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Original Article

miR-145 and miR-143 expression differ between Uygur and Han women with endometrial cancer

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Abstract: The aim of this study was to compare the clinicopathological significance of miR-145/143 expression in Uygur and Han women with endometrial cancer in Xinjiang and assess their effects and ethnic factor in the occurrence of endometrial cancer. miR-145 and miR-143 expression in 62 Uygur and 74 Han endometrial cancers was determined by qRT-PCR. Functional assays including cell proliferation, migration, and invasion assays were used to determine the role of miR-145 and miR-143 in endometrial cancer cells. Uygur women with endometrial cancer were significantly less than Han women in Xinjiang Cancer Hospital and Uygur patients had significantly prolonged survival ($P < 0.05$). miR-145 and miR-143 increased in Uygur patients but decreased in Han patients. Furthermore, in the Uygur group miR-145/143 increased significantly in non-endometrioid carcinomas (NEECs) ($P < 0.05$) and there was a trend that NEECs exhibiting favorable clinicopathological factors exhibited higher miR-145/143 expression. However, in the Han group, miR-145/143 decreased significantly in endometrioid carcinomas (EECs) ($P < 0.05$) and EECs with worse clinicopathological variables had lower expression although without statistical significance. Additionally, miR-145 or miR-143 upregulation was significantly associated with a favorable survival in the whole cohort ($P < 0.05$). Enforced miR-145 expression inhibited proliferation, migration and invasion in endometrial cancer cells whereas miR-143 only affected migration and invasion properties. Our findings suggest that miR-145/143 expression in endometrial cancer might be associated with ethnic factors and tumor subtype in Xinjiang women. miR-145/143 upregulation may possess favorable impacts that might be related to the mild behavior of endometrial cancer in Uygur women.

Keywords: miR-145, miR-143, uygur women, Han women, endometrial carcinoma

Introduction

Endometrial carcinoma is the most common malignancy of the female genital tract. The most common basis for determining the risk of aggressive disease has been the categorization of endometrial cancer into two subtypes. Type I tumors (approximately 80%) are endometrioid carcinomas (EECs) and are usually low grade and follow a favorable course. In contrast, type II (10%-20%) tumors are non-endometrioid carcinomas (NEECs) with high grade and poor prognosis [1, 2].

Xinjiang Uygur Autonomous Region is located in the northwest part of China. Many groups of people, such as Uygur (46%), Han (39%), and Kazakh people (7%), intermingle in this area. The Uygur people are different from Han people at many aspects such as culture, lifestyle, and genetic background [3]. Interestingly, we observed that the number of Uygur women with endometrial cancer admitted in Xinjiang Cancer Hospital was significantly less than that of Han women. In fact, biological differences of endometrial cancer between the two ethnic groups have not been studied extensively.

MicroRNAs are endogenous non-coding single strand RNAs with 19-25 nucleotides. They widely exist in eukaryotic cells and participates in the regulation of gene expression by inducing degradation or inhibiting translation of target mRNA [4]. miR-145 and miR-143 have frequently been reported as being downregulated in various malignancies and considered to act as broad tumor suppressors [5-7]. In previous study, we found that miR-145 and miR-143

were downregulated in endometrial cancer of Han women and downregulation of these two miRNAs were associated with worse prognosis in EEC [8]. However, expression patterns of these two miRNAs and their roles in Uygur women with endometrial cancer have not been evaluated. Therefore, in this study we compared specific expression of miR-145 and miR-143 in endometrial cancer tissues from Uygur and Han women residing in Xinjiang Uygur Autonomous Region, and analyzed the relationship between miR-145 and miR-143 expression and endometrial cancer.

Material and methods

Patients and samples

A total of 598 endometrial cancer patients were admitted in Xinjiang Cancer Hospital from 2009 to 2015 including 92 cases of Uygur women and 506 cases of Han women. Formalin-fixed, paraffin-embedded (FFPE) tissue samples of 136 endometrial carcinomas (62 Uygur and 74 Han) were obtained from the Surgical Pathology files of the Xinjiang Cancer Hospital for miRNA expression analysis. All 136 samples had sufficient viable tissue available for RNA extraction. Ten unmatched proliferative endometrial samples from Uygur or Han women served as normal controls respectively. Additionally, inclusion criteria for the cases included the absence of any treatment prior to surgery. The endometrial carcinoma cases were reviewed and classified using the 2014 World Health Organization criteria [9]. Tumors were staged according to the 2009 International Federation of Gynecology and Obstetrics (FIGO) guidelines [10]. Follow-up information was obtained for 94 patients (34 Uygur and 60 Han) in the 136 selected cases for miRNA expression analysis. The median follow-up in Uygur patients was 28 months (range, 1-66 months) and 25 months in Han patients (range, 4-108 months). The study was approved by the Institutional Research Ethic Committee.

