Contents lists available at ScienceDirect

Translational Oncology

The controversial role and therapeutic development of the m6A demethylase FTO in renal cell carcinoma

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ARTICLE INFO

ABSTRACT

Fat mass and obesity-associated (FTO) protein, the first m^{6}A demethylase identified in 2011, regulates multiple aspects of RNA biology including splicing, localization, stability, and translation. Accumulating data show that FTO is involved in numerous physiological processes and is implicated in multiple cancers including renal cell carcinoma (RCC). However, the exact role of FTO in RCC remains controversial. Some studies demonstrated that decreased FTO expression was associated with aggressive clinical features and shorter overall survival in clear cell RCC (ccRCC) patients, while others found that FTO inhibition selectively reduced the growth and survival of VHL-deficient ccRCC cells *in vitro* and *in vivo*. Here, we review the evidence supporting either a promoting or suppressive role of FTO in kidney cancers, the mechanisms of action of FTO, and recent progress in developing FTO inhibitors.

Introduction

The global prevalence and mortality rate of renal cell carcinoma (RCC) has significantly increased over the past decades. In the U.S., it is estimated that 79,000 people will be diagnosed with cancers in the kidney and renal pelvis resulting in 13,920 deaths in 2022 [1]. Targeted therapies, including tyrosine kinase inhibitor-based anti-angiogenic therapies and immune checkpoint inhibitor-based immunotherapies, have been the mainstay for RCC [2]. However, metastatic RCC remains incurable not only because of its insensitivity to conventional radio- and chemo-therapies, but also due to its intrinsic and acquired resistance to targeted therapies [3]. Moreover, the efficacy, response rate, and adverse event rate of first-line therapies are not satisfactory, under-scoring the urgency to develop new therapeutic targets and strategies for metastatic RCC [4]. Only through improving our understanding of the underlying molecular mechanisms involved in the disease process will we be better equipped to fight the global burden of kidney cancer.

Fat mass and obesity-associated (FTO) gene, so-named after genome-wide association studies established a strong correlation between single-nucleotide polymorphisms in FTO and human obesity almost a decade after FTO was first cloned [5], has recently been implicated in multiple RCC subtypes, with clear cell RCC (ccRCC) being the most well studied.

N^6-methyladenosine (m^{6}A), one of the most abundant epitranscriptomic modifications of messenger RNA (mRNA) and non-coding RNA (ncRNA) in eukaryotic cells, is its main substrate, making FTO the first m^{6}A demethylase identified [6]. m^{6}A modifications regulate almost every stage of RNA metabolism [7], which in turn affects many biological processes including metabolism [8], innate immunity [9], DNA repair [10], and programmed cell death [11], as well as various diseases including obesity [12] and many types of cancers [13]. Not surprisingly, FTO, as one of only two known m^{6}A erasers [14], plays an important role in all of these biological processes and human diseases, particularly in cancers [15]. The protein structure, molecular targets, and biological functions of FTO across cell lines and tissues, especially its context-dependent specificity toward its substrates, have been reviewed in detail [16].

FTO plays context-dependent tumor-suppressive or oncogenic roles in various solid cancers, usually in an m^{6}A-dependent manner, through modulating a variety of cellular processes including metabolism, cancer stem cell self-renewal, epithelial-mesenchymal transition, immune response, and drug resistance [15,17]. In diverse cancer types, FTO can either promote or suppress tumor progression; however, in a subset of cancers including RCC, its exact role is still controversial (Table 1).

We will focus mainly on studies pertaining to ccRCC, as it is the most

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prevalent subtype of RCC (80% of diagnoses), while briefly highlighting the available data relating to less common subtypes, papillary RCC (pRCC) and chromophobe RCC (chRCC). To date, 9 studies on the role of FTO in ccRCC have been published, and all within the last three years. Out of the nine studies discussed in detail in the next sections, six indicated that FTO is tumor-suppressive [18–23], while three suggest it is oncogenic [24–26]. Here, we summarize current evidence supporting a promoting or suppressing role of FTO in RCC progression in pre-clinical models, as well as its value as a prognostic marker in RCC patients. Finally, we will discuss the ongoing efforts to develop selective and potent FTO inhibitors and their potential as therapeutic agents in RCC.

