XXYLT1 Methylation Contributes to the Occurrence of Lung Adenocarcinoma

Hui Zeng
Zhejiang Cancer Hospital
ying wang
zhejiang chinese medical university
ying wang
Zhejiang Cancer Hospital
yongjun zhang (zhangyj@zjcc.org.cn)
https://orcid.org/0000-0002-2326-7870

Research article

Keywords: Lung adenocarcinoma, DNA methylation, Gene expression, Promoter methylation, Notch signaling pathway

Posted Date: January 30th, 2020

DOI: https://doi.org/10.21203/rs.2.22275/v1

Abstract

Objective: 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.

Methods: Thirty patients with lung adenocarcinoma (fifteen men and fifteen women) were recruited in this study. Cancer tissues and para-carcinoma tissues were obtained from each of the patients. The expression levels of *XXYLT1* mRNA were determined, and the DNA methylation status was analyzed by MassARRAY Spectrometry. The methylation data of individual units were generated by EpiTyper v1.0.5 software.

Results: Among the male patients, the expression level of *XXYLT1* mRNA was significantly higher in the para-carcinoma tissues compared to the cancer tissues. Meanwhile, among the male patients, the methylation rates of three CpG units (CpG_23, CpG_25, and CpG_60.61.62.63.64.65) within the *XXYLT1* gene were lower in the para-carcinoma tissues compared to the cancer tissues.

Conclusions: Our results show that *XXYLT1* mRNA was down-regulated and methylation rates were increased in lung adenocarcinoma tissues than in para-carcinoma tissues. These suggested that methylation of XXYLT1 may have significance in the pathogenesis of lung adenocarcinoma. Additional research is required to elucidate this aspect.

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]. 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 therapeutics 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 (NECD) 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 (GT8) [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 GT8 family. It has been reported that the C3orf21 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, 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.

Materials And Methods

Study Subjects and Sample Collection

This study was conducted at Zhejiang Cancer Hospital, China, between July 2015 and July 2016. A total of thirty patients with a confirmed diagnosis of lung adenocarcinoma who underwent surgical treatment were recruited, and without any anti-tumor treatment (including radiotherapy, chemotherapy, targeted therapy and immunotherapy, etc) before operation in this study. From the patients diagnosed with lung adenocarcinoma, a total of thirty cancer (40 mg) and thirty para-carcinoma (40 mg) tissues were obtained from the participating patients during the surgical intervention. The collected tissues 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.

Quantitative Reverse Transcription–Polymerase Chain Reaction (qRT-PCR)

Total RNA (1 μg) was isolated from the tumors and adjacent nontumorous tissues 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′-CTGGCAGGAAACTGTCAAAT-3′. For glyceraldehyde 3-phosphate dehydrogenase (GAPDH), the forward and reverse primers were 5′-GGAGTCCACTGGCGTCTTC-3′ and 5′-GCTGATGATCTTGAGGCTGTTG-3′, respectively. The reaction conditions for the PCR were 95 °C for 2 min followed by 40 cycles at 95 °C for 15 s, 58 °C for 20 s, and 72 °C for 20 s. Finally, a cycle at 72 °C for 5 min was performed. The level of mRNA was analyzed using a gel imaging system (Bio-Rad Gel Doc 2000, USA). The data were analysied using the $2^{-\Delta\Delta CT}$ gene quantification method. All the assays reported in this study were repeated three times.

Methylation of the XXYLT1 Gene

DNA was extracted from the tumors and adjacent nontumorous tissues 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′-AGGAAGAGAGTTTTGGTGAATATTATTAGTAGGTGGT-3′ and reverse 5′-CAGTAATACGACTCACTATAGGGAGAAGGCTATACCCTTAAAACCTAAAACCCAAC-3′; primers for the CpG island of XXYLT1 were used to amplify the bisulfite-treated DNA with 20 cycles at 95 °C for 30 s and 50 °C for 15 min. The reaction conditions used for the PCR program were as follows: 95 °C for 4 min and then 72 °C for 3 min, followed by 45 cycles at 95 °C for 20 s, 56 °C for 30 s, and 72 °C for 1 min. 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).

