Abstract. Abnormal methylation of the TNFRSF10C and TNFRSF10D genes has been observed in numerous types of cancer; however, no studies have investigated the methylation of these genes in non-small cell lung cancer (NSCLC). The aim of the present study was to investigate the association between TNFRSF10C and TNFRSF10D methylation and NSCLC. Methylation levels of 44 pairs of NSCLC tumor tissues and distant non-tumor tissues were analyzed using quantitative methylation specific PCR and methylation reference percentage values (PMR). The methylation levels of the TNFRSF10C gene in NSCLC tumor tissue samples were significantly higher compared with those in the distant non-tumor tissues (median PMR, 2.73% vs. 0.75%; P=0.013). Subgroup analysis demonstrated that the methylation levels of TNFRSF10C in tumor tissues from male patients were significantly higher compared with those in distant non-tumor tissues (median PMR, 2.73% vs. 0.75%; P=0.041). The levels of TNFRSF10C methylation were also higher in the tumor tissues of patients who were non-smokers compared with their distant non-tumor tissues (median PMR, 2.50% vs. 0.63%; P=0.013). TNFRSF10C methylation levels were higher in the tumor tissues from male patients compared with those from female patients (median PMR, 2.50% vs. 0.63%; P=0.031). However, no significant differences in the methylation levels of the TNFRSF10D gene were observed between the sexes. Using the cBioPortal and The Cancer Genome Atlas lung cancer data, it was demonstrated that TNFRSF10C methylation levels were inversely correlated with TNFRSF10C mRNA expression levels (r=-0.379; P=0.008). In addition, demethylation of lung cancer cell lines A549 and NCI-H1299 using 5'-aza-deoxycytidine further confirmed that TNFRSF10C hypomethylation was associated with significant upregulation of TNFRSF10C mRNA expression levels [A549 fold-change (FC)=8; P=1.0x10^-4; NCI-H1299 FC=3.163; P=1.143x10^-5]. A dual luciferase reporter gene assay was also performed with the insert of TNFRSF10C promoter region, and the results revealed that the TNFRSF10C gene fragment significantly enhanced the transcriptional activity of the reporter gene compared with that in the control group (FC=1.570; P=0.032). Overall, the results of the present study demonstrated that hypermethylation of TNFRSF10C was associated with NSCLC.

Introduction

Lung cancer is the leading cause of cancer-associated mortality among males and the second leading cause of cancer-associated mortality among females globally, with an estimated 1.8 million new cases, and 1.6 million mortalities in 2012 (1). In the past 30 years, the number lung cancer-associated deaths in China has increased by 464.84%, and ~600,000 individuals die of lung cancer each year (2). In recent years, with the advances in early diagnosis and clinical treatment techniques, lung cancer treatment and diagnosis has improved (3). However, the number of patients with lung cancer in China has grown at a rate of 0.9% per year between 2000 and 2011 (2). Due to China's large population, such a growth rate causes a huge economic burden on public health.

Non-small cell lung cancer (NSCLC) includes large cell lung cancer, adenocarcinoma and squamous cell carcinoma, accounting for ≤85% of lung cancer cases (4). For NSCLC, surgery is the best treatment (5). However, only 25-30% of patients are suitable for potentially curative resection (5). At present, the 5-year survival rates of patients with stage Ia and IIIa NSCLC undergoing resection are 75 and 25%, respectively (5). Therefore, the development of new clinical
changes (6-8). Epigenetic changes include DNA methylation, histone modifications and expression of non-coding RNA (9). Tumor development is primarily associated with the inactivation of tumor suppressor genes and the activation of oncopgenes (8). DNA methylation in the promoter region can prevent the activation of a gene, leading to a downregulation of its expression (10). Previous studies have indicated that the primary markers of lung cancer include the inactivation of various tumor suppressor gene promoters by methylation. For example, aberrantly methylated genes associated with homeoboxes are frequently observed in early stage lung cancer, including SIX homeobox (SIX), LIM homeobox (LHX), paired box (PAX) and distal-less homeobox (DLX) (11). Hypermethylated tumor suppressor genes commonly found in advanced non-small cell lung cancer include: Helicase-like transcription factor (HLTF), adenovirus E1B 19 kDa interacting protein 3 (BNIP3), member X in H2A histone family (H2AFX), calcium voltage-gated channel subunit alpha 1 G (CACNA1G), TGFβ-induced factor homebox 1 (TGIF), inhibitor of DNA binding 4 (ID4) and calcium voltage-gated channel subunit alpha 1A (CACNAIA) (12). The present study aimed to identify aberrantly methylated genes in tumors, and then to develop diagnostic markers for NSCLC in plasma or other body fluids.

