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Original Article

CpG island methylation of the CADM1 gene correlates with cervical carcinogenesis in the Uighur and Han populations of Xinjiang, China

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Abstract: Aberrant DNA hypermethylation in gene promoter regions is one of most well-fined epigenetic alterations in tumors and is associated with gene silencing. Cell adhesion molecule 1 (CADM1), an immunoglobulin, could lead to tumor invasion or metastasis with the inhibition of gene expression mainly induced by promoter methylation. We used a MassARRAY platform to test methylation of 10 CpG sites in the promoter region of the CADM1 gene and used MALDI-TOF mass spectrometry to draw the gene mass spectrogram. HPV16 infection was detected by PCR. Results showed a trend of the methylation rate of the CADM1 gene promoter increasing with severity of the disease, and aberrant methylation of CADM1 was present in a higher proportion of invasive cervical carcinoma (ICC) clinical samples compared with normal samples in Uighurs and Han populations. Among the four pathologic lesions formed during the progression of cervical cancer, ICC showed the highest methylation rate and a stronger correlation between CpG sites and lesion grades in Uighurs than in Hans. Methylation of specific CpG sites in the CADM1 gene promoter region was observed between HPV16-positive and -negative groups. Altogether, these results indicate that DNA methylation of CADM1 may play an important role in the carcinogenesis of cervical cancer, and the differential methylation of CADM1 may partially explain the disparity in cervical cancer incidence between Uighur and Han women.

Keywords: Cervical cancer, methylation, CADM1, HPV, Uighur, Han

Introduction

Cervical cancer remains the second most common type of cancer among women. According to the 2012 GLOBOCAN estimates, about 528,000 new cases were recorded worldwide; of which, 130,000 cases were recorded in China, accounting for one-third of global new cases [1]. Uighurs, an ethnic group living in Xinjiang, has the highest reported morbidity of cervical cancer [2]. The incidence rate of cervical cancer among Uighurs is 622 per 100,000 in Xinjiang in 2008 [3], which is much higher than the average rate of 126 per 100,000 in Hans [4], indicating an obvious difference in regional ethnic characteristics.

Epidemiologic and molecular studies have showed that persistent infections of high-risk types of human papillomavirus (HR-HPV) are essential for cervical carcinogenesis [5]. The currently accepted carcinogenic mechanism of cervical cancer is that HPV E6 protein loses its tumor suppressor function by the degradation and inactivation of p53 protein, resulting in cell malignant transformation. However, in recent years, a large number of studies have reported that positive rates of HPV are not related to p53 protein expression in cervical cancer. Some cervical cancer tissues infected with HR-HPV still have p53 protein accumulation [6]. Furthermore, patients with HPV infection regressed spontaneously and only a small portion of these patients developed persistent infection and cancer, suggesting that additional genetic and epigenetic alterations are required for the pathogenesis of cervical cancer [7]. Research has shown that CpG methylation of

gene promoters, an important epigenetic mechanism for gene silencing, is the earliest and most common molecular change in the multistep carcinogenesis process [8]. Recent advances in epigenome analysis have provided evidence that almost all cancers carry hundreds of genes with an abnormal hypermethylation status [9]. Thus, specific methylation patterns among tumor types may provide a range of opportunities for tumor diagnosis and prognosis [10].

Many cervical cancer studies have found that CpG island methylation of multiple tumor suppressor gene promoters occurs in different developmental stages of cervical intraepithelial neoplasia (CIN) and the process of evolution to invasive cervical carcinoma (ICC). Cell adhesion molecule 1 (CADM1) was first observed in patients with non-small cell lung carcinoma (NSCLC), which was mapped on chromosome 11q23 [11]. CADM1 could suppress tumor growth through anti-proliferative and proapoptotic activity, and loss of its expression could lead to tumor invasion or metastasis [12-14]. It is now known that CADM1 is frequently silenced in a variety of human carcinomas [15]. Research shows that CADM1 promoter hypermethylation is one of the main mechanisms of expression silencing [14]. Overmeer et al. found that the density of CADM1 methylation accounts for a high proportion in CIN3 and SCC cases, and the frequency of CADM1 methylation increased with the severity of cervical dysplasia associated with HR-HPV infection; it is thought that DNA methylation of CADM1 could be used as a diagnostic marker of CIN3 and cervical cancer [16].

