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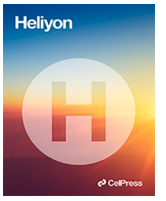

Research article

Oncogenic and immunological role of EDIL3 in human tumours: From pan-cancer analysis to validation in gastric cancer

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ABSTRACT

Background: Epidermal growth factor-like repeats and discoidin I-like domains 3 (EDIL3) is a secreted extracellular matrix protein implicated in diverse physiological and pathological processes including embryonic development, angiogenesis, and anti-inflammatory responses. Recent reports have indicated that EDIL3 play critical roles in carcinogenesis and progression of many cancers. Herein, we performed a pan-cancer investigation to study the potential functions of EDIL3 in various cancers and experimentally validate its function in gastric cancer (GC).

Methods: We analysed EDIL3 expression profiles in different tumours using The Cancer Genome Atlas database. The Kaplan–Meier Plotter was used to investigate the prognostic value of EDIL3, while receiver operating characteristic curve was performed to analyze its diagnostic efficacy. Several bioinformatics tools were used to study the association between EDIL3 and promoter methylation, gene enrichment analysis, immune infiltration, immune-related genes, and drug sensitivity. Molecular biology experiments were conducted to validate the tumorigenic effects of EDIL3.

Results: EDIL3 is variably expressed in different cancers and is closely associated with clinical outcomes. An inverse correlation between EDIL3 and DNA methylation has been observed in 13 cancers. Enrichment analysis indicated that EDIL3 is correlated with many cellular pathways such as extracellular matrix receptor interactions and focal adhesion. EDIL3 was tightly associated with immune infiltration and immune checkpoints. EDIL3 knockdown can promote GC calls apoptosis while preventing proliferation, migration, and invasion *in vitro*.

Conclusion: EDIL3 is a promising prognostic, diagnostic, and immunological biomarker in various cancers, which could be applied as a new target for cancer therapy.

1. Introduction

Cancer is a major human health problem worldwide, resulting in 9.9 million mortalities every year [1]. Despite advancements were made in cancer diagnosis and treatment, the therapeutic effect of advanced cancer is still unsatisfactory. Invasion and metastasis of tumour are the leading causes of mortality in most patients with cancer [2]. Thus, revealing the molecular mechanisms of tumour initiation and development and identifying novel diagnostic and therapeutic targets is critical.

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Recent researches have demonstrated that extracellular matrix (ECM) proteins are involved in carcinogenesis, development, and metastasis [3,4]. Epidermal growth factor-like repeats and discoidin I-like domains 3 (EDIL3) are 52kDa ECM glycoproteins that were first identified in 1998 [5]. Structurally, EDIL3 comprises two discoidin I-like repeats and three EGF-like repeats that interact with integrin to modulate endothelial cell function [6,7]. Recent studies have reported that EDIL3 plays critical roles in tumourigenesis, metastasis, and angiogenesis [8–12]. For instance, EDIL3 is overexpressed in hepatocellular carcinoma and promotes angiogenesis, invasion, and migration *in vitro* [13]. Furthermore, EDIL3 expression is elevated in breast cancer (BC) and miR-496 may target EDIL3 to inhibit BC cell proliferation [14]. In contrast, EDIL3 is downregulated in human lung carcinoma cells [15]. These studies suggest that EDIL3 expression and function varies in different cancers.

Recently, various databases have been constructed and widely used in cancer research [16,17]. Using public databases for pan-cancer genomic analysis aids in clarifying the underlying molecular mechanisms of tumour initiation and progression and identifying novel diagnostic and treatment targets [18]. In the present study, we analysed the expression, prognostic value, diagnostic significance, DNA methylation, potential regulatory networks, correlation with immune infiltration, and drug sensitivity of EDIL3 in various cancer types. According to our results, EDIL3 could be used as a prognostic and therapeutic marker for human pan-cancer.

2. Materials and methods

2.1. EDIL3 expression analysis

Genotype-Tissue Expression (GTEx) and The Cancer Genome Atlas (TCGA) data were obtained from UCSC XENA (https://xenabrowser.net) [16,17,19]. EDIL3 expression in 33 TCGA tumour and non-tumour tissues was analysed (Table S1). We plotted boxplots using the 'ggplot2' R package. EDIL3 expression in multiple organs was analysed using the Human Protein Atlas (HPA) (https://www.proteinatlas.org/) [20]. The Cancer Cell Line Encyclopedia (CCLE) (https://portals.broadinstitute.org/ccle) was utilized to analyze EDIL3 expression in different human cancer cell lines [21].

2.2. Survival analysis

A Cox regression analysis was conducted to assess the association between EDIL3 expression and overall survival (OS). The 'survminer' and 'survival' packages were utilized to conduct analysis. Forest graphs were utilized to analyze the association between EDIL3 expression and prognosis of different types of cancer. The 'Survival Analysis' module of Gene Expression Profiling Interactive Analysis 2 (GEPIA2) (http://gepia2.cancer-pku.cn) [22] was utilized to analyze the prognostic significance of EDIL3 in 33 different cancers.

2.3. Receiver operating characteristic (ROC) analysis

ROC analysis was employed to assess the effectiveness of EDIL3 expression in distinguishing tumours from normal tissues by using TCGA cohort. The 'pROC' and 'ggplot2' packages were used to compute area under the curve (AUC) and draw ROC curve, respectively. AUC >0.9 indicates high accuracy, 0.7–0.9 indicates moderate accuracy, and 0.5–0.7 indicates low accuracy [23].

2.4. DNA methylation analysis

The University of ALabama at Birmingham CANcer data analysis Portal (UALCAN) (http://ualcan.path.uab.edu) [24] and DNA methylation interactive visualization database (DNMIVD) (http://119.3.41.228/dnmivd/) [25] was utilized to analyze DNA methylation of EDIL3. Additionally, we analysed the EDIL3 promoter methylation levels as well as relationship between promoter methylation and EDIL3 expression in pan-cancer, and survival analysis was performed to evaluate whether EDIL3 promotes methylation and prognosis.

2.5. EDIL3-related genes enrichment analysis

STRING (https://cn.string-db.org/) [26] was used to predict the interacting protein partners of EDIL3 (*Homo sapiens*) and to form a protein–protein interaction network. BioGRID (https://thebiogrid.org/) [27] was utilized to construct the EDIL3-protein interaction network. GEPIA2's 'Similar Gene Detection' module was employed to identify the top 100 EDIL3-related genes. The 'Correlation Analysis' module was utilized to analyze a pairwise gene correlation in 33 different cancers. A Venn diagram was drawn to screen for the overlapping members of the STRING, BioGRID, and GEPIA2 results. Gene Ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were performed using 'ggplot2', 'org.Hs.eg.db', and 'clusterProfler' packages.

2.6. Immune infiltration analysis

Systematic and comprehensive analysis of different cancers immune infiltration was estimated using the immune-related characteristics of EDIL3 determined via the Immunedeconv package [28], including the CIBERSOR, EPIC, MCPCOUNTER, QUANTISEQ, TIMER, and XCELL algorithms. The results are presented as a heatmap. TIMER2.0 (http://timer.compgenomics.org/) [29] was utilized to investigate the relationship between EDIL3 and cancer-associated fibroblasts (CAFs) infiltration.

2.7. Correlation analysis between EDIL3 and immunotherapy

The relationship between EDIL3 and immune checkpoint genes, mismatch repair (MMR) genes, microsatellite instability (MSI) status, and tumour mutation burden (TMB) status was assessed with Spearman's correlation test. CD274, HAVCR2, TIGIT, LAG3, SIGLEC15, CTLA4, PDCD1, and PDCD1LG2 are classic immune checkpoints. MLH1, MSH2, MSH6, PMS2, and EPCAM are the main MMR proteins [30]. MSI data were acquired from Bonneville et al. 's study [31]. TMB was derived from Thorsson et al. 's study [32].

2.8. Drug sensitivity analysis

The correlation between EDIL3 expression and drug sensitivity was assessed using Genomics of Drug Sensitivity in Cancer (GDSC) (https://guolab.wchscu.cn/GSCA/#/drug) [33]. The half maximal inhibitory concentration (IC_{50}) for gastric cancer (GC) patients from TCGA dataset were calculated using the 'pRRophetic' package. Ten commonly used drugs in GC treatment were selected for analysis.

2.9. Patients and specimens

Fifteen pairs of GC and corresponding non-tumour tissues were obtained from Tianjin Medical University Cancer Hospital. All samples were frozen at −80 °C for further analysis.

2.10. Western blotting and qRT-PCR analysis

The protein was isolated utilising RIPA buffer (Beyotime, China). Samples were separated by SDS-PAGE gel and transferred onto PVDF membranes. Membranes were probed with anti-EDIL3 (Abcam, UK) and anti-GAPDH (Proteintech, China) antibody. The RNA was isolated using the TRIZOL method (Invitrogen, USA). qRT-PCR was conducted on an ABI 7900 system (Life Technologies, USA). The following primers were used: EDIL3 gene forward, 5′-TGACAGATGGCCGTGGATT-3′, reverse 5′-TCCTCTTGGCTCCTTGGGTAA-3'. GAPDH gene forward, 5′-GCACCGTCAAGGCTGAGAAC-3′, reverse 5′-TGGTGAAGACGCCAGTGGA-3'. The $2^{-\triangle\triangle Ct}$ method was used to analyze EDIL3 mRNA expression.

