Int J Clin Exp Pathol 2017;10(6):6395-6404
www.ijcep.com /ISSN:1936-2625/IJCEP0051705

Original Article

Methylation of *Jagged1* and *Notch1* promotes breast carcinogenesis and progression of Uighur women and comparing with Han

Xiaoming Du[1*], Wei Li[1*], Na Zhang[1,2*], Yixiao Li[1], Liuhua Wang[1], Xiaowen Chen[1], Zemin Pan[3], Yuqin Zheng[4], Zhenzhu Sun[5], Yuwen Cao[1,4], Feng Li[1,6]

Departments of [1]Pathology, Key Laboratory of Xinjiang Endemic and Ethnic Diseases (Ministry of Education), [3]Biochemistry and Molecular Biology, School of Medicine, Shihezi University, Shihezi, Xinjiang, China; [2]Department of Pathology, The Affiliated Xiangyang City Central Hospital of Hubei Liberal Arts College, Xiangyang, Hubei, China; [4]Department of Pathology, The First Affiliated Hospital, Medicine College of Shihezi University, Shihezi, Xinjiang, China; [5]Department of Pathology, Xinjiang Uygur Autonomous Region People's Hospital, Urumqi, Xinjiang, China; [6]Department of Pathology, Beijing Chaoyang Hospital, Capital Medical University, Beijing, China. [*]Equal contributors.

Received February 26, 2017; Accepted April 25, 2017; Epub June 1, 2017; Published June 15, 2017

Abstract: Breast cancer is a complex and heterogeneous disease occurring in women of various races/ethnicities. DNA methylation plays an essential role in the occurrence and development of breast cancer. There are no studies have examined methylation changes on these genes in Uighur women and comparison between Uighur and Han. We employed MassARRAY spectrometry to quantify methylation of Jagged1 and Notch1 genes in 74 Uighur women with breast cancer and 27 adjacent normal breast tissues. Moreover, we further assessed the differences of methylation in breast tissues from Uighur and Han women. In Uighur breast samples, the overall methylation level of Jagged1 and Notch1 genes, especially at CpG_1 on Jagged1 gene and some CpG sites on Notch1 gene, were significantly decreased in cancer tissues and obviously related to multiple clinicopathological features. Moreover, Notch1 hypomethylation was significantly more sensitive for determination of Uighur cancer risk. In addition, the hypomethylation of Jagged1 was more sensitive as a predictor for Han breast cancer. These findings suggested that hypomethylation of Jagged1 and Notch1 genes and specific CpG sites may be indicators for both Uighur and Han breast carcinogenesis and progression and may also represent the "ethnicity-sensitive" biomarkers for breast cancer occurrence and aggressiveness in different races.

Keywords: Jagged1, Notch1, methylation, breast cancer, Uighur

Introduction

Breast cancer is a complex, heterogeneous disease [1] and one of the leading causes of death among women. The incidence and distribution patterns of breast cancer vary widely among individuals of different racial or ethnic backgrounds [2, 3]. Among multiracial/multiethnic populations in the Xinjiang region of China, the Uighur race is a dominant group that has been shown to have a high incidence and generally poor prognosis in breast cancer [4], second only to individuals of the Han ethnicity.

Breast carcinogenesis and development is a multistep process resulting from the accumulation of genetic and epigenetic alterations, including DNA methylation. Notch signaling genes, particularly the genes encoding ligand *Jagged1* and receptor *Notch1*, are involved in the tumorigenesis and progression of breast cancer [5-10]. Moreover, our previous studies have shown that DNA methylation of *Jagged1* and *Notch1* genes is disrupted in breast cancer tissues from Han women in the Xinjiang region of China [11, 12].

Differences in breast cancer epidemiological features between Uighur and Han ethnic groups can be largely explained by differences in risk factors, and different risk factors are associated with variations in DNA methylation [13, 14],

which can affect the occurrence and aggressiveness of breast cancer [15]. Because race-related differences in methylation may be associated with differences in race-related breast cancer susceptibility [16-18], it is possible that the methylation levels of *Jagged1* and *Notch1* genes may differ among Uighur and Han women and may be associated with the ethnic differences in tumorigenesis and biological behaviors in breast cancer.

As an extension of our earlier work examining the methylation statuses of the *Jagged1* and *Notch1* genes in Han women [12], in this study, we investigated changes of the methylation statuses on the *Jagged1* and *Notch1* genes in breast cancer and matched normal breast tissues from Uighur women, and analyzed the associations between DNA methylation and clinicopathological features. Furthermore, we compared the DNA methylation levels in breast tissues from Uighur and Han ethnicities and assessed the contributions of DNA methylation to the clinicopathological characteristics in both ethnicities. Our results may facilitate the identification of methylated genes that could have applications as "ethnicity-sensitive" biomarkers for the occurrence and progression of breast cancer.