RNA extraction and quantitative RT-PCR

The hematoxylin and eosin (H&E) stained slides were checked by a pathologist to identify the tumor region. Tumor samples from corresponding FFPE blocks were cut into 20-μm fragments and total RNA samples were then extracted from the corresponding FFPE tissues using RNeasy FFPE kit (Qiagen, Crawley, UK). RNA extraction and qRT-PCR (quantitative real-time polymerase chain reaction) were performed as described previously [8]. The quality of total RNA was measured using the NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington DE, USA). The Taqman microRNA RT kit (Applied Biosystems, Foster City, CA, USA) was used for reverse transcription. The single-tube TaqMan miRNA assays were used to detect and quantify mature miRNAs (miR-143: ABI No.002249, miR-145: ABI No.002278). U6 small nuclear RNA (Ambion, Austin, TX, USA) was used as an internal normalization control. The relative quantity (RQ) of each miRNA was calculated by the comparative CT ($2^{-\Delta\Delta Ct}$) method [11], in which ΔΔCt was calculated as follows: ΔΔCt = (Ct miR-of-interest - Ct U6)$_{cancer}$ - (Ct miR-of-interest - Ct U6)$_{control}$. Expression levels higher than that of normal controls were considered as upregulation of miR-145 or miR-143, and vice versa. All experiments were run in triplicate.

Cell culture and transient transfection

Human endometrial cancer cell lines Ishikawa (well differentiated) and HEC-1B (moderately differentiated) were kindly provided by Dr. LH Wei (Department of Gynecology and Obstetrics, Peking University People's Hospital, China). The KLE (poorly differentiated) cells were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). All cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM)/F12 (HyClone, UT, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco, Carlsbad, CA, USA), 100 units/mL penicillin, and 100 μg/mL streptomycin at 37°C in a humidified atmosphere of 5% CO_2. The miR-145 and miR-143 mimics (Ambion) were used to achieve the transient overexpression of miR-145 and miR-143. Cells were transfected with lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions.

Cell proliferation assay

Cells (3000 cells/well) were seeded in 96-well plates. At specified time points, 10 μl CCK-8 (Cell Counting Kit-8) reagent (Dojindo, Tokyo, Japan) was added to each well and then the cells were incubated at 37°C for an additional 2 hours. The absorbance was measured at 450 nm.

Cell migration and invasion assays

Migration and invasion assays were carried out using transwell system (24-well, 8 μm pore size;

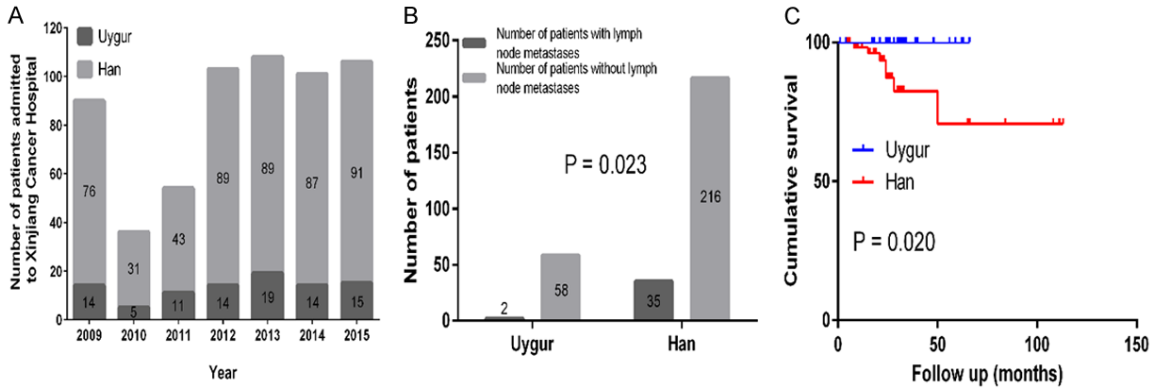

Figure 1. Comparison of Uygur and Han women with endometrial cancer in Xinjiang Cancer Hospital. A. The number of Uygur women with endometrial cancer was significantly less than that of Han women. B. Uygur women had lower lymph node metastasis rate than Han women. C. Uygur women had a better survival than Han women.