The evolution of cervical tissue lesions needs 10-15 years from the first infection of HR-HPV, and analyzing gene methylation events in the multi stage process of the carcinogenesis of cervical epithelial cells is important to predict the development of CIN and to screen this new marker for early diagnosis of cervical cancer. Uighurs women living in Xinjiang have the highest reported morbidity of cervical cancer in China, which differs from various other ethnic groups, such as Han and other population groups in China. The study on the methylation status of CADM1 among Uighurs and Han patients with different stages of cervical lesions is not yet reported and the research regarding the association between special CpG sites and HPV infection has not been reported. In this study, we used the MassARRAY EpiTYPER DNA methylation analysis techniques to analyze the CpG methylation status of the CADM1 promoter in ICCs, CIN1s, CIN2/3s and normal groups among Uighur and Han patients, to explore the association between the methylation status of the CADM1 promoter and the high incidence of cervical cancer among Uighurs, and the relationship between CADM1 methylation and HPV16 infection, with the hope to identify molecular markers of ICC for early diagnosis.

Materials and methods

Ethics statement

Written informed consent was obtained from each participant prior to enrollment in the study. Study procedures were approved by the Research Ethics Boards at the First Affiliated Hospital of Shihezi University School of Medicine.

Specimens

We collected 260 cervical tissue samples including 49 ICC, 34 CIN2/3, 11 CIN1, and 20 control Uighur samples and 49 ICC, 45 CIN2/3, 28 CIN1, and 24 control Han samples from three hospitals in Xinjiang from 2001-2010. All samples were formalin-fixed and paraffin-embedded, and the diagnoses of all original hematoxylin-eosin (HE)-stained slides were histologically confirmed by experienced pathologist review. Control samples were normal cervical tissues that came from total hysterectomies of patients who were suffering from uterine leiomyoma.

DNA extraction, bisulfate modification

Total genomic DNA was isolated from the 260 FFPE samples, using the standard phenol/chloroform extraction method. Then, DNA was bisulfite-treated using an EZ DNA Methylation Kit (Zymo Research), following manufacturer instructions. Genomic DNA was stored at -20°C for further polymerase chain reaction (PCR) analysis.

HPV16 E6 DNA analysis

HPV16 E6 DNA was detected by PCR, using forward primer 5'-GACCCAGAAAGTTACCACAG-3'

and reverse primer 5'-CACAACGGTTTGTTGTATTG-3' (primers synthesis from Shanghai Health Industrial). Reactions were set up for positive, negative, and blank controls; CaSki cell DNA was used as eth positive control, the samples without specific fragment by PCR amplification was negative control and high pressure disinfection of double distilled water replacing DNA template was blank control. PCR reactions were as follows: pre-degeneration at 94°C, 4 min; degeneration at 94°C, 45 sec; annealing at 54°C, 45 sec; and extension at 72°C, 1 min, for a total of 35 cycles.

Quantitative MassARRAY analysis of gene methylation status

The MassARRAY platform (SEQUENOM) was used to perform quantitative methylation analysis of CADM1 enzymatic cleavage with MALDI-TOF mass spectrometry, a highly accurate, sensitive, and high-throughput method for the quantitative analysis of DNA methylation at CpG sites [PMID: 16243968]. The robustness of this approach for quantifying methylated and unmethylated DNA has been demonstrated by the Sequenom groups [PMID: 18353987]. The primer sequences of CADM1 designed by Epidesigner (http://www.epidesigner.com; Sequenom) were as follows: forward primer 5'-AGGAAGAGAGAGGGGTAGGGGAGATTAGATAAGTT-3'; reverse primer 5'-CAGTAATACGACTCACTATAGGGAGAAGGCTCAAAAATAACTACACCCACACCTTC-3'.

In brief, the 5-μl PCR mixture contained 10 ng bisulfate-treated DNA, 25 mM dNTP, 0.5 U of Hot Start Taq DNA polymerase (Sequenom, Sequenom Inc., San Diego, CA, U.S.), and a 1-μM mixture of forward and reverse primers. The PCR mixture was pre-heated for 4 min at 94°C and then incubated for 45 cycles of 94°C for 20 s, 56°C for 30 s, and 72°C for 1 min, followed by 72°C for 3 min. The Shrimp-alkaline Phosphatase (SAP) mixture (2 μl), containing 1.7 μl DNase-free water and 0.3 μl (1.7 U) of SAP (Sequenom), was added to digest redundant dNTPs with the following program: 37°C for 20 min, 85°C for 5 min, then maintained at 4°C. Subsequently, 2 μl of the PCR reaction mixture was incubated for 3 h at 37°C with 5 μl of Transcleave mixture (3.15 μl RNase-free water, 0.89 μl 5 × T7 polymerase buffer, 0.24 μl T cleavage mixture, 0.22 μl DTT (100 μmol/L), 0.44 μl T7 RNA/DNA polymerase, 0.06 μl RNase A) for concurrent *in vitro* transcription and T cleavage. After the addition of 20 μl H_2O and 6 mg CLEAN resin (Sequenom), 15 μl of the cleavage reactions were dispensed onto silicon chips preloaded with matrix (SpectroCHIPS, Sequenom). Mass spectra were collected using a MassARRAY mass spectrometer (Bruker-Sequenom) and analyzed using proprietary peak picking and signal-to-noise calculations (Sequenom EpiTYPER v1.0.5 software).

