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Potential involvement of cuproptosis induced by m6A-modified autophagy gene ATG10 in KICH

Cuproptosis induced by m6A-modified ATG10 in KICH

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Abstract

Kidney Chromophobe (KICH) is the third most prevalent renal malignancy, with research challenges due to a dearth of cell lines and clinical samples. There is no specific treatment regimen tailored exclusively for KICH. This study employed gene expression analysis, immunohistochemistry (IHC), Spearman's correlation, immune cell infiltration assessment, and molecular network construction to investigate the autophagy gene ATG10 in KICH. ATG10 was uniquely downregulated in KICH, predominantly regulated by RNA m6A methylation. This downregulation correlated with patient survival, suggesting a potential tumor-regulatory role. ATG10's involvement in the protein lipidation pathway, essential for cuproptosis, was identified. B cells and CD8 + T cells were key immune cells in KICH tumorigenesis associated with ATG10. Examination of molecular networks identified several key molecules and mechanisms, including ceRNA, interplaying proteins, and transcription factors. Additionally, drug targeting analysis pointed to specific amino acids and metabolites as potential therapeutic agents. This study elucidates the significance of ATG10 in KICH, implicating m6A methylation and cuproptosis as novel targets for therapeutic intervention. The identification of B cells and CD8 + T cells as key immune components, along with specific amino acids, suggests that a combination of targeted immune therapies and dietary interventions could provide a multifaceted approach to KICH treatment. Given the limited understanding of KICH pathogenesis, our analysis has unveiled new theoretical insights and potential clinical significances for KICH, expected to broaden the research horizon in this field.

Keywords ATG10, Chromophobe, Cuproptosis, Kidney cancer, m6A

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Introduction

Kidney Chromophobe (KICH), a distinct subtype of renal cell carcinoma (RCC), accounts for about 5% of all RCC cases, representing a significant clinical entity in oncology [1]. Epidemiological data suggest that KICH is more frequently diagnosed in the fifth decade of life, with a slight female predominance [2]. The tumor is often discovered in earlier stages (I or II) due to its tendency to present as a large, solitary mass without necrosis or calcification, which may contribute to its relatively favorable prognosis compared to other RCC subtypes [2]. However, up to 10% of patients with KICH will develop metastatic disease, highlighting the need for a better understanding of the molecular mechanisms underlying this aggressive phenotype [3]. Regrettably, KICH remains an understudied subtype within the broader RCC research landscape. The rarity of KICH has limited the scope of large-scale clinical trials, and no standardized treatment for metastatic disease could be identified. The therapeutic approaches for KICH are observed to share similarities with those utilized for clear cell kidney carcinoma. However, it is important to note that, as of now, there exists no unique or exclusive treatment regimen specifically tailored for KICH. What is worse, the lack of commercial cell lines has limited the study of gene function and molecular mechanisms. Indeed, while the pathology of KICH is not extensively characterized, certain significant clinical insights have emerged regarding the mTOR pathway. Specifically, mutations in this pathway have been linked to a poorer prognosis, suggesting that they might serve as indicators for patient responsiveness to mTOR inhibitors, as reported in the studies [4] (meeting abstract, Journal of Clinical Oncology. 2020; 38 (6). https://doi.org/10.1200/JCO.2020.38.6_suppl.7). Furthermore, targeted therapy, including the mTOR inhibitor temsirolimus, has shown a durable partial response in clinical settings [5]. In contrast, the impact of mTOR inhibitors on renal clear cell carcinoma has been less than satisfactory, as noted in another study [6]. Additionally, the exploration of molecular pathologic mechanisms underlying KICH remains in its infancy, with only a limited number of studies having been conducted to date [7, 8]. So, ongoing research efforts are especially crucial for this cancer type.

The Autophagy-related 10 (ATG10) gene is a new member of the autophagy-related protein family and plays a pivotal role in the process of autophagy, a cellular mechanism for the degradation and recycling of cellular components [9]. During autophagy, ATG12 is conjugated to ATG5 by ATG7 (an E1-like protein) and ATG10 (an E2-like protein), creating a complex that is crucial for the extension of the autophagosome membrane [10, 11]. The expression level of ATG10 has been found to correlate with the susceptibility, malignancy, therapeutic response, and prognosis of various tumors [12–15]. High expression of ATG10 is associated with a poorer prognosis in some cancer types, potentially due to its role in promoting autophagy and enhancing cell survival in tumor cells [16, 17]. In renal cell carcinoma, research data are scarce and it only shows that, ATG10 is associated with characteristic eosinophilic cytoplasmic inclusions (ECIs) formation [18]. Still, the role of ATG10 in KICH has not been reported.

To overcome the technical limitations of experimental research on KICH, we here fully utilized public data resources to study the role of ATG10 in KICH. We found that ATG10 is significantly downregulated in KICH, and its expression may be regulated by RNA m6A modification, which may inhibit KICH growth by inducing cellular cuproptosis, and its special expression is beneficial for patient survival. Examination of molecular networks revealed several key molecules and mechanisms, including ceRNA (competing endogenous RNA), associated proteins, and transcription factors, that may be integral to ATG10's function in KICH. Analysis of immune cell infiltration identified B lymphocytes and CD8 + T cells as the predominant immune cell types associated with ATG10 in the context of KICH tumorigenesis. A targeted drug analysis highlighted a spectrum of amino acids and their metabolites with potential therapeutic relevance. These findings provide new insights into the pathology of KICH and the potential use of copper-based agents, cellular immunotherapy, and/or targeted dietary interventions for the treatment of KICH.

Materials and methods

Analysis of ATG10 expression patterns

Pan-cancer analysis of ATG10 expression patterns was performed using UALCAN (The University of ALabama at Birmingham CANcer data analysis Portal) [19] (https://ualcan.path.uab.edu/index.html) and TIME2 [20] (http://timer.cistrome.org/) based on The Cancer Genome Atlas (TCGA) database. Relationship data of ATG10 expression with DNA methylation level were extracted from UALCAN. Employing the GEPIA2 (Gene Expression Profiling Interactive Analysis) tool [21], we conducted Spearman correlation analyses to assess the associations between ATG10 expression and the expression levels of genes implicated in DNA methylation/demethylation, RNA m6A methylation/demethylation as well as cuproptosis pathways. coRNA (co-expression RNA) genes were extracted from GEPIA, and RBP (RNA-binding protein) genes were obtained from ENCORI (Encyclopedia of RNA Interactomes) [22]. RNA modification sites were parsed by SRAMP (Sequence-based RNA

Adenosine Methylation site Predictor) [23] and SangerBox tool.

Immunohistochemical examination of ATG10 expression in KICH

Tissue samples from two patients diagnosed with KICH were obtained from our hospital. These included samples of KICH and corresponding adjacent non-neoplastic tissue. A certified pathologist conducted a microscopic examination to ascertain the presence or absence of malignant cells. Informed consent was obtained from all participants, and the study protocol was reviewed and approved by the Ethics Committee of Gongli Hospital, ensuring compliance with ethical standards for biomedical research. The pathology laboratory processed the tissue sections, and immunohistochemical (IHC) assays were conducted in accordance with established laboratory protocols [24]. We utilized specific antibodies directed against the human ATG10 protein to detect its expression within KICH tissues. The antibodies were raised against a synthesized peptide derived from the human ATG10 protein (Accession Number Q9H0Y0), corresponding to amino acid residues I182-P220. In short, normal goat serum was utilized for blocking, followed by an overnight incubation at 4 °C for the primary antibody (Affinity Biosciences; DF8366; RRID: AB_2841630) at a dilution ratio of 1:200. And a subsequent 1-h incubation at room temperature for the secondary antibody. To visualize the ATG10 expression levels, the chromogenic substrate 3,3'-diaminobenzidine (DAB) was employed as the colorimetric developer. Subsequently, the slides were counterstained with hematoxylin to delineate the cell nuclei and facilitate the assessment of negative staining in the IHC analysis.

