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RESEARCH PAPER

Elucidating the role of MICAL1 in pan-cancer using integrated bioinformatics and experimental approaches

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ABSTRACT

Molecule interacting with CasL 1 (MICAL1) is a crucial protein involved in cell motility, axon guidance, cytoskeletal dynamics, and gene transcription. This pan-cancer study analyzed MICAL1 across 33 cancer types using bioinformatics and experiments. Dysregulated expression, diagnostic potential, and prognostic value were assessed. Associations with tumor characteristics, immune factors, and drug sensitivity were explored. Enrichment analysis revealed MICAL1's involvement in metastasis, angiogenesis, metabolism, and immune pathways. Functional experiments demonstrated its impact on renal carcinoma cells. These findings position MICAL1 as a potential biomarker and therapeutic target in specific cancers, warranting further investigation into its role in cancer pathogenesis.

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Bioinformatics; immune pathways; MICAL1; pan-cancer; tumor immune microenvironment

Introduction

Cancer is a complex disease affecting millions globally. Despite advancements in treatment modalities like surgery, chemotherapy, radiotherapy, targeted therapy, and immunotherapy, patients continue to experience drug resistance, adverse effects, and other complications [1]. Therefore, identifying novel biomarkers or therapeutic targets is imperative to enhance cancer diagnosis and treatment.

MICAL1 (Molecule Interacting with CasL 1) is a protein that is encoded by the MICAL1 gene. It belongs to the MICAL (Molecule Interacting with CasL) family of proteins, which are involved in various cellular processes, including cytoskeletal organization, cell migration, and vesicle trafficking [2]. MICAL1 has been shown to possess monooxygenase activity, which is involved in the oxidation of actin filaments and microtubules, leading to cytoskeletal rearrangements [3]. MICAL1 has been implicated in the regulation of cell morphology, cell adhesion, and cell signaling. It has been shown to interact with various proteins and participate in different cellular processes. For example, MICAL1 interacts with the focal adhesion protein Paxillin and is involved in regulating focal adhesion dynamics and cell migration [4]. It also interacts with the small GTPase Rac1 and modulates its activity, which affects actin cytoskeleton remodeling [5]. Furthermore, MICAL1 has been implicated in neuronal development and synaptic plasticity. It is expressed in the developing nervous system and has been shown to play a role in neurite outgrowth and axon guidance [6]. MICAL1 has also been implicated in the regulation of dendritic spine morphology and synaptic function [7]. Overall, MICAL1 is a multifunctional protein that plays important roles in various cellular processes, including cytoskeletal organization, cell migration, and neuronal development. The multifunctional protein MICAL1 has stepped into the spotlight as an emerging force in cancer biology [8]. This dynamic protein harbors specialized domains that enable it to engage in diverse molecular interactions, allowing it to choreograph key cellular processes such as cell migration, division, and signaling [5,9]. However, when MICAL1 is overexpressed or underexpressed, it can lead to potential adverse effects. Aberrant expression of MICAL1 has been implicated in enabling cancer progression and other diseases [3,10–13]. Exciting new evidence reveals that MICAL1 is particularly adept at

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driving pancreatic cancer forward. Studies show MICAL1 is upregulated in pancreatic tumors, where it activates the WNT/β-catenin pathway, a central switchboard controlling proliferation [12]. At the same time, analyses in breast cancer models reveal that silencing MICAL1 expression disrupts the ability of cells to invade and migrate [13]. Findings from pancreatic and breast cancer research converge to indicate that MICAL1 sits at the helm of signaling pathways that steer the hallmark capabilities of cancer. Bringing MICAL1 expression into balance could therefore offer therapeutic benefits. As a dynamic protein conductor sitting at the center of a complex molecular network, MICAL1 represents an emerging target for halting cancer in its tracks.

Despite the importance of pan-cancer analysis of tumorigenesis and progression, studies exploring MICAL1's role in pan-cancer are lacking. We aimed to address this gap by comprehensively analyzing the MICAL1 expression–prognosis relationship across 33 cancer types using bioinformatics. We also investigated the correlation between MICAL1 expression, tumor immune microenvironment, and biological functions in Kidney Renal Clear Cell Carcinoma (KIRC) cell lines. Our findings suggest MICAL1 May be a potential prognostic biomarker, with its expression correlating with the tumor immune microenvironment across cancers. Additionally, our study elucidates the molecular mechanisms underlying MICAL1 functions in KIRC cells, potentially contributing to new therapeutic strategies for cancer treatment. Further studies are warranted to validate these findings and explore MICAL1's clinical utility in cancer diagnosis and treatment.

Materials and methods

Analysis of MICAL1 transcript and protein levels in pan-cancer

Data on the expression of the MICAL1 gene in tumors and their corresponding normal samples were obtained from The Cancer Genome Atlas database (TCGA, https://portal.gdc.cancer.gov/) via UCSC Xena (https://xena.ucsc.edu/) [14,15]. Clinical characterization, mutation data, and tumor stemness data for all samples were also downloaded. We analyzed MICAL1 expression differences between cancer and normal tissues across cancer types, along with expression-stage correlations. MICAL1 protein expression levels were investigated using the UALCAN (http://ualcan.path.uab.edu/analysis-prot.html) portal's CPTAC analysis module, comparing total MICAL1 protein between tumors and normal tissues across 10 cancer types – Breast invasive carcinoma (BRCA), ovarian serous cystadenocarcinoma (OV), colon adenocarcinoma (COAD), KIRC, uterine corpus endometrial carcinoma (UCEC), lung adenocarcinoma (LUAD), pancreatic adenocarcinoma (PAAD), head and neck squamous cell carcinoma (HNSC), Glioblastoma multiforme (GBM), and liver hepatocellular carcinoma (LIHC) [16].

Analysis of the diagnostic and prognostic value of MICAL1 in pan-cancer

To assess the pan-cancer diagnostic potential of MICAL1, receiver operating characteristic curves were generated and the area under the curve (AUC) determined. Higher AUC indicates greater diagnostic accuracy. AUCs below 0.7, 0.7–0.9, and above 0.9 indicate poor, moderate, and high diagnostic accuracy, respectively [17]. Kaplan–Meier analysis evaluated overall survival (OS) of TCGA cohort patients. Univariate Cox regression analyses assessed the prognostic value of MICAL1 in predicting overall survival, disease-specific survival (DSS), disease-free interval (DFI), and progression-free interval (PFI) across cancers.

Functional enrichment and gene set enrichment analyses

STRING database (https://string-db.org/) provided MICAL1-interacting proteins for protein–protein interaction network analysis [18]. Genes with similar functions to MICAL1 were predicted using GeneMANIA (http://genemania.org/) [19]. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes enrichment analyses were performed on MICAL1-associated genes from STRING and GeneMANIA via Enrichr (https://maayanlab.cloud/Enrichr/) [20]. Samples were categorized into high and low MICAL1 expression groups, and Kyoto Encyclopedia of Genes and Genomes pathway enrichment in each group was analyzed by gene set enrichment analysis (GSEA) using clusterProfiler. $p < .05$ was significant.

Correlations of MICAL1 expression with tumor mutation burden (TMB), microsatellite instability (MSI), stemness, and mismatch repair (MMR) genes

Tumor mutation burden, microsatellite instability, mRNAsi/mDNAsi stemness scores, and mismatch repair genes are important biomarkers [21]. Spearman's correlations analyzed associations between MICAL1 andTMB, MSI, mRNAsi/mDNAsi, and MMR genes.

Correlation of MICAL1 expression with the immune microenvironment

The stromal score, immune score, and ESTIMATE score for each tumor sample were calculated using ESTIMATE. The correlation between MICAL1 and immune cells was analyzed using the R package 'UCSCXenaShiny' [22]. Tumor immune regulation is closely associated with immune checkpoint genes. The relationship between immune checkpoint gene expression and MICAL1 expression was analyzed. Additionally, the correlation between MICAL1 and immune regulatory genes was investigated.

Drug sensitivity analysis

To investigate the impact of MICAL1 on drug sensitivity, gene expression and drug sensitivity data were obtained from CellMiner (https://discover.nci.nih.gov/cellminer/) [23]. Only Food and Drug Administration (FDA)-approved drugs were included. Spearman's correlation analysis was performed with a p-value cutoff of 0.05.