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 two groups were assessed using a paired two-tailed Student's t-test. $P < 0.05$ was the cut-off level for statistical significance.

Results

Clinical Characteristics of the Subjects

A total of 30 patients were enrolled in this study. The patients consisted of 15 men and 15 women, all of whom were diagnosed with lung adenocarcinoma. Ten of the fifteen men were smokers, while all of the women were nonsmokers. The cancer and para-carcinoma tissues were successfully obtained from each of the 30 patients recruited in this study.

The expression of XXYLT1 mRNA between cancer tissues and para-carcinoma tissues

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 = 0.179). 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 = 0.662). This study further revealed that in the male study subjects, the gene expression level of XXYLT1 in the para-carcinoma and cancer tissues was 1.00 ± 0.10 and 0.88 ± 0.24, respectively (p = 0.017) (Table 1 and Fig. 1).

Table 1
Comparison of the XXYLT1 mRNA expression between groups

	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	0.179	0.662	0.017
CA, cancer tissues; CP, para-carcinoma tissues; P value, CA compared with CP.			

XXYLT1 Methylation Status in cancer tissues and para-carcinoma tissues

MassARRAY was used to analyze the data obtained from 30 cancer and 30 para-carcinoma tissues. The outcome of the XXYLT1 gene methylation studies showed that the methylation rate of the 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) did not exhibit a significant difference between the cancer and para-carcinoma tissues in all patients

enrolled in this study. Interestingly, 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 = 0.03, p = 0.02, and p = 0.02, respectively) (Table 2 and Fig. 2).

Table 2
Comparison of the methylation rates of CpG units between groups

		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.(
A	CA	3.87 ± 0.86	0 ± 0	2.30 ± 0.60	2.43 ± 0.68	7.90 ± 1.60	26.47 ± 3.69	0 ± 0	3.53 ± 0.82	13.87 ± 1.20	3.27 ± 0.94
	CP	3.77 ± 0.82	0 ± 0	2.43 ± 0.63	2.33 ± 0.66	7.37 ± 1.69	25.27 ± 3.20	0 ± 0	3.33 ± 0.71	13.40 ± 2.37	3.03 ± 0.56
	P	0.60	-	0.40	0.59	0.18	0.13	0	0.25	0.38	0.230
F	CA	3.73 ± 0.88	0 ± 0	2.20 ± 0.56	2.47 ± 0.74	7.40 ± 1.72	25.4 0 ± 4.42	0 ± 0	3.47 ± 0.83	13.20 ± 0.86	3.13 ± 0.92
	CP	3.8 ± 0.77	0 ± 0	2.40 ± 0.63	2.33 ± 0.62	7.47 ± 1.88	25.27 ± 3.95	0 ± 0	3.27 ± 0.59	13.67 ± 2.97	3.00 ± 0.65
	P	0.81	-	0.42	0.61	0.91	0.92	-	0.38	0.60	0.58
M	CA	4.00 ± 0.85	0 ± 0	2.40 ± 0.63	2.40 ± 0.63	8.40 ± 1.35	27.53 ± 2.50	0 ± 0	3.60 ± 0.83	14.53 ± 1.13	3.40 ± 0.99
	CP	3.73 ± 0.88	0 ± 0	2.47 ± 0.64	2.33 ± 0.72	7.27 ± 1.53	25.27 ± 2.37	0 ± 0	3.40 ± 0.83	13.13 ± 1.64	3.07 ± 0.46
	P	0.33	-	0.75	0.81	0.03	0.02	-	0.46	0.02	0.29

A, all patients; F, female patients; M, male patients; CA, cancer tissues; CP, para-carcinoma tissue.

