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Original Article

MORC2 facilitates cholangiocarcinoma progression through cell cycle acceleration and immune microenvironment modification

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Abstract

This study explored a novel therapeutic target, MORC2 (Microrchidia family CW-type zinc finger 2), for patients with unresectable advanced Cholangiocarcinoma (CCA), a lethal epithelial cell malignancy lacking effective treatments. Utilizing bioinformatics analysis, we examined MORC2's role in CCA progression. The focus was on its association with the cell cycle and its involvement in the tumor's immunosuppressive microenvironment. MORC2 was found to accelerate CCA cell proliferation by promoting cell cycle progression through the activation of TNF-α signaling via the NFKB signaling pathway. Furthermore, the downregulation of MORC2 induced cell cycle arrest and might facilitate neutrophil infiltration by upregulating CCL3, indicating its pivotal role in modifying the immunosuppressive tumor microenvironment. Our findings suggest that MORC2 plays a crucial role in both the proliferation of CCA cells and the modification of the tumor microenvironment. Targeting MORC2 presents a novel potential therapeutic approach for patients with advanced CCA.

Keywords: **C**holangiocarcinoma, MORC2, **C**ell cycle regulation, **T**umor immune microenvironment, CCL3

1. Introduction

Cholangiocarcinoma (CCA) is a highly lethal epithelial cell malignancy categorized into intrahepatic CCA (iCCA), perihilar CCA (pCCA), and distal CCA (dCCA) based on anatomical location[1]. CCA ranks as the second most prevalent primary liver cancer after hepatocellular carcinoma (HCC), comprising approximately 15% of all primary liver cancers[2]. Globally, the incidence and mortality rates of CCA continue to rise, predominantly attributed to iCCA[2, 3]. Well-established risk factors for CCA include chronic hepatitis B and C infections, cirrhosis, primary sclerosing cholangitis, and choledocholithiasis[4-6]. Additionally, non-alcoholic fatty liver disease and metabolic syndrome contribute to an escalating risk of CCA in Western nations[7, 8].

The majority of CCAs manifest as well, as moderately, or poorly differentiated adenocarcinomas[9, 10]. Early-stage CCA patients may qualify for resection or liver transplantation[11], yet most cases are diagnosed in advanced stages[12]. Identifying an ideal biomarker remains imperative for treating advanced CCA.

Human MORC2 (Microrchidia family CW-type zinc finger 2), characterized by a CW-type zinc finger and three coiled-coil domains, belongs to the MORC protein family and predominantly localizes in the nucleus[13, 14]. Recent studies have elucidated MORC2's role in gene transcription repression[14, 15], chromatin remodeling during DNA damage response[15], and regulation of lipogenesis[16]. Elevated MORC2 expression correlates with an aggressive phenotype in clinical gastric cancer and shortened overall survival. Increased MORC2 levels promote tumor cell differentiation and proliferation via C/EBPα-mediated SUMOylation modification, thereby affecting protein stability and inducing cell proliferation and tumorigenesis[17]. Additionally, MORC2 regulates cell cycle checkpoints through an acetylation-dependent mechanism in breast cancer[18]. Moreover, MORC2 enhances cancer stemness and tumorigenesis by facilitating DNA methylation-dependent silencing of Hippo signaling, presenting a potential molecular target for cancer therapeutics[19].

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Previous research has shown that MORC2 promotes CCA cell proliferation and tumorigenesis[20]. This study aims to further investigate MORC2's role in CCA progression, suggesting its potential as a therapeutic target for CCA.

2. Material and Methods

2.1. Data Collection

RNA sequencing (RNA-Seq) data from GSE26566 and GSE76297 were retrieved from the Gene Expression Omnibus (GEO) repository database (https://www.ncbi.nlm.nih.gov/gds/).

2.2. Cell Lines

The human cell line HuCCT1 was procured from the Chinese Academy of Sciences' Shanghai Cell Bank. All cell lines were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin). Cells were maintained in an incubator at 37 °C with 5% CO2.

2.3. Western Blot

Cells were lysed in a radioimmunoprecipitation assay buffer containing protease and phosphatase inhibitors. Samples were separated using SDS–PAGE and transferred to polyvinylidene fluoride membranes. Following blocking with 5% skimmed milk at room temperature for 1 h, membranes were incubated with primary antibodies overnight. Subsequently, membranes were incubated with secondary antibodies for 1 h at room temperature, and the bands were visualized using an enhanced chemiluminescence kit. The primary antibody used in this study was MORC2 (NBP3-05127).

2.4. TIMER2.0 Database Exploration of MORC2 Expression and Tumor Features in TCGA

The TIMER2.0 database (http://timer.cistrome.org/)[21]enables the investigation of differential expression between tumor and adjacent normal tissues for the MORC2 gene across all TCGA tumors. Box plots depict distributions of MORC2 expression levels, with statistical significance assessed using the Wilcoxon test and annotated with stars (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$). The database facilitates the identification of up-regulated or down-regulated MORC2 expression in tumors compared to normal tissues for each cancer type, with normal data displayed in gray columns when available.