Cell lines and cell culture

The RCC cell lines ACHN (Adenocarcinoma of the Human Kidney), 786-O (786-O Renal Cell Carcinoma) and Caki-1 (Caki-1 Renal Cell Carcinoma), and normal kidney cell line HK-2 (Human Kidney-2) (ATCC) were cultured at 37°C with 5% CO2. ACHN and 786-O were grown in RPMI 1640 (Gibco) while Caki-1 was cultured in McCoy's 5A medium (Gibco). HK-2 was maintained in DMEM/F12 (Gibco). All media were supplemented with 10% fetal bovine serum (FBS) (Hyclone) and 1% penicillin-streptomycin.

Small interfering RNA (siRNA) transfection and lentivirus transfection

Small interfering RNAs (siRNAs) targeting MICAL1 and non-targeting negative controls (NC) were obtained from Shanghai GenePharma Co., Ltd (GenePharma) and transfected into Clear Cell Renal Cell Carcinoma (ccRCC) cells using Lipofectamine 3000 (Invitrogen) following the manufacturer's instructions. The MICAL1 siRNA target sequences are provided in Additional file 1: Tables S1. For lentivirus transfection, lentivirus-mediated MICAL1 overexpression and negative control vector were transfected with Lipofectamine 3000 (Invitrogen).

RNA isolation and quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was isolated from ccRCC cell lines using the RNA-easy isolation reagent (Thermo Fisher Scientific) and reverse transcribed into cDNA using the PrimeScript RT Master Mix (Takara Bio, Inc) per the manufacturer's protocol. Quantitative real-time PCR was performed to examine gene expression using the Taq Pro Universal SYBR qPCR Master Mix (Vazyme, Q712–02). For normalization, β-actin was utilized as an internal control and the 2−ΔΔCt method was used to analyze the relative expression levels of target genes. Primer sequences are provided in Additional file 1: Tables S2.

Cell proliferation and transwell assays

Cell proliferation was measured by CellTiter 96 Aqueous One Solution Cell Proliferation Assay (Promega). 1 × 103 cells per well were seeded in 96-well plates and treated with assay solution. Absorbance at 490 nm was read every 24 h for 5 d using a SpectraMax M5 plate reader (Molecular Devices) [24]. Transwell migration assays were performed using transwell inserts (Corning). 1 × 105 cells were seeded in serum-free medium in upper chambers. After 24 h, migrated cells in lower chambers were fixed with methanol, stained with crystal violet, and counted in 5 random fields under a microscope [25].

Results

MICAL1 Expression Analysis in pan-cancer

Firstly, we analyzed MICAL1 expression levels across various cancers, ranking them from lowest to highest (Figure 1a). MICAL1 was expressed in all tumors, with the highest levels observed in acute myeloid leukemia (LAML) and the lowest levels seen in adrenocortical carcinoma (ACC). We then evaluated MICAL1 expression in TCGA pan-cancer. The results revealed elevated MICAL1 expression in 11 tumor types: BRCA, cholangiocarcinoma (CHOL), COAD, HNSC, KIRC, Kidney renal papillary cell carcinoma (KIRP), LIHC, pheochromocytoma and paraganglioma (PCPG), rectum adenocarcinoma (READ), Stomach adenocarcinoma (STAD), and Thyroid carcinoma (THCA). In contrast, reduced MICAL1 expression was observed in six tumor types: bladder urothelial carcinoma (BLCA), kidney chromophobe (KICH), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), PAAD, and UCEC (Figure 1b). In the examination on the tumor stage relevance, we discovered that lower MICAL1

expression was associated with more advanced stages in KIRP, LUAD, and TGCT. Conversely, higher MICAL1 expression was linked to later stages in KIRC (Figure 1c). Finally, we investigated MICAL1 protein expression patterns across normal tissues and cancers using the UALCAN database. The results illustrated significantly elevated MICAL1 protein levels in BRCA, KIRC, PAAD, HNSC, GBM, and LIHC tissues compared to matched normal tissues. In contrast, OV, UCEC, and LUAD tissues displayed markedly reduced MICAL1 protein levels relative to corresponding normal tissues (Figure 1d). In summary, these analyses of multiple datasets revealed heterogeneous MICAL1 expression patterns across cancers. MICAL1 levels differed between cancer types and correlated with tumor stage in certain cancers. This suggests MICAL1 May play varied roles in different malignancies.

Diagnostic and prognostic significance of MICAL1 in pan-cancer

Taking into account the distinctive expression pattern of MICAL1 in diverse cancer types, our endeavor was to explore the diagnostic and prognostic significance of MICAL1 across these malignancies. ROC curve analyses were conducted to evaluate the diagnostic potential of MICAL1 in TCGA pan-cancer. ROC curves demonstrated high diagnostic value for MICAL1 in KIRP (AUC = 0.902), KIRC (AUC = 0.926), CHOL (AUC = 0.994), and PCPG (AUC = 0.996) (Figure 1e). We further evaluated the prognostic value of MICAL1 expression in cancer patients. Univariate Cox regression analyses revealed that high MICAL1 expression was associated with favorable OS in PAAD, LUAD, BRCA, and HNSC. In contrast, high MICAL1 expression was associated with poor OS in KIRC, ACC, uveal melanoma (UVM), mesothelioma (MESO), and LAML (Figure 2a). For DSS, high MICAL1 expression was prognostic of better outcome in PAAD, KIRP, PRAD, and LUAD, but worse outcome in KIRC, ACC, UVM, and MESO (Figure 2b). Regarding DFI, high MICAL1 expression was associated with favorable DFI in PAAD and PCPG, but poorer DFI in COAD (Figure 2c). For PFS, high MICAL1 predicted better PFI in PAAD, CHOL, and BLCA, but worse PFI in ACC, KIRC, and UVM (Figure 2d). Kaplan–Meier analyses further revealed that high MICAL1 expression was correlated with inferior OS in LAML, ACC, KIRC, MESO, and UVM, but superior OS in LUAD, BRCA, PAAD, and HNSC (Figure 3). High MICAL1 expression also associated with shorter PFI in ACC, MESO, COAD, LUSC, and READ (Figure 4a–e), but longer PFI in PAAD (Figure 4f). Regarding DSS, elevated MICAL1 predicted poor outcome in ACC, KIRC, and MESO (Figure 4g–i), while low expression predicted poor survival in PAAD (Figure 4j). Finally, high MICAL1 expression associated with worse DFI in COAD (Figure 4k).

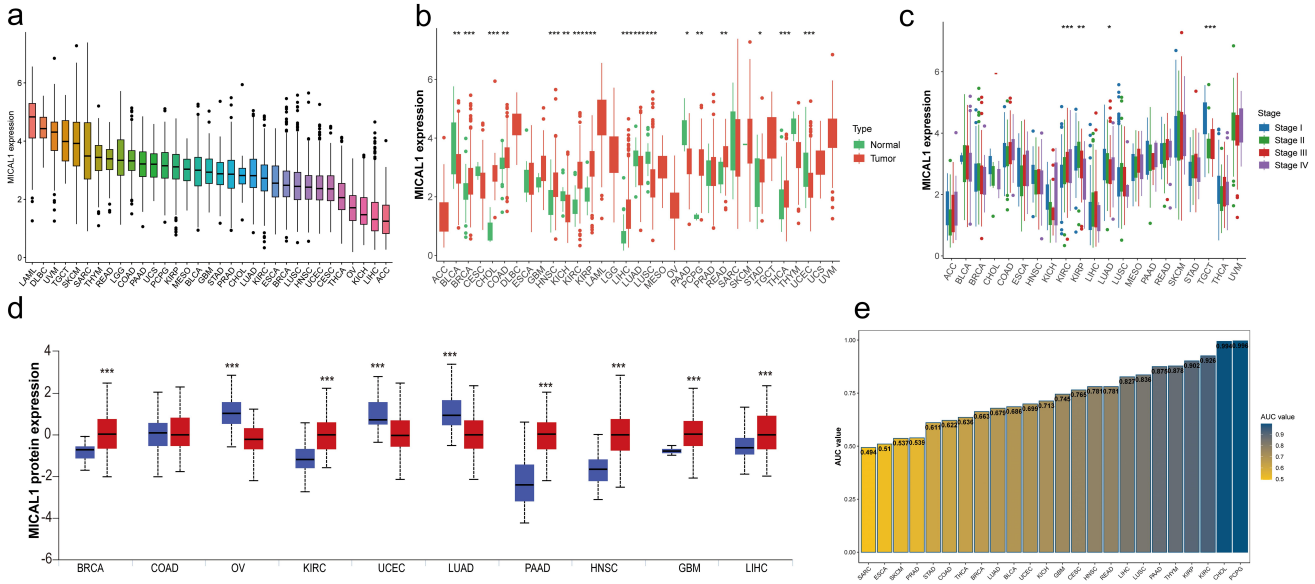

Figure 1. MICAL1 expression and diagnostic value in pan-cancer. (a) MICAL1 mRNA expression ranked from high to low across multiple cancer types; (b) MICAL1 mRNA expression in tumor vs normal tissues for selected cancer types; (c) MICAL1 mRNA expression by cancer stage for selected cancer types; (d) ROC curve analysis of MICAL1 expression for distinguishing tumor from normal tissues; (e) MICAL1 protein expression in tumor vs normal tissues for selected cancer types. ***$p < .001$, **$p < .01$, *$p < .05$.