All experiments were performed in triplicate. The quantitative results are referred to as CpG units (units contain either one individual CpG site or multiple consecutive CpG sites). The methylation level is expressed as the percentage of methylated cytosines divided by the total number of methylated and unmethylated cytosines. Non-applicable readings and corresponding sites were eliminated in calculation.

Statistical analysis

CADM1 methylation data were analyzed using EpiTYERv1.05 software. Statistical analyses were performed using Fisher's Exact test and Wilcoxon test (two-sided) in GraphPad Prism version 5.0 (GraphPad Software, Inc., La Jolla, CA). A *t*-test was used to compare differences between the CADM1 methylation of the ICC, CIN2/3, CIN1, and control groups, and to compare DNA methylation levels between patients who were positive and negative for HPV16 infection in ICCs. Two-way ANOVA was performed to compare different methylation levels between groups. All values were represented as mean ± SD. Significance was set at a P value of <0.05.

Results

DNA methylation status of the CADM1 gene in normal controls, CIN1s, CIN2/3s and ICCs among Uighur and Han samples

We used the MassARRAY platform to perform quantitative methylation analysis of CADM1 gene. The CADM1 gene amplicon contained 15 CpG sites, and was divided into 10 CpG units (units contained either one individual CpG site or multiple consecutive CpG sites). Then, we used an unsupervised two-way hierarchical clustering of the CpG unit methylation in the CIN1s, CIN2/3s, ICCs, and normal controls

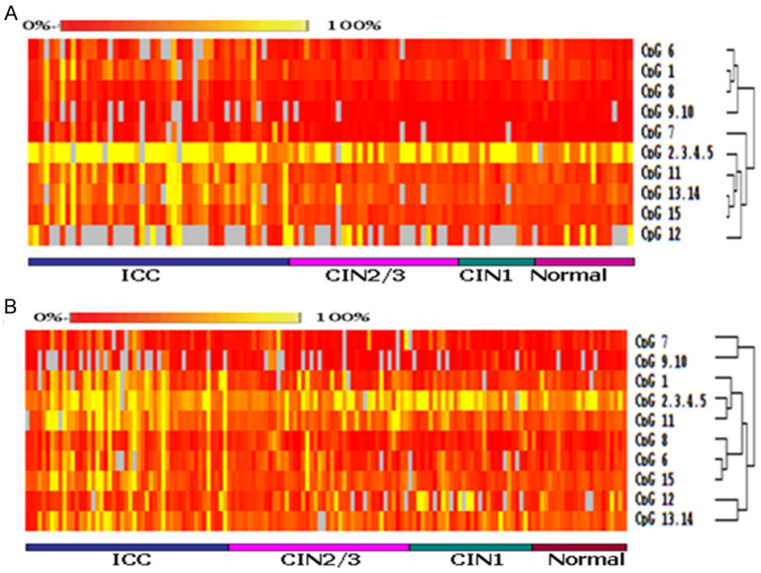

Figure 1. DNA methylation status of CADM1 in the Uighur and Han samples. A. Clustering analysis diagram of ICC, CIN2/3, CIN1, and normal groups in the Uighurs. B. Clustering analysis diagram of ICC, CIN2/3, CIN1, and normal groups in the Hans. Each row represents a sample. Each column represents a CpG unit, which is a single CpG site or a combination of CpG sites. Color coding reflects the degree of methylation with yellow being 100% and red being 0%; gray, no data.

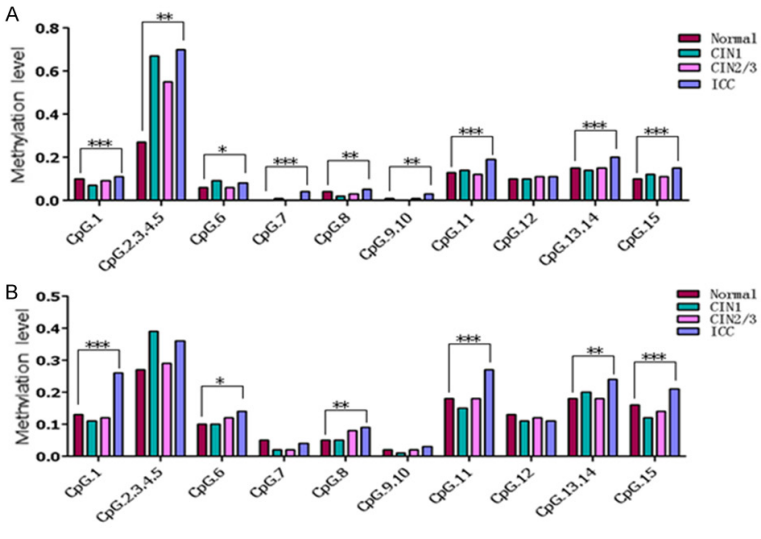

Figure 2. Methylation level of each CpG site with differences between the four lesion grades. A. Histogram depicting the methylation level of each CpG site with differences between the four lesion grades in the Uighur samples. B. Histogram indicating the different methylation levels of each CpG site between the four lesion grades in the Hans. **P*<0.05, ***P*<0.01, ****P*<0.001.