2.11. Cell culture and transient transfection

Human GC cell lines BGC-823 and AGS were obtained from the Chinese Academy of Science (Shanghai, China). Specific shRNAs against EDIL3 (sh-EDIL3: 5′-GGAGGTTGCATCAGATGAAGA-3′) and a negative control (sh-NC: 5′-TTCTCCGAACGTGTCACGT-3′) were purchased from GeneChem (Shanghai, China). The knockdown efficiency was assessed using qRT-PCR.

2.12. Cell counting kit-8 (CCK-8) assay

Cells were cultured in 96-well plates and treated with CCK-8 reagent (Abcam, UK). The absorbance was determined at 450 nm and cells growth curve was drawn.

2.13. Cell apoptosis assay

For apoptosis assay, cells were stained with an Annexin V/PI apoptosis kit (BD, USA), and apoptosis rate was analysed using flow cytometry.

2.14. Wound-healing assay

Cells were cultured in six-well plates, then a line wound was made using pipette tips. The wound areas were photographed and recorded at 0 and 48 h. The distance of the injury line was measured using ImageJ software.

2.15. Transwell assay

A chamber coated with Matrigel was used for invasion testing. The cells were cultured in the upper chamber of serum-free DMEM, while the lower chamber was filled with 20 % FBS in DMEM. After 24 h culture, invasive cells on the bottom side were fixed, stained, and counted.

2.16. Statistics analysis

Statistical analyses were conducted using R, version 4.1.3 and GraphPad Prism 8. Student's t-test was performed to test for differences between two groups. Differences were defined as significance at $P < 0.05$.

3. Results

3.1. EDIL3 expression in pan-cancer

The TCGA and GTEx data were combined with examining the expression patterns of EDIL3 in human cancers. As shown in Fig. 1A and S1, EDIL3 expression indicated low tumour specificity. Expression of EDIL3 was markedly upregulated in 14 types of cancer (BRCA, CHOL, DLBC, ESCA, HNSC, KIRP, KIRC, LAML, LIHC, PAAD, STAD, TGCT, THCA, and THYM). Conversely, EDIL3 expression in ACC, BLCA, COAD, CESC, GBM, KICH, LUSC, LUAD, OV, PRAD, READ, SKCM, UCS, and UCEC was downregulated.

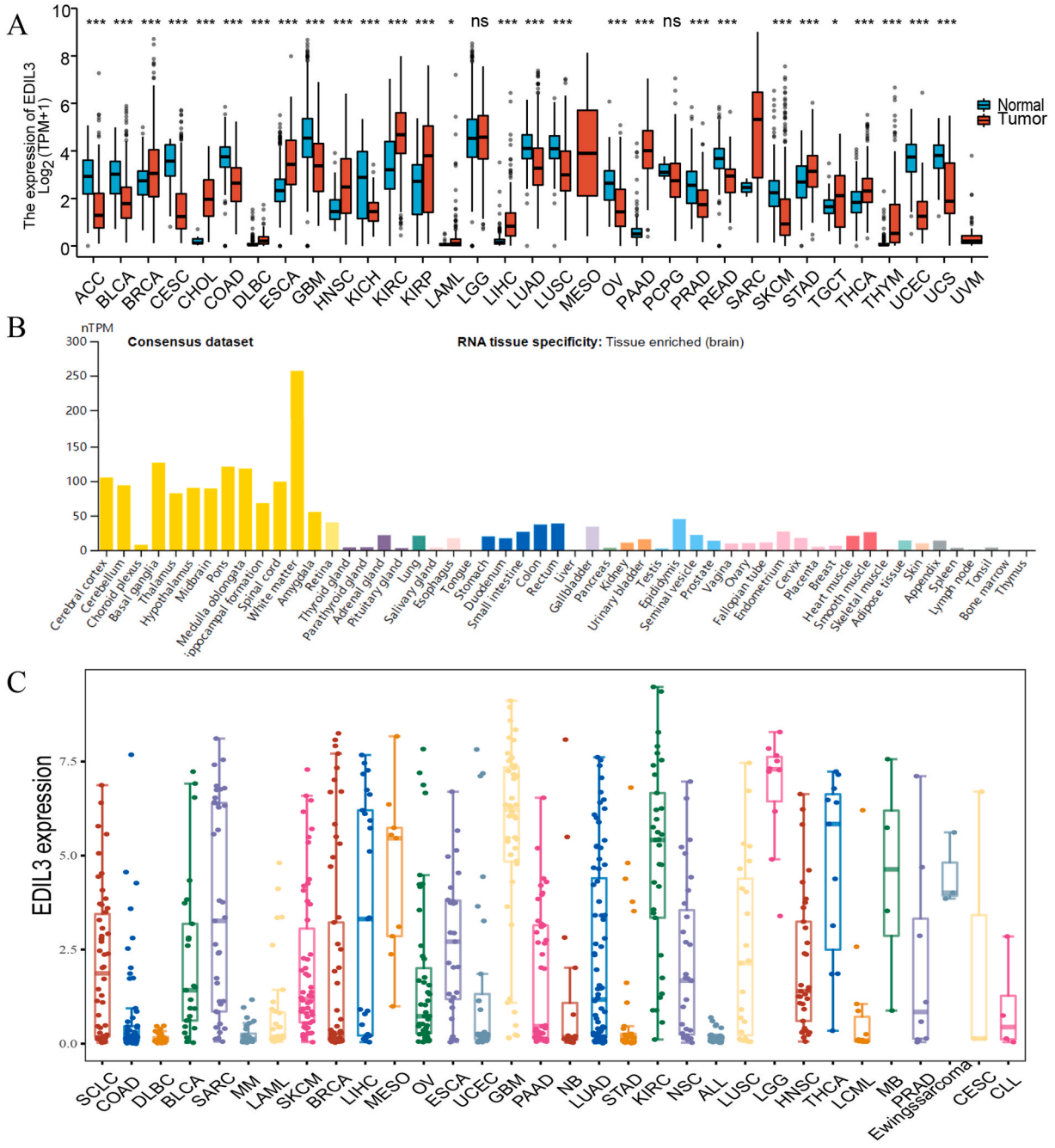

Fig. 1. Pan-cancer analysis of EDIL3 expression. (A) Pan-cancer differential EDIL3 expression between tumour and non-tumour samples from the TCGA datasets. (B) EDIL3 expression in different normal tissues from the HPA consensus datasets. (C) EDIL3 expression in different tumour tissues from the CCLE datasets. (*$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$).

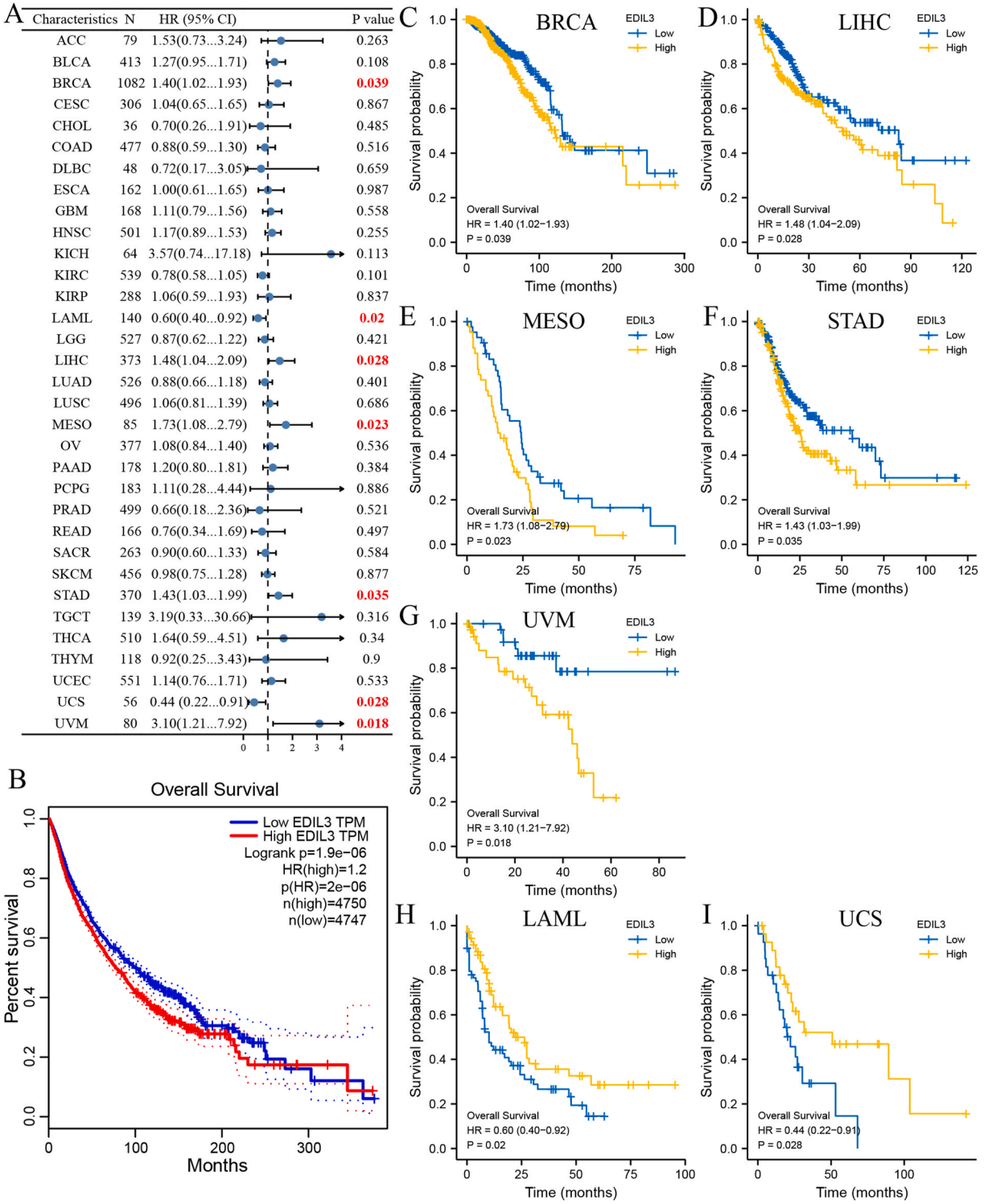

Fig. 2. Associating prognosis with EDIL3 expression. (A) Forest map of the association between EDIL3 expression and OS in multiple tumours from the TCGA dataset. (B) High expression of EDIL3 was associated with poor prognosis in 33 cancer types from GEPIA. (C–I) Kaplan–Meier survival curves are statistically significant for seven cancer types (BRCA, LIHC, MESO, STAD, UVM, LAML, and UCS) from the TCGA dataset.