Materials and methods

Patients and samples

A total of 101 specimens, including 74 breast cancer and 27 adjacent normal breast tissues, were collected from women of the Uighur ethnicity by surgical resection, along with corresponding clinicopathological data, at the Xinjiang Uygur Autonomous Region People's Hospital from January 2009 to June 2013. All samples were randomly collected under multistage cluster sampling, and none of the patients received therapy before sample collection. The acquired breast tissues were fixed in 10% neutral formalin and embedded in paraffin after patients underwent modified radical mastectomy. We gathered clinical and pathological data, such as lymph node metastasis, tumor-node-metastasis (TNM) stage, histological grade, and estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) statuses, from the hospital medical records. The clinical stage was classified according to TNM staging system (American Joint Committee on Cancer classification) [19], while the histological type and grade were classified according to World Health Organization (WHO) classification [20]. Two experienced pathologists independently evaluated the expression statuses of ER, PR, and HER2. We considered ER and PR expression as positive at least 1% of cells exhibited nuclear staining. We scored membranous immunostaining for HER2 on a scale of 0 to 3+, and HER2 expression was considered as positive when scores 3+ by Immunohistochemistry or HER2 gene amplification with a ≥2.2-fold increase determined by fluorescence in situ hybridization (FISH). We also collected normal breast tissues at least 5 cm away from the tumor site. Informed consent was obtained from all the patients before enrollment in the study, this study was approved by the Research Ethics Committee of Xinjiang Uygur Autonomous Region People's Hospital, this study was prospectively performed and approved by the institutional Ethics Committees of above-mentioned hospital and conducted in accordance with the ethical guidelines of the Declaration of Helsinki [12].

Matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF-MS)-based DNA methylation analysis

Quantitative DNA methylation analysis of *Jagged1* and *Notch1* genes was performed using the MassARRAY platform (Sequenom, San Diego, CA, USA) as described previously [12]. Briefly, formalin-fixed, paraffin-embedded tissue blocks were cut to 5-mm-thick slices and placed in sterile 2.0-ml EP tubes. Genomic DNA was isolated using a DNA extraction kit (Qiagen Inc., Valencia, CA, USA). The sequences of CpG islands on promoter region in Jagged1 gene and on exon 25 in *Notch1* gene, as well as the primer sets for methylation analysis, were described previously [12]. Polymerase chain reaction (PCR) amplification and dephosphorylation of unincorporated dNTPs by shrimp alkaline phosphatase (Sequenom) were carried out. Transcription and digestion were performed simultaneously, and samples were cleaved by RNase A and then dispensed onto silicon chips preloaded with matrix (Spectro-CHIPS, Sequenom). The mass spectra data were collected by MassARRAY Compact MALDI-TOF (Sequenom), and the methylated portions for each single or combined CpG site were analyzed

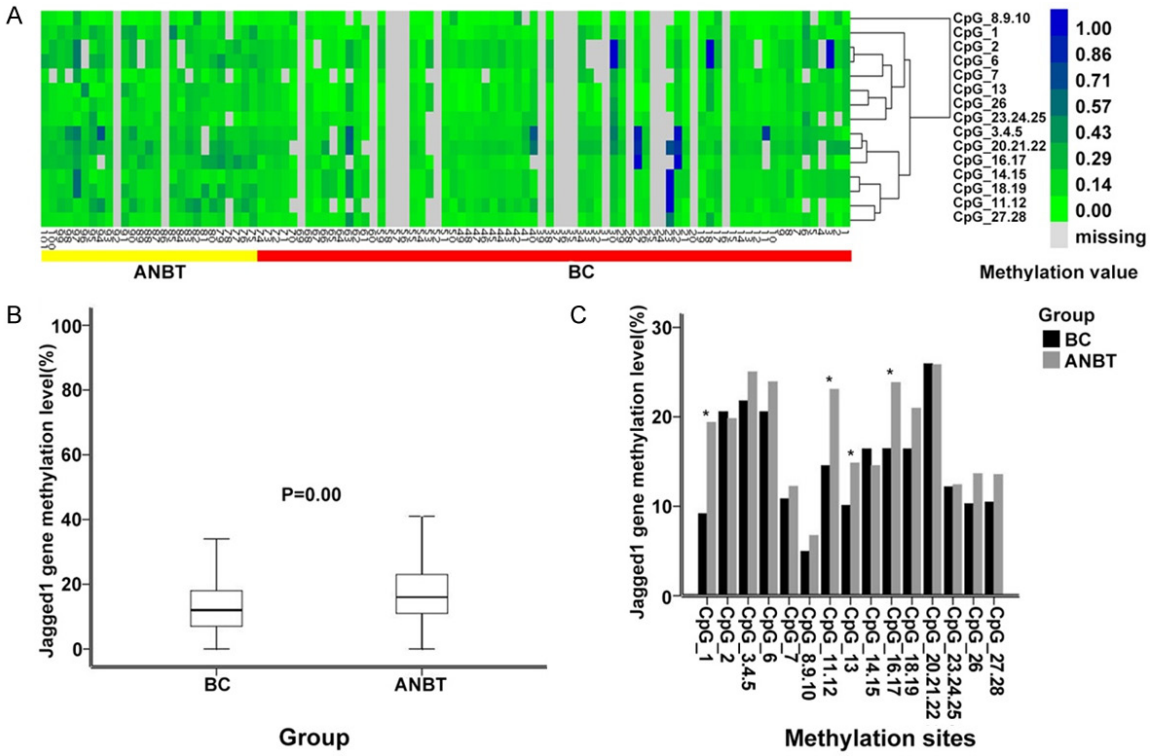

Figure 1. Methylation patterns of the Jagged1 gene in breast cancer (BC) and adjacent normal breast tissue (ANBT) samples from Uighur women. A. Two-way hierarchical cluster analysis of methylation levels. The green to blue colors indicate changes from low to high methylation status. B. The overall methylation profiles in BC and ANBT groups. C. Methylation levels of 15 individual CpG sites in BC and ANBT specimens. *P<0.05.

using Epityper software v.1.0.5 (Sequenom). Non-applicable reads and corresponding sites were eliminated during calculation.