(**Figure 1**). The patterns we observed in Uighur samples (**Figure 1A**) and Han samples (**Figure 1B**) in the cluster analyses showed that methylation patterns of ICCs were notably different from those observed in other lesion grades, and also showed a trend that the methylation rate increased with disease severity. This result indicates that DNA methylation status of the CADM1 gene positively correlated with cervical carcinogenesis.

Methylation levels at individual CpG sites along the CADM1 promoter region among Uighurs and Han samples

Based on the map data collected by MassARRAY mass spectrometer and analysis by proprietary peak picking and signal-to-noise calculations, we can accurately position these to the methylation status of each CpG unit and use methylation rate to show the quantification of each CpG unit. We found heterogeneity between individual CpG units in the different stages of cervical lesions. In Uighur samples, nine CpG units showed increased methylation in ICCs compared with normal controls. As shown in **Figure 2A**, the methylation level was significantly different between ICC samples and the other lesion grades at CpG_1, CpG_2.3.4.5, CpG_6, CpG_7, CpG_8, CpG_9.10, CpG_11, CpG_13.14 and CpG_15. *P* values of each comparison are provided in the supplementary material, Table S1. However, In Han samples, the methylation level was different between ICC samples and the other lesion grades at CpG_1, CpG_6, CpG_8, CpG_11, CpG_13.14 and CpG_15 (**Figure 2B**). *P* values of each comparison are provided in the supplementary material, Table S2.

Table 1. Pairwise comparison of lesion grades in Uighur samples

Sites	Lesion Grades	Normal	CIN1	CIN2/3	ICC
CpG_1	Normal	1			
	CIN1	0.771	1		
	CIN2/3	0.786	0.925	1	
	ICC	0.002**	0.004**	0.000***	1
CpG_7	Normal	1			
	CIN1	0.763	1		
	CIN2/3	0.713	0.974	1	
	ICC	0.000***	0.005**	0.000***	1
CpG_8	Normal	1			
	CIN1	0.637	1		
	CIN2/3	0.719	0.827	1	
	ICC	0.016*	0.015*	0.001**	1
CpG_9.10	Normal	1			
	CIN1	0.556	1		
	CIN2/3	0.567	0.87	1	
	ICC	0.011*	0.007**	0.000***	1
CpG_11	Normal	1			
	CIN1	0.894	1		
	CIN2/3	0.556	0.738	1	
	ICC	0.001**	0.005**	0.000***	1
CpG_13.14	Normal	1			
	CIN1	0.962	1		
	CIN2/3	0.613	0.653	1	
	ICC	0.000***	0.005**	0.000***	1
CpG_15	Normal	1			
	CIN1	0.951	1		
	CIN2/3	0.767	0.861	1	
	ICC	0.000***	0.006**	0.000***	1

Note: There is a significant heterogeneity between ICC and other lesion grades in these CpG sites in the Uighur samples. *P<0.05, **P<0.01, ***P<0.00.

Next, we performed a pairwise comparison of the four lesion grades to identify whether there is heterogeneity in methylation of CpG sites between the four types of lesions. In Uighur samples, we observed striking differences between ICCs and the other lesion grades at CpG_1, CpG_7, CpG_8, CpG_9.10, CpG_11, CpG_13.14, and CpG_15 (**Table 1**). In Han samples, a statistically significant difference was observed between ICC and the other lesion grades at CpG_1, CpG_8, CpG_11, CpG_13.14, and CpG_15 (**Table 2**). Interestingly, no differences were observed between the normal groups and CIN1 or CIN2/3, or between CIN1 and CIN2/3 whether in Uighur or Han samples.

Association between CADM1 methylation and histological grade between Uighurs and Han samples

Our results showed similar CADM1 methylation profiles between Uighur and Han patients, however, research indicates that the Uighurs have an increased incidence of cervical cancer compared with the Hans [17]. We hypothesized that Uighur women may have different genetic factors making them more susceptible to ICC compared with Han women living in the same region. To achieve this, we performed a comparison of the CADM1 DNA methylation profiles between Uighur and Han patients among four lesion grades. As shown in **Figure 3**, CpG_1, CpG_7, CpG_8, CpG_13.14, and CpG_15 in the normal groups (**Figure 3A**); CpG_1, CpG_2.3.4.5, CpG_6, CpG_7, CpG_8, CpG_11, and CpG_15 in the CIN2/3 groups (**Figure 3C**); and CpG_1, CpG_2.3.4.5, CpG_6, CpG_8, CpG_11, CpG_12, and CpG_15 in the ICC groups (**Figure 3D**) were different between Uighur and Han samples. The comparison of CpG methylation was not statistically significant in the CIN1 groups (**Figure 3B**). *P* values are provided in the supplementary material, Table S3.