Table 1. Clinicopathological features of the 598 endometrial carcinoma patients

Variables	No. of cases		
	Uygur	Han	P
N	92	506	
Age	54.3 ± 8.2	53.7 ± 9.1	0.450
Times of pregnancy			
< 5	80 (87.0%)	491 (97.0%)	< 0.001
≥ 5	12 (13.0%)	15 (3.0%)	
Type			
EEC	75 (81.5%)	437 (86.4%)	0.223
NEEC	17 (18.5%)	69 (13.6%)	
Grade (EEC)			
Low	63 (84.0%)	348 (79.6%)	0.380
High	12 (16.0%)	89 (20.4%)	
Myometrial invasion			
< 1/2	71 (77.2%)	397 (78.5%)	0.783
≥ 1/2	21 (22.8%)	109 (21.5%)	
Lymph node metastasis[a]			
No	58 (96.7%)	216 (86.1%)	0.023
Yes	2 (3.3%)	35 (13.9%)	
Vessel invasion			
No	85 (92.4%)	472 (93.3%)	0.756
Yes	7 (7.6%)	34 (6.7%)	
Stage			
I & II	77 (83.7%)	411 (81.2%)	0.574
III & IV	15 (16.3%)	95 (18.8%)	

[a]60 Uygur and 251 Han women received lymphadenectomy. EEC: endometrioid carcinoma; NEEC: non-endometrioid carcinoma.

Corning-Costar, New York, NY, USA). For the migration assay, 5 × 10^4 cells were plated in the top chamber in 200 µl serum-free DMEM/F12. For the invasion assay, 1 × 10^5 cells were seeded in the top chamber coated with Matrigel (BD Biosciences, Franklin Lakes, NJ, USA) in 200 µl serum-free DMEM/F12. Culture medium containing 10% FBS was added to the lower chamber as a chemoattractant. After 24 hours of incubation, cells successfully translocated were fixed by methanol for 30 minutes, stained with 0.1% crystal violet for 30 minutes, and counted under a light microscope.

Western blotting

Proteins from cells were separated by SDS-PAGE and transferred to PVDF membrane (Millipore, Billerica, MA, USA). The membrane was blocked with 5% non-fat milk and then incubated with the primary antibody overnight at 4°C. After three washes in TBST (triethanolamine-buffered saline solution with Tween), the membrane was incubated with horseradish peroxidase-conjugated secondary antibody at room temperature for 1 hour, and detected using the ECL (Enhanced Chemiluminescence) reagent (Millipore). Antibodies used include anti-HER2, anti-PIK3CA, anti-AKT and anti-phospho-AKT (Ser473) (Cell Signaling, Danvers, MA, USA).

Statistical analysis

Differences between the variables examined were statistically evaluated using the Student's *t*-test and Chi-square test. Survival analysis was performed using Kaplan-Meier curves with log-rank test. The Cox model was used to analyze the independent prognostic factors. A p value < 0.05 was considered to

Table 2. Clinicopathological features of 62 Uygur and 74 Han endometrial carcinomas

Variables	No. of cases		
	Uygur	Han	p
N	62	74	
Age			
Median age (range)	54 (33-77)	55 (37-80)	
Times of pregnancy			
< 5	55 (88.7%)	73 (98.6%)	0.023
≥ 5	7 (11.3%)	1 (1.4%)	
Type			
EEC	49 (79.0%)	53 (71.6%)	0.320
NEEC	13 (21.0%)	21 (28.4%)	
Grade (EECs)			
Low	43 (87.8%)	46 (86.8%)	0.884
High	6 (12.2%)	7 (13.2%)	
Myometrial invasion[a]			
< 1/2	35 (72.9%)	55 (74.3%)	0.863
≥ 1/2	13 (27.1%)	19 (25.7%)	
Lymph node metastasis[b]			
No	40 (97.6%)	34 (79.1%)	0.015
Yes	1 (2.4%)	9 (20.9%)	
Vessel invasion[a]			
No	47 (97.9%)	70 (94.6%)	0.647
Yes	1 (2.1%)	4 (5.4%)	
Stage[a]			
I & II	40 (83.3%)	61 (82.4%)	0.898
III & IV	8 (16.7%)	13 (17.6%)	