EDIL3 is also widely distributed in normal tissues, similar to its distribution in tumour tissues. The HPA consensus datasets (HPA, GTEx, and FANTOM5) revealed that EDIL3 was highly expressed in nervous tissues, such as the white matter, basal ganglia, and pons (Fig. 1B and S2). The CCLE dataset revealed that EDIL3 was aberrantly expressed in different tumour cell lines (Fig. 1C). SNU.349, KMRC.3, and SNU.489 were the top three cell lines with highest EDIL3 expression (Table S2). Overall, these findings indicate that EDIL3 is differentially expressed in various cancers.

3.2. Prognostic and predictive potential of EDIL3 in pan-cancer

The relationship between EDIL3 and overall survival (OS) was assessed in pan-cancer based on TCGA dataset (Fig. 2A). We found that EDIL3 overexpression correlated with unfavorable OS in all TCGA cancer types (Fig. 2B, hazard ratio [HR] = 1.2, $P < 0.001$). The results demonstrated that high EDIL3 expression was significantly associated with worse OS in BRCA, LIHC, MESO, STAD, and UVM (Fig. 2C–G; HR > 1.0, $P < 0.05$). Conversely, EDIL3 had protective function in LAML (Fig. 2H; HR = 0.60, $P = 0.02$) and UCS (Fig. 2I;

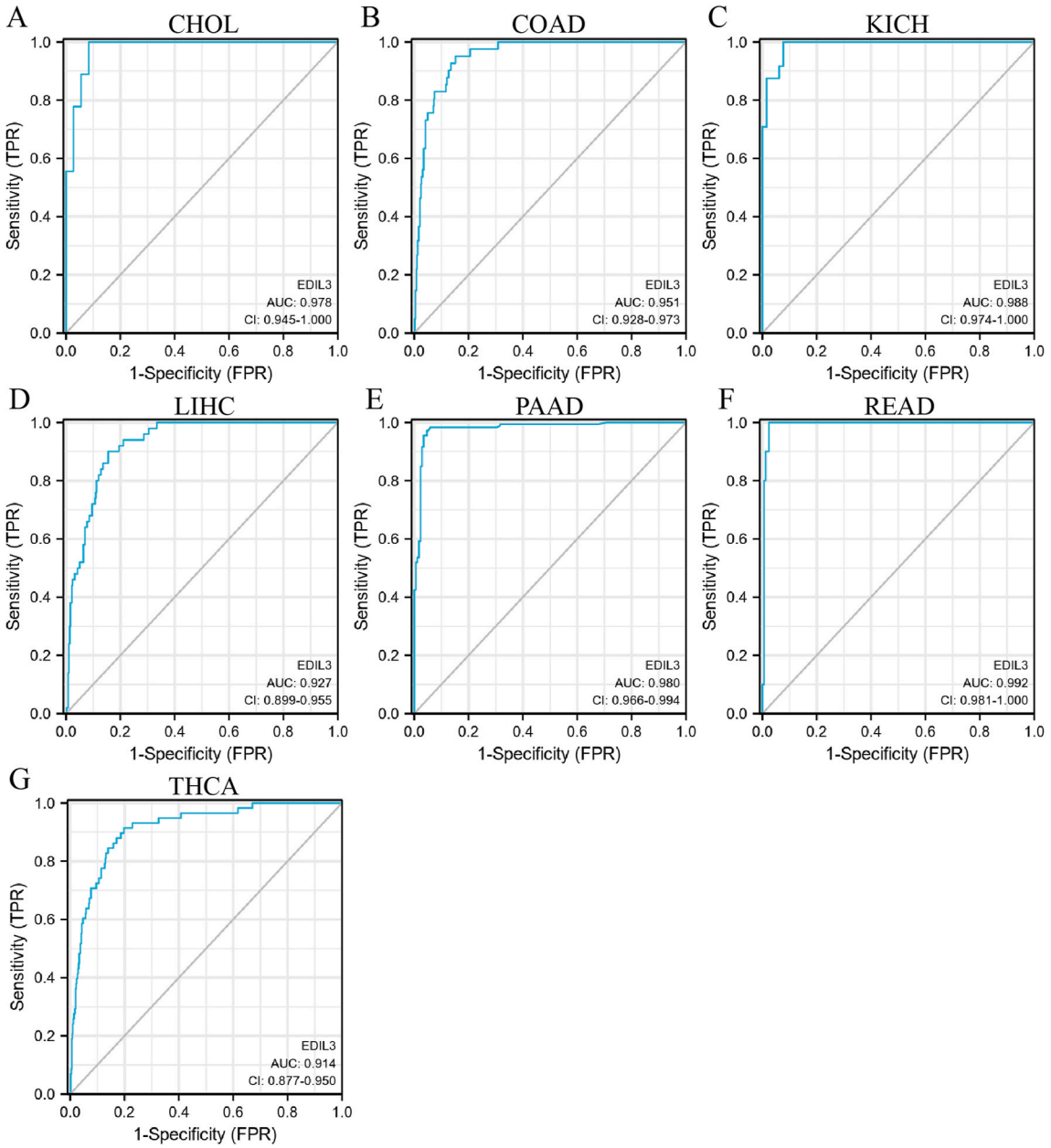

Fig. 3. ROC curves of EDIL3 expression. EDIL3 had high diagnostic efficiency for CHOL (A), COAD (B), KICH (C), LIHC (D), PAAD (E), READ (F), and THCA (G). The AUC value of the ROC curves was >0.9.

HR = 0.44, $P = 0.028$). These findings demonstrate that the prognostic value of EDIL3 varies significantly between cancer types.

In addition, the results ROC curve showed that EDIL3 could discriminate between patients with cancer and those without in 32 cancer types (Fig. 3and S3; AUC >0.5). The ROC curves showed that EDIL3 had high diagnostic efficiency for CHOL, COAD, KICH,