Statistical analysis

SPSS version 17.0 software was employed for statistical analyses. Two-way hierarchical clusters were used to show Jagged1 and Notch1 gene methylation profiles in cancer and normal tissues. T-tests or Mann-Whitney U-tests were performed to assess the methylation levels of the overall and individual CpG site in Jagged1 and Notch1 genes. Receiver operating characteristic (ROC) curves were used to calculate methylation sensitivity and specificity, and chi-square tests were conducted to further compare the sensitivity and specificity of *Jagged1* and *Notch1* genes in different tissues and different ethnicities. Mann-Whitney U-tests or Kruskal-Wallis H tests were used to evaluate the relationships between methylation and clinicopathological parameters. All statistical analyses were two-sided, and *P* values of less than 0.05 were considered statistically significant.

Results

DNA methylation patterns of Jagged1 and Notch1 genes in breast samples from Uighur women, and comparison with Han ethnicity

First, we analyzed 15 CpG sites on the promoter sequence of *Jagged1* gene [12], which encodes a ligand protein involved in *Notch1* signaling pathway, in 74 breast cancer and 27 matched adjacent normal breast tissues from Uighur women. Hierarchical cluster analysis showed that decreased methylation levels of Jagged1 CpG sites in cancer (**Figure 1A**). The overall methylation level of the *Jagged1* gene was significantly lower (P<0.001) in breast cancer samples (mean methylation rate: 14.55%) than normal controls (mean methylation rate: 18.14%; **Figure 1B**). Most single CpG sites were hypomethylated in cancer tissues, particularly CpG_1 (*P*<0.001), CpG_11.12 (*P*=0.005), CpG_13 (*P*=0.025), and CpG_16.17 (*P*=0.023; **Figure 1C**).

Next, we evaluated differences in DNA methylation levels of *Jagged1* gene between Uighur

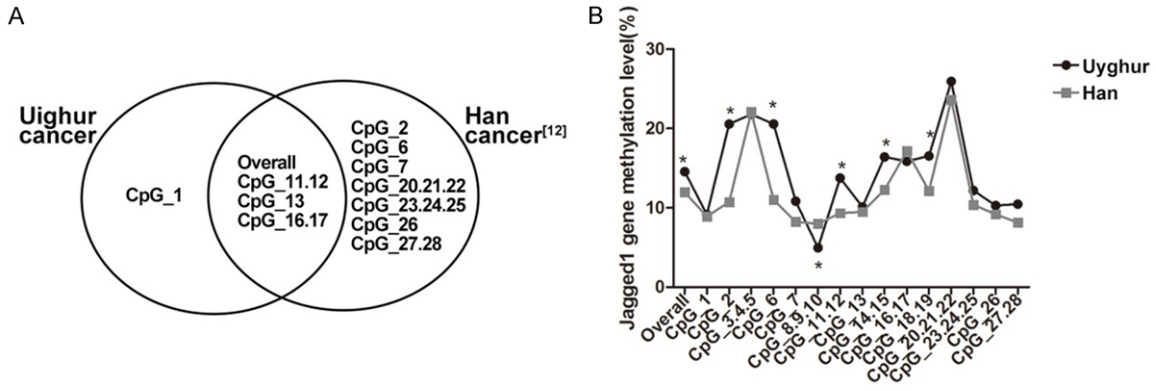

Figure 2. A. Overlap of the methylation on Jagged1 gene in Uighur and Han breast cancer. B. Comparison of the methylation on Jagged1 gene between Uighur and Han breast cancer. *P<0.05.