Gene ontology (GO), Kyoto Encyclopedia of Genes and genomes (KEGG) and GSEA pathway analyses

The protein–protein interaction networks of MICAL1 were visualized using the STRING online database (Figure 5a). The MICAL1 associated genes were identified with the GeneMANIA online database (Figure 5b). The GO and KEGG pathway enrichment analysis revealed that MICAL1 and its functional partners are involved in various biological processes, cellular components, molecular functions, and signaling pathways relevant to cancer and immunity (Figure 5c–f). These results suggest MICAL1 plays a crucial role in regulating cell adhesion, cell motility, and axon guidance. It also participates in modulating focal adhesions and the cytoskeleton, and possesses GTP binding and GTPase activity. Furthermore, MICAL1 is implicated in key signaling pathways including Fc gamma R-mediated phagocytosis, tight junctions, and AMPK signaling, which are vital for cancer and immunity. Gene set enrichment analysis using the differentially expressed genes between low- and high-MICAL1 subgroups in each cancer type highlighted MICAL1-associated cancer hallmarks. We found MICAL1 expression was highly relevant to immune-related pathways such as antigen processing and presentation, cytokine-cytokine receptor interaction, chemokine signaling, complement and coagulation cascades, Fc gamma R-mediated phagocytosis, hematopoietic cell lineage, intestinal immune network for IgA production, JAK-STAT signaling, natural killer cell cytotoxicity, NOD-like receptor signaling, primary immunodeficiency, RIG-I-like receptor signaling, T cell receptor signaling, Toll-like receptor signaling, and type I diabetes mellitus (Figure 5g). These results indicate MICAL1 May have

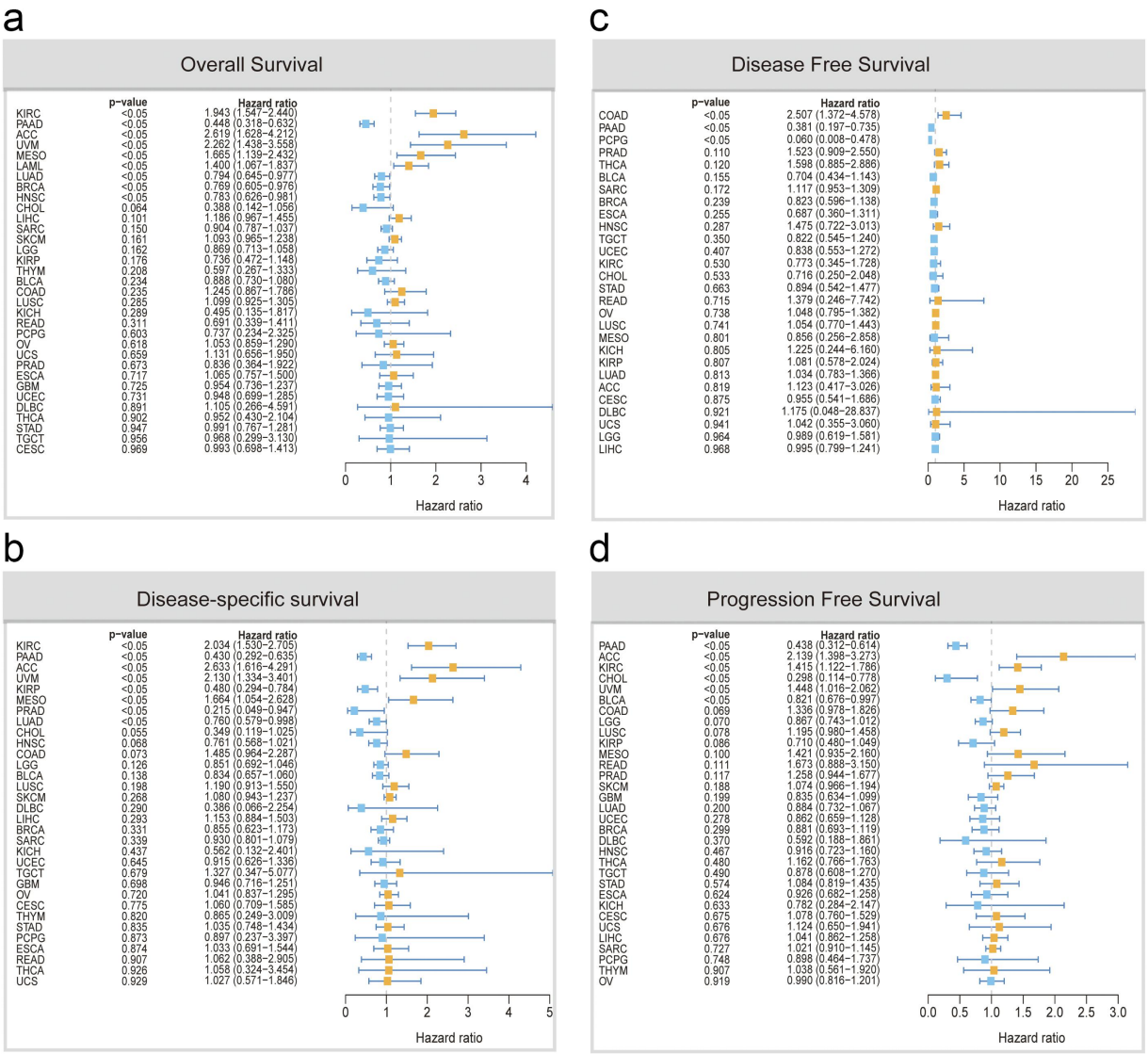

Figure 2. Association between MICAL1 expression and survival outcomes. (a) Overall survival; (b) disease-specific survival; (c) disease-free survival; (d) progression-free survival.

