**International Journal of Biology and Life Sciences**
ISSN: 2957-9511 | Vol. 13, No. 3, 2026

# Mechanism of miR-135a-5p influencing KRT8 expression and epithelial-mesenchymal transition in clear cell renal carcinoma via targeted regulation of TFAP2A

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**Abstract:** Clear cell renal carcinoma (ccRCC) is the most prevalent pathological subtype of renal cell carcinoma. Early symptoms are often insidious, and advanced-stage patients have low sensitivity to radiotherapy and chemotherapy, resulting in poor prognosis. Epithelial-mesenchymal transition (EMT) is the core driver of its invasion and metastasis. Aberrant expression of microRNAs (miRNAs), transcription factors, and keratin is involved in the malignant progression of ccRCC. Among these, miR-135a-5p plays a regulatory role in various tumors, but its systemic regulatory relationship with transcription factor TFAP2A, keratin KRT8, and EMT in ccRCC remains unclear. This article reviews the basic biological characteristics, expression patterns, and biological functions of miR-135a-5p, TFAP2A, and KRT8 in ccRCC, based on recent research progress. The regulatory network of these three genes is analyzed: miR-135a-5p is lowly expressed in ccRCC and exhibits a tumor suppressor effect by inhibiting TFAP2A expression through targeting the 3'-UTR; TFAP2A, as a tumor suppressor gene, is lowly expressed in ccRCC and can target and regulate the expression of KRT8 and EMT-related genes; abnormal KRT8 expression is correlated with the malignancy of ccRCC and affects cell invasion and metastasis by regulating EMT. These three genes form the miR-135a-5p/TFAP2A/KRT8 regulatory axis, jointly participating in the regulation of ccRCC cell proliferation, apoptosis, invasion, metastasis, and prognosis. This article also points out current research limitations and looks forward to future research directions, providing theoretical reference for the molecular mechanism research, early diagnosis, and novel targeted therapies of ccRCC.

**Keywords:** Clear Cell Renal Cell Carcinoma; MiR-135a-5p; TFAP2A; KRT8; Epithelial-Mesenchymal Transition.

## 1. Introduction

Clear cell renal cell carcinoma (ccRCC) is the most prevalent pathological subtype of renal cell carcinoma, accounting for 70%-80% of all renal cell carcinoma cases. Its early symptoms are often insidious, and most patients are diagnosed at an intermediate or advanced stage, accompanied by local invasion or distant metastasis, severely impacting prognosis. Surgical resection is the preferred treatment for localized ccRCC, with a 5-year survival rate exceeding 95% for T1 stage patients. However, advanced-stage patients have low sensitivity to radiotherapy and chemotherapy. While targeted and immunotherapy combined with targeted therapy have made progress, problems such as drug resistance and high recurrence rates remain [1]. Therefore, there is an urgent need to explore effective early diagnostic biomarkers and novel targeted therapies. Epithelial-mesenchymal transition (EMT) is a core event in tumor invasion and metastasis, referring to the loss of polarity and intercellular connections in epithelial cells, which then acquire the ability to migrate and invade mesenchymal cells. EMT participates in the entire process of ccRCC metastasis and is closely related to malignant progression and poor prognosis. Furthermore, the spatial gradient expression of EMT-related genes is associated with ccRCC invasion patterns [2]. MicroRNAs (miRNAs) are 18-25 nucleotide non-coding small RNA molecules that regulate post-transcriptional expression by targeting the 3'-UTR of target gene mRNAs, and are widely involved in tumor pathophysiology. miR-135a-5p is abnormally expressed in various malignant tumors and is associated with tumor invasion, metastasis, and prognosis [3]. Previous studies have suggested its involvement in ccRCC regulation, but its regulatory associations with transcription factors TFAP2A, keratin KRT8, and EMT remain fragmented and have not formed a systematic network. This article reviews the expression characteristics, biological functions, and regulatory relationships of these three factors in ccRCC, based on recent research progress, providing theoretical reference for the study of ccRCC molecular mechanisms, diagnosis, and targeted therapy.