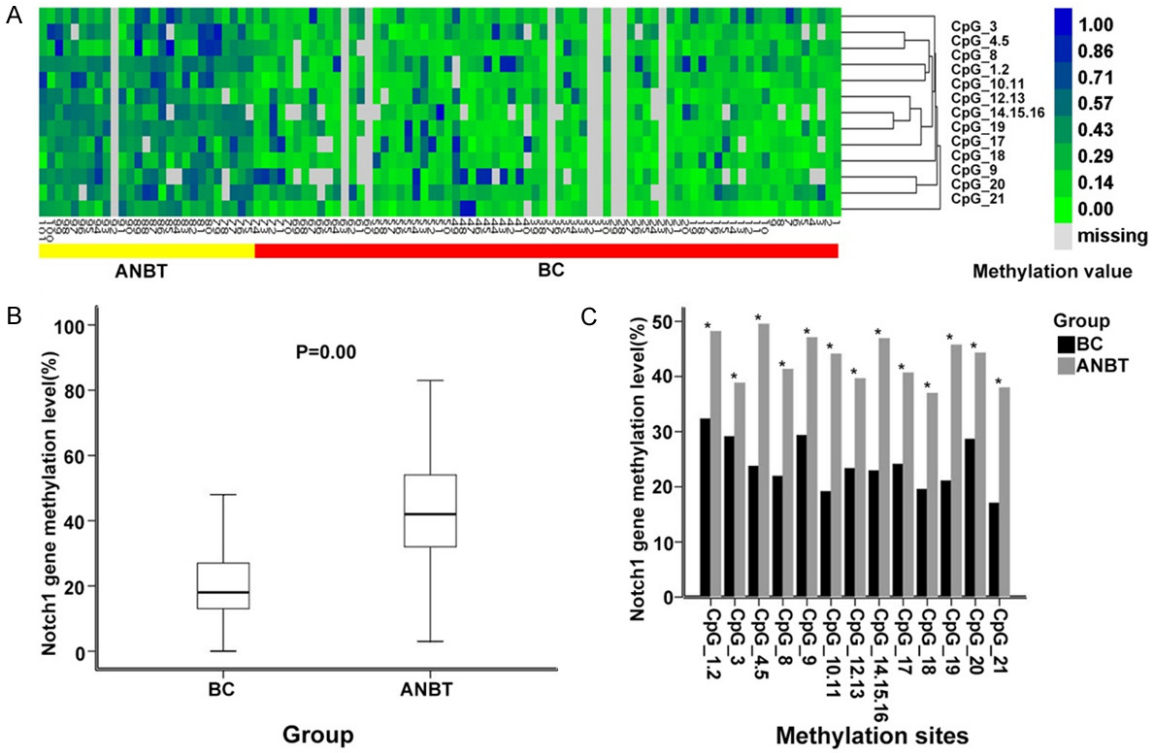

Figure 3. Methylation profiles of Notch1 gene in breast cancer (BC) and adjacent normal breast tissue (ANBT) samples from Uighur patients. A. Methylation changes by two-way hierarchical clustering. The green to blue colors indicate changes from low to high methylation levels. B. Overall methylation patterns in BC and ANBT groups. C. Methylation levels of 13 individual CpG sites in BC and ANBT samples. *P<0.05.

and Han breast samples. In our previous report, we had showed that methylation of overall and CpG-2, CpG-6, CpG_7, CpG_11.12, CpG_13, CpG_16.17, CpG_20.21.22, CpG_23.24.25, CpG_26, and CpG_27.28 sites on *Jagged1* were significantly lower in cancer than normal tissues from Han individuals [12]. So, together Han and Uighurs, we found the similar methylation profiles of overall and CpG_11.12, CpG_13, CpG_16.17 sites in cancer from both Uighur and Han (**Figure 2A**). However, the diversity of methylation in the two ethnics showed that overall (P<0.001) and CpG_2 (P<0.001), CpG_6 (P<0.001), CpG_11.12 (P=0.025), CpG_14.15 (P=0.018) and CpG_18.19 (P=0.010) were significantly higher in Uighur than in Han patients, whereas that of CpG_8.9.10 (P<0.001) was lower (**Figure 2B**). In normal tissue, the overall

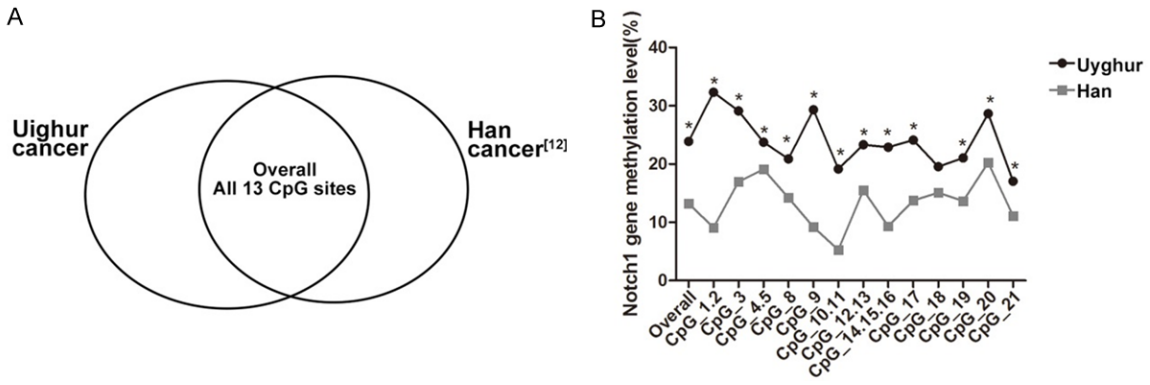

Figure 4. A. Overlap of Notch1 gene methylation in Uighur and Han breast cancer. B. Comparison of methylation levels on Notch1 gene between Uighur and Han breast cancer. *P<0.05.

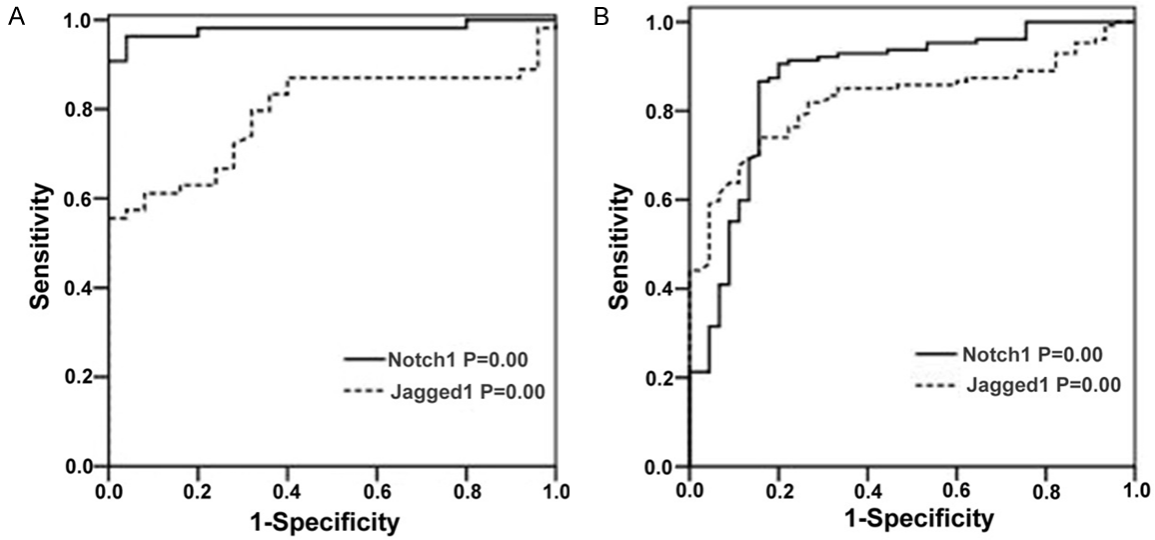

Figure 5. ROC analysis of the methylation statuses on Jagged1 and Notch1 genes in breast samples from a Uighur women and b both Uighur & Han women.

