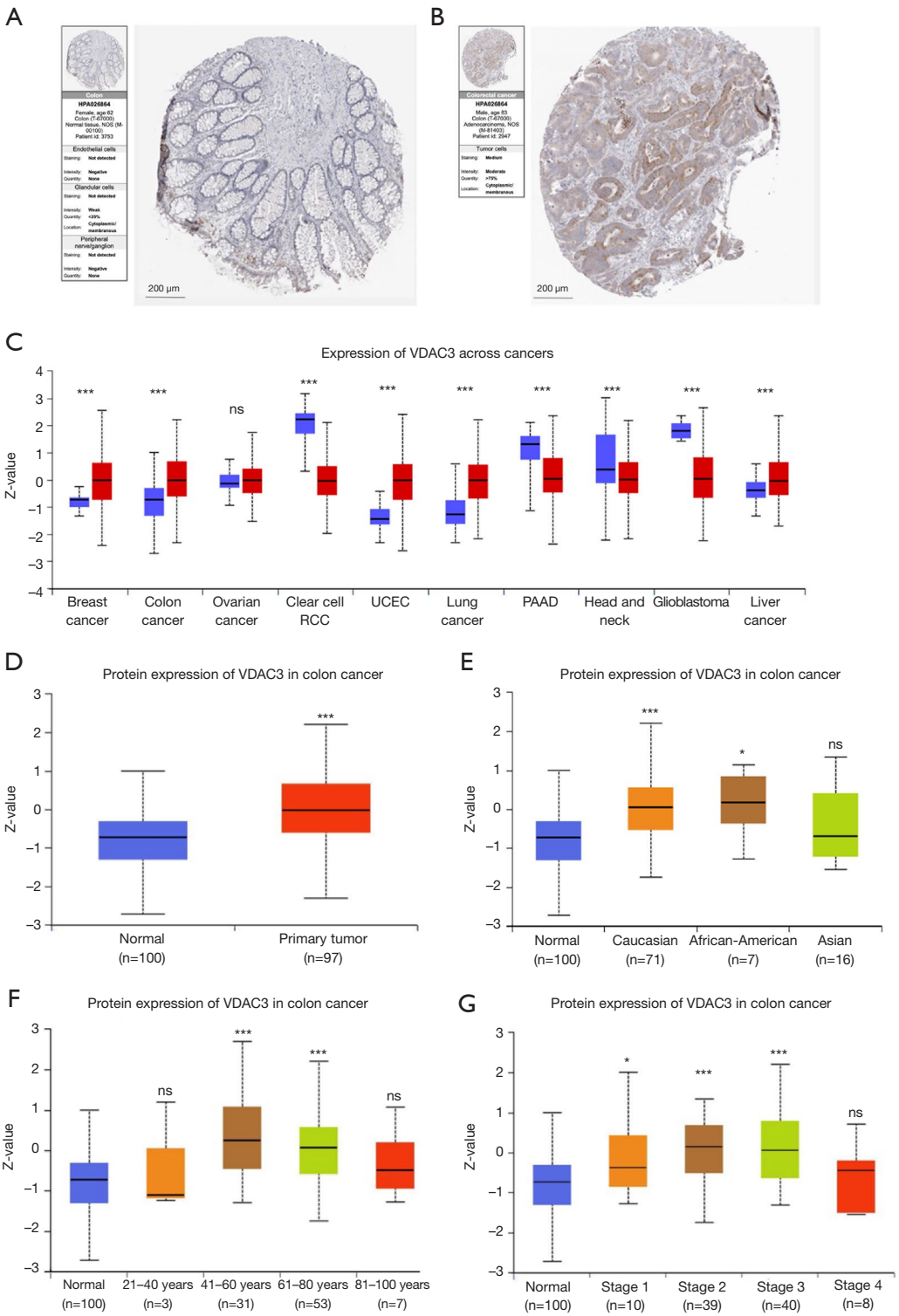

Figure 2 Total protein expression level of VDAC3 in COAD. (A,B) Representative immunohistochemistry images of VDAC3 in both normal and COAD tissues sourced from the Human Protein Atlas database (https://www.proteinatlas.org/). Image credit goes to the Human Protein Atlas. The following links were VDAC3 expression of normal and tumor tissue (https://www.proteinatlas.org/ENSG00000078668-VDAC3/tissue/colon#imid_6773678; https://www.proteinatlas.org/ENSG00000078668-VDAC3/pathology/colorectal+cancer#img).

Scale bar, 200 μm. (C) The expression of VDAC3 protein in pan-cancer. ***, P<0.001; ns, not statistically significant. (D) VDAC3 protein expression in normal colon tissues or COAD tissues. ***, P<0.001. (E) Expression of VDAC3 protein in normal tissues and COAD tissues of different races. *, P<0.05; ***, P<0.001; ns, not statistically significant. (F) Expression of VDAC3 protein in normal tissues and COAD tissues of patients at different ages. ***, P<0.001; ns, not statistically significant. (G) Expression of VDAC3 protein in normal colon tissues and COAD tissues of patients with different pathological stages. Z-values represent standard deviations from the median across samples for the given cancer type. *, P<0.05; ***, P<0.001; ns, not statistically significant. HPA, Human Protein Atlas; NOS, not otherwise specified; RCC, renal cell carcinoma; UCEC, uterine corpus endometrial carcinoma; PAAD, pancreatic adenocarcinoma; VDAC3, voltage-dependent anion channel 3; COAD, colorectal adenocarcinoma.

