Mechanism of interaction between TM4SF1 and jak2-stat3 signaling pathway in lung cancer cells and expression analysis in non-small cell lung cancer

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Research article

Keywords: Transmembrane 4 superfamily member 1 (TM4SF1); non-small cell lung cancer(NSCLC); A549; migration; JAK2-STAT3 signaling pathway; clinical characteristics

Posted Date: May 7th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-23716/v1

Abstract

Background According to the latest data released in 2018, it is estimated that there will be 18.1 million new cancer cases worldwide (excluding 1.7 million non-melanoma skin cancers) and 9.6 million cancer deaths (excluding 950 non-melanoma skin cancers) Million cases). Among them, the incidence of lung cancer (11.6% of the total number of cases) and mortality (18.4% of the total number of cancer deaths, which are expected to cause 1.8 million deaths) are the first. In recent years, studies have found TM4SF1 play an important role in the development process of many tumors.

Methods Sixty-one patients with NSCLC who underwent surgical resection of cancer tissues, para-carcinoma tissues, and 10 normal lung tissues removed from benign lung disease (Jun/2018-Dec/2018) were collected. Real-time immunofluorescence quantitative PCR (qRT-PCR) and Western blot were used to detect the expression of TM4SF1 in NSCLC tissues (CT), para-carcinoma tissue (PCT), and normal lung tissues(NLT). TM4SF1 gene was overexpressed in lung cancer A549 cells using lentiviral transfection technology, qRT-PCR and Western blot were used to detect whether TM4SF1 gene was successfully expressed in lung cancer A549 cells, and Transwell was used to detect the effect of TM4SF1 overexpression on A549 migration. JAK2-STAT3 signal pathway interference reagent AG490 was used to analyze the expression levels of Stat3 and downstream Sox2 genes in the overexpression group, blank group, negative control group and their corresponding treatment groups TM4SF1, JAK2-STAT3 signal pathway using real-time qRT-PCR. Analyze the relevance of these three indicators at the same time.

Results The expression levels of TM4SF1 mRNA and protein in cancer tissues were significantly higher than those in adjacent cancer tissues ($P<0.05$) and normal lung tissue specimens ($P <0.05$). The expression of TM4SF1 was not significantly associated with the age and sex of patients, but was associated with tumor size, degree of differentiation, lymph node metastasis, and clinical stage were related ($P<0.05$). TM4SF1 was successfully overexpressed in A549 cells. After overexpressing TM4SF1, the ability to migrate of A549 cells was significantly enhanced, and the expression levels of Stat3 and downstream Sox2 in the JAK2-STAT3 signaling pathway were up-regulated. The expression of TM4SF1, Stat3 and Sox2 at the mRNA level showed a positive correlation trend ($P<0.01$).

Conclusion TM4SF1 is highly expressed in NSCLC, and its expression level is closely related to many clinical staging indicators. Overexpression of this gene can promote the migration of A549 cells and up-regulate the expression levels of Stat3 and downstream Sox2 in the JAK2-STAT3 signaling pathway. The expressions of TM4SF1, Stat3 and Sox2 were positively correlated in A549 cells. TM4SF1 may promote the occurrence, development and distant metastasis of NSCLC through this pathway. TM4SF1 may become a potential therapeutic target for NSCLC.

1. Background

Lung cancer is the most common cancer in humen and the leading cause of death worldwide[1]. In 2019, malignant neoplasms of the lung continue to be the principal cancer-related cause of death in Spain, with

a survival rate of only 10.7%[2]. The results indicated that an estimated 4292,000 new cancer cases and 2814,000 cancer deaths would occur in China in 2015, with lung cancer being the most common incident cancer and the leading cause of cancer death[3]. More than 85% of these patients are diagnosed with non-small cell lung cancer (NSCLC). The traditional treatment methods for lung cancer include surgery, radiation therapy, and chemotherapy. Recently emerging targeted treatment methods has also significantly improved the therapeutic effect of lung cancer, especially NSCLC. Despite targeted therapy has increased the treatment options in the latest years, the overall 5-year survival rate is less than 5 %[4]. But treatment for late stage lung cancer, especially NSCLC with distance metastasis, remains challenging. It is urgent to better understand the cancer migration/metastasis mechanism and identify novel gene targets for the treatment of late stage NSCLC[5].

Transmembrane 4 superfamily member 1 (TM4SF1), also known as tumor-associated antigen L6 (tumor-associated antigen L6), are members of the four-transmembrane protein superfamily. Recent studies have found that TM4SF1 gene plays an important role in the progression of various tumors [6], and affecting the growth, migration, infiltration and metastasis of the tumor cells [7-9]. Recent studies have confirmed[10] that TM4SF1 may regulate the expression level of downstream Sox2 through the JAK2-STAT3 signaling pathway and thus affect the prognosis and distant metastasis of breast cancer. However, it is a lack of study on TM4SF1 in NSCLC and its relationship with clinicopathology.