Table 1. The sensitivity and specificity of Jagged1 and Notch1 methylation between Uighur and Han nationality

Gene	Race	Sensitivity (No. of cancer)				Specificity (No. of normal)			
		All	+	-	*P*	All	+	-	*P*
Jagged1	Uighur	54	30	24	0.001	25	25	0	0.05
	Han	73	60	13		20	17	3	
Notch1	Uighur	54	52	2	0.002	25	24	1	0.09
	Han	73	56	17		20	16	4	

methylation level did not differ between the two ethnics.

We then analyzed DNA methylation on exon 25 of *Notch1* gene [12], which encodes a receptor protein in *Notch1* signaling pathway, using the same panel of samples from Uighur women. The methylation levels of 13 CpG sites were markedly decreased in cancer samples (**Figure 3A**). Similarly to hierarchical cluster analysis, the overall level of methylation was significantly lower (P< 0.001) in cancer than normal tissues (**Figure 3B**) with obvious hypomethylation on all 13 individual CpG sites (*P*<0.001, *P*=0.025, *P*<0.001, *P*=0.001, *P*<0.001, *P*<0.001, *P*<0.001, *P*<0.001, *P*<0.001, *P*<0.001, *P*<0.001, *P*<0.001 and *P*<0.001, respectively) in breast cancer (**Figure 3C**).

Furthermore, we performed ethnic-related analysis of methylation on *Notch1* gene in can-

Table 2. Relationship of Jagged1 gene methylation with clinicopathological parameters in Uighur BC patients

Parameter	Jagged1 (methylation %) in Uighur							
	No.	Overall	CpG_1	CpG_8.9.10	CpG_13	CpG_16.17	CpG_23.24.25	CpG_26
Lymph node metastasis								
- (LNN)	43	15.82*	9.09	7.64*	11.31	15.78	13.93	11.50
+ (LNP)	31	12.78	9.27	1.96	9.00	15.96	10.16	8.77
TNM stage								
I	31	17.84*	9.48	6.74	13.05	19.94*	14.55	14.05*
II	27	13.12	10.65	4.60	9.17	15.95	11.14	8.26
III	16	11.40	6.29	3.07	7.93	10.38	10.23	7.93
Histological grade								
1	30	16.11*	11.10*	6.84	10.70	19.00	12.89	11.00
2	27	13.96	10.90	4.70	10.61	16.09	11.24	10.13
3	17	12.79	3.93	2.79	9.20	11.38	12.64	9.53
Receptor status								
ER(+) PR(+) Her2(-)	40	15.57*	9.23	6.93	12.63*	15.00	14.46*	13.47*
ER(-) PR(-) Her2(+)	28	13.19	9.11	2.92	7.75	16.73	9.73	6.86

*Represent P<0.05.

cer and controls from Uighur and Han. We had reported that the overall and all 13 individual CpG sites of *Notch1* gene exhibited significantly decreased methylation in Han breast cancer [12]. Moreover, the hypomethylation of overall and all 13 CpG sites were also observed in Uighur cancer samples (**Figure 4A**). In addition, the methylation level of overall and 12 CpG sites ($P<0.001$, $P<0.001$, $P=0.001$, $P<0.001$, $P<0.001$, $P<0.001$, $P=0.011$, $P<0.001$, $P<0.001$, $P=0.002$, $P=0.001$ and $P=0.007$, respectively) were slightly but significantly higher in Uighur than Han patients (**Figure 4B**). Then, in normal breast tissues, there were no significant difference in overall methylation between Uighur and Han women.

Sensitivity and specificity of DNA methylation on Jagged1 and Notch1 in breast samples from Uighur and Han race

Following, we compared the sensitivities and specificities of DNA methylation on *Jagged1* and *Notch1* in the two ethnics. The areas under the ROC curves were 0.793 with 55.6% sensitivity and 100.0% specificity for *Jagged1* and 0.979 with 96.3% sensitivity and 96.0% specificity for *Notch1* in Uighur women (**Figure 5A**). The results of Han women [12] were similar to Uighur women. Combination of the data from both races (Uighur+Han) showed the areas under the ROC curves were 0.825 with 74.0% sensitivity and 84.4% specificity for *Jagged1* and 0.871 with 86.6% sensitivity and 84.4% specificity for *Notch1* (**Figure 5B**).

From the above-mentioned differences in DNA methylation and ROC curves of the two genes in breast samples from the two ethnics, we further compared the differences in methylation among Uighur and Han race. The cut-off values of DNA methylation could be obtained from ROC analysis, and cut-off values of *Jagged1* gene were 11.44% for Uighur and 14.29% for Han, and cut-off values of *Notch1* were 35.12% for Uighur and 32.22% for Han. Therefore, cancer tissues with DNA methylation rate lower than the cut-off value were defined as positive, otherwise, higher as negative. Further statistical analysis using chi-square tests showed that hypomethylation of the *Jagged1* gene was significantly more sensitive for predicting diagnosis in Han breast cancer ($P=0.001$), whereas hypomethylation of *Notch1* was a more sensitive indicator for predicting diagnosis in Uighur breast cancer ($P=0.002$; **Table 1**). There was no significant difference in the specificities of *Jagged1* and *Notch1* DNA methylation between Uighur and Han individuals.