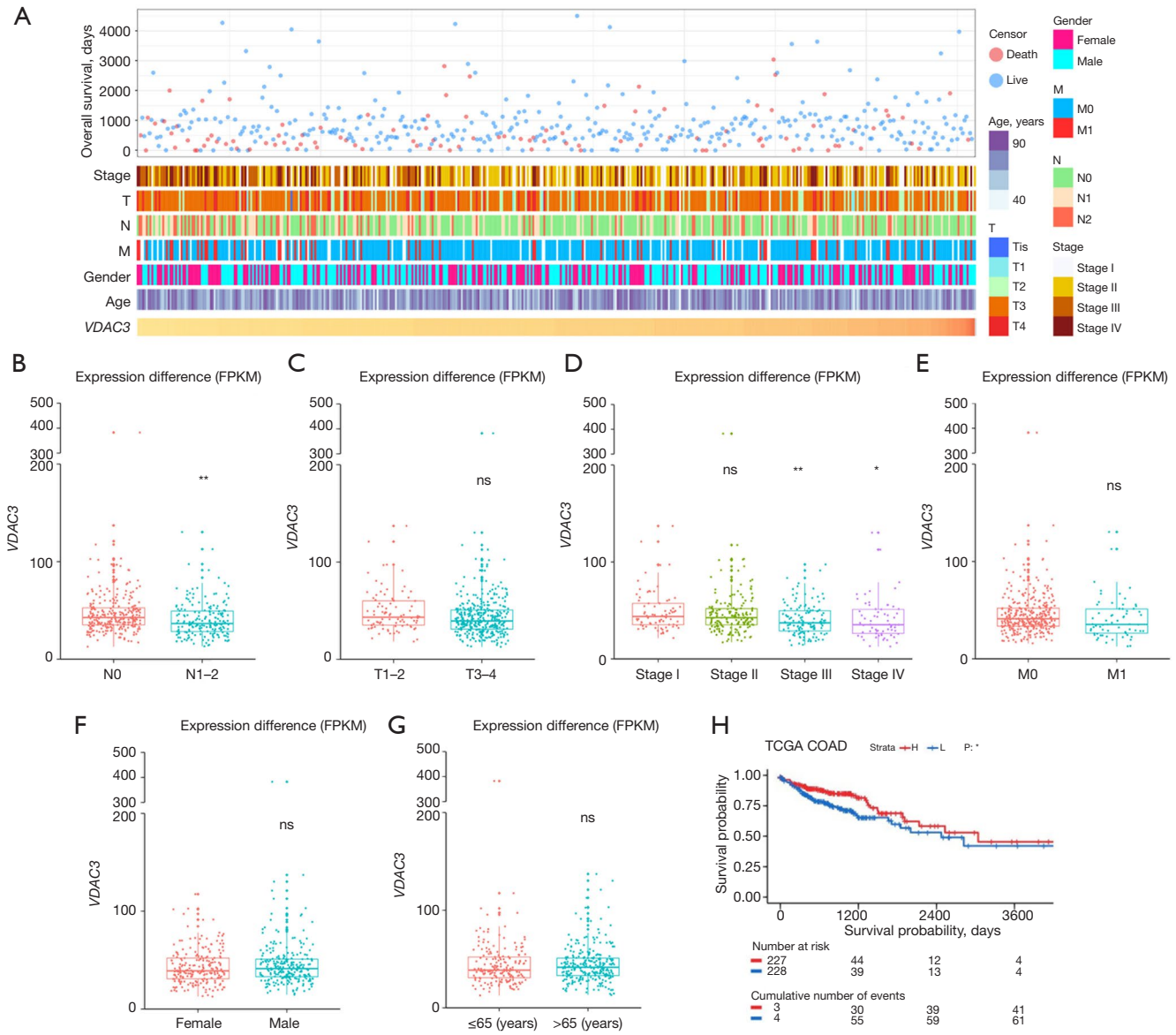

Figure 3 Relationship between *VDAC3* and clinicopathological features of COAD. (A) Prospect of clinicopathological features associated with *VDAC3* in colon adenocarcinoma from TCGA database. Relationship between *VDAC3* expression and clinicopathological parameters. (B) N, (C) T, (D) phase, (E) M, (F) gender, (G) age. *, P<0.05; **, P<0.01; ns, not statistically significant. (H) Kaplan-Meier analysis of *VDAC3* expression in the TCGA database, and the significance of prognostic value was tested by log-rank test. *, P<0.05. *VDAC3*, voltage-dependent anion channel 3; FPKM, fragments per kilobase million; TCGA, The Cancer Genome Atlas; COAD, colorectal adenocarcinoma.

some clinical data were missing, we only analyzed the clinical data available in TCGA. In the TCGA database, our findings indicated a significant association between *VDAC3* expression and N-stage (*Figure 3B*), as well as a trend with T-stage (P=0.06) (*Figure 3C*) and pathological stage (*Figure 3D*) in COAD. Utilizing GEPIA2 for analysis, the results demonstrated a significant correlation between VDAC3 expression and pathological staging (Figure S1B). However, *VDAC3* expression did not exhibit significant differences in M-stage (*Figure 3E*), gender (*Figure 3F*), or age (*Figure 3G*). It is important to note that our data statistics do not contain missing data information. To elucidate the prognostic value of *VDAC3* in patients, K-M analyses were conducted utilizing TCGA databases. Patients with lower *VDAC3* expression exhibited significantly shorter overall survival compared to those with higher *VDAC3* expression in the COAD database (*Figure 3H*). Survival analyses were conducted using GEPIA2, and the results indicated that patients with lower VDAC3 expression also have a poorer prognosis (Figure S1C). These findings suggest that *VDAC3* gene expression is generally elevated in cancer patients compared to normal individuals. However, as the disease progresses, *VDAC3* expression tends to decrease, and low *VDAC3* expression in cancer patients is indicative of a poorer prognosis.

