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XXYLT1 methylation contributes to the occurrence of lung adenocarcinoma
Methylation and lung adenocarcinoma

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Abstract

Background: There is evidence that DNA methylation play major roles in lung cancer. In our previously study, C3 or f21 , also referred to as *XXYLT1*, rs2131877 polymorphism is associated with a reduced risk of lung adenocarcinoma. So, we explored the role of *XXYLT1* methylation in lung adenocarcinoma.

Methods: This study was conducted in 2 steps. In the first step, we recruited 15 patients with lung adenocarcinoma. Cancer tissues and para-carcinoma tissues were obtained from each of the patients. In the second step, 150 patients with lung adenocarcinom were enrolled, and cancer and normal lung tissue were obtained from each patients, respectively. The expression levels of *XXYLT1* mRNA were determined, the deoxyribonucleic acid methylation status was analyzed by MassARRAY Spectrometry. The methylation data of individual units were generated by EpiTyper v1.0.5 software.

Results: The XXYLT1 mRNA expression was significantly lower in cancer tissues than in para-carcinoma and normal lung tissues. Meanwhile, the methylation rates of three CpG units (CpG_23, CpG_25, and CpG_60.61.62.63.64.65) within the *XXYLT1* gene were higher in cancer tissues compared to the para-carcinoma and the normal lung tissues. This difference was particularly significant in male patients.

Conclusions: Our results suggested that methylation of XXYLT1 may have significance in the pathogenesis of lung adenocarcinoma.

Abbreviations: DNA = deoxyribonucleic acid, EGF = epidermal growth factor-like, RNA = ribonucleic acid, XXYLT1 = xyloside xylosyltransferase 1.

Keywords: Lung adenocarcinoma, DNA methylation, gene expression, XXYLT1

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1. Introduction

Lung cancer has an increasing prevalence and has emerged as a main cause of cancer-related deaths in both genders.[1] It has a higher mortality rate than other cancer types such as breast, colorectal, and prostate cancers, and it is the second most common cause of mortality in women.[2] Smoking addiction is the main predominant risk feature for the development of lung cancer, and the overall survival rate of lung cancer is only 15%. Therefore, lung cancer is a great global health concern. In the USA, lung cancer accounts for 13.18% of the total diagnosed cancers and has become the second most prevalent malignancy; it is responsible for 25.94% of cancer-related deaths.[3] In 2015, Chen et al reported that lung cancer was also the most prevalent cancer and the main cause of mortality in China.[4,5]

Currently, the cancer survival rate has increased due to the involvement of advanced technology in both early diagnosis and therapy, especially in targeted therapy. Despite the technological advancements in diagnosis and the timely availability of treatment, it has been shown that greater than 50% of cancer cases are still diagnosed at an advanced stage, when the therapeutic approaches are palliative rather than curative.[6,7] Further exploration of the molecular rationale of the causative factors of cancer may lead to the discovery of newer molecular targets.

The Notch signaling pathway operates in several cell types in a highly conserved manner. It has shown essential functions in the development of all metazoans as well as in adult tissue

homeostasis, and defective patterns in this signaling pathway can cause numerous types of cancer.[8–11] The existence of tandem epidermal growth factor-like (EGF) repeats in the extracellular domain of the Notch architecture leads to substantial modification of a variety of O-linked glycans via an S_Ni-like mechanism. Varying degrees of O-linked glycosylation (addition of an O-linked glucose moiety) to the Notch extracellular domain regulates Notch activation.[12] It has been reported that Notch activation occurs by the addition of an O-glucose moiety to multiple EGF repeats and occurs repeatedly across the NECD. Mechanistically, glucoside-xylosyltransferases 1/2 (GXYLT1/2) initially donate one 1,3-linked xylose motif to mammalian O-glycosylated Notch EGF repeats,[13] while xyloside xylosyltransferase 1 (XXYLT1) can shift the subsequent xylose moiety through catalytic addition to O-glycosylated EGF repeats of Notch.[14]

The catalytic domain of the XXYLT1 type II membrane protein, located in the endoplasmic reticulum,[15] extends into the luminal region.[16] XXYLT1 is a key member of glycosyltransferase family 8.[17] During the biochemical transformation, the stereochemistry of XXYLT1 remains unchanged after the catalytic addition of the α-linked xylose moiety donated by the UDP-xylose molecule. XXYLT1 also plays a negative regulatory role in the Notch signaling pathway, and reduced activity of XXYLT1 would lead to enhanced Notch signaling.[18] XXYLT1 participated in the biosynthesis of Glc-O-type sugar chains and encodes xylosyltransferase activity.[19] After XXYLT1 activities, it transfers the second xylose to O-glucosylated EGF repeats of Notch, and modulated Notch activation.[16] Furthermore, chromosome 3 open reading frame 21 (C3orf21), located on chromosome 3q29 and also referred to as XXYLT1, is a member of the glycosyltransferase family 8 family. It has been reported that the chromosome 3 open reading frame 21 polymorphism rs2131877 is associated with a reduced risk of lung adenocarcinoma.[20] We previously reported that lung adenocarcinoma is closely associated with the ablation of XXYLT1.[21] In malignant cancer, deoxyribonucleic acid (DNA) methylation is closely associated with aberrant gene expression. Moreover, DNA methylation has been associated with the therapeutic outcome and disease prognosis in a few types of cancer.[22–24] To date, XXYLT1 gene methylation has not been investigated; therefore, the pathogenic potential of methylated XXYLT1 in lung cancer still remains unknown. This study aimed to observe the methylation levels and mRNA expression of XXYLT1 and to further analyze their possible correlation with the risk of lung adenocarcinoma.