Association of the methylation frequencies on Jagged1 and Notch1 genes with clinicopathological characteristics in Uighur breast cancer, and comparison with Han race

Finally, we assessed the correlations between methylation status of the two genes and clinico-

Table 3. Comparison of Jagged1 methylation with clinicopathological features between Uighur and Han patient

Parameter	Jagged1 gene methylation status	
	Uighur	Han[12]
Lymph node metastasis	Overall CpG_8.9.10	Overall CpG_8.9.10
TNM stage	Overall CpG_16.17 CpG_26	Overall CpG_8.9.10 CpG_23.24.25
Histological grade	Overall CpG_1	Overall CpG_11.12
Receptor status	Overall CpG_13 CpG_23.24.25 CpG_26	

Table 4. Relationship between Notch1 gene methylation and clinicopathological features in Uighur patients

Parameter	Notch1 (methylation %) in Uighur							
	N	Overall	CpG_4.5	CpG_8	CpG_12.13	CpG_19	CpG_20	CpG_21
Lymph node metastasis								
- (LNN)	43	26.39*	26.63*	21.81	24.57	25.76*	30.03	19.84
+ (LNP)	31	20.49	20.00	19.62	21.54	14.86	26.74	13.36
TNM stage								
I	31	27.98*	27.58	20.52	32.80*	24.46*	27.8	20.00*
II	27	23.66	21.73	24.28	16.22	23.52	31.88	18.56
III	16	17.96	20.75	16.13	18.4	12.13	24.87	10.25
Histological grade								
1	30	28.45*	26.65*	22.44	28.32	26.58*	35.30*	22.04
2	27	22.33	26.54	20.42	18.79	20.33	24.17	13.58
3	17	18.57	14.8	19.07	22.14	12.67	23.14	13.93
Receptor status								
ER(+) PR(+) Her2(-)	40	24.77	25.19	23.03*	21.51	23.74	31.05*	17.05
ER(-) PR(-) Her2(+)	28	22.64	21.73	17.77	25.88	17.30	25.33	17.04

*Represent P<0.05.

pathologic parameters in breast cancer from the two races. The overall hypomethylation of *Jagged1* in primary cancer tissues was associated with lymph node metastasis (P=0.018), advanced stage (P<0.001), high grade (P< 0.001), and HER2 overexpression subtype (ER-, PR-, HER+; P=0.019). The hypomethylation of CpG_1 was observed in high-grade primary tumors (P<0.001). The hypomethylation of CpG_8.9.10 in primary cancer was more frequent in cases with lymph node metastasis (P=0.005). CpG_13, CpG_23.24.25 and CpG_ 26 were significantly hypomethylated in HER2 overexpression subtype (P=0.014, P=0.007 and P=0.003, respectively). The hypomethylation of CpG_16.17 and CpG_26 in primary cancer was associated with advanced stage (P=0.039 and P=0.044, respectively) (**Table 2**).

We further compared the relationship of *Jagged1* gene methylation with clinicopathological features between Uighur and Han patients. This analysis showed that methylation of the overall and CpG_8.9.10 was associated with lymph node metastasis in both ethnicities (**Table 3**).

We also analyzed the correlations between *Notch1* methylation and clinicopathological features in Uighur patients (**Table 4**). The overall low methylation in primary cancer samples was significantly related to lymph node metastasis (P<0.001), advanced stage (P<0.001), and high grade (P<0.001). CpG_4.5 was significantly hypomethylated in primary tumors with lymph node metastasis (P=0.024) and higher grade (P=0.001). The hypomethylation of CpG_8 in primary cancer was associated with HER2 overexpression subtype (P=0.037). CpG_12.13 was low methylated with advanced stage (P=0.017). CpG_19 methylation was significantly decreased in primary tumors with

Table 5. Comparison of Notch1 methylation with clinicopathological features between Uighur and Han patient

Parameter	Notch1 gene methylation status	
	Uighur	Han[12]
Lymph node metastasis	Overall CpG_4.5 CpG_19	Overall CpG_14.15.16 CpG_4.5 CpG_10.11
TNM stage	Overall CpG_12.13 CpG_19 CpG_21	Overall CpG_14.15.16 CpG_18
Histological grade	Overall CpG_4.5 CpG_19 CpG_20	Overall CpG_1.2 CpG_12.13
Receptor status	CpG_8 CpG_20	Overall CpG_14.15.16 CpG_3 CpG_8

lymph node metastasis (P=0.004), advanced stage (P=0.009), and high grade (P=0.008). The low methylation of CpG_20 was observed in primary cancer with high grade (P=0.026) and the HER2 overexpression (P=0.042). The hypomethylation of CpG_21 in primary tumor was associated with advanced stage (P=0.025).

In both Uighur and Han patients (**Table 5**), the overall hypomethylation of the *Notch1* gene was associated with lymph node metastasis, advanced stage, and high grade. The low methylation on CpG_4.5 related to lymph node metastasis. CpG_12.13 hypomethylation was correlated with clinical stage in Uighur and histological grade in Han patients.

