Fat mass and obesity-associated protein promotes the tumorigenesis and development of liver cancer

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Abstract. Liver cancer is the fourth leading cause of cancer-associated mortality worldwide. Statistics indicate that the incidence of liver cancer has been increasing and that its prognosis remains poor. Fat mass and obesity-associated protein (FTO) is a demethylase that is involved in N6-methyladenosine (m6a) RNA modification; however, to the best of our knowledge, its role in tumorigenesis and development of liver cancer remains unknown. In the present study, cell proliferation, colony formation, apoptosis, Transwell and wound healing assays of small interfering (si)RNA-FTO HepG2 cells were performed, and the levels of m6A RNA methylation were assessed. Additionally, the prognostic value of FTO in liver cancer was analyzed using immunohisto-chemistry analysis. The results from the EpiQuik m6A RNA methylation quantitative assay revealed that knockdown of FTO increased the total m6A methylation level. Notably, FTO promoted the proliferation and migration of liver cancer cells. Additionally, FTO expression was upregulated in patients with liver cancer and was associated with a high Edmondson Grade, which served as an independent prognostic factor for liver cancer. Results from the Kaplan-Meier survival analysis revealed that low expression levels of FTO predicted a good prognosis. The 5-year overall survival of the low FTO expression group was 68% compared with 48% in the high FTO expression group (P=0.077). In conclusion, the present study suggested that FTO regulates the tumorigenesis and development of liver cancer.

Introduction

Liver cancer is the fifth and ninth most commonly diagnosed cancer globally in men and women, respectively (1). It was the fourth leading cause of cancer-associated mortality worldwide and was estimated to cause ~800,000 global deaths in 2015 (2). Liver cancer is frequently diagnosed at an advanced stage and is often characterized by poor prognosis (3). Consequently, only a small proportion of patients are eligible for potentially curative therapies (4). Various therapeutic strategies are currently used in the management of liver cancer, including surgery, liver transplantation, chemotherapy, radiotherapy and immunotherapy (5-12). However, liver cancer is frequently unresponsive to chemotherapy and radiotherapy, making its clinical outcomes poor.

The existence of chemically modified RNA species has been documented during the past decades (13). One of the most common chemical modifications on RNA molecules is the N6-methyladenosine (m6A) modification on mRNAs and long non-coding RNAs, which serve a crucial role in gene expression (14). This reversible RNA modification is catalyzed by the adenosine methyltransferases family of enzymes (15) and is reversed by demethylases (16). Members of the m6A methyltransferase family include methyltransferase like (METTL)3, METTL14, WT1 associated protein (WTAP), RNA binding motif protein 15 and vir like m6A methyltransferase associated (15). The m6A demethylase group of enzymes includes the fat mass and obesity-associated protein (FTO) and AlkB homolog 5 (16). Additionally, it has been demonstrated that

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m6A can be selectively recognized by proteins, including heterogeneous nuclear ribonucleoprotein C, heterogeneous nuclear ribonucleoprotein A2/B1, YTH N6-methyladenosine RNA binding protein (YTHDF)2, YTHDF1 and eukaryotic translation initiation factor 3 subunit A (14). FTO has been demonstrated to modulate multiple RNA modifications, including m6A and N6,2-O-dimethyladenosine (17), and it has been recently reported that the activity of this enzyme is oncogenic in certain types of cancer (18,19). Furthermore, FTO has been implicated in the induction of resistance to chemo-radiotherapy in cervical cancer by influencing the function of β-catenin via mRNA demethylation (20). Additionally, FTO upregulation has been associated with breast cancer progression via the PI3K/AKT signaling pathway (21). Overall, the aforementioned studies suggest that FTO may have various oncogenic roles in numerous types of cancer by modulating different cell signaling pathways.

The role of m6A methylation in the development of liver cancer has been explored in a few studies. For example, it has been reported that through a m6A-YTHDF2-dependent mechanism, METTL3 promotes tumorigenicity and metastasis of liver cancer *in vitro* and *in vivo* (22). Additionally, it has been proposed that WTAP serves an important role in the progression of liver cancer via m6A-HuR-dependent epigenetic silencing of the ETS proto-oncogene 1 (23). However, there is a lack of similar studies on the role of FTO in liver cancer. The aim of the present study was to explore the biological functions of FTO and its clinical relevance in liver cancer.