2. Materials and methods

2.1. Study subjects and sample collection

This study was conducted at Zhejiang Cancer Hospital, China. In the first step, we recruited 15 patients with a confirmed diagnosis of lung adenocarcinoma who underwent surgical treatment between July 2015 and July 2016. A total of 15 cancer (40 mg) and 15 para-carcinoma (40 mg) tissues were obtained from the participating patients during the surgical intervention. In the second step, 150 cancer (40 mg) and 150 normal lung tissue (40 mg) samples, which were obtained from 150 patients with lung adenocarcinoma who underwent surgical treatment in the same time period, were enrolled from the Zhejiang Cancer Hospital biological sample bank, and to validate what we found in the first

step. All patients in this study were not received any antitumor therapy before surgery (including chemotherapy, radiotherapy, targeted therapy, immunotherapy, etc). All tissue samples were kept at −80°C until further analysis. This study followed the guidelines of the Helsinki declaration, and the protocols were approved by the Medical Ethics Committee of Zhejiang Cancer Hospital. Detailed information was provided to all study subjects, and a written consent was obtained from each participant before the commencement of the study.

2.2. Quantitative reverse transcription–polymerase chain reaction (qRT-PCR)

Total ribonucleic acid (RNA) (1 μg) was isolated from the tumors and adjacent nontumorous tissues at the first step, and tumors and lung tissues at the second step using TRIzol reagent (Invitrogen, USA), according to the manufacturer's instructions, and then reverse transcribed using a Transcriptor First Strand cDNA Synthesis Kit (Roche, Switzerland) with DnaseI (RNase-free, Fermentas, USA). qRT-PCR was conducted using qPCR with a SYBR Premix EX Taq kit (Takara, China) and the StepOnePlus Real-Time PCR System (ABI, USA). The reverse transcription of RNA and PCR of cDNA were combined to measure the mRNA expression of XXYLT1. The primers for XXYLT1 were designed by the EpiDesigner website (http://www.epidesigner.com/) as follows: forward, 5′-TGCTGTGCTGACG-GATAAG-3′ and reverse, 5′-CTGGCAGGAAA- CTGTCAAAT-3′. For glyceraldehyde 3-phosphate dehydrogenase (GAPDH), the forward and reverse primers were 5′-GGAGTC-CACTGGCGTCTTC-3′ and 5′-GCTGATGATCTT-GAGGCTGTTG-3′, respectively. The reaction conditions for the PCR were 95°C for 2 minutes followed by 40 cycles at 95°C for 15 seconds, 58°C for 20 seconds, and 72°C for 20 seconds. Finally, a cycle at 72°C for 5 minutes was performed. The level of mRNA was analyzed using a gel imaging system (Bio-Rad Gel Doc 2000, USA). The data were analyzed using the $2^{-\Delta\Delta CT}$ gene quantification method. All the assays reported in this study were repeated 3 times.

2.3. Methylation of the XXYLT1 gene

DNA was extracted from the tumors and adjacent nontumorous tissues at the first step, and tumors and lung tissues at the second step using a DNA extraction kit (Tiangen, China). The sequence of the XXYLT1 gene was obtained from the University of California, Santa Cruz Genome Biological Information Network (http://genome.ucsc.edu/). The DNA was purified and converted by bisulfite treatment using an EZ DNA Methylation Kit (Zymo Research, USA). The CpG islands were located in the promoter region of XXYLT1 (Fig. 1). Primers for the CpG island of XXYLT1 were used to amplify the bisulfite-treated DNA. The primers for XXYLT1 were designed using EpiDesigner software, which is available online (www.epidesigner.com, Agena Bioscience, USA). Forward DNA was modified by sodium bisulfite using the following reaction conditions: 5′-AGGAAGA-GAGTTTTGGTGAATATTATTAGTAGGTGGT-3′ and reverse 5′-CAGTAATACGACTCACTATAGGGAGAAGGCTA-TACCCTTAAAACCTAAAACCCAAC-3′; primers for the CpG island of XXYLT1 were used to amplify the bisulfite-treated DNA with 20 cycles at 95°C for 30 seconds and 50°C for 15 minutes. The reaction conditions used for the PCR program were as follows: 95°C for 4 minutes and then 72°C for 3 minutes,
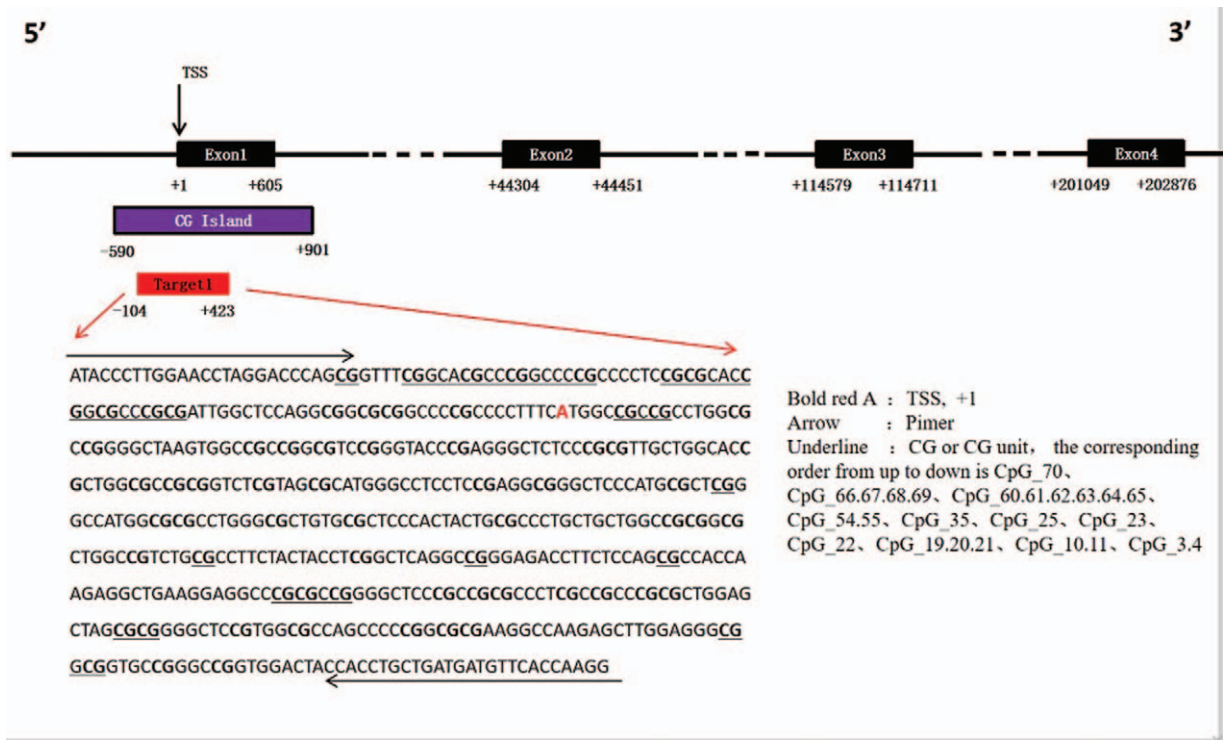
Figure 1. The CpG islands location in the promoter region of XXYLT1 .