VDAC3 is an independently predictive element of COAD

After establishing the prognostic significance of *VDAC3* expression through survival analysis, we conducted univariate Cox regression analyses to further investigate the impact of *VDAC3* on COAD prognosis (Table S1). The results of the univariate Cox analysis demonstrated significant associations between overall survival in COAD patients and *VDAC3* expression, age, M-stage, N-stage, T-stage, and pathological stage (P<0.05) (*Figure 4A*). As depicted in *Figure 4B*, the subsequent multivariate Cox analysis revealed that *VDAC3* remains an independent predictor of overall survival in COAD. Collectively, our findings indicate that *VDAC3* expression stands as an independent prognostic factor in COAD.

GO and signaling pathway enrichment analysis of VDAC3

To investigate the biological functions associated with *VDAC3*, we identified genes strongly correlated with *VDAC3* through Pearson correlation analysis using TCGA databases. Subsequently, we performed GO and KEGG analyses based on these gene sets. In the TCGA database, the analysis revealed that *VDAC3* was most closely linked to several biological processes, including mitochondrial translation, DNA-dependent DNA replication, ribosomal large subunit biogenesis, and iron-sulfur cluster assembly (*Figure 5A*). Furthermore, *VDAC3* was predominantly localized within the cellular components of the mitochondrial inner membrane and nucleoplasm (*Figure 5B*). Regarding signaling pathways, *VDAC3* exhibited strong associations with amyotrophic lateral sclerosis (25), Prion-disease, and Huntington disease pathways (*Figure 5C*). Molecular functions attributed to *VDAC3* include RNA binding and structural constituent of ribosome (*Figure 5D*). GSVA in the TCGA databases was used to determine the enrichment score of the biological process. GSVA analysis revealed that *VDAC3* gene expression was associated with mitochondrial function and mitochondrial diseases such as Parkinson's disease (26) and Alzheimer's disease (27) (*Figure 5E*).

Subsequently, we built a PPI network by leveraging the STRING database to investigate the underlying interacting proteins of *VDAC3*, as shown in *Figure 5F*. Ten genes (*VDAC2*, *VDAC1*, *TOMM70A*, *TOMM20*, *TOMM40*, *RHOT1*, *CYCS*, *PPIF*, *TSPO*, *COX4I1*) were significantly correlated with *VDAC3* function (*Figure 5G*). These findings collectively suggest that *VDAC3*, as a mitochondrial membrane protein, plays a pivotal role in mitochondrial biological functions and is closely intertwined with the onset and progression of mitochondrial diseases.

Mitochondria play a role in the development and progression of COAD, prompting numerous studies to construct mitochondrial-related prognostic models for COAD (28-30). In this study, we conducted a correlation analysis between VDAC3 and genes within these prognostic models. The results indicated significant associations between VDAC3 and many of the genes included (Table S2).

Relationship between VDAC3 mutation and prognosis in COAD

We conducted an investigation into the prevalence of *VDAC3* mutations in cancer using the cBioPortal database. Our analysis revealed that *VDAC3* alterations were present in approximately 4.2% of CRC patients (*Figure 6A*), and the specific mutation sites are illustrated in *Figure 6B*. K-M analysis demonstrated that patients with *VDAC3* gene mutations exhibited worse OS, DSS, and PFS outcomes