FTO expression and prognostic value in RCC

FTO expression in ccRCC

We and others have analyzed 539 ccRCC samples and 72 normal kidney samples from The Cancer Genome Atlas (TCGA) and found that FTO mRNA expression was significantly increased in ccRCC with VHL deletions/mutations or intact VHL compared to normal kidney tissue [25,26]. Examination of the E-MTAB- 6692 dataset, a meta-dataset comprising a total of 347 samples including both primary tumors and tumor-free renal tissues from six independent GEO datasets, confirmed FTO overexpression in ccRCC compared to normal renal tissues [25]. Additionally, analysis of transcriptomic and proteomic data of

treatment-naive ccRCC and paired normal tissues in the Clinical Proteomic Tumor Analysis Consortium (CPTAC) containing 110 treatment-naive ccRCC and 84 matched normal samples revealed that FTO is overexpressed at both the transcriptomic and protein levels in tumor tissues relative to normal tissues [25]. Immunohistochemical analysis of FTO expression in a tissue microarray containing 30 pairs of ccRCC and adjacent tissue further confirmed that FTO protein levels are increased in ccRCC compared to normal tissue [25]. Finally, in another six pairs of fresh ccRCC specimens and adjacent tissue, FTO expression was increased in ccRCC compared to normal tissue as determined by qPCR and western blot [25]. These studies concur that FTO expression is increased in ccRCC relative to normal kidney tissue.

However, Zhuang et al. reported that FTO transcript levels were decreased in ccRCC by qRT-PCR in a cohort of 35 ccRCC and adjacent normal tissues, and FTO protein level was reduced in 4 pairs of ccRCC tissues compared with adjacent normal tissues by western blot [22]. Moreover, Strick et al. observed decreased FTO protein expression in 147 ccRCC compared to 30 normal renal tissues by immunohistochemistry, while FTO transcript levels were similar between 166 ccRCC and 106 normal renal tissues by qRT-PCR [19]. The basis of these divergent findings regarding relative levels of FTO transcripts and protein in ccRCC compared to normal kidney is unclear, although most point to increased expression of FTO in ccRCC. Consistent with these findings, Hua et al. reported that in 402 Wilms tumor patients and 1198 healthy controls, the rs8047395 A allele polymorphism in FTO was significantly correlated with elevated FTO expression and increased risk

Table 1
The role of FTO in different cancer types.

Cancer	Role	Expression in cancer	Proliferation/ apoptosis	Migration/ invasion	Tumor growth	Metastasis	Survival	Target
BC	Pro[28, 29]	↑		↑		↑	shorter, and higher grade	miR-181b-3p
BlaC	Pro[13, 30]	↑	↑	↑	↑		shorter, and higher stage	miR-576, PYCR1
CerC	Pro[31, 32]	↑	↑	↑				HOXC13
EC	Pro[33]	↑		↑	↑	↑		HOXB13
ESCC	Pro[34, 35]	↑	proliferation↓/ Apoptosis↑		↑			LINC00022, SIM2
GC	Pro [36–39]	↑	proliferation↑	↑			shorter	ITGB1, CAV1
Glioma	Pro[40]		proliferation↑					MYC
HNSCC	Pro[41]	↑	proliferation↑	↑			shorter	CTNNB1
LC	Pro [42–46]	↑	proliferation↑/ Apoptosis↑	↑	↑	↑	shorter	E2F1
Melanoma	Pro[47]	↑	proliferation↑	↑	↑			PD-1, CXCR4, SOX10
OSCC	Pro [48–52]	↑	proliferation↑	↑	↑		shorter	PD-L1, MYC, YAP1, eIF4G1, CCND1
PCa	Sup [53–55]	↓	proliferation↓	↓	↓		NC, but lower grade and stage	MC4R
PTC	Sup[56]	↓	proliferation↓		↓	↓		APOE
CC	Sup [57–59] Pro[60, 61]	↓ ↑	proliferation↓ proliferation↑	↓	↑	↓	longer	PGC-1α, MTA1 MZF1/c-MYC, PD-L1
HC	Pro[62] Sup [63–65]	↑ ↓	proliferation↑ proliferation↓/ Apoptosis↑	↑ ↓	↓	↓	shorter, and higher grade longer	GNAO1
OC	Pro[66] Sup[67]	↑ ↓	proliferation↑/ Apoptosis↓ proliferation↓		↑ ↓			AKT PDE1C, PDE4B
PC	Pro[68] Sup[69]	↑ ↓	proliferation↑ proliferation↓	↓			longer, and lower stage	PJA2
ccRCC	Pro[24-26] Sup [18–23]	↑ ↓	proliferation↑ proliferation↓/ Apoptosis↓	↑	↑ ↓	↑	longer	SLC1A5, BRD9 PGC-1α