followed by 45 cycles at 95°C for 20 seconds, 56°C for 30 seconds, and 72°C for 1 minute. The PCR products were reacted with shrimp alkaline phosphatase followed by a uracil-specific cleavage reaction using a MassCLEAVE Reagent Kit (Agena, USA). The Agena MassARRAY platform was used to analyze the methylation levels of specific CG loci or CG units of the CpG island in the *XXYLT1* gene promoter. According to the manufacturer's protocol, a cluster of consecutive CpG sites was defined as a CpG unit. Methylation data of individual CG loci or CG units were generated by EpiTYPER software, v1.2 (Agena, USA).

2.4. Statistical analysis

The data were analyzed by SPSS, version 17.0 (SPSS Inc., Chicago, USA), and corresponded to a normal distribution. Data are represented as the mean ± standard error. Differences between the 2 groups were assessed using a paired 2-tailed Student *t*-test. $P < .05$ was the cut-off level for statistical significance.

3. Results

3.1. Clinical characteristics of the subjects

In this study, all patients were diagnosed with lung adenocarcinoma. At the first step, a total of 15 patients were enrolled in this study. The patients consisted of 8 men and 7 women. The average patient age was 64.07 ± 5.44 years old (the average ages of the male and female patients were 63.87 ± 5.57 years old and 64.29 ± 5.74 years old, respectively). Three of the 8 men were smokers, while all of the women were nonsmokers. At the second step, 150

patients were nonsmoker (75 male and 75 female). The average patient age was 57.97 ± 8.67 years old (the average ages of the male and female patients were 58.13 ± 9.28 years old and 57.81 ± 8.08 years old, respectively).

3.2. The expression of XXYLT1 mRNA between cancer tissues and para-carcinoma tissues and normal lung tissues.

At the first step, in order to compare the expression of *XXYLT1* in cancer and para-carcinoma tissues, the mRNA levels of *XXYLT1* were measured in both tissue types. In all patients, the results showed that there was no significant difference between the cancer and para-carcinoma tissues (0.95 ± 0.21 vs 1.00 ± 0.14, $P = .179$). We then carried out subgroup analysis according to the gender. In the female study subjects, the expression value of *XXYLT1* mRNA was 1.03 ± 0.18 and 1.00 ± 0.16 in the cancer and para-carcinoma tissues, respectively ($P = .662$). This study further revealed that in the male study subjects, the gene expression level of *XXYLT1* in the para-carcinoma and cancer

Table 1

Comparison of the *XXYLT1* mRNA expression between groups (15 samples).

	All patients	Female patients	Male patients
CA	0.95 ± 0.21	1.03 ± 0.18	0.88 ± 0.24
CP	1.00 ± 0.14	1.00 ± 0.16	1.00 ± 0.10
P value	.179	.662	.017