Assessment of tumor-infiltrating immune cell scores using the TIMER algorithm

Utilizing the TIMER algorithm (R package IOBR) in an online analytical platform (SangerBox) designed for the comprehensive evaluation of tumor-infiltrating immune cell populations, we conducted a reassessment of immune cell infiltration scores. This analysis encompassed B cells, CD4 + T cells, CD8 + T cells, neutrophils, macrophages, and dendritic cells (DCs) within individual tumors, stratified by gene expression data. Our study encompassed a substantial cohort, with infiltration scores derived from 528 samples of kidney renal clear cell carcinoma (KIRC), 65 samples of KICH, and 285 samples of kidney renal papillary cell carcinoma (KIRP). Subsequently, we employed the corr.test function within the R package psych (version 2.1.6) to compute the Spearman's rank correlation coefficient for genes and their corresponding immune cell infiltration scores across each tumor type. This statistical approach facilitated the identification of immune infiltration scores that demonstrated significant correlation with gene expression profiles.

Integrative network analyses utilizing bioinformatics tools

We conducted a series of network analyses to elucidate the interplay among proteins, genes, and their interactions within the biological context of our study. The NetworkAnalyst tool was employed for this purpose, leveraging multiple databases to construct and analyze the networks [25]. For Protein–Protein Interactions (PPIs), we utilized the STRING Interactome database with a confidence score threshold set at 900, ensuring that only interactions with high reliability and required experimental validation were included. Additionally, computationally predicted Host-Microbiome PPIs were inferred from the Domain-Domain binding MicrobioLink database.

ceRNA network was constructed by gene-miRNA interplays. Gene-miRNA interactions were identified using miRTarBase v8.0, a repository that compiles comprehensive, experimentally validated miRNA-gene interaction data. Transcription factor and gene target associations were extracted from the ENCODE ChIP-seq dataset, focusing on interactions with a peak intensity signal of less than 500 and a predicted regulatory potential score of less than 1, as determined by the BETA Minus algorithm. Protein-chemical interactions were curated from the Comparative Toxicogenomics Database (CTD). Furthermore, clinical drugs that target the genes of interest were sourced from a consortium of databases including DrugBank, ChEMBL, BindingDB, and PharmGKB, along with pertinent references indexed in PubMed. The resulting network data were visualized using Cytoscape software, which facilitated the graphical representation of the complex interactions within our dataset.

Results

Pan-cancer analysis of ATG10 expression patterns has unveiled significant alterations specifically within KICH

Utilizing TCGA database, we assessed ATG10 expression across a spectrum of 24 out of 32 tumor types. A pronounced upregulation of ATG10 was observed in the majority of these tumors (Fig. 1A). Notably, within the context of renal cancers, ATG10 exhibited a distinct expression pattern; while it remained relatively stable in the more aggressive subtypes, KIRC and KIRP, it was significantly downregulated in the comparatively less malignant KICH. This finding was substantiated through the application of an additional bioinformatic tool (Fig. 1B). Immunohistochemistry examination of ATG10 protein confirmed its decreased expression in KICH (Fig. 1C and D).

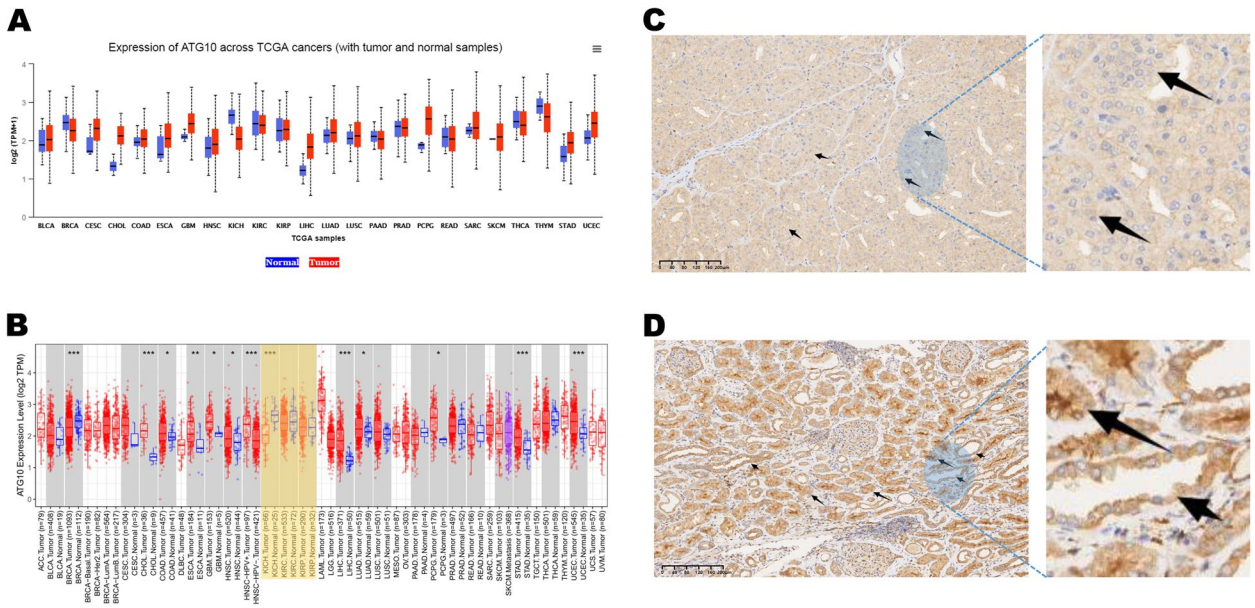

Fig. 1 Comprehensive analysis of ATG10 expression across diverse tumor types. **A** Pan-cancer analysis of ATG10 expression patterns among 24 out of 32 tumor types by UALCAN. **B** ATG10 shows significant differential expression specifically in Kidney Chromophobe (KICH), but not in Kidney Renal Clear Cell Carcinoma (KIRC) or Kidney Renal Papillary Carcinoma (KIRP), by TIME2. **C** Low expression of ATG10 protein in KICH examined by immunohistochemistry (IHC,10x). Long arrow indicates tumor cells in KICH. **D** Hight expression of ATG10 protein in the normal tissue adjacent to the carcinoma examined by immunohistochemistry (IHC, 10x). The long arrow points to the renal proximal tubule, while the short arrow denotes the collecting duct. UALCAN and TIME2 are resource-based tools

The modulation of ATG10 expression appears to be independent of gene-specific DNA methylation

In an effort to dissect the regulatory mechanisms governing ATG10 expression, we scrutinized the DNA methylation patterns within the promoter region of ATG10 in KICH. A discernible negative correlation emerged between promoter DNA methylation and ATG10 expression, in concordance with theoretical predictions. However, upon examining genes exhibiting methylation and demethylation, a positive correlation with ATG10 expression was noted, with minimal variance in correlation coefficients. This indicates a dynamic interplay within the methylation dynamics of ATG10, which maintains its methylation status, complicating the identification of the underlying mechanisms driving ATG10 expression changes (Fig. 2).