## 2. Research Progress on the Correlation between Clear Cell Renal Carcinoma and Epithelial-Mesenchymal Transition

### 2.1. Molecular Biological Characteristics of Clear Cell Renal Carcinoma

Clear cell renal cell carcinoma (ccRCC) is a common malignant tumor of the urinary system, with its incidence increasing globally year by year. The incidence is significantly higher in men than in women, and the age of onset is mainly concentrated between 50 and 70 years. As the most common pathological subtype of renal cell carcinoma, ccRCC accounts for 70%-80% of all renal cell carcinoma cases. Its typical pathological features include round or polygonal tumor cells with clear cytoplasm (due to the rich glycogen and lipid content in the cytoplasm, which is dissolved during routine HE staining). In lower nuclear grades (Fuhrman nuclear grade I-II), the nucleoli are

indistinct or slight, and the tumor boundaries are clear, often forming nested or acinar structures. In higher nuclear grades (Fuhrman grade III-IV), the tumor cells show significant atypia, increased mitotic figures, and a tendency to invasively grow. The development of ccRCC is a complex, multi-gene, multi-step process involving both genetic and environmental factors. Among the genetic factors, VHL (von Hippel-Lindau) gene abnormalities are the core molecular characteristic of ccRCC. Approximately 90% of sporadic ccRCC patients have VHL gene inactivation (including gene mutations, deletions, or methylation), leading to the accumulation of hypoxia-inducible factor (HIF)-α protein [4]. This, in turn, activates the expression of downstream genes such as VEGF and PDGF, promoting tumor angiogenesis and malignant progression. This is the core biological basis for current targeted therapy for ccRCC. Besides VHL, mutations or abnormal expression of genes such as MET, PI3K, AKT, and mTOR are also common. These genes participate in the development of ccRCC by regulating signaling pathways such as cell proliferation, apoptosis, and angiogenesis.

The invasion and metastasis of ccRCC is a complex, multi-stage process, involving multiple steps such as tumor cell detachment from the primary tumor, invasion of surrounding tissues and blood vessels, metastasis to distant organs via the bloodstream or lymphatic system, and colonization and proliferation in metastatic lesions. Its key molecular mechanisms mainly involve three aspects: abnormal signaling pathways, regulation of the tumor microenvironment, and abnormal gene expression [5]. Regarding abnormal signaling pathways, in addition to the aforementioned VHL-HIF-VEGF pathway, abnormal activation of the PI3K/AKT/mTOR signaling pathway is also common. This pathway promotes ccRCC invasion and metastasis by regulating tumor cell proliferation, apoptosis, and migration [6]. Furthermore, MAPK/ERK, Hippo, and Wnt/β-catenin signaling pathways also exhibit varying degrees of abnormality, synergistically regulating the malignant progression of ccRCC. Regarding the regulation of the tumor microenvironment, the ccRCC tumor microenvironment is mainly composed of tumor cells, immune cells, tumor-associated fibroblasts (CAFs), vascular endothelial cells, and extracellular matrix. CAFs can secrete cytokines such as TGF-β and IL-6, promoting tumor cell EMT transformation and invasion/metastasis. Immune cells, such as tumor-associated macrophages (TAMs) and regulatory T cells (Tregs), provide favorable conditions for tumor cell invasion and metastasis by suppressing the body's anti-tumor immune response. Furthermore, recent spatial transcriptomics studies have revealed significant spatial heterogeneity in the ccRCC tumor microenvironment, with strong interactions between tumor cells and immune cells in the invasion border zone, facilitating tumor cells' evasion of immune surveillance and their invasion and spread. In terms of abnormal gene expression, in addition to miRNAs, the abnormal expression of various protein-coding genes also participates in ccRCC invasion and metastasis, such as EMT-related genes like E-cadherin, N-cadherin, and vimentin, as well as genes of particular interest in this study, such as TFAP2A and KRT8. The abnormal expression of these genes collectively constitutes the molecular basis of ccRCC invasion and metastasis. Since invasion and metastasis of ccRCC is a major cause of poor patient prognosis, in-depth research into its molecular mechanisms of invasion and metastasis and the discovery of key molecular targets are of great clinical value for improving patient prognosis [7].

### 2.2. The Mechanism of Epithelial-Mesenchymal Transition (EMT) in Clear Cell Renal Carcinoma

EMT is the process by which epithelial cells lose epithelial characteristics and acquire mesenchymal characteristics under physiological/pathological conditions [8]. It was initially discovered during embryonic development, and its abnormal activation under pathological conditions participates in tumor invasion and metastasis. EMT occurs in three stages: loss of epithelial polarity and disruption of intercellular connections (downregulation of E-cadherin); cytoskeleton reorganization, resulting in a spindle-shaped morphology; and upregulation of mesenchymal markers (N-cadherin, vimentin), enhancing migration and invasion capabilities. Transcription factors such as Twist, Snail, and the ZEB family are core regulators of EMT, promoting EMT progression by inhibiting epithelial markers and activating mesenchymal markers.