We studied the expression of TM4SF1 in 61 NSCLC and found TM4SF1 expression in cancer tissues is significantly higher than para-carcinoma tissues and normal lung tissues that indicating the gene may have played a role in the development of NSCLC. To confirm this, we constructed a cellular model over-expressing the TM4SF1and found that its high expression affects the migration ability of A549 cells, in particular, via the JAK2-STAT3[11]. Our study reveals novel mechanism for NSCLC infiltration and metastasis, and potential new treatment idea.

2. Methods

2.1 Lung specimens

A total of 61 patients with NSCLC who underwent surgical resection in the Second Affiliated Hospital of Guangxi Medical University from June to November 2018 were collected. Including 45 males and 16 females, aged 32 to 81 (58.08 ± 11.81) years old. The patients have not received any radiotherapy and chemotherapy before the surgery. Pathological examination was performed by board certified pathologist CH. A small tumor associated normal lung tissue (>5 cm away from the tumor margin) was also collected from each patient. All pathological examinations were primary non-small cell lung cancer, and the corresponding adjacent tissues were normal lung tissues> 5 cm from the edge of the cancer tissue. Degree of differentiation: 17 cases of low differentiation, 44 cases of moderate-high differentiation; clinical staging: 16 cases of stage I, 28 cases of stage II, 16 cases of stage III, and 1 case of stage IV. In addition, normal lung tissue specimens excised from benign lung diseases of 10 patients were also collected. There were 7 males and 3 females, aged 25 to 56 (40.53 ± 12.45) years old; 4 cases

of benign granulomas, 2 cases of fungal transfection, 3 cases of inflammatory pseudotumor, and 1 case of pulmonary bullae. All specimens were stored in a refrigerator at 80 ° C within 20 minutes of ex vivo. The study was approved by the hospital ethics committee(IRB) and all patients were fully consented.

2.2 culture cell

Human lung cancer cells A549 were purchased from the Shanghai Cell Bank of the Chinese Academy of Sciences, TM4SF1 lentivirus overexpression vector was purchased from Shanghai Jikai Gene Technology Co., Ltd., and AG490 was purchased from MCE. Human lung cancer A549 cells were quickly thawed in a 37 ℃ thermostatic water bath, and the cells were seeded in 1640 medium containing 10% fetal bovine serum (Vicent Biotechnology (Nanjing) Co., Ltd.) (Vicent Biotechnology (Nanjing) Limited Company), add 1% penicillin-streptomycin double antibody (Dalian Meilun Biotechnology Co., Ltd.) to the culture medium, and culture the cells in a 37 ℃, 5% CO2 incubator, passaging once every 2-3 days, digest with trypsin (Dalian Meilun Biotechnology Co., Ltd.) for 1 min, passage cells at 1: 3, and take log-grown cells for experiment.

2.2.1 lentiviral transfection

Overexpression lentiviral vector was synthesized and constructed by Shanghai Jikai Gene Company. Design three parallel experimental groups. The first group was the TM4SF1 overexpression group (OG), the second group was the vehicle group(VG), and the third group was the untreated group (UG). The transfection process was performed according to the company's instructions. A day before transfection, A549 cells were seeded into 6-well plates at 4×10^4 cells/ml, and transfection began when the cell fusion reached 30% -50% on the day of transfection. The old medium was aspirated, washed twice with PBS, and transfected with MOI values of 10 and 100. The total liquid volume was 1 ml/well. Twelve hours after transfection, the medium in the six-well plate was changed to 1640 medium containing 10% fetal bovine serum and the culture was continued. At 72 h after transfection, multiple visual fields were randomly selected to count the number of visible cells under fluorescence, and the transfection efficiency under different transfection conditions was compared. At the time of transfection MOI = 100, the fluorescence was the strongest at 72 h after transfection. At this time, cells were harvested for the next experiment.

2.3 PCR、qRT-PCR、

DMSO (Beijing Solarbio) was used to configure the AG490 solution, and the appropriate amount of medium containing 10% fetal bovine serum was added to dilute, so that the final concentration of DMSO in the diluted solution was <0.1%, and the concentration of AG49 was configured to 40 μM. Cells in logarithmic growth phase were tested. Cells containing AG490 were used to culture cells in the OG,VG, and UG. After the drug treatment cells for 48 h ,extracted mRNA of cells. The three cells were divided into the drug treatment group and Control group. The mRNA column extraction kit (purchased from Shenggong Co., Ltd.) was used to extract the total mRNA of the specimen and cells in a low temperature environment according to the instructions. The spectrometer was used to evaluate the quality of the