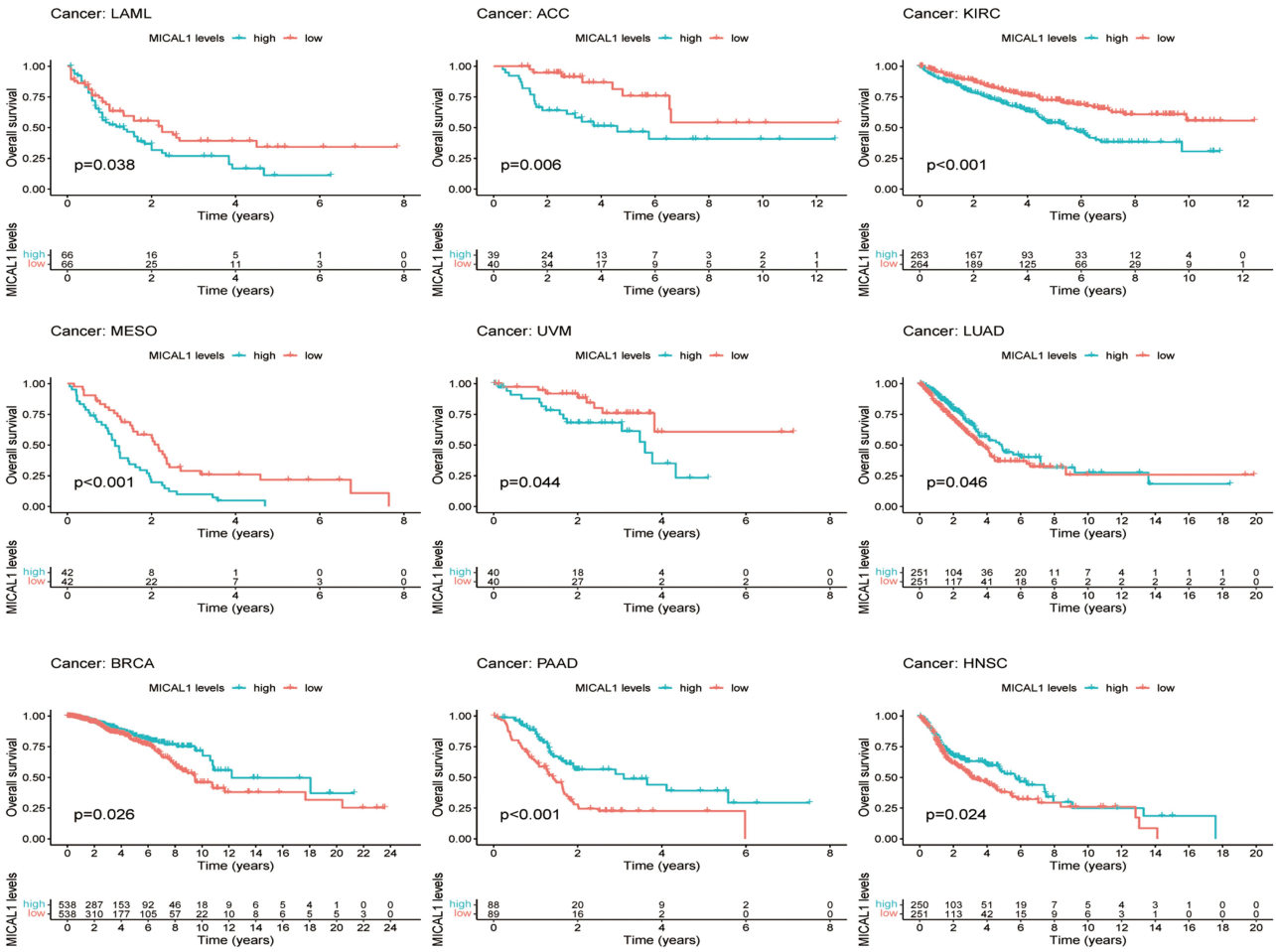

Figure 3. Kaplan-Meier curves showing overall survival of cancer patients stratified by MICAL1 expression levels. In LAML, ACC, KIRC, MESO, and UVM patients, high MICAL1 expression was associated with significantly worse overall survival compared to low expression. In LUAD, BRCA, PAAD and HNSC patients, low MICAL1 expression was associated with significantly worse overall survival compared to high expression.

a critical role in cancer progression and immune response modulation.

MICAL1 expression is related to TMB, MSI, tumor stemness and mismatch repair

TMB and MSI within the tumor microenvironment are associated with anti-tumor immunity and can predict efficacy of immunotherapy. Our analysis revealed that MICAL1 expression was significantly inversely correlated with MSI status in COAD, ACC, THYM, TGCT, STAD, OV. In contrast, MICAL1 expression positively correlated with MSI status in HNSC, LUAD, LUSC, PRAD, BRCA, CESC, THCA (Figure 6a). Regarding TMB, we found that MICAL1 expression positively correlated with TMB in BRCA, CESC, THCA, PRAD, LUAD, LUSC, HNSC. In contrast, MICAL1 expression was inversely correlated with TMB in OV, STAD, TGCT, THYM, ACC, COAD ((Figure 6b). Stemness acquisition is a major driver of tumor progression, with stemness index reflecting the extent of similarity between tumor cells and stem cells. Our analysis revealed that MICAL1 expression positively correlated with DNA stemness score in TGCT, PCPG, THCA, BRCA and PRAD. In contrast, MICAL1 expression was inversely correlated with DNA stemness score in THYM, KICH, DLBC, LUSC, CESC, Sarcoma (SARC), UCEC, PAAD, HNSC, BLCA, and LGG ((Figure 6c). With regard to RNA stemness score, we found that MICAL1 expression positively correlated with this metric in THYM, SKCM, SARC, LGG. In contrast, MICAL1 expression was inversely correlated with RNA stemness score in BRCA, COAD, UCEC, HNSC, LAML, KIRC, BLCA, CESC, THCA, LUAD, OV, UVM, PRAD, DLBC, LUSC, KICH (Figure 6d). Mismatch repair (MMR) genes

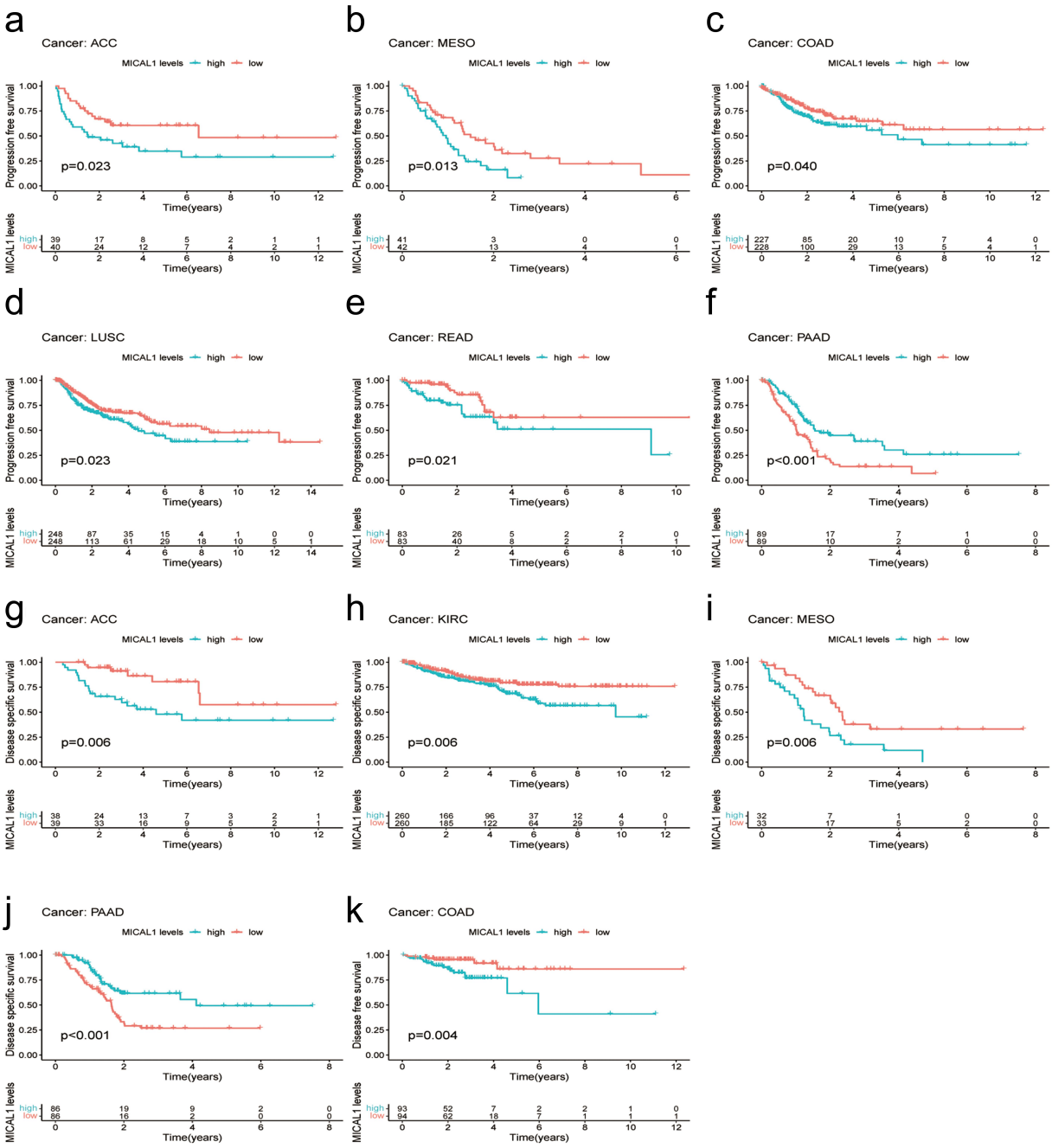

Figure 4. MICAL1 expression is associated with survival outcomes across multiple cancer types. (a-f) Kaplan-Meier curves of progression free survival based on MICAL1 expression levels in ACC, MESO, COAD, LUSC, READ and PAAD;(g-j) Kaplan-Meier curves of disease specific survival based on MICAL1 expression levels in ACC, KIRC, MESO, PAAD; (h) Kaplan-Meier curves of disease-free survival based on MICAL1 expression levels in COAD.

maintain genomic integrity by recognizing and correcting DNA replication errors involving mismatched nucleotide bases. We analyzed the association between MICAL1 expression and five key MMR genes in pan-cancer. As shown in Figure 6e, MICAL1 expression significantly correlated with MMR in all but ACC and DLBC. Overall, the analysis showed that MICAL1 expression is correlated with various factors in the tumor microenvironment, including MSI status, TMB, stemness index, and MMR genes.

