M6A Demethylase FTO Plays a Tumor Suppressor Role in Thyroid Cancer

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The purpose of this study was to investigate the expression and clinical significance of N6-methyladenosine demethylase FTO in thyroid cancer. Bioinformatic analysis showed that FTO expression was downregulated in thyroid cancer tissues and correlated with lymph node metastasis in thyroid cancer patients. We conducted experimental verification by collecting Asian samples. The results of quantitative reverse transcription-PCR showed that the mRNA expression of FTO in the blood of 30 thyroid cancer patients was lower than that of the control population. At the same time, we found that FTO expression was negative in tissues of 16/56 (28.57%) thyroid cancer cases and 4/40 (10.00%) nontumor thyroid cases through the immunohistochemical method, indicating a lower FTO expression in thyroid cancer tissues than nontumor thyroid tissues ($p < 0.05$). In addition, the protein expression of FTO was significantly related to the tumor grade and lymph node metastasis in thyroid cancer patients ($p < 0.05$), but not to other clinicopathological features. Multivariate logistic regression analysis showed that FTO expression was an independent risk factor for tumor grade. Survival analysis showed no significant difference in the disease-free survival time of thyroid cancer patients between high expression and low expression groups of FTO. Furthermore, bioinformatic analysis found that promoter DNA methylation and copy number variation might cause downregulated FTO and then affect TP53 pathways in thyroid cancer. We found that FTO expression was downregulated in thyroid cancer tissues and related to the progression of thyroid cancer, suggesting a tumor suppressor role of FTO in thyroid cancer.

Keywords: FTO, m6A modification, thyroid cancer, metastasis, prognosis

Introduction

IN THE PAST 50 YEARS, the incidence of thyroid cancer has been rising worldwide (Li *et al.*, 2020b). Thyroid cancer is the fifth most common cancer among women in the United States, and in 2020, it was estimated that there were more than 52,890 new cases of men and women (Siegel *et al.*, 2020). From 1990 to 2020, the global incidence of thyroid cancer has continued to rise (Wild *et al.*, 2020). Although the incidence rate has steadily increased, the mortality rate of thyroid cancer has changed little in most countries and regions (Vaccarella *et al.*, 2016; Dal Maso *et al.*, 2018). Compared with high-income countries (19%), the relative growth rate of thyroid cancer in low-income countries (33%) is higher. The reason for this manifestation mainly reflects the increasingly stricter examination of the thyroid by ultra-

sound and other diagnostic techniques (Li *et al.*, 2020a). When comparing different regions of the world, the incidence of thyroid cancer seems to be very different due to differences in diagnostic methods, environmental exposure, and individual risk factors (Li *et al.*, 2020b). Because thyroid cancer has the characteristics of strong concealment and slow progress, some patients have tumor metastasis at the initial diagnosis (Ancker *et al.*, 2017). According to reports in the literature, the occurrence of thyroid cancer is related to activation and deregulation of oncogenes, so revealing thyroid cancer-related genes and regulatory mechanisms is of great significance for the treatment of thyroid cancer (Wang *et al.*, 2020).

Studies have shown that N6-methyladenosine (m6A) is the most abundant RNA modification in eukaryotic and human RNA (Desrosiers *et al.*, 1974). Similar to DNA and

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protein modification, the RNA methylation process is continuous and reversible. In recent years, m6A has attracted more and more scientists' attention due to its great biological significance, prognosis, and therapeutic potential (Cao *et al.*, 2016). The m6A modification can work under the coordinated action of methyltransferase (writers), demethylase (erasers), and reader proteins (Yue *et al.*, 2015). The M6A writer complex comprises METTL3, WTAP, RBM15, METTL14, KIAA1429, and ZC3H13 (Roignant and Sollier, 2017; Lan *et al.*, 2019). Demethylase FTO and ALKBH5 constitute m6A erasers (Fitzsimmons and Batista, 2019). M6A readers include YTHDF1, YTHDF2, YTHDC1, YTHDC2, and HNRNPC, which can recognize m6A modifications and thus directly guide alternative splicing, localization, translation, and stability of RNA and other processes (Arguello *et al.*, 2017; Wang *et al.*, 2018).