Tumor necrosis factor (TNF) receptor superfamily member 10c (TNFRSF10C) and TNFRSF10D are decoy receptors for the TNF-related apoptosis-inducing ligand (TRAIL), a member of the TNF family (13). TNFRSF10C and TNFRSF10D bind to TRAIL and do not contain the death domain necessary for apoptosis; thus, they inhibit TRAIL-induced apoptosis by competing with the TRAIL receptors (TRAIL-R) death receptor 4 (DR4) and DR5 for binding TRAIL (13). TRAIL and its associated receptors have been used as targets for anticancer therapeutics (14). The tolerability and safety of TRAIL was determined in clinical phase I studies, and no dose-limiting toxicity was observed (15,16). TRAIL-R agonists can be used as a single agent or in combination with conventional chemotherapy drugs for advanced NSCLC (17), colorectal cancer (18), B-cell lymphoma (19) and advanced or metastatic solid tumors or non-Hodgkin's lymphoma (NHL) (20). TRAIL ligands are widely expressed in tumor tissues and have attracted attention due to their ability to selectively induce tumor cell apoptosis (21).

The methylation levels of TNFRSF10C and TNFRSF10D in lung cancer have been studied in lung adenocarcinoma (22). A previous study explored tumor-associated genes, in which TNFRSF10C methylation levels were higher in patients with lung adenocarcinoma who were non-smokers compared with those who were smokers (22). However, the aforementioned study did not compare the methylation levels of TNFRSF10C and TNFRSF10D genes between lung cancer and normal tissues. In the present study, the association between TNFRSF10C and TNFRSF10D methylation levels and NSCLC was investigated by analyzing NSCLC and distant non-tumor tissues from patients from The First Affiliated Hospital of Fujian Medical University (Fuzhou, China) and The Cancer Genome Atlas (TCGA) database. The aim of the present study was to identify the contribution of methylation of the aforementioned genes to NSCLC pathogenesis.

Materials and methods

Tissue sample collection. A total of 44 pairs of tumor tissues and distant non-tumor tissues were collected from June 2017 to September 2018. These samples were obtained from 44 patients with NSCLC at The First Affiliated Hospital of Fujian Medical University (Fuzhou, China). Patient clinicopathological information was collected, including age, sex, family history. The average age of the patients was 62.91±9.05 years (mean ± SD; age range, 42-87 years), including 23 female and 21 male patients. There were 32 cases of adenocarcinoma, 12 cases of squamous cell carcinoma and 2 inflammatory pseudotumors. The research protocol was approved by The Ethics Committee of the First Affiliated Hospital of Fujian Medical University (Fuzhou, China; approval number, 2017-KY-068). All subjects provided written informed consent. Samples were taken during resection and the sampling procedure was performed under the guidance of a pathologist to distinguish between tumor tissues and distant non-tumor tissues (>10 cm away from the lesions).

