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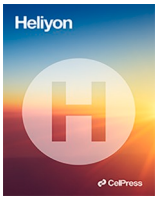

FOXM1/KIF20A axis promotes clear cell renal cell carcinoma progression via regulating EMT signaling and affects immunotherapy response

Kai Fang, Min Gong, Dong Liu, Shengjie Liang, Yang Li, Weicong Sang, Rujian Zhu *

Department of Urology, Shanghai Pudong Hospital, Fudan University Pudong Medical Center, Shanghai, China

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ABSTRACT

Background: The correlation between FOXM1 and KIF20A has not been revealed in clear cell renal cell carcinoma (ccRCC).
Methods: Public data was downloaded from The Cancer Genome Atlas (TCGA) database. R software was utilized for the execution of bioinformatic analysis. The expression levels of specific molecules (mRNA and protein) were detected using real-time quantitative PCR (qRT-PCR) and Western blot assays. The capacity of cell growth was assessed by employing CCK8 and colony formation assay. Cell invasion and migration ability were assessed using transwell assay.
Results: In our study, we illustrated the association between FOXM1 and KIF20A. Our results indicated that both FOXM1 and KIF20A were associated with poor prognosis and clinical performance. The malignant characteristics of ccRCC cells can be significantly suppressed by inhibiting FOXM1 and KIF20A, as demonstrated by in vitro experiments. Moreover, we found that FOXM1 can upregulate KIF20A. Then, EMT signaling was identified as the underlying pathway FOXM1 and KIF20A are involved. WB results indicated that FOXM1/KIF20A axis can activate EMT signaling. Moreover, we noticed that FOXM1 and KIF20A can affect the immunotherapy response and immune microenvironment of ccRCC patients.
Conclusions: Our results identified the role of the FOXM1/KIF20A axis in ccRCC progression and immunotherapy, making it the underlying target for ccRCC.

1. Introduction

Clear cell renal cell carcinoma (ccRCC) is the predominant subtype of kidney cancer in adults, comprising approximately 70 %–80 % of all cases of renal cell carcinoma (RCC) [1]. ccRCC is often asymptomatic in the early stages, and many cases are incidentally detected during imaging for other conditions [2]. The disease is notoriously resistant to radiation and chemotherapy, making surgical resection the primary treatment option for localized disease [3]. However, approximately one-third of patients present with metastatic disease at diagnosis, and a significant portion of those treated for localized disease will relapse and develop metastasis [4]. In recent years, significant advances have been made in the understanding of the molecular genetics of ccRCC. It is now known that ccRCC is often associated with the inactivation of the VHL and HIF, and subsequent overexpression of genes that promote angiogenesis, cell

* Corresponding author.
E-mail address: 20667@shpdh.org (R. Zhu).

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proliferation, and survival [5]. This insight has led to the development of targeted therapies and immunotherapies, which have improved outcomes for patients with advanced disease. However, resistance to these therapies often develops, underlining the need for continued research in this field.

FOXM1, a transcription factor, is essential for the progression of the cell cycle, repair of DNA damage, and proliferation of cells [6]. The excessive expression of FOXM1 has been detected in various types of human malignancies, such as breast cancer, lung cancer, and hepatocellular carcinoma, among other types [7–9]. FOXM1 promotes tumorigenesis through several mechanisms. In addition, it can inhibit apoptosis, promote angiogenesis, and contribute to the acquisition of metastatic and drug-resistant phenotypes in cancer cells [10]. FOXM1 has thus emerged as a promising therapeutic target, and various strategies to inhibit its function are currently under investigation. Nevertheless, further investigation is required to gain a comprehensive understanding of the intricate function of FOXM1 in cancer and to formulate efficacious treatments that specifically target this protein. According to reports, KIF20A is known to have significant functions during mitosis, specifically in the creation of the mitotic spindle and cytokinesis [11]. The abnormal functioning of KIF20A has been linked to various types of human cancers, where its excessive expression frequently associates with unfavorable prognosis [12,13]. In cancer cells, KIF20A has been suggested to promote cell proliferation, migration, and invasion, contributing to tumor growth and metastasis [14,15]. Some previous studies have illustrated the association between FOXM1 and KIF20A in cancer. For instance, Yu et al. found that FOXM1 could regulate KIF20A, further affecting the docetaxel resistance in prostate cancer [16]. Khongkow et al. noticed that FOXM1 can regulate KIF20 expression and influence the paclitaxel resistance of breast cancer [17]. However, the association between FOXM1 and KIF20A in ccRCC remains unclear.

Here, we illustrated the association between FOXM1 and KIF20A. Our results indicated that both FOXM1 and KIF20A were linked to unfavorable prognosis and clinical outcomes. The malignant characteristics of ccRCC cells can be significantly suppressed by inhibiting FOXM1 and KIF20A, as demonstrated by in vitro experiments. Moreover, we found that FOXM1 can upregulate KIF20A. Then, EMT signaling was identified as the underlying pathway FOXM1 and KIF20A are involved. WB results indicated that the FOXM1/KIF20A axis can activate EMT signaling. Moreover, we noticed that FOXM1 and KIF20A can affect the immunotherapy response and immune microenvironment of ccRCC patients.

2. Methods

2.1. Acquisition of open-accessed data

Data from the TCGA database (TCGA-KIRC, accessed time: 2023/5/26) was acquired, which included transcriptome and clinical data [18]. The clinical data includes the survival status, survival time and some clinical features (**Supplementary file 1**). The original transcriptional profile file is in STAR-Counts format. Then, the R code is used for data conversion and consolidation. The genome annotation file is GRCh38.gtf obtained from the Ensembl website. Clinical data was obtained in the bcr-xml format and then arranged using the Perl code. The fluorescence data for subcellular localization is directly obtained from The Human Protein Atlas (HPA) database [19].

2.2. Gene set enrichment analysis (GSEA)

Biological enrichment analysis was performed using the GSEA analysis (fgsea package) [20]. In our analysis, gene sets with a nominal p-value less than 0.05 and a false discovery rate (FDR) below 25 % were considered to be statistically significant. The reference gene set is the Hallmark, gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) gene sets.

2.3. Tumor immune dysfunction and exclusion (TIDE)

The TIDE algorithm was employed to quantify the response rate of patients on immunotherapy based on the expression profile [21].

2.4. Immune microenvironment analysis and genomic features

The infiltration level of specific immune cells was quantified using the CIBERSORT algorithm [22]. The input file of this algorithm is the expression profile data. The Tumor Mutation Burden (TMB) and Microsatellite Instability that reflect the genomic instability were downloaded from the TCGA database.

2.5. Cell lines

The human tubular epithelial cell line HK-2 and three RCC cell lines (786-O, Caki-1 and Caki-2) were routine laboratory storage. Cells are incubated at 37 °C in a humidified atmosphere with 5 % CO_2. Caki-1 cells use DMEM medium, and other cells use 1640 medium for cell culture.