ATG10 expression regulation may be subject to RNA m6A methylation

Further delineation of the expression regulatory mechanisms led us to examine the m6A methylation status of ATG10 RNA. A robust positive correlation was identified between the expression of RNA methyltransferases and demethylases with that of ATG10, underscoring the dynamic nature of ATG10 RNA methylation (Fig. 3A-D). Notably, the m6A eraser FTO and the reader HNRNPC demonstrated a significantly more pronounced correlation with ATG10 expression, suggesting that demethylation processes may exert a more potent influence on ATG10 expression levels. Intersection analysis of the positively co-expressed genes of ATG10 in KICH with its RNA-binding proteins confirmed the presence of both FTO and HNRNPC, while the methyltransferase METTL3/14 was conspicuously absent (Fig. 3E). Detailed analysis of the m6A modification sites within ATG10 identified five sites with high and one with exceptionally high modification levels (Fig. 3F). Further, we comprehensively parsed the m1C, m5C and m6A RNA modifications of ATG10 using key proteins of RNA modification regulation. The results confirmed the m6A modification of ATG10 (Fig. 3G).

ATG10 expression may exert an effect on the survival rates of patients with KICH

To ascertain the clinical relevance of these molecular findings, we performed a survival analysis integrating ATG10 expression with that of RNA m6A modification-associated genes FTO and HNRNPC. The results indicated a negative correlation between low ATG10 expression and improved patient survival, positing ATG10 as a potential tumor regulator of significance (Fig. 4A and D). Although diminished expression levels of FTO and HNRNPC in tumor tissues were not significantly associated with overall survival, low expression of

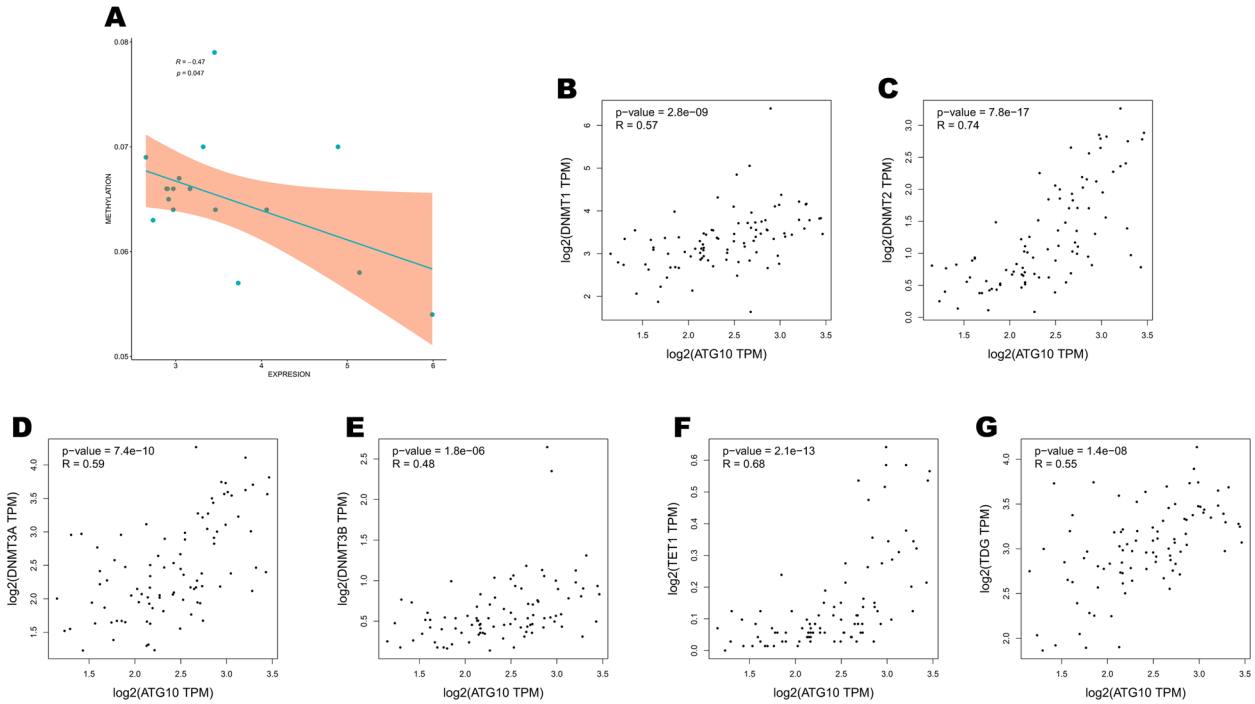

Fig. 2 Correlative analysis of ATG10 expression and DNA methylation patterns in KICH. Correlative analyses, utilizing the Spearman's Rank Correlation Coefficient, were conducted through the GEPIA platform. **A** ATG10 expression inversely correlates with DNA methylation level. **B**-**E** ATG10 expression positively correlates with DNA methylators, DNMT1, DNMT2, DNMT3A and DNMT3B. **F**, **G** ATG10 expression positively correlates with DNA demethylators, TET1 and TDG

these genes was consistently predictive of a favorable survival outcome (Fig. 4B and E, C and F).

ATG10 may precipitate cellular cuproptosis

In our quest to unravel the underlying mechanisms, we initially explored the Gene Ontology (GO) functional pathways associated with ATG10 and identified a notable lipidation category (Fig. 5A). Given the pivotal role of protein lipidation in copper homeostasis, we postulated a possible link between ATG10 and the process of cellular cuproptosis. Analysis of 10 key cuproptosis genes (CDKN2A, FDX1, DLD, DLAT, LIAS, GLS, LIPT1, MTF1, PDHA1 and PDHB) within the KICH clinical expression database yielded a significant correlation between ATG10 and these genes. Five of them had a high positive correlation index, greater than 0.5. (Figure 5B-F, Supplementary Fig. 1A-E), suggesting that ATG10 may potentially exert its regulatory effects on KICH through the mediation of cuproptosis.

Immunoinfiltration profiling of ATG10 and its associated m6A and cuproptosis genes in KICH

In this segment of our research, we undertook a targeted examination of the above mentioned eight genes (ATG10, FTO, HNRNPC, FDX1, GLS, LIPT1, MTF1, and PDHB) integral to autophagy and m6A regulation, as well as cuproptosis. Our selection was predicated on evidence from the innateDB database, which illustrates the potential for ATG10 to interact with a triad of autophagic proteins, namely ATG5, ATG7, and ATG12, to form a complex that is likely crucial for immune response modulation (Fig. 6A). This prompted an in-depth immunoinfiltration analysis of the aforementioned genes within the context of three predominant renal cancer subtypes: KICH, KIRC, and KIRP (Fig. 6B-I). Our analysis yielded intriguing findings, with all eight genes exhibiting the most robust correlations with B lymphocytes and CD8 + T cells within the KICH cohort, surpassing those observed in KIRC and KIRP. These correlations suggest a significant immunological interplay between B cells/CD8 + T cells and tumor cells in the immunoinfiltration, particularly in the context of ATG10-mediated pathways.

Delineation of central molecules through the integration of multitiered molecular networks

In our quest to elucidate the molecular underpinnings of the ATG10 gene's role in carcinogenesis, we employed a comprehensive systems biology approach.