Quantitative methylation-specific PCR (qMSP) assay. Primer sequences were designed based on the region of the TNFRSF10C and TNFRSF10D genes rich in CG sites. The primer sequences were as follows: TNFRSF10C forward, 5′-AGGTTGCAGTATTGATTAG-3′ and reverse, 5′-CGA TAACGCAGCACTT5′; TNFRSF10D forward, 5′-CGA TATGAAACGACGAAT-3′ and reverse, 5′-AACCAACCA CATAACTCTTAACC-3′. The conditions for qMSP were as follows: Initial denaturation at 95°C for 10 min, denaturation at 95°C for 20 sec, annealing at 56°C for 20 sec and extension at 72°C for 30 sec for 45 cycles. The melting curve program included 95°C for 15 sec, 60°C for 60 sec and then from 60°C to 95°C at a speed of 0.1°C/sec.

DNA was extracted from patient tissue samples according to the instructions of the QIAamp DNA FFPE Tissue kit (Qiagen GmbH). The extracted samples were measured for purity and concentration using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Inc.). The DNA concentration of all samples was >25 ng/µl, and the purity of DNA was 1.8-2.0 based on the absorbance ratio at 260 and 280 nm. The DNA was subjected to bisulfite modification using the EZ DNA Methylation-Gold™ kit according to the manufacturer’s instructions (Zymo Research Corp.). The primers and the bisulfite-modified DNA were then subjected to qMSP. The total reaction system was 10 µl, containing 5 µl SYBR mixture, 4 µl double-distilled H2O, 0.5 µl each of the forward and reverse primers and 0.5 µl modified DNA. To avoid errors in sample loading, ACTB was used as an internal reference for each sample. The primer sequences of ACTB were as follows: Forward, 5′-TGGTGATGGAGGTGGTTGTTAAGT-3′ and reverse, 5′-AACCAATAAACACCTACTCCTCCCTTA-3′. A total of 100% M-Sss I (New England BioLabs, Inc.) treated sperm DNA was used as the positive control and nuclease-free
water was used as the negative control for each group. The qMSP product was then subjected to Qsep100 DNA fragment analysis (BiOptic, Inc.) and visualized. Three randomly picked qMSP products of each gene were Sanger sequenced for validation.

ACTB is a housekeeping gene that is often stably expressed in most healthy and tumor cells; thus, it is commonly used as an internal reference for quantitation of mRNA expression levels and qMSP assays (23). Completely methylated DNA referred to human sperm DNA treated with methylated-SsSI, which allowed all C sites in the DNA to be methylated, including CG sites, and thus was used as a positive control. The formula for quantifying methylation levels was used to calculate the relative methylation level by the comparison between the target gene and the ACTB gene (24,25). For each sample, the relative methylation value was determined using the 2⁻ΔΔCq method. The sample methylation reference percentage (PMR) was calculated using the following formula: PMR=2⁻ΔΔCq x 100%; ΔΔCq =sample DNA (Cq gene-CqACTB)-fully methylated DNA (Cq gene-CqACTB). Luciferase reporter gene assay. A recombinant plasmid pGL3-TNFRSF10C containing the selected fragment (-121 to +363 bp of TNFRSF10C, 485 bp) was constructed. The target plasmid pGL3-TNFRSF10C was co-transfected with the pRL-SV40 plasmid carrying the Renilla luciferase gene to provide an internal control. Cells co-transfected with the pGL3-Basic plasmid and the pRL-SV40 plasmid were used as negative references in each experiment. The pGL3-Promoter plasmid and the pRL-SV40 plasmid were also co-transfected as a positive control each time. The plasmids were separately transferred into 293T cells (10% FBS, 37°C, 5% CO₂; China Center for Type Culture Collection) using FuGENE6® HD Transfection Reagent (Promega Corporation), according to the manufacturer’s protocol. The target plasmid (1 µg) and the Renilla luciferase plasmid (0.1 µg) were transfected into 293T cells at a ratio of 10:1 using FuGENE6® HD. After 24 h of transfection, luciferase activity was detected using a dual luciferase assay kit (Promega Corporation), according to the manufacturer’s protocol. The luciferase reaction intensity was measured at a wavelength of ~560 nm, using a SpectraMax 190 microplate reader (Molecular Devices) by adding the Luciferase Assay Reagent II. After the fluorescence reaction intensity was measured, the Stop&Glo Reagent was added to the same sample to quench the aforementioned reaction, and the Renilla luciferase reaction was simultaneously initiated for the second measurement. Luciferase activity was finally calculated using the Dual Luciferase Reporter Assay system (Promega Corporation) and normalized to Renilla luciferase activity.