The obesity and fat-related protein called α -ketoglutarate-dependent dioxygenase is an enzyme encoded by the human FTO gene (Li *et al.*, 2019a). Animal model studies have shown that FTO deficiency can prevent obesity and cause growth retardation (Fischer *et al.*, 2009; Gao *et al.*, 2010), and overexpression of FTO can lead to increased food intake and obesity (Church *et al.*, 2010). In humans, growth retardation and various malformations or even premature death may be caused by FTO gene mutation or loss of function (Boissel *et al.*, 2009). By demethylating the target mRNA, FTO is involved in the regulation of various tumors (Li *et al.*, 2017; Wang *et al.*, 2017). However, the role of FTO in human thyroid cancer is still unknown.

Through clarifying the expression and clinical significance of FTO in thyroid cancer, we aim to explore the biological function of FTO in thyroid cancer and provide new clues for molecular targeted therapy of thyroid cancer.

Materials and Methods

Blood samples

Thirty blood samples in the case group were collected from patients with thyroid cancer diagnosed by pathological and clinical methods in the First Affiliated Hospital of Guilin Medical University from July to December 2019. These patients had no thyroid surgery, radiotherapy, or chemotherapy before. At the same time, 30 normal control blood samples were collected from the Department of Physical Examination in Guilin People's Hospital. The control group was matched with the case group according to age and gender and had no thyroid diseases. All the samples were from Asians. This study was based on the 1975 Declaration of Helsinki and was approved by the Ethics Committee of Guilin Medical University (GLMC2014003).

Bioinformatic analysis

Data of FTO expression in thyroid cancer tissues were obtained from the National Center for Biotechnology Information Gene Expression Omnibus (NCBI GEO) and The Cancer Genome Atlas (TCGA) databases through the GDC platform according to the previous method (Guo *et al.*, 2020). High and low expression groups of FTO were set by the median of the FTO expression value. We used the Kaplan–Meier (KM) plotter for survival analysis. KM analysis was used for relapse-free survival time from TCGA's thyroid cancer patients. FTO DNA methylation and copy number alterations (CNA) in TCGA-thyroid carcinoma (THCA) were studied using cBioPortal for Cancer Genomics. We used the Whistle–Epitranscriptome online tool to predict the m6A methylation site of the TP53 gene (Chen *et al.*, 2019).

FIG. 1. Expression of N 6-methyladenosine RNA methylation regulators in thyroid cancer in TCGA database. The Wilcoxon test was used to determine different gene expression levels between the tumor group and normal group; *** $p < 0.001$. TCGA, The Cancer Genome Atlas. Color images are available online.

Quantitative reverse transcription–polymerase chain reaction

RNA was isolated from blood samples using the QIAamp RNA Blood Mini Kit (QIAGEN, Germantown, MD) and subsequently used for cDNA synthesis with the FastQuant RT Kit (Tiangen, Beijing, China), according to the manufacturers' protocols. Then, quantitative reverse transcription (qRT)-PCR was performed using the StepOne/StepOnePlus™ Real-Time PCR System with SYBR Green PCR Master mix (Thermo Fisher Scientific, Waltham, MA). The primer sequence for FTO was the following: forward, 5'-GGGTTCATCCTACAACGGACA-3', and reverse, 5'-AGGTTCTCTTT CAGGTATGGC-3'. The GAPDH primers used were the following: forward, 5'-GACCACTTTGTCAAGCTCATTTTC-3',

and reverse, 5'-CTCTCTTCCTCTTGTGCTCTTG-3'. An optimal reaction was obtained with the following thermocycling conditions: initial denaturation at 95°C for 10 min; 45 cycles of denaturation at 95°C for 15 s, annealing at 60°C for 1 min, and elongation at 72°C for 1 min; and a final extension at 72°C for 10 min.