2.5. Correlation Analysis of MORC2 Expression and Tumor Features in GEO Datasets

We assessed the differential expression of MORC2 in tumors compared to normal tissues for cholangiocarcinoma in the GSE26566 and GSE76297 datasets. Up-regulated or down-regulated MORC2 expression levels were identified, with statistical significance denoted by asterisks (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$).

2.6. Gene Set Enrichment Analysis

Gene set enrichment analysis (GSEA)[22]was conducted using the GSEA website (http://software.broadinstitute.org/gsea/index.jsp). Samples were categorized into high- and low-expression groups based on risk score and viral expression levels. We utilized the c2.cp kegg.v7.4.symbols.gmt sub-aggregate from the Molecular Signatures Database[23](http://www.gsea-msigdb.org/gsea/downloads.jsp) to assess related pathways and molecular mechanisms. Gene expression profiles and phenotypic grouping guided the setting of minimum and maximum gene sets to 5 and 5,000, respectively, with 1,000 times resampling. Statistical significance was determined by $p < 0.05$ and false discovery rate (FDR) < 0.25.

2.7. Correlation Analysis between MORC2 and HALLMARKS Signaling Pathways

We computed HALLMARKS signaling pathway scores through single-sample GSEA (ssGSEA) analysis using the combined GEO microarray dataset comprising GSE26566 and GSE76297. Pearson's correlation analysis was employed to evaluate the relationship between MORC2 expression and hallmarks signaling pathways, encompassing the mitotic spindle, coagulation, G2M checkpoint, and DNA repair within the combined GEO microarray dataset.

2.8. GO and KEGG Enrichment Analyses

For functional enrichment analysis of gene sets, we utilized Gene Ontology (GO)[24]annotations of genes from the "org.Hs.eg.db" R software package as the background to map genes into the background set. Subsequently, enrichment analysis was conducted using the "clusterProfiler" R software package. The minimum and maximum gene sets were defined as 5 and 5,000, respectively, with statistical significance set at $p < 0.05$ and FDR < 0.25.

For gene set functional enrichment analysis, we employed the KEGG[25] REST API (https://www.kegg.jp/kegg/rest/keggapi.html) to obtain the latest KEGG pathway gene annotations for mapping genes into the background set. Enrichment analysis was then performed using the "clusterProfiler" R software package, with the minimum and maximum gene sets set at 5 and 5000, respectively. Statistical significance was determined at $p < 0.05$ and FDR < 0.25.

2.9. Immune Cell Infiltration Levels and Immune-Related Functional Analysis Associated with MORC2

IOBR[26]serves as a computational tool for immunotumor biology research. Utilizing our expression profile of the combined GEO microarray dataset, the R package "IOBR" selected ESTIMATE and quanTIseq methods to calculate the immune-infiltrating cell score of each sample. Pearson's correlation analysis was employed to assess the relationship between MORC2 and immune-infiltrating cell scores.

Cell cycle-related scores, TNF-α signaling via NFKB score, inflammation-related scores, and ICPScore were determined through ssGSEA analysis in the combined GEO microarray dataset. Pearson's correlation analysis was utilized to explore the relationship between inflammation-related scores and ICPScore and immune-infiltrating cells. Analysis of the single-cell sequencing dataset from GSE125449 was conducted using the TISCH database (http://tisch.comp-genomics.org/).

2.10. Statistical Analysis

Statistical analysis was performed using Sangerbox 3.0 (http://www.sangerbox.com/tool) and GraphPad Prism 9.00 (GraphPad Software, La Jolla, CA, USA). Most of the data analysis was performed using the Sangerbox 3.0 (http://www.sangerbox.com/tool). Student's t-test was

employed for comparisons between the two groups. Pearson's correlation analysis was utilized to determine linear relationships. Results were considered statistically significant at $p < 0.05$ (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$).

3. Results

3.1. Association of MORC2 with Cholangiocarcinoma Progression

The expression of MORC2 displayed dysregulation across various tumor types, with notably higher levels observed in several tumor tissues, including cholangiocarcinoma (CCA) (Figure 1A). Elevated MORC2 expression was consistently observed in CCA tumor tissues, validated by analyses of patient samples from GEO datasets (Figure 1B). The combined GEO microarray dataset, comprising GSE26566 and GSE76297, was analyzed by GSEA, revealing enrichment of gene sets associated with malignant progression-related signaling pathways, such as the mitotic spindle, G2M checkpoint, and DNA repair, in CCA patients with high MORC2 levels. Conversely, gene sets linked to coagulation were enriched in CCA patients with low MORC2 levels (Figure 1C). Furthermore, utilizing the combined GEO microarray dataset, single-sample Gene Set Enrichment Analysis (ssGSEA) was performed to calculate the hallmarks signaling pathway scores of CCA patients. Regression analysis demonstrated a positive correlation between MORC2 expression and the levels of the mitotic spindle, G2M checkpoint, and DNA repair pathways, while the expression of MORC2 exhibited a negative correlation with coagulation levels (Figure 1D). These findings collectively suggest that elevated MORC2 expression is implicated in CCA progression.