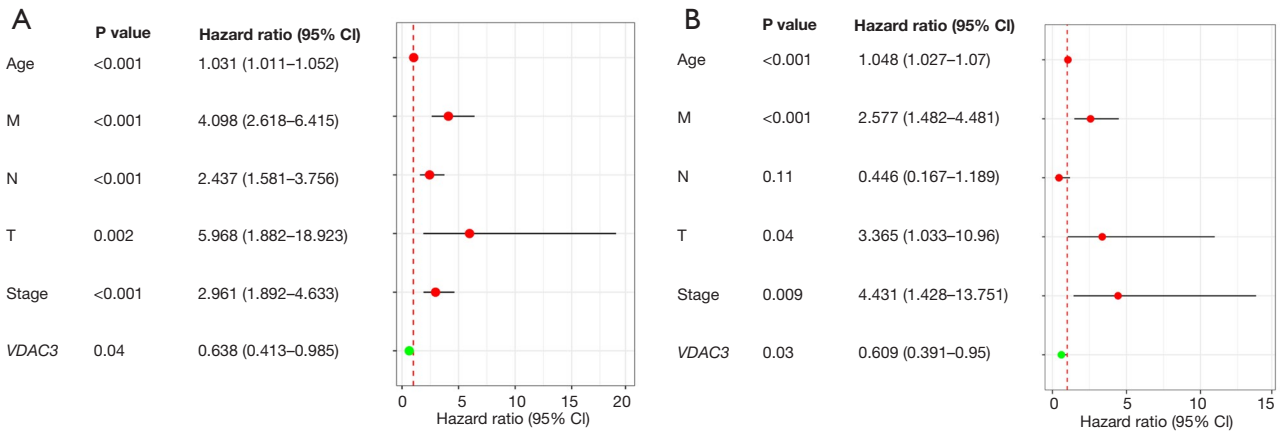

Figure 4 Univariate and multivariate Cox analysis of *VDAC3* in COAD. (A) Univariate Cox analysis was used to analyze the relationship between *VDAC3* expression and clinicopathological variables in COAD in TCGA database. (B) Multivariate Cox analysis in TCGA database was used to analyze the relationship between *VDAC3* expression and clinicopathological variables in COAD. CI, confidence interval; *VDAC3*, voltage-dependent anion channel 3; COAD, colorectal adenocarcinoma; TCGA, The Cancer Genome Atlas.

compared to those without *VDAC3* gene mutations (*Figure 6C-6E*). However, it's worth noting that mutations in *VDAC3* in the context of COAD were not found to have a statistically significant correlation with DFS (*Figure 6F*). These findings contribute to our understanding of the impact of *VDAC3* mutations in CRC and may have implications for future research in this field.

Relationship between genomic heterogeneity and VDAC3 expression

Our study encompassed extensive analyses aiming at elucidating the intricate interplay between genomic heterogeneity and *VDAC3* gene expression. Specifically, we delved into the potential connections between *VDAC3* and various genomic parameters, encompassing HRD, MSI, MATH, TMB, ploidy, and LOH. Notably, our results unveiled a compelling and statistically significant relationship between *VDAC3* expression and HRD, as demonstrated in *Figure 7A*. HRD is a pivotal concept within our study, denoting impairments in the cellular genome repair mechanisms (31,32). HRD status is of paramount importance in oncology, serving as a crucial determinant for treatment selection and prognosis. It is notably closely linked with the sensitivity of certain therapeutic interventions. These findings contribute valuable insights into the role of *VDAC3* in CRC, particularly its relationship with genomic heterogeneity and HRD, which could have implications for future research and clinical applications in this field (33). However, it is noteworthy that *VDAC3* expression did not show associations with MSI, MATH, TMB, ploidy, or LOH in the context of COAD (*Figure 7B-7F*).

Relevance of VDAC3 expression to anticancer drug sensitivity

We conducted an investigation into the association between *VDAC3* expression and susceptibility to antineoplastic agents using the CellMiner database. This analysis pinpointed three antineoplastic agents that displayed remarkable correlations with *VDAC3* expression. As depicted in *Figure 8*, our findings revealed a substantial positive correlation between *VDAC3* and both chelerythrine and cladribine. We also identified a significant negative correlation between *VDAC3* and Ergenyl (*Figure 8A-8F*). These findings provide valuable insights into the potential influence of *VDAC3* expression on the response to these specific antineoplastic agents, offering implications for tailored approaches to cancer treatment.

Discussion

In recent years, the incidence of COAD has been on the rise among digestive system cancers (34). Despite significant advancements in the diagnosis and treatment of COAD, it remains a malignancy with high mortality and a low 5-year survival rate. Consequently, the identification

