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Original Contribution

FTO mRNA expression in the lower quartile is associated with bad prognosis in clear cell renal cell carcinoma based on TCGA data mining

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ABSTRACT

Fat mass and obesity associated (FTO) is a protein-coding gene, also known as the obesity gene. It has been reported previously to be associated with a variety of malignant cancers, such as breast, thyroid and acute myeloid leukemia. The aim of the present study was to investigate the FTO mRNA expression in human clear cell renal cell carcinoma and its clinical value. FTO mRNA expression and its prognostic value were investigated by bioinformatic analysis of the data from The Cancer Genome Atlas (TCGA, https://cancergenome.nih.gov/). The Kaplan-Meier analysis showed that FTO mRNA expression in the lower quartile is significantly associated with poor survival in clear cell renal cell carcinoma patients ($P < 0.0001$). This study indicated that higher FTO mRNA expression may have a protective role and it may be a vital molecular marker in the prognosis of clear cell renal cell carcinoma patients.

1. Introduction

Renal cell carcinoma (RCC) is among the 10 most common cancers in both men and women, representing 3.7% of all new cancer cases [1]. Clear cell renal cell carcinoma (ccRCC) is the main type of the histological classification of RCC, which has a worse prognosis compared with the papillary RCC (pRCC - typeI and II) and chromophobe RCC (chRCC) even after stratification for stage and grade [2,3]. The overall prognosis of ccRCC is still poor, especially for patients who present with high-stage disease, although the 5-year survival rates have shown some improvement [1,4].

Tumor stage (TNM) is recognized as one of the strongest prognostic factors in the clinical outcome of patients with RCC [5]. The 5-year recurrence free-survival of patients with stage I is over 92%, whereas the rate of recurrence for those with stage II and III disease is up to 40% [6,7]. The University of California Los Angeles Integrated Staging System (UISS) has been developed for classification of patients into low-risk, intermediate-risk, and high-risk prognostic groups based on stage, Fuhrman nuclear grade, and Eastern Cooperative Oncology Group performance status [8]. Such prognostic score is established and used for balancing and comparing groups of patients in clinical studies, but with less usefulness for individualized prediction. Analysis of the association between different gene signatures and clinical outcomes has been explored to provide further information beyond traditional clinicopathologic parameters. The incorporation of gene-based prognostic tools into routine clinical practice, however, awaits further study.

Studies in ccRCC have been revealing and leading to the concept that ccRCC is a metabolic disease [9,10]. CcRCC is generally accompanied by reprogramming of glucose, fatty acid metabolism, as well as the tricarboxylic acid cycle, and these changes may provide opportunities for new therapeutic strategies and biomarkers [11,12]. FTO was known to be associated with body mass and obesity in humans [13] and its over-expression affected the energy metabolism of cancer cells [14]. As the first identified RNA demethylase that regulates the demethylation of target mRNAs, FTO has been reported to regulate dopaminergic signaling in brain [15]. Recently, FTO has been proved to play a critical oncogenic role in acute myeloid leukemia (AML) [16]. In the present study, we used the data from TCGA of ccRCC to explore the relationship between FTO mRNA expression and the survival, which may provide a new potential molecular marker in the prognosis of ccRCC patients.

2. Materials and methods

2.1. Collection and processing of RNA-Seq and the clinical data

In creating the data set, raw RNA-Sequencing reads and the corresponding clinical information for a total of 536 ccRCC and 72 normal samples were downloaded from the TCGA Data Portal. All the RNA expression levels of the samples had been processed and normalized with the Bioconductor packages in an R statistical environment (version

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Table 1
Survival difference between stages (TNM) in ccRCC samples.

Stages	*P* value (log-rank test)
Stage I vs stage II	No significance**
Stage I vs stage III	$P < 0.0001$***
Stage I vs stage IV	$P < 0.0001$***
Stage II vs stage III	$P = 0.0105$*
Stage II vs stage IV	$P < 0.0001$***
Stage III vs stage IV	$P < 0.0001$***

* $P < 0.05$.
** $P > 0.05$.
*** $P < 0.001$.

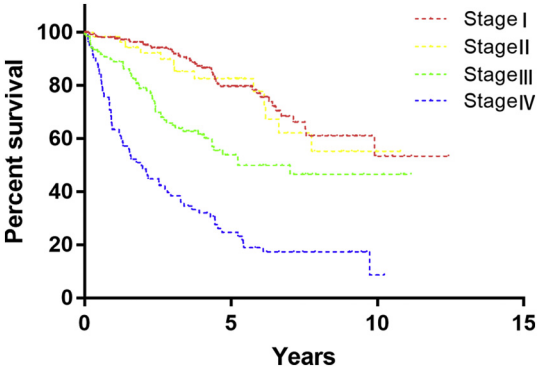

Fig. 1. Survival analysis of different stages (TNM) in ccRCC samples.