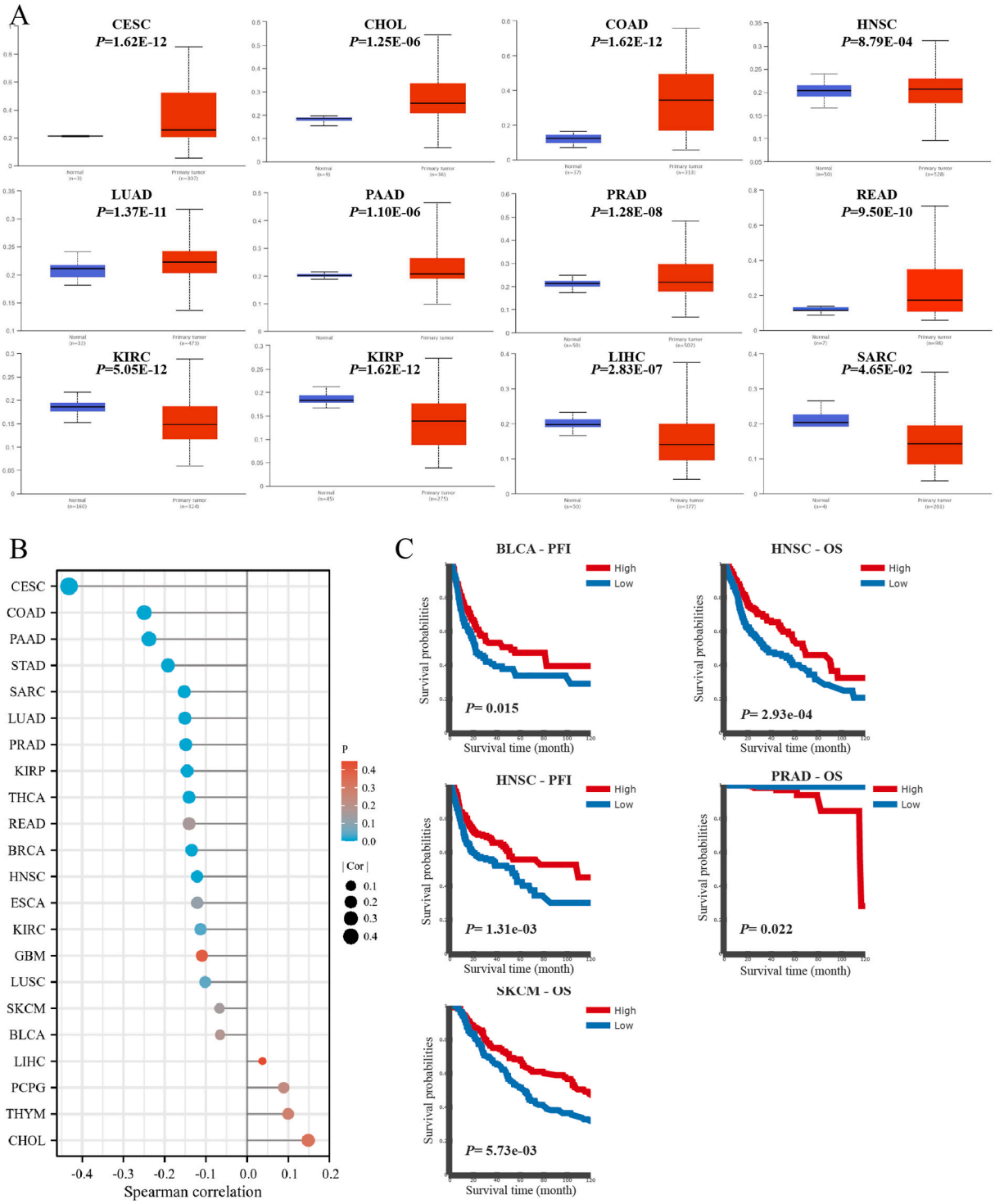

Fig. 4. Promoter methylation analysis of EDIL3 in pan-cancer. (A) Promoter methylation for EDIL3 in different cancers. (B) The relationship between promoter methylation and EDIL3 expression in pan-cancer. (C) The relationship between EDIL3 promoter methylation and prognosis in different cancers.

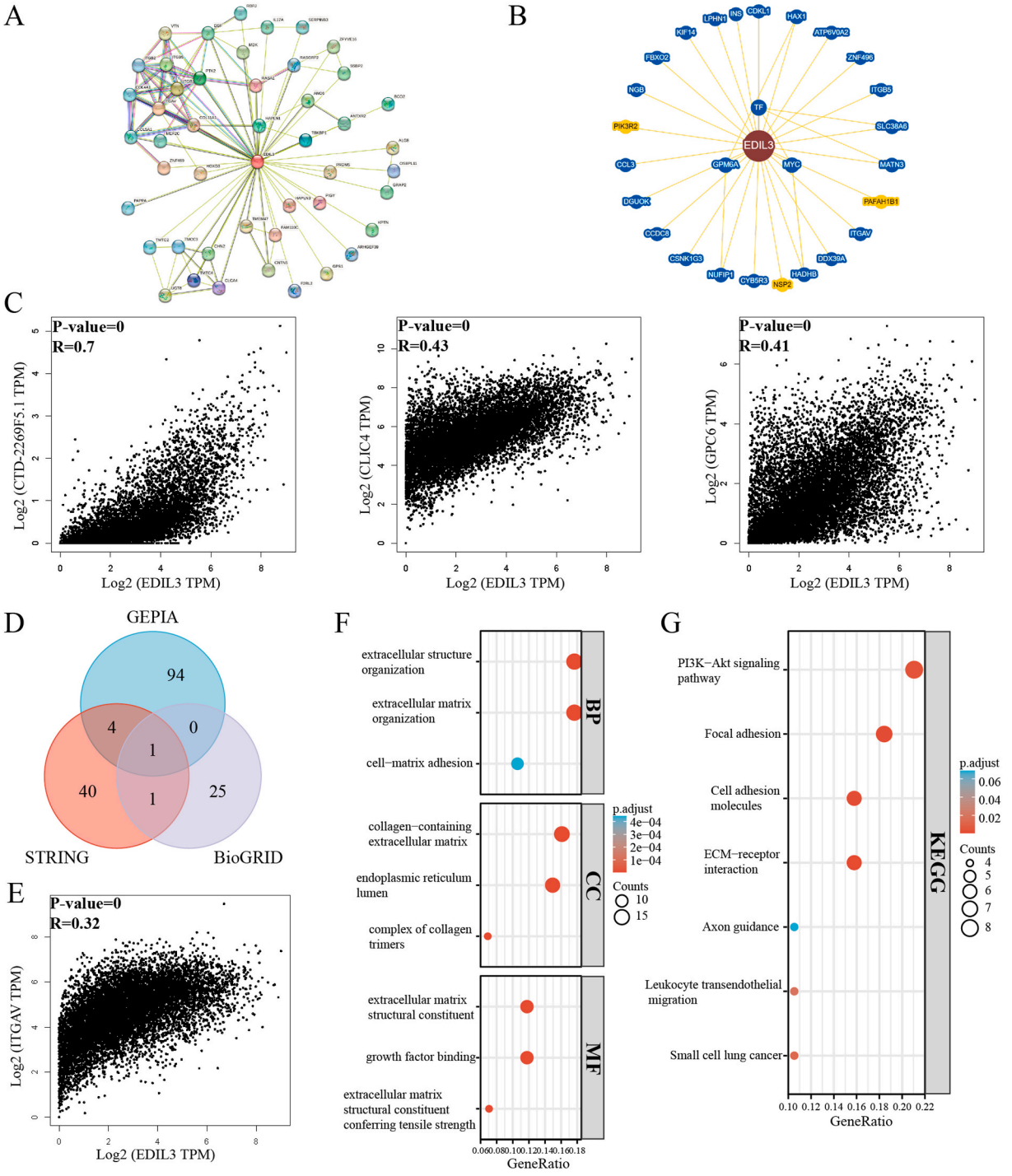

Fig. 5. Gene enrichment analysis of EDIL3 in cancers. (A) The PPI network of EDIL3 was obtained by STRING. (B) EDIL3-protein interactions were determined by BioGRID. (C) The correlation between EDIL3 and the top three EDIL3-related genes in different cancers according to GEPIA2. (D) The Venn diagram showing the common member of EDIL3-binding and associated genes. (E) The correlation between EDIL3 and ITGAV in cancers. GO enrichment (F) and KEGG pathway (G) analyses were performed based on the top 100 EDIL3-related genes obtained by the GEPIA2.

LIHC, PAAD, READ, and THCA (AUC >0.9). Overall, our findings suggest that EDIL3 could be considered a predictive factor for multiple cancers (see).

3.3. DNA methylation of EDIL3 in pan-cancer

EDIL3 methylation levels in different cancers were analysed using UALCAN. EDIL3 methylation levels were markedly elevated in eight cancer types: CESC, COAD, CHOL, HNSC, LUAD, PRAD, PAAD, and READ (Fig. 4A; $P < 0.001$). Conversely, EDIL3 methylation levels were significantly lower in five cancer types: KIRC, KIRP, LIHC, SARC, and TGCT ($P < 0.05$).

As shown in Tables S3 and 26 CpG sites of EDIL3 and their information was identified. The DNMIVD dataset revealed that EDIL3 expression was negatively associated with DNA methylation levels in 13 types of cancer (BRCA, COAD, CESC, HNSC, KIRP, KIRC, LUSC, LUAD, PRAD, PAAD, SARC, STAD, and THCA) (Fig. 4B and Table S4). In contrast, there was no markedly positive correlation between EDIL3 expression and methylation levels in any tumour type. These findings indicate that promoter methylation is critical for EDIL3 expression regulation in many tumours. Furthermore, we explored the correlation between EDIL3 methylation and survival in diverse tumour types. The results revealed that higher EDIL3 methylation levels were associated with prolonged progression free interval (PFI) in HNSC and BLCA (Fig. 4C, $P < 0.05$). Additionally, EDIL3 had a high DNA methylation level that associated with higher