Immunohistochemical detection

The immunohistochemical (IHC) method was performed after tissue specimens were integrated into a tissue chip by the Fanpu company (Guilin, China). After IHC standard steps for baking, dewaxing, and antigen repair, peroxidase blocking, and goat serum incubation, the tissue chip was

FIG. 2. Expression of FTO in thyroid cancer in the online database. (A–D) Data from the GEO dataset. (E, F) Data from TCGA dataset. The *t*-test or paired *t*-test was used to determine different gene expression levels between the tumor group and normal group; ***p* < 0.01 and ****p* < 0.001. GEO, Gene Expression Omnibus. Color images are available online.

incubated with anti-rabbit polyclonal antibody against FTO (Atlas Antibodies, Bromma, Sweden) overnight at 4°C. Then, the anti-rabbit secondary antibody was added and incubated at 37°C for 30 min. Finally, the diaminobenzidine solution was added to develop color and hematoxylin was

added to restaining. The IHC results were determined under a microscope by two professional pathologists. The average staining intensity and percentage of positively stained cells in five visual fields were recorded and counted. The scores for percentage of positively stained cells were 0 for none, 1

FIG. 3. Experimental verification of FTO expression in patients with thyroid cancer. **(A)** The expression of FTO in the blood of thyroid cancer patients and nonthyroid cancer patients matched by age and sex was analyzed by qRT-PCR. The paired *t*-test was used to determine different gene expression levels between the tumor group and normal group; **p* < 0.05. **(B)** Representative FTO protein expression was detected in thyroid cancer tissues and nontumor thyroid tissues by IHC analysis at 400× magnification. **(C)** In the GEO database, comparison of the expression of FTO after cancer classification with the normal control group; ***p* < 0.01. **(D)** In TCGA database, comparison of the expression of FTO after cancer classification with the normal control group; ****p* < 0.001. IHC, immunohistochemical; qRT, quantitative reverse transcription. Color images are available online.

for $\leq 10\%$, 2 for 11–50%, 3 for 51–75%, and 4 for $>75\%$, respectively. The scores for staining strength were 0 for no staining, 1 for light brown, 2 for medium brown, and 3 for dark brown. Finally, the scores for staining strength and percentage of positively stained cells were multiplied to obtain the total expression score. An expression score ≤ 4 was defined as negative expression and >4 was defined as positive expression.

Gene set enrichment analysis

Gene set enrichment analysis (GSEA) first generated an ordered list of all genes based on their correlation with FTO expression and then we conducted GSEA to clarify the significant differences observed between the high and low expression groups of FTO. The genome was aligned 1000 times per analysis. The expression level of FTO was used as a phenotypic marker. Nominal p -value and normalized enrichment score (NES) were used to classify the enrichment pathways in each phenotype.

Statistical analysis

All data were analyzed using SPSS 24.0. The expression of FTO between thyroid cancer and paired nontumor thyroid tissues was compared by the chi-square test. The relationship between the expression level of FTO and clinicopathological features of thyroid cancer patients was analyzed by the chi-square test and multivariate logistic regression analysis. The KM method was used for survival analysis. $p < 0.05$ was considered as statistically significant.

Results

Expression of m6A-associated factors in thyroid cancer

According to literature reports, genes of the m6A family play important biological roles in a variety of cancers, but there are few relevant studies in thyroid cancer (Wang *et al.*, 2020; Zhao *et al.*, 2020). To screen out the deregulated m6A-associated factors, we used TCGA database to analyze data of the expression of m6A family transcriptome in thyroid cancer. We chose 14 identified m6A-associated factors in the analysis, METTL3, METTL14, METTL5, ALKBH5, FTO, YTHDC1, YTHDC2, YTHDF1, YTHDF2, HNRNPC, WTAP, KIAA1429, ZC3H13, and RBM15. As shown in Figure 1, except the m6A-binding protein, YTHDF2, all other 13 m6A-associated factors were differentially expressed in thyroid cancer tissues ($p < 0.01$). This result is consistent with previous research (Xu *et al.*, 2020). Among these 13 m6A-associated factors, the expression levels of binding proteins, HNRNPC and methyltransferase METTL5, in thyroid cancer tissues were higher than those in adjacent nontumor tissues. Compared with the adjacent nontumor tissues, FTO and the remaining 10 genes of the m6A family were significantly downregulated in thyroid cancer tissues.