Next, we performed Spearman analysis in order to confirm whether there was an association between CADM1 methylation at every CpG site among the four lesion grades. In Uighur samples, we found correlation at CpG_1, CpG_2.3.4.5, CpG_7, CpG_8, CpG_9.10, CpG_11, CpG_13.14, and CpG_15 sites. In contrast, such correlation was found at CpG_1, CpG_6, CpG_7, CpG_8, CpG_11, and CpG_15 in Han samples (**Table 3**).

Association between CADM1 methylation and HPV16 infection status

Persistent infection with HR-HPV can lead to the occurrence of cervical cancer; methylation of CpG islands in gene promoter regions can predict disease progression [18]. To explore the association between methylation of CpG islands in the CADM1 promoter region and HPV16 infection status, we compared DNA

Table 2. Pairwise comparison of lesion grades in Han samples

Sites	Lesion Grades	Normal	CIN1	CIN2/3	ICC
CpG_1	Normal	1			
	CIN1	0.647	1		
	CIN2/3	0.447	0.183	1	
	ICC	0.001**	0.000***	0.001**	1
CpG_8	Normal	1			
	CIN1	0.840	1		
	CIN2/3	0.230	0.304	1	
	ICC	0.003**	0.003**	0.026*	1
CpG_11	Normal	1			
	CIN1	0.656	1		
	CIN2/3	0.172	0.357	1	
	ICC	0.000***	0.000***	0.000***	1
CpG_13.14	Normal	1			
	CIN1	0.616	1		
	CIN2/3	0.781	0.383	1	1
	ICC	0.013*	0.042*	0.001**	
CpG_15	Normal	1			
	CIN1	0.767	1		
	CIN2/3	0.644	0.408	1	
	ICC	0.000***	0.000***	0.000***	1

Note: There is a significant heterogeneity between ICC and other lesion grades at these CpG sites in Han samples. *P<0.05, **P<0.01, ***P<0.001.

methylation levels between patients who were positive and negative for HPV16 infection in ICCs. We observed that the methylation level of each CpG unit was numerically higher in samples of HPV16 (-) than HPV16 (+), and was statistically significance at CpG_1, CpG_8, CpG_11, CpG_13.14 and CpG_15 between HPV16 (+) and HPV16 (-) (P<0.05) in Uighur samples (**Figure 4A**). In contrast, we found that methylation levels of all CpG units were numerically higher in samples of HPV16 (+) than HPV16 (-), which was significantly different at CpG_11 and CpG_15 between HPV16 (+) and HPV16 (-) (P<0.05) in Han samples (**Figure 4B**). Our results indicated an association between CADM1 methylation and HPV16 infection in Uighur and Han samples.

Discussion

Cervical cancer is one of the most common malignant tumors in the female reproductive system. HR-HPV infection is a major cause of cervical cancer, but only a small proportion of women with HR-HPV infection will develop cervical cancer [19]. The progression of CIN developing into invasive cervical cancer mediated by HPV may involve many factors, such as HR-HPV persistent infection, abnormal regulation of HPV E6 and E7, allelic loss of oncogene and anti-oncogene chromosomes, and promoter methylation [20-22].

CADM1, formerly referred to as TSLC1, was first found in NSCLC. Earlier studies identified a special region of approximately 700 kb in human chromosome 11q23.2, of which deletion can completely inhibit the malignant phenotype of lung cancer cell line A549; functional complementation studies have found that the inhibitory effect is mainly dependent on a 100-kb region, and CADM1 is the only gene in this region [23]. CADM1 belongs to the immunoglobulin superfamily. The main biological functions of CADM1 include cell adhesion, cell movement, signal transduction, immune regulation, and tumor suppression. Many scholars have found that the CADM1 gene showed a loss of expression in cervical cancer, breast cancer, nasopharyngeal carcinoma, and many other malignant tumors and the extent of the deletion played a negative role in the occurrence and development of tumors [16, 24, 25]. The main mechanism of CADM1 inactivation was promoter methylation and loss of heterozygosity. The present study showed that inhibition of the CADM1 gene expression was closely related to gene promoter methylation [26, 27]. In the study of cervical cancer tumor cells and primary cancer tissues, the high methylation of CADM1 gene promoter was identified as the main cause of such inhibition [28]. In 2004, Steenbergen et al. first reported that the inhibition of the CADM1 gene expression was related to the occurrence of cervical cancer. In early cervical precancerous lesions, the methylation of CADM1 gene promoter methylation may inhibit transcription, resulting in gene downregulation or functional inactivation, and loss of negative regulatory function, making cells more susceptible to malignant transformation. Furthermore, they found that the frequency of methylation in the CADM1 gene promoter region was increased with progression of cervical lesions [27]. Yang et al. found that the meth-