Table 2
Survival difference between different quartiles of FTO mRNA expression in ccRCC samples.

Different quartiles	*P* value (log-rank test)
Lower quartile vs second quartile	$P = 0.0024$**
Lower quartile vs third quartile	$P = 0.0006$***
Lower quartile vs upper quartile	$P = 0.0013$**
Second quartile vs third quartile	No significance*
Second quartile vs upper quartile	No significance
Third quartile vs upper quartile	No significance

* $P > 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

3.4.0). FTO mRNA expression data matrix and clinical information files were matched by sample ID for each sample.

2.2. Statistical analyses

Firstly, the samples were divided into different groups and did the survival analysis according to tumor stages (TNM). Secondly, the total samples were assigned into four groups based on the expression level of FTO mRNA and did the survival analysis with different cutting-off points (the lower quartile, second quartile, third quartile, and upper quartile). Thirdly, correlation between FTO mRNA expression and the overall survival of the patients in different stages was assessed using the lower quartile FTO mRNA expression value as a cutting-off point. GraphPad Prism 7.0 was used for statistical analyses. The Kaplan-Meier method and the log-rank test were used to compare the overall survival rate between the FTO high-expression group and the low-expression group. We did multivariate analysis of FTO mRNA and tumor stage to evaluate their prognostic value in the survival of ccRCC patients by Cox regression model. $P < 0.05$ was considered to indicate a statistically significant difference.

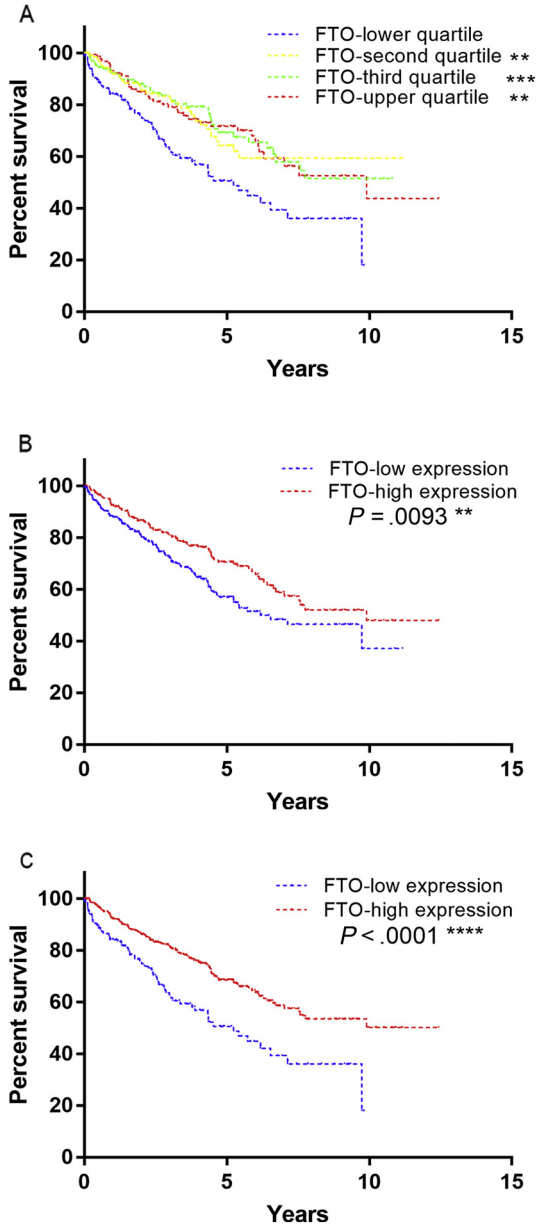

Fig. 2. Kaplan-Meier analysis of FTO mRNA expression and overall survival in total ccRCC samples. (A) Kaplan-Meier analysis of FTO mRNA expression and overall survival between different quartiles. Kaplan-Meier analysis of FTO mRNA expression and overall survival when using the median value (B) or lower quartile (C) as the cutting-off point.