Abnormal activation of endogenous tumor metastasis (EMT) is a key driver of invasion and metastasis in ccRCC. The expression of related genes is closely related to the malignancy, clinical stage, and prognosis of ccRCC. Spatially gradient-expressed EMT-related genes (dEMT) are widely distributed in the invasive borderline region; higher scores indicate worse prognosis and higher stage. EMT regulatory factors include: in the tumor microenvironment, TGF-β secreted by cancer cells (CAFs) is a major inducing factor, regulating EMT transcription factors through activation of the Smad pathway; HIF-α accumulation under hypoxic conditions simultaneously regulates angiogenesis and EMT-related genes. Regarding signaling pathways, abnormal activation of PI3K/AKT, MAPK/ERK, and Wnt/β-catenin can all promote EMT. For example, the PI3K/AKT pathway regulates Snail and Twist through phosphorylation, inhibiting E-cadherin [9]. In terms of gene regulation, miRNAs and lncRNAs participate in ccRCC EMT transformation by targeting and regulating EMT-related genes. Furthermore, EMT is also associated with drug resistance in ccRCC; cells undergoing EMT show reduced sensitivity to targeted and chemotherapeutic drugs, which is an important reason for poor treatment outcomes in advanced stages.

## 3. Research Progress of miR-135a-5p in Clear Cell Renal Cell Carcinoma

### 3.1. Basic Biological Characteristics of miR-135a-5p

miR-135a-5p is an important member of the miRNA family and one of the mature forms of miR-135a. Its gene is located on human chromosomes 1q32.1 and 7q11.23, encoded by two independent genes, MIRN135A1 and MIRN135A2. These two gene sequences are highly conserved and both can be transcribed to produce the pre-miR-135a, which is approximately 70 nucleotides long and has a typical hairpin structure. Under the action of the nuclease Dicer, it is further cleaved into the mature miR-135a-5p and miR-135a-3p, with miR-135a-5p being the mature form that performs the main biological function. The mature sequence of miR-135a-5p is highly conserved across different species, with sequence similarity exceeding 90% in humans, mice, and rats, suggesting an important biological function during evolution.

The gene encoding and transcriptional processing of miR-135a-5p exhibit clear molecular characteristics, as shown in Figure 1. This figure clearly illustrates the gene location, transcription, and processing flow of miR-135a-5p: the MIRN135A1 gene is located in the 1q32.1 chromosome region, and the MIRN135A2 gene is located in the 7q11.23 chromosome region. Both genes contain a promoter region, a coding region, and a terminator region. The promoter region can bind to RNA polymerase II to initiate gene transcription. The transcribed primary miRNA (pri-miR-135a) is approximately several hundred to several thousand nucleotides in length and contains a hairpin structure region. In the cell nucleus, it is cleaved by the nuclease Drosha, removing the non-hairpin structure sequences at both ends to generate pre-miR-135a. Pre-miR-135a is processed by Exp. Mediated by ortin-5, miR-135a-5p is transported from the nucleus to the cytoplasm. There, under the action of Dicer nuclease, the loop portion of the hairpin structure is cleaved, generating mature miR-135a-5p (approximately 22 nucleotides in length) and miR-135a-3p. Mature miR-135a-5p binds to the Argonaute (Ago) protein, forming the RNA-induced silencing complex (RISC). This RISC exerts post-transcriptional regulatory effects by targeting the 3'-UTR region of target gene mRNA. Its regulatory mechanisms primarily include two types: when miR-135a-5p binds to the target gene mRNA in a completely complementary manner, it induces the degradation of the target gene mRNA; when the binding is incompletely complementary, it inhibits the translation of the target gene mRNA, thereby reducing the expression level of the target gene protein and thus regulating various biological behaviors of the cell.