Discussion

DNA methylation has been reported to be associated with breast cancer incidence and invasion [21, 22]. In this study, we first analyzed the potential role of aberrant DNA methylation in *Jagged1* and *Notch1* gene on Uighur breast cancer occurrence and progression. Our results demonstrated that both genes at overall and some CpG sites were significantly hypomethylated in cancer samples, which were consistent with our findings in Han breast cancer [11, 12] and other researches in other tumors [23, 24]. The results suggested the low methylation may be an indicator for breast cancer risk. The high sensitivity and specificity of the two genes implied the potential important role facilitating to Uighur breast carcinogenesis. Moreover, the hypomethylation of overall and some CpG sites in *Notch1* and *Jagged1* genes were associated with various clinicopathological parameters, highlighting the importance of *Jagged1* and *Notch1* in breast cancer progression.

Interestingly, variations of methylation frequency in different ethnic breast samples became more and more epidemic [25, 26]. On the basis of our previous results of *Jagged1* and *Notch1* methylation in Han breast samples [12], we further identified the regulatory roles of common and race-related methylation on occurrence and progression for Uighur and Han breast cancer. The overlap of methylation status of the overall level on *Jagged1* gene as well as the overall and CpG_4.5, CpG_12.13 on *Notch1* gene in the both two ethnics may be essential markers for both Uighur and Han breast carcinogenesis and aggressiveness no matter racial diversity. Although the methylation of the two genes did not differ in normal tissues among Uighur and Han, it should be noted that the number of normal samples were not enough and the population structure was not analyzed. Despite the similarities among Han and Uighur patients, the differences of the two genes between Uighur and Han had be identified as "ethnicity-sensitive" potential biomarkers for breast cancer occurrence and development. Our data showed that Jagged1 hypomethylation was significantly more sensitive as a predictor for Han breast cancer, whereas *Notch1* hypomethylation for Uighur breast cancer. Furthermore, the racial-diversity of methylation level on specific CpG sites, such as CpG_1 on *Jagged1* and some CpG sites on *Notch1* for Uighur patients, as well as CpG_23.24.25 on Jagged1 and major CpG sites on *Notch1* for Han women, were significantly decreased in cancer tissues and markedly associated with clinicopathological characteristics. These data suggested the hypomethylation on the specific sites may take most responsibility for ethnics-related breast cancer risk and invasiveness.

In summary, this is the first report describing the methylation profiles of *Jagged1* and *Notch1* genes in Uighur breast samples and comparison with Han ethnic. The methylation profiles at overall and special CpG sites of *Jagged1* and *Notch1* gene may be predictive markers for both Uighur and Han breast cancer occurrence and aggressiveness. The different sensibility of

methylation and diversity of some CpG sites in the two genes could represent the race-related biomarkers for breast cancer risk and development.

Acknowledgements

We sincerely appreciate Xinjiang Uygur Autonomous Region People's hospital provides Uighur patients specimen and informed consent text. This work was supported by the National Natural Science Foundation of China (No. 81560433) and Scientific Research Innovation Project of Xinjiang Graduate (XJGR12-014065).

Disclosure of conflict of interest

None.

Address correspondence to: Yuwen Cao, Department of Pathology, Key Laboratory of Xinjiang Endemic and Ethnic Diseases, School of Medicine, Shihezi University, Shihezi 832000, Xinjiang, China; Department of Pathology, The First Affiliated Hospital, Shihezi University Medicine College, Shihezi 832000, Xinjiang, China. E-mail: cywwb2013@126.com; Feng Li, Department of Pathology, Key Laboratory of Xinjiang Endemic and Ethnic Diseases, School of Medicine, Shihezi University, Shihezi 832000, Xinjiang, China; Department of Pathology, Beijing Chaoyang Hospital, Capital Medical University, Beijing 100020, China. E-mail: fengli7855@126.com

References

[1] Daly B and Olopade OI. A perfect storm: how tumor biology, genomics, and health care delivery patterns collide to create a racial survival disparity in breast cancer and proposed interventions for change. CA Cancer J Clin 2015; 65: 221-238.

[2] Warner ET, Tamimi RM, Hughes ME, Ottesen RA, Wong YN, Edge SB, Theriault RL, Blayney DW, Niland JC, Winer EP, Weeks JC and Partridge AH. Racial and ethnic differences in breast cancer survival: mediating effect of tumor characteristics and sociodemographic and treatment factors. J Clin Oncol 2015; 33: 2254-2261.

[3] Sineshaw HM, Gaudet M, Ward EM, Flanders WD, Desantis C, Lin CC and Jemal A. Association of race/ethnicity, socioeconomic status, and breast cancer subtypes in the national cancer data base (2010-2011). Breast Cancer Res Treat 2014; 145: 753-763.

[4] Ding J, Huang X, Yu B and GS. The analysis of clinicopathological characteristics and prognosis in Uygurs women with breast cancer of Xinjiang. Cancer Research and Clinic 2006; 8: 550-552.

[5] Cao YW, Wan GX, Sun JP, Cui XB, Hu JM, Liang WH, Zheng YQ, Li WQ and Li F. Implications of the Notch1-Snail/Slug-epithelial to mesenchymal transition axis for lymph node metastasis in infiltrating ductal carcinoma. Kaohsiung J Med Sci 2015; 31: 70-76.

[6] Cao YW, Li WQ, Wan GX, Li YX, Du XM, Li YC and Li F. Correlation and prognostic value of SIRT1 and Notch1 signaling in breast cancer. J Exp Clin Cancer Res 2014; 33: 97.

[7] Dickson BC, Mulligan AM, Zhang H, Lockwood G, O'Malley FP, Egan SE and Reedijk M. High-level JAG1 mRNA and protein predict poor outcome in breast cancer. Mod Pathol 2007; 20: 685-693.