3. Results

3.1. Survival analysis of different stages (TNM) in ccRCC samples

Time to death was plotted in a Kaplan-Meier curve for those cases exhibiting in different tumor stages (Stages I–IV). Except for the survival rate between Stage I and Stage II, all the rest showed significant differences between each other (Table 1). At the same time, the survival rate decreased with the increase of stages (Fig. 1), which was a further confirmation of tumor stage as one of the strongest prognostic factors in the patients with ccRCC.

3.2. Prognostic value of FTO mRNA expression in total ccRCC samples

The total samples were assigned into four groups based on the

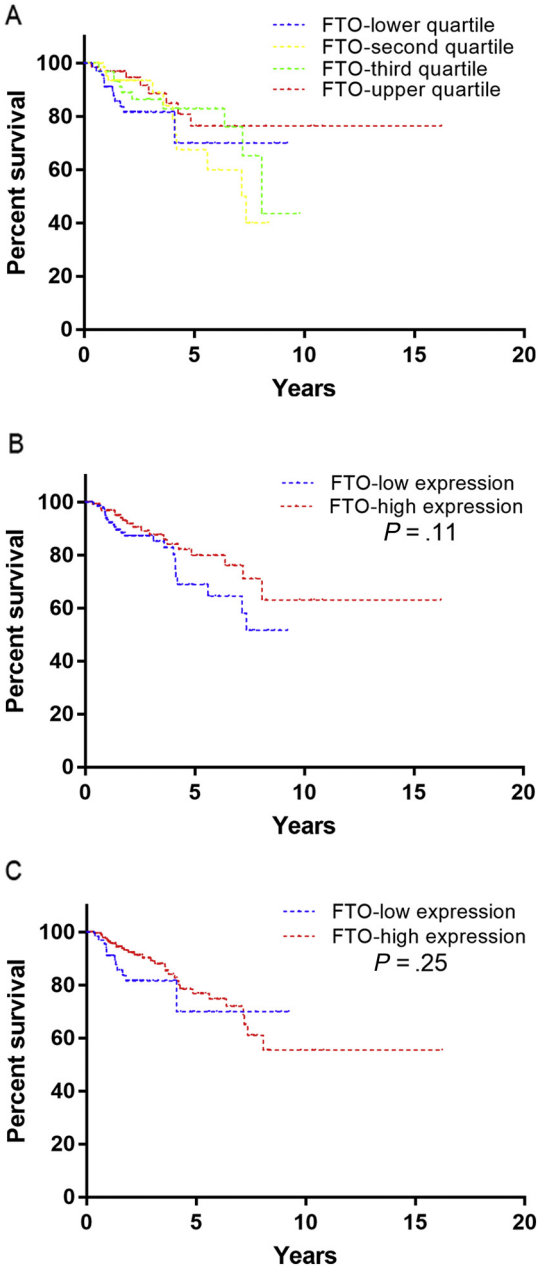

Fig. 3. Kaplan-Meier analysis of FTO mRNA expression and overall survival in total pRCC samples. (A) Kaplan-Meier analysis of FTO mRNA expression and overall survival between different quartiles. Kaplan-Meier analysis of FTO mRNA expression and overall survival when using the median value (B) or lower quartile (C) as the cutting-off point.