CA = cancer tissues, CP = para-carcinoma tissues, *P* value = CA compared with CP.
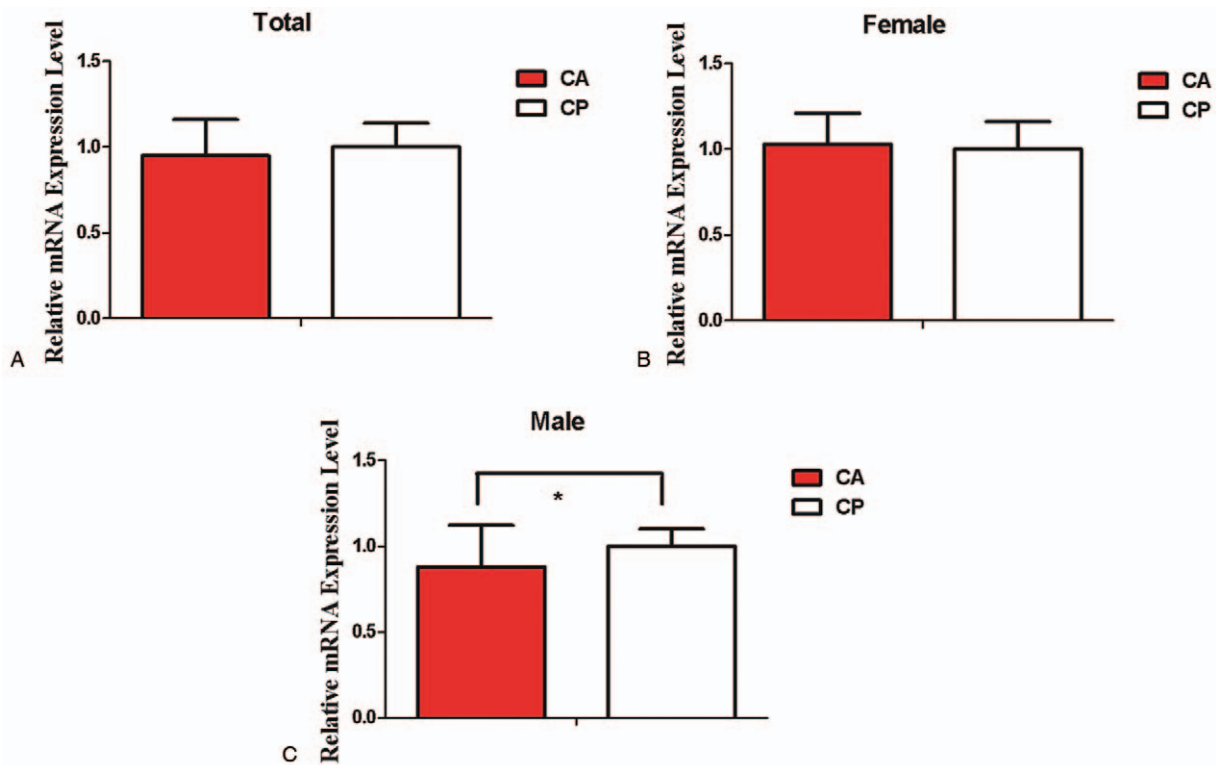
Figure 2. The expression level of *XXYLT1* mRNA (15 patients). (A) The relative mRNA expression level of the *XXYLT1* gene among different tissue samples. (B) The relative mRNA expression level of the *XXYLT1* gene among different tissue samples from female subjects. (C) The relative mRNA expression level of the *XXYLT1* gene among different tissue samples from male subjects.

tissues was 1.00 ± 0.10 and 0.88 ± 0.24, respectively ($P = .017$) (Table 1 and Fig. 2).

At the second step, we found a consistent trend. In all patients, the XXYLT1 mRNA expression level was significant difference between the cancer and normal lung tissues (0.93 ± 0.25 vs 1.00 ± 0.18, $P < .001$). In the female patients, the expression value of XXYLT1 mRNA was 0.97 ± 0.25 and 1.00 ± 0.20 in the cancer and normal lung tissues, respectively ($P = .064$). In the male patients, the expression level of XXYLT1 mRNA in the cancer tissues and normal lung tissues was 0.90 ± 0.25 and 1.00 ± 0.15, respectively ($P < .001$) (Table 2 and Fig. 3).

3.3. XXYLT1 methylation status in cancer tissues and para-carcinoma tissues and normal lung cancer

The methylation of eleven CpG units (CpG_3.4, CpG_10.11, CpG_19.20.21, CpG_22, CpG_23, CpG_25, CpG_35, CpG_54.55, CpG_60.61.62.63.64.65, CpG_66.67.68.69, and

CpG_70) were detected in this study. Three CpG units (CpG_10.11, CpG_35, and CpG_70) were removed because no methylation levels were detected, and 8 CpG units' methylation rate was enrolled in date analysis. MassARRAY was used to analyze the data. At the first step, The outcome of the *XXYLT1* gene methylation studies showed that the methylation rate of the CpG units (CpG_3.4, CpG_19.20.21, CpG_22, CpG_23, CpG_25, CpG_54.55, CpG_60.61.62.63.64.65, CpG_66.67.68.69) did not exhibit a significant difference between the cancer and para-carcinoma tissues in all patients. A similar trend was noticed in all of the female subjects. In contrast, in the male patients, the methylation rate of the CpG units (CpG_23, CpG_25, and CpG_60.61.62.63.64.65) was higher in the cancer tissues than in the para-carcinoma tissues. The methylation rates of CpG_23, CpG_25, and CpG_60.61.62.63.64.65 were 8.40 ± 1.35, 27.53 ± 2.50, and 14.53 ± 1.13, respectively, in the cancer tissues; however, in the para-carcinoma tissues, the methylation rates were 7.27 ± 1.53, 25.27 ± 2.37, and 13.13 ± 1.64 ($P = .03$, $P = .02$, and $P = .02$, respectively) (Table 3 and Fig. 4). At the second step, The methylation rates of the CpG units CpG_23, CpG_25 and CpG_60.61.62.63.64.65 were 7.81 ± 1.3, 25.98 ± 3.09, and 13.99 ± 1.11 in cancer tissues, and 7.47 ± 1.48, 25.33 ± 3.08, and 13.33 ± 2.22 in normal lung tissues in all patients. There is a significant difference between the cancer and normal lung tissues ($P = .023$, $.039$, and $.002$, respectively). A similar trend was noticed in the male patients, the methylation rate of the CpG units (CpG_23, CpG_25, and CpG_60.61.62.63.64.65) was higher in the cancer tissues than in the normal lung tissues. The methylation rates of CpG_23, CpG_25, and CpG_60.61.62.63.64.65 were

Table 2

Comparison of the *XXYLT1* mRNA expression between groups (150 samples).