extracted mRNA according to OD260/OD280, and the measured value was used to calculate the mRNA concentration. Dilute the extracted mRNA according to the concentration and use the reverse transcription kit (Monad Biotechnology Co., Ltd.) to synthesize cDNA by reverse transcription. MonAmp™ SYBR Green qPCR Mix (High Rox) (Monad Biotechnology Co., Ltd.) qRT-PCR technology was used to detect the expression of TM4SF1 in the specimens. The primers were designed and synthesized by Biotech Biotechnology (Shanghai) Co., Ltd. The primer sequences are shown in the table 1. Reaction conditions: pre-denaturation at 95 ℃ for 30 s, denaturation at 95 ℃ for 5 s, annealing at 60 ℃ for 10 s, extension at 72 ℃ for 30 s, a total of 40 cycles. $2^{-\Delta\Delta Ct}$ quantification[12] was used to calculate the expression levels of TM4SF1 and Sox2 in tissues and cells. Set 3 replicates in each group and calculate the average. Experiments were repeated 3 times independently.

2.4 Western blot

Use protein lysate to lyse (Biyuntian Co., Ltd.) three kinds of tissues and cells, extract total protein from the cells, and determine the protein concentration using BCA method and add 5 × SDS protein loading buffer (Biyuntian Co., Ltd.). Using 12% SDS-PAGE (Beijing Solibao Technology Co., Ltd.) to separate the proteins. After the electrophoresis, transfer the protein bands to the PVDF membrane (Beijing Solibao Technology Co., Ltd.) and block with 5% skim milk powder for 1 h at room temperature. Put the bands into rabbit anti-human TM4SF1 polyclonal primary antibody (Abcam, USA) and β-actin monoclonal primary antibody (both working volume dilution ratio is 1: 1000), and react at 4 ℃ overnight; add after washing the membrane Fluorescent secondary antibody (1: 5000 dilution of working solution), react for 1 h at room temperature, wash the membrane, and then use the Odyssey bid-color infrared fluorescence imaging system to analyze protein expression levels. The relative expression level of TM4SF1 protein = the gray value of the target gene band / the gray value of the internal reference band. Each group of experiments was repeated 3 times independently.

2.6 Transwell

Three groups of cells were collected and counted 72 h after transfection. Cells were added to the transwell upper chamber at 2×10^5/well. The upper chamber was 200 μl of 1640 serum-free medium and the lower chamber was 500 μl of complete medium. After incubating in the incubator for 12 h, wipe the cells on the surface of the upper chamber with a cotton swab. Cells on the surface of the lower chamber were stained with crystal violet for 2 min. Five fields were randomly selected to observe and count under the microscope, and the average number of transmembrane cells was calculated. Each experiment was repeated three times.

2.7 statistical analysis

SPSS 26.0 software was used for analysis. The t-test was used to compare the means between the two groups. The one-way analysis of variance was used to compare the means between the two groups. The LSD method was used for the pairwise comparison. Statistical results are expressed as s, and $P < 0.05$ indicates that the difference is statistically significant.

3. Results

3.1 Expression of TM4SF1 in CT, PCT and NLT

The expression level of TM4SF1 mRNA in CT,PCT and NLT was statistically significant (F=18.864, P=0.000<0.001), while expression levels of para-carcinoma tissues and normal lung tissues were not statistically significant (t=0.109, P>0.05,Table 2) . The expression level of TM4SF1 mRNA in CT was 4.90 times as much as PCT and 5.17 times as much as NLT. The increase of TM4SF1 mRNA expression in CT was related to the clinical stage, tumor size, lymph node metastasis, and degree of pathological differentiation (P <0.05). However, no correlation was found with the patient's gender, age, and histological type (P> 0.05, Table 3). The relative gray value of the target gene in CT, PCT, and NLT were 3.74 ± 0.16, 0.92 ± 0.013, and 0.82 ± 0.045, indicating that the expression level of TM4SF1 protein in CT was higher than that in PCT and NLT. The difference was statistically significant (F=924.039, P=0.000<0.001); while the levels of PCT and NLT were not different, and the results were not statistically significant. (P=0.172> 0.05,Fig.2A-B).

3.2 A549 cells were transfected with lentiviral vector TM4SF1

To understand the relationship of elevated expression of TM4SF1 to the progression of NSCLC, we overexpressed the gene in A549 cell line. The fluorescence microscopy was used to observe the transfected cells, and the transfection efficiency of the lentivirus-transfected cells was measured. This indicates that the TM4SF1 overexpression lentiviral vector successfully transfected cells. (Fig.3A-B).