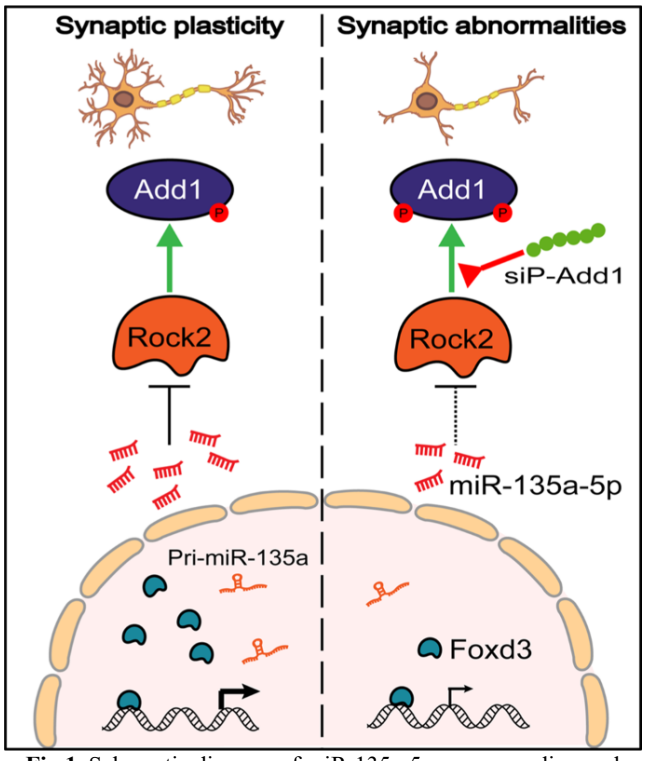


**Fig 1.** Schematic diagram of miR-135a-5p gene encoding and transcriptional processing

miR-135a-5p is widely expressed in normal tissues such as the brain, kidneys, and liver, regulating normal cell proliferation, differentiation, and apoptosis, and maintaining tissue physiological functions [10]. For example, in normal kidneys, it is expressed in renal tubular epithelial cells, regulating renal tubular function; in the brain, it participates in neuronal development and synapse formation. Abnormal expression can lead to cellular dysfunction and participate in the development of diseases such as malignant tumors.

## 3.2. Expression Characteristics of miR-135a-5p in Clear Cell Renal Carcinoma

In recent years, with the continuous development of miRNA research technology, more and more studies have focused on the expression characteristics of miR-135a-5p in ccRCC. Most studies currently show that the expression level of miR-135a-5p in ccRCC tissues and cell lines is significantly lower than that in normal kidney tissues and normal renal tubular epithelial cells, exhibiting a clear tumor suppressor gene expression characteristic. Furthermore, its expression level is closely related to the clinicopathological features of ccRCC. For example, a clinical study involving 60 ccRCC patients found that the expression level of miR-135a-5p in ccRCC tissues was significantly lower than that in adjacent normal kidney tissues ($P<0.05$), and its expression level was closely related to tumor size, clinical stage, lymph node metastasis, and distant metastasis in ccRCC patients: the larger the tumor volume, the higher the clinical stage, and the presence of lymph node metastasis or distant metastasis, the lower the expression level of miR-135a-5p in their ccRCC tissues ($P<0.05$); while there was no significant correlation with the patient's age, sex, or Fuhrman nuclear grade ($P>0.05$). Another in vitro cell experiment found that the expression level of miR-135a-5p in ccRCC cell lines (such as 786-O, ACHN, and Caki-1) was significantly lower than that in the normal renal tubular epithelial cell line (HK-2) ($P<0.05$), with the lowest expression level observed in highly invasive ccRCC cell lines (such as Caki-1), further confirming the low expression characteristic of miR-135a-5p in ccRCC.

Regarding the mechanism of low miR-135a-5p expression in ccRCC, current research suggests it is mainly related to gene methylation, lncRNA regulation, and transcription factor regulation. Regarding gene methylation, abnormal methylation of the promoter regions of MIRN135A1 and MIRN135A2 genes is one of the main reasons for the low expression of miR-135a-5p. Studies have found that the methylation level of the promoter region of the MIRN135A1 gene in ccRCC tissue is significantly higher than that in normal kidney tissue, and the methylation level is negatively correlated with the expression level of miR-135a-5p ($r=-0.62$, $P<0.05$). When ccRCC cell lines are treated with methylation inhibitors (such as 5-Aza-CdR), the expression level of miR-135a-5p is significantly increased ($P<0.05$), suggesting that gene methylation inhibits the transcription of miR-135a-5p. Regarding lncRNA regulation, various lncRNAs can adsorb miR-135a-5p through sponge binding, leading to a decrease in its expression level. For example, lncRNA H19 is highly expressed in ccRCC tissues; it can adsorb miR-135a-5p through sponge binding, inhibiting miR-135a-5p expression and thus promoting the invasion and metastasis of ccRCC cells. Furthermore, lncRNAs such as MALAT1 and NEAT1 can also regulate miR-135a-5p expression in ccRCC through similar mechanisms. Regarding transcription factor regulation, some transcription factors can bind to the promoter regions of MIRN135A1 and MIRN135A2 genes, inhibiting their transcription and leading to low miR-135a-5p expression. For example, the transcription factor Snail is highly expressed in ccRCC; it can bind to the promoter region of the MIRN135A1 gene, inhibiting miR-135a-5p