Correlation between MICAL1 expression and tumor microenvironment features in diverse cancers

In order to gain a deeper understanding of MICAL1's involvement in the tumor microenvironment across various cancer types, we investigated its relationship

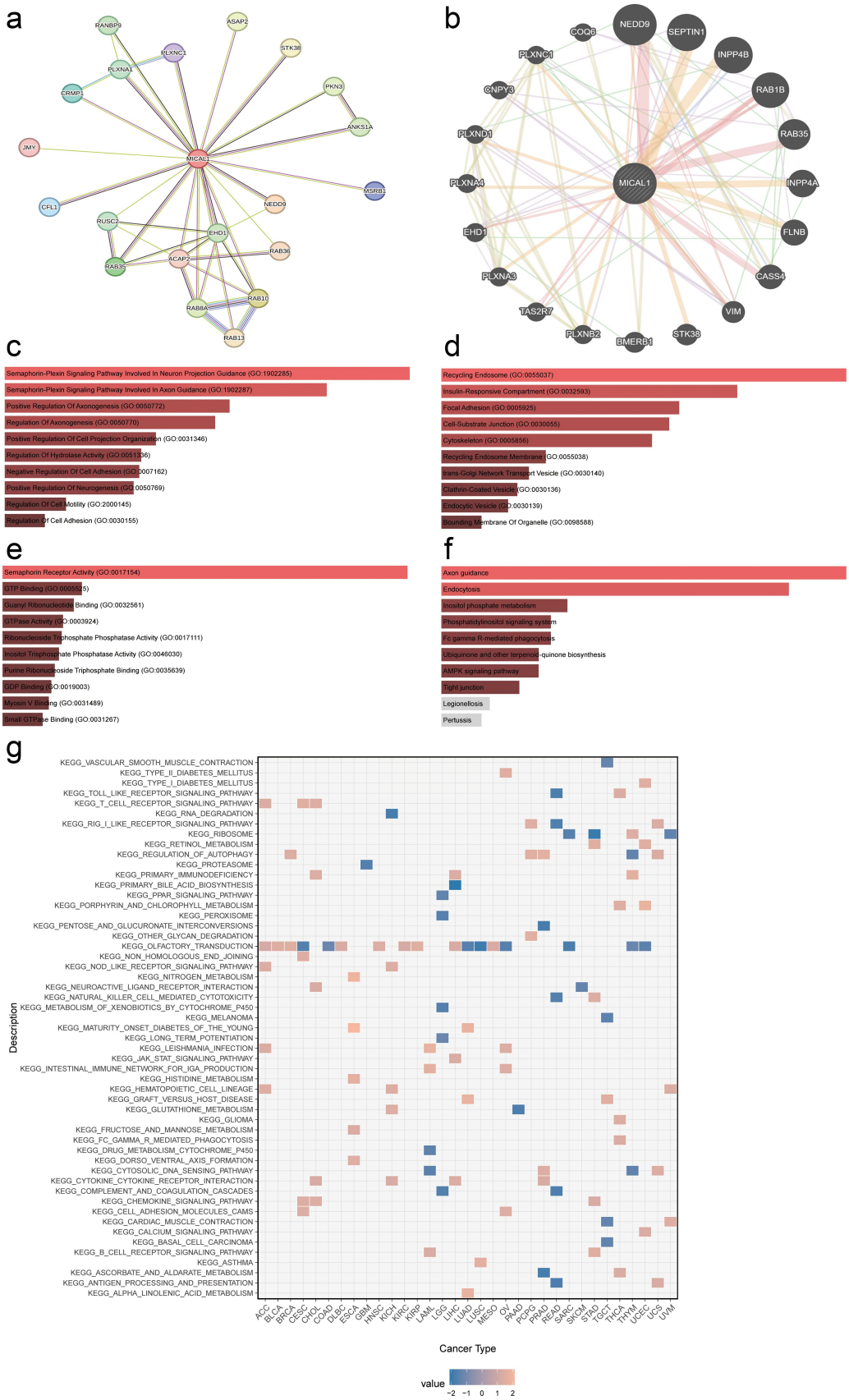

Figure 5. MICAL1 expression is associated with genomic and transcriptomic instability across cancer types. (a) Association between MICAL1 expression and microsatellite instability; (b) association between MICAL1 expression and tumor mutation burden; (c) association between MICAL1 expression and DNA stability scores; (d) association between MICAL1 expression and RNA stability scores; (e) correlations between MICAL1 expression and mismatch repair genes. ***$p < .001$, **$p < .01$, *$p < .05$.

Figure 6. Functional analysis of MICAL1 in various cancers (a) the protein – protein interaction network of MICAL1 from STRING; (b) the gene–gene interaction network of MICAL1 from GeneMANIA; (c) biological process (BP) functional analysis of MICAL1 and its functional partners; (d) cellular components (CC) functional analysis of MICAL1 and its functional partners; (e) molecular function (MF) functional analysis of MICAL1 and its functional partners; (f) Kyoto Encyclopedia of Genes and genomes (KEGG) pathway analysis of MICAL1 and its functional partners; (g) gene Set Enrichment analysis of MICAL1 in pan-caner.

with tumor microenvironment (TME) scores, immune cell infiltration, and immunosuppressive molecules. TME is a complex system of various cell types. To assess the correlation between MICAL1 and TME features, we analyzed stromal, immune, and ESTIMATE scores across cancers. MICAL1 expression significantly correlated with these scores in most cancer types (Figure 7a–c). In KICH, MICAL1 expression levels positively correlated with stromal/immunity scores, while in SARC, MICAL1 expression levels negatively correlated with such tumor microenvironment components. To understand relationships between immune cells and MICAL1 in various tumors, abundance of 22 immune cell types was calculated. As depicted in Figure 7d, MICAL1 expression exhibited variable correlations with immune cell types across different cancers. Correlations between MICAL1 and immune checkpoints varied among cancer types. For instance, in LIHC, MICAL1 positively correlated with most checkpoints, whereas in COAD, MICAL1 negatively correlated or did not correlate with the checkpoints (Figure 7e).

Drug sensitivity analysis

MICAL1 expression correlated with sensitivity to 13 drugs in CellMiner (Figure 8). Positive correlations were seen with several agents including okadaic acid ($r = 0.368$, $p = .004$), rebimastat ($r = 0.363$, $p = .004$), ABT-199 ($r = 0.314$, $p = .015$), vemurafenib ($r = 0.293$, $p = .023$), megestrol acetate ($r = 0.293$, $p = .023$), temsirolimus ($r = 0.277$, $p = .032$), carboplatin ($r = 0.276$, $p = .033$), calusterone ($r = 0.274$, $p = .034$), ixazomib citrate ($r = 0.264$, $p = .042$), wortmannin ($r = 0.257$, $p = .048$), and PD-98059 ($r = 0.257$, $p = .048$). A significant negative correlation was evident between MICAL1 and agents such as the byproduct of CUDC-305 ($r = -0.372$, $p = .003$) and docetaxel ($r = -0.318$, $p = .013$). These findings indicate a potential role for MICAL1 in modulating response to specific treatments and warrant further investigation.

MICAL1 knockdown inhibited the malignant behaviors in ccRCC cells

Using qRT-PCR, we found that MICAL1 was expressed in all four ccRCC cell lines tested (Figure 9a). We observed relatively higher expression of MICAL1 in ACHN, CAKI-1, and 786-O cells compared to HK cells. To determine the role of MICAL1 in ccRCC cell proliferation and migration, we utilized two siRNAs to knock down MICAL1 expression in ACHN, CAKI-1, and 786-O cells and measured knockdown efficiency using qRT-PCR (Figure 9b–d). Subsequently, we performed Cell Counting Kit-8 (CCK8) assays to evaluate cell proliferation. The results demonstrated that MICAL1 knockdown significantly inhibited the proliferation rates of ACHN, CAKI-1, and 786-O cells (Figure 9e–g). Additionally, MICAL1 silencing substantially impaired the migratory ability of ccRCC cells as evidenced by Transwell migration assays (Figure 9h,i).