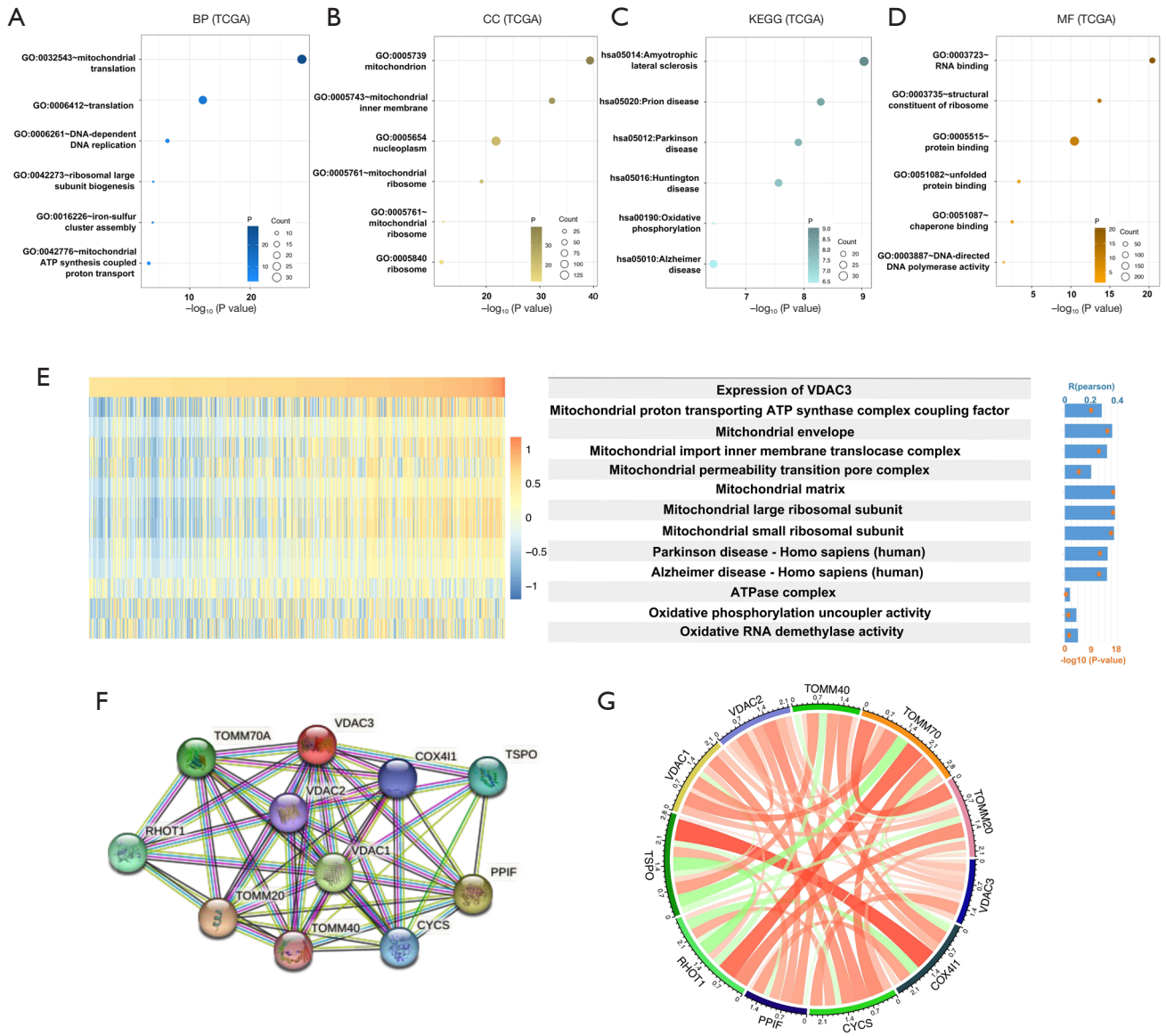

Figure 5 *VDAC3* is closely related to the biological activities and functions of mitochondria. (A-D) BP, CC, and MF are mostly related to *VDAC3* in the TCGA database. KEGG analysis of *VDAC3* in the TCGA database. (E) In the COAD data, enrichment scores for *VDAC3* expression and mitochondrial related biological activities were obtained for each patient. Samples were arranged in ascending order of *VDAC3* expression. The bar and line graphs on the right show the R and P values from the correlation analysis. (F) PPI network associated with *VDAC3*. (G) Pearson correlation between *VDAC3* and related genes. BP, biological processes; TCGA, The Cancer Genome Atlas; CC, cellular components; KEGG, Kyoto Encyclopedia of Genes and Genomes; MF, molecular function; GO, Gene Ontology; ATP, adenosine triphosphate; *VDAC3*, voltage-dependent anion channel 3; COAD, colorectal adenocarcinoma; PPI, protein-protein interaction.

of prognostic markers and therapeutic targets for COAD has become a crucial focus in medical research (35,36). *VDAC3* plays a crucial role in cellular biology, managing molecular exchange between cells and regulating various cellular processes such as apoptosis, metabolism, and ion homeostasis. Although *VDAC3*'s association with other tumor types like melanoma and hepatocellular carcinoma has been extensively studied, its connection with COAD