Bioinformatic analysis of FTO expression in thyroid cancer

To further verify the expression of FTO in thyroid cancer, we next conducted bioinformatic analysis to measure the

expression of FTO in thyroid cancer patients using TCGA and GEO databases. Compared with the normal thyroid cancer tissues, the expression of FTO was significantly lower in thyroid cancer tissues in GSE27155, GSE3467, GSE60542, and TCGA databases ($p < 0.01$, Fig. 2A–C and E). In addition, in GSE82208, FTO expression in follicular thyroid carcinoma was lower than that in follicular thyroid adenoma ($p < 0.01$, Fig. 2D). Especially in TCGA database, we further paired the samples to perform a paired t -test. As shown in Figure 2F, the expression of FTO in thyroid cancer tissues was still lower than that in adjacent nontumor tissues ($p < 0.01$). These online analyses suggest that FTO has a downregulated expression in thyroid cancer tissues.

Expression of FTO in patients with thyroid cancer

We collected blood and tissue samples from thyroid cancer patients to further detect the expression of FTO in thyroid cancer and to verify the results of online databases. As shown in Figure 3A, the mRNA expression of FTO in the blood of thyroid cancer patients was lower than that of the matched normal control population ($p < 0.05$). The IHC method was used to detect the expression of FTO protein in thyroid cancer tissues. Results showed that the FTO protein was mainly located in the nucleus (Fig. 3B). Moreover, 28.57% (16/56) of thyroid cancer tissues had negative FTO expression and 10.00% (4/40) of nontumor thyroid tissues had negative FTO expression. Statistical analysis showed that the negative expression of FTO in thyroid cancer tissues was higher than that in nontumor thyroid tissues ($p < 0.05$, Table 1). These results further indicate that FTO is downregulated in thyroid cancer tissues.

We further classified thyroid cancer and found that in TCGA database, the expression of FTO in each type of cancer tissue was lower than that of normal thyroid tissue ($p < 0.001$, Fig. 3D). Similarly, in GSE27155, FTO expression in thyroid gland papillary carcinoma and tall cell variant thyroid gland papillary carcinoma was also lower than that in normal thyroid tissues ($p < 0.01$, Fig. 3C).

The relationship between FTO expression and clinicopathological features of thyroid cancer patients

We also analyzed the relationship between FTO expression and clinical characteristics of thyroid cancer patients through online databases and IHC results. From TCGA database, it was found that the expression of FTO was related to lymph

TABLE 1. FTO EXPRESSION IN THYROID CANCER TISSUES AND ADJACENT NONTUMOR TISSUES BY IMMUNOHISTOCHEMICAL DETECTION

FTO expression	Adjacent nontumor tissues	Thyroid cancer tissues	χ^2	p
Negative	4	16	4.879	0.027
Positive	36	40		
Total	40	56		

Bold values indicate significance.

FIG. 4. The relationship between FTO expression and clinicopathological features of thyroid cancer patients analyzed from TCGA database. (A) The relationship between FTO expression and tumor grade of thyroid cancer patients. (B) The relationship between FTO expression and lymph node metastasis in patients with thyroid cancer; * $p < 0.05$ is based on the t -test. Color images are available online.

node metastasis and tumor grade in patients with thyroid cancer ($p < 0.05$, Fig. 4A, B). According to IHC results, 56 patients with thyroid cancer were divided into FTO-positive and -negative expression groups. As shown in Table 2, the expression of FTO was significantly correlated with age, lymph node metastasis, and tumor grade ($p < 0.05$), but not with other clinicopathological features (such as gender and tumor diameter), in patients with thyroid cancer. Multiple logistic regression analysis further showed that the expression of FTO was related to the tumor grade of thyroid cancer and its odds ratio was 0.38 ($p < 0.01$, Table 3). These results indicate that FTO expression is associated with lymph node metastasis and tumor grade in patients with thyroid cancer.

TABLE 2. ASSOCIATION BETWEEN THE EXPRESSION OF FTO AND CLINICOPATHOLOGICAL FEATURES OF THYROID CANCER PATIENTS BASED ON IMMUNOHISTOCHEMICAL RESULTS

Variables	Total	FTO staining		χ^2	p
		Negative	Positive		
Gender					
Female	30	6	24	2.326	0.127
Male	26	10	16		
Age, years					
≤ 50	34	4	30	11.979	0.001
> 50	22	12	10		
Tumor diameter (cm)					
≤ 4	44	10	34	3.436	0.064
> 4	12	6	6		
Tumor grade					
I+II	48	8	40	23.333	<0.001
III+IV	8	8	0		
Lymphatic node metastasis					
None	44	9	35	6.629	0.010
Presence	12	7	5		

Bold values indicate significance.