3.3 Expression of TM4SF1 proteins in A549 cells.

The relative gray values of TM4SF1 in the OG, UG and VG were 1.38±0.02, 0.99±0.03, and 1.00±0.02, respectively. The TM4SF1 protein level in the OG was significantly higher than that of VG and UG (P=0.001<0.01,Fig.4A-B). After transfection, the expression levels of TM4SF1 and Sox2 mRNA in the cells were significantly higher than those in VG and UG(P=0.003<0.05, Table 4), which proved that the lentiviral vector successfully stably transfected lung cancer A549 cells and let TM4SF1 high expression in cells.

3.4 Expression levels of Stat3 and Sox2 after AG490 treatment of cells

After treatment with AG490, the expression levels of Sox2 in the UG and VG both decreased slightly, but there was no significant difference between the two groups. The expression levels of Sox2 in the OG significantly decreased after treatment, which was lower than that in the positive control group (P=0.006<0.05,Table 5). Spearman rank correlation analysis showed that the expressions of TM4SF1, Stat3 and Sox2 showed a positive correlation (P <0.01, Table 6). Transwell migration experiments were performed on the three groups of cells. The experimental results showed that the cell migration rate of the OG was significantly higher than that of VG and UG (F=74.607, P <0.01), but there was no significant difference between the latter two groups (P=0.434> 0.05), indicating that TM4SF1 overexpression can significantly promote the migration of lung cancer cells A549 cells (Fig.5A-B).

4. Discussion

At present, lung cancer is still the number one cancer in terms of morbidity and mortality in the world, and the incidence of lung cancer has been increasing year by year in China, and NSCLC accounts for the vast majority. At this stage, the treatment of NSCLC is still surgery-based, supplemented by radiotherapy, chemotherapy, targeted and biological therapy. However, due to the occurrence and development of NSCLC and long-term migration, the overall survival rate of patients is not ideal [13], and the 5-year survival rate of advanced lung cancer is only 20% -30% [14-15].

TM4SF1 (Transmembrane-4-L-six-family-1) is also known as tumor-associated antigen L6 (tumor-associated antigen L6). The molecular weight of TM4SF1 is 21kd. , Followed by two potential N-glycosylated hydrophilic regions and a -COOH terminal transmembrane region. These transmembrane regions may be involved in multiple signal transduction pathways. Many tumor cell experiments have shown that TM4SF1 plays a very important role in tumor cell growth, migration, invasion and metastasis [16]. TM4SF1 is widely expressed in malignant tumors of epithelial origin. In recent years, many domestic and foreign studies have confirmed that TM4SF1 has abnormally high expression in colon cancer [17], pancreatic cancer[18], breast cancer[19], ovarian cancer[20] and other tissues. It is closely related to the migration of tumor cells and the prognosis of patients.

And some studies have shown that[21] During the occurrence and development of NSCLC, the activation of the JAK2-STAT3 signaling pathway has an important role in the proliferation and metastasis of tumor cells.

A large number of experiments have shown that[22-23], the JAK-STAT signaling pathway is closely related to many tumorigenesis-related pathways, and is the aggregation site of these transduction pathways, and the persistently high expression status of the JAK-STAT signaling pathway has been confirmed can induce normal cell canceration to become tumor cells, and STAT3 has been identified as an oncogene as a key target[24].

Disruption of JAK2-STAT3 pathway activation in many cancers will curb the malignant progression of cancer. STAT3 is another important signaling molecule in the tumor microenvironment. It is a key signaling molecule for various cytokines and growth factors to play a role. It not only mediates the process of extracellular signal transmission to the nucleus, but also directly mediates gene regulation[25].

However, studies on the expression of TM4SF1 at different levels in the same specimen of NSCLC and its relationship with clinicopathology are rare. Therefore, this study used qRT-PCR and Western blot to detect the different expression of TM4SF1 in CT and PCT (distance from cancer tissues>5 cm) , and the expression of TM4SF1 in 10 NLT was detected at the same time to avoid errors caused by different specimens detection. The study confirmed that the mRNA and protein expression levels of TM4SF1 in CT were significantly higher than those in PCT and NLT, indicating that the expression of TM4SF1 was consistent at the transcription and translation levels. The expression level of TM4SF1 was closely related

to the tumor size ,clinical stage and lymph node metastasis, suggesting that TM4SF1 may participate in the occurrence, development and long-term migration of NSCLC through some mechanism.