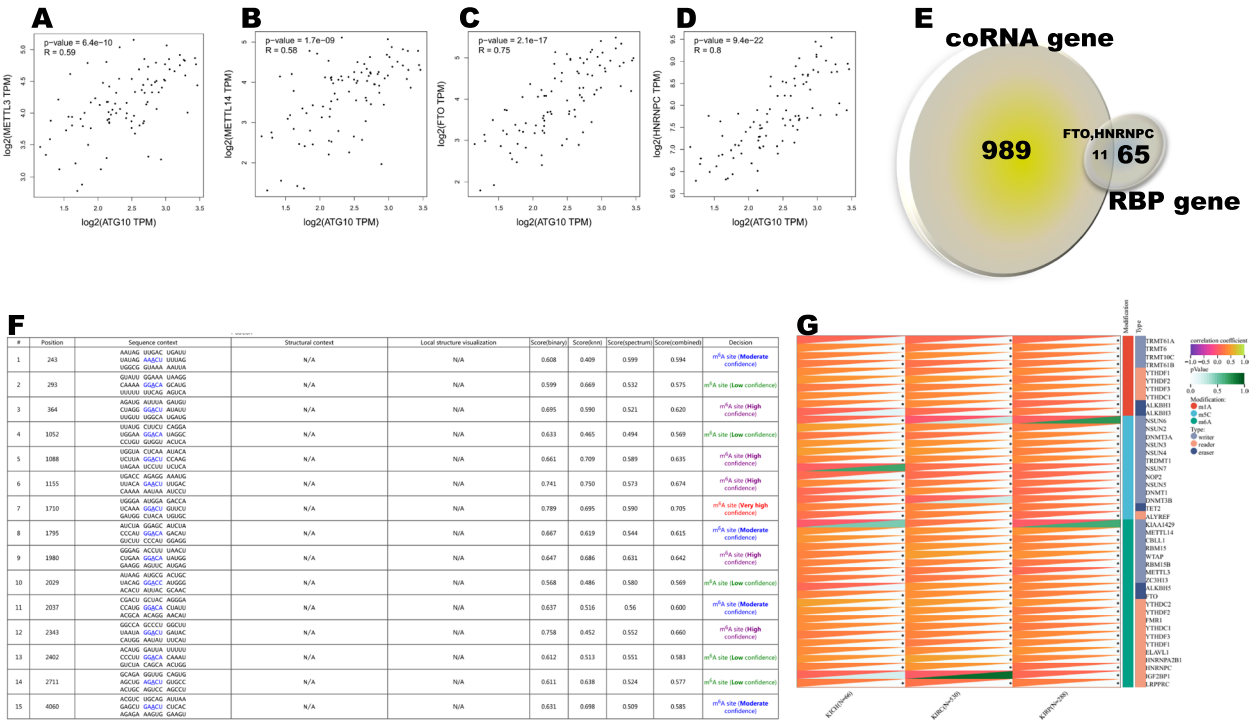

Fig. 3 Potential regulation of ATG10 expression by m6A modification. Correlative analyses, utilizing the Spearman's Rank Correlation Coefficient, were conducted through the GEPIA platform. **A**, **B** Correlations of ATG10 with RNA m6A methyltransferase genes. **C**, **D** Correlations of ATG10 with RNA m6A demethylase and reader genes. **E** Intersection of coRNA (co-expression RNA) genes and RBP (RNA-binding protein) genes using ENCORI. **F** The mapping of m6A modification sites within the ATG10 transcript reveals the presence of six sites with high to very high confidence levels. **G** Various RNA modifications (m1A, m5C, and m6A) of ATG10 was systematically analyzed based on writer, reader, and eraser, in three major renal cancers. KICH, Kidney Chromophobe; KIRC, Kidney Renal Clear Cell Carcinoma; KIRP, Kidney Renal Papillary Carcinoma

Utilizing a cohort of eight genes centered around ATG10, we established a ceRNA (competing endogenous RNA) network for ATG10. This analysis led to the identification of 31 pivotal miRNAs, which we have designated as hub molecules (depicted in the middle circle, Fig. 7A). Further exploration of the PPI (protein–protein interaction) network associated with ATG10 uncovered 17 critical proteins that may serve as hubs within this interactive framework (represented in the outer circle, Fig. 7B). Examination of the TF (transcription factor) regulatory network for ATG10 yielded 27 additional hub transcription factors (also shown in the middle circle, Fig. 7C). Our investigation was extended to the TF-microbiome network, where the central role of the aforementioned eight genes was confirmed, highlighting their significance in the broader biological context (represented by red molecules in the inner circle, Fig. 7D). These findings suggest that the identified hub miRNAs, interacting proteins, and transcription factors are likely to be integral components in the mechanistic pathways through which ATG10 contributes to the process of tumorigenesis.

Targeted identification of chemicals and pharmaceuticals acting on the ATG10-centric molecular network

In our targeted exploration of the therapeutic potential of the ATG10-centric molecular network, we systematically identified chemicals that specifically target the eight genes surrounding ATG10. This comprehensive analysis facilitated the construction of a chemical interaction network that underscored the central role of these genes within the network (depicted in the central circle, Fig. 8A). Our findings revealed that, with the exception of FTO and FDX1, the expression of the remaining six genes is modulated by copper ions (Fig. 8A). An in-depth examination of drugs that target the ATG10-centric m6A-related genes (MRGs) and cuproptosis-related genes (CRGs) yielded a list of 37 FDA-approved medications (Fig. 8B-E). Furthermore, our drug mining efforts, aimed at identifying compounds that target the broader spectrum of these eight genes, successfully identified an additional inferred 23 FDA-approved drugs (Fig. 8F-G). Interestingly, our analysis also identified a range of amino acids and their metabolites, including L-glutamine, glutamic acid, arginine, D-tyrosine, cysteine,

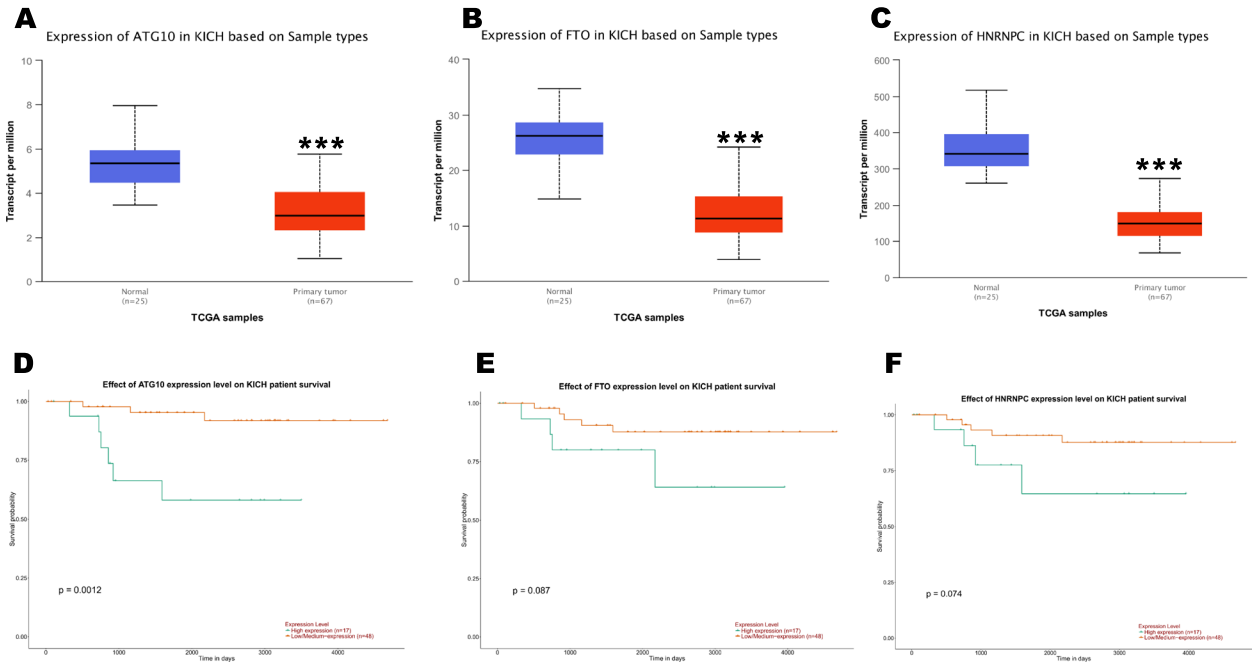

Fig. 4 Influence of ATG10, FTO, and HNRNPC expression on survival in KICH patients. The analyses were performed by UALCAN. **A**, **B** Downregulated expression of ATG10, FTO, and HNRNPC in KICH Tissues. **C**-**F** Negative association between declined levels of ATG10, FTO, and HNRNPC expression and enhanced patient survival rates. Effect of gene expression on patient survival was showed by Kaplan meier plot (High expression patients show expression value > 3rd quartile). Significance of survival impact is measured by log rank test

gamma-aminobutyric acid (GABA), asparagine, ornithine, and taurine. These findings suggest that dietary interventions, potentially enriched with these specific amino acids and metabolites, may offer a novel therapeutic strategy for KICH patients.