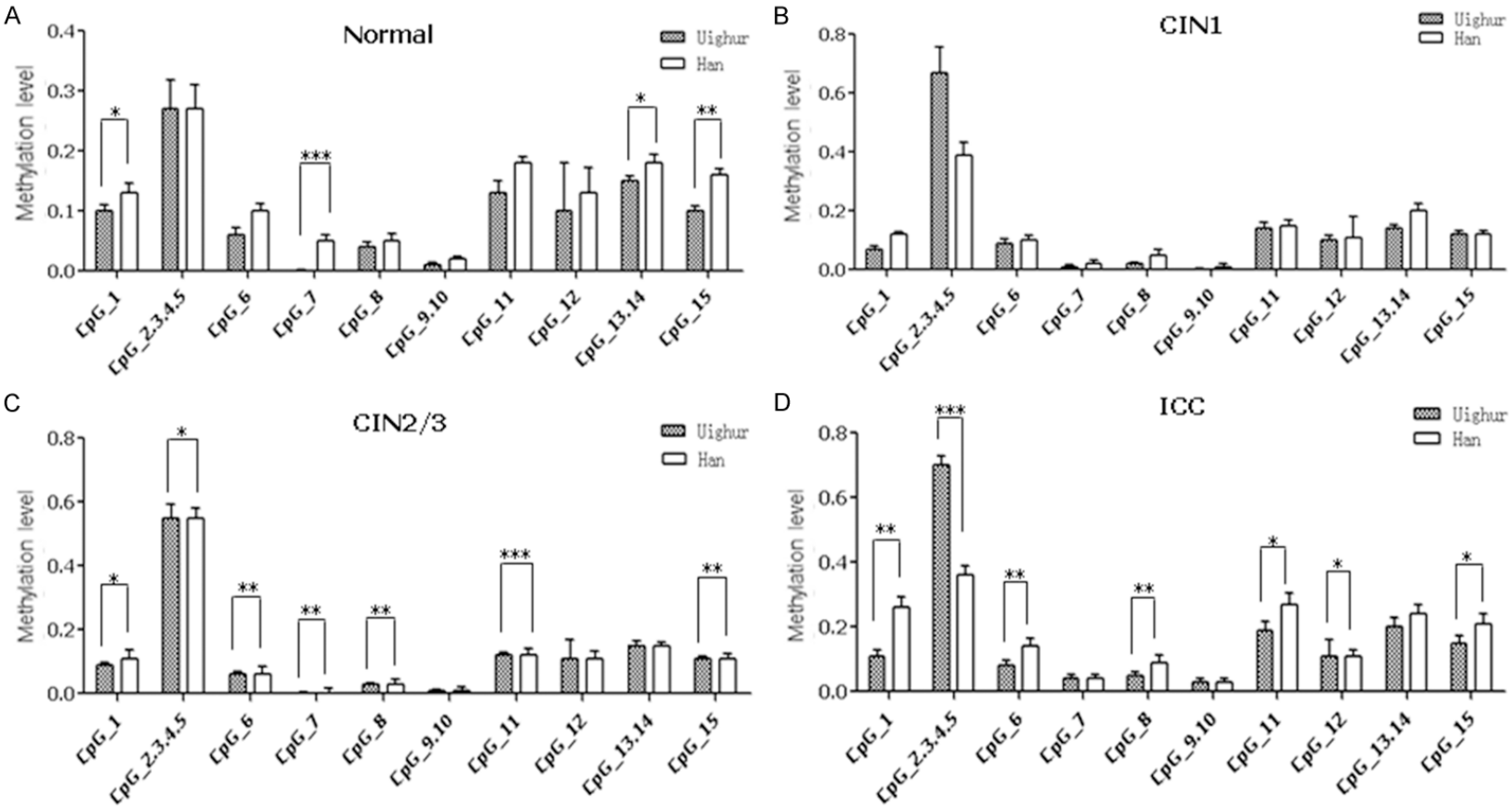

Figure 3. Comparison between CADM1 DNA methylation of Uighur and Han samples among four lesion grades. *P<0.05, **P<0.01, ***P<0.00.

Table 3. Correlation between methylation of each CpG site and lesion grades in Uighur and Han samples

		CpG_1	CpG_2.3.4.5	CpG_6	CpG_7	CpG_8	CpG_9.10	CpG_11	CpG_12	CpG_13.14	CpG_15
Lesion grades (r)	Uighur	0.311**	0.351***	0.151	0.503***	0.306**	0.266**	0.383***	0.048	0.459***	0.312**
	Han	0.305***	0.041	0.235**	0.094	0.301***	0.111	0.386***	-0.074	0.129	0.314***

Note: Uighur samples have a stronger correlation between methylation of each CpG site and lesion grade. R value is the correlation coefficient. *P<0.05, **P<0.01, ***P<0.001.