At the cellular level, we selected lentivirus as the TM4SF1 overexpression vector. Western blot and qRT-PCR tests showed that the expression vector was successfully transferred into the cells. Transwell experiments showed that the upregulation of TM4SF1 expression can enhance the migration of lung cancer cell A549 ability, suggesting that the high expression of TM4SF1 is closely related to the metastasis of lung cancer. TM4SF1 may play an important functional role in the metastatic process of lung cancer. Gao[26] and other researchers recently discovered a new signal pathway for breast cancer multi-target organ metastasis. TM4SF1 couples collagen receptor DDR1 with tyrosine protein kinase activity and activates downstream JAK2-STAT3 signaling by recruiting syntenin 2 and PKC pathway, which in turn promotes the expression of downstream transcription factors such as Sox2. This new DDR1 signal transduction pathway promotes breast cancer metastasis to target organs such as lung, bone, and brain. In this study, we detected that TM4SF1 overexpression in lung cancer cells A549 can mediate the up-regulation of Stat3 and Sox2. After the addition of AG490, a JAK2-STAT3 signaling pathway inhibitor, the expression levels of Stat3 and Sox2 downstream of the pathway were significantly suppressed, proving that TM4SF1 promotes the expression of downstream Sox2 factors through the JAK2-STAT3 signaling pathway. Recent studies have shown[27] that DDR1 and its downstream target ERK/Akt-mTOR interaction may be the mechanism by which TM4SF1 enhances the sensitivity of NSCLC chemotherapy. However, the mechanism by which TM4SF1 may promote the transfer of NSCLC by upregulating Sox2 downstream of the JAK2-STAT3 signaling pathway is still unclear. In the next part of this study, DDR1 and its downstream targets ERK/Akt-mTOR Possible mechanisms such as interactions can be further verified.

5. Conclusion

The high expression of TM4SF1 in lung cancer may indicate that TM4SF1 is related to the generation, progression and metastasis of non-small cell lung cancer, and may find a new idea for the diagnosis and treatment of non-small cell lung cancer. Overexpression of TM4SF1 can significantly improve the migration ability of lung cancer A549 cells. TM4SF1, which is highly expressed in lung cancer A549 cells, can up-regulate Sox2 expression downstream through the JAK2-STAT3 pathway, and may affect the long-term development and metastasis of lung cancer through this pathway.

Declarations

Ethics approval and consent to participate

The study was approved by the Second Affiliated Hospital of Guangxi Medical University. Written informed consent was obtained from all individual participants included in the study.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and/or analysed during the current study are not publicly available due to the terms agreed with the local ethics committee. They are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was partially supported by Scientific Research and Technology Development Program of Guangxi (S2017011). The funding body agreed to the design of the study.

Author contributions

N.YM, the first author, is responsible for writing articles and implementing main experiments. X.L revised the article. L.LP and D.HL collected clinical specimens. C.WK and W.YX did part of experiment.All authors have read and approved the manuscript.

Acknowledgements

Not applicable.

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Tables

Table 1 Primer sequences in RT-qPCR.

Gene name	Sequence（5′-3′）
TM4SF1	Forward：CTCAGCCGCTTCGTGTGGTTC
	Reverse: CAGCAGCCACAGCAGTCATCC
Sox2	Forward： GCTCGCAGACCTACATGAACGG
	Reverse： AGCTGGCCTCGGACTTGACC
Stat3	Forward： CACCAAGCGAGGACTGAGCATC
	Reverse： AGCCAGACCCAGAAGGAGAAGC
GAPDH	Forward： CAGGAGGCATTGCTGATGAT
	Reverse： GAAGGCTGGGGCTCATTT

Table.2 Comparative expression of TM4SF1 mRNA in CT, PCT and NLT（`$x \pm s$）

group	TM4SF1mRNA（ΔCt）	ΔΔCt	$2^{-\Delta\Delta Ct}$	*t*	*P*
CT&PCT	3.57±2.10&5.86±2.34	-2.29	4.89	5.752	0.000
CT&NLT	3.57±2.10&5.94±0.40	-2.37	5.17	3.541	0.001
PCT&NLT	5.86±2.34&5.94±0.40	——	——		

Table.3 Relationship between TM4SF1 mRNA expression and clinicopathological characteristics in NSCLC（`$x \pm s$）

clinicopathological characteristics		Number of cases	mRNA of TM4SF1	*t*	*P*
Gender	Male	44	3.40±2.09	-1.005	0.319
	Female	17	4.00±2.07		
Age	≤60	31	3.49±2.19	-0.279	0.781
	>60	30	3.64±2.01		
Degree of pathological differentiation	Medium-high differentiation	44	2.67±1.76	2.138	0.037
	Poorly differentiated	17	3.91±2.12		
Tumor size	≤3cm	24	1.72±1.28	F=15.574[a]	0.000
	>3cm&≤5cm	25	3.13±1.70		
	>5cm	12	4.94±1.90		
Lymph node metastasis	N	36	2.69±1.82	-2.887	0.005
	P	25	4.17±2.07		
Histology type	Adenocarcinoma	44	3.47±226	-0.580	0.564
	Other types	17	3.82±1.59		
TNM	I~II	44	1.68±1.07	5.285	0.000
	III~IV	17	4.30±1.92		

Note：a.Tumor size analysis of variance between three groups, mRNA expression levels were different between the three groups, $P<0.05$.