Cell culture and gene expression detection. The embryonic cells A549 and NCI-H1299 were purchased from China Center for Type Culture Collection, and the cells were treated with 5 µmol/l 5'-azadeoxycytidine (5-Aza; A3656, Sigma-Aldrich; Merck KGaA) for 24 h at 37°C (25). RNA was extracted by TRIzol® (Invitrogen; Thermo Fisher Scientific, Inc.) before and after 5-Aza treatment, and cDNA was obtained by using a PrimeScript RT kit (Takara Biotechnology Co., Ltd.). The quantitative PCR primers sequences (Sangon Biotech Co., Ltd.) were as follows: TNFRSF10C forward, 5'-TGCACA GAGGGTGTGATTAC-3' and reverse, 5'-ATTCCGGAA GGTGCTCTCTT-3'; ACTB forward, 5'-AGCACAGAGCCT CGCCTTT-3' and reverse, 5'-AGGGTGAGGATGCTCTCT TC-3'. The Real-time PCR system was configured using the Takara TB Green kit (Takara Biotechnology Co., Ltd.), and the mRNA expression levels of TNFRSF10C were detected using a StepOne Plus real-time PCR instrument (Applied Biosystems; Thermo Fisher Scientific, Inc.). The thermocycling conditions for qPCR were as follows: Initial denaturation at 95°C for 10 min, 45 cycles of denaturation at 95°C for 20 sec, annealing at 56°C for 20 sec, extension at 72°C for 30 sec and a final extension at 72°C for 10 min. Relative expression levels were calculated using the 2⁻ΔΔCq method (25) and normalized to internal reference gene β-actin. A negative reference was also prepared using nuclease-free water.

Bioinformatics analysis. A total of 312 pairs of lung cancer tumor tissues and distant non-tumor tissues were obtained from TCGA database (https://tcga.xenahubs.net) for the analysis of differential TNFRSF10C and TNFRSF10D gene methylation levels. The correlation between methylation and mRNA expression levels of the TNFRSF10C gene (49 samples) were analyzed and charted using cBioPortal (http://www.cbioportal.org). TNFRSF10C DNA methylation and TNFRSF10C expression data from the lung adenocarcinoma (LUAD) project were obtained by downloading the data of 568 TCGA lung cancer samples from National Cancer Institute GDC Data Portal (https://portal.gdc.cancer.gov/repository).

Statistical analysis. Statistical analysis was performed using SPSS version 20.0 (IBM Corp). Nonparametric Wilcoxon signed-rank tests were used to determine the differences in methylation between tumor tissues and distant non-tumor tissues. The methylation level is expressed as the median. The nonparametric Wilcoxon rank-sum test was used to analyze the methylation differences between tumor tissues and distant non-tumor tissues in TCGA database. The correlation between TNFRSF10C methylation levels and mRNA expression levels was determined using Spearman's test. Luciferase reporter gene activities were analyzed using the Kruskal Wallis test with Dunn's post hoc test. P<0.05 was considered to indicate a statistically significant difference.

Results

Selected promoter fragment in the current methylation assay. Corresponding primers for the CG-rich region of TNFRSF10C and TNFRSF10D were selected for analysis (Fig. 1A). The target fragments of both genes were detected using capillary electrophoresis amplification, and the amplified fragment was confirmed using Sanger sequencing (Fig. 1B and C). There were 7 and 15 cytosines (including 5 CG and 1 CG) in the primer sequences of TNFRSF10C and TNFRSF10D, respectively. The qMSP melting curves indicated that the qMSP products of both genes were homogeneous (data not shown).