[a]48 Uygur and 74 Han women received hysterectomy. [b]41 Uygur and 43 Han women received lymphadenectomy. EEC: endometrioid carcinoma; NEEC: non-endometrioid carcinoma.

be statistically significant. Statistical analysis was performed using the SPSS 19.0 (Chicago, IL, USA).

Results

The clinicopathological features of Uygur and Han patients

The 598 endometrial cancer patients admitted in Xinjiang Cancer Hospital from 2009 to 2015 included 92 cases of Uygur women and 506 cases of Han women. The number of Uygur patients was significantly less than that of Han patients (**Figure 1A**). The data also show significant differences in times of pregnancy and childbirth between the two ethnic groups. Compared with Han women, Uygur women had more times of pregnancy and childbirth ($P < 0.001$; **Table 1**). In addition, lymph node metastasis occurred more often in Han women (13.9%) than in Uygur women (3.3%) ($P = 0.023$; **Table 1**; **Figure 1B**). No significant difference existed in other clinicopathological factors between the two ethnic groups.

Expression of miR-145 and miR-143 differ between Uygur and Han women with endometrial carcinoma and their relationship with patient survival

As mentioned above we observed that the number of Uygur women with endometrial cancer in Xinjiang Cancer Hospital was significantly less than that of Han women. Furthermore, in an earlier study, we found miR-145 and miR-143 decreased significantly in EECs with worse clinicopathological factors in Han women. Therefore, in this cohort miR-145 and miR-143 expression was compared between Uygur and Han women with endometrial cancer and assess their roles in different ethnic groups.

The clinicopathological features of the 136 selected cases for miRNA expression analysis (62 Uygur and 74 Han patients) are shown in **Table 2**. More times of pregnancy and childbirth ($P = 0.023$) and lower lymph node metastasis rate ($P = 0.015$) were also observed in these Uygur women. In addition, Kaplan-Meier curves demonstrated favorable survival in Uygur patients compared with Han patients with statistical significance ($P = 0.020$; **Figure 1C**).

Expression of miR-145 and miR-143 was analyzed in 62 Uygur and 74 Han endometrial cancer tissues with 10 proliferative endometrial samples as normal controls respectively. Expression of the two miRNAs was significantly different between the Uygur and Han group (all $P < 0.001$). The expression level of miR-145/143 increased in Uygur patients ($\log_2$RQ = 2.40 ± 0.49, 0.84 ± 0.44) but decreased in Han patients ($\log_2$RQ = -1.78 ± 0.42, -2.64 ± 0.34) compared to normal controls.

Furthermore, in the Uygur group, these two miRNAs increased significantly in NEECs ($\log_2$RQ = 3.95 ± 0.83, 2.04 ± 0.73) compared with normal controls ($P < 0.001$, $P = 0.040$), but in EECs only miR-145 approached significance