Table.4 Comparison of the relative expression of TM4SF1, Stat3 and Sox2 mRNA in three groups（$\bar{x}\pm s$）

Gene	TM4SF1 mRNA	*P*	Sox2 mRNA	*P*	Stat3 mRNA	*P*
Group						
UG	0.94±0.83	0.006[a]	1.09±1.04	0.003[a]	1.17±0.13	0.000[a]
VG	1.14±0.96	0.007[a]	1.00±0.75	0.003[a]	1.02±0.10	0.000[a]
OG	8.05±0.41	——	7.12±0.92	——	7.56±0.21	——

Note:a.Compared with the OG,P<0.05.

Table.5 Expression levels of TM4SF1, Stat3 and Sox2 mRNA in the treatment group($\bar{x}\pm s$)

Gene	Sox2 mRNA	P	Stat3 mRNA	P
Group				
OG	7.12±0.92	——	7.56±0.21	——
Treatment of UG	-0.55±0.20	——	-0.64±0.04	——
Treatment of VG	-0.64±0.13	——	-0.70±0.02	——
Treatment of OG	-1.43±0.14	0.006[a]	-1.50±0.12	0.000[a]

Note:a.Comparison of overexpression treatment group with OG,P<0.05.

Table.6 Analyzed the correlation of mRNA expression levels of TM4SF1, Stat3 and Sox2($\bar{x}\pm s$)

Gene	TM4SF1	Stat3	Sox2
Correlation coefficient			
mRNA	8.05±0.41	7.56±0.21	7.12±0.92
r	0.991[a]	0.964[b]	0.937[c]
P	0.000[a]	0.000[b]	0.002[c]

Note:a.compared with Stat3, b.compared with Sox2, c.compared with TM4SF1,P<0.01.

Figures

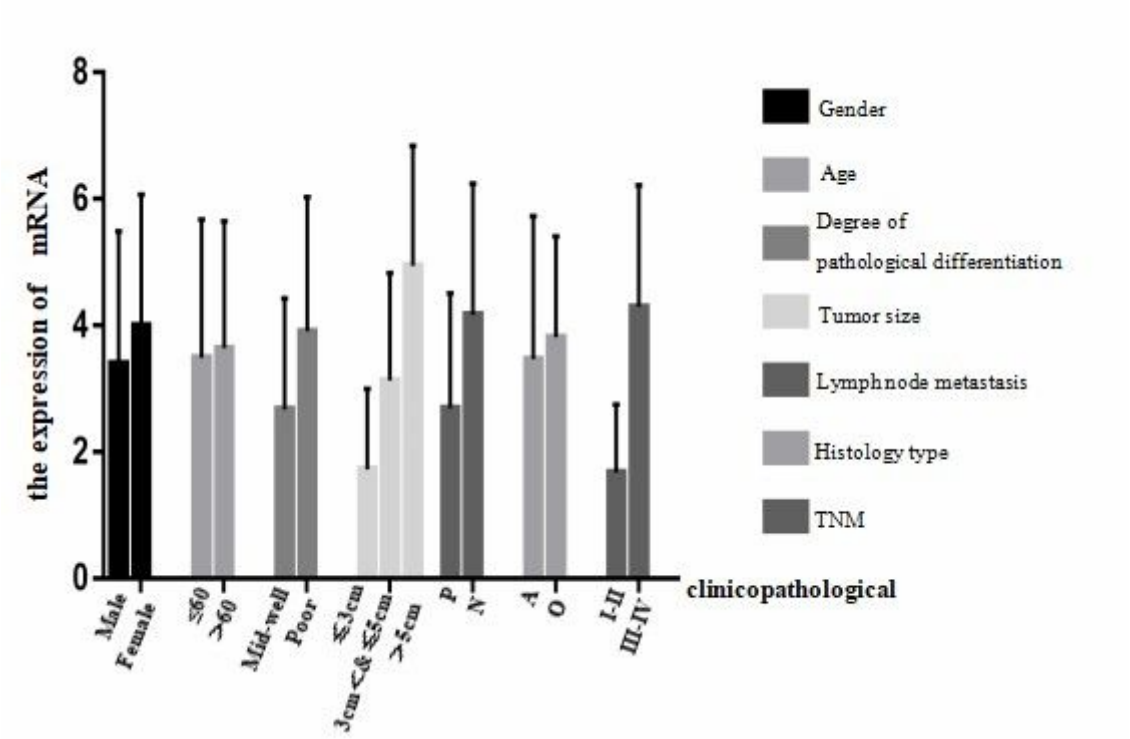

Figure 1

comparison of relative expression levels of TM4SF1 mRNA among different clinicopathological features