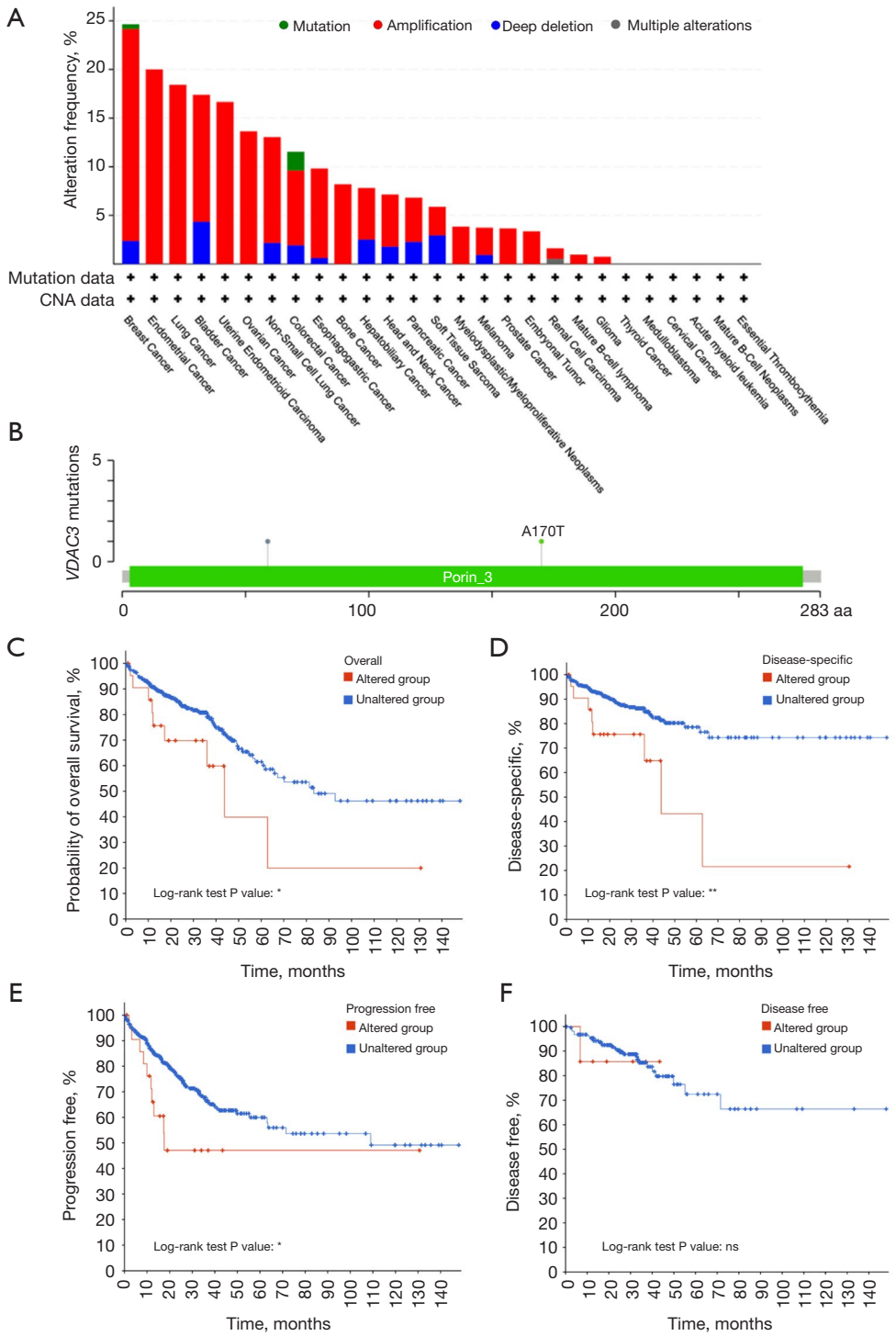

Figure 6 Analyses the mutational profile of *VDAC3* and its prognosis in COAD using the cBioPortal database. (A) Change frequency of *VDAC3* mutation types in pan-cancer in TCGA database. (B) Mutation sites of *VDAC3* in COAD in TCGA database. (C) Kaplan-Meier survival analysis of *VDAC3* mutations on OS. *, P<0.05. (D) Kaplan-Meier survival analysis of *VDAC3* mutations on DSS. **, P<0.01. (E) Kaplan-Meier survival analysis of *VDAC3* mutations on PFS. *, P<0.05. (F) Kaplan-Meier survival analysis of *VDAC3* mutations on DFS. ns, not statistically significant. CNA, copy number alteration; *VDAC3*, voltage-dependent anion channel 3; COAD, colorectal adenocarcinoma; TCGA, The Cancer Genome Atlas; OS, overall survival; DSS, disease-specific survival; PFS, progression-free survival; DFS, disease-free survival.