[8] Reedijk M, Pinnaduwage D, Dickson BC, Mulligan AM, Zhang H, Bull SB, O'Malley FP, Egan SE and Andrulis IL. JAG1 expression is associated with a basal phenotype and recurrence in lymph node-negative breast cancer. Breast Cancer Res Treat 2008; 111: 439-448.

[9] Reedijk M, Odorcic S, Chang L, Zhang H, Miller N, McCready DR, Lockwood G and Egan SE. High-level coexpression of JAG1 and NOTCH1 is observed in human breast cancer and is associated with poor overall survival. Cancer Res 2005; 65: 8530-8537.

[10] Mutvei AP, Fredlund E and Lendahl U. Frequency and distribution of Notch mutations in tumor cell lines. BMC Cancer 2015; 15: 311.

[11] Zhang N, Sun ZZ, Li F, Cao YW, Zhao CX, Liang WH, Sun HP, Li HA and Fu XG. [Detection and clinical significance of Notch1 methylation in breast cancer and intraductal proliferative breast lesions]. Zhonghua Bing Li Xue Za Zhi 2011; 40: 324-329.

[12] Cao Y, Li Y, Zhang N, Hu J, Yin L, Pan Z, Li Y, Du X, Zhang W and Li F. Quantitative DNA hypomethylation of ligand Jagged1 and receptor Notch1 signifies occurrence and progression of breast carcinoma. Am J Cancer Res 2015; 5: 1897-1910.

[13] Tao MH, Marian C, Shields PG, Nie J, McCann SE, Millen A, Ambrosone C, Hutson A, Edge SB, Krishnan SS, Xie B, Winston J, Vito D, Russell M, Nochajski TH, Trevisan M and Freudenheim JL. Alcohol consumption in relation to aberrant DNA methylation in breast tumors. Alcohol 2011; 45: 689-699.

[14] Tao MH, Marian C, Nie J, Ambrosone C, Krishnan SS, Edge SB, Trevisan M, Shields PG and Freudenheim JL. Body mass and DNA promoter methylation in breast tumors in the western New York exposures and breast cancer study. Am J Clin Nutr 2011; 94: 831-838.

[15] Lehmann U. Aberrant DNA methylation of microRNA genes in human breast cancer-a critical appraisal. Cell Tissue Res 2014; 356: 657-664.
[16] Lee JS, Lo PK, Fackler MJ, Argani P, Zhang Z, Garrett-Meyer E and Sukumar S. A comparative study of Korean with Caucasian breast cancer reveals frequency of methylation in multiple genes correlates with breast cancer in young, ER, PR-negative breast cancer in Korean women. Cancer Biol Ther 2007; 6: 1114-1120.
[17] Xia YY, Ding YB, Liu XQ, Chen XM, Cheng SQ, Li LB, Ma MF, He JL and Wang YX. Racial/ethnic disparities in human DNA methylation. Biochim Biophys Acta 2014; 1846: 258-262.
[18] Ambrosone CB, Young AC, Sucheston LE, Wang D, Yan L, Liu S, Tang L, Hu Q, Freudenheim JL, Shields PG, Morrison CD, Demissie K and Higgins MJ. Genome-wide methylation patterns provide insight into differences in breast tumor biology between American women of African and European ancestry. Oncotarget 2014; 5: 237-248.
[19] Edge SB and Compton CC. The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. Ann Surg Oncol 2010; 17: 1471-1474.
[20] Lakhani SR. WHO classification of tumours of the breast. International Agency for Research on Cancer 2012.
[21] Fleischer T, Edvardsen H, Solvang HK, Daviaud C, Naume B, Borresen-Dale AL, Kristensen VN and Tost J. Integrated analysis of high-resolution DNA methylation profiles, gene expression, germline genotypes and clinical end points in breast cancer patients. Int J Cancer 2014; 134: 2615-2625.
[22] Barrow TM, Barault L, Ellsworth RE, Harris HR, Binder AM, Valente AL, Shriver CD and Michels KB. Aberrant methylation of imprinted genes is associated with negative hormone receptor status in invasive breast cancer. Int J Cancer 2015; 137: 537-547.
[23] Leshchenko VV, Kuo PY, Shaknovich R, Yang DT, Gellen T, Petrich A, Yu Y, Remache Y, Weniger MA, Rafiq S, Suh KS, Goy A, Wilson W, Verma A, Braunschweig I, Muthusamy N, Kahl BS, Byrd JC, Wiestner A, Melnick A and Parekh S. Genomewide DNA methylation analysis reveals novel targets for drug development in mantle cell lymphoma. Blood 2010; 116: 1025-1034.
[24] Gao S, Krogdahl A, Eiberg H, Liu CJ and Sorensen JA. LOH at chromosome 9q34.3 and the Notch1 gene methylation are less involved in oral squamous cell carcinomas. J Oral Pathol Med 2007; 36: 173-176.
[25] Mehrotra J, Ganpat MM, Kanaan Y, Fackler MJ, McVeigh M, Lahti-Domenici J, Polyak K, Argani P, Naab T, Garrett E, Parmigiani G, Broome C and Sukumar S. Estrogen receptor/progesterone receptor-negative breast cancers of young African-American women have a higher frequency of methylation of multiple genes than those of Caucasian women. Clin Cancer Res 2004; 10: 2052-2057.
[26] Wang S, Dorsey TH, Terunuma A, Kittles RA, Ambs S and Kwabi-Addo B. Relationship between tumor DNA methylation status and patient characteristics in African-American and European-American women with breast cancer. PLoS One 2012; 7: e37928.