2.6. Cell transfection

The lipofectamine 3000 reagent was utilized to perform cell transfection following the standard protocols. The designed plasmids were purchased from the GenePharma (Shanghai, China). The used shRNA including: sh-FOXM1-1, 5′-GCTGGGATCAAGATTATTA-3′,

sh-FOXM1-2, 5′-GGCCACCCTACTCTTACAT-3′, sh-FOXM1-3, 5′-CTGCCCAACAGGAGTCTAA-3’; sh-KIF20A-1, 5′-GAGGGCCAGAA-GAATATAA-3′, sh-KIF20A-2, 5′-CAGGAGGTTAAAGCTAAAT-3′, sh-KIF20A-3, 5′-CAGGCCTTGATGATAT-3’.

2.7. Real-time quantitative PCR (qRT-PCR)

Total RNA was extracted using the total RNA extraction kit and then reverse transcribed into cDNA for further experiments. The cDNA is mixed with a PCR master mix containing DNA polymerase, nucleotides, buffer, and SYBR Green dye. Primers specific to the gene of interest are added. The primer used were as follows: FOXM1, forward, 5′-CGTCGGCCACTGATTCTCAAA-3′, reverse, 5′-GGCAGGGGATCTCTTAGGTTC-3’; KIF20A, forward, 5′-TGCTGTCCGATGACGATGTC-3′, reverse, 5′-AGGTTCTTGCGTACCACAGAC-3’; GAPDH, forward, 5′-TTGTCTCCTGCGACTTCAACAG-3′, reverse, 5′-GGTCTGGGATGGAAATTGTGAG-3’.

2.8. CCK8, colony formation, and transwell assays

CCK8, colony formation, and transwell assays were used to assess the proliferation, invasion and migration ability of RCC cells,

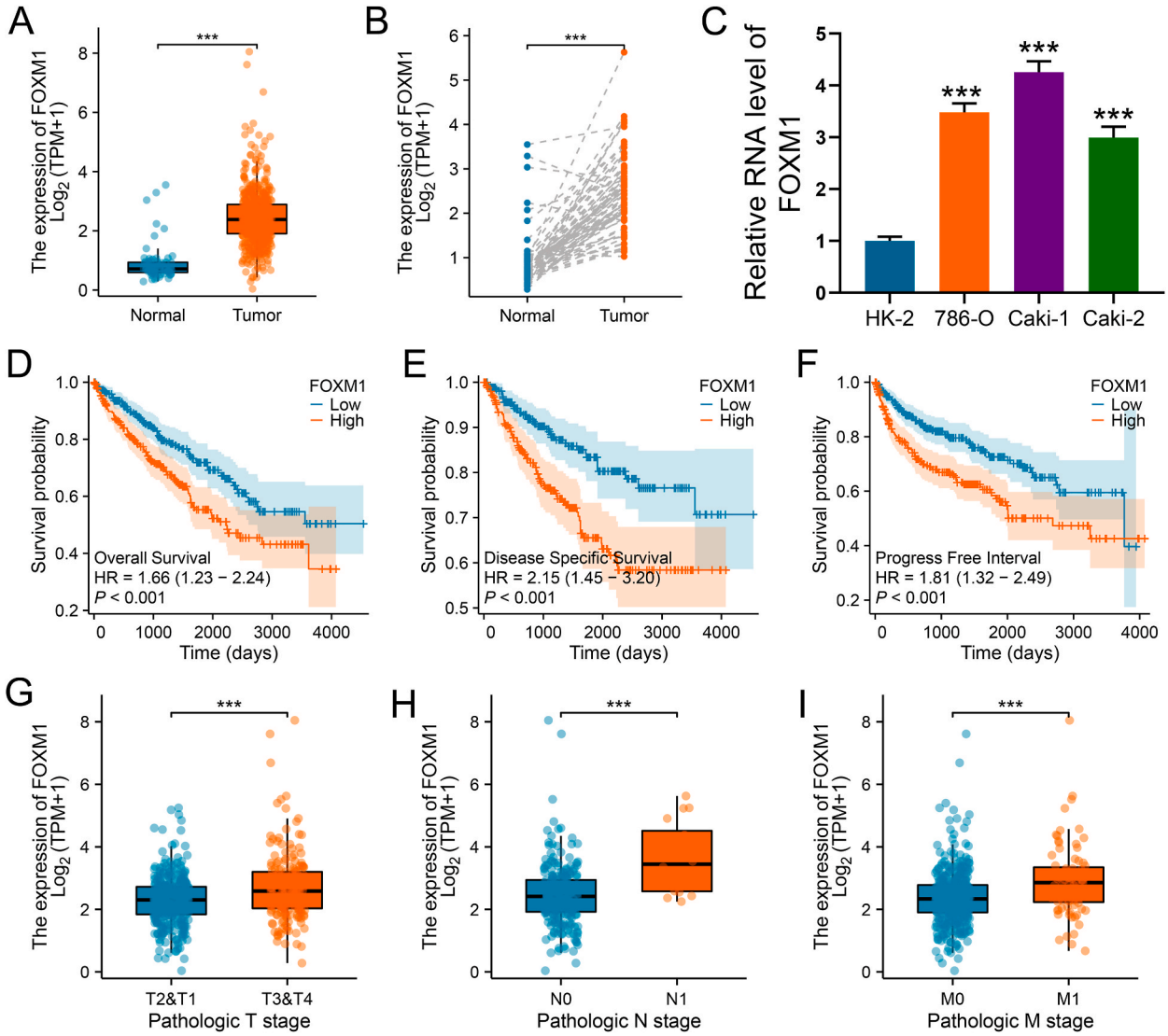

Fig. 1. Expression pattern and clinical role of FOXM1 in ccRCC
Notes: A: Expression level of FOXM1 in ccRCC and normal tissue; B: Expression level of FOXM1 in the paired ccRCC and normal tissue; C: Expression level of FOXM1 in different cell lines; D: KM curve of patients with high and low FOXM1 expression (OS); E: KM curve of patients with high and low FOXM1 expression (DSS); F: KM curve of patients with high and low FOXM1 expression (PFS); G: Expression level of FOXM1 in T1-2 and T3-4 patients; H: Expression level of FOXM1 in N0 and N1 patients; I: Expression level of FOXM1 in M0 and M1 patients.

which were conducted according to the previously published papers.

2.9. Western blot

The protein sample is extracted from cells or tissues using a protein extraction kit (Beyotime, Shanghai). The sample is often sonicated or homogenized to ensure complete lysis, and then centrifuged to separate the protein-containing supernatant. Protein concentration in the lysate is determined using a protein assay, such as the BCA assay. Next, Western blot was performed based on the standard procedure. The primary antibody was purchased from Protentech, including anti-FOXM1 (13147-1-AP, 1:2000), anti-KIF20A (15911-1-AP, 1:2000), anti-E-cadherin (20874-1-AP, 1:20000), anti-N-cadherin (22018-1-AP, 1:2000), anti-vimentin (10366-1-AP, 1:2000) and anti-GAPDH (10494-1-AP, 1:5000).