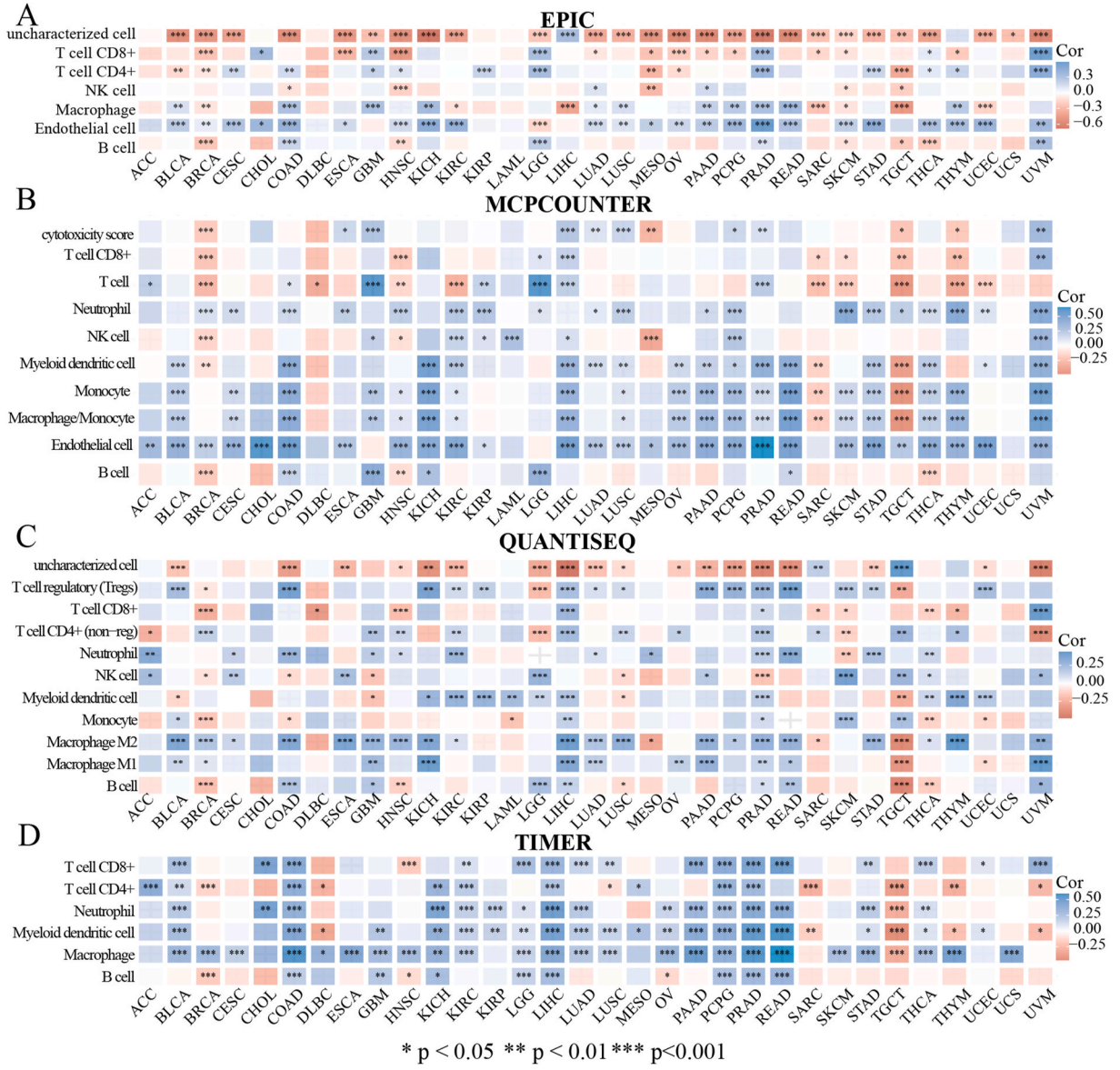

Fig. 6. Relationship between EDIL3 expression and immune infiltrates analysed using the immunedeconv package. Immune cell infiltration was analysed using the EPIC (A), MCPCOUNTER (B), QUANTISEQ (C), and TIMER (D) algorithms. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

OS in HNSC and SKCM ($P < 0.005$). Conversely, higher EDIL3 methylation levels associated with lower OS in PRAD ($P < 0.005$).

3.4. Enrichment analysis of EDIL3-related gene in pan-cancer

According to the STRING tool, 46 EDIL3-binding proteins were identified using the STRING database (Table S5). Fig. 5A shows the interaction networks of these proteins. These genes were enriched for ECM-receptor interactions and focal adhesions (Table S6). Based on BioGRID4.4, 27 proteins interacted with EDIL3 (Table S7), and EDIL3 physically interacted with TF, GPM6A, and MYC (Fig. 5B). Moreover, GEPIA2 was utilized to acquire the top 100 EDIL3 expression-associated genes from 33 cancer types (Table S8). The top three positively correlated genes were CTD-2269F5.1 (R = 0.70), CLIC4 (R = 0.43), and FERMT2 (R = 0.43) (Fig. 5C; $P < 0.001$). Interaction analysis identified one common member, ITGAV (Fig. 5D). Correlation analyses revealed that EDIL3 expression was closely correlated with ITGAV expression in all 33 cancers (Fig. 5E, R = 0.32, $P < 0.001$) (see Fig. 6).

To further investigate EDIL3 pan-cancer biological function, we identified 293 GO terms and 15 enriched KEGG pathways (Table S9). As shown in Fig. 5F, biological process (BP) analysis indicated that EDIL3 might correlated with extracellular structure organisation, ECM organisation, and cell-matrix adhesion. Cellular component (CC) analysis revealed that EDIL3-related genes were involved in the response to collagen-containing ECM, endoplasmic reticulum lumen, and collagen trimer complex. Molecular function (MF) analysis indicated that EDIL3-associated genes were enriched in growth factor binding, ECM structural constituents, and ECM structural constituents conferring tensile strength. KEGG analysis revealed that PI3K-Akt signalling pathway, focal adhesion, and cell

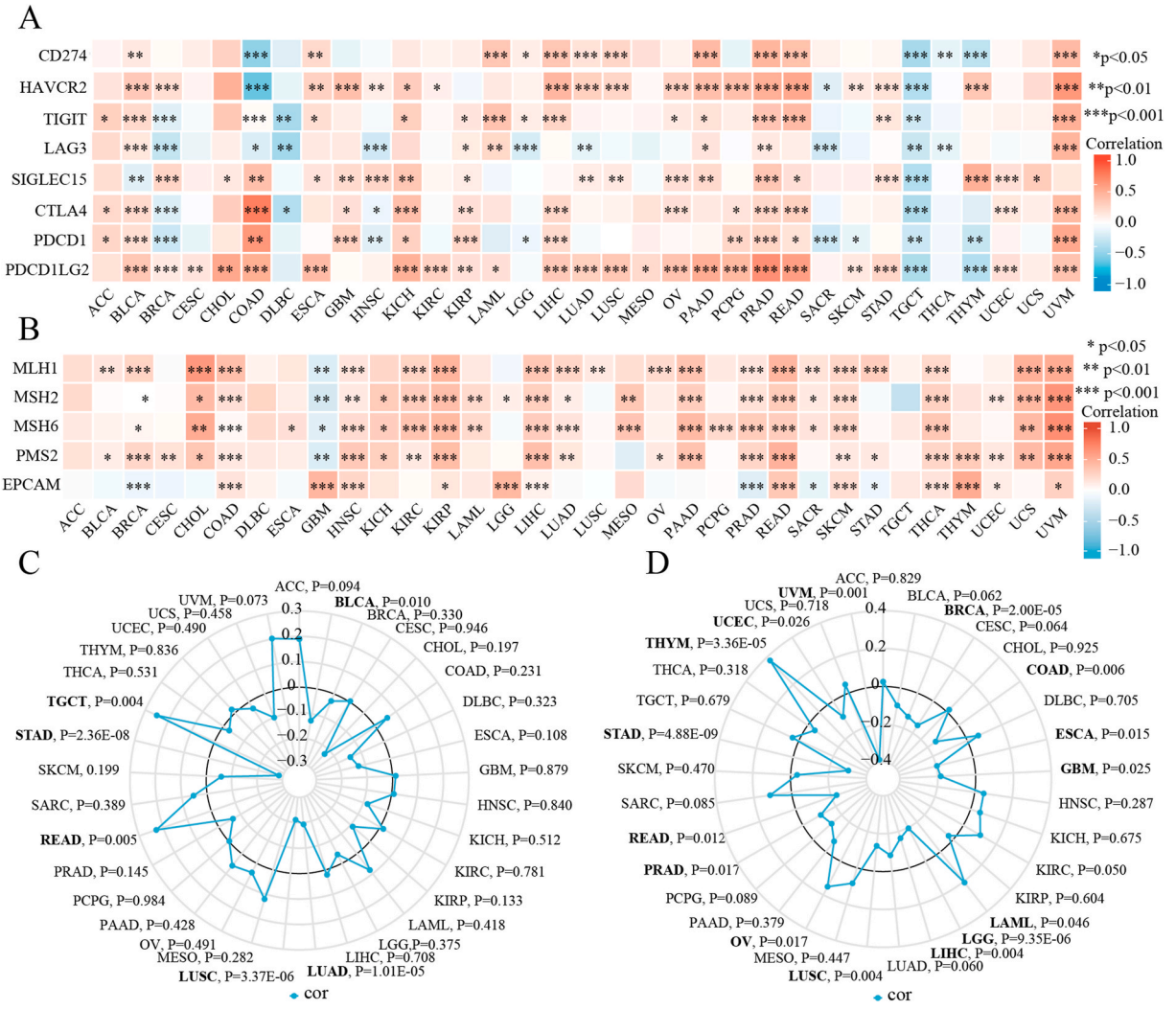

Fig. 7. Relationship between EDIL3 expression and immune checkpoints, MMR genes, MSI, and TMB in pan-cancer. (A) Correlation between EDIL3 expression and immune checkpoints in pan-cancer. (B) Correlation between EDIL3 expression and MMR genes in pan-cancer. (C) Correlation between EDIL3 expression and MSI in pan-cancer. (D) Correlation between EDIL3 expression and TMB in pan-cancer. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

adhesion molecules were the three most enriched pathways (Fig. 5G). Collectively, these results indicated that EDIL3 regulates tumour initiation and development by regulating cell adhesion and ECM formation.

3.5. EDIL3 correlation with immune infiltration in pan-cancer

To clarify the association between EDIL3 and immune cell infiltration, the 'immunedeconv' package was utilized to quantify immune cells, which integrates six algorithms (Fig. 6 and S4). The results demonstrate that EDIL3 was closely correlated with multiple immune cells. EDIL3 expression showed a remarkable relationship with $CD4^+$ T cells in BRCA, MESO, TGCT, THYM, and UVM. EDIL3 was strongly correlated with $CD8^+$ T cells in HNSC and UVM. EDIL3 was significantly associated with B cells in BRCA and HNSC. EDIL3 was closely correlated with M1/M2 macrophages in BRCA, GBM, and READ groups. The relationship between EDIL3 and CAFs infiltration was also studied in various cancers. The results indicated that EDIL3 was significantly associated with CAFs infiltration in many human cancers, including BRCA, COAD, OV, HNSC, PAAD, and STAD (Fig. S5). Overall, these findings suggested that EDIL3 might participate in controlling the tumour microenvironment (TME).