transcription and thus promoting the malignant progression of ccRCC.

### 3.3. Biological Functions of miR-135a-5p in Clear Cell Renal Carcinoma (ccRCC)

MiR-135a-5p mainly plays a tumor-suppressive role in ccRCC, regulating cell proliferation, invasion and metastasis, cell cycle, and apoptosis. Its effect on migration ability is controversial. In vitro experiments showed that overexpression of miR-135a-5p inhibited ccRCC cell proliferation and colony formation, while silencing promoted them. In vivo experiments showed that the volume and weight of xenografts in nude mice were significantly reduced in the overexpression group ($P<0.05$), and Ki-67 expression was decreased. Its mechanism involves upregulating cell cycle repressor genes such as p21 and p27, and downregulating cell cycle promoter genes such as Cyclin D1 and CDK4, arresting cells in the G0/G1 phase; simultaneously, it inhibits the PI3K/AKT pathway, indirectly arresting the cell cycle.

Most studies have shown that miR-135a-5p can inhibit ccRCC cell invasion and metastasis. Overexpression reduces the number of cells that penetrate the membrane and the scratch healing rate in Transwell assays ($P<0.05$), while silencing enhances these effects; in an in vivo lung metastasis model, the number and volume of metastatic lesions were significantly reduced in the overexpression group ($P<0.05$). The mechanism involves upregulating E-cadherin and downregulating N-cadherin, vimentin, and Twist and Snail, inhibiting EMT transformation. A few studies have shown that it has no significant effect on ACHN cell migration ($P>0.05$), which is speculated to be related to cell line differences and expression levels.

MiR-135a-5p can promote apoptosis in ccRCC cells. Overexpression increases the apoptosis rate ($P<0.05$), while silencing decreases it. The mechanism involves inhibiting Bcl-2, upregulating Bax and Caspase-3/9, activating the apoptosis pathway, and simultaneously inhibiting the PI3K/AKT pathway to promote apoptosis. Its core regulatory pathways include PI3K/AKT, ERK1/2, and Hippo, which synergistically regulate cell behavior: the PI3K/AKT pathway is the core, and its inhibition can suppress proliferation and invasion, and promote apoptosis; inhibition of the ERK1/2 pathway can inhibit proliferation and invasion; and activation of the Hippo pathway can promote apoptosis and inhibit invasion. Furthermore, low expression of miR-135a-5p was an independent risk factor for poor prognosis in ccRCC (HR=2.35, 95%CI: 2.28-4.32, $P<0.05$). The 5-year overall survival (OS) rate (75.0%) and progression-free survival (PFS) rate (67.5%) in the high-expression group were significantly higher than those in the low-expression group (42.5%, 35.0%, respectively).

## 4. Research Progress of TFAP2A in Clear Cell Renal Cell Carcinoma

### 4.1. Basic Biological Characteristics of TFAP2A

TFAP2A is a core member of the AP-2 transcription factor family. Its gene is located at 6p24.3, with a full-length cDNA of 2.8 kb, encoding a 435-amino acid, 48 kDa nuclear transcription factor. It is widely expressed in human tissues and participates in biological processes such as cell proliferation and differentiation, playing a crucial role in embryonic development and tumor progression.

The TFAP2A protein contains four functional domains (Figure 2): the N-terminal activation domain (amino acids 1-50), rich in acidic amino acids, recruits co-activators such as p300, and enhances transcriptional activity; the DNA-binding domain (positions 100-200), which forms an HTH structure and specifically binds to the consensus sequence of the promoter of the target gene (5'-GCCNNNGGC-3'), and is the core functional domain; the dimerization domain (positions 180-220), which overlaps with the DNA-binding domain, mediates the formation of homo/heterodimers, and is a prerequisite for transcriptional regulation; and the C-terminal regulatory domain (positions 300-435), rich in proline and serine, which can be phosphorylated by PKA and PKC to regulate nuclear localization and transcriptional activity, and can also bind to co-repressors such as HDACs to negatively regulate transcription.