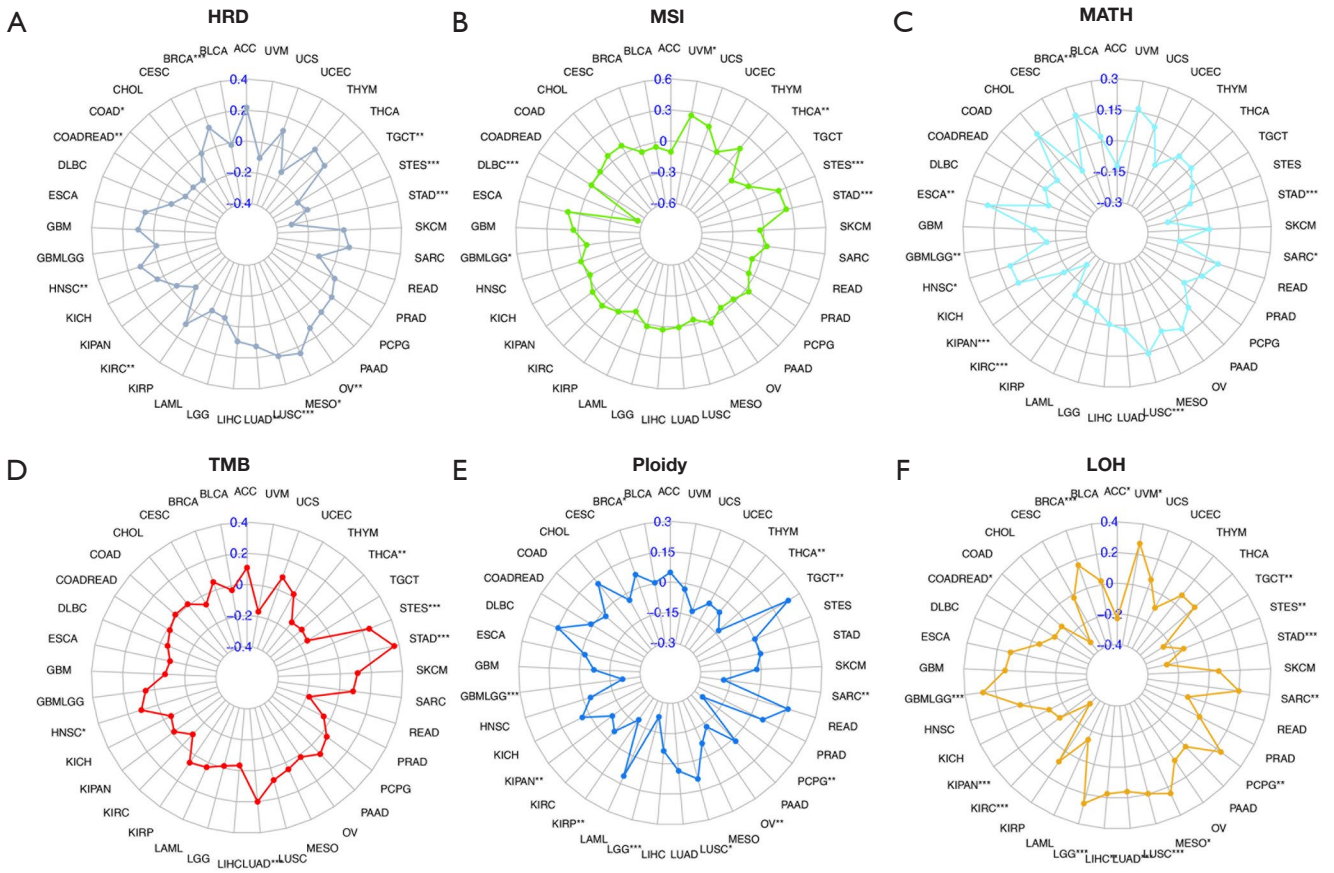

Figure 7 The relationship between *VDAC3* and HRD, MSI, MATH, TMB, Ploidy, LOH. *, P<0.05; **, P<0.01; ***, P<0.001. (A) Association of *VDAC3* with HRD. (B) Association of *VDAC3* with MSI. (C) Association of *VDAC3* and MATH. (D) Association of *VDAC3* with TMB. (E) Association of *VDAC3* with Ploidy. (F) Association of *VDAC3* with LOH. HRD, homologous recombination deficiency; MSI, microsatellite instability; MATH, mutant-allele tumor heterogeneity; TMB, tumor mutational burden; LOH, loss of heterozygosity; *VDAC3*, voltage-dependent anion channel 3.

prognosis has been inadequately explored (37,38).

In our study, utilizing TCGA-COAD and GEO databases, we conducted GSVA and batch Cox regression analysis, revealing a close association between *VDAC3* and COAD development. Specifically, in TCGA database, *VDAC3* expression significantly increased in COAD tissues, a result validated in the GEO database. Immunohistochemical microarray analysis of *VDAC3* protein expression confirmed a significant elevation in COAD tissues compared to non-cancerous colon tissues, highlighting abnormal *VDAC3* expression and reinforcing its potential role in cancer development. Further analysis indicated that *VDAC3* expression correlated closely with COAD's N stage and histopathology. K-M survival curve analysis revealed a correlation between low *VDAC3* expression and poorer patient survival rates. Interestingly, *VDAC3* expression showed a significant increase in the early stages of COAD, followed by a declining trend with disease progression. This suggests dynamic changes in *VDAC3* expression at different stages, providing new insights for COAD staging and prognosis. Univariate and multivariate Cox regression analysis of clinical data from TCGA database identified *VDAC3* as an independent factor influencing COAD prognosis. Approximately 4.2% of COAD patients exhibited *VDAC3* gene alterations, correlating with poorer outcomes in OS, DSS, and PFS.

To delve into the mechanistic role of *VDAC3* in COAD, we performed GO analysis, KEGG analysis, and GSVA enrichment analysis (39-41). Results indicated a close association of *VDAC3* with mitochondria-related biological