MICAL1 overexpression promoted aggressive behaviors in ccRCC cells

To further explore the pivotal role of MICAL1 in ccRCC, CAKI-1 cells were transfected with an MICAL1 overexpression vector alongside a negative control. Following transfection with the MICAL1 overexpression vector, a notable upregulation of MICAL1 expression was observed in CAKI-1 cells (Figure 10a). Cell proliferation assay revealed a significant augmentation in the proliferative capacity of Caki-1 upon MICAL1 overexpression (Figure 10b). Transwell assay demonstrated a marked increase in the migratory cell population in MICAL1-overexpressing CAKI-1 cells compared to the negative control (Figure 10c,d).

Discussion

In this study, we analyzed MICAL1's expression, prognostic value, and potential functional role across diverse cancer types and immune infiltration statuses by comprehensive bioinformatics analyses. Our results demonstrate MICAL1 is differentially expressed in tumors compared to normal tissues, with elevated expression in 11 cancers, including BRCA, CHOL, COAD, HNSC, KIRC, KIRP, LIHC, PCPG, READ, STAD, and THCA, and reduced expression in 6 cancers, including BLCA, KICH, LUAD, LUSC, PAAD, and UCEC. The aberrant expression patterns suggest MICAL1 dysregulation may promote tumorigenesis. Interestingly, we found MICAL1's prognostic value varies among cancers. High MICAL1 expression associated with favorable prognosis in certain cancers like LUAD, BRCA, PAAD, and HNSC, yet with poorer prognosis in other cancers including LAML, ACC, KIRC, MESO, and UVM. Overall, we found MICAL1 upregulated in KIRC versus normal tissue and associated with poor survival, implying it likely serves as an oncogene therein. Conversely, in PAAD and LUAD, MICAL1 expression was downregulated in tumors compared to normal tissue and high expression

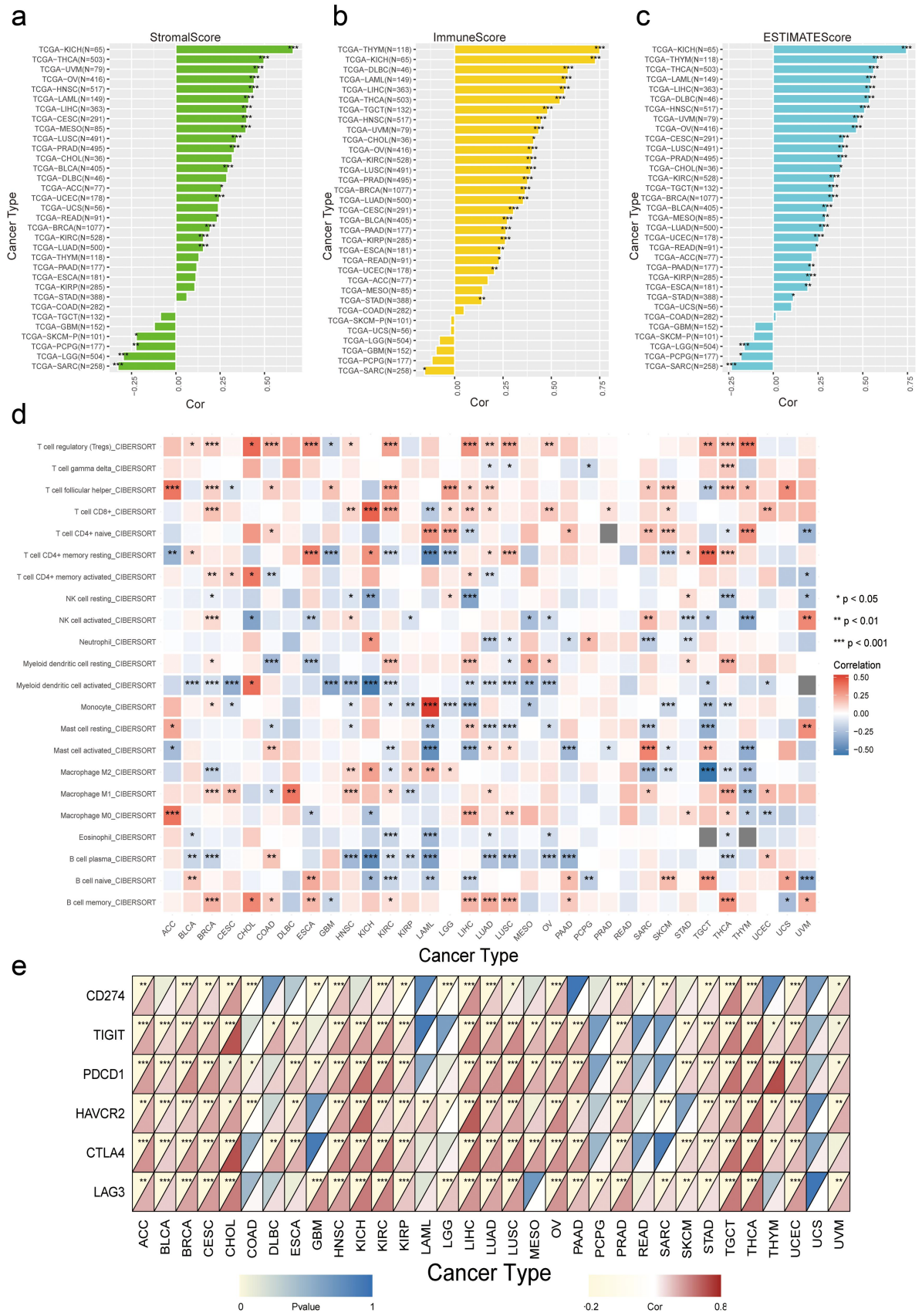

Figure 7. Correlation of MICAL1 expression with immune infiltration and immunosuppression across different cancer types. (a-c) correlation between MICAL1 expression and stromal score, immune score, and ESTIMATE score levels based on ESTIMATE algorithm; (d) correlation between MICAL1 expression and immune cell fractions estimated by CIBERSORT algorithm; (e) correlation between MICAL1 expression and expression of immunosuppressive molecules including LAG3, CTLA4, HAVCR2, PDCD1, TIGIT, and CD274. ***$p < .001$, **$p < .01$, *$p < .05$.

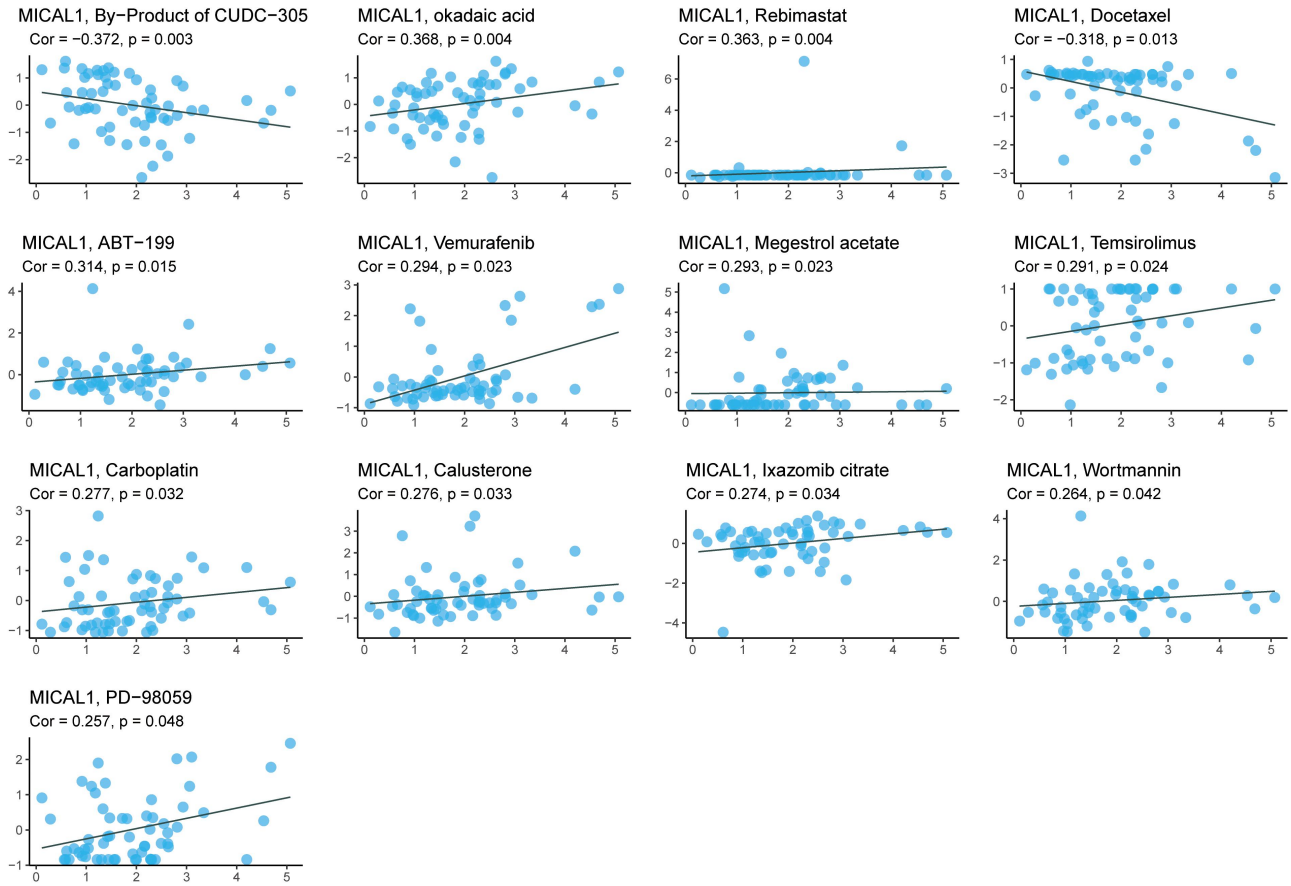

Figure 8. Correlation between MICAL1 expression and drug response.