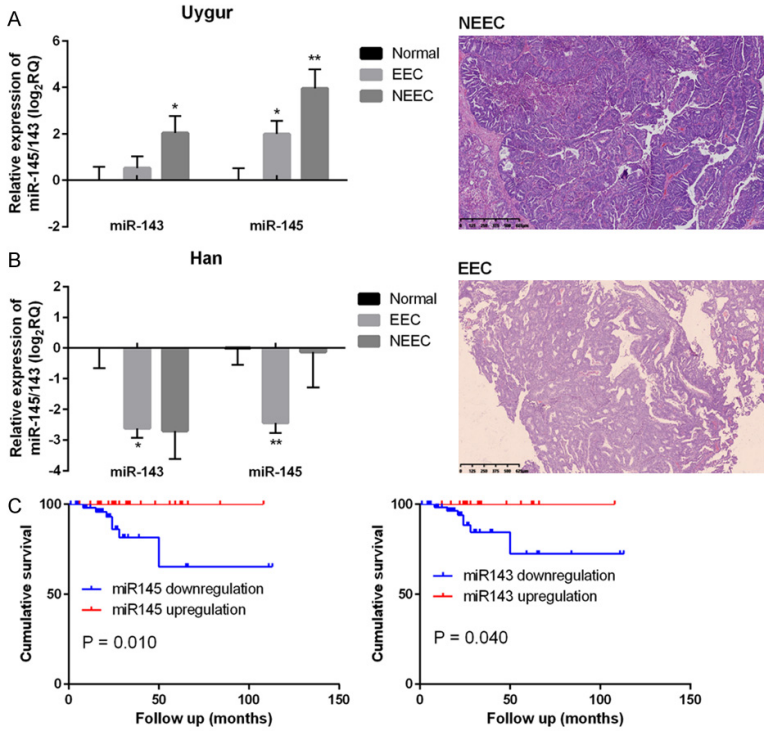

Figure 2. Expression of miR-145 and miR-143 differ between Uygur and Han women with endometrial carcinoma and correlate with favorable survival. A. In Uygur women, miR-145/143 expression increased significantly compared with normal controls, especially in NEEC. B. In contrast, in Han women miR-145/143 expression decreased compared with normal controls, especially in EEC. Data were presented as mean ± SE. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with normal controls. C. Upregulation of miR-145 or miR-143 significantly correlated with favorable prognosis.

(log_2RQ = 1.99 ± 0.56, P = 0.013), while miR-143 did not increase significantly (log_2RQ = 0.52 ± 0.51, P = 0.504) (**Figure 2A**). In addition, there was a trend that NEECs exhibiting favorable clinicopathological factors including superficial myometrial invasion, no lymph node metastasis and no vessel invasion exhibited higher miR-145 and miR-143 expression, although it was not statistically significant (**Tables 3**, **4**). However, early stage NEECs did not show higher miR-145/143 expression levels compared with the late stage ones and this may be due to the very limited number of the late stage cases (4 Stage III, 4 Stage IV).

In contrast, in the Han group, miR-145 and miR-143 decreased significantly in EECs (**Figure 2B**) and EECs with worse chinicopathological variables had lower miR-145 and miR-143 expression levels, although the associations did not reach statistical significance (P > 0.05). However, such trend did not exist in NEECs (**Tables 3**, **4**).

In this cohort, considering the follow-up was limited in the Uygur group as well as in the Han group, miR-145 and miR-143 had similar protective effects in the two ethnic groups, two ethnic groups were pooled together. The Kaplan-Meier curve demonstrated a significantly favorable survival for patients with miR-145 or miR-143 upregulation (P = 0.010, P = 0.040; **Figure 2C**). However, such associations did not persist in multivariate analysis (P > 0.05).

Restoration of miR-145/143 inhibits cellular proliferation, migration and invasion in endometrial cancer cell lines

For the gain of function assays, the miR-145 and miR-143 mimics were transiently transfected into Ishikawa, HEC-1B and KLE cell lines respectively. Cell proliferation was assessed using the CCK-8 assay. Ectopic miR-145 expression significantly reduced proliferation of all the cell lines, whereas the phenomena were not observed after transfection of miR-143 (**Figure 3A**). In addition, after transfection of miR-145 or miR-143 mimics, almost all the three cell lines showed reduced migration and invasion capabilities significantly except that the KLE cell line did not reach statistical significance with miR-145 mimics transfection (**Figure 3B**).

Restoration of miR-145 or miR-143 inhibits HER2 expression or downregulates PI3K pathway activity

Using the target gene prediction algorithms, including TargetScan (www.targetscan.org), RNA22 (https://cm.jefferson.edu/rna22/Precomputed) and TarBase v7.0 (http://www.microrna.gr/tarbase), HER2 (human epidermal growth factor receptor 2) was identified as potential target of miR-145. PIK3CA, the gene

Table 3. miR-145 expression differs between Uygur and Han women with endometrial cancer