In aggregate, our bioinformatics analysis has revealed a molecular network, with ATG10 at its core, linked to genes involved in m6A methylation and cuproptosis. This network has potentially facilitated the identification of the critical functions of B lymphocytes and CD8 + T cells within the immune response, the dissection of central miRNAs, interacting proteins, and transcription factors, and the pinpointing of candidate therapeutic drugs and amino acids (Fig. 9). The discoveries made are of relevance to advancing our understanding and treatment strategies for KICH.

Discussion

Within the spectrum of renal carcinomas, KICH emerges as the third most prevalent variant. Despite its prevalence, clinical investigation into KICH is impeded by a paucity of available clinical samples [26], leading to a significant gap in clinical research. The overall treatment for KICH is found to be analogous to that of clear cell carcinoma. Regrettably, only a minuscule fraction of patients has access to clinical trials [27]. There is an urgent call for the development of KICH-specific clinical trials and the formulation of tailored treatment strategies to address this unmet need in the therapeutic landscape. The absence of readily accessible commercial cell lines further complicates the elucidation of its pathological underpinnings [28]. These obstacles have led to a persistent lack of clarity regarding the etiopathogenesis of KICH. All these mean that the research on the molecular basis of KICH is more urgent.

Current, albeit limited, evidence points towards two plausible underpinnings: hyperactivation of the mTORC1 pathway due to mutations in the PTEN pathway and mitochondrial dysfunction culminating in increased oxidative stress [7, 8]. Recent discoveries have identified potential therapeutic targets, including pathways involving innate lymphoid cells/IL-15 and cysteine homeostasis/ferroptosis [8]. These preliminary findings have intrigued us to delve deeper into the pathological mechanisms underlying KICH.

In this investigation, we have harnessed publicly available resources to perform a preliminary exploration into the potential regulatory mechanisms governing the growth of KICH. Our findings, though at an early stage, suggest a significant role for ATG10 in modulating KICH proliferation via the induction of cuproptosis, a process that is orchestrated through the m6A demethylation pathway. Given the burgeoning interest in m6A methylation as a pivotal epigenetic modulator, which has

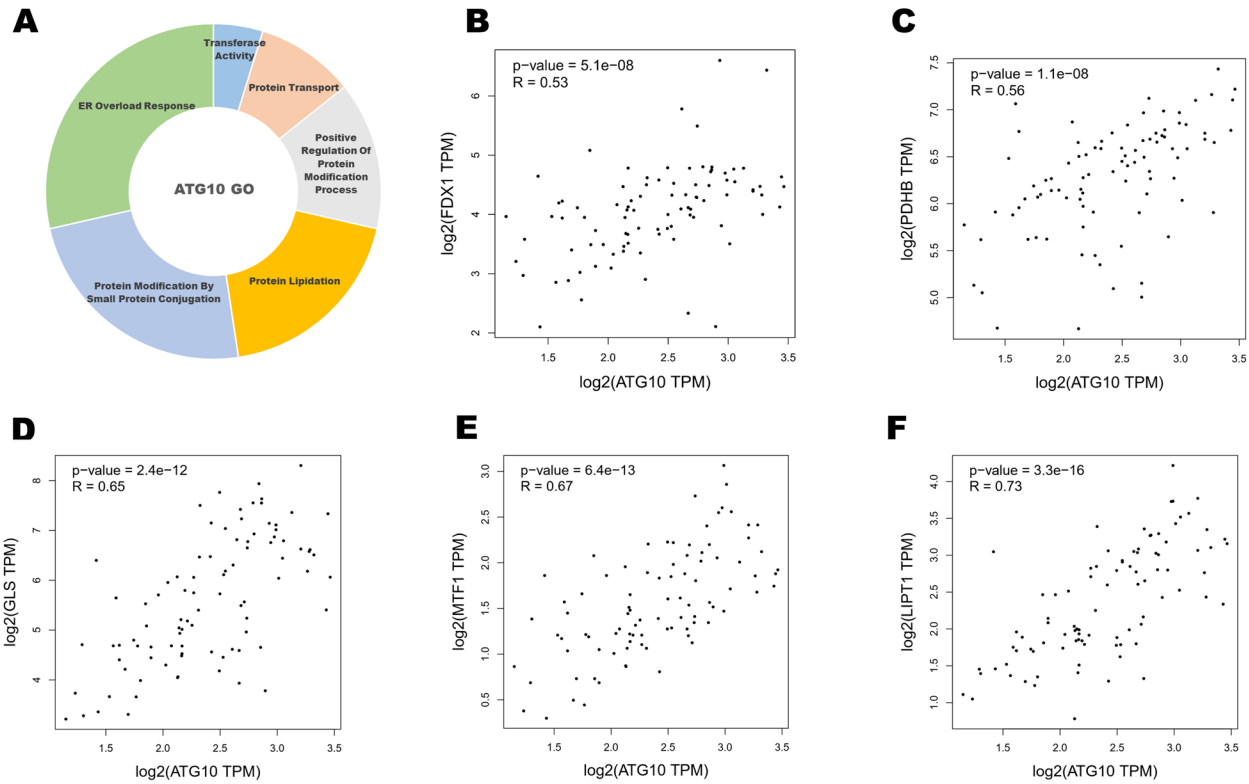

Fig. 5 Potential role of ATG10 in mediating cuproptosis in KICH. Correlative analyses, utilizing the Spearman's Rank Correlation Coefficient, were conducted through the GEPIA platform. **A** Gene Ontology (GO) Enrichment Analysis of ATG10 elucidates the significant association of ATG10 with the process of protein lipidation. **B**-**F** Significant positive correlations were observed between ATG10 and key cuproptosis-associated genes, including FDX1, PDHB, GLS, MTF1, and LIPT1

emerged as a central player in a myriad of physiological processes and disease etiologies in recent years [24, 29, 30], our study contributes to the expanding body of literature in this domain. We conducted an analysis of the putative m6A modification sites within ATG10, a strategy that paves the way for the targeted selection of candidates for future experimental validation and potential drug target intervention, as illustrated in Fig. 3F. Furthermore, the correlation analysis of genes associated with m1A, m5C, and m6A modifications, as depicted in Fig. 3G, has laid a theoretical foundation that is instrumental for the advancement of subsequent mechanistic and interventional studies. While we have performed a correlation analysis between DNA methylation, RNA methylation, and the expression of the ATG10 gene, it is important to acknowledge the inherent complexity of the dynamic interplay between methylation and demethylation processes. Additionally, bioinformatics analyses, with the exception of Mendelian analyses which can suggest causal relationships, typically reveal correlations rather than establishing causality. Consequently, our methylation analysis serves as a preliminary reference that should be further explored through validation and application in subsequent studies. Concurrently, the discovery of cuproptosis as a novel form of programmed cell demise, only recently characterized [31, 32], adds a layer of complexity and novelty to our understanding of cellular demise mechanisms. The implications of our research are twofold: firstly, it sheds light on the potential molecular underpinnings of KICH, which may be critical for advancing our comprehension of its pathogenesis; and secondly, it paves the way for the potential development of targeted therapeutic interventions employing epigenetic modulators and copper-based agents. Given the current paucity of understanding regarding the pathological mechanisms of KICH, our study, which concentrates on ATG10, m6A methylation, and cuproptosis, is poised to offer potentially novel or complementary insights into the pathogenesis of KICH.