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. Based on our analysis, the expression of XXYLT1 mRNA in male patients with lung adenocarcinoma displayed a lower level in the cancer tissues compared with the para-carcinoma tissues. In addition, we noticed that 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 of the male patients. These results suggested that the XXYLT1 gene could be an anti-oncogene 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 type. Notch signaling takes place through cell-cell communication in 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, GXYLT1/2 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. 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 anti-oncogene. 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 CDKN2A gene was not 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. 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 TGFBI expression levels were markedly downregulated in tumor and normal lung tissues that were methylated in the TGFBI promoter [40]. The results of this study suggest that hypermethylation of the XXYLT1 promoter may be associated with the pathogenesis of adenocarcinoma and that XXYLT1 methylation is a key mechanism responsible for XXYLT1 downregulation.

In the present study, the degree of XXYLT1 methylation was notably higher in the cancer tissues than in the para-carcinoma tissues among males. However, there was no remarkable difference in the degree of XXYLT1 methylation between the cancer tissues and para-carcinoma tissues among the female patients. To justify this observed trend, there are two possibilities. Firstly, of the fifteen recruited male study subjects, ten subjects were smokers. Tobacco smoking can impact the DNA methylation of lung cancer-related genes [41]. Secondly, the occurrence of DNA methylation may be gender specific. Recently, the escape from X-inactivation tumor suppressor genes have been proposed to describe a resulting difference in the expression levels between males and females [42]. For example, Bi et al. found that the CHST7 methylation status in colorectal cancer was different between the genders [43]. However, our study sample size was low, and our results should be verified in a larger cohort.

Conclusions

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

Abbreviations

CA: Cancer Tissues, CP: Para-Carcinoma Tissues

Declarations

Ethics approval and consent to participate

This study followed the guidelines of the Helsinki declaration, and the protocols were approved by the Medical Ethics Committee of Zhejiang Cancer Hospital

Consent for publication

Not applicable

Competing interests

None

Funding

This study was supported by grants from the Zhejiang Provincial Medical and Health Scientific and Technical Fund (No. 2017171306 and No. 2020369964).

Authors' contributions

Hui Zeng, Ying Wang, and Yongjun Zhang conceived the experiments, collected and analyzed the data, search literatures, generated figures and table. Ying Wang and Yongjun Zhang wrote the manuscript. Hui Zeng, Ying Wang, Ying Wang and Yongjun Zhang performed the experiments, generated and collected the data. All authors had final approval of the submitted and published version.

Acknowledgements

Thanks to the zhejiang cancer hospital’s tumor biological specimen bank for providing the specimens for this study.

Availability of data and materials

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

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68:394-424.
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin. 2016;66:7-30.
3. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA Cancer J Clin. 2019;69:7-34.
4. Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, et al. Cancer statistics in China, 2015. CA Cancer J Clin. 2016;66:115-132.
5. Torre LA, Siegel RL, Ward EM, Jemal A. Global cancer incidence and mortality rates and trends—an update. Cancer Epidemiol Biomarkers Prev. 2016;25:16-27.
6. McDermott U1, Iafrate AJ, Gray NS, Shioda T, Classon M, Maheswaran S, et al. Genomic alterations of anaplastic lymphoma kinase may sensitize tumors to anaplastic lymphoma kinase inhibitors. Cancer Res. 2008;68:3389-3395.
7. Philip Ong and David Ost. Lung Cancer Epidemiologic Changes: Implications in Diagnosis and Therapy. Jose Pable. Diaz-Jimenez and Alicia N. Rodriguez, editors. Interventions in Pulmonary Medicine. Cham: Springer International Publishing, 2nd ed, 2018. pp. 323-32,