	All patients	Female patients	Male patients
CA	0.93 ± 0.25	0.97 ± 0.25	0.90 ± 0.25
LT	1.00 ± 0.18	1.00 ± 0.20	1.00 ± 0.15
P value	$< .001$	$.064$	$< .001$

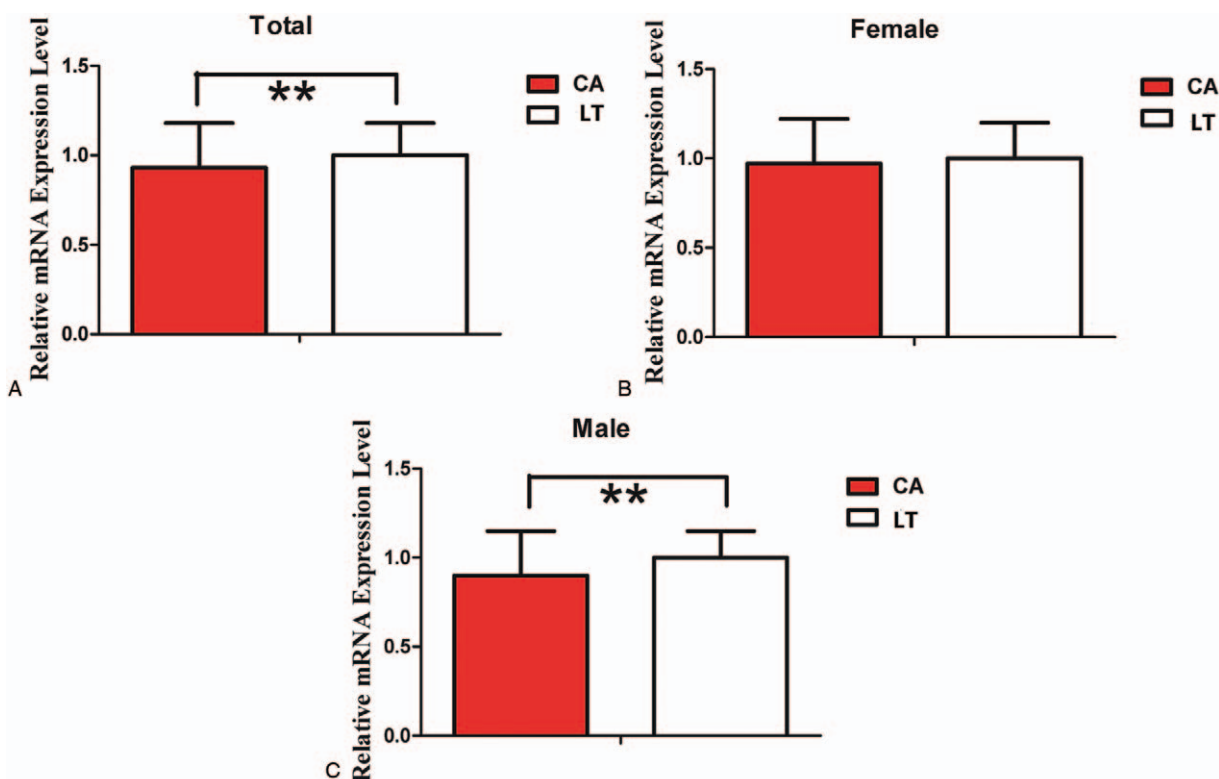CA = cancer tissues, LT = lung tissues, *P* value = CA compared with LT.

Figure 3. The expression level of *XXYLT1* mRNA (150 patients). (A) The relative mRNA expression level of the XXYLT1 gene among different tissue samples. (B) The relative mRNA expression level of the XXYLT1 gene among different tissue samples from female subjects. (C) The relative mRNA expression level of the XXYLT1 gene among different tissue samples from male subjects.

7.88 ± 0.90, 26.35 ± 1.36, and 13.68 ± 0.70, respectively, in the cancer tissues; however, in normal lung tissues, the methylation rates were 7.27 ± 1.49, 25.27 ± 2.31, and 13.13 ± 1.60 ($P = .001$, $P = .001$, and $P = .005$, respectively). But in the female patients, the methylation rate of CpG_60.61.62.63.64.65 was different between cancer and norml lung tissue (14.29 ± 1.33 and 13.53 ± 2.70, $P = .045$) (Table 4 and Fig. 5).

4. Discussion

In addition to lung adenocarcinoma, the amplification of *XXYLT1* has been reported in several types of cancer.[25,26] In malignant cancer, DNA methylation is closely related to aberrant gene expression. Moreover, DNA methylation has been associated with the therapeutic outcome and disease prognosis in a few cancer types. However, to date, *XXYLT1* gene methylation has not been investigated. In this study, we carried out a preliminary exploratory study, in the first, we found that the expression of *XXYLT1* mRNA in male patients displayed a lower level in the cancer tissues compared with the para-carcinoma tissues, and the methylation rates of CpG_23, CpG_25, and CpG_60.61.62.63.64.65 were higher in the cancer tissues than in the para-carcinoma tissues in the male patients with lung adenocarcinoma. To verify this preliminary findings, we further analyzed 150 patients with lung adenocarcinoma (75 men and 75 women) and found that the XXYLT1 mRNA expression was significantly higher in normal lung tissues than in cancer tissues, but, the XXYLT 1methylation rates lower in

Table 3

Comparison of the methylation rates of CpG units between groups.