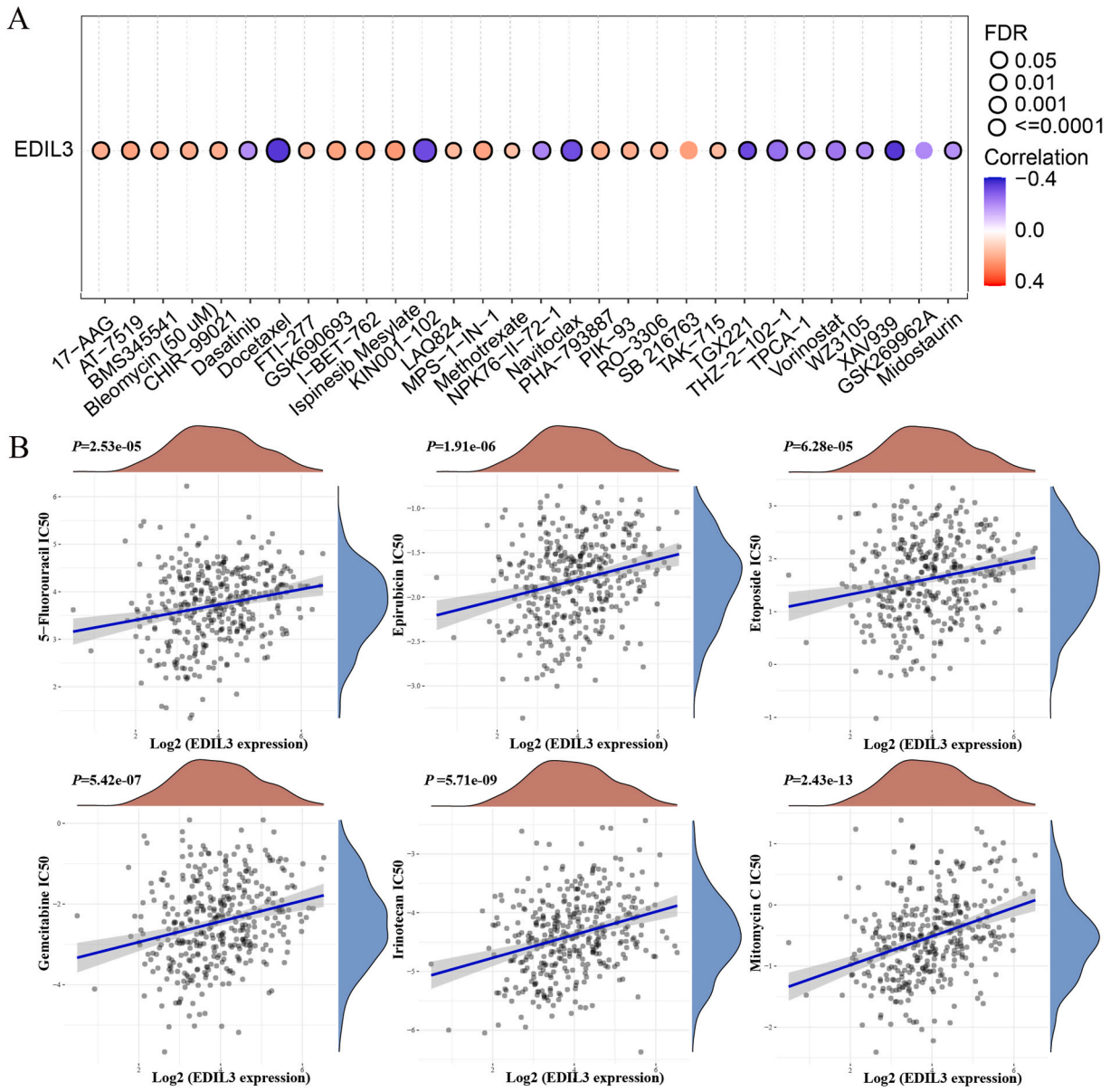

Fig. 8. Drug sensitivity analysis. (A) The correlation between EDIL3 and multiple drugs in pan-cancer based on the GDSC database. (B) EDIL3 expression in GC with drug sensitivity analysis (IC_{50}).

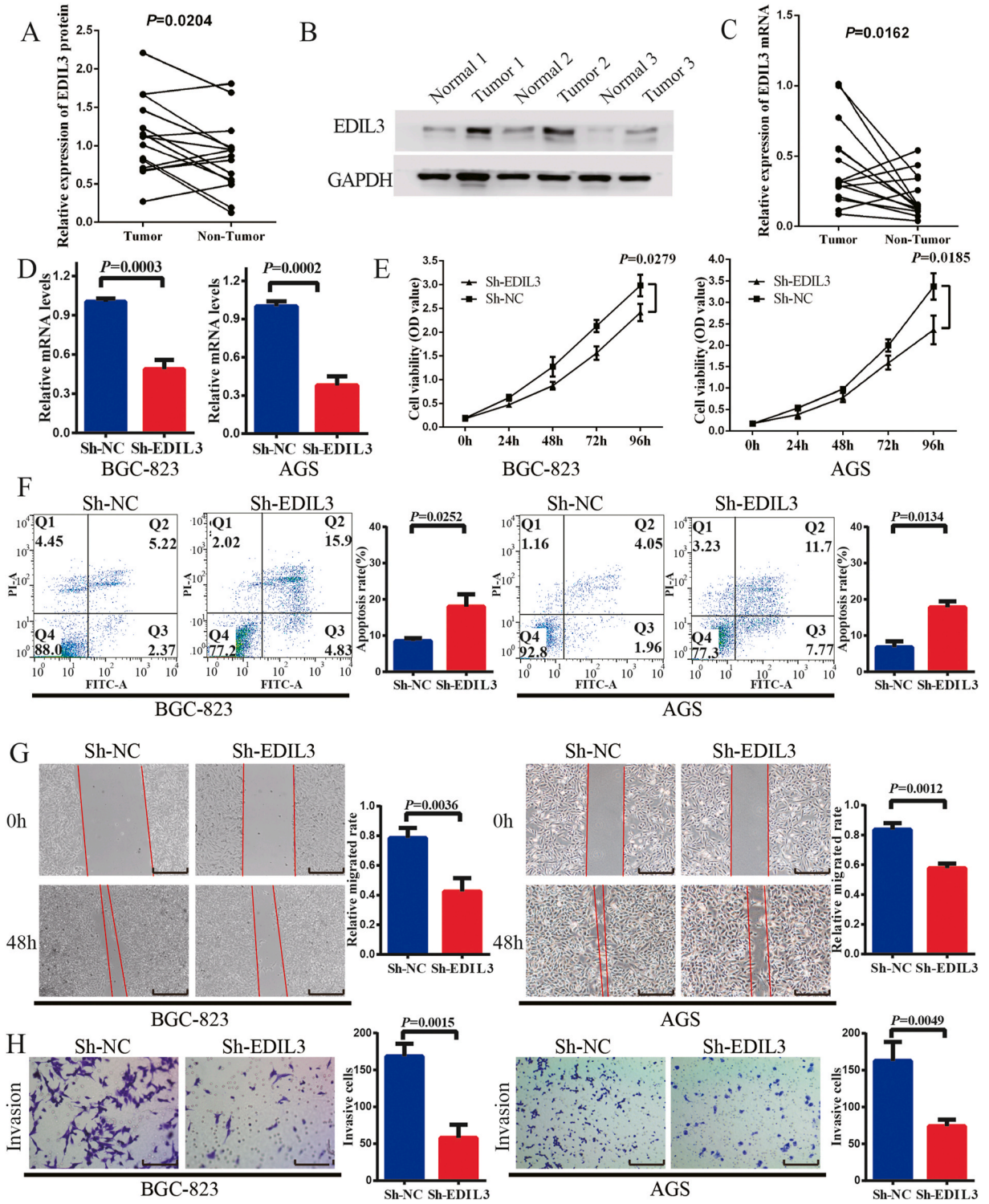

Fig. 9. Expression and cellular functions of EDIL3 in GC. (A) Relative protein expression of EDIL3 in GC tissues and corresponding non-tumour tissues. (B) Representative immunoblots of EDIL3 expression in the three paired samples. (The original image is provided in the Supplementary Fig. S7). (C) Relative mRNA expression of EDIL3 in GC tissues and corresponding non-tumour tissues. (D) mRNA expression of EDIL3 in BGC-823 and AGS cells after sh-EDIL3 transfection. (E) After EDIL3 knockdown, CCK-8 assay was used to measure cell viability. (F) Cell apoptosis was determined by Annexin V/PI double-staining assay. (G) Wound-healing assay was used to determine cell migration after transfection. (H) Transwell assay was conducted to assess invasion capacity after transfection. Scale bars, 100 μm.

3.6. Association between EDIL3 and immunotherapy in pan-cancer

A correlation analysis between EDIL3 and several representative markers was performed to investigate the association between EDIL3 and immunotherapy. First, a co-expression analysis was carried out to assess the association between EDIL3 and eight checkpoints in different cancers (Fig. 7A). The results indicated that EDIL3 was strongly correlated with most checkpoints in multiple tumours, particularly BLCA, KICH, KIRP, LIHC, PAAD, PRAD, READ, and UVM. We then examined the association between EDIL3 and MMR genes (Fig. 7B). The results revealed that EDIL3 was positively correlated with MMR genes in multiple cancers, particularly COAD, HNSC, KIRP, LIHC, SKCM, THCA, and UVM. Finally, correlation analysis between EDIL3 and MSI/TMB was conducted. The relationship between MSI and EDIL3 was positive in READ and TGCT, but negatively in BLCA, LUSC, LUAD, and STAD (Fig. 7C). TMB was positively correlated with EDIL3 in LAML, OV, and THYM, but negatively correlated with EDIL3 in BRCA, COAD, ESCA, GBM, LGG, LUSC, LIHC, PRAD, READ, STAD, THYM, UCEC, and UVM (Fig. 7D). In summary, our results suggested that EDIL3 can be used to predict immunotherapy efficacy in certain cancers.