predicted improved survival, suggesting it may function as a tumor suppressor in these cancers. The above analysis indicates MICAL1 May play distinct context-dependent roles across different cancers.

Our analysis revealed intriguing connections between MICAL1 expression and TMB, MSI, cancer stemness, and MMR. We found that MICAL1 expression correlated both positively and negatively with TMB and MSI status depending on the cancer type. Higher MICAL1 expression associated with increased TMB and MSI in breast, cervical, head and neck, lung, prostate, and thyroid cancers. In contrast, MICAL1 expression inversely correlated with TMB and MSI in ovarian, stomach, testicular, thymoma, adrenocortical, and colorectal cancers. TMB and MSI promote immunogenicity, thereby improving response to immune checkpoint inhibitors [26]. The variable correlations suggest complex interplay between MICAL1, TMB, MSI, and anti-tumor immunity in different contexts. Regarding cancer stemness, MICAL1 expression also exhibited bidirectional correlations with mRNA and DNA stemness indices across cancers. Positive associations occurred in prostate, thyroid, breast, and skin cancers for DNA stemness and in glioma, sarcoma, and skin cancers for RNA stemness. Negative correlations were evident in bladder, cervical, colorectal, head and neck, lung, ovarian, and uterine cancers for DNA stemness and in bladder, breast, colorectal, head and neck, leukemia, ovarian, uterine cancers for RNA stemness. These results position MICAL1 as a regulator of stemness and differentiation states in cancer cells. We also uncovered widespread correlations between MICAL1 expression and MMR genes, suggesting MICAL1's involvement in maintaining genomic integrity. In summary, our analysis reveals that MICAL1 expression correlates variably with major determinants of immunogenicity and genomic integrity, including TMB, MSI, stemness, and MMR. The cancer-specific nature of these associations points to complex, context-dependent interplay between MICAL1 and the tumor immune microenvironment. By influencing TMB, MSI, differentiation, and DNA repair, MICAL1 May regulate anti-tumor immune responses.

GO, KEGG and GSEA analyses provide critical insights into the biological processes, molecular functions, signaling pathways, and gene programs associated with MICAL1 in cancer. GO analysis demonstrates MICAL1's inextricable links to cell

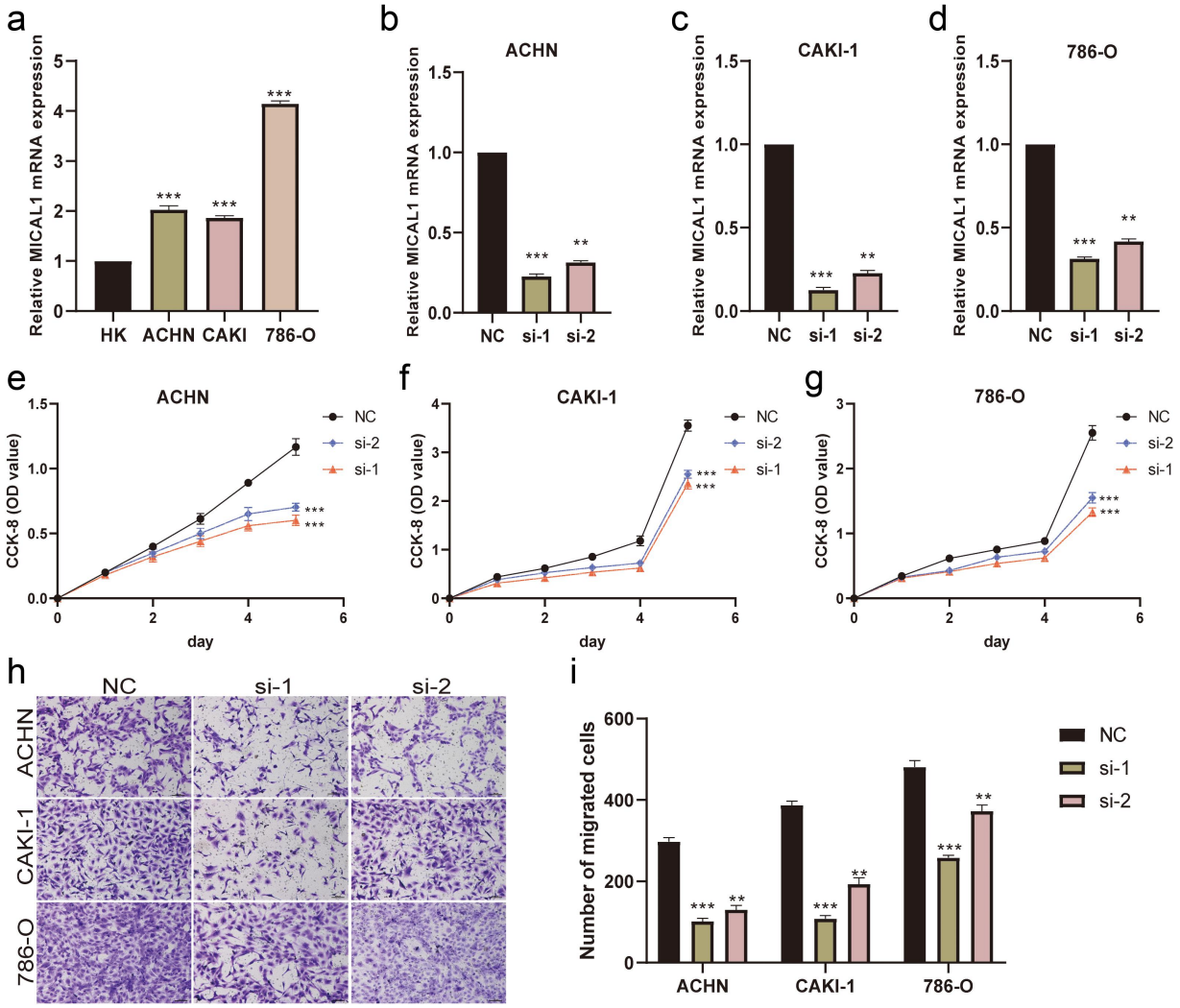

Figure 9. MICAL1 knockdown suppressed the proliferation and migration in clear cell renal cell carcinoma cell lines. (a) MICAL1 expression increases in clear cell renal cell carcinoma cell lines; (b-d) qRT-PCR results verified the knockdown effect of MICAL gene mediated by siRnas in ACHN, CAKI-1 and 786-O cell lines, respectively; (e-g) CCK-8 assay results showed that knockdown of MICAL1 gene inhibited the proliferative ability of ACHN, CAKI-1 and 786-O cell lines;(G-I) the migration number of ACHN, CAKI-1 and 786-O cells significantly decreased after MICAL1 knockdown. ***$p < .001$, **$p < .01$.

adhesion, motility, cytoskeletal organization, and GTPase activity – key cellular processes dysregulated in metastatic progression. KEGG analysis further reveals MICAL1's involvement in pathways highly relevant to cancer pathogenesis and the tumor microenvironment, including focal adhesion, actin regulation, AMPK signaling, phagocytosis, and tight junctions [27–31]. The focal adhesion and cytoskeletal pathways confirm MICAL1's adhesion/motor functions [32]. AMPK and phagocytosis signaling modulate metabolism and immune cell function, respectively, while the tight junction pathway governs metastasis-related permeability and polarity [33,34]. Most intriguingly, enrichment analysis showed significant associations between MICAL1 expression and diverse immune-related features across cancer types. Tumors with high MICAL1 levels were enriched for antigen presentation, chemokines, complement, natural killer cells, T/B-cell signaling, and Toll-like receptor pathways – implying that MICAL1 levels substantially impact anti-tumor immunity [35]. This enrichment analysis strongly supports an integral role for MICAL1 in the numerous biological processes and signaling cascades driving tumor progression and shaping anti-tumor immunity. MICAL1 emerges as a key link between cytoskeletal dynamics, adhesion, motility, and invasion with metabolism, immune function, and intracellular signaling.