The immunological landscape of KICH remains largely uncharted, with research hindered by a paucity of clinical samples and established cell lines. Our immuno-infiltration analysis has identified a potential role for B cells and CD8 + T cells in KICH, particularly in the context of ATG10 expression, which is linked to autophagy—a process known to influence immune responses [33]. While

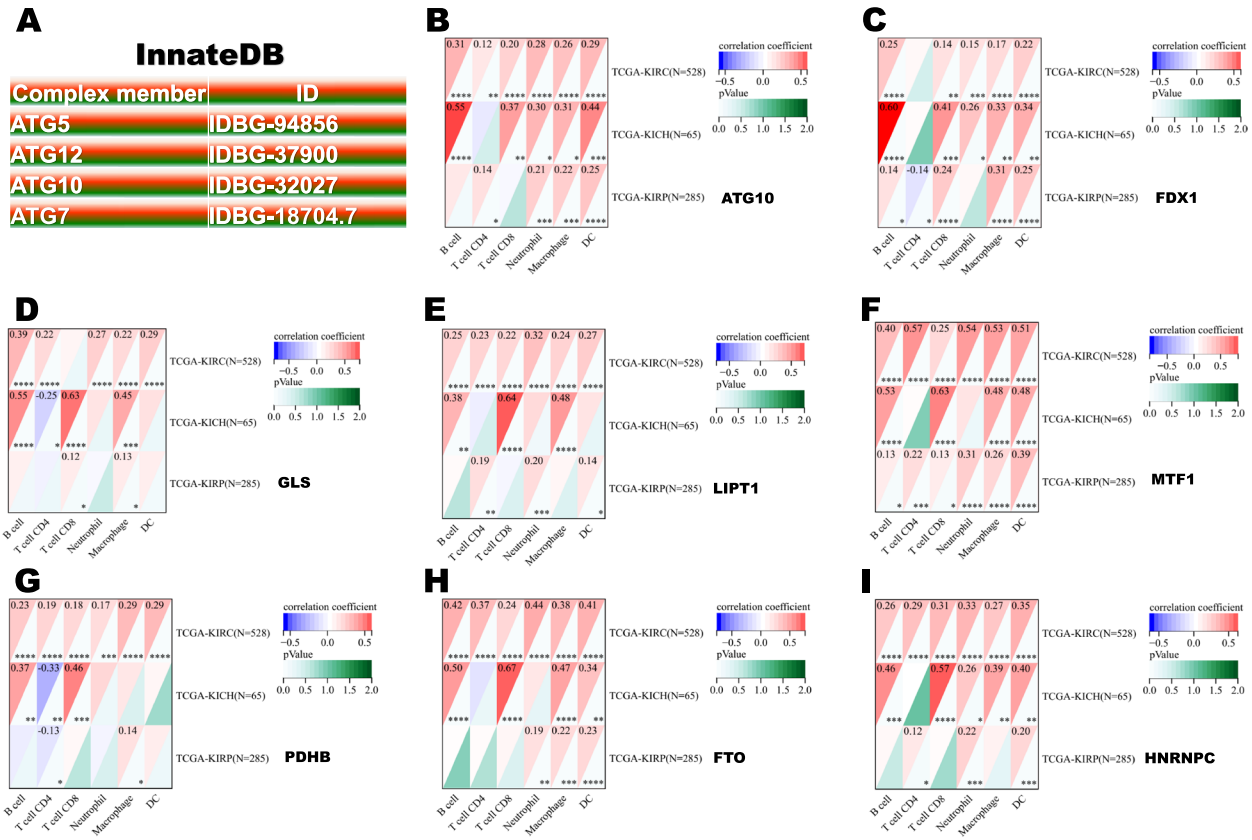

Fig. 6 Immunoinfiltration analysis of ATG10-circled MRGs and CRGs in three major renal cancers. Immunoinfiltration analysis is conducted using SANGERBOX, which features an integrated TIMER algorithm. B cells, CD4, CD8, neutrophil, macrophage, DC (dendritic cell) are parsed. **A** Annotation of immune protein complex including ATG10 in the InnateDB database. **B**-**I** Immune infiltration analysis of ATG10 in three major renal cancers. MRGs, m6A-related genes; CRGs, cuproptosis-related genes; KICH, Kidney Chromophobe; KIRC, Kidney Renal Clear Cell Carcinoma; KIRP, Kidney Renal Papillary Carcinoma

specific functions of these immune cells in KICH are not yet fully elucidated, their presence suggests a complex interplay within the tumor microenvironment (TME). In the broader RCC context, CD8 + T cells are recognized for their cytotoxic properties, and B cells are known to modulate immune responses [34], indicating their potential significance in KICH as well. The role of ATG10 and its impact on immune cell function in KICH warrant further investigation, as does the development of novel models to better recapitulate KICH pathology and facilitate more in-depth immunological studies. Despite the limited research on KICH, insights from other RCC subtypes may provide valuable context and reveal new therapeutic opportunities.

Our research has identified a set of amino acids and their metabolites including L-glutamine, glutamic acid, arginine, D-tyrosine, GABA, asparagine, ornithine, and taurine. This selection process highlights their potential role in modulating gene expression and, consequently, the biological significance of KICH. For example, L-glutamine's role in fueling cancer cell proliferation suggests its strategic importance in the disease's progression [35]. Arginine, with its involvement in immune function and nitric oxide production, is another key player in the complex interplay between cancer cell biology and the immune system [36, 37]. The therapeutic potential of these amino acids and metabolites is further underscored by their impact on TME. Taurine, for instance, has demonstrated anti-inflammatory and antioxidant properties that could significantly influence the TME and immune response to cancer [38]. GABA's regulatory function in cellular stress responses also positions it as a promising target for novel cancer therapies, given its associations with cell cycle arrest and apoptosis [39]. Controlling the levels of several AAs (e.g., cysteine, methionine, and leucine) and lipids was important for the anticancer activity of the diets in mice with RCC [40]. The involvement of cysteine uptake in exchange for glutamate in cancer cell ferroptosis, a new cell death model, complicates its role in tumors [41]. Asparagine enhances LCK signaling