3.7. Association between EDIL3 and drug sensitivity

The correlation between EDIL3 and IC_{50} data for 251 drugs or small molecules was evaluated using the GSCA database (Table S10). EDIL3 was positively associated with sensitivity to drugs, such as 17-AAG, PIK-93, and vorinostat, whereas EDIL3 was negatively correlated with certain drugs, including docetaxel, dasatinib, and pazopamob (Fig. 8A). We then analysed the relationship between EDIL3 and IC_{50} data of 10 drugs in TCGA-STAD samples. The results indicated that EDIL3 was positively associated with drug response in patients treated with 5-fluorouracil, epirubicin, etoposide, gemcitabine, irinotecan, and mitomycin C (Fig. 8B). Additionally, there was no correlation between EDIL3 and anticancer drugs cisplatin, docetaxel, paclitaxel, and lapatinib (Fig. S6). Thus, EDIL3 can be used as a biomarker to predict GC drug therapy.

3.8. Expression and function of EDIL3 in GC

GC was selected as a representative human cancer to confirm the expression and function of EDIL3. First, we investigated EDIL3 expression in GC and non-tumour tissues. Western blotting demonstrated that expression of EDIL3 was elevated in GC tissues ($P = 0.0204$; Fig. 9A and B). qRT-PCR results were in agreement with western blotting analysis (Fig. 9C). Then, shRNA was applied to knockdown expression of EDIL3 in GC cell lines (Fig. 9D). CCK-8 results indicated that EDIL3 knockdown suppressed GC cell proliferation (Fig. 9E). In addition, apoptosis assays indicated that EDIL3 knockdown significantly increased apoptosis of GC cells (Fig. 9F). EDIL3 knockdown markedly reduced GC cell migration and invasion (Fig. 9G and H). Altogether, EDIL3 is overexpression in GC tissues and facilitates malignant behaviour of GC *in vitro*.

4. Discussion

The prognosis of cancer remains unsatisfactory, and millions of people die of cancer annually [1]. Hence, identify novel diagnostic and therapeutic targets for cancer is imperative. Recently, EDIL3 function in several cancers has been studied. However, most studies focused on its role in specific tumours, whereas its function in all human cancers remains unknown. The purpose of this research was to investigate EDIL3's the potential function of EDIL3 in pan-cancer through comprehensive analysis.

Herein, we analysed EDIL3 expression using TCGA datasets. We found that EDIL3 expression was markedly elevated in 14 cancer types, including LIHC, PAAD, and STAD. Conversely, EDIL3 expression was downregulated in 14 types of cancer, including ACC, LUAD, and OV. Previous studies have indicated that EDIL3 is overexpressed in BRCA [8,14], LIHC [13], PAAD [11], and STAD [12], and expressed weakly in LUAD [15,34]. These findings indicated that EDIL3 may have varying functions in different types of cancers. Several researches have reported that EDIL3 overexpression was associated with poor BRCA and LIHC prognosis [8,13,35]. Our results also indicate that increased EDIL3 expression predicts poor OS in all cancer patients. However, the influence of EDIL3 on prognosis of different cancer types varies. Elevated EDIL3 expression was associated with worse prognosis in certain cancer types, such as BRCA, LIHC, and STAD, whereas it was correlated with better prognosis in other cancer types, such as LAML and UCS. The prognostic significance of EDIL3 in different tumours still need further exploration and validation. Additionally, the ROC curve analysis indicated that EDIL3 expression could accurately distinguish patients with cancer from most cancer types. Our findings demonstrated that EDIL3 has potential prognostic and diagnostic value in multiple cancers.

Recent studies have demonstrated that epigenetic changes such as DNA methylation play important functions in tumourigenesis and progression of multiple cancer types [36]. The UALCAN and DNMIVD datasets were used to assess EDIL3 methylation levels across all TCGA cancers. The methylation levels of EDIL3 were significantly different among different tumour types. EDIL3 methylation levels were markedly elevated in eight cancer types; however, EDIL3 expression levels were downregulated in 14 types of cancer. Therefore, additional mechanisms may participate in regulation of EDIL3 in various cancers. EDIL3 methylation level was correlated with prognosis in several cancers, and EDIL3 promoter methylation levels can be used as prognostic markers in certain cancers. Further researches are needed to elucidate the potential function of EDIL3 methylation in different cancers.

Using STRING, BioGRID, and GEPIA2, we integrated and analysed information on EDIL3-related genes and proteins. We also identified a common component of ITGAV. ITGAV is a membrane protein that is widely participated in various biological process, including cancer initiation and progression [37,38]. EDIL3 and ITGAV expression were positively correlated in various cancers. Enrichment analyses suggested that EDIL3 may be involved in multiple cancer-related pathways. Likewise, previous reports

demonstrated that EDIL3 was overexpressed in cancer cells and plays critical functions in cell adhesion regulation [39,40]. In conclusion, EDIL3 may perform different functions in different cancer types. Further research is required to investigate its functions in cancer. Additionally, the predicted EDIL3-related proteins must be verified.

Recent reports revealed that TME plays critical functions in tumour progression and influences final prognosis [41]. Furthermore, reports have demonstrated that TME is associated with various cancer genes [42]. Our results indicated that EDIL3 was highly associated with the levels of various tumour-infiltrating immune cells in multiple cancers. Additionally, EDIL3 expression is significantly associated with CAFs in most cancers. These findings suggested that EDIL3 may play a significant role in remodelling tumour immune microenvironment in cancer. Recently, immune checkpoint inhibitors (ICIs) have been extensively utilized in cancer therapy [43]. Checkpoint gene levels, MMR status, MSI status, and TMB are considered representative predictors of immunotherapy response [44]. Generally, MSI-high, TMB-high, and deficient MMR are correlated with better response to ICIs [45]. Our findings indicated that EDIL3 was negatively associated with MSI and TMB in BLCA, LUSC, and STAD. Furthermore, EDIL3 is positively correlated with MMR and checkpoint genes in various cancers. Therefore, EDIL3 can serve as a predictor of cancer immunotherapy response in certain cancers. The molecular mechanism by which EDIL3 affects tumour immune microenvironment requires further experimental verification.

Drug resistance directly reduces chemotherapy efficacy and leads to poor prognosis. Our results indicated that EDIL3 was closely associated with multiple antitumour drugs sensitivity. Further validation of GC demonstrated that elevated EDIL3 expression was correlated with poorer clinical response to 5-fluorouracil, epirubicin, etoposide, gemcitabine, irinotecan, and mitomycin C. These findings suggested that EDIL3 could act as a potential predictor of cancer chemotherapy, although further studies are needed to verify this relationship.

Bioinformatic analysis indicated that EDIL3 exhibits high diagnostic and prognostic value for GC, and its expression may be correlated with immunotherapy efficacy in GC. Our previous study demonstrated that EDIL3 is overexpressed in GC and correlated with unfavorable clinical characters [46]. Here, we validated EDIL3 function in GC using molecular biological methods. We confirmed that expression of EDIL3 was elevated in GC tissues. EDIL3 knockdown promotes apoptosis while preventing proliferation, migration, and invasion of GC cells. The results showed that EDIL3 functioned an oncogenic role in GC and has therapeutic potential. Further molecular researches are required to explore the underlying mechanisms of action of EDIL3 in GC.

This study has some limitations. First, most of the results presented were derived from public databases, and more clinical and laboratory experiments are required to confirm these conclusions. Second, we only explored the role of EDIL3 in GC, and further validation is required in other cancers. Third, we revealed that EDIL3 is closely associated with malignant behaviors of GC cells; however, underlying mechanism requires further investigation.

5. Conclusion

To our knowledge, this is the first comprehensive pan-cancer examination of EDIL3. These findings revealed that EDIL3 expression is heterogeneous in different malignant tumours and that EDIL3 could be a potential marker for prognosis and diagnosis of various cancers. Moreover, EDIL3 expression correlates with DNA methylation, immune infiltrates, TMB, and MSI in various human cancers. Furthermore, we confirmed that EDIL3 is overexpressed in GC and contributes to its malignant behaviour; these findings aid in elucidating the role of EDIL3 in cancer. Thus, EDIL3 has the potential to serve as a valuable marker for predicting clinical outcomes and therapeutic effect in certain patients.

Data availability statement

The original data of this study are available from the corresponding authors.

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Ethics approval and consent to participate

This study was approved by the Ethics Committee of Tianjin Medical University Cancer Institute and Hospital (bc2023054). All participants signed patients' informed consent.

CRediT authorship contribution statement

Bin Ke: Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization. **Peng Jin:** Methodology, Investigation. **Xue-Jun Wang:** Validation, Supervision, Methodology. **Ning Liu:** Visualization, Methodology. **Han Liang:** Writing – review & editing, Supervision. **Ru-Peng Zhang:** Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e32291.