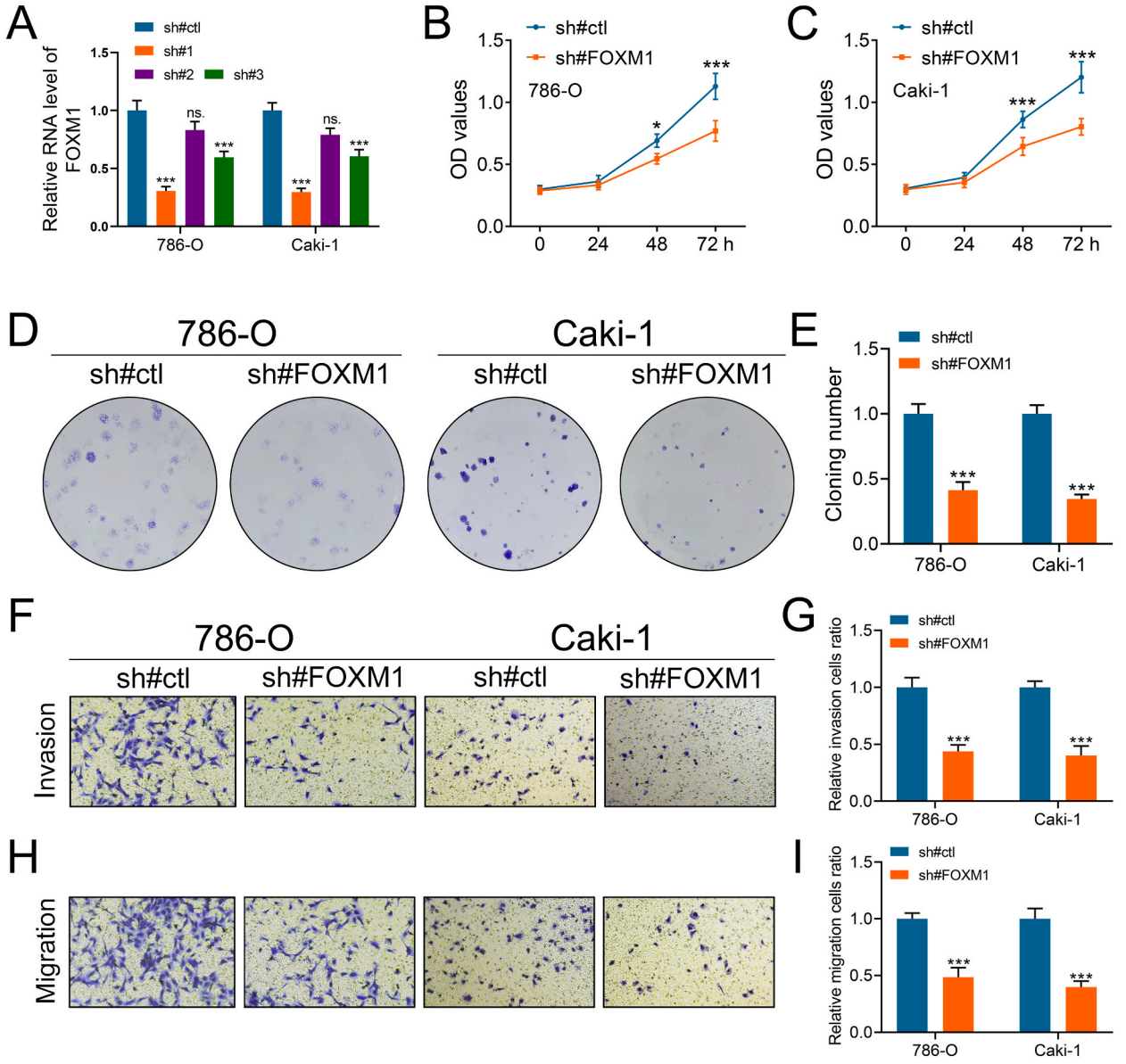

Fig. 2. FOXM1 promotes ccRCC proliferation, invasion and migration. Notes: A: qRT-PCR was used to detect the knockdown efficiency of FOXM1 in 786-O and Caki-1 cells; B–C: CCK8 assay was performed in the FOXM1 knockdown and control cells; D–E: Colony formtaion assay was performed in the FOXM1 knockdown and control cells; F–I: Transwell assay was performed in the FOXM1 knockdown and control cells.

2.10. Statistical analysis

R and GraphPad Prism 8 software were used for all analysis. A significance level of 0.05 was established for all comparisons. Various statistical techniques are employed for varying forms of data distribution.

3. Results

3.1. Expression pattern and clinical role of FOXM1 in ccRCC

Firstly, we evaluated the expression pattern of FOXM1 in ccRCC and normal tissue using data from TCGA-KIRC, as well as paired ccRCC and para-carcinoma tissue (Fig. 1A and B). The findings indicated that FOXM1 is overexpressed in the ccRCC tissue (Fig. 1A and B). Additionally, we assessed the cellular expression level of FOXM1 in various cell lines. The findings revealed that FOXM1 is highly expressed in the RCC cells in comparison to the normal HK-2 cells (Fig. 1C). Kaplan-Meier (KM) survival curves indicated that the