8. Ntziachristos P, Lim JS, Sage J, Aifantis I. From fly wings to targeted cancer therapies: A centennial for notch signaling. Cancer Cell. 2014;25:318-334.
9. Klinakis A, Lobry C, Abdel-Wahab O, Oh P, Haeno H, Buonamici S, et al. A novel tumour-suppressor function for the Notch pathway in myeloid leukaemia. Nature. 2011;473:230-233.
10. Wang NJ, Sanborn Z, Arnett KL, Bayston LJ, Liao W, Proby CM, et al. Loss-of-function mutations in Notch receptors in cutaneous and lung squamous cell carcinoma. Proc Natl Acad Sci USA. 2001;108:17761-17766.
11. Agrawal N, Frederick MJ, Pickering CR, Bettegowda C, Chang K, Li RJ, et al. Exome sequencing of head and neck squamous cell carcinoma reveals inactivating mutations in NOTCH1. Science. 2011;333:1154-1157.
12. Takeuchi H, Haltiwanger RS. Significance of glycosylation in Notch signaling. Biochem Biophys Res Commun. 2014;453:235-242.
13. Sethi MK, Buettner FF, Krylov VB, Takeuchi H, Nifantiev NE, Haltiwanger RS, et al. Identification of glycosyltransferase 8 family members as xylosyltransferases acting on O-glucosylated notch epidermal growth factor repeats. J Biol Chem. 2010;285:1582-1586.
14. Cantarel BL, Coutinho PM, Rancurel C, Bernard T, Lombard V, Henrissat B. The Carbohydrate-Active EnZymes database (CAZy): An expert resource for glycogenomics. Nucleic Acids Res. 2009;37(Database issue):D233-238.
15. Lombard V, Golaconda Ramulu H, Drula E, Coutinho PM, Henrissat B. The carbohydrate-active enzymes database (CAZy) in 2013. Nucleic Acids Res. 2014;42(Database issue):D490-495.
16. Sethi MK, Buettner FF, Ashikov A, Krylov VB, Takeuchi H, Nifantiev NE, et al. Molecular cloning of a xylosyltransferase that transfers the second xylose to O-glucosylated epidermal growth factor repeats of notch. J Biol Chem. 2012;287:2739-2748.
17. Lairson LL, Henrissat B, Davies GJ, Withers SG. Glycosyltransferases: structures, functions, and mechanisms. Annu Rev Biochem. 2008;77:521-555.
18. Lee TV, Sethi MK, Leonardi J, Rana NA, Buettner FF, Haltiwanger RS, et al. Negative regulation of notch signaling by xylose. PLoS Genet. 2013;9:e1003547.
19. Sethi MK, Buettner FF, Ashikov A, Bakker H In vitro assays of orphan glycosyltransferases and their application to identify Notch xylosyltransferases. Methods Mol Biol. 2013;1022:307-320.
20. Zhang Y, Gu C, Shi H, Zhang A, Kong X, Bao W, et al. Association between C3orf21, TP63 polymorphisms and environment and NSCLC in never-smoking Chinese population. Gene. 2012;497:93-97.
21. Yang L, Wang Y, Fang M, Deng D, Zhang Y. C3orf21 ablation promotes the proliferation of lung adenocarcinoma, and its mutation at the rs2131877 locus may serve as a susceptibility marker. Oncotarget. 2017;8:33422-33431.
22. Plimack ER, Kantarjian HM, Issa JP. Decitabine and its role in the treatment of hematopoietic malignancies. Leuk Lymphoma. 2007;48:1772-1480.
23. Jacinto FV, Esteller M. MGMT hypemethylation: aprognostie foe, a predictive friend. DNA Repair (Amst). 2007;6:1155-1160.