		CpG_3.4	CpG_19.20.21	CpG_22	CpG_23	CpG_25	CpG_54.55	CpG_60.61.62. 63.64.65	CpG_66.67. 68.69
A	CA CP	3.87 ± 0.86	2.30 ± 0.60	2.43 ± 0.68	7.90 ± 1.60	26.47 ± 3.69	3.53 ± 0.82	13.87 ± 1.20	3.27 ± 0.94
		3.77 ± 0.82	2.43 ± 0.63	2.33 ± 0.66	7.37 ± 1.69	25.27 ± 3.20	3.33 ± 0.71	13.40 ± 2.37	3.03 ± 0.56
	P	.60	.40	.59	.18	.13	.25	.38	.230
F	CA CP	3.73 ± 0.88	2.20 ± 0.56	2.47 ± 0.74	7.40 ± 1.72	$25.4 0 \pm 4.42$	3.47 ± 0.83	13.20 ± 0.86	3.13 ± 0.92
		3.8 ± 0.77	2.40 ± 0.63	2.33 ± 0.62	7.47 ± 1.88	25.27 ± 3.95	3.27 ± 0.59	13.67 ± 2.97	3.00 ± 0.65
	P	.81	.42	.61	.91	.92	.38	.60	.58
M	CA CP	4.00 ± 0.85	2.40 ± 0.63	2.40 ± 0.63	8.40 ± 1.35	27.53 ± 2.50	3.60 ± 0.83	14.53 ± 1.13	3.40 ± 0.99
		3.73 ± 0.88	2.47 ± 0.64	2.33 ± 0.72	7.27 ± 1.53	25.27 ± 2.37	3.40 ± 0.83	13.13 ± 1.64	3.07 ± 0.46
	P	.33	.75	.81	.03	.02	.46	.02	.29

A = all patients, F = female patients, M = male patients, CA = cancer tissues, CP = para-carcinoma tissue.
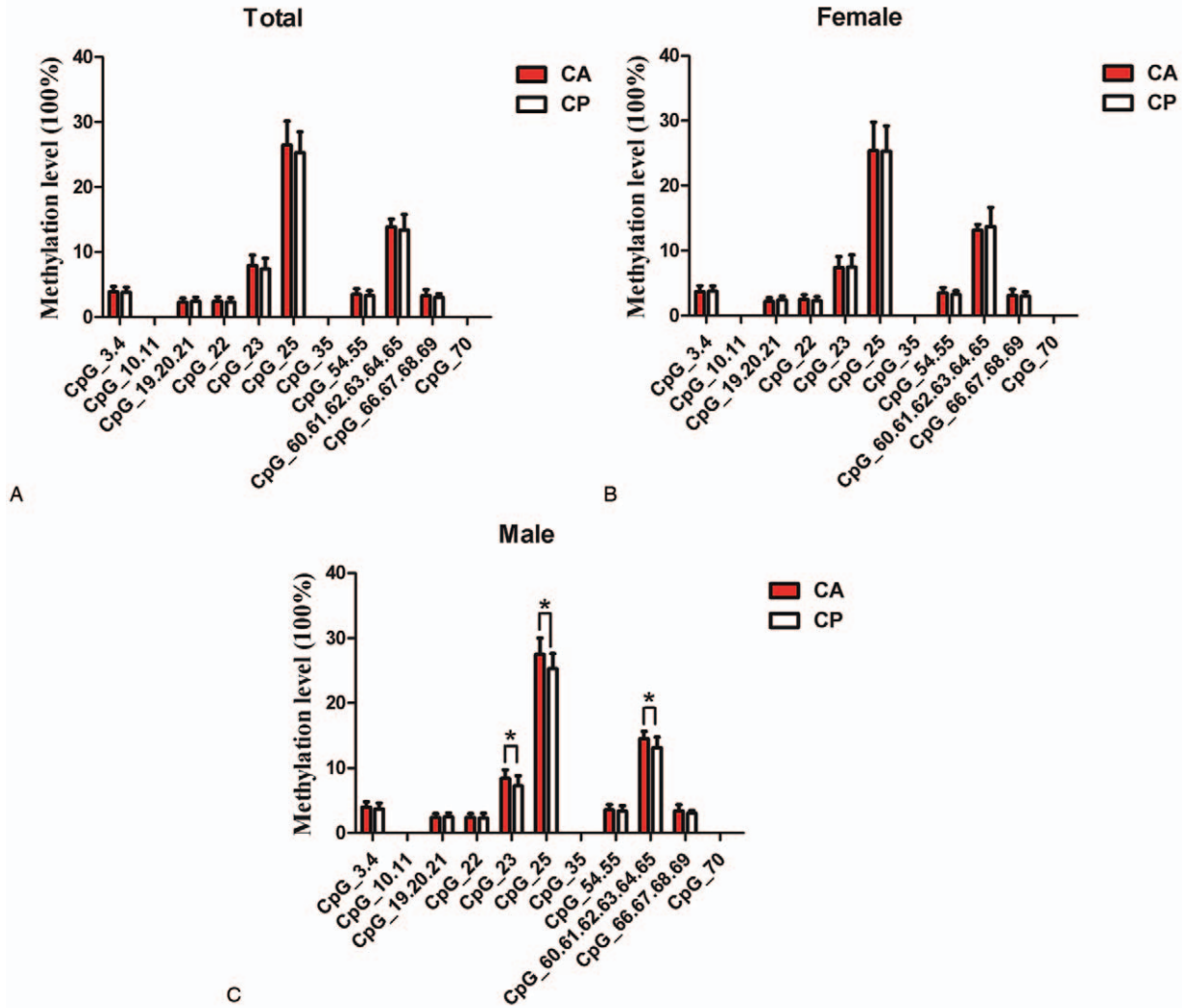
Figure 4. The methylation status of *XXYLT1* (15 patients). (A) Methylation level of the *XXYLT1* gene CpG units among the different tissue samples. (B) Methylation level of the *XXYLT1* gene CpG units among the different tissue samples from female subjects. (C) Methylation level of the *XXYLT1* gene CpG units among different tissues from the male subjects.

normal lung tissues than in cancer tissues. This difference was particularly significant in male patients. These results suggested that the *XXYLT1* gene could be an antioncogene and that its higher expression could inhibit the development of lung cancer.