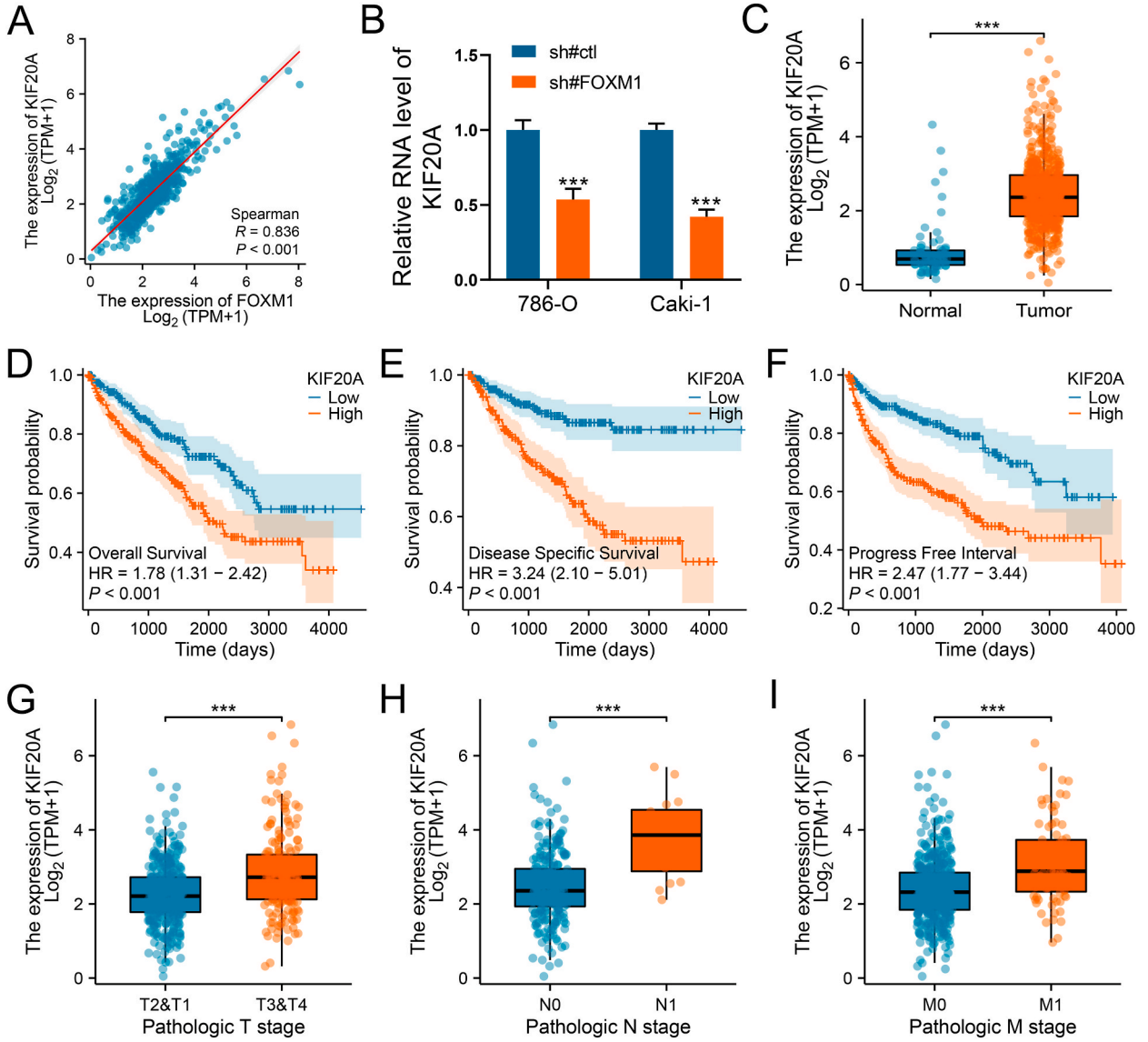

Fig. 3. Expression pattern and clinical role of KIF20A in ccRCC
Notes: A: Correlation between FOXM1 and KIF20A in ccRCC; B: Expression level of KIF20A in the FOXM1 knockdown and control cells; C: Expression level of KIF20A in the ccRCC and normal tissue; D: KM curve of patients with high and low KIF20A expression (OS); E: KM curve of patients with high and low KIF20A expression (DSS); F: KM curve of patients with high and low KIF20A expression (PFS); G: Expression level of KIF20A in T1-2 and T3-4 patients; H: Expression level of KIF20A in N0 and N1 patients; I: Expression level of KIF20A in M0 and M1 patients.

patients with high FOXM1 expression tend to have a worse survival performance (Fig. 1D–F). Meanwhile, we found that FOXM1 is associated with worse clinical features (Fig. 1G–I, T3/4 compared to T1-2; N1 compared to N0, M1 compared to M0).

3.2. FOXM1 promotes ccRCC proliferation, invasion, and migration

Next, our focus was to investigate the impact of FOXM1 on ccRCC cells. We knocked down the FOXM1 in 786-O and Caki-1 cells due to their relatively high expression level of FOXM1, and the knockdown efficiency was evaluated using the qRT-PCR assay. Results indicated that the sh-FOXM1#1 has the best knockdown efficiency and therefore was selected for further analysis (Fig. 2A). The proliferation ability of ccRCC cells was significantly suppressed by inhibiting FOXM1, as shown by the results of CCK8 and colony formation assays (Fig. 2B–E). Meanwhile, the transwell assay showed that the knockdown of FOXM1 can remarkably inhibit the invasion and migration ability of ccRCC cells (Fig. 2F–I). The publicly available FISH results indicate that FOXM1 is mainly localized in the nucleoplasm (Fig. S1).

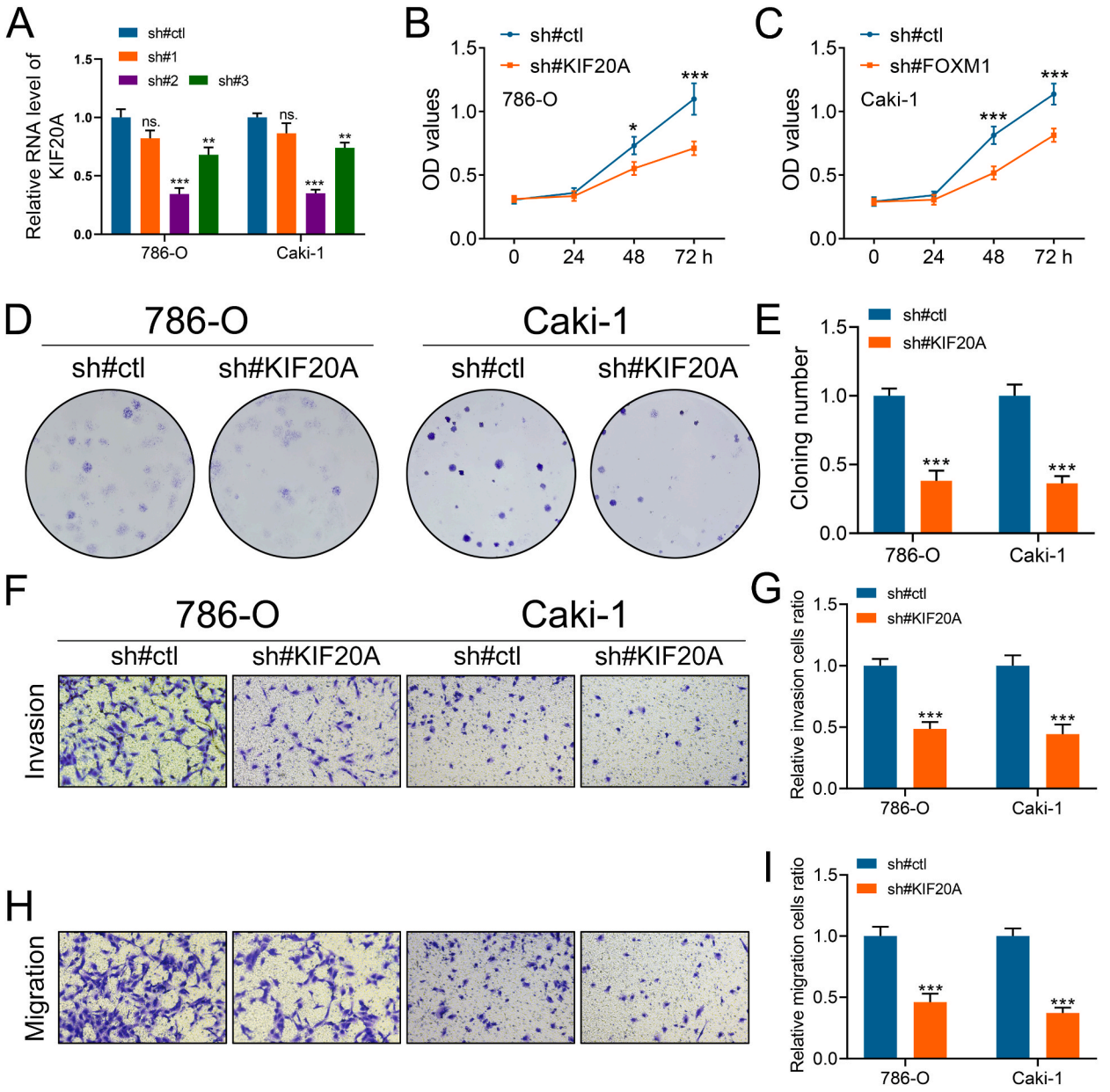

Fig. 4. KIF20A enhances the ccRCC malignant biological behaviors. Notes: A: qRT-PCR was used to detect the knockdown efficiency of KIF20A in 786-O and Caki-1 cells; B–C: CCK8 assay was performed in the KIF20A knockdown and control cells; D–E: Colony formation assay was performed in the KIF20A knockdown and control cells; F–I: Transwell assay was performed in the KIF20A knockdown and control cells.