Differences in TNFRSF10C methylation levels in tumor tissues and distant non-tumor tissues. Methylation levels of the TNFRSF10C and TNFRSF10D genes between tumor and
distant non-tumor tissues were compared in 44 patients with NSCLC. The results demonstrated that the methylation levels of *TNFRSF10C* in tumor tissues were significantly higher compared with those in distant non-tumor tissues (P=0.013; Fig. 2A). Further subgroup analysis of the clinicopathological data revealed a significant association between *TNFRSF10C* methylation levels and NSCLC in male patients and in non-smokers (Table I). In male patients, the methylation levels of *TNFRSF10C* in tumor tissues were higher compared with those in distant non-tumor tissues (P=0.041; Fig. 2B), and the methylation levels in tumor tissues from male patients were significantly higher compared with those in tumor tissues from female patients (P=0.013; Fig. 2B). In patients who were non-smokers, *TNFRSF10C* methylation levels were higher in tumor tissues compared with distant non-tumor tissues (P=0.031; Fig. 2C). However, in female patients who were non-smokers there were no significant differences in the DNA methylation levels of *TNFRSF10C* between tumor tissues
and distant non-tumor tissues (P=0.46; Table I). In male patients who were non-smokers, the methylation levels in tumor tissues were significantly lower compared with those in distant non-tumor tissues (P=0.03; Table I). In male patients who were smokers, no significant difference was observed in the methylation levels between tumor tissues and distant non-tumor tissues (P=0.225; Table I). These results indicated that the association of TNFRSF10C hypomethylation with lung cancer was only observed in male patients who were non-smokers. Due to the limited number of patients in each subgroup, this conclusion needs to be interpreted with caution.

The levels of methylation of TNFRSF10D were not significantly different in the paired samples (P=0.198; Fig. 2D). Furthermore, subgroup analysis of the clinicopathological data did not reveal any significant associations between TNFRSF10D methylation levels and NSCLC.

To ensure that the data of the inflammatory pseudotumor did not affect the results of the experiment, the data from the two inflammatory pseudotumors were removed from analysis.

Figure 2. Differences in TNFRSF10C/TNFRSF10D methylation levels in tumor tissues and distant non-tumor tissues. (A) Differences in the level of methylation of TNFRSF10C in tumor and distant non-tumor tissues. (B) Difference in methylation levels of TNFRSF10C in tumor tissues between male and female patients. (C) Difference between TNFRSF10C methylation in NSCLC tissues and distant non-tumor tissues was significant for non-smokers. (D) Differences in the levels of methylation of TNFRSF10D in tumor tissues and distant non-tumor tissues. PMR, methylation reference percentage; TNFRSF10C, tumor necrosis factor receptor superfamily member 10c; TNFRSF10D, tumor necrosis factor receptor superfamily member 10d.

TCGA data analysis also failed to identify any significant association between TNFRSF10D and lung cancer (data not shown). Overall, these results suggested no association between TNFRSF10D methylation levels and lung cancer.

Clinicopathological, TNFRSF10C DNA methylation and TNFRSF10C expression data from the lung adenocarcinoma (LUAD) project were downloaded from TCGA database. From the gene promoter region (-2,000 to +500 bp), 10 CG sites closest to the qMSP amplicon presented in Fig. 4A were selected, and between-group comparison and association analyses were performed. Based on the comparison between cancer and non-cancerous tissues, it was demonstrated that multiple CG sites, such as cg08309809, were significantly associated with LUAD (Fig. 4B). Further subgroup analysis revealed an association between cg08309809 and LUAD in male patients (Fig. 4C), female patients (Fig. 4D) and patients who were smokers (Fig. 4E). There was no significant association between cg08309809 and LUAD in the non-smoking group (Fig. 4F); however, this may be due to the small number of non-tumor tissues (n=2). The methylation and expression levels of multiple CG sites in the TNFRSF10C gene were significantly inversely correlated (r=-0.408~0.146; P=1.00x10^{-13}~2.65x10^{-4} for different CG sites; Fig. 4G), suggesting that TNFRSF10C promoter methylation may be associated with gene silencing.