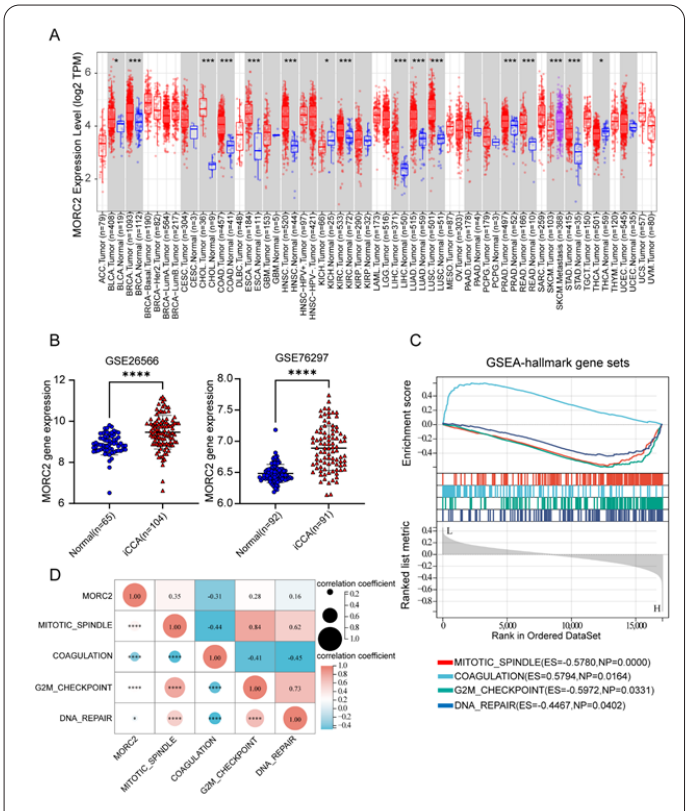

Fig. 1. MORC2 was associated with cholangiocarcinoma progression (**A**) Distributions of MORC2 expression levels in pan-cancer were displayed using box plots. The statistical significance computed by the Wilcoxon tests was annotated by the number of stars. (**B**) The expression of MORC2 in cholangiocarcinoma is based on the datasets of GSE26566 and GSE76297. (**C**) Based on the combined GEO microarray dataset of GSE26566 and GSE76297, the GSEA enrichment analysis in the patients with cholangiocarcinoma. (**D**) The correlation analysis between the expression of MORC2 and hallmarks signaling pathway. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

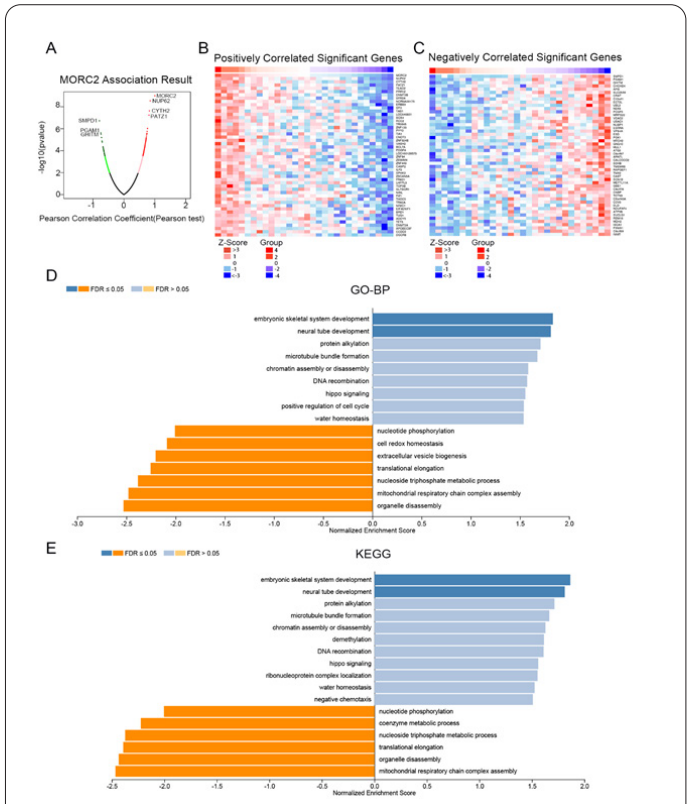

Fig. 2. MORC2 was associated with the regulation of the cell cycle (**A**) The volcano plot showed the genes related to MORC2 in patients with cholangiocarcinoma. (**B-C**) The heatmap showed the top 50 genes of positively correlated significant genes with MORC2 and negatively correlated significant genes with MORC2. (**D**) GO functional analysis for MORC2 co-expressed genes in Biological Process (BP). (**E**) KEGG enrichment analysis for MORC2 co-expressed genes.