Materials and methods

Human samples, tissue microarray and cell lines. A total of 330 liver cancer tissues and 187 adjacent non-cancerous tissues (≥5 cm from the edge of the tumor tissue), were obtained from 330 patients at Zhejiang Provincial People's Hospital (Hangzhou, China). Written informed consent was obtained from all participants. The collected tissues were analyzed by FTO immunohistochemistry (IHC) and microarray analysis. The liver cancer tissue microarray was purchased from Shanghai BioChip Co., Ltd, and was performed according to the manufacturer's protocol. Ethical approval for the present study was obtained from the Ethics Committee of Zhejiang Provincial People's Hospital. The enrolled patients consisted of 268 males and 62 females, with a median age of 56 years (range, 25-91 years) at the time of surgery. Patient follow-up was performed for ≥5 years and the survival time was calculated from the date of surgical intervention to death. The human liver cancer HepG2 cell line was purchased from the American Type Culture Collection. HepG2 cells were cultured in DMEM supplemented with 10% FBS (both Gibco; Thermo Fisher Scientific, Inc.), 100 μg/ml penicillin and 0.1 mg/ml streptomycin at 37°C with 5% CO_2 in a humidified incubator.

Small interfering RNA (siRNA) transfection. HepG2 cells (1x10^6 cells/well) were seeded into 6-well plates and cultured with DMEM supplemented with 10% FBS for 48 h. Subsequently, cells were transfected with a mixture of siRNA (100 nM) and Lipofectamine® 2000 reagent (Thermo Fisher Scientific, Inc.), according to the manufacture's protocol. The sequences of

siRNAs used were as follows: siRNA-FTO-1, 5'-GGATGACTC TCATCTCGAA-3'; siRNA-FTO-2, 5'-GCTGAAATATCCTA AACTA-3'; siRNA-FTO-3, 5'-GTCACGAATTGCCCGA ACA-3'; and control siRNA, 5'-UUCUCCGAACGUGUCA CGU-3'. Cells were collected 24 or 48 h post-transfection for subsequent experimentation.

Western blot analysis. Western blot analysis was performed as previously described (24). The primary antibodies used in the present study were anti-FTO (1,1000; ab124892; Abcam) and anti-β-actin (1:200; ab115777; Abcam). Immunoreactive products were visualized using the ChemiDoc™ Touch Imaging system (Bio-Rad Laboratories, Inc.) and semi-quantified by densitometry using ImageJ software (version 1.50; National Institutes of Health).

Cell proliferation assay. The cell proliferation assays were performed using the Cell Counting Kit-8 (CCK-8; cat. no. CK04; Dojindo Molecular Technologies, Inc.) according to the manufacturer's protocol (23). Briefly, 200 μl HepG2 cells or siRNA-FTO HepG2 cells (2x10^4) were seeded into 96-well cell culture-treated plates. Cells were then cultured for 48, 72 or 96 h, after which 10 μl CCK-8 was directly added into the culture medium in each well. Subsequently, cells were incubated at 37°C for 2 h, and the absorbance was read at 450 nm using a microplate reader. Cell proliferation was measured in five wells for each experimental group.

Colony formation assay. A total of 1x10^2 HepG2 or siRNA-FTO HepG2 cells were seeded into each well of a 6-well cell culture plate and cultured for 7 days. Subsequently, cells were washed with PBS and fixed with 4% paraformaldehyde, and then stained with 0.5% crystal violet. Cells were observed under an inverted light microscope (magnification, x10).

Apoptosis analysis. HepG2 or siRNA-FTO HepG2 cells (1x10^5 cells/well) were seeded in 24-well plates and cultured for 48 h. After being washed with PBS twice and trypsinization, cells were resuspended in 500 μl binding buffer supplemented with 5 μl Annexin V-FITC (Nanjing KeyGen Biotech Co., Ltd.) and 5 μl propidium iodide according to the manufacturer's protocol. Finally, the fluorescence intensity of the samples was determined by flow cytometry (EPICS XL-MCL; Beckman Coulter, Inc.). The number of apoptotic cells in each sample was analyzed using FCS Express version 3.0 (De Novo Software).

Transwell assay and wound healing assay. Matrigel (BD Biosciences) was thawed at 4°C overnight and diluted with DMEM. A total of 60 μl diluted Matrigel was added in the upper chambers of a 24-well Transwell insert and incubated at 37°C for 30 min. A total of 200 μl HepG2 cells or siRNA-FTO HepG2 cells (2x10^4 cells/well) in serum-free DMEM were seeded into the upper chamber. DMEM supplemented with 10% FBS (600 μl) was added into the lower chamber and incubated for 24 h at 37°C. Non-migrating cells on the top of the Transwell insert were removed using a cotton swab. The cell migration rate was analyzed using methanol and 0.3% crystal violet staining. Wound healing experiments were performed to investigate the effect of FTO on the migration ability of HepG2 cells. The initial HepG2 or siRNA-FTO HepG2 cell seeding

density was $2x10^5$ cells/cm^2. A scratch wound was made using a 10-μl pipette tip. The cells were washed twice with PBS and then incubated with serum-free DMEM for 24 or 48 h at 37°C. The cells were visualized under an inverted light microscope. The amount of wound healing was quantified using ImageJ software (version 1.50; National Institutes of Health).