OSCC-Oral squamous cell carcinoma GC-Gastric cancer PCa-Prostate cancer PTC-Papillary thyroid cancer BlaC-bladder cancer BC-Breast cancer CC-Colon cancer HC-hepatocellular carcinoma HNSCC-Head and neck squamous cell carcinoma CerC-Cervical cancer ESCC- Esophageal squamous cell carcinoma OC-Ovarian cancer ccRCC-Clear cell renal cell carcinoma LC-Lung cancer PC-Pancreatic cancer EC-Endometrial cancer NC-no correlation Pro: proliferation; Sup: suppression.

for Wilms tumor, the most common pediatric renal malignancy [27]. Although the expression level of FTO in ccRCC tissues remains divisive, these results suggest FTO might play an important oncogenic role in kidney cancer in both children and adults.

FTO as a prognostic marker in ccRCC

In the TCGA ccRCC dataset, we found that FTO mRNA expression levels, while elevated, did not vary significantly across stages I-IV VHL-deficient ccRCC tumors [25]. Chen et al. reported that 5 of the 16 m^{6}A RNA methylation regulators were prognostic in the TCGA ccRCC dataset; however, FTO expression was not associated with overall survival (OS) [26]. In contrast, two other studies described a positive correlation of FTO transcript levels with both OS and disease-free survival using the TCGA ccRCC dataset [18,22]. Similarly, Wen et al. showed that FTO mRNA expression in the lower quartile of the TCGA ccRCC cohort was associated with poor prognosis [20]. Moreover, Strick et al. revealed that lower FTO transcript levels were correlated with shorter OS and cancer-specific survival (CSS) in 166 ccRCC patients, while FTO protein levels did not predict OS in 147 ccRCC patients [19]. It is difficult to resolve the underlying reasons why different results can come from analysis of the same TCGA dataset of >500 ccRCC samples, because several of these studies have not included information on how patients were stratified into comparison groups. Unfortunately, only a single study has investigated FTO protein levels and their association with disease outcomes, meaning that additional study of protein and RNA levels will be necessary to test whether FTO can serve as a prognostic biomarker in ccRCC.

FTO expression and clinical significance in pRCC and chRCC

pRCC and chRCC show varying FTO mRNA and protein expression levels in the few studies published on these subtypes. Analysis of the TCGA dataset showed pRCC had significant upregulation of FTO mRNA, though not as high as ccRCC, while chRCC expressed the lowest FTO mRNA levels out of the three subtypes [31]. However, in a separate cohort utilizing primary RCC tumor tissue with 40 patients representing each subtype, pRCC and chRCC both had significantly lower FTO mRNA levels than ccRCC, with pRCC displaying the lowest FTO expression [31]. Immunohistochemistry staining showed chRCC had the lowest FTO protein expression, while pRCC showed high protein levels [18]. Thus, while transcript levels of FTO in pRCC are low, its protein levels are high, pointing towards the involvement of post-transcriptional modifications in FTO expression in pRCC. In concordance with these results, Strick et al. found pRCC had the highest FTO protein levels of the three main subtypes, whereas chRCC showed a lower FTO protein level compared to benign tissue [19]. As the studies of FTO expression in non-ccRCC are limited, there is currently no decisive conclusion that can be drawn regarding differential gene or protein expression in these subtypes.