In cancer, the Notch signaling pathway is a remarkable and fascinating oncology target; however, the consequences of Notch signaling on the tumor response mainly depend on the cancer. Notch signaling takes place through cell-cell communication in

Table 4

Comparison of the methylation rates of CpG units bewteen groups.

		CpG_3.4	CpG_19.20.21	CpG_22	CpG_23	CpG_25	CpG_54.55	CpG_60.61.62. 63.64.65	CpG_66.67. 68.69
A	CA	3.80 ± 0.79	2.40 ± 0.61	2.43 ± 0.67	7.81 ± 1.30	25.98 ± 3.09	3.41 ± 0.64	13.99 ± 1.11	3.17 ± 0.79
	LT	3.81 ± 0.81	2.48 ± 0.63	2.33 ± 0.65	7.47 ± 1.48	25.33 ± 3.08	3.33 ± 0.60	13.33 ± 2.22	3.07 ± 0.51
	P	.934	.231	.214	.023	.039	.201	.002	.141
F	CA	3.73 ± 0.86	2.31 ± 0.59	2.47 ± 0.72	7.73 ± 1.61	25.61 ± 4.13	3.36 ± 0.65	14.29 ± 1.33	3.13 ± 0.89
	LT	3.80 ± 0.75	2.45 ± 0.64	2.33 ± 0.60	7.67 ± 1.46	25.40 ± 3.71	3.27 ± 0.58	13.53 ± 2.70	3.00 ± 0.64
	P	.567	.146	.234	.775	.688	.310	.045	.199
M	CA	3.87 ± 0.72	2.49 ± 0.62	2.40 ± 0.62	7.88 ± 0.90	26.35 ± 1.36	3.47 ± 0.62	13.68 ± 0.70	3.21 ± 0.68
	LT	3.81 ± 0.87	2.51 ± 0.62	2.33 ± 0.70	7.27 ± 1.49	25.27 ± 2.31	3.39 ± 0.61	13.13 ± 1.60	3.13 ± 0.34
	P	.636	.88	.567	.001	.001	.426	.005	.434

A = all patients, F = female patients, M, male patients, CA = cancer tissues, LT = lung tissue.
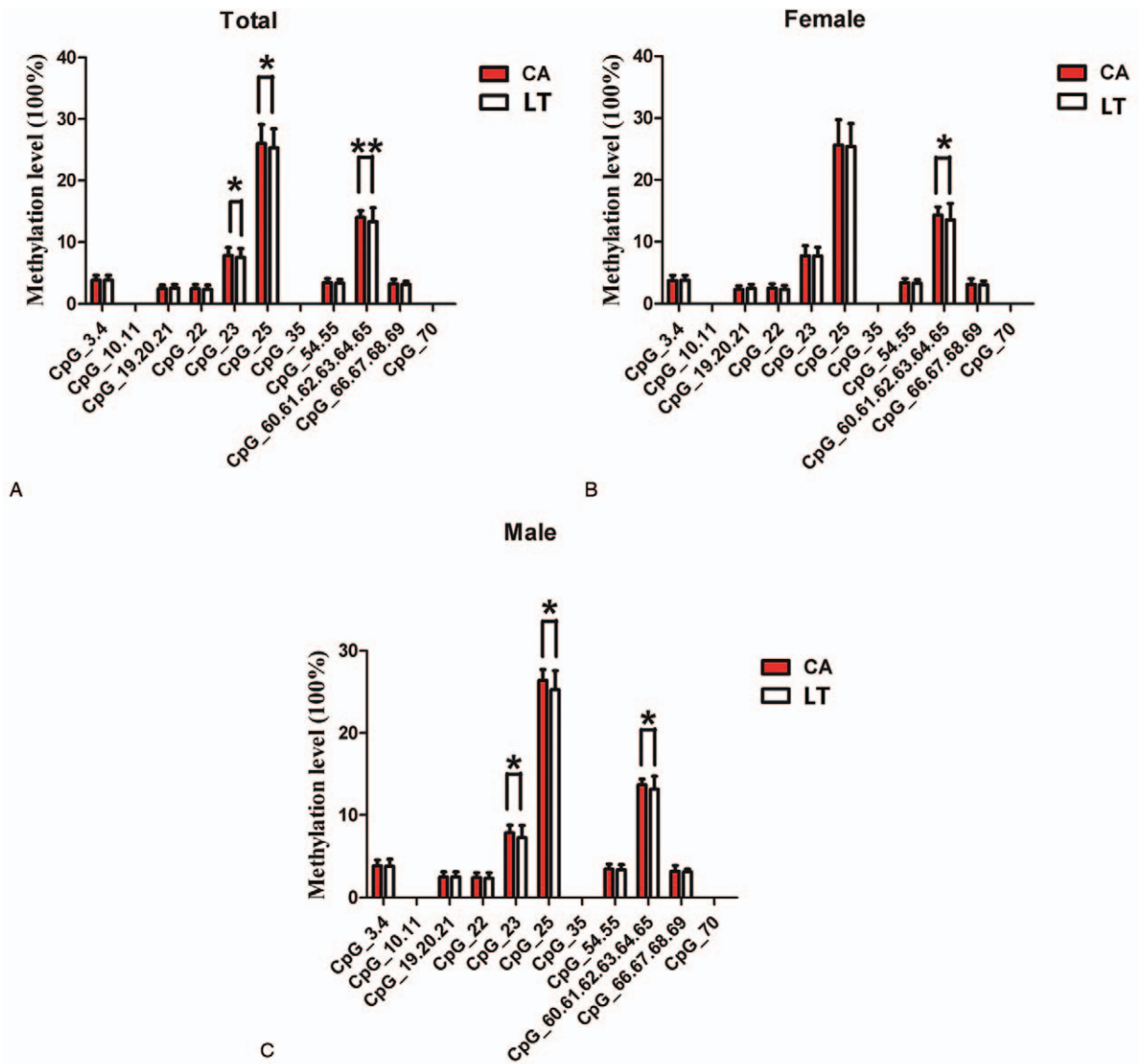
Figure 5. The methylation status of *XXYLT1* (150 patients). (A) Methylation level of the *XXYLT1* gene CpG units among the different tissue samples. (B) Methylation level of the *XXYLT1* gene CpG units among the different tissue samples from female subjects. (C) Methylation level of the *XXYLT1* gene CpG units among different tissues from the male subjects.