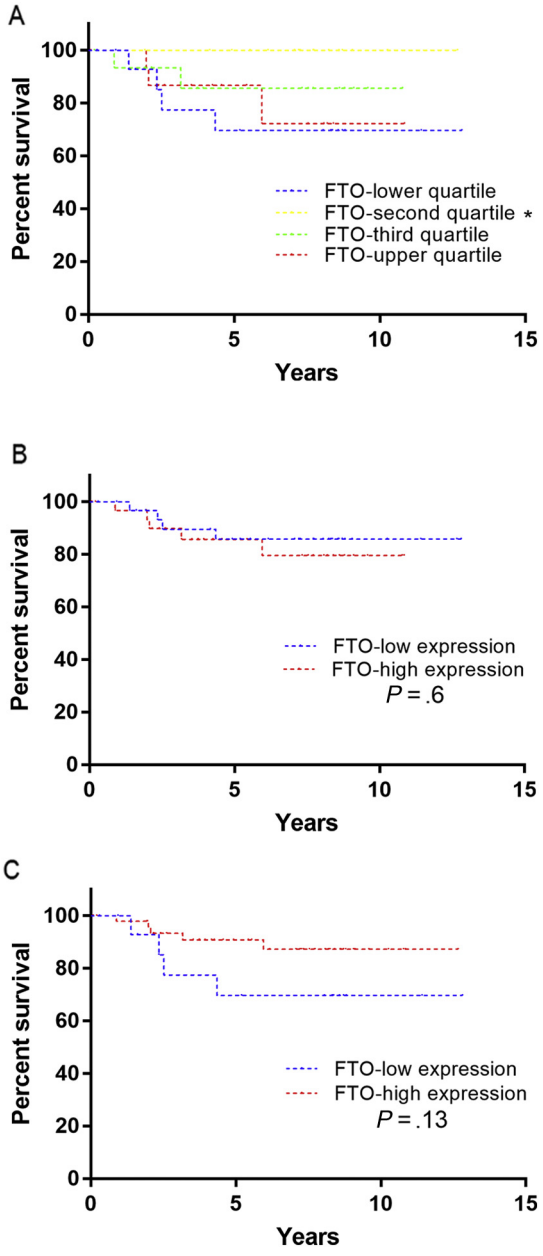

Fig. 4. Kaplan-Meier analysis of FTO mRNA expression and overall survival in total chRCC samples. (A) Kaplan-Meier analysis of FTO mRNA expression and overall survival between different quartiles. Kaplan-Meier analysis of FTO mRNA expression and overall survival when using the median value (B) or lower quartile (C) as the cutting-off point.

expression level of FTO mRNA and did the survival analysis between each other (the lower quartile, second quartile, third quartile and upper quartile). There were significant differences between the lower quartile with all the other three quartiles (Table 2, Fig. 2A). Higher expression of FTO mRNA was related with good prognosis in ccRCC when using the lower quartile or median value as the cutting-off point (Fig. 2B–C). However, this phenomenon was not seen in the papillary RCC ($P > 0.05$, Fig. 3A–C) and chromophobe RCC ($P > 0.05$, Fig. 4A–C).

Using univariate analysis, FTO mRNA and TNM tumor stage are important prognostic parameter. We also did multivariate analysis of FTO mRNA and tumor stage to evaluate their prognostic value in the survival of ccRCC patients by Cox regression model. The results showed that FTO mRNA value was the independent prognostic factors ($P = 0.003$, Table 3).

Table 3
Multivariate analysis of prognostic value of FTO-low expression (FTO mRNA expression in the lower quartile) in the survival of ccRCC patients by Cox regression model.

Parameters	HR (95% CI)	P value
FTO-low expression	1.620 (1.181–2.221)	$P = 0.003$**
Tumor stage	1.868 (1.635–2.135)	$P < 0.001$***