3.2. Association of MORC2 with Cell Cycle Regulation

Analysis of Linkedomics datasets revealed significant correlations between MORC2 expression and a multitude of genes in CCA patients. Specifically, MORC2 exhibited positive correlations with 1844 genes and negative correlations with 922 genes (Figure 2A-C). GO and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses unveiled that MORC2 co-expressed genes were enriched in processes such as microtubule bundle formation, chromatin assembly or disassembly, DNA recombination, and positive regulation of the cell cycle in CCA patients. This enrichment profile suggests that MORC2-coexpressed genes predominantly participate in the regulation of the cell cycle (Figure 2D-E).

3.3. MORC2 Facilitates Cholangiocarcinoma Progression by Promoting Cell Cycle Advancement

To delve deeper into the involvement of MORC2 in cell cycle progression, we employed shRNA to knock down MORC2 expression in the human CCA cell line HuCCT1. Subsequent RNA sequencing allowed for the identification of differentially expressed genes (Figure 3A-B). Hallmarks enrichment analysis revealed that MORC2

downregulation led to decreased expression of genes associated with the cell cycle and the MTORC1 signaling pathway. Interestingly, the suppression of MORC2 appeared to potentiate the anti-tumor immune response by activating the coagulation and complement signaling pathway (Figure 3C). Moreover, GO enrichment analysis indicated that downregulated MORC2 influenced cytoskeleton organization, cell cycle processes, mitotic cell cycles, and cell division (Figure 3D-F). These findings collectively suggest that MORC2 promotes cholangiocarcinoma progression by accelerating cell cycle advancement.

3.4. MORC2 Regulates Cell Cycle Progression via TNF-α Signaling through NFKB

Utilizing ssGSEA, we established cell cycle-related scores based on differentially expressed genes by MORC2 knocking down, associated with E2F targets, the G2M checkpoint, and the mitotic spindle (Figure 4A). The previous results suggested that decreased MORC2 could regulate the TNF-α signaling via NFKB signaling pathway which was associated with cell cycle progression (Figure 3C). Then, we calculated the TNF-α signaling via NFKB enrichment score from KEGG signaling pathways using ssGSEA analysis. Linear analysis revealed a negative correlation between MORC2 expression and TNF-α signaling via NFKB, as well as a negative correlation between cell cycle-related scores and TNF-α signaling via NFKB (Figure 4B). Furthermore, we established the TNF-α signaling via NFKB score using differentially expressed genes between the sh-MORC2 group and the Control group related to TNF-α signaling via NFKB (Figure 4C). Intriguingly, both MORC2 and cell cycle-related scores exhibited a negative correlation with the TNF-α signaling via NFKB score (Figure 4D). These results suggest that MORC2 regulates cell cycle progression through TNF-α signaling via NFKB.

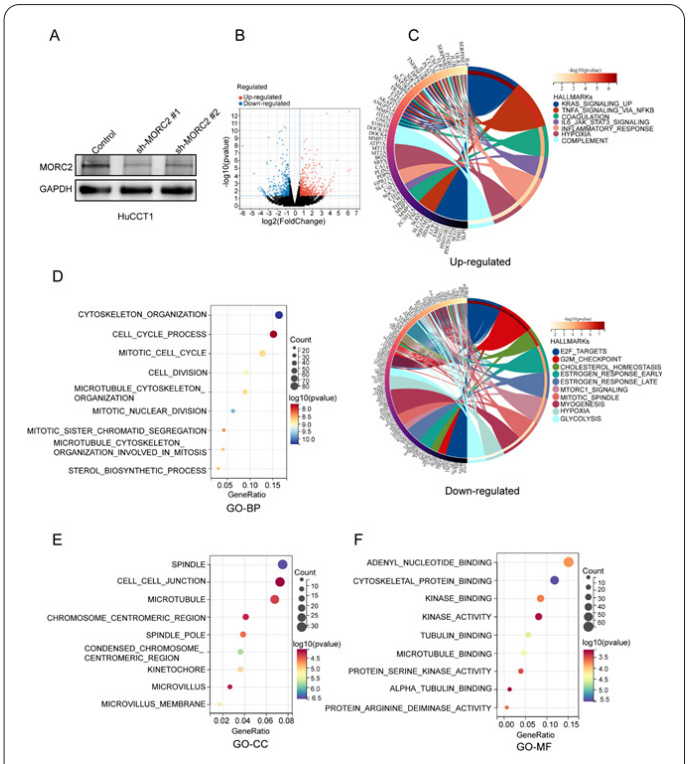

Fig. 3. MORC2 promoted the progression of cholangiocarcinoma via accelerating cell cycle progression (**A**) Western blots show MORC2 knockdown by shRNA. (B) The heatmap of differently expressed genes related to MORC2 knockdown. (**C**) The hallmarks enrichment analysis revealed the signaling pathways related to the differently expressed genes in the human cell line HuCCT1 with MORC2 knockdown. (**D-F**) GO functional analysis for down-regulated genes in Biological Process (BP), Cellular Component (CC), and Molecular Function (MF).