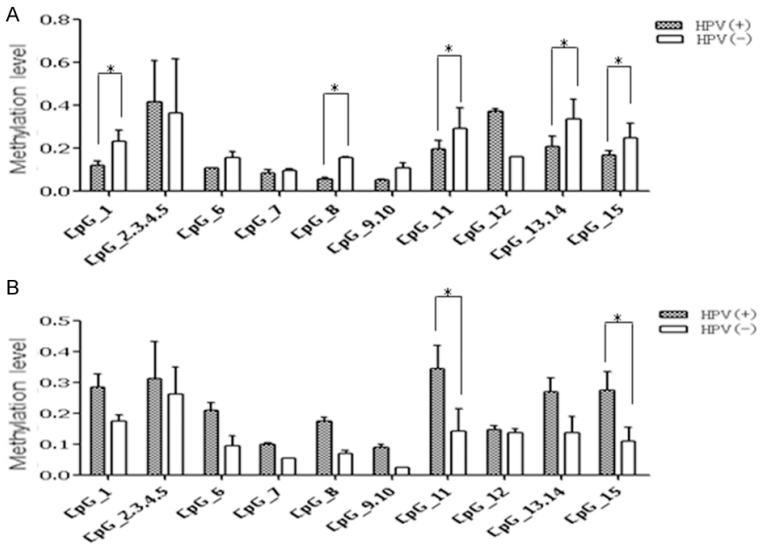

Figure 4. Average methylation of the CpG units of CADM1 in patients positive and negative for HPV16 infection in ICCs among Uighur and Han samples. A. Methylation of CpG units of CADM1 in Uighur patients positive and negative for HPV16 infection. B. Methylation of the CpG units in Han patients positive and negative for HPV16 infection. Error bars represent standard error.

ylation rate of the CADM1 gene promoter increased gradually in normal groups, CIN1s, CIN2s, CIN3s and cervical cancer tissues, and speculated that the methylation rate of the CADM1 gene is a molecular marker for the identification of CIN and early diagnosis of cervical cancer [29].

In the present study, we used the MassARRAY EpiTYPER DNA methylation detection technology to test DNA methylation status of the CADM1 gene promoter in ICCs, CIN2/3s, CIN1s, and normal groups among Uighur and Han samples. Consistent with the results of Steenbergen et al., our results showed that the methylation rate of the CADM1 gene promoter increased with the severity of the disease. Research has shown that CpG promoter methylation is involved in the carcinogenic process [30], therefore we tested individual CpG sites along the CADM1 promoter region among Uighur and Han samples. Results showed that the mean methylation rate of the CpG_2.3.4.5 site was higher than other sites, and the mean methylation rate of CpG_9.10 was lower than other sites. This indicated that different CpG sites in the CADM1 gene promoter region showed different degrees of methylation. Furthermore, In Uighur samples, the methylation level of CpG_1, CpG_7, CpG_8, CpG_9.10, CpG_11, CpG_13.14, and CpG_15 in ICCs was significantly higher than CIN2/3, CIN1 and normal groups, and in Han samples, methylation was statistically significant at CpG_1, CpG_8, CpG_11, CpG_13.14, and CpG_15 sites. However, no differences were observed between the normal groups and CIN1 or CIN2/3 or between CIN1 and CIN2/3 in both Uighur and Han samples. From these results, we speculated that the methylation rate of CpG sites in the CADM1 gene promoter was lower in normal squamous epithelium and CIN. However, as CIN progressed to ICC, the methylation rate of some specific CpG sites increased significantly, which may lead to decreased or complete inhibition of CADM1 gene expression, causing the loss of cell adhesion and tumor suppression function, thereby promoting tumor malignant transformation.

Uighurs, an ethnic group living in Xinjiang, have the highest reported morbidity of cervical cancer in China [2], of which the incidence of ICC was 622 of 100,000 in 2008 [3], much higher than the incidence in the Han [4]. Our effort to detect the methylation status of the CADM1 gene promoter between Uighurs and Hans showed some differences in methylation patterns between Uighurs and Hans, which may be related to genetic characteristics of ethnic groups. We performed a comparison of CADM1 DNA methylation profiles between Uighur and Han patients. Results suggested that some CpG sites were methylated in both Uighurs and Hans, whereas certain CpG sites were differently methylated in these populations during the evolution of cervical cancer. It can be inferred that the research on commonly methylated CpG sites in gene promoters of Uighurs and Hans may help us to further understand the pathogenesis of cervical cancer, whereas the study of unique changes of CpG sites in the two populations may be helpful for further understanding the ethnic differences existing in the pathogenesis of cervical cancer. However,

our study found that the methylation rate of some CpG sites in in the ICCs, CIN2/3s, CIN1s, and control groups in Uighurs were lower than that in corresponding groups of the Han population. In particular, partial CpG sites in Uighurs were always lower than that in the Hans in the development process of normal cervical squamous epithelium (CIN1 CIN2/3, and ICC); these results cannot explain the phenomenon of the high incidence of cervical cancer in Uighur.