Xiang et al. generated in silico models of pRCC and chRCC based on m^{6}A methylation patterns and pathological tissue typing. Their study showed pRCC, which had the highest FTO expression of the subtypes in the model, yielded poorer prognosis than chRCC [48]. Yet, in a TCGA analysis, increased FTO expression at the mRNA level led to a greater progression free survival and OS in pRCC [18]. Although pRCC and chRCC are less common than ccRCC, as the global incidence of RCC continues to rise, so too do the diagnoses of these subtypes. Further investigation into the role of FTO in these subtypes will prove necessary to better understand their disease mechanisms.

Role of FTO in ccRCC progression

Additional controversy surrounds the role of FTO in the biology of ccRCC progression. One study, published in 2018, demonstrated that ectopic overexpression of FTO inhibited growth and induced apoptosis

in 786-O and 769-P, two commonly used ccRCC cell lines, by increasing oxidative stress [22]. FTO-overexpressing ccRCC cells also grew significantly slower *in vivo* in a subcutaneous xenograft model, suggesting a tumor-suppressive role of FTO in ccRCC [22]. Later, the same group found that miR-155 knockdown attenuated cell proliferation and induced apoptosis in 786-O cells by upregulating FTO, confirming the tumor-suppressive role of FTO in ccRCC [23]. However, we recently showed that inhibition of FTO either genetically or pharmacologically selectively reduced the growth, survival, and clonogenic capability of VHL-deficient 786-OM1A cells, but not isogenic VHL-reconstituted cells, in a HIF-independent manner *in vitro* and inhibited orthotopic xenograft growth *in vivo*, suggesting that FTO plays an oncogenic role only in the context of VHL-deficient ccRCC cells [25]. Consistent with our findings, Zhang et al. reported that FTO knockdown significantly inhibited 786-O cell proliferation and clonogenic capability, albeit the magnitude of inhibition was smaller in HIF2α wild type cells than in HIF2α knockdown cells, suggesting the oncogenic function of FTO is more significant in the absence of than in the presence of HIF2α in ccRCC cells [24]. Clearly, FTO plays a vital role in ccRCC progression which appears to be affected by loss of VHL, and its exact role will be defined as more data is available.

Targets of FTO in ccRCC

As one of the two m^{6}A erasers, FTO affects the expression of several important genes involved in RCC development and progression. We performed an integrated analysis of transcriptome-wide m^{6}A-seq and mRNA-seq analysis and identified the glutamine transporter SLC1A5 as a FTO target that promotes metabolic reprogramming and survival of VHL-deficient ccRCC cells [25]. Our study further confirmed that SLC1A5 mRNA and protein levels were decreased upon FTO knockdown in VHL-deficient 786-O and UMRC2 ccRCC cells, and the level of m^{6}A at the 5' UTR and 3' UTR of SLC1A5 was increased in these cells [25]. Moreover, SLC1A5 knockdown recapitulated the phenotype of FTO knockdown including decreased glutamine consumption in VHL-deficient ccRCC cells, suggesting that FTO regulates metabolic reprogramming of VHL-deficient ccRCC cells by targeting SLC1A5 [25]. In another study, overexpression of FTO reduced the level of m^{6}A PGC-1α mRNA and upregulated PGC-1α protein expression in 769-P cells, suggesting PGC-1α is another direct target of FTO [22]. Overexpression of FTO restored mitochondrial activity, induced oxidative stress and reactive oxygen species (ROS) production, and impaired tumor growth through increasing expression of PGC-1α that was largely reversed by PGC-1α knockdown, indicating PGC-1α is a functionally important target of FTO that facilitates its tumor-suppressive activity [22]. This is consistent with a recent PAN-cancer study mining 102 transcriptomic datasets for the expression of 29 m^{6}A-RNA methylation regulators, including FTO, in 41 diseases and cancers, which demonstrated that 40 out of 165 ROS regulators were modulated by FTO [70]. BRD9 has also been identified as a direct target of FTO. In the Caki-2 ccRCC cell line, which expresses low levels of HIF2α, RNA immunoprecipitation revealed direct binding of BRD9 pre-mRNA with FTO [22]. In these HIF2α-low but not HIF2α-high ccRCC cells, FTO knockdown stabilized BRD9 mRNA and increased its protein expression level, in turn facilitating Caki-2 cell growth and rendering the cells sensitive to BRD9 inhibitors. These results indicate that BRD9 is an effective target for treating HIF2α-low ccRCC [22]. Additional targets of FTO will likely emerge as candidate genes identified by large-scale screening, such as transcriptome-wide m^{6}A-seq and mRNA-seq analysis, are validated experimentally.