References

[1] H. Sung, J. Ferlay, R.L. Siegel, et al., Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA A Cancer J. Clin. 71 (3) (2021) 209–249.
[2] D. Hanahan, Hallmarks of cancer: new dimensions, Cancer Discov. 12 (1) (2022) 31–46.
[3] A. Corona, G.C. Blobe, The role of the extracellular matrix protein TGFBI in cancer, Cell. Signal. 84 (2021) 110028.
[4] S.S. Hassanein, A.L. Abdel-Mawgood, S.A. Ibrahim, EGFR-dependent extracellular matrix protein interactions might light a candle in cell behavior of non-small cell lung cancer, Front. Oncol. 11 (2021) 766659.
[5] C. Hidai, T. Zupancic, K. Penta, et al., Cloning and characterization of developmental endothelial locus-1: an embryonic endothelial cell protein that binds the alphavbeta3 integrin receptor, Gene Dev. 12 (1) (1998) 21–33.
[6] J. Zhong, B. Eliceiri, D. Stupack, et al., Neovascularization of ischemic tissues by gene delivery of the extracellular matrix protein Del-1, J. Clin. Invest. 112 (1) (2003) 30–41.
[7] C. Hidai, M. Kawana, H. Kitano, et al., Discoidin domain of Del1 protein contributes to its deposition in the extracellular matrix, Cell Tissue Res. 330 (1) (2007) 83–95.
[8] S.J. Lee, J. Lee, W.W. Kim, et al., Del-1 expression as a potential biomarker in triple-negative early breast cancer, Oncology 94 (4) (2018) 243–256.
[9] J. Gasca, M.L. Flores, R. Jimenez-Guerrero, et al., EDIL3 promotes epithelial-mesenchymal transition and paclitaxel resistance through its interaction with integrin alphaVbeta3 in cancer cells, Cell death discovery 6 (2020) 86.
[10] Y.X. Wei, J.H. Han, H.M. Shen, et al., Highly sensitive fluorescent detection of EDIL3 overexpressed exosomes for the diagnosis of triple-negative breast cancer, Nanotechnology 33 (42) (2022) 425204.
[11] S.H. Jiang, Y. Wang, J.Y. Yang, et al., Overexpressed EDIL3 predicts poor prognosis and promotes anchorage-independent tumor growth in human pancreatic cancer, Oncotarget 7 (4) (2016) 4226–4240.
[12] L. Zhang, K.W. Peng, B. Wang, et al., EDIL3 regulates gastric cancer cell migration, invasion and epithelial-mesenchymal transition via TGF-beta1/XIST/miR-137 feedback loop, Transl. Cancer Res. 9 (10) (2020) 6313–6330.
[13] H. Xia, J. Chen, M. Shi, et al., EDIL3 is a novel regulator of epithelial-mesenchymal transition controlling early recurrence of hepatocellular carcinoma, J. Hepatol. 63 (4) (2015) 863–873.
[14] S.J. Lee, J.H. Jeong, J. Lee, et al., MicroRNA-496 inhibits triple negative breast cancer cell proliferation by targeting Del-1, Medicine 100 (14) (2021) e25270.
[15] S.H. Lee, D.Y. Kim, F. Jing, et al., Del-1 overexpression potentiates lung cancer cell proliferation and invasion, Biochem. Biophys. Res. Commun. 468 (1–2) (2015) 92–98.
[16] K. Tomczak, P. Czerwinska, M. Wiznerowicz, The Cancer Genome Atlas (TCGA): an immeasurable source of knowledge, Contemp. Oncol. 19 (1A) (2015) A68–A77.
[17] G.T. Consortium, Human genomics. The Genotype-Tissue Expression (GTEx) pilot analysis: multitissue gene regulation in humans, Science 348 (6235) (2015) 648–660.
[18] I.T.P.-C.A.o.W.G. Consortium, Pan-cancer analysis of whole genomes, Nature 578 (7793) (2020) 82–93.
[19] M.J. Goldman, B. Craft, M. Hastie, et al., Visualizing and interpreting cancer genomics data via the Xena platform, Nat. Biotechnol. 38 (6) (2020) 675–678.
[20] M. Uhlen, L. Fagerberg, B.M. Hallstrom, et al., Proteomics. Tissue-based map of the human proteome, Science 347 (6220) (2015) 1260419.
[21] M. Ghandi, F.W. Huang, J. Jane-Valbuena, et al., Next-generation characterization of the cancer cell line Encyclopedia, Nature 569 (7757) (2019) 503–508.
[22] Z. Tang, B. Kang, C. Li, et al., GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis, Nucleic Acids Res. 47 (W1) (2019) W556–W560.
[23] A.K. Akobeng, Understanding diagnostic tests 3: receiver operating characteristic curves, Acta Paediatr. 96 (5) (2007) 644–647.
[24] D.S. Chandrashekar, S.K. Karthikeyan, P.K. Korla, et al., UALCAN: an update to the integrated cancer data analysis platform, Neoplasia 25 (1) (2022) 18–27.
[25] W. Ding, J. Chen, G. Feng, et al., DNMIVD: DNA methylation interactive visualization database, Nucleic Acids Res. 48 (D1) (2020) D856–D862.
[26] D. Szklarczyk, A.L. Gable, K.C. Nastou, et al., The STRING database in 2021: customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets, Nucleic Acids Res. 49 (D1) (2021) D605–D612.
[27] R. Oughtred, J. Rust, C. Chang, et al., The BioGRID database: a comprehensive biomedical resource of curated protein, genetic, and chemical interactions, Protein Sci. : a publication of the Protein Society 30 (1) (2021) 187–200.
[28] G. Sturm, F. Finotello, M. List, Immunedeconv: an R package for unified access to computational methods for estimating immune cell fractions from bulk RNA-sequencing data, Methods Mol. Biol. 2120 (2020) 223–232.
[29] T. Li, J. Fu, Z. Zeng, et al., TIMER2.0 for analysis of tumor-infiltrating immune cells, Nucleic Acids Res. 48 (W1) (2020) W509–W514.
[30] D.J. McGrail, J. Garnett, J. Yin, et al., Proteome instability is a therapeutic vulnerability in mismatch repair-deficient cancer, Cancer Cell 37 (3) (2020) 371–386 e312.
[31] R. Bonneville, M.A. Krook, E.A. Kautto, et al., Landscape of microsatellite instability across 39 cancer types, JCO precision oncology 2017 (1) (2017) 1–15.
[32] V. Thorsson, D.L. Gibbs, S.D. Brown, et al., The immune landscape of cancer, Immunity 51 (2) (2019) 411–412.
[33] C.J. Liu, F.F. Hu, G.Y. Xie, et al., GSCA: an integrated platform for gene set cancer analysis at genomic, pharmacogenomic and immunogenomic levels, Briefings Bioinf. 24 (1) (2023) 1–10.
[34] D. Jeong, S. Ban, S. Oh, et al., Prognostic significance of EDIL3 expression and correlation with mesenchymal phenotype and microvessel density in lung adenocarcinoma, Sci. Rep. 7 (1) (2017) 8649.
[35] J.C. Sun, X.T. Liang, K. Pan, et al., High expression level of EDIL3 in HCC predicts poor prognosis of HCC patients, World J. Gastroenterol. 16 (36) (2010) 4611–4615.
[36] C. Chen, Z. Wang, Y. Ding, et al., DNA methylation: from cancer biology to clinical perspectives, Front. Biosci. 27 (12) (2022) 326.

[37] M. Kemper, A. Schiecke, H. Maar, et al., Integrin alpha-V is an important driver in pancreatic adenocarcinoma progression, J. Exp. Clin. Cancer Res. : CR 40 (1) (2021) 214.
[38] E. Brzozowska, S. Deshmukh, Integrin alpha v beta 6 (alphavbeta6) and its implications in cancer treatment, Int. J. Mol. Sci. 23 (20) (2022) 12346.
[39] J.X. Niu, W.J. Zhang, L.Y. Ye, et al., The role of adhesion molecules, alpha v beta 3, alpha v beta 5 and their ligands in the tumor cell and endothelial cell adhesion, Eur. J. Cancer Prev. : the official journal of the European Cancer Prevention Organisation 16 (6) (2007) 517–527.
[40] M. Li, D. Zhong, G. Li, Regulatory role of local tissue signal Del-1 in cancer and inflammation: a review, Cell. Mol. Biol. Lett. 26 (1) (2021) 31.
[41] A. Sheik, M.R. Farani, E. Kim, et al., Therapeutic targeting of the tumor microenvironments with cannabinoids and their analogs: update on clinical trials, Environ. Res. 231 (Pt 1) (2023) 115862.
[42] R. Zhang, W. Wang, N. Zhang, et al., Systematic pan-cancer analysis identifies RBM39 as an immunological and prognostic biomarker, J. Cell Mol. Med. 26 (18) (2022) 4859–4871.
[43] S. Tan, D. Day, S.J. Nicholls, et al., Immune checkpoint inhibitor therapy in oncology: current uses and future directions: JACC: CardioOncology state-of-the-art review, JACC. CardioOncology 4 (5) (2022) 579–597.
[44] B. Li, H.L. Chan, P. Chen, Immune checkpoint inhibitors: basics and challenges, Curr. Med. Chem. 26 (17) (2019) 3009–3025.
[45] Y. Shiravand, F. Khodadadi, S.M.A. Kashani, et al., Immune checkpoint inhibitors in cancer therapy, Curr. Oncol. 29 (5) (2022) 3044–3060.
[46] B. Ke, Z.K. Liang, B. Li, et al., EDIL3 is a potential prognostic biomarker that correlates with immune infiltrates in gastric cancer, PeerJ 11 (2023) e15559.