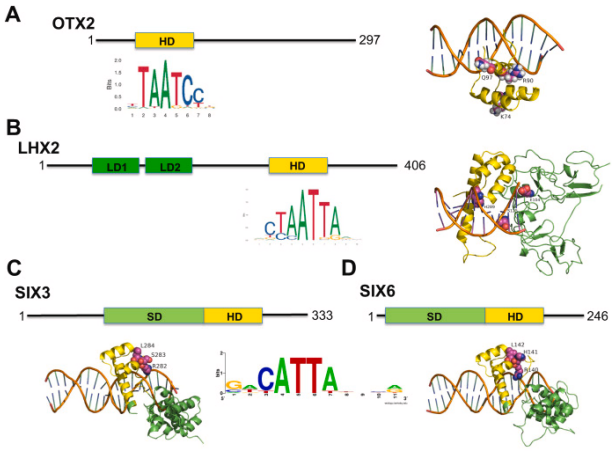


**Fig 2.** Schematic diagram of the protein structure of transcription factor TFAP2A

### 4.2. Expression Characteristics of TFAP2A in Clear Cell Renal Cell Carcinoma (ccRCC)

In recent years, an increasing number of studies have focused on the expression characteristics of TFAP2A in ccRCC. Most current research indicates that the expression level of TFAP2A in ccRCC tissues and cell lines is significantly lower than that in normal kidney tissues and normal renal tubular epithelial cells, and its expression level is closely related to the clinicopathological features and prognosis of ccRCC, exhibiting characteristics of a tumor suppressor gene. For example, a clinical study involving 70 ccRCC patients found that the mRNA and protein expression levels of TFAP2A in ccRCC tissues were significantly lower than those in adjacent normal kidney tissues ($P<0.05$), and its protein expression level was closely related to tumor size, clinical stage, Fuhrman nuclear grade, and lymph node metastasis in ccRCC patients: the larger the tumor volume, the higher the clinical stage, the higher the Fuhrman nuclear grade, and the more lymph node metastasis the patient, the lower the TFAP2A protein expression level in their ccRCC tissues ($P<0.05$); however, there was no significant correlation with the patient's age or gender ($P>0.05$).

In vitro cell experiments yielded similar conclusions. In ccRCC cell lines (such as 786-O, ACHN, Caki-1, and OS-RC-2), the mRNA and protein expression levels of TFAP2A were significantly lower than those in the normal renal tubular epithelial cell line (HK-2) ($P<0.05$). The lowest expression levels were observed in highly invasive and malignant ccRCC cell lines (such as Caki-1 and OS-RC-2), further confirming the low expression characteristic of TFAP2A in ccRCC.

Furthermore, immunohistochemical experiments showed that TFAP2A was mainly expressed in the nucleus of ccRCC cells, with a small amount expressed in the cytoplasm. In contrast, in normal renal tubular epithelial cells, TFAP2A was mainly highly expressed in the nucleus, suggesting that abnormal nuclear localization of TFAP2A may also be involved in the development and progression of ccRCC. Downregulation of TFAP2A expression and abnormal cytoplasmic localization in some ccRCC cells prevent it from performing its normal transcriptional regulatory function.

The mechanisms underlying the low expression of TFAP2A in ccRCC are currently believed to be mainly related to gene methylation, miRNA regulation, and gene mutation. Regarding gene methylation, abnormal methylation of the TFAP2A gene promoter region is one of the main reasons for its low expression. Studies have found that the methylation level of the TFAP2A gene promoter region in ccRCC tissues is significantly higher than that in normal kidney tissues ($P<0.05$), and the methylation level is negatively correlated with the mRNA expression level of TFAP2A ($r=-0.58$, $P<0.05$). When ccRCC cell lines are treated with methylation inhibitors (such as 5-Aza-CdR), both the mRNA and protein expression levels of TFAP2A are significantly increased ($P<0.05$), suggesting that gene methylation inhibits the transcription of TFAP2A. Regarding miRNA regulation, various miRNAs can inhibit the mRNA and protein expression of TFAP2A by targeting and binding to the 3'-UTR region. Besides miR-135a-5p, which is the focus of this study, miR-21, miR-18a, and miR-92a can also target and inhibit TFAP2A expression in ccRCCs. For example, miR-21 is highly expressed in ccRCC tissues; it can bind to TFAP2A mRNA through sponge-like structures, inhibiting its translation and leading to downregulation of TFAP2A protein expression, thereby promoting the invasion and metastasis of ccRCC cells. Regarding gene mutations, a few studies have found gene mutations in the TFAP2A gene in ccRCC tissues (such as missense mutations and deletion mutations), but the mutation rate is low (approximately 5%-10%), mainly occurring in the DNA-binding domain and dimerization domain. This results in abnormal TFAP2A protein structure, preventing the formation of dimers and binding to target gene promoter regions, thus losing transcriptional regulatory function and leading to reduced expression levels and activity.