** $P < 0.01$.
*** $P < 0.001$.

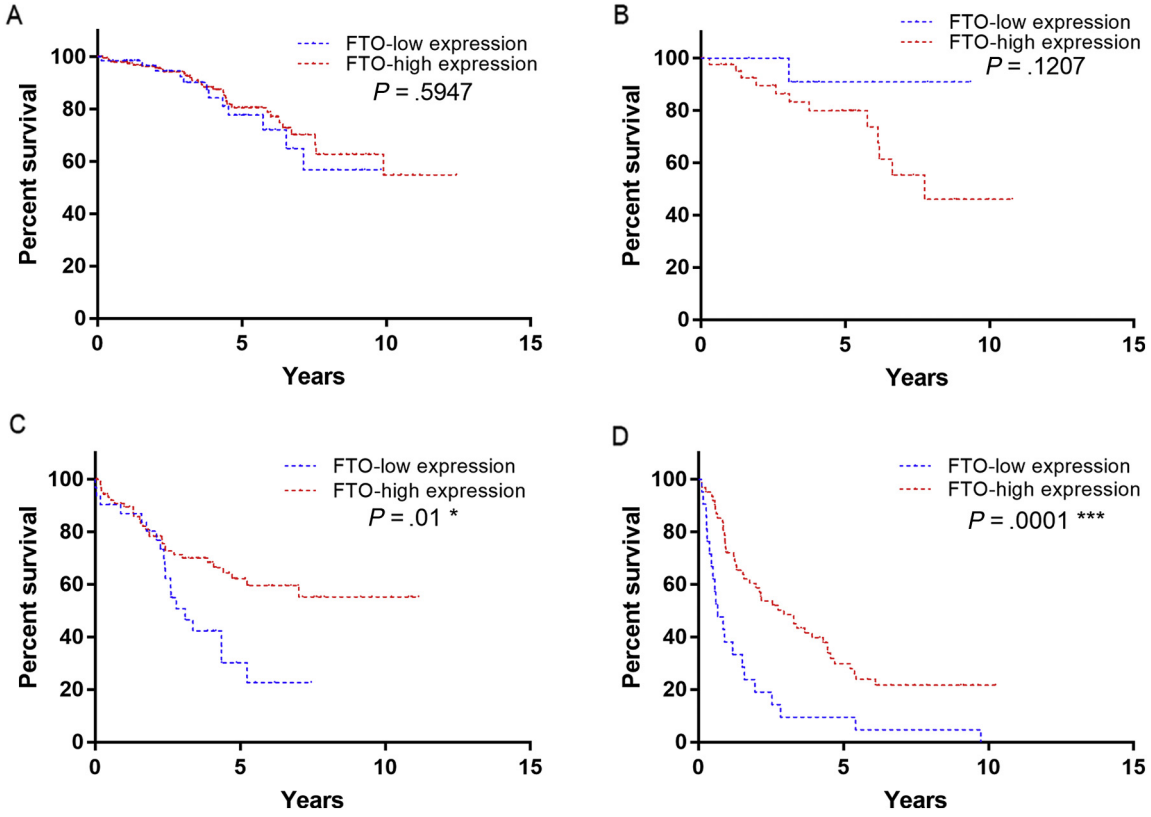

Fig. 5. Kaplan-Meier analysis of FTO mRNA expression and survival in different stages (TNM) of ccRCC samples. (A) Stage I. (B) Stage II. (C) Stage III. (D) Stage IV.

3.3. Prognostic value of FTO mRNA expression in different stages (TNM) of ccRCC samples

The correlation between FTO mRNA expression and the overall survival of the patients in different stages was assessed using the lower quartile (the first quartile) FTO mRNA expression value as the cut-off. There was no statistical significance in the relation of FTO mRNA expression level with the survival in stage I and stage II (Fig. 5A–B). Higher expression of FTO was related with good prognosis in stage III and stage IV (Fig. 5C–D). The results showed that the prognostic value of FTO mRNA expression in the lower quartile increased with the increase of tumor stage.

4. Discussion

We did the survival analysis of different stages (TNM) in ccRCC samples with the data from TCGA. The results illustrated that, except for the survival rate between Stage I and Stage II, all the rest showed significant differences between each other and the survival rate decreased with the increase of stages. All these above were a solid confirmation of tumor stage as one of the strongest prognostic factors in the patients with ccRCC. At the same time, it proved that the methods we applied in the data analysis were believable. The total samples were assigned into four groups based on the expression level of FTO mRNA and did the survival analysis between each other (the lower quartile, second quartile, third quartile and upper quartile). During this process, we found that the lower quartile of FTO mRNA expression was the best cutting-off point to show the prognosis value of FTO. Higher expression of FTO mRNA was related with good prognosis in ccRCC. According to the results above, higher expression of FTO was related with good prognosis in stage III and stage IV when the lower quartile (the first quartile) FTO mRNA expression value was used as the cut-off. These results showed that the prognostic value of FTO mRNA expression in the lower quartile increased with the increase of tumor stages.

Obesity, measured as body mass index (BMI), waist circumference (WC), waist-to-hip ratio (WHR) or increase in weight, is a well-established risk factor for ccRCC in both women and men [17,18]. Although obesity is associated with an increased incidence of ccRCC, patients of ccRCC with obesity show longer survival than non-obese patients [19]. In a single-centre clinical study of 2119 patients with ccRCC, obese patients were less likely to present with cancer of higher stage and grade compared with normal weight patients [20]. Choi et al. demonstrated that high BMI was associated with significantly longer cancer-specific survival (CSS) in a meta-analysis of 14 studies [21]. In addition, Donin et al. identified that obese patients had a statistically significant overall survival (OS) advantage over the normal body weight ones in a large, prospective and international study of patients with ccRCC [22]. The phenomenon of an increased risk of disease but improved survival with obesity is known as “obesity paradox”. Such paradoxes have been described in type 2 diabetes [23] and congestive heart failure [24]. However, the mechanisms underlying this phenomenon are poorly understood.

In conclusion, FTO was known to be associated with body mass and obesity in humans. We found that FTO mRNA expression in the lower quartile is significantly associated with bad prognosis in ccRCC patients ($P < 0.0001$) and the prognostic value of FTO mRNA expression in the lower quartile increased with the increase of tumor stages. We supposed that FTO may play an important role in the biological association between obesity and the survival of ccRCC patients. Further studies are needed to explore the mechanisms underlying the relationship.

Conflict of interest

The authors declare that they have no competing interests.

Acknowledgments

Not applicable.

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