which the transmembrane ligands on a particular cell normally trigger the transmembrane receptors on a juxtaposed cell. The Notch signaling pathway has shown essential functions in the development of metazoans and in adult tissue homeostasis.[27,28] Many cancers and disorders especially pertaining to developmental stages are caused by defective patterns in the Notch signaling pathway. [29–31]Varying degrees of *O*-linked glycosylations (the addition of an *O*-linked glucose moiety) to the NECD regulates Notch activation.[32] It has been reported that Notch activation by the addition of an *O*-glucose moiety to multiple epidermal growth factors occurs repeatedly across the NECD. Mechanistically, glucoside- xylosyltransferases 1/2 initially donate one 1,3-linked xylose motif to mammalian *O*-glycosylated Notch EGF repeats,[14] while XXYLT1 can shift the subsequent xylose moiety through catalytic addition to the *O*-glycosylated EGF repeats of Notch.[15]

In our current study, we found that the *XXYLT1* mRNA expression was lower in the cancer tissues than in the para-carcinoma tissues in male patients in the first step, and significantly lower than that in lung normal tissues in the second step. The *XXYLT1* expression was found to be negatively regulated by the Notch pathway, and the attenuated activity of XXYLT1 resulted in the elevation of Notch signaling.[20] Furthermore, high expression levels of the *Notch1* and *Notch3* genes have been reported to be significantly associated with a poor prognosis of lung adenocarcinoma.[32,33] Combined with these studies, our study revealed a correlation between a decreased *XXYLT1* expression and lung cancer and indicated that *XXYLT1* could be an antioncogene. Delightfully, this outcome is consistent with our previous findings that were determined in an in vitro study showing that *XXYLT1* mRNA expression was associated with lung cancer risk and its ablation

promoted lung cancer cell proliferation, inhibited apoptosis, and accelerated cell migration.[21]

DNA hypermethylation is primarily an early incident of carcinogenesis in lung cancer.[34] Aberrant DNA methylation facilitates carcinogenesis through promoter methylation of tumor suppressor genes and silencing their expression and functions.[35,36] The cyclin dependent kinase inhibitor 2A (CDKN2A) gene was the first tumor suppressor gene that was found to be inactivated in lung cancer through aberrant hypermethylation,[37] but it is the most-studied tumor suppressor gene in lung carcinogenesis.[38] In another report, DNA methylation has been described in a large number of genes associated with lung cancer.[39] In this study, we evaluated the methylation level of 11 CpG sites in XXYLT1 and found that there was a significant increase in the methylation level of three CpG islands (CpG_23, CpG_25, and CpG_60.61.62.63.64.65) in cancer tissues, compared to that of para-carcinoma tissues in male patients in the first step, and that of lung normal tissues in the second step. We also investigated whether methylation of the XXYLT1 gene is correlated with gene silencing in cancer tissues and para-carcinoma tissues. The results indicated that the methylation level was inversely related with the mRNA expression level. Our results are in close agreement with a published study establishing that the transforming growth factor beta induced expression levels were markedly downregulated in tumor and normal lung tissues that were methylated in the transforming growth factor beta induced promoter.[40] The results of this study suggest that hypermethylation of the XXYLT1 promoter may be associated with the pathogenesis of nonsmall cell lung cancer and that XXYLT1 methylation is a key mechanism responsible for XXYLT1 downregulation.

In the present study, the difference of XXYLT1 methylation rate in cancer tissues and para-carcinoma tissue and normal lung tissues was notably in males patients than in female patients. Our results are consistent with those of Vaissière et al. They found that the methylation levels of ras association domain family 1A were influenced by sex, with males showing higher levels of methylation.[41] This trend also has been observed in other tumors and genes. For example, Bi et al found that the carbohydrate sulfotransferase 7 methylation status in colorectal cancer was different between the genders.[42] In addition, Lin et al demonstrated that there are differences in the DNA methylation patterns between men and women with B cell chronic lymphocytic leukemia.[43] Recently, the escape from X-inactivation tumor suppressor genes has been proposed to describe a resulting difference in the expression levels between males and females.[44] These studies suggest that the occurrence of DNA methylation may be gender-specific. However, the present study does have certain limitations. For example, our study was a single center, retrospective study, and the sample size was low. So, our results should be verified by multicenter studies with larger cohorts in the future.

5. Conclusions

We found that XXYLT1 in patients with lung cancer was hypermethylated and exhibited lower mRNA expression levels, especially in male patients, which may in turn contribute to the onset of lung cancer. Our data provide evidence that the methylation level of XXYLT1 may be a useful biomarker for an increased risk of lung cancer and that XXYLT1 may be a potential novel target for the development of lung cancer therapeutics.

Author contributions

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