Measurement of total m6A level. Total RNA from HepG2 cells was extracted and purified using the RNeasy Mini kit (Qiagen GmbH), and the level of m6A RNA methylation was assessed using the EpiQuik M6A RNA Methylation Quantification kit (EpiGentek Group, Inc.) according to the manufacturer's protocol.

IHC analysis. The tissues were fixed in 10% buffered formalin for 6-12 h at room temperature, embedded in paraffin and cut into 4-μm-thick sections. Sections were deparaffinized in xylene, rehydrated using a gradient of ethanol concentrations (100, 95, 85 and 75%) and boiled in 1 mM TE buffer using a high-pressure cooker ($\geq$100°C) for 3 min for antigen retrieval. Subsequently, the sections were blocked with 3% hydrogen peroxide for 15 min at room temperature to inhibit endogenous peroxidase activity and incubated with 10% goat non-immune serum (Invitrogen; Thermo Fisher Scientific, Inc.) for 20 min at room temperature. The sections were then incubated with anti-FTO antibody (dilution, 1:100; cat. no. ab124892; Abcam) overnight at 4°C and with goat anti-rabbit IgG H&L (HRP) (1:2,000; cat. no. ab205718; Abcam) at room temperature for 15 min. This was followed by development using a DAB Substrate kit (Dako; Agilent Technologies, Inc.). IHC staining of FTO was scored by two independent pathologists using a light microscope (magnification, x200), based on the intensity and the proportion of positively stained cells. Specifically, staining intensity was evaluated with a grading system: 0, negative; 1, weak; 2, moderate; and 3, strong. The percentage of positive cells was scored as follows: 0, no staining; 1, 1-25% cells stained; 2, 26-50% cells stained; 3, 51-75% cells stained; and 4, >75% cells stained. The final score was obtained by multiplying the scores for intensity and percentage of positive cells.

FTO mRNA expression analysis. UALCAN (http://ualcan.path. uab.edu) is an online tool that uses The Cancer Genome Atlas RNA-sequencing and clinical data from 31 types of cancer. Additionally, it analyzes the relative expression levels of a query gene in various tumor sub-groups based on individual cancer stage, tumor grade, body weight or other clinicopathological features (25). In the present study, UALCAN was used to evaluate the mRNA expression levels of FTO in cancer and normal liver samples.

Statistical analysis. All statistical analyses were conducted using SPSS v13.0 (SPSS Inc.) and data are presented as the mean ± SD of at least three individual experiments. An unpaired t-test was used to compare the differences between two groups. Comparisons among multiple groups were analyzed using one-way ANOVA followed by Tukey's post hoc test (all data met the assumption of homogeneity of variance). A χ^2-test was used to assess the association between FTO expression and clinicopathological parameters. A multivariate

survival analysis was performed to identify the factors associated with prognosis according to the Cox proportional hazards regression model. The association between FTO expression and overall survival (OS) was analyzed by the Kaplan-Meier method with a log-rank test. P<0.05 (two-tailed) was considered to indicate a statistically significant difference.

Results

FTO expression is increased in liver cancer tissues. To investigate the role of FTO in liver cancer oncogenesis, the expression profile of FTO was characterized in liver cancer tissues and was compared with that in adjacent normal liver tissues by tissue microarrays. FTO scores were calculated with scores of 0-5 and 6-12 representing the low and high expression groups, respectively. The results of the IHC analysis revealed high FTO expression in 330 liver cancer tissues (126/330; 38.2%), which was significantly higher than high FTO expression in 187 normal liver tissues (46/187; 24.6%; χ^2=10.765; P=0.001; data not shown). These results were in agreement with the outcome of an analysis using UALCAN that revealed that FTO expression was higher in liver cancer tissues compared with in normal liver tissues (P<0.001; Fig. 1A). Overall, these observations suggested that FTO upregulation may be associated with the development of liver cancer.