24. Hegi ME, Diserens AC, Godard S, Dietrich PY, Regli L, Ostermann S, et al. Clinical trial substantiates the prddictive value of O.-6-methylguanine-DNA methyltransferase promoter methylation in glioblastoma patients treated with temozolomide. Clin Cancer Res. 2004;10:1871-1874.
25. Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov. 2012;2:401-404.
26. Yu H, Takeuchi M, LeBarron J, Kantharia J, London E, Bakker H, et al. Notch-modifying xylosyltransferase structures support an SNilike retaining mechanism. Nat Chem Biol. 2015;11:847-854.
27. Guida V, Sinibaldi L, Pagnoni M, Bernardini L, Loddo S, Margiotti K, et al. A de novo proximal 3q29 chromosome microduplication in a patient with oculo auriculo vertebral spectrum. Am J Med Genet A. 2015;167A(4):797-801.
28. Artavanis-Tsakonas S, Muskavitch MA. Notch: the past, the present, and the future. Curr Top Dev Biol. 2010;92:1-29.
29. Penton AL, Leonard LD, Spinner NB. Notch signaling in human development and disease. Semin Cell Dev Biol. 2012;23:450-457.
30. Louvi A, Artavanis-Tsakonas S. Notch and disease: A growing field. Semin Cell Dev Biol. 2012;23:473-480.
31. South AP, Cho RJ, Aster JC. The double-edged sword of Notch signaling in cancer. Semin Cell Dev Biol. 2012;23:458-464.
32. Ye YZ, Zhang ZH, Fan XY, Xu XL, Chen ML, Chang BW, et al. Notch3 overexpression associates with poor prognosis in human non-small-cell lung cancer. Med Oncol. 2013;30:595.
33. Donnem T, Andersen S, Al-Shibli K, Al-Saad S, Busund LT, Bremnes RM. Prognostic impact of Notch ligands and receptors in nonsmall cell lung cancer: coexpression of Notch-1 and vascular endothelial growth factor-A predicts poor survival. Cancer. 2010;116:5676-5685.
34. Belinsky SA. Gene-promoter hypermethylation as a biomarker in lung cancer. Nat Rev Cancer. 2004;4:707-717.
35. Esteller M. Epigenetics in cancer. N Engl J Med. 2008;358:1148-1159.
36. Heyn H, Esteller M. DNA methylation profiling in the clinic: applications and challenges. Nat Rev Genet. 2012;13:679-692.
37. Merlo A, Herman JG, Mao L, Lee DJ, Gabrielson E, Burger PC, et al. 5'CpG island methylation is associated with transcriptional silencing of the tumour suppressor p16/CDKN2/MTS1 in human cancers. Nat Med.(1995;1:686-92.
38. Lukas J, Parry D, Aagaard L, Mann DJ, Bartkova J, Strauss M, et al. Retinoblastoma-protein-dependent cell-cycle inhibition by the tumour suppressor p16. Nature. 1995;375:503-506.
39. Duruisseaux M, Esteller M. Lung cancer epigenetics: From knowledge to applications. Semin Cancer Biol. 2018;51:116-128.
40. Seok Y, Lee WK, Park JY, Kim DS. TGFBI Promoter Methylation is Associated with Poor Prognosis in Lung Adenocarcinoma Patients. Mol Cells. 2019;42:161-165.
41. Gao X, Zhang Y, Breitling LP, Brenner H. Tobacco smoking and methylation of genes related to lung cancer development. Oncotarget. 2016;7:59017-59028.