3.3. FOXM1 can upregulate the expression level of KIF20A

Earlier research has indicated that FOXM1 can control the expression of KIF20A in different types of tumors [17,23,24]. Next, we attempt to investigate whether FOXM1 can regulate the expression of KIF20A in ccRCC. Based on the public ccRCC data from the TCGA database, we performed correlation analysis and found that FOXM1 was positively correlated with the KIF20A at the tissue level (Fig. 3A, cor = 0.836). Subsequently, qRT-PCR indicated a lower expression level of KIF20A in the FOXM1 knockdown group

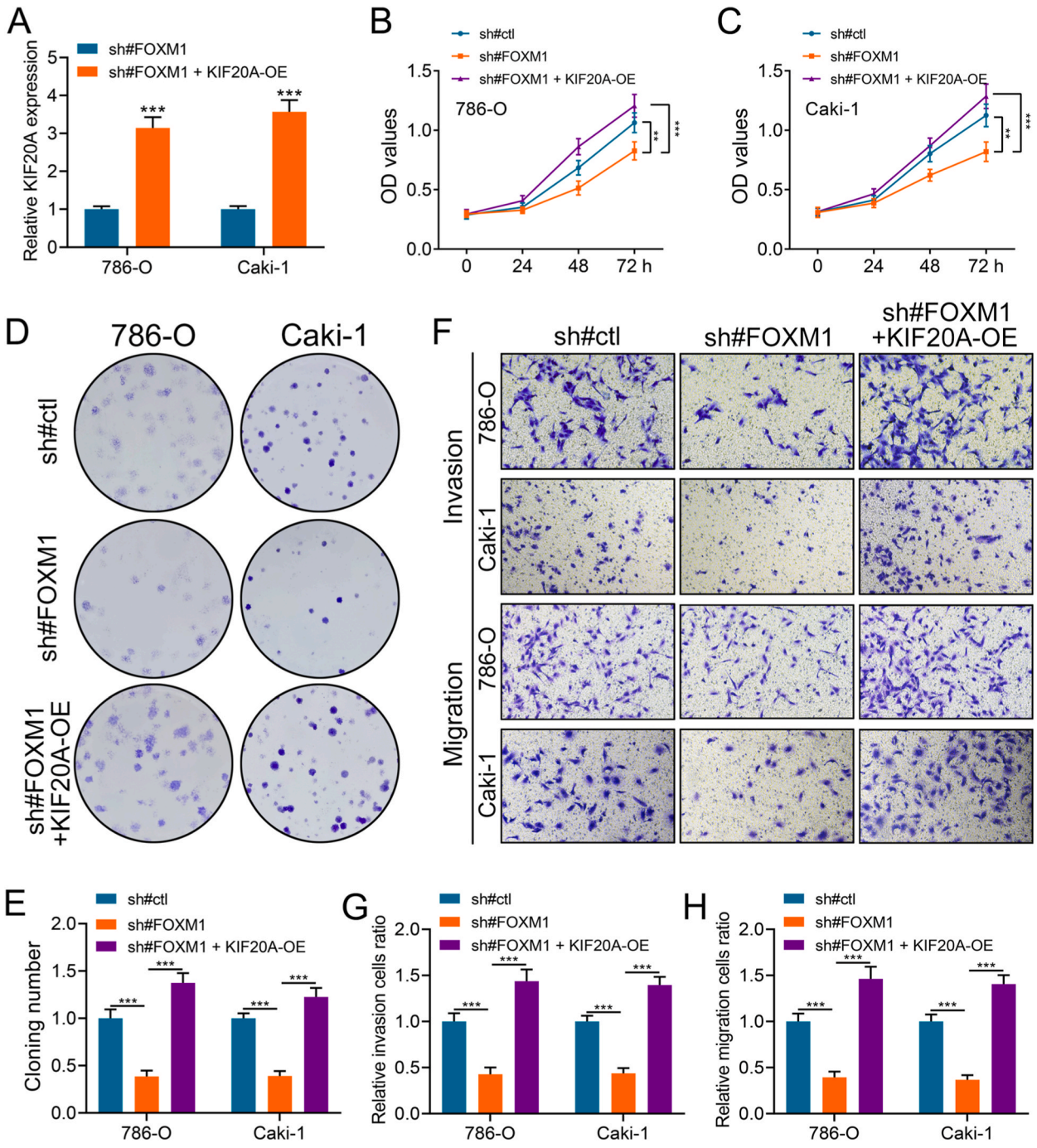

Fig. 5. FOXM1 promotes malignant phenotype by upregulating KIF20A. Notes: A: qRT-PCR was used to detect the overexpression efficiency of KIF20A in FOXM1 knockdown cells; B–C: CCK8 assay was performed in the sh#ctl, sh#FOXM1 and shFOXM1 + KIF20A-OE cells; D–E: Colony formation assay was performed in the sh#ctl, sh#FOXM1 and shFOXM1 + KIF20A-OE cells; F–H: Transwell assay was performed in the sh#ctl, sh#FOXM1 and shFOXM1 + KIF20A-OE cells.

compared with the control group (Fig. 3B). Moreover, we found that KIF20A was upregulated in the ccRCC tissue (Fig. 3C). KM survival curves demonstrated that the patients with high KIF20A expression tend to have a worse survival performance (Fig. 3D–F, OS: HR = 1.78, P < 0.001; DSS, HR = 3.24, P < 0.001; PFS, HR = 2.47, P < 0.001). Same with FOXM1, KIF20 is also correlated with worse clinical features (Fig. 3G–I).

3.4. KIF20A enhances the ccRCC malignant biological behaviors

Afterward, we attempted to ascertain if KIF20A played a role in the cancer-promoting impact of FOXM1. Therefore, we further knock down KIF20A in the 786-O and Caki-1 cells (Fig. 4A). The proliferation ability of ccRCC cells was significantly inhibited by the knockdown of FOXM1, as demonstrated by CCK8 and colony formation assays (Fig. 4B–E). In the meantime, the transwell assay demonstrated that suppressing FOXM1 significantly hinders the invasion and migration capacity of ccRCC cells (Fig. 4F–I). The publicly available FISH results indicate that KIF20A is mainly localized in the nucleoplasm (Fig. S2).