The evolution process of CIN2/3 into ICC with HPV infection was often observed in CADM1 gene silencing induced by promoter methylation; however, CADM1 mRNA in non-tumor HPV-immortalized cell lines was highly expressed, suggesting that the expression silencing of CADM1 gene may be associated with the transformation of HPV immortalization into the oncogenic phenotype [27]. Comparing the methylation rate of the CADM1 gene of the HPV16-positive and -negative groups showed that the methylation rate of CpG_1, CpG_8, CpG_11, CpG_13.14, and CpG_15 in ICCs with HPV16 infection were significantly higher than those with HPV16-negative groups in Uighurs. On the contrary, the methylation rate of CpG_11 and CpG_15 in HPV16-positive groups was higher than that in the HPV16-negative groups, suggesting that the methylation of special CpG sites in the CADM1 gene promoter may promote the occurrence and development of ICC together with HPV16 infection.

In conclusion, CADM1 hypermethylation may play an important role in the tumorigenesis of cervical cancer. The difference in CADM1 methylation between Uighur and Han patients may contribute to high incidence of cervical cancer in Uighur and the disparity between the two ethnicities. Furthermore, the methylation of certain specific CpG sites in the CADM1 gene promoter region may promote the occurrence and development of ICC together with HPV16 infection. Further research into the role of CADM1 gene in the pathogenesis of cancer, may lead to the identification of the CADM1 gene as a sensitive marker of early stage cervical cancer, which could be used in early diagnosis and improved prognosis of cervical cancer.

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Disclosure of conflict of interest

None.

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Table S1. Comparison of CADM1 methylation between the four lesion grades in the Uighur patients

	CpG_1	CpG_2.3.4.5	CpG_6	CpG_7	CpG_8	CpG_9.10	CpG_11	CpG_12	CpG_13.14	CpG_15
F	8.049	4.141	3.108	9.526	5.036	5.781	9.676	0.424	7.538	7.842
P	<0.001***	0.008**	0.03*	<0.001***	0.003**	0.001**	<0.001***	0.736	<0.001***	<0.001***

Note: *P* values were obtained from the comparisons of methylation level of each CpG site between the four cervical lesion grades in Uighur patients. **P*<0.05, ***P*<0.01, ****P*<0.001.

Table S2. Comparison of CADM1 methylation between the four lesion grades in Han patients

	CpG_1	CpG_2.3.4.5	CpG_6	CpG_7	CpG_8	CpG_9.10	CpG_11	CpG_12	CpG_13.14	CpG_15
F	7.978	0.9	3.8	0.847	4.577	1.868	11.02	3.529	4.396	10.232
P	<0.001***	0.443	0.012*	0.47	0.004**	0.139	<0.001***	0.17	0.005**	<0.001***

Note: *P* values were obtained from comparison of methylation levels of each CpG site between the four cervical lesion grades in Han patients. **P*<0.05, ***P*<0.01, ****P*<0.001.

Table S3. Differences in methylation levels of the CADM1 gene among four lesiongrades

Lesion grade		CpG_1	CpG_2.3.4.5	CpG_6	CpG_7	CpG_8	CpG_9.10	CpG_11	CpG_12	CpG_13.14	CpG_15
Normal	t	-2.125	0.319	-1.935	-4.483	-1.680	-0.781	-0.803	1.102	-2.294	-3.623
	P	0.040*	0.752	0.060	<0.001***	0.100	0.440	0.426	0.278	0.027*	0.001**
CIN1	t	-1.810	0.700	-0.571	-1.702	-1.573	-1.899	-1.420	-1.150	-1.595	-1.381
	P	0.079	0.488	0.572	0.097	0.124	0.066	0.164	0.261	0.119	0.176
CIN2/3	t	-2.569	2.339	-2.770	-2.779	-3.509	-1.886	-4.119	1.646	-0.630	-3.134
	P	0.012*	0.022*	0.007**	0.007**	0.001**	0.064	<0.001***	0.105	0.530	0.002**
ICC	t	-3.165	4.891	-2.676	-0.365	-3.059	-0.473	-2.381	2.624	0.087	-2.037
	P	0.002**	<0.001***	0.009**	0.716	0.003**	0.637	0.019*	0.011*	0.931	0.044*

Note: Comparison of methylation levels of the CADM1 gene between Uighur and Han patients in four cervical lesion grades.**P*<0.05, ***P*<0.01, ****P*<0.001.