TABLE 3. MULTIVARIABLE LOGISTIC REGRESSION ANALYSIS OF FTO EXPRESSION IN THYROID CANCER PATIENTS

Variables	B	Wald	p	OR ^a	95% CI for OR	
					Lower	Upper
FTO	-0.967	7.480	0.006	0.380	0.190	0.760

Bold values indicate significance.

^aOR adjusted for age and gender.

CI, confidence interval; OR, odds ratio.

The relationship between FTO expression and prognosis of thyroid cancer patients

Because the prognosis of patients with thyroid cancer is better, we used the KM method to analyze the relationship between FTO expression and the prognosis of thyroid cancer patients online. As shown in Figure 5, according to TCGA database, there was no significant difference in tumor-free survival time between high and low expression groups of FTO after tumor surgery.

Possible mechanism of FTO expression in thyroid cancer

We also explored the potential mechanisms of FTO downregulation in thyroid cancer from the perspective of genetic and epigenetic alterations. Among thyroid cancer patients with complete mRNA, CNA, and methylation data ($n = 497$) in TCGA-THCA dataset, two patients had FTO shallow deletion and 13 patients had FTO copy gain (low-level amplification). FTO copy gain was significantly related to FTO mRNA overexpression ($p < 0.05$, Fig. 6B). Besides, we evaluated the association between FTO expression and its DNA methylation. Linear regression analysis showed a significant correlation between FTO mRNA expression and

FIG. 5. The relationship between FTO expression and prognosis of thyroid cancer patients from TCGA database. Color images are available online.

FIG. 6. Possible regulatory mechanism of FTO expression in thyroid cancer. **(A)** Correlation analysis between FTO mRNA expression and FTO DNA methylation. **(B)** Comparison of FTO mRNA expression in different CNA groups. **(C)** GSEA using TCGA-THCA dataset. FTO silence was significantly correlated with thyroid cancer. **(D)** Correlation between TP53 and FTO in transcriptome data after TPM correction. $**P < 0.01$. CNA, copy number alteration; GSEA, gene set enrichment analysis; THCA, thyroid carcinoma; TPM, transcripts per million. Color images are available online.

the total DNA methylation level (Pearson's $r = -0.15$, $p < 0.01$, Fig. 6A). Moreover, we established a link between the methylation site and expression of FTO. The results showed that the methylation sites, cg12495954, cg01485549, cg06392242, and cg27021131, would affect the expression of FTO (Supplementary Fig. S1).

To identify signaling pathways that FTO may participate in for thyroid cancer, we conducted GSEA between low and high expression datasets of FTO. GSEA revealed significant differences in enrichment of KEGG (FDR $q < 0.05$, NOM $p < 0.01$). We selected the most significant enriched signaling pathways based on their NES (Fig. 6C and Table 4). After the GSEA, we found that the expression of FTO might affect the KEGG THYROID CANCER pathway. We tried a correlation between FTO and the key gene, TP53, in the KEGG THYROID CANCER pathway in patients with thyroid cancer and found that FTO and TP53 were positively correlated. Online prediction of the m6A methylation site of TP53 through the Whistle website found that there

were several m6A methylation sites on TP53 (Supplementary Table S1). Therefore, we speculate that FTO may affect the metastasis of thyroid cancer by affecting the KEGG THYROID CANCER signaling pathway through targeting m6A methylation of TP53 (Fig. 6D).