3.5. FOXM1 exerts its role by upregulating KIF20A and epithelial-mesenchymal transition (EMT)

To determine whether the cancer-promoting effect of FOXM1 partly depends on KIF20A, we re-overexpressed KIF20A in the FOXM1 knockdown cells (Fig. 5A). CCK8 and colony formation assays showed that the knockdown of FOXM1 can significantly inhibit the ccRCC proliferation, yet the re-overexpression of KIF20A can remedy this effect (Fig. 5B–E). Transwell assay indicated that the inhibition of FOXM1 can significantly suppress the ccRCC invasion and migration, whereas the re-overexpression of KIF20A can

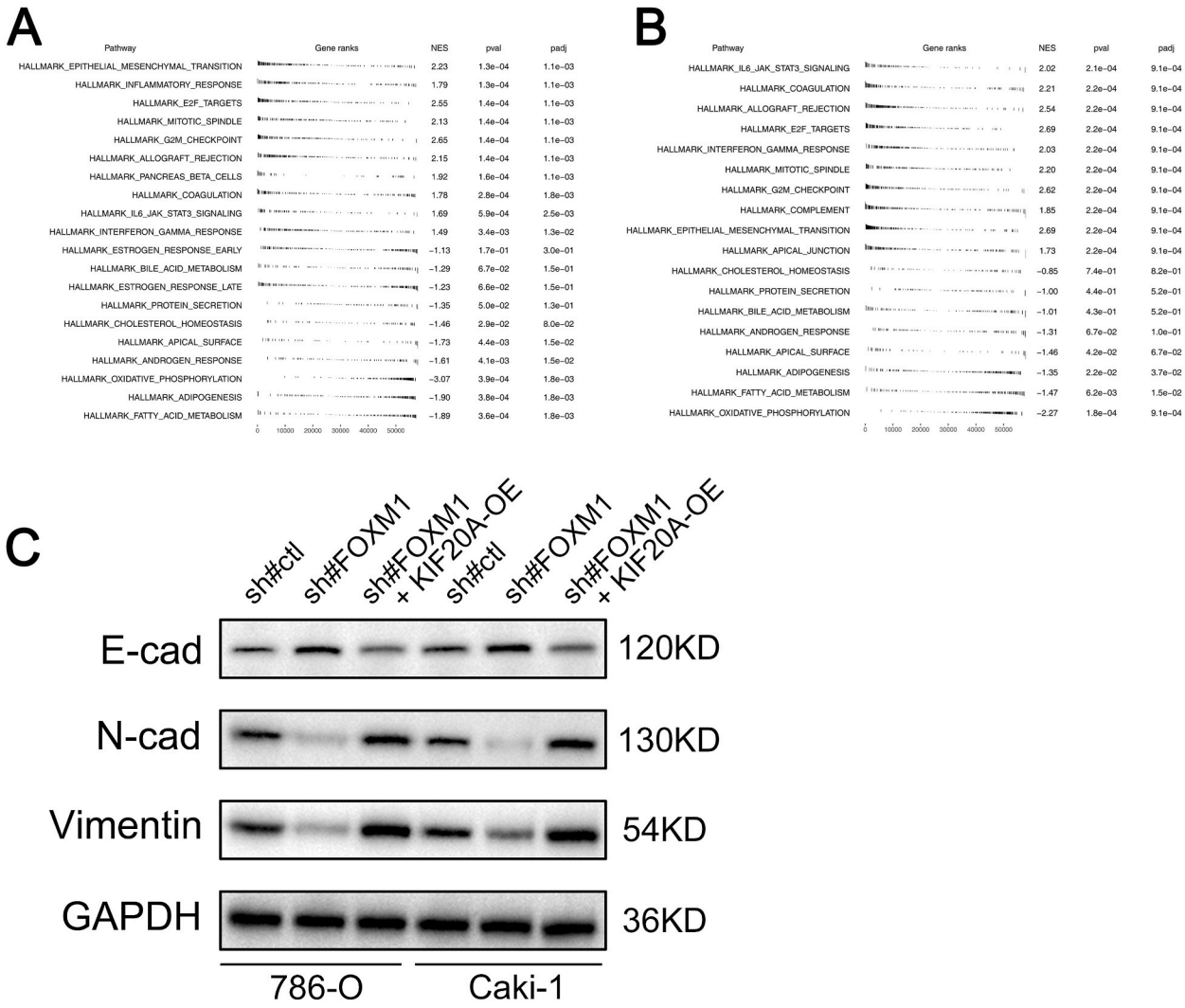

Fig. 6. FOXM1/KIF20A axis can affect the activity of EMT signaling. Notes: A: GSEA analysis of FOXM1 based on Hallmark gene set; B: GSEA analysis of KIF20A based on Hallmark gene set; C: WB assay was conducted to illustrate the effect of FOXM1 and KIF20A on EMT pathway.

remedy this effect (Fig. 5F–H). Furthermore, we performed GSEA analysis utilizing the Hallmark gene set to illustrate the underlying biological pathways FOXM1 and KIF20 are involved. We noticed that in patients with high FOXM1 or KIF20A expression, the EMT pathway was both significantly activated (Fig. 6A and B and **Supplementary file 2–3**). GSEA analysis based on GO gene set showed that KIF20A was positively correlated with immunoglobulin complex, T cell receptor complex and immunoglobulin receptor binding,