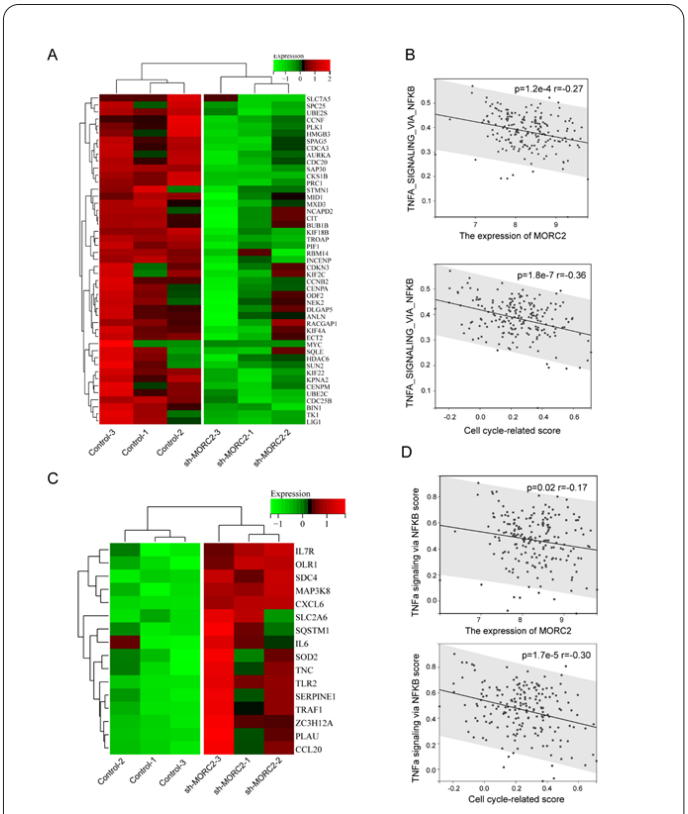

Fig. 4. MORC2 regulated cell cycle progression by TNF-α signaling via NFKB (**A**) The heatmap showed differently expressed genes between the sh-MORC2 group and the Control group related to cell cycles signaling pathways, including E2F targets, G2M checkpoint, and mitotic spindle in the HuCCT1 cell line. (**B**) The linear analysis of TNF-α signaling via NFKB, MORC2, and cell cycle-related scores. (**C**) The heatmap showed differently expressed genes between the sh-MORC2 group and the Control group related to TNF-α signaling via NFKB. (**D**) The linear analysis of TNF-α signaling via NFKB score, MORC2, and cell cycle-related scores. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

3.5. Role of MORC2 in the Tumor Immune Microenvironment

We conducted an immune infiltration analysis utilizing the combined GEO microarray dataset. ESTIMATE analysis revealed a negative correlation between MORC2 expression and the ESTIMATE Score, Stromal Score, and Immune Score, suggesting a potential association of MORC2 with microenvironment modification (Figure 5A). Furthermore, quanTIseq immune infiltration analysis demonstrated a negative correlation between MORC2 and various immune cell types, including B cells, Macrophages M2, Neutrophils, NK cells, and CD8+ T cells (Figure 5B). Targeting MORC2 could potentially enhance anti-tumor immune responses by promoting the infiltration of inflammatory cells. A heatmap displayed that the downregulation of MORC2 increased the expression of genes related to TNF-α signaling via NFKB, inflammatory response, coagulation, and complement in the HuCCT1 cell line

(Figure 5C). Linear analysis based on the combined GEO microarray dataset showed a negative correlation between MORC2 and the expression of certain differential genes, including IL7R, IL6, THBD, C8G, SLC7A2, DOCK10, and GNG2 (Figure 5D). Inflammation-related scores were established using these genes, demonstrating a positive correlation with various immune cell types, including B cells, Macrophages M1, Macrophages M2, Neutrophils, NK cells, CD4+ T cells, CD8+ T cells, Tregs, and dendritic cells (Figure 5E). The intersection of immune cells between MORC2 and Inflammation-related scores included B cells, Macrophages M2, Neutrophils, NK cells, and CD8+ T cells, suggesting a potential association of MORC2 with immune microenvironment modification.