FTO promotes in vitro proliferation and mobility of the human liver cancer cell line HepG2. FTO may be a modulator of m6A RNA demethylation and may promote liver cancer oncogenesis (17). siRNAs were used to knock down FTO expression in the human liver cancer HepG2 cell line. FTO expression downregulation was confirmed by western blotting (Fig. 1B and C). In addition, the m6A RNA level in the total RNA pool was quantified and it was observed that FTO knockdown was accompanied by a significant increase in the level of m6A RNA (P=0.032; Fig. 1D), suggesting an m6A demethylation function for FTO that may serve a role in liver cancer carcinogenesis. However, the exact mechanism needs to be further elucidated. Subsequently, a cell proliferation assay was performed using CCK-8. This analysis indicated that downregulation of FTO expression suppressed cell proliferation (Fig. 1E).

To further explore the role of this gene in liver cancer carcinogenesis, a colony formation assay was performed and it was observed that downregulation of FTO expression impaired the ability of HepG2 cells to form colonies (Fig. 2A and B). To establish the mechanism by which loss of FTO expression impaired these processes, an apoptosis assay was performed. Although transfection with siRNA-FTO appeared to decrease apoptosis, there was no significant difference in apoptosis between siRNA-NC and siRNA-FTO HepG2 cells, suggesting that FTO knockdown did not affect the apoptosis of HepG2 cells (Fig. 2C and D). Cell migration and wound healing assays suggested that FTO promoted the invasion and migration of liver cancer cells, as this was suppressed with FTO knockdown (Fig. 3). Overall, these findings indicated that FTO may promote the proliferation and migration of liver cancer cells *in vitro*.

Prognostic value of FTO in patients with liver cancer. The potential clinical prognostic value of FTO was investigated.
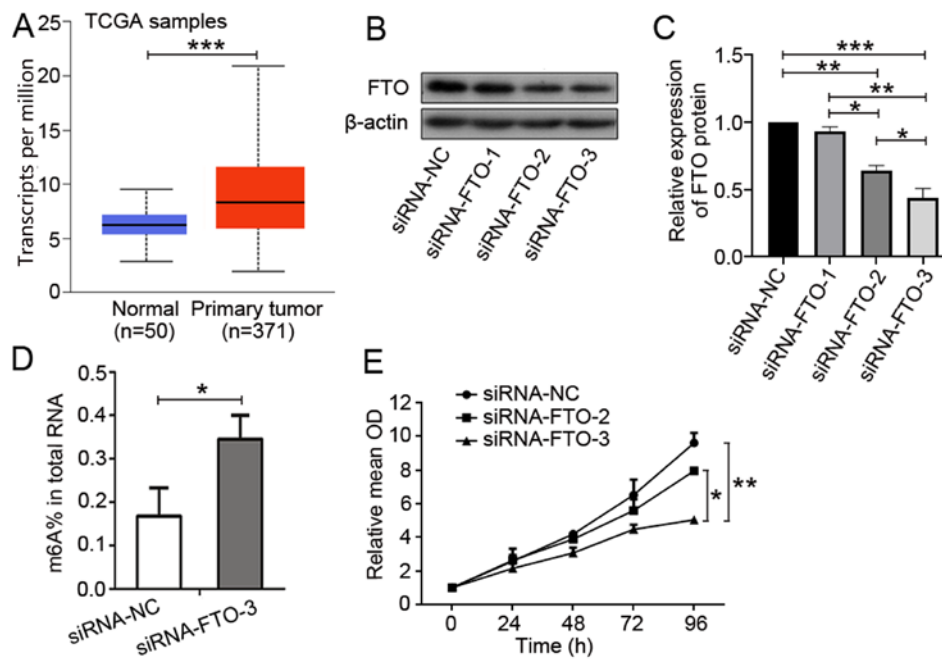
Figure 1. FTO promotes the proliferation of the HepG2 liver cancer cells *in vitro*. (A) TCGA database analysis by UALCAN revealed that FTO is highly expressed in liver cancer tissues compared with in normal liver tissues. (B) FTO protein downregulation was confirmed by western blotting. (C) Semi-quantified data of relative expression of FTO in siRNA-transfected cells. (D) Knockdown of FTO significantly upregulated the total RNA of m6A in HepG2 cells (P=0.032). (E) Knockdown of FTO inhibited the proliferation of HepG2 cells, as determined by CCK-8 assay. *P<0.05; **P<0.01; ***P<0.001. TCGA, The Cancer Genome Atlas; NC, negative control; FTO, fat mass and obesity-associated protein; siRNA, small interfering RNA.
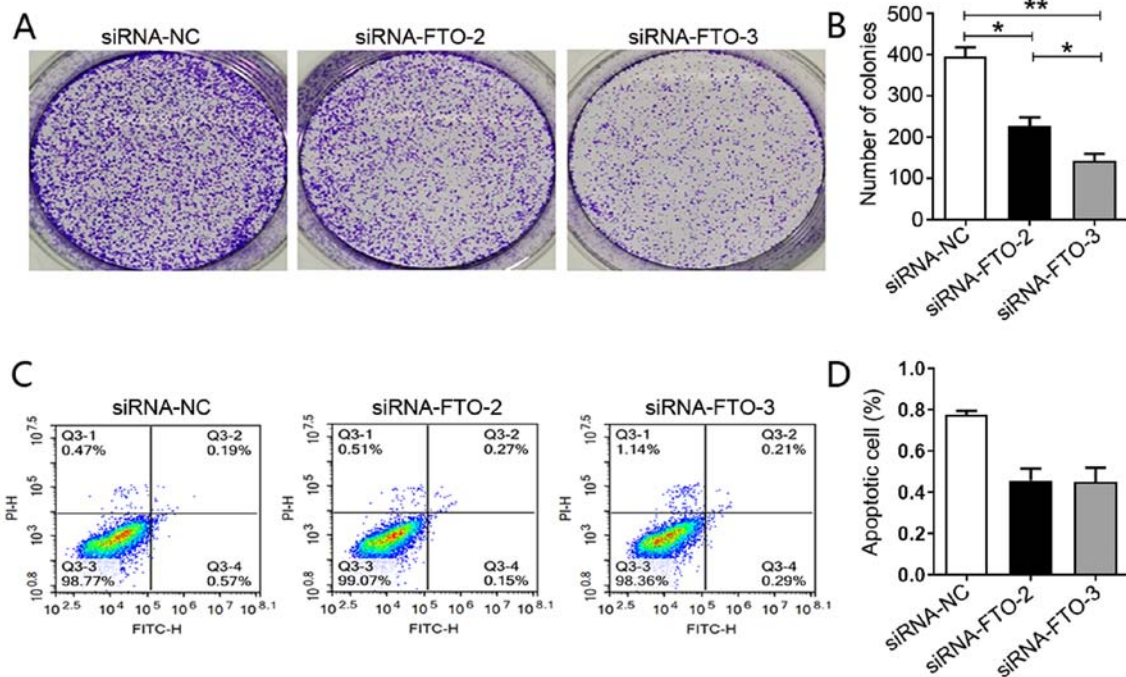