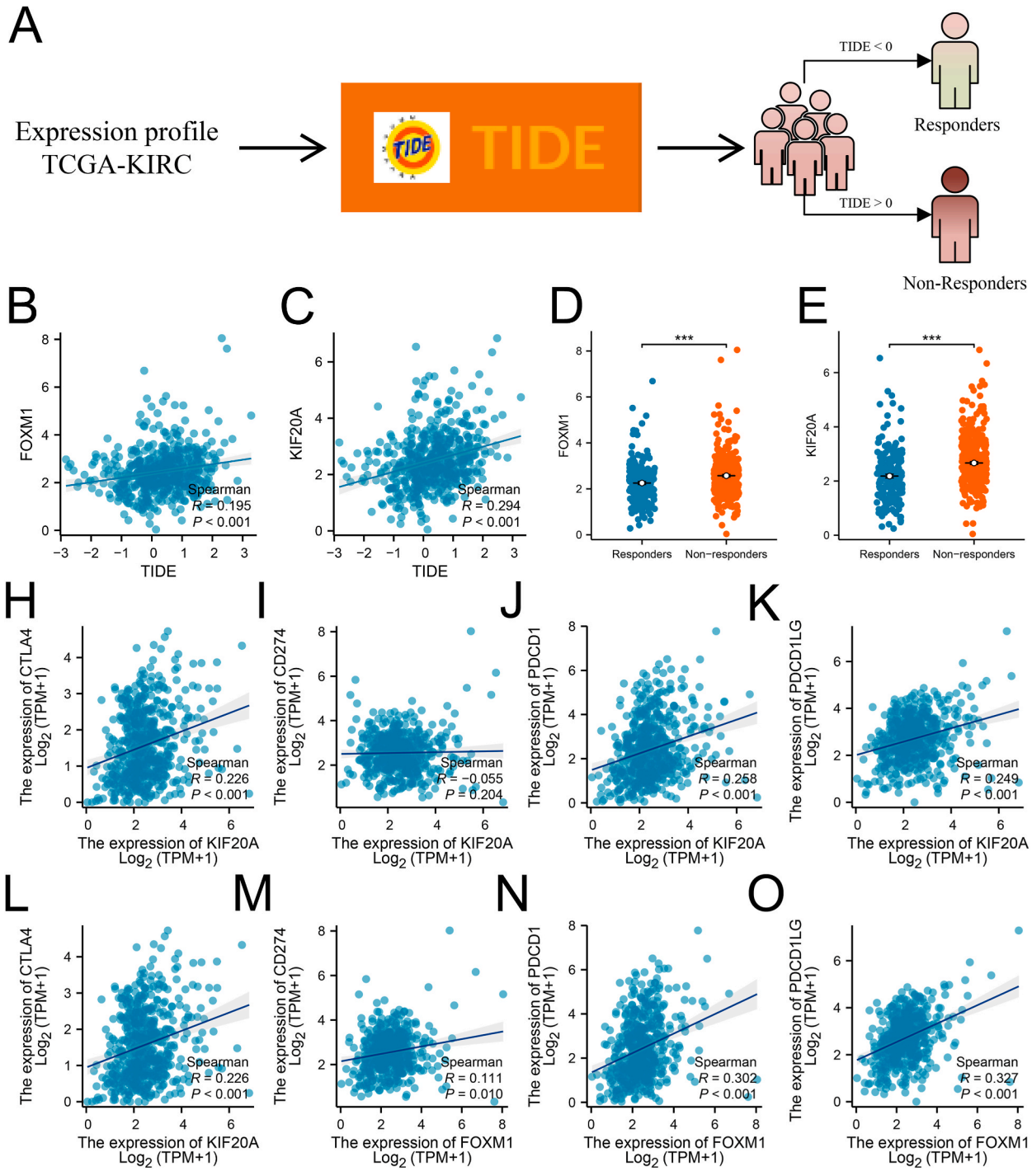

Fig. 7. FOXM1 and KIF20A can affect the immunotherapy response. Notes: A: TIDE algorithm was performed to quantify the immunotherapy response of ccRCC; B–C: The correlation between TIDE score and FOXM1, as well as KIF20A; D–E: The expression level of FOXM1 and KIF20A in the immunotherapy responders and non-responders; H–K: The correlation between KIF20A and key immune checkpoints; L–O: The correlation between FOXM1 and key immune checkpoints.

but negatively correlated with inner mitochondrial protein complex, mitochondrial protein containing complex and regulation of Ph (Fig. S3A). GSEA analysis based on KEGG gene set indicated that KIF20A was cytokine cytokine receptor interaction, Ecm receptor interaction and cell cycle, but negatively correlated with oxidative phosphorylation, vibrio cholerae infection and parkinsons disease (Fig. S3B). GSEA analysis based on GO gene set revealed that FOXM1 was positively correlated with immunoglobulin complex, T cell receptor complex and immunoglobulin receptor binding, but negatively correlated with inner mitochondrial protein complex, mitochondrial protein containing complex and respirasome (Fig. S3C). GSEA analysis based on KEGG gene set showed that FOXM1 was positively correlated with cell cycle, maturity onset diabetes of the young and Ecm receptor interaction, but negatively correlated with oxidative phosphorylation, parkinsons disease and huntingtons disease (Fig. S3D). Subsequently, the WB analysis revealed a substantial increase in the protein expression of E-cadherin, while N-cadherin and vimentin exhibited a notable decrease in the FOXM1 knockdown cells. However, the effect was effectively remedied through the overexpression of KIF20A (Fig. 6C). Collectively, our findings demonstrated that the interaction between FOXM1 and KIF20A can enhance the malignant characteristics of ccRCC cells through the activation of EMT signaling.

3.6. *FOXM1 and KIF20A can affect the immunotherapy response of ccRCC*

Then, we used the TIDE algorithm to quantify the immunotherapy response of ccRCC patients (Fig. 7A). We noticed that both

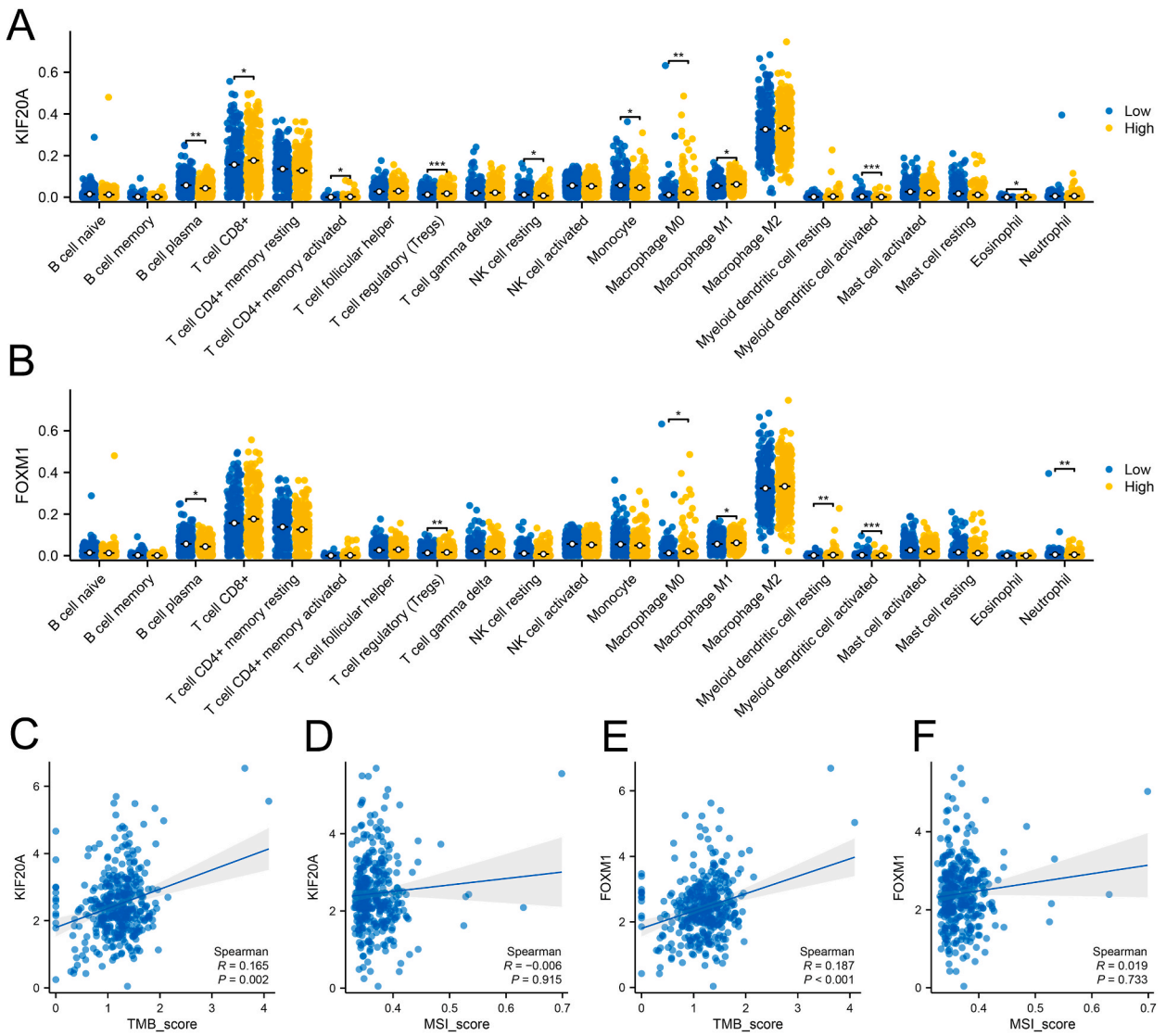

Fig. 8. Effect of FOXM1 and KIF20A on ccRCC immune microenvironment and genome instability. Notes: A: The infiltration level of immune cells in patients with high and low KIF20A expression; B: The infiltration level of immune cells in patients with high and low FOXM1 expression; C: The correlation between KIF20A and TMB, as well as MSI; D: The correlation between FOXM1 and TMB, as well as MSI.