Discussion

In all RNA modification processes, RNA methylation accounts for more than 60%, and m6A is recognized as one of the most common types of eukaryotic mRNA methylation modifications (Li *et al.*, 2019b). At present, three enzymes have been identified to participate in the m6A methylation process, including demethylase, methylase, and methylation recognition enzyme (Bartosovic *et al.*, 2017). Among them, m6A demethylase comprises FTO and ALKBH5 (Zhao *et al.*, 2020). As the first RNA demethylase identified, FTO is reported to regulate many cellular processes, such as adipogenesis, mRNA splicing, and cancer development (Iles

TABLE 4. GENE SETS ENRICHED IN PHENOTYPE WITH HIGH FTO EXPRESSION

Name	Size	NES	NOM p-val	FDR q-val
KEGG_WNT_SIGNALING_PATHWAY	150	-2.16318	0	0.001723
KEGG_TGF_BETA_SIGNALING_PATHWAY	85	-2.1385	0	0.003128
KEGG_PROSTATE_CANCER	89	-2.10176	0	0.005823
KEGG_INSULIN_SIGNALING_PATHWAY	137	-2.08895	0	0.004367
KEGG_COLORECTAL_CANCER	62	-2.08428	0	0.003494
KEGG_ADHERENS_JUNCTION	73	-2.08084	0	0.002911
KEGG_ERBB_SIGNALING_PATHWAY	87	-2.07179	0	0.002496
KEGG_INOSITOL_PHOSPHATE_METABOLISM	54	-2.07178	0	0.002184
KEGG_LYSINE_DEGRADATION	44	-2.07048	0	0.001941
KEGG_VASOPRESSIN_REGULATED_WATER_REABSORPTION	44	-2.06472	0	0.002094
KEGG_MTOR_SIGNALING_PATHWAY	52	-2.05036	0	0.002697
KEGG_ADIPOCYTOKINE_SIGNALING_PATHWAY	67	-2.00841	0	0.004424
KEGG_PHOSPHATIDYLINOSITOL_SIGNALING_SYSTEM	76	-1.98014	0.002012	0.006921
KEGG_LONG_TERM_POTENTIATION	70	-1.96732	0	0.008028
KEGG_ENDOMETRIAL_CANCER	52	-1.95239	0.00207	0.009158
KEGG_DORSO_VENTRAL_AXIS_FORMATION	24	-1.94849	0	0.009134
KEGG_UBIQUITIN_MEDIATED_PROTEOLYSIS	134	-1.94066	0.004032	0.009273
KEGG_NEUROTROPHIN_SIGNALING_PATHWAY	126	-1.93574	0.006048	0.009454
KEGG_PATHWAYS_IN_CANCER	325	-1.92563	0	0.009923
KEGG_VASCULAR_SMOOTH_MUSCLE_CONTRACTION	115	-1.92032	0.001992	0.010206
KEGG_MELANOGENESIS	101	-1.91057	0	0.010899
KEGG_RENAL_CELL_CARCINOMA	70	-1.91011	0.002041	0.010493
KEGG_PANCREATIC_CANCER	70	-1.90877	0.004	0.010174
KEGG_SPHINGOLIPID_METABOLISM	39	-1.90253	0	0.010619
KEGG_GNRH_SIGNALING_PATHWAY	101	-1.89992	0	0.010654
KEGG_SMALL_CELL_LUNG_CANCER	84	-1.89944	0.00396	0.010328
KEGG_MAPK_SIGNALING_PATHWAY	267	-1.89907	0.002058	0.009997
KEGG_HEDGEHOG_SIGNALING_PATHWAY	56	-1.89773	0.004115	0.00973
KEGG_ENDOCYTOSIS	181	-1.89215	0.004115	0.010083
KEGG_FOCAL_ADHESION	199	-1.88785	0.00409	0.010008
KEGG_ABC_TRANSPORTERS	44	-1.87848	0	0.011053
KEGG_REGULATION_OF_AUTOPHAGY	35	-1.86332	0.001953	0.013473
KEGG_N_GLYCAN_BIOSYNTHESIS	46	-1.86282	0.00198	0.013245
KEGG_GAP_JUNCTION	90	-1.85028	0.002101	0.014413
KEGG_O_GLYCAN_BIOSYNTHESIS	30	-1.84649	0.003984	0.014426
KEGG_PROGESTERONE_MEDIATED_OOCYTE_MATURATION	85	-1.83695	0.006036	0.014921
KEGG_TYPE_II_DIABETES_MELLITUS	47	-1.83182	0.001969	0.015452
KEGG_AXON_GUIDANCE	129	-1.81561	0.010661	0.017908
KEGG_OOCYTE_MEIOSIS	112	-1.81074	0.008282	0.01847
KEGG_THYROID_CANCER	29	-1.80713	0.003992	0.018437
KEGG_CHRONIC_MYELOID_LEUKEMIA	73	-1.79938	0.008333	0.019905
KEGG_GLYCOSYLPHOSPHATIDYLINOSITOL_GPI_ANCHOR_BIOSYNTHESIS	25	-1.7958	0.005703	0.02003
KEGG_REGULATION_OF_ACTIN_CYTOSKELETON	213	-1.79179	0.008097	0.020411
KEGG_NON_SMALL_CELL_LUNG_CANCER	54	-1.79158	0.008197	0.019998