Dual luciferase assay to detect promoter activity of TNFRSF10C. A dual luciferase reporter plasmid containing the promoter fragment of TNFRSF10C (-121 to +363 bp) was constructed. The results demonstrated that the inserted gene fragment significantly enhanced the transcriptional activity of the reporter gene compared with the control group [fold-change (FC)=1.570, P=0.032; Fig. 5A]. The purpose
of the luciferase experiment was to confirm whether the promoter fragment regulated gene expression levels. By cloning the fragment into the reporter vector, it was demonstrated that the fragment enhanced the expression levels of the reporter gene, suggesting that the fragment regulated gene expression levels. Therefore, the methylation of this fragment may serve a role in regulating the \textit{TNFRSF10C} gene expression levels. To determine the relationship between \textit{TNFRSF10C} promoter methylation and its transcriptional expression levels in lung cancer cells, 5'-aza-deoxycytidine was used to treat lung cancer cells A549 and NCI-H1299. The results demonstrated that the mRNA expression levels of \textit{TNFRSF10C} in the two lung cancer cell lines were significantly increased following demethylation treatment (A549, FC=8.000, \(P=0.0001\); NCI-H1299, FC=3.163, \(P=1.143\times10^{-5}\); Fig. 5B).

**Discussion**

Decreased gene expression levels of \textit{TRAIL} decoy receptor gene \textit{TNFRSF10C} caused by hypermethylation have been observed in glioblastoma (26), prostate (27) and breast (28) cancer. The results of the present study also demonstrated that the levels of methylation of \textit{TNFRSF10C} in NSCLC tissues were higher compared with those in distant non-tumor tissues, which was consistent with the aforementioned studies. The present study also demonstrated that hypermethylation of \textit{TNFRSF10C} was specific to tumor tissues in male patients.
Figure 4. The Cancer Genome Atlas analysis of the association between TNFRSF10C methylation levels, lung adenocarcinoma and TNFRSF10C gene expression. (A) TNFRSF10C gene structure. ▲, testing CG sites. (B) Differences in the methylation levels of CG sites between tumor and para-tumor. (C) Differences in the methylation levels of CG sites between tumor and para-tumor in female patients. (D) Differences in the methylation levels of CG sites between tumor and para-tumor in male patients. (E) Differences in the methylation levels of CG sites between tumor and para-tumor in patients who were smokers. (F) Differences in the methylation levels of CG sites between tumor and para-tumor in patients who were non-smokers. (G) Association of the methylation levels of each CG site with TNFRSF10C expression. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. F, forward primer; R, reverse primer; TNFRSF10C, tumor necrosis factor receptor superfamily member 10c.
and patients who were non-smokers. **TNFRSF10C** methylation levels in tumor tissues of male patients were higher compared with those of female patients. These results demonstrated that **TNFRSF10C** methylation levels were associated with NSCLC.

Previous studies have reported that the relationship between **TNFRSF10C** gene hypermethylation and downregulated expression levels has been observed in human prostate cancer and pancreatic cancer (27,29). In the present study, the levels of **TNFRSF10C** methylation were inversely correlated with mRNA expression levels in lung cancer tissue data from the cBioPortal database. 5'-Aza-deoxycytidine was used to treat two lung cancer cell lines, A549 and NCI-H1299, and the mRNA expression levels before and after treatment were compared. The results demonstrated that the mRNA expression levels of **TNFRSF10C** were significantly increased after 5'-aza-deoxycytidine-induced demethylation. These results indicated that the expression of **TNFRSF10C** was regulated by promoter methylation.