FOXM1 and KIF20A were positively correlated with TIDE score (Fig. 7B and C, FOXM1, Cor = 0.195, P < 0.001; KIF20A, Cor = 0.294, P < 0.001). Moreover, based on the TIDE results, we found that the immunotherapy non-responders have higher FOXM1 and KIF20A expression (Fig. 7D and E). Furthermore, we tried to explore the correlation between immune checkpoints and FOXM1, as well as KIF20A (Fig. 7H-O). Results revealed that both FOXM1 and KIF20A were positively correlated with CTLA-4, PDCD1, and PDCD1LG2. These results indicate that FOXM1 and KIF20A can affect the immunotherapy response of ccRCC.

3.7. Effect of FOXM1 and KIF20A on tumor microenvironment and genomic instability

Then, we explored the effect of FOXM1 and KIF20A on ccRCC microenvironment and genomic instability. The results showed that the infiltration level of $CD8^+$ T cell, activated memory $CD4^+$ T cell, Tregs and M1 macrophage were higher, while plasma B cell, resting NK cell, monocyte and activated myeloid dendritic cell were lower in patients with high KIF20A expression (Fig. 8A). Moreover, the plasma B cell, Tregs, M1 macrophages and activated myeloid dendritic cell have the same trend in patients with high FOXM1 expression (Fig. 8B). The results of genomic instability showed that FOXM1 and KIF20A were positively correlated with TMB score, but not MSI score (Fig. 8C–F, KIF20A- TMB: cor = 0.165, P = 0.002; KIF20A – MSI: cor = −0.006, P = 0.915; FOXM1 – TMB: cor = 0.187, P < 0.001; FOXM1 – MSI: cor = 0.019, P < 0.733).

4. Discussion

ccRCC is the most common type of kidney cancer, accounting for about 75 % of all cases [25]. The prognosis for advanced ccRCC is poor due to resistance to radiotherapy, chemotherapy, and targeted therapies [3]. Therefore, it is crucial to discover novel treatment objectives to enhance the well-being of patients. The discovery of novel targets for ccRCC will not only enhance our understanding of the disease's molecular mechanisms but also provide new avenues for treatment. Identifying such targets can lead to the development of more effective and specific drugs with fewer side effects, offering new hope for patients who are resistant or refractory to current therapies [26]. Furthermore, these targets can also serve as biomarkers for early detection, prognostic assessment, and monitoring of treatment response, thereby improving patient management. Given the heterogeneity of ccRCC, personalized therapies based on specific targets could be a promising direction for future research and clinical practice.

In our study, we illustrated the association between FOXM1 and KIF20A. Our results indicated that both FOXM1 and KIF20A were linked to unfavorable prognosis and clinical outcomes. The malignant characteristics of ccRCC cells can be significantly suppressed by inhibiting FOXM1 and KIF20A, as demonstrated by in vitro experiments. Moreover, we found that FOXM1 can upregulate KIF20A. Then, EMT signaling was identified as the underlying pathway FOXM1 and KIF20A are involved. WB results indicated that FOXM1/KIF20A axis can activate EMT signaling. Moreover, we noticed that FOXM1 and KIF20A can affect the immunotherapy response and immune microenvironment of ccRCC patients.

FOXM1 is a star molecule that serves a regulatory role in the vast majority of malignant tumors. For example, Yao et al. conducted a comprehensive review and revealed that FOXM1 is a promising target for cancer drug resistance [27]. Zhang et al. found that ALKBH5, an m6A demethylase, sustains the expression of FOXM1 and the cell proliferation program, thereby maintaining the tumorigenicity of glioblastoma stem-like cells [28]. Chen et al. observed that the activation of the Wnt/β-catenin pathway by stabilizing FOXM1 could aid in the progression of pancreatic cancer, with USP28 playing a facilitating role [29]. Varghese et al. discovered that FOXM1 can regulate 5-FU resistance in colon cancer by modulating TYMS expression [30]. Liu et al. analyzed the effect pattern of FOXM1 and STMN1 in cancer. The researchers discovered that the unusually elevated activation of a FOXM1-STMN1 pathway may play a role in the advancement and development of FOXM1-induced tumors [31]. Yi et al. indicated that RNF26 can promote bladder cancer progression, which was regulated by FOXM1 [32].

EMT is a biological phenomenon where a polarized cell of epithelial origin undergoes various biochemical alterations, leading to the acquisition of a mesenchymal cell phenotype [33]. EMT is defined by the disruption of cell-cell adhesion and the acquisition of migratory and invasive characteristics [34]. This transformative process is a critical driver of cancer metastasis. EMT plays a pivotal role in the progression and metastasis of the disease. EMT allows cells to become more mobile, invasive, and resistant to apoptosis, facilitating their spread to other parts of the body [35]. In renal cancer, Xu et al. demonstrated that the EMT signaling was regulated by the SLC27A2/CDK3 axis, further influencing renal cancer progression [36].

Despite the significant findings, this study is not without limitations. Firstly, our analysis was largely based on bioinformatic analysis of publicly available databases, which may lack specific clinical details and include inherent biases of retrospective studies. Secondly, while we identified a potential regulatory relationship between FOXM1 and KIF20A, further experiments are required to definitively establish causality. Thirdly, although we found evidence that FOXM1 might activate the EMT pathway through KIF20A, the precise underlying molecular mechanisms remain to be elucidated. Lastly, our findings are yet to be validated in in vivo models and clinical samples. Thus, further research, including preclinical and clinical studies, is needed to solidify these findings and explore their potential clinical implications.

Ethics statement

All the cell lines were purchased from the Cell Bank of Culture of the Chinese Academy of Sciences.

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Data availability

The open-accessed data in this study can be directly downloaded from TCGA dabase (https://portal.gdc.cancer.gov/). Due to the need for subsequent experiments and ownership of R code, we did not disclose all data. However, based on reasonable requirements and scientific needs, all data can be obtained through email from the corresponding author.

CRediT authorship contribution statement

Kai Fang: Writing - original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Min Gong:** Writing - original draft, Software, Methodology, Formal analysis. **Dong Liu:** Validation, Formal analysis, Data curation. **Shengjie Liang:** Methodology, Formal analysis, Data curation. **Yang Li:** Software, Formal analysis. **Weicong Sang:** Writing - original draft, Validation, Methodology. **Rujian Zhu:** Methodology, Funding acquisition, Conceptualization.

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.

Acknowledgements

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e22734.

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