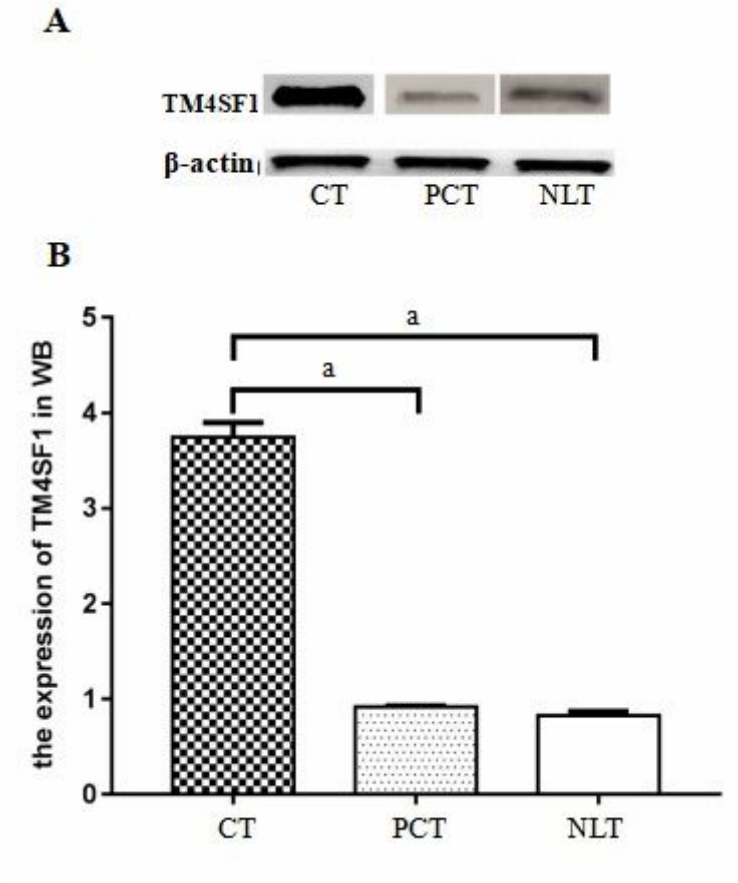

Figure 2

Electrophoresis results (A) and histogram (B) of TM4SF1 protein expression in CT, PCT, and NLT detected by WB a. P <0.05 The difference in relative gray value of the target gene between CT and PCT and NLT.

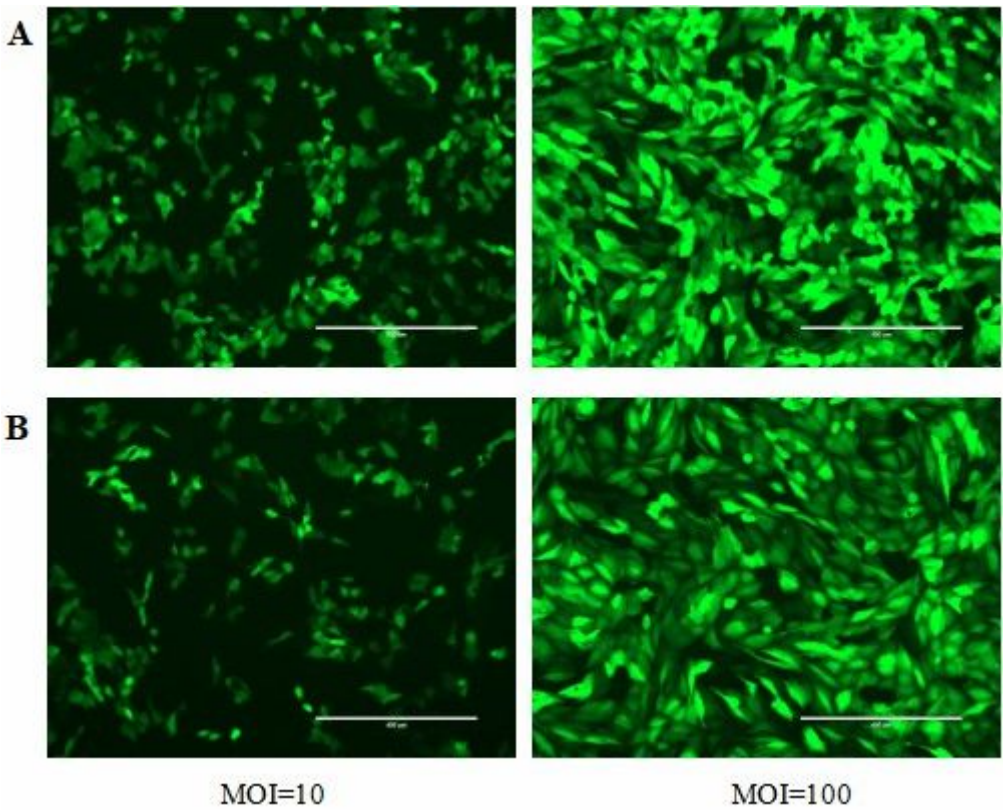

Figure 3

TM4SF1 (A) and negative control virus (B) transfect lung cancer cells.