Gene sets with NOM *p*-val <0.01 and FDR *q*-val <0.5 are considered as significant.
FDR, false discovery rate; NES, normalized enrichment score; NOM, nominal.

et al., 2013; Ben-Haim *et al.*, 2015; Li *et al.*, 2017). At present, we understand that m6A methylation can affect the occurrence and development of tumors (Yue *et al.*, 2015). After searching relevant literature, we found that FTO has some biological functions in lung cancer, colorectal cancer, breast cancer, and gastric cancer (Tan *et al.*, 2015; Li *et al.*, 2019a, 2019c; Liu *et al.*, 2019). However, thyroid cancer-related research has not been found. In addition, the pathogenesis of thyroid cancer is complex. From the perspective of molecular pathology, its occurrence and development are similar to other malignant tumors and it is related to the abnormal expression of oncogenes and tumor suppressor genes at different stages (Ramirez-Moya *et al.*, 2018).

In this study, we first analyzed TCGA and GEO databases and found that FTO mRNA levels in thyroid cancer tissues were relatively lower than in normal tissues. Because there were few Asian patients in TCGA and GEO databases, we collected Asian samples from the hospital and conducted qRT-PCR/IHC experiments. Results showed that FTO mRNA levels in the blood of patients with thyroid cancer were downregulated. Subsequent IHC results showed that the FTO protein was expressed in the nucleus. Compared with nontumor tissues, the protein expression of FTO in thyroid cancer tissues was also reduced. In addition, by analyzing the clinical information of thyroid cancer patients in TCGA database, we concluded that the expression of

FTO had a negative relationship with the tumor grade. Moreover, the expression of FTO in patients with lymph node metastasis was relatively lower than that in patients without lymph node metastasis. After multivariate logistic regression analysis, we found that the expression of FTO was an independent risk factor for tumor grade of thyroid cancer. Among patients collected for IHC detection, patients with lymph node metastasis also had a lower positive rate of FTO protein than patients without lymph node metastasis. Consistent with the bioinformatic results, as the tumor grade increased, the expression of FTO protein also decreased relatively.

From results of the KM survival analysis using data of TCGA database, we can see that disease-free survival in the low expression group of FTO was generally lower compared with the high expression group of FTO, although the difference between the two groups was not significant. We assume this phenomenon might be due to the reasons that the general prognosis of thyroid cancer is good and it is more difficult to track disease-free survival, which causes more data loss.

As we expected, CNV and DNA methylation both affect FTO expression. Although this association is relatively weak, it also provides a certain direction for subsequent research. Based on the above results, we speculated that the expression of FTO might decrease with progression and metastasis of thyroid cancer. Therefore, through GSEA, we tried to find the possible regulatory mechanism of FTO in thyroid cancer. From results of the GSEA, we can see that the expression of FTO may be involved in regulation of many cancer-related pathways. We screened the most relevant thyroid cancer pathway, KEGG THYROID CANCER, and found that this pathway was related to metastasis of thyroid cancer. Through correlation analysis of the corrected transcriptome data, we concluded that the key protein, TP53, in this pathway was positively correlated with FTO, which further proved our speculation. However, whether and how FTO participates in regulation of these pathways need more in-depth research.

Conclusion

In conclusion, we found downregulated expression of FTO in thyroid cancer and the expression of FTO may be a potential prognostic molecular marker for thyroid cancer. Further experimental verification should be conducted to prove the biological impact of FTO.

Disclosure Statement

No competing financial interests exist.

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Supplementary Material

Supplementary Figure S1

Supplementary Table S1

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