Additionally, we observed an association between MORC2 and the expression of immune checkpoint genes (Figure 6A). Utilizing immune checkpoint genes related to MORC2, we established the ICPScore using ssGSEA analysis. QuanTIseq immune infiltration analysis indicated a positive correlation between MORC2 and B cells,

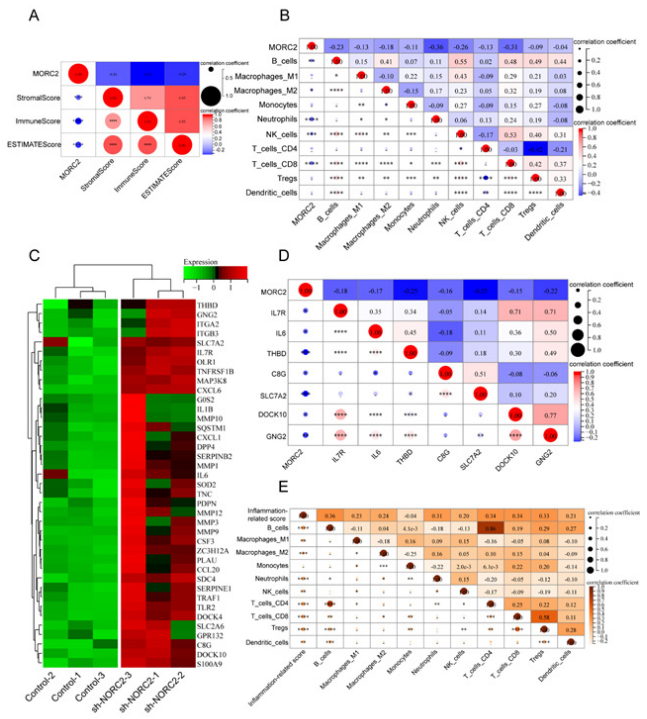

Fig. 5. The role of MORC2 in tumor immune environment characterization (**A**) The ESTIMATE analysis showed the correlation between MORC2 and the ESTIMATE Score, Stromal Score, and Immune Score in the combined GEO microarray dataset of GSE26566 and GSE76297. (**B**) The quanTIseq immune infiltration analysis showed the correlation between MORC2 and immune cells in the combined GEO microarray dataset. (**C**) The heatmap showed differently expressed genes between the sh-MORC2 group and the Control group related to TNF-a signaling via NFKB, inflammatory response, coagulation, and complement signaling pathways in the HuCCT1 cell line. (**D**) The correlation analysis between MORC2 and genes related to TNF-a signaling via NFKB, inflammatory response, coagulation, and complement signaling pathways in the combined GEO microarray dataset. (**E**) The correlation analysis between inflammation-related scores generated by ssGSEA analysis and immune cells in the combined GEO microarray dataset. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

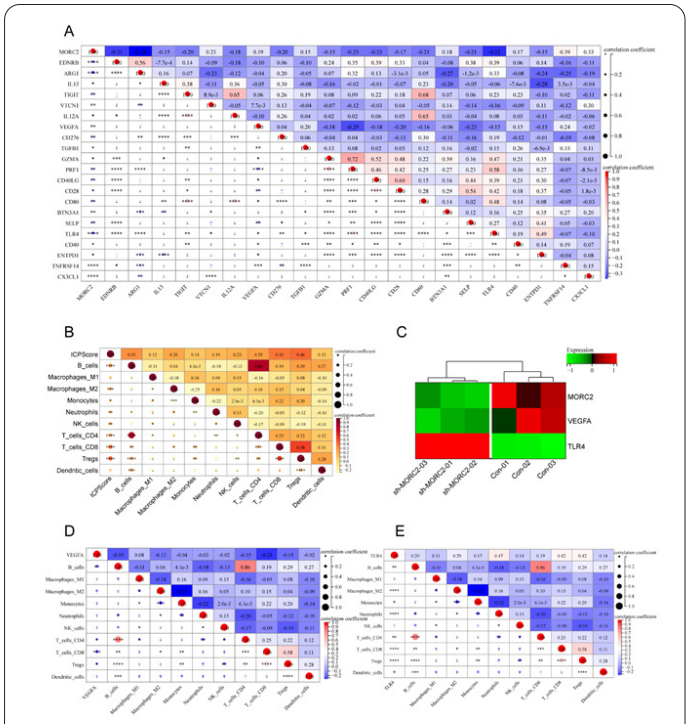

Fig. 6. MORC2 influenced tumor immune microenvironment via regulating immune checkpoint (**A**) The correlation analysis between MORC2 and immune checkpoint in the combined GEO microarray dataset. (**B**) The quanTIseq immune infiltration analysis showed the correlation between ICPScore by ssGSEA analysis and immune cells in the combined GEO microarray dataset. (**C**) The heatmap showed differently expressed VEGFA and TLR4 between the sh-MORC2 group and the Control group in the HuCCT1 cell line. (**D-E**) The correlation between VEGFA and TLR4 and immune cells in the combined GEO microarray dataset. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

Macrophages M2, Neutrophils, NK cells, CD8+ T cells, and Tregs (Figure 6B). RNA-seq analysis revealed that downregulation of MORC2 decreased the expression of VEGFA and increased the expression of TLR4 in HuCCT1 cells. TLR4, known for its role in promoting inflammatory cell infiltration[27], showed a positive correlation with B cells, Macrophages M2, Neutrophils, CD4+ T cells, and CD8+ T cells, as well as Tregs (Figure 6C-E). The intersection of immune cells in MORC2, Inflammation-related scores, ICPScore, and TLR4 included B cells, Macrophages M2, Neutrophils, NK cells, and CD8+ T cells, further supporting the involvement of MORC2 in immune microenvironment modification.