Figure 2. Knockdown of FTO inhibits the colony forming ability of HepG2 cells *in vitro*. (A) Downregulation of FTO expression suppressed the ability of HepG2 cells to form colonies. (B) Quantified data of the colony formation assay. (C) Knockdown of FTO expression had no effect on apoptosis of HepG2 cells. (D) Quantified data of the apoptosis assay. *P<0.05; **P<0.01. NC, negative control; FTO, fat mass and obesity-associated protein; siRNA, small interfering RNA; PI, propidium iodide; FITC, fluorescein isothiocyanate.

The expression levels of FTO in patients with liver cancer at Zhejiang Provincial People's Hospital were analyzed using IHC and the association between FTO expression and 5-year OS was explored. The results indicated a significantly higher FTO expression in liver cancer tissues compared

with adjacent normal tissues (Fig. 4A; data not shown). Additionally, a significant positive association was identified between FTO expression and Edmonson Grade (4) (χ^2=10.523; P=0.001; Table I), which was an established prognostic indicator for liver cancer in multivariate analysis (coefficient, 0.990;
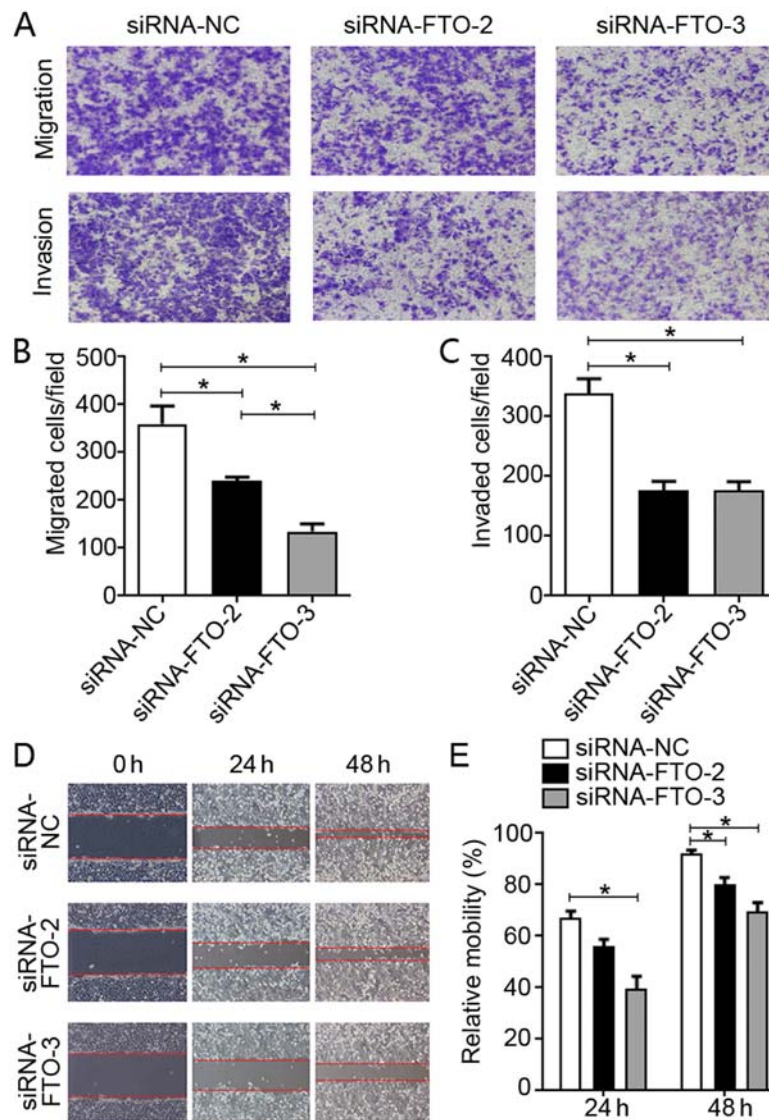
Figure 3. FTO promotes the migration of liver cancer cells. (A) Knockdown of FTO inhibited the invasion and migration of HepG2 cells (Transwell assay). Magnification, x10. Quantified data of the (B) migration and (C) invasion assays. (D) Downregulation of FTO expression inhibited the migration of HepG2 cells (wound healing assay). Magnification, x10. (E) Quantified data of the wound healing assays. *P<0.05. NC, negative control; FTO, fat mass and obesity-associated protein; siRNA, small interfering RNA.

P=0.006; Table II). The Kaplan-Meier analysis suggested that reduced FTO expression was indicative of a good prognosis. Although not statistically significant, a 68% 5-year OS was associated with low FTO expression compared with a 48% 5-year OS for high FTO expression (P=0.077; Fig. 4B).

Discussion