Unraveling the intricate interplay between tumor cells and the surrounding microenvironment is imperative for deciphering cancer pathogenesis and designing optimal therapies [36]. Our pan-cancer analyses provide novel evidence that MICAL1 expression

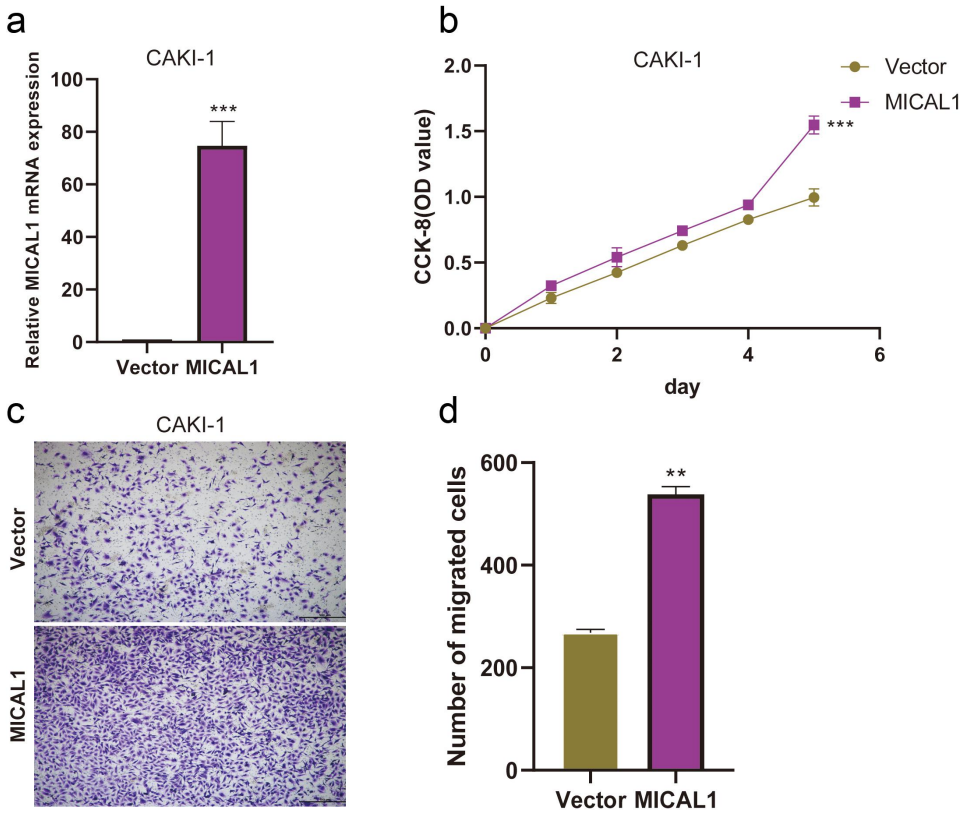

Figure 10. MICAL1 overexpression promoted the proliferation and migration in clear cell renal cell carcinoma in vitro. (A) qRT-PCR of MICAL1 mRNA expression in vector-control cells (vector) and MICAL1-overexpressing cells (MICAL1); (B) MICAL1 overexpression significantly enhanced the proliferative activity as evidenced using CCK-8 assay. (C-D) transwell migration assay revealed that MICAL1 overexpression increased the cell numbers of migration. ***$p < .001$, **$p < .01$.

extensively correlates with major compositional and functional attributes of the tumor microenvironment across diverse malignancies. We discovered a significant positive correlation between MICAL1 expression and stromal scores in most cancers, except SARC. The stromal score reflects the presence of non-malignant cells in the tumor microenvironment including immune cells, fibroblasts, and endothelial cells [37]. This suggests elevated MICAL1 associates with an enriched stromal component, which can modulate cancer progression either through pro-tumorigenic inflammation or anti-tumor immunity. Additionally, we found positive correlations between MICAL1 levels and immune scores in many cancer types, except SARC. The immune score denotes the infiltration of immune cell subpopulations [38]. Our results indicate heightened MICAL1 expression corresponds to intensified immune infiltration, implying it may be linked to cancer immunity. However, the specific immune contexture elicited by MICAL1 remains to be elucidated. Further analysis revealed variable correlations between MICAL1 and distinct immune cell types and immune checkpoints across cancers. This cancer-specific connection suggests the immunomodulatory functions of MICAL1 May be context-dependent, either synergizing with or antagonizing immune pathways.

Our analysis provides novel insights into associations between MICAL1 expression and drug sensitivity across cancer cell lines. We identified significant correlations between MICAL1 levels and 13 anti-cancer agents, including both positive and negative associations. The negative correlations with microtubule-targeting agents like docetaxel suggest that MICAL1 expression may modulate cytoskeletal dynamics in a manner that impacts sensitivity to these drugs [39]. This aligns with MICAL1's known role in actin disassembly and interactions with microtubules. Intriguingly, we also found positive correlations between MICAL1 and targeted therapies like vemurafenib and ABT-199 that inhibit oncogenic signaling pathways in melanomas and certain hematological malignancies [40,41]. It will be important to determine if higher MICAL1 levels enhance dependence on these pathways, thereby increasing vulnerability to agents like vemurafenib that suppress them.

The cell experiments in this study provide direct evidence further elucidating the tumor-promoting role of MICAL1 in ccRCC. We first confirmed the high expression of MICAL1 across multiple ccRCC cell lines using qRT-PCR. This is consistent with our bioinformatics analysis results, which found high MICAL1 expression associated with poor prognosis in KIRC patients. We endeavored to suppress the expression of MICAL1 and observed a substantial diminishment in the proliferative and migratory capacities of ccRCC cells. Conversely, heightened expression led to a notable escalation in both proliferation and migratory prowess. This indicates MICAL1 May exert its pro-cancer effects by promoting tumor cell proliferation and metastasis. More importantly, these findings uncover for the first time that MICAL1 is a potential oncogenic driver and therapeutic target in ccRCC.

Our study has identified two major limitations that should be acknowledged. First, the in vitro evaluation was limited to a single cancer type, which may restrict the generalizability of our findings. While our research provides valuable insights into the dysregulated expression patterns and potential functional implications of MICAL1 in that specific cancer type, it remains essential to investigate MICAL1 in multiple cancer types. This broader evaluation will help determine whether the dysregulation of MICAL1 is a common phenomenon across various malignancies or if it is specific to certain types of cancer. Second, the lack of further mechanistic studies is another limitation of our study. Although our research offers computational evidence for the potential functional impact of MICAL1 dysregulation, it is crucial to conduct additional mechanistic studies to elucidate the underlying molecular mechanisms. These studies would provide a more comprehensive understanding of how MICAL1 influences cellular processes and contributes to cancer development and progression.

In summary, this preliminary study provides crucial discoveries regarding MICAL1's expression patterns, clinical significance, and biological functions in cancer. Our integrated approach yielded important insights, but further validation is required before considering clinical translation of MICAL1 as a cancer biomarker or therapeutic target. Detailed elucidation of its tissue-specific roles could ultimately allow leveraging MICAL1 for personalized cancer management.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

The TCGA belongs to public database. The patients involved in the database have obtained ethical approval. Users can download relevant data for free for research and publish relevant articles. Our study is based on open-source data, so there are no ethical issues and other conflicts of interest.

Ethical approval and consent to participate

All of the animal care and experimental procedures were approved by the Institutional Animal Care and Use Committee of Sun Yat-sen University. The methods were carried out in accordance to the approved guidelines.

Author contributions

Canxuan Li, Yunfei Xiao, Jianqiu Kong, Zhuohang Li, and Weibin Xie designed the research. Cong Lai and Zhiliang Chen performed the research and collected the data. Canxuan Li, Yunfei Xiao, and Jianqiu Kong analyzed the data. Canxuan Li drafted the manuscript. All authors contributed to the article and approved the submitted version.

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