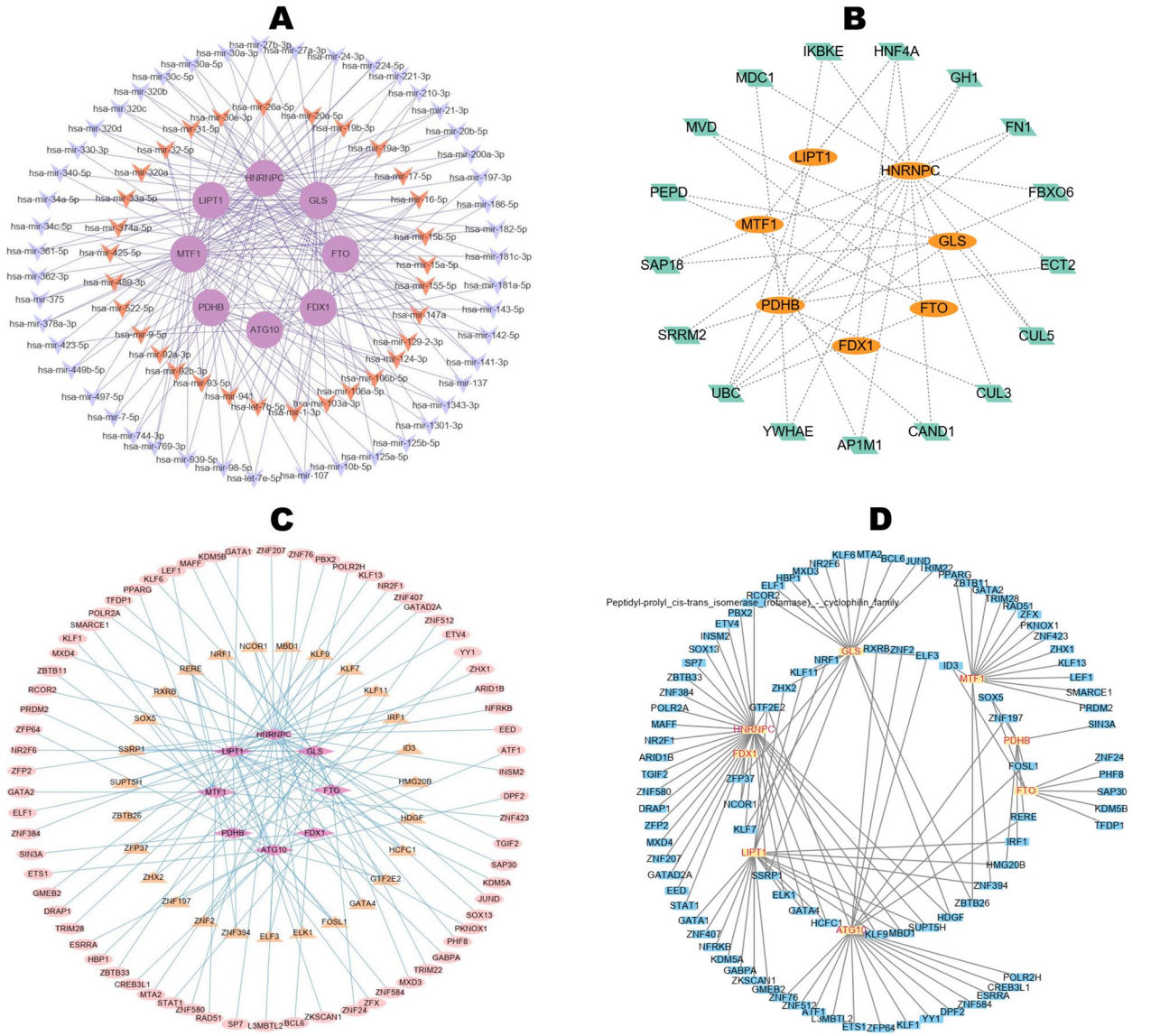

Fig. 7 Identify hub genes/molecules by constructing networks based on MRGs and CRGs. **A** ceRNA network of ATG10. **B** PPI network of ATG10. **C** TF network of ATG10. **D** TF-microbiome network of ATG10. ceRNA, competing endogenous RNA. PPI, protein–protein interaction. TF, transcription factor. MRGs, m6A-related genes. CRGs, cuproptosis-related genes

to potentiate CD8 + T-cell activation and anti-tumour responses [42]. Aliphatic polyamines, putrescine, spermidine and spermine, are a family of polycationic molecules derived from decarboxylation of ornithine. Aliphatic polyamines play an essential role in rapidly dividing cells and are also involved in carcinogenesis, suggesting a target in cancer therapy [43]. These findings underscore the need for a deeper investigation into the role of amino acid metabolism in KICH. Future research should explore the effects of these specific amino acids and metabolites on the clinical outcomes of KICH patients and the broader implications for cancer treatment. Understanding the metabolic pathways affected by these molecules could reveal new therapeutic targets and strategies for managing KICH. Furthermore, given that these amino acids and metabolites were identified from ATG10-centric MRGs and CRGs, it implies that they play a role in modulating the broader molecular network. This is instrumental in unraveling novel mechanisms underlying the pathology of KICH.

The expression analysis results of ATG10 in KICH present a contradiction with the survival analysis results (Fig. 4). The relationship between gene expression and survival rates can be quite intricate and is influenced by a

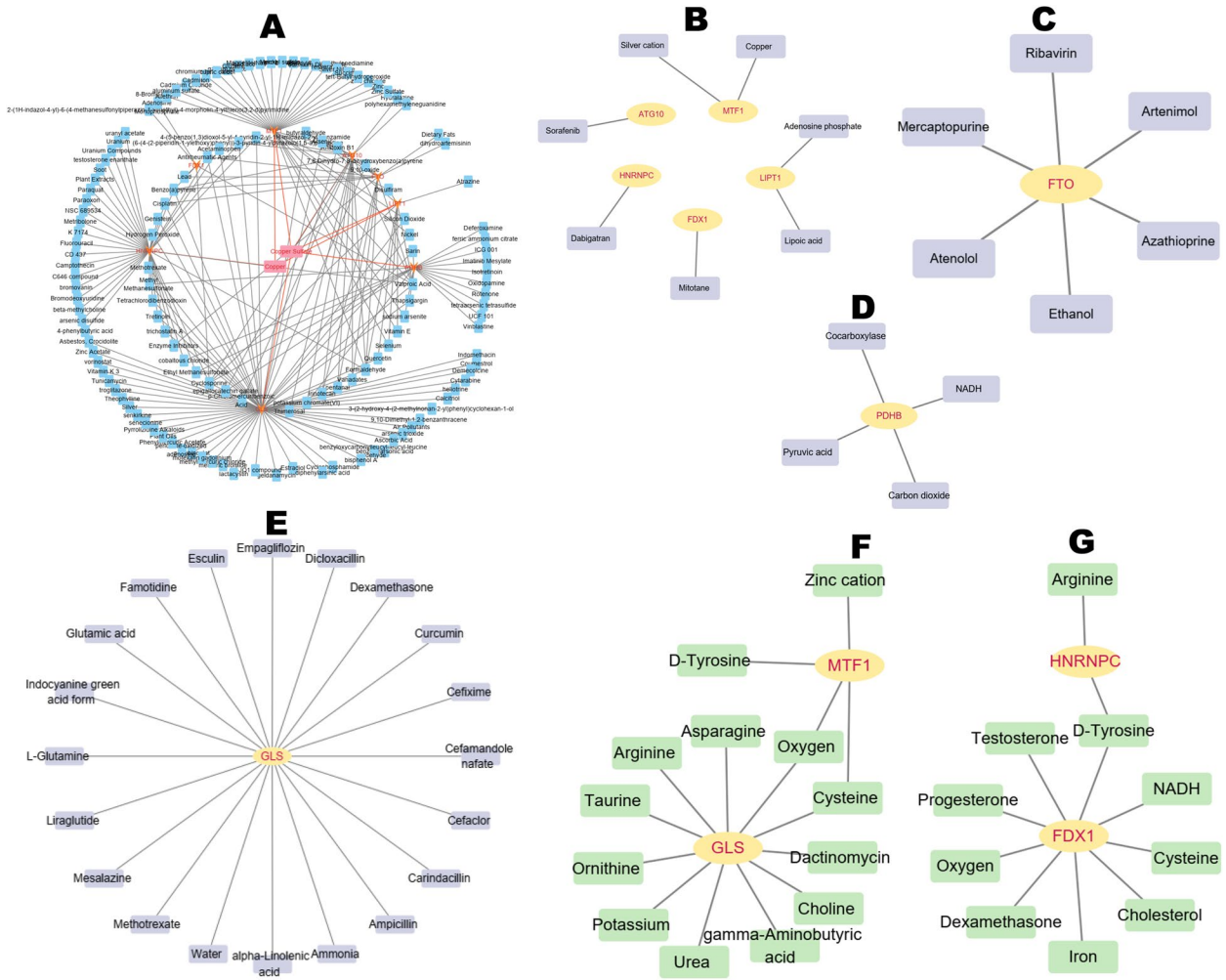

Fig. 8 Excavate chemicals and drugs targeting ATG10-circled molecules (MRGs and CRGs). **A** Chemical network targeting ATG10-circled MRGs and CRGs. **B**-**F** Confirmed FDA-approved drugs targeting ATG10-circled MRGs and CRGs. **G** Inferred FDA-approved drugs targeting ATG10-circled MRGs and CRGs. MRGs, m6A-related genes. CRGs, cuproptosis-related genes