The present study reports a critical role of FTO in liver cancer tumorigenesis. The present analyses indicated that upregulation of FTO expression was frequently observed in liver cancer tissues. Knockdown of FTO significantly suppressed proliferation and migration of cultured liver cancer cells *in vitro*. Additionally, FTO knockdown led to a significant elevation of m6A methylation, suggesting that FTO-mediated m6A demethylation may contribute to liver cancer. However, the mechanism of this process remains unclear and merits further investigation. The IHC analysis revealed that FTO expression was markedly elevated in liver cancer tissues compared with

adjacent normal tissues. Furthermore, a positive association between FTO expression and Edmonson Grade was observed, suggesting that FTO may be a potential prognostic indicator for liver cancer. The Kaplan-Meier analysis suggested that low FTO expression was indicative of good prognosis.

RNA methylation has recently emerged as an important modulator of tumorigenesis. m6A methylation is one of the most common modifications found in eukaryotic mRNA and is a critical event in RNA metabolism (26). It is becoming increasingly evident that the 'writers', 'erasers' and 'readers' of m6A serve critical roles in tumorigenesis (27). FTO has been identified as a m6A 'eraser' and is associated not only with increased body mass and obesity (28), but also with carcinogenesis (29). The association between FTO single nucleotide polymorphisms and tumorigenesis has been previously investigated in prostate, pancreatic, breast and colorectal cancer (29,30). However, the m6A demethylase role of FTO in cancer development has only recently emerged. It has been previously demonstrated that FTO expression is enriched in

Table I. FTO expression in liver cancer tissues.

Clinical parameters	Total no.	FTO expression, n		χ^2	P-value
		Low	High		
Age, years				0.190	0.663
<55	128	81	47		
≥55	202	123	79		
Sex				0.456	0.500
Male	268	168	100		
Female	62	36	26		
Size, cm				1.328	0.249
≤5	192	123	69		
>5	130	75	55		
NA	8				
Tumour number				0.730	0.393
Single	270	164	106		
Multiple	60	40	20		
Edmondson grade				10.523	0.001[a]
I+II	205	139	66		
III	119	59	60		
NA	6				
Metastasis				0.525	0.469
M0	297	186	111		
M1	27	15	12		
NA	6				
Microvascular invasion				3.449	0.063
Absence	124	83	41		
Presence	121	67	54		
NA	85				
HBs antigen				1.135	0.287
Negtive	63	35	28		
Positive	261	164	97		
NA	6				
Cirrhosis				0.231	0.631
Negative	110	66	44		
Positive	220	138	82		
AFP, μg/l				2.056	0.152
<50	145	92	53		
≥50	124	68	56		
NA	61				