42. Dunford A, Weinstock DM, Savova V, Schumacher SE, Cleary JP, Yoda A, et al. Tumor-suppressor genes that escape from X-inactivation contribute to cancer sex bias. Nat Genet. 2017;49:10-16.
43. Bi H, Liu Y, Pu R, Xia T, Sun H, Huang H, et al. CHST7 Gene Methylation and Sex-Specific Effects on Colorectal Cancer Risk. Dig Dis Sci. 2019;64:2158-2166.

Figures

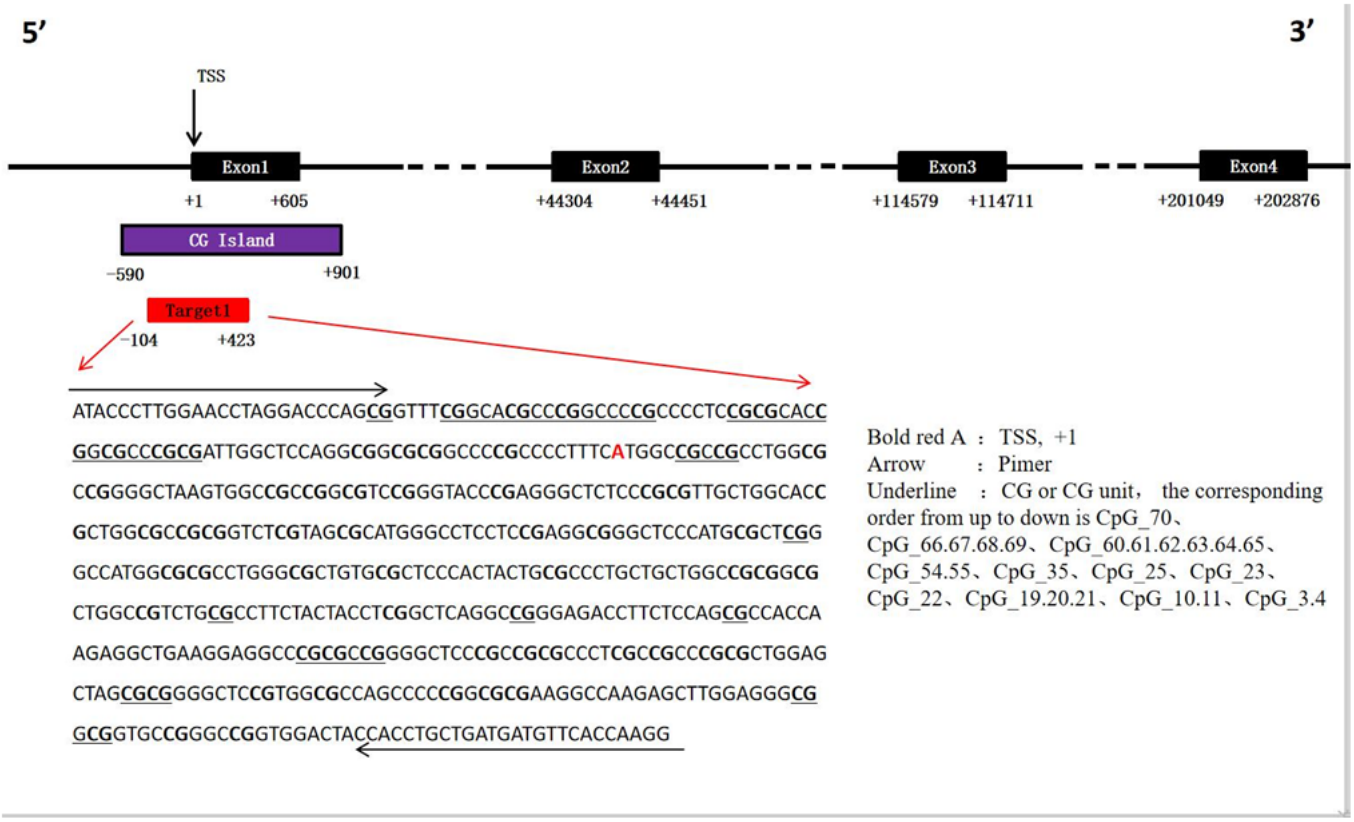

Figure 1

The CpG islands location in the promoter region of XXYLT1

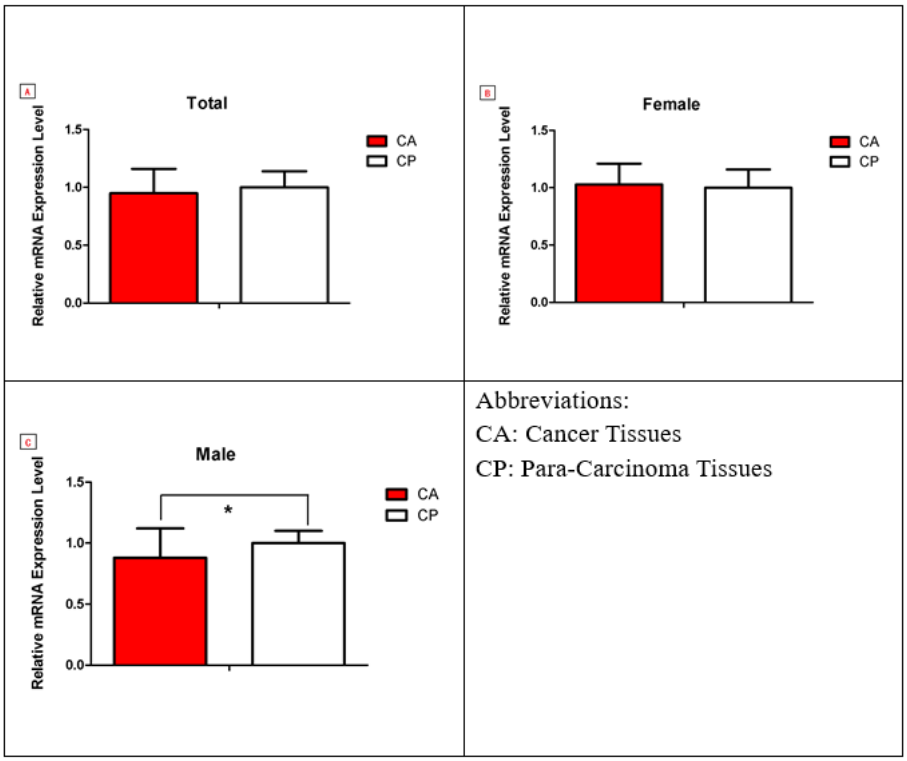

Figure 2

The expression level of XXYLT1 mRNA.

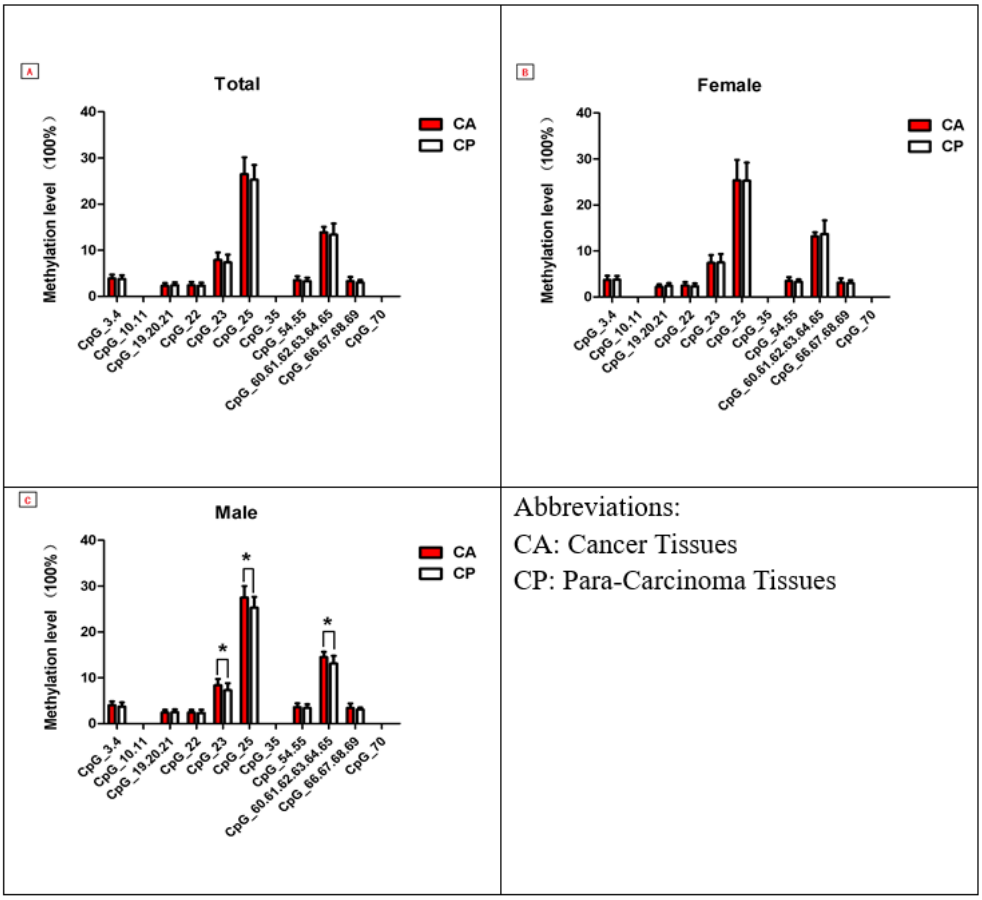

Figure 3

The methylation status of XXYLT1.