Regulation of FTO expression in ccRCC

How FTO expression is regulated in cells is largely unknown, and only two studies have investigated regulation of FTO expression in ccRCC. FTO belongs to the family of Fe(II)–α-ketoglutarate (αKG)–

dependent dioxygenases that require iron and ascorbate for enzymatic activity. Zhang et al. showed that an increased intracellular α-ketoglutarate–to-succinate ratio in HIF2α-low Caki-2 ccRCC cells led to activation of FTO, which in turn demethylated and stabilized BRD9 mRNA [24]. In addition, Yang et al. used a bioinformatic approach to identify miR-155 as a negative regulator of FTO expression through direct binding to the 3′UTR of FTO mRNA [23]. They also showed that overexpression of miR-155 increased global mRNA m^6A levels, enhanced tumor cell proliferation *in vitro* and *in vivo*, and decreased apoptosis in a FTO-dependent manner, demonstrating the functional importance of miR-155 in regulating FTO expression and global mRNA m^6A levels in ccRCC [23]. In normal human kidney HEK293 cells, FTO mRNA and protein levels are dramatically downregulated by essential amino-acid deficiency but not by deprivation of nonessential amino acids [71]. It will be interesting to determine whether these regulation mechanisms of FTO expression are employed by ccRCC cells.

Therapeutic inhibition of FTO

Given the important clinical applications that effective FTO inhibitors could have in human diseases, particularly in obesity and cancer [5,72], efforts have been made to identify and develop selective FTO inhibitors. This work has been facilitated by the resolution of the crystal structure of FTO in 2010 which showed strong $Fe2^+$- and αKG-dependent activity as a dioxygenase at its N-terminal and, later, as an m^6A demethylase [6,73]. The structural insights into FTO, strategies to achieve selective inhibition of FTO, methods of screening FTO inhibitors, and currently known inhibitors of FTO as well as their mode of action have been reviewed in detail [72]. Most FTO inhibitors show some off-target effects due to non-selective inhibition of related enzymes. For instance, rhein, a natural product that inhibits FTO by competitively binding to the substrate binding site of FTO, also inhibits αKG-dependent dioxygenase B (AlkB) by binding to its 2-oxoglutarate (2OG) binding site [74]. Moreover, the efficacy of the known FTO inhibitors is sub-optimal with an IC50 in the micromolar range. However, two small molecule inhibitors, CS1 and CS2, have been identified using an *in silico* structure-based screen and were validated as showing efficacy in inhibiting the growth of human leukemic cell lines. These inhibitors have an IC50 ranging from 22 to 410 nM depending on the cell line tested [75]. Whether these candidate inhibitors are effective against solid tumors is unknown, and their mechanisms of action have not been defined. Clearly, additional work will be necessary to identify novel potent and selective FTO inhibitors with low toxicity.