FTO expression was divided into low and high expression groups based on immunohistochemistry scores of 0-5 and 6-12, respectively. [a]$P<0.05$. FTO, fat mass and obesity-associated protein; HBs, hepatitis B surface; AFP, α-fetoprotein; NA, not available.

the tumor area and that it promotes proliferation, migration and lymph node metastasis in gastric cancer (19). In addition, Li *et al* (31) revealed that FTO expression is associated with an overall decrease in cancer survival. It has also been reported that FTO significantly enhances proliferation while inhibiting apoptosis in lung cancer cell lines (32).

Mechanistically, FTO has been demonstrated to suppress the m6A methylation of ubiquitin specific peptidase 7 while increasing mRNA stability via mRNA demethylation (33). These results indicate that FTO may promote oncogenesis and that suppressing its expression may offer a beneficial therapeutic strategy for various types of cancer. The results of the present study revealed that FTO may promote proliferation and migration of the liver cancer HepG2 cell line *in vitro*. However, the wound healing assay was inconclusive as it could not exclude the possibility that the suppressed

Table II. Univariate and multivariate Cox regression analyses for the clinicopathological parameters in patients with liver cancer.

Parameters	No.	Univariate analysis				Multivariate analysis			
		Coefficient	HR	95% CI	P-value	Coefficient	HR	95% CI	P-value
Age (<55/≥55 years)	128/202	-0.427	0.653	0.412-1.034	0.069	-0.275	0.759	0.381-1.514	0.435
Sex (male/female)	268/62	0.414	1.512	0.888-2.577	0.128	-0.194	0.823	0.384-1.766	0.618
Tumor size (≤50/>50 mm)	192/130	0.714	2.042	1.283-3.248	0.003[a]	0.448	1.565	0.729-3.356	0.250
Tumor number (single/multiple)	270/60	0.160	1.173	0.644-2.138	0.601	0.860	2.364	0.982-5.692	0.055
Edmondson grade (I+II/III)	205/119	1.018	2.769	1.733-4.423	0.000[a]	0.990	2.691	1.319-5.490	0.006[a]
Metastasis (M0/M1)	297/27	1.402	4.063	2.173-7.596	0.000[a]	1.424	4.153	1.481-9.625	0.007[a]
Microvascular invasion (-/+)	124/121	0.637	1.891	1.136-3.148	0.041[a]	-0.170	0.844	0.382-1.862	0.674
HBs antigen (-/+)	63/261	0.125	1.133	0.633-2.030	0.674	-0.173	0.841	0.267-2.652	0.768
Cirrhosis (-/+)	110/220	0.167	1.182	0.717-1.948	0.513	0.781	2.183	0.881-5.406	0.092
AFP (<50/≥50 μg/l)	145/124	0.837	2.310	1.315-4.058	0.004[a]	0.504	1.655	0.801-3.418	0.174
FTO (-/+)	185/124	0.412	1.150	0.950-2.399	0.081	0.451	1.570	0785-3.141	0.202