multitude of factors. It is not uncommon in gene expression survival analyses to encounter seemingly paradoxical results where a gene is under-expressed in tumors, yet its over-expression correlates with poorer survival rates. Potential reasons for this contradiction include the complexity of gene functions, intricate network interactions, and the impact of various confounding factors such as the age and gender of the samples, tumor staging, and treatment modalities. This indicates the need for further stratification and investigation of this type of tumor. However, due to the rarity of samples, reaching a clear conclusion may be a protracted process.

While our findings are indeed comprehensive and intriguing, it is imperative to exercise caution when interpreting our conclusions, which are derived from informatics analysis, given the absence of supporting cell and animal experimental data. Recognizing the necessity for further investigation, we are actively engaged in the establishment and popularization of KICH cell lines, an indispensable tool for dissecting the molecular mechanisms at play. This endeavor is poised to serve as a cornerstone for future research, with the ultimate aim of fostering significant progress in the field of KICH research and clinical management.

Conclusion

Despite the research barriers in KICH due to the scarcity of commercial cell lines and limited clinical samples, this study utilized public databases and online tools to investigate the autophagy gene ATG10's role in KICH development. ATG10 exhibited a unique expression pattern in KICH, contrasting with other renal carcinomas like KIRC and KIRP, and could be predominantly regulated by RNA m6A methylation rather than

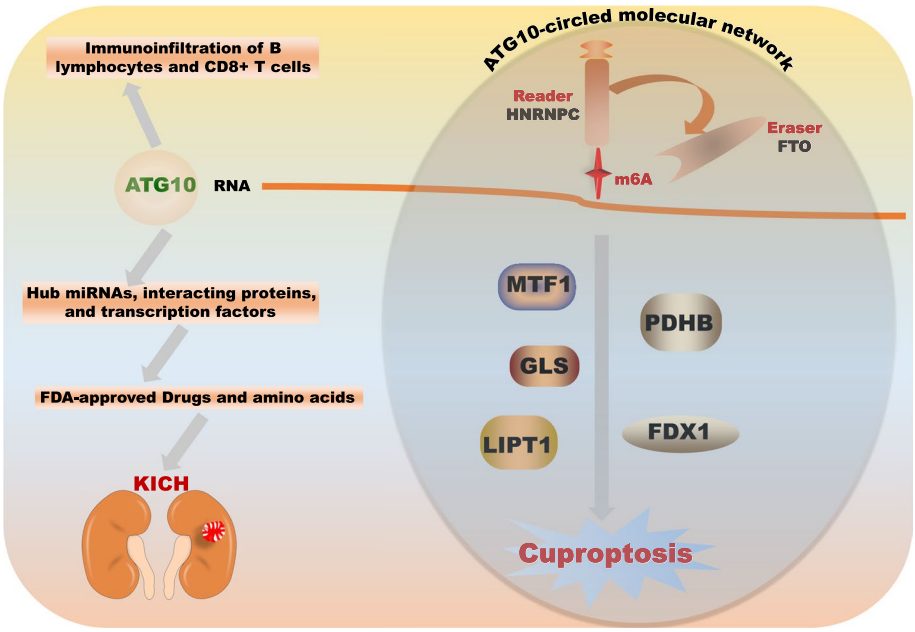

Fig. 9 Schematic Summary of the Core Findings of This Study. A molecular network has been outlined, with ATG10 at its core, and is intricately linked to m6A modification and genes implicated in cuproptosis. Through this network analysis, the significant roles of B cells and CD8 + T cells in immune infiltration have been revealed. It has defined hub miRNAs, interacting proteins, and transcription factors. Furthermore, potential targeted drugs and amino acids for therapeutic intervention have been identified. These findings are of considerable significance, offering new avenues for the research and treatment of KICH

DNA methylation. Low ATG10 expression levels were significantly correlated with improved patient survival, suggesting a potential tumor-regulatory function. The gene's integral role in the protein lipidation pathway, essential for cuproptosis, was established through Spearman's correlation and gene-copper interaction network analysis, indicating a regulatory impact on this novel cell death mechanism. Furthermore, the study revealed a complex interplay of molecular networks, including ceRNA mechanisms, associated proteins, and transcription factors, central to ATG10 function in KICH. Immune cell infiltration analysis identified B lymphocytes and CD8 + T cells as key components in KICH tumorigenesis related to ATG10. A targeted drug analysis pointed to specific amino acids and metabolites with therapeutic potential. In light of the limited understanding of the pathological mechanisms underlying KICH, our comprehensive, multi-faceted mechanistic study introduces a novel theoretical framework for deciphering the complexities of KICH pathogenesis. Collectively, this study pioneers the examination of m6A methylation and cuproptosis in KICH, presenting potential new avenues for therapeutic intervention. It highlights the potential of leveraging immune cell interactions and targeted dietary interventions as innovative therapeutic strategies for KICH patients.

Abbreviations

KICH	Kidney Chromophobe
ceRNA	Competing endogenous RNA
IHC	Immunohistochemistry
RCC	Renal cell carcinoma
ATG10	Autophagy-related 10
ECIs	Eosinophilic cytoplasmic inclusions
UALCAN	The University of ALabama at Birmingham CANcer data analysis Portal
TCGA	The Cancer Genome Atlas
GEPIA2	Gene Expression Profiling Interactive Analysis
coRNA	Co-expression RNA
RBP	RNA-binding protein
ENCORI	Encyclopedia of RNA Interactomes
SRAMP	Sequence-based RNA Adenosine Methylation site Predictor
DAB	3,3′-Diaminobenzidine
DCs	Dendritic Cells
PPIs	Protein-Protein Interactions
CTD	Comparative Toxicogenomics Database
KIRC	Kidney renal clear cell carcinoma
KIRP	Kidney renal papillary cell carcinoma
GO	Gene Ontology
TF	Transcription factor
MRGs	M6A-related genes
CRGs	Cuproptosis-related genes
GABA	Gamma-aminobutyric acid
TME	Tumor microenvironment

Supplementary Information

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Supplementary Material 1.

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Authors' contributions
Conception and design: Xiangqi Li; Administrative support: All authors; Provision of study materials: Qingyun Zhu, Daiquan Fu, Zhaohui Zhu, Jian Wu, Chuan Chen, Yanxiang Li; Collection and assembly of data: Qingyun Zhu, Daiquan Fu, Zhaohui Zhu; Data analysis and interpretation: All authors; Manuscript writing: Xiangqi Li; Final approval of manuscript: All authors.

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Data availability
All data generated or analysed during this study are included in this published article [and its supplementary information files].

Declarations

Ethics approval and consent to participate
Informed consent was obtained from the patients. The study protocol was reviewed and approved by the Ethics Committee of Gongli Hospital. All procedures performed in studies involving human participants were in accordance with the ethical standards of institutional and/or research committee, and with the 1975 Declaration of Helsinki, as revised in 2013.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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