### 4.3. Biological Functions and Regulatory Roles of TFAP2A in Clear Cell Renal Cell Carcinoma

TFAP2A exerts an antitumor effect in ccRCC, inhibiting proliferation, invasion, and metastasis, arresting the cell cycle, and promoting apoptosis. Its expression is negatively correlated with malignancy. In in vitro experiments, overexpression of TFAP2A inhibited ccRCC cell proliferation and colony formation, while silencing it promoted these effects. In in vivo experiments, the overexpression group showed significantly reduced tumor volume and weight in nude mice ($P<0.05$), and decreased Ki-67 expression. The mechanism involves binding to p21 and p27 promoters to promote their expression, inhibiting Cyclin D1 expression, and arresting cells in the G0/G1 phase; it also inhibits the PI3K/AKT pathway, indirectly arresting the cell cycle.

TFAP2A inhibits ccRCC cell invasion and metastasis. Overexpression reduces the number of transwell cells and the scratch healing rate ($P<0.05$), while silencing it enhances these effects. In an in vivo lung metastasis model, the overexpression group showed a significant reduction in metastatic lesions ($P<0.05$). The mechanism involves binding to the E-cadherin promoter to promote its expression, inhibiting interstitial markers and EMT transcription factors, while simultaneously inhibiting MMP-2 and MMP-9, reducing extracellular matrix degradation. Furthermore, it promotes apoptosis in ccRCC cells; overexpression increases the apoptosis rate ($P<0.05$). This is achieved by promoting Bax and Caspase-3/9 expression, inhibiting Bcl-2 and Bcl-xL, activating the endogenous apoptosis pathway, and regulating the TNF-α and Fas pathways to enhance apoptosis.

TFAP2A regulates ccRCC cell behavior by targeting gene promoters. This binding effect can be verified by ChIP experiments. Downstream target genes include those related to cell cycle, apoptosis, EMT, and angiogenesis. NPHS2 is a key target gene, encoding the podocin protein, which maintains kidney function. TFAP2A promotes its expression and inhibits ccRCC progression. In addition, TFAP2A can synergistically regulate target genes with p53 and interact with pathways such as PI3K/AKT. Its transcriptional activity can be precisely regulated by phosphorylation modification with PKC (enhancing) and PKA (inhibiting).

## 5. Research Progress of KRT8 in Clear Cell Renal Cell Carcinoma

### 5.1. Basic Biological Characteristics of KRT8

KRT8 is an important member of the keratin family. Keratin is the core of the epithelial cell cytoskeleton, maintaining cell morphology, mechanical stability, and intercellular connections, and participating in cell proliferation and differentiation. The KRT8 gene is located at 12q13.13, with a full-length cDNA of 2.2kb, encoding a type I acidic keratin with 483 amino acids and a molecular weight of 52kDa. It often forms heterodimers with KRT18 and KRT19, constituting intermediate filaments in epithelial cells.

The KRT8 protein contains three domains: a central α-helical rod-shaped domain (core, 310 amino acids), which forms a dimer with type II keratin and aggregates to form intermediate filaments, maintaining cell morphology; and N-terminal and C-terminal non-helical domains, rich in glycine and serine, which interact with proteins such as desmoplakin, stabilizing intercellular connections. These domains can be phosphorylated to regulate their polymerization state and function. KRT8 is widely expressed in monolayered epithelial tissues such as the gastrointestinal tract, liver, gallbladder, and kidneys. In normal kidney tissue, it is mainly expressed in renal tubular epithelial cells, maintaining the structural integrity and physiological function of the renal tubules. Abnormal expression is involved in the progression of epithelial tumors.