The total number of patients for some parameters was <330 due to incomplete pathological data. [a]P<0.05. FTO, fat mass and obesity-associated protein; HBs, hepatitis B surface; AFP, α-fetoprotein; HR, hazard ratio.
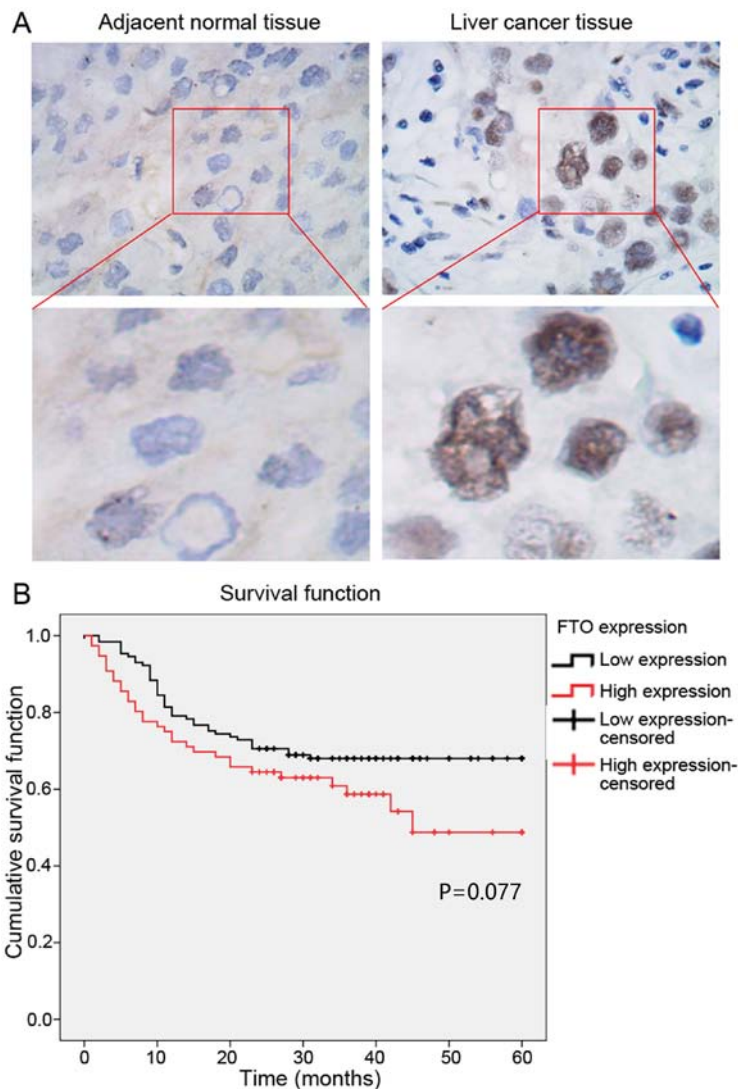

Figure 4. Prognostic value of FTO in liver cancer. (A) Representative IHC staining of FTO in specimens from patients with liver cancer. Magnification, x200. (B) Kaplan-Meier survival curves of patients with liver cancer with 'low FTO expression' (IHC score, 0-5) and 'high FTO expression' (IHC score, 6-12). FTO, fat mass and obesity-associated protein; IHC, immunohistochemistry.

migration observed upon FTO knockdown was not caused by a reduction in cell proliferation. Results from the Transwell assay supported the aforementioned observation that reduced FTO expression may inhibit invasion and migration of HepG2 cells. Although FTO seemed to promote proliferation of HepG2 cells, it was also observed that this does not significantly affect apoptosis. Huang *et al* (33) demonstrated that FTO depletion decreases the expression levels of the mitotic checkpoint complex and G2/M regulators in mouse GC-1 cells. Wu *et al* (34) observed that FTO knockdown markedly decreases the expression levels of cyclin A2 and CDK2, both crucial cell cycle regulators, leading to delayed entry of methylene diphenyl diisocyanate-induced cells into G2 phase. However, whether FTO-knockdown affects the cell cycle of HepG2 cells is unclear, and the mechanism of FTO promoting proliferation of HepG2 cells is likely more complex than just through cell cycle arrest. Therefore, these mechanisms need to be further clarified. By suggesting a potential role of FTO in liver cancer, the results of the present study strengthened earlier reports of a role of FTO in oncogenesis. However, further research is required to elucidate the mechanism by which FTO-mediated modulation of m6A demethylation affects liver cancer oncogenesis.

The role served by m6A methylation in the development of liver cancer has been previously explored. It has been reported that the methyltransferase METTL3 promotes tumorigenicity and metastasis of liver cancer *in vitro* and *in vivo* by suppressing the expression of suppressor of cytokine signaling 2 via an m6A-YTHDF2-dependent mechanism (22). The m6A methyltransferase WTAP has also been reported to serve a crucial role in the development of liver cancer via the HuR-ETS1-p21/p27 axis (23). Although more evidence is required to support a role of FTO in liver cancer, it has been reported that FTO suppresses the proliferation and migration of intrahepatic cholangiocarcinoma cells by impairing the mRNA stability of the TEA domain transcription factor 2 oncogene (35). However, data from the present study revealed that FTO could promote the progression of liver cancer while downregulating m6A RNA methylation levels.

In conclusion, the present study revealed that FTO may contribute to liver cancer oncogenesis via the downregulation of m6A RNA methylation levels. Further investigations are required to better understand the association between the development of liver cancer and m6A methylation. The present study reported FTO as a potential prognostic indicator for liver cancer and a potential novel therapeutic target for the management of liver cancer.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

ZY, SW and ZX designed the study. ZY, SW, WC, XZ, JC, JJ, MW and LZ performed the data collection and collation. All the authors were involved in the analysis and interpretation of data. ZY wrote the paper, with the help of the co-authors. SW, WC and XZ reviewed and revised the manuscript. All authors agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Zhejiang Provincial People's Hospital (Hangzhou, China). Written informed consent was obtained from all participants.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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