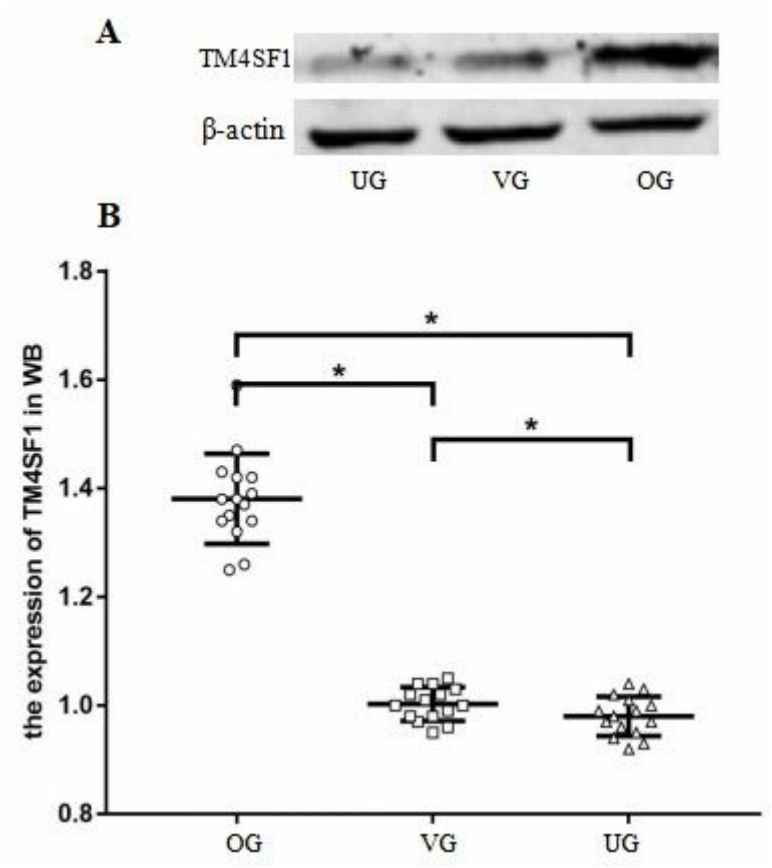

Figure 4

WB method to detect TM4SF1 protein expression levels in OG, UG and VG (A) and violin plot (B) *The difference between overexpression group and negative control group and blank group is statistically significant, P <0.05

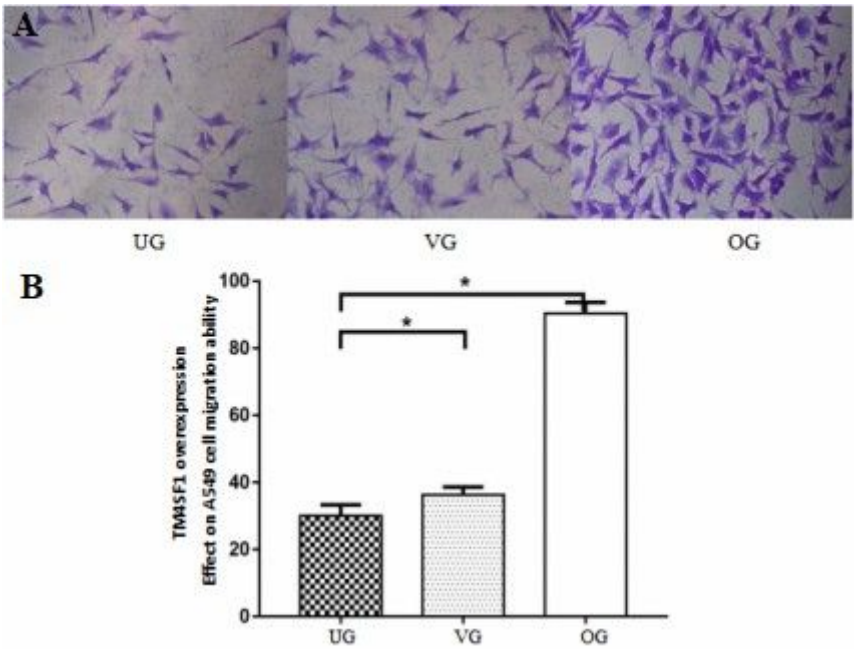

Figure 5

Cell migration and invasion experiment (A) detection of the effect of TM4SF1 on cell migration and results analysis histogram (B) * The cell migration rate of OG is higher than that of VG and UG, and the results are statistically significant, P <0.05