Hypermethylation of the **TNFRSF10C** promoter initiates the binding of TRAIL with tumor necrosis factors DR4 and DR5, and thus mediates the TRAIL-R signaling pathway (30). Although TRAIL-R induces apoptosis in a variety of cancer types, several types of tumor, such as cholangiocarcinoma and leukemia have recently been demonstrated to be resistant to TRAIL-R signaling (31,32). In addition, TRAIL-R can activate the NF-kB signaling pathway and promote the proliferation and migration of cancer cells, and thus TRAIL-R is associated with the development of cholangiocarcinoma and leukemia (31,32).

Although smoking can cause epigenetic changes that induce cancer development, especially lung cancer, smoking is not the only cause of lung cancer (33). **TNFRSF10C** has been demonstrated to promote the epidermal growth factor receptor (EGFR)-KRAS signaling pathway-mediated tumor development (34). A previous study reported that patients who were non-smokers harbored a higher frequency of EGFR mutations compared with smokers (35). Downregulation of **TNFRSF10C** expression via methylation promotes TRAIL-R deactivation in the EGFR-KRAS signaling pathway and promotes cancer progression and invasion (34). The results of the present study demonstrated that the **TNFRSF10C** methylation levels were higher in tumor tissues compared with distant non-tumor tissues in non-smokers; however, no significant differences were observed between the groups in smokers. These results may provide novel evidence supporting the epigenetic changes occurring in lung cancer in patients who are non-smokers.

The incidence of lung cancer in China is high in males at present (2). In the present study, the methylation levels of **TNFRSF10C** in tumor tissues from male patients were higher compared with distant non-tumor tissues, and the methylation levels in tumor tissues from male patients were significantly higher compared with those from female patients. These findings may provide novel evidence supporting the effects of sex on **TNFRSF10C** methylation levels in lung cancer.

**TRAIL-R** can mediate the inhibition of tumor development (36). **TNFRSF10C** and **TNFRSF10D** lack a cytoplasmic domain and do not contain transmembrane domains; thus, they are unable to participate in the transduction of apoptotic signals (37). Hypermethylation of **TNFRSF10C** promotes the binding of TRAIL to apoptotic receptors, thereby promoting apoptosis in cancer cells and preventing tumorigenesis (38). However, **TNFRSF10D** has a truncated death domain and hence is not capable of inducing apoptosis but protects against TRAIL-mediated apoptosis (39). The results of the present study indicated that **TNFRSF10D** methylation was not associated with lung cancer.

The present study primarily examined the methylation levels of the **TNFRSF10C** and **TNFRSF10D** genes in lung tumor and distant non-tumor tissues. The results demonstrated that **TNFRSF10C** hypermethylation was associated with NSCLC, especially in non-smokers and male patients. The...
underlying molecular mechanisms of the hypermethylation of TNFRSF10C in the development and progression of NSCLC need further investigation. DNA methylation of tissue biopsy may be used as a standard indicator of current pathological diagnosis (40,41). However, tissue biopsy is inconvenient and traumatic. Liquid biopsy, such as circulating tumor cells, circulating tumor DNA and exosomes can provide more comprehensive disease information (42,43). Furthermore, investigating the methylation levels of TNFRSF10C and TNFRSF10D genes of patients' blood samples, and combining data from both tissues and blood may allow their development as novel diagnostic biomarkers of lung cancer.

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Availability of data and materials

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

Authors' contributions

YQ and LQ collected tissue samples and patient data, analyzed the data and wrote the manuscript. MQ and MZ carried out the experiments. SD designed the study and critically revised the manuscript for intellectually important content. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by The Ethics Committee of the First Affiliated Hospital of Fujian Medical University (Fuzhou, China; approval number: 2017-KY-068). All patients provided written informed consent for participation in this study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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