3.6. Targeting MORC2 Induced Elevation of CCL3 Associated with Increased Neutrophil Infiltration

RNA-seq analysis revealed MORC2's involvement in the regulation of cytokine-cytokine receptor interactions. Specifically, downregulation of MORC2 led to increased expression of the inflammatory chemokine CCL3 (Figure 7A). CCL3 exhibited positive correlations with macrophages M1, monocytes, neutrophils, and CD8+ T cells (Figure 7B). Furthermore, analysis of single-cell sequencing data from GSE125449 demonstrated that MORC2 was highly enriched in malignant tumor cells but less so in inflammatory cells, whereas CCL3 showed the opposite pattern, being less enriched in malignant tumor cells but highly enriched in inflammatory cells (Figure 7C).

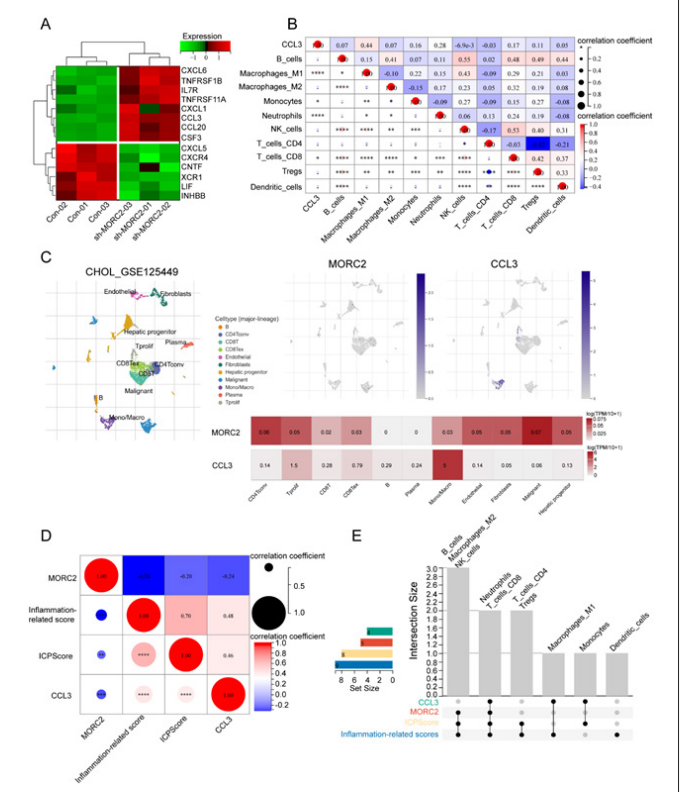

Fig. 7. Targeting MORC2 enhanced neutrophil infiltration through CCL3 upregulation (**A**) The heatmap showed differently expressed genes related to cytokine-cytokine receptor interaction between the sh-MORC2 group and the Control group in the HuCCT1 cell line. (**B**) The correlation between CCL3 and immune cells in the combined GEO microarray dataset. (**C**) Based on the TISCH database, the single-cell sequencing dataset of GSE125449 analysis showed the expression of MORC2 and CCL3 in different cells. (**D**) The correlation analysis of MORC2 with Inflammation-related scores, ICPScore, and CCL3. (**E**) The intersection of immune cells related to MORC2, Inflammation-related scores, ICPScore, and CCL3 based on the quanTIseq immune infiltration analysis. **p*<0.05; ***p*<0.01; ****p*<0.001; *****p*<0.0001.

Linear analysis indicated a negative correlation between MORC2 and inflammation-related scores, ICPScore, and CCL3 (Figure 7D). Notably, the intersection of immune cells in MORC2, inflammation-related scores, ICPScore, and CCL3 highlighted neutrophils and CD8+ T cells, with neutrophils being the most relevant immune cell (Figure 7E, Table 1).

Therefore, targeting MORC2 could significantly enhance neutrophil infiltration, thereby promoting an anti-tumor immune response by upregulating CCL3 expression. In summary, these findings demonstrate MORC2's role in modifying the immune microenvironment and suggest that targeted MORC2 interventions may improve the tumor immune microenvironment by enhancing neutrophil infiltration through CCL3 upregulation.

4. Discussion

Our prior research has demonstrated MORC2's role in promoting cholangiocarcinoma cell proliferation[20]. In this subsequent study, we aimed to delve into the mechanisms underlying MORC2's promotion of cholangiocarcinoma proliferation. GSEA enrichment analysis revealed that patient samples exhibiting high MORC2 levels in cholangiocarcinoma were enriched in genes associated with the mitotic spindle, G2M checkpoint, and DNA repair pathways. Furthermore, MORC2 expression positively correlated with the expression levels of genes related to these pathways. Additionally, Linkedomics analysis indicated that MORC2 co-expressed genes primarily participated in regulating the cell cycle. Our experimental results strongly supported the hypothesis that MORC2 promotes CCA cell proliferation by modulating the cell cycle.