Several FTO specific inhibitors have been developed in recent years, including FB23-2, one of the most potent and selective FTO inhibitors that has shown efficacy in mouse models of cancer [72]. Three newly identified FTO inhibitors were reported in the past two years. In 2021, using information from crystal structures of FTO complexed with 2OG and substrate mimics, Shishodia et al. designed and synthesized two series of FTO inhibitors, including a highly potent inhibitor 14a (IC50 80 nM). With a selective binding interaction spanning the FTO 2OG and substrate binding sites, this compound warrants further optimization for *in vivo* studies to determine its activity in combating FTO-mediated diseases [76]. In a second study, Prakash et al. used a novel approach of merging fragments of previously reported FTO inhibitors and synthesized compound 11b with an IC50 of 87 nM. Treatment of AML cells with a prodrug of 11b decreased cell viability, increased global m^6A levels, and induced downregulation of MYC and upregulation of RARA, two known FTO target genes [77]. Huff et al. combined structure-based drug design and molecular docking using the Schrödinger software suite to target the MA binding site of FTO. They identified 20 small molecules with low micromolar IC50s and significantly higher specificity toward FTO compared to ALKBH5, a Fe(II)–α-ketoglutarate (αKG)– dependent dioxygenase family member [78]. One of these competitive inhibitors, FTO-04, increased m^6A and m^6A(m) levels in glioblastoma stem cells (GSCs) and prevented neurosphere formation in patient-derived GSCs

without inhibiting the growth of healthy neural stem cell-derived neurospheres, suggesting FTO-04 is a potential new lead for treatment of glioblastoma [78]. In 2022, Xie et al. developed two small molecule inhibitors of FTO (18077 and 18097) by conducting virtual screenings and structural optimization. Specifically, 18097 (IC50 0.64 μM) bound to the active site of FTO, resulting in an increased m^6A level of suppressor of cytokine signaling 1 (SOCS1) mRNA. This led to the recruitment of IGF2BP1 to increase mRNA stability of SOCS1, activating the p53 signaling pathway. Activation of p53 inhibited cell cycle progression and decreased the migration of breast cancer cells *in vitro* and tumor growth and lung metastasis *in vivo*. In addition, 18097 suppressed cellular lipogenesis via downregulation of peroxisome proliferator -activated receptor gamma (PPARγ), CCAAT/enhancer-binding protein alpha (C/EBPα), and C/EBPβ, suggesting 18097 modulates multiple FTO-regulated cellular processes in breast cancer [79]. Whether these novel inhibitors will serve as potent anti-cancer agents in RCC needs to be investigated.

Conclusions

FTO plays an important but controversial role in RCC, and additional work will be necessary to precisely define its function in this disease. FTO has been implicated as both oncogenic and tumor suppressive in multiple RCC subtypes, even when the same dataset or model systems are used, which may be attributed to differences in data analysis methods and experimental procedures. Very likely, the differences in the effects of FTO are context-specific, such as the synthetic lethality observed in VHL-null ccRCC. FTO is aberrantly expressed and associated with clinical prognosis in ccRCC. Its dysregulation in ccRCC is associated with altered cell proliferation, invasion, and metastasis both *in vitro* and *in vivo*. Mechanistically, FTO exerts its activity through the regulation of m^6A levels of target genes including SLC1A5, BRD9, and PGC-1α in ccRCC. Significant progress has been made in the development of FTO inhibitors, however, novel strategies such as molecular degrader-induced targeted protein degradation [80] have not been tested in therapeutic inhibition of FTO. Further investigations are needed to better understand the role of FTO in RCC to help determine the potential of developing FTO inhibitors as novel therapeutic agents to treat RCC.

CRediT authorship contribution statement

Dalin Zhang: Writing – original draft. **Sarah Wornow:** Writing – review & editing. **Donna M. Peehl:** Writing – review & editing. **Erinn B. Rankin:** Writing – review & editing. **James D. Brooks:** Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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