### 5.2. Expression Characteristics of KRT8 in Clear Cell Renal Carcinoma (ccRCC)

The expression characteristics of KRT8 in ccRCC remain controversial, but most studies indicate that abnormal expression is associated with ccRCC progression. Some studies show that KRT8 expression in ccRCC tissues and cell lines is significantly higher than in normal kidney tissue and HK-2 cells ($P<0.05$), and is positively correlated with tumor stage, Fuhrman nuclear grade, and invasion and metastasis ($P<0.05$). Patients with high expression have a poorer prognosis. Other studies show that its expression is reduced in poorly differentiated ccRCC, which may be related to the

loss of tumor epithelial phenotype. The mechanisms of abnormal KRT8 expression mainly include transcriptional regulation and post-translational modification: TFAP2A can target and bind to the KRT8 gene promoter, promoting its transcriptional expression; low TFAP2A expression in ccRCC can lead to abnormal KRT8 expression. Furthermore, miR-135a-5p can indirectly regulate KRT8 expression by inhibiting TFAP2A, forming a miR-135a-5p/TFAP2A/KRT8 regulatory axis. Phosphorylation modification can also regulate KRT8 function; PKC, MAPK, etc., can phosphorylate its serine sites, affecting its polymerization state and interactions with other proteins, participating in ccRCC progression. Immunohistochemistry shows that KRT8 is mainly expressed in the cytoplasm of ccRCC cells, with a small amount expressed in the cell membrane; abnormal expression localization may affect its function.

### 5.3. Biological Function of KRT8 in Clear Cell Renal Carcinoma and its Relationship with EMT

The biological function of KRT8 in ccRCC is related to its expression level; high expression mainly plays a pro-cancer role, while low expression may be involved in epithelial phenotype loss. High expression of KRT8 promotes the proliferation of ccRCC cells. In vitro experiments showed that overexpression of KRT8 enhanced cell proliferation and colony formation ($P<0.05$), while silencing it inhibited proliferation. The mechanism is the regulation of Cyclin D1 and p21 expression, which promotes cell cycle progression. KRT8 also promotes ccRCC cell invasion and metastasis; overexpression enhances transwell penetration and scratch healing rate ($P<0.05$). This mechanism is closely related to EMT. High expression of KRT8 inhibits E-cadherin and promotes N-cadherin and vimentin expression, thus promoting EMT transformation. It also upregulates MMP-9, accelerating extracellular matrix degradation. A few studies have shown that low expression of KRT8 inhibits ccRCC cell proliferation and promotes apoptosis, possibly related to epithelial cell cytoskeleton disruption and loss of cell polarity, leading to increased apoptosis sensitivity. Furthermore, KRT8 can serve as a potential prognostic biomarker for ccRCC. High expression in patients significantly shortened progression-free survival and overall survival ($P<0.05$). Multivariate regression analysis showed it could be used as an independent prognostic indicator (HR=2.96, 95%CI: 2.03-3.73, $P<0.05$). Combining it with miR-135a-5p and TFAP2A can further improve the accuracy of prognostic assessment.

## 6. Conclusion

Invasive metastasis of ccRCC is a major cause of poor prognosis in patients, with EMT being its core driving force. miR-135a-5p, TFAP2A, and KRT8 are all abnormally expressed in ccRCC, forming a complex regulatory network: miR-135a-5p is lowly expressed in ccRCC and regulates its expression by targeting and inhibiting TFAP2A. TFAP2A, as a tumor suppressor gene, can target and regulate KRT8 and EMT-related genes. Abnormal KRT8 expression further regulates EMT transformation and malignant behavior in ccRCC cells. All three participate in the development, invasion, metastasis, and prognostic regulation of ccRCC. Current research still has limitations: the specific binding site and regulatory details of miR-135a-5p targeting TFAP2A need further verification; the expression characteristics of KRT8 in ccRCC are controversial, and a larger sample size is needed to clarify its association with clinicopathological features; the in vivo validation of the regulatory axis of these three genes in ccRCC and their synergistic effects with other signaling pathways require further investigation. In the future, we can focus on the molecular mechanisms of the three regulatory axes, explore novel treatment strategies targeting these axes, and verify the clinical value of the three as early diagnostic, prognostic, and targeted therapeutic targets for ccRCC, providing new directions for precision treatment of ccRCC.

## Acknowledgments

2025 Innovation Project of Youjiang Medical University for Nationalities Graduate Education (YXCXJH2025017).

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