Further mRNA sequencing analysis revealed that genes downregulated by sh-MORC2 were implicated in the cell cycle and MTORC1 signaling pathway, with these pathways predominantly regulating cell proliferation and metabolism. Moreover, GO enrichment analysis highlighted that downregulated genes were enriched in processes such as the cell cycle, mitotic cell cycle, and cell division in cholangiocarcinoma. We elucidated that MORC2 regulates cell cycle progression via the TNF-α signaling pathway through NFKB in CCA. Targeting MORC2 presents a promising avenue for novel therapeutic strategies in treating CCA.

Another noteworthy discovery was the association between MORC2 and the tumor immune microenvironment. ESTIMATE analysis revealed a negative correlation between MORC2 and the ESTIMATE Score, Stromal Score, and Immune Score, indicating a potential link between MORC2 and immune cell infiltration. Vascular endothelial growth factor A (VEGFA) is secreted by various cell types, including endothelial cells and tumors.

Table 1. The correlation analysis of the immune cells with MORC2, Inflammation-related scores, ICPScore, and CCL3.

	Correlation analysis			
quanTIseq	**MORC2**	**Inflammation-related Score**	**ICPScore**	**CCL3**
B cells	Negative (**)	Positive (****)	Positive (****)	-
Macrophages M1	-	Positive (**)	-	Positive (****)
Macrophages M2	Negative (*)	Positive (***)	Positive (***)	-
Monocytes	-		Positive (*)	Positive (*)
Neutrophils	Negative (****)	Positive (****)	Positive (**)	Positive (****)
NK cells	Negative (***)	Positive (**)	Positive (**)	-
T_cells_CD4	-	Positive (****)	Positive (****)	-
T_cells_CD8	Negative (****)	Positive (****)	Positive (****)	Positive (*)
Tregs	-	Positive (****)	Positive (****)	-
Dendritic cells	-	Positive (**)	-	-

*p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

Initially identified as an endothelial growth factor and regulator of vascular permeability[28, 29], VEGFA has been shown to promote angiogenesis in cholangiocarcinoma, thereby facilitating tumor growth[30]. Our investigation also demonstrated a decrease in VEGFA expression in HUCCT1 cells following MORC2 knockdown.

Toll-like receptor 4 (TLR4) is a single transmembrane cell-surface receptor pivotal in the innate immune system. While predominantly expressed in myeloid-derived immune cells such as monocytes, macrophages, and dendritic cells (DCs), TLR4 is also found in non-immune cells like endothelial cells[31]. TLR4 induces inflammation in monocytes/macrophages through interactions with TLR6 and the receptor CD86[27].

The quanTIseq immune infiltration analysis revealed an intersection of immune cells involving MORC2, inflammation-related scores, ICPScore, and TLR4. This intersection included B cells, M2 macrophages, neutrophils, NK cells, and CD8+ T cells, indicating MORC2's involvement in modifying the immune microenvironment.

Additionally, we observed that decreased MORC2 expression led to increased levels of the inflammatory chemokine CCL3, known for its inflammatory and chemokinetic properties. Analysis of the single-cell sequencing dataset GSE125449 revealed MORC2 to be highly enriched in malignant tumor cells but less so in inflammatory cells. Conversely, CCL3 exhibited low enrichment in malignant tumor cells but high enrichment in inflammatory cells. Particularly noteworthy was the intersection of immune cells in MORC2, inflammation-related scores, ICPScore, and CCL3, with neutrophils and CD8+ T cells being identified, with neutrophils being the most relevant immune cell. Thus, targeted inhibition of MORC2 may significantly enhance neutrophil infiltration, thereby promoting an anti-tumor immune response through the upregulation of CCL3 expression. Consequently, MORC2 emerges as a promising therapeutic target for CCA.

This study has several limitations. First, it primarily relies on cellular and genomic analyses, lacking experimentation at the tissue and animal levels for additional validation. Second, the absence of survival and clinicopathological data for Cholangiocarcinoma limits our ability to investigate the correlation between MORC2 expression and clinical outcomes, thereby hindering its potential for guiding clinical treatment strategies.

5. Conclusion

In summary, our study illustrates that MORC2 regulates cell cycle progression through TNF-α signaling via NFKB, thereby facilitating the progression of CCA. Additionally, MORC2 significantly influences the tumor immune microenvironment. Targeting MORC2 could notably enhance neutrophil infiltration, promoting an anti-tumor immune response by upregulating CCL3 expression. Thus, MORC2 emerges as a promising therapeutic target for CCA.

Author Contributions

G Liao, S Tai: Conception and design; G Liao: Administrative support; S Huang, Z Li, H Wu: Provision of study materials or patients; D Tang, Z Xiao: Collection and assembly of data; Y Liu, X Jing: Data analysis and interpretation; Manuscript writing: All authors; Final approval of manuscript: All authors.

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Data availability

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

Ethics approval and consent to participate

Not applicable.

Competing interests

The authors report no conflicts of interest in this work.

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