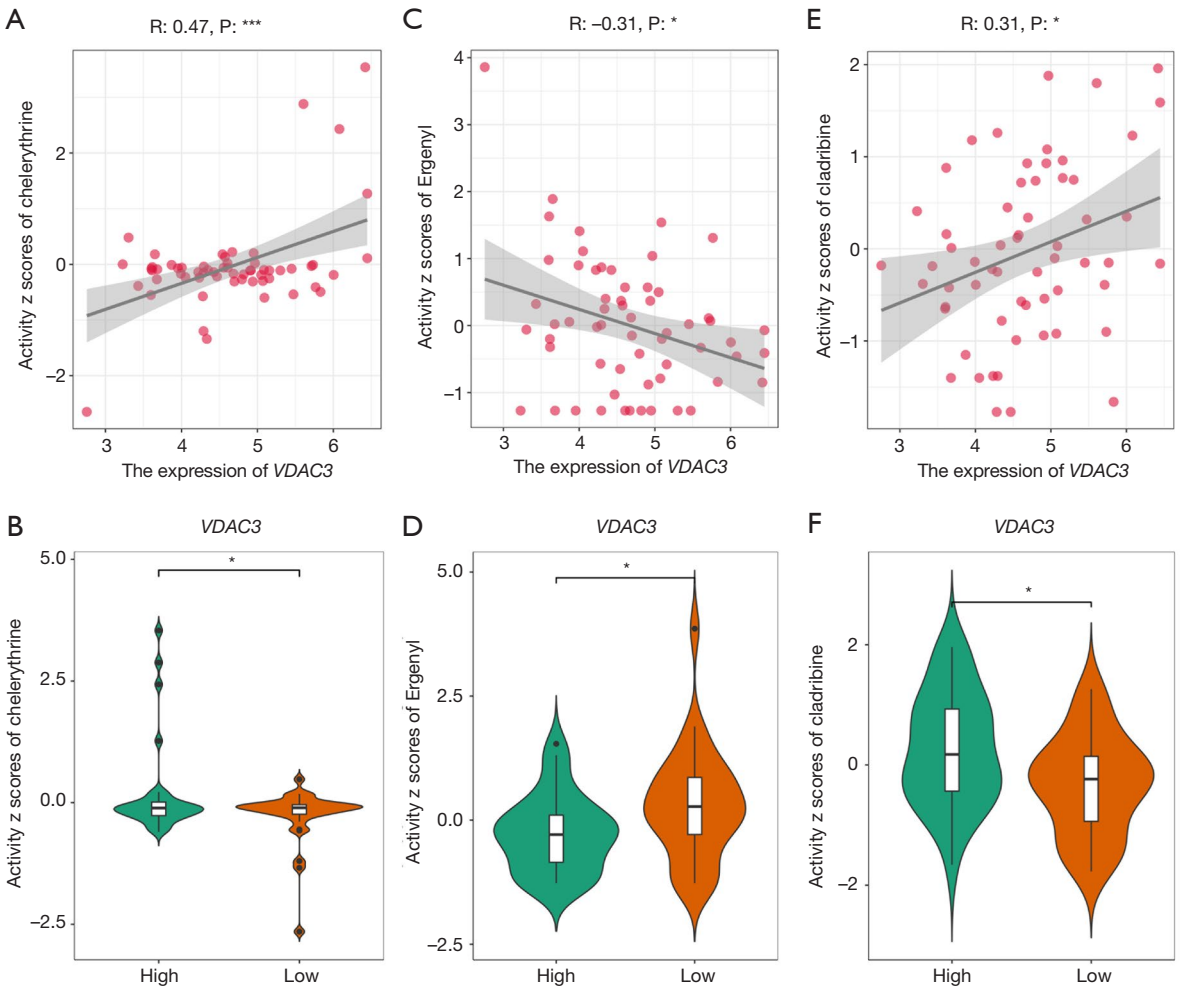

Figure 8 Relationship between *VDAC3* expression and tumor drug sensitivity. (A,B) Relationship between *VDAC3* expression and sensitivity to chelerythrine drugs. *, P<0.05; ***, P<0.001. (C,D) Relationship between *VDAC3* expression and sensitivity to Ergenyl drugs. *, P<0.05. (E,F) Relationship between *VDAC3* expression and sensitivity to cladribine drugs. *, P<0.05. *VDAC3*, voltage-dependent anion channel 3.

processes, implicating its involvement in the occurrence and development of mitochondria-related diseases. Mitochondria play a crucial role in COAD development, especially in cellular metabolism and apoptosis (42,43). As an OMM protein, *VDAC3* may play a vital role in COAD development through its impact on mitochondria. Studies have shown that *VDAC3* is associated with cancer (44-46). The antineoplastic agent erastin induces rapid, oxidative, non-apoptotic death of human tumor cells harboring oncogenes *HRAS*, *KRAS* or *BRAF* mutations. On the mitochondrial outer membrane, Erastin can bind to *VDAC3*. The expression of *VDAC3* was strongly reduced after Erastin treatment, but the exact mechanism remains unclear (11). Constructing a PPI network for *VDAC3* revealed significant correlations with mitochondrial outer membrane proteins like *TOMM40* and *TOMM20*. Studies suggest that upregulation of *TOMM20* in COAD tissues is associated with cell cycle dysregulation and cancer cell invasiveness (47-49). This further confirms the pivotal role of mitochondrial membrane-related proteins, represented by *VDAC3*, in the development of cancer.

Lastly, using the CellMiner database, we explored the connection between *VDAC3* expression levels and sensitivity to anticancer drugs (50). *VDAC3* expression positively correlated with chelerythrine and cladribine, while negatively correlating with Ergenyl. Research suggests the potential of chelerythrine as an anticancer drug in colon cancer treatment (51). Our analysis indicated that

cancer patients with high *VDAC3* expression were more sensitive to chelerythrine chemotherapy.

In conclusion, our study establishes a significant correlation between abnormal *VDAC3* expression and the occurrence and development of COAD. Its involvement in mitochondria-related biological functions suggests it as one of the mechanisms influencing CRC. However, there are several limitations to our study. Due to the partial absence of clinical data in the TCGA database and the limited number of non-cancerous colon tissue samples, some biases may be introduced. Additionally, the single-center nature of the study may limit the generalizability of the results. Future research should include larger and more diverse sample cohorts and be validated across multiple research centers to enhance the reliability and validity of *VDAC3* as a biomarker. Furthermore, we recommend further exploration of the specific mechanisms by which *VDAC3* is involved in disease progression and how it affects the prognosis of the disease. This includes, but is not limited to, how *VDAC3* participates in key biological processes such as apoptosis, energy metabolism, and signal transduction. By employing advanced molecular biology techniques, such as *CRISPR-Cas9* gene editing, high-throughput sequencing, and proteomic analysis (52), we can more accurately determine the role of *VDAC3* in the disease. Ultimately, we believe that in-depth research on *VDAC3* will provide new perspectives for the diagnosis and treatment of COAD, such as targeted therapies against *VDAC3*, which may offer more effective treatment options for patients.

Conclusions

In summary, our study establishes a significant link between abnormal *VDAC3* expression and COAD development. *VDAC3* emerges as an independent prognostic factor, showcasing dynamic changes in expression across COAD stages. Mechanistically, it influences mitochondria-related processes, impacting cellular metabolism and apoptosis crucial for cancer progression. Our exploration of *VDAC3*'s association with drug sensitivity hints at potential personalized treatment approaches. Our findings offer valuable insights and potential biomarkers for COAD diagnosis and prognosis, paving the way for targeted therapeutic strategies.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-402/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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