Variables	miR-145 Log_2 (Relative Quantity), Mean ± SE			
	Uygur		Han	
	EEC	NEEC	EEC	NEEC
	1.99 ± 0.56	3.95 ± 0.83	-2.43 ± 0.34	-0.13 ± 1.16
p[a]	0.013	< 0.001	0.006	0.959
Grade (EECs)				
Low	3.03 ± 1.15	-	-1.92 ± 0.47	-
High	1.22 ± 0.46	-	-3.00 ± 0.48	-
p	0.154		0.113	
Myometrial invasion				
< 1/2	1.40 ± 0.76	5.98 ± 1.13	-2.26 ± 0.36	-0.72 ± 1.21
≥ 1/2	0.65 ± 0.99	2.87 ± 1.08	-2.90 ± 0.83	1.76 ± 3.07
p	0.704	0.104	0.493	0.375
Lymph node metastasis				
No	1.61 ± 0.87	4.08 ± 1.07	-1.85 ± 0.69	-0.60 ± 1.33
Yes	0.51 ± 0.74	1.86	-2.51 ± 1.05	-3.23 ± 1.71
p	0.681	0.545	0.640	0.201
Vessel invasion				
No	1.24 ± 0.75	3.92 ± 1.05	-2.35 ± 0.37	-0.03 ± 1.43
Yes	1.62 ± 1.23	3.88 ± 1.73	-2.81 ± 0.88	-0.56 ± 0.48
p	0.849	0.985	0.619	0.729
Stage				
I & II	1.37 ± 0.72	3.43 ± 0.66	-2.27 ± 0.36	-0.20 ± 1.17
III & IV	0.51 ± 0.74	4.57 ± 2.06	-3.51 ± 0.97	0.04 ± 3.01
p	0.726	0.620	0.218	0.928

[a]Compared with normal controls. EEC: endometrioid carcinoma; NEEC: non-endometrioid carcinoma.

encoding the p110a catalytic subunit of PI3K (phosphoinositide 3-kinase), and AKT were predicted to be potential targets of miR-143. Therefore, protein levels of HER2 and downstream PI3K pathway targets were analyzed after enforced miR-145 and miR-143 expression to assess the effect of these two miRNAs on HER2-PI3K signaling activity. After transfection of miR-145 mimics, inhibition of HER2 expression was marked in Ishikawa and HEC-1B cells, but moderate in KLE cells. miR-143 mimics just slightly reduced HER2 expression in Ishikawa and KLE cells. Enforced miR-143 expression significantly reduced PI3KCA protein expression in HEC-1B and KLE cells and this occurred slightly in Ishikawa cells (**Figure 3C**). Moreover, miR-143 mimics induced AKT and p-AKT inhibition in all the three cell lines (**Figure 3C**). However, PI3K-AKT pathway activity did not change so much after miR-145 mimics transfection.

Discussion

Xinjiang Uygur Autonomous Region is located in the northwest part of China adjacent to Mongolia in the east, Russia in the north, Pakistan and India in the south, and Kazakhstan, Kirgizstan and Tajikistan in the west. People of different ethnic groups including Uygur (46%), Han (39%), and Kazakh (7%), intermingle in this area [12, 13]. Originating from inter-marriage between Caucasians and Mongolians, the Uygur people have their own language, culture, genetic background, lifestyle, and dietary habits. The Uygur women usually have early marriage, multiple times of pregnancy and childbirth compared with Han women [3, 14]. In the present study, we observed similar phenomenon in Uygur and Han women with regard to endometrial cancer. Furthermore, to our knowledge, we report for the first time the distinct occurrence of endometrial cancer between Uygur and Han women as well as different microRNA expression between the two ethnic groups.

The number of Uygur women with endometrial cancer admitted to Xinjiang Cancer Hospital was 3-6 times less than that of Han women. Xinjiang Cancer Hospital is the only cancer center in this area with 1500 beds for inpatients, located in Urumqi, the capital city of Xinjiang Uygur Autonomous Region. Considering their culture and dietary habits, most Uygur cancer patients prefer to be treated in Xinjiang Cancer Hospital. Therefore, at some extent the patients admitted in this hospital represent the occurrence and characteristic of cancer in Xinjiang.

The data also imply that multiple times of pregnancy and childbirth might be important exter-

Table 4. miR-143 expression in Uygur and Han women with endometrial cancer

Variables	miR-143 Log_2 (Relative Quantity), Mean ± SE			
	Uygur		Han	
	EEC	NEEC	EEC	NEEC
	0.52 ± 0.51	2.04 ± 0.73	-2.61 ± 0.32	-2.70 ± 0.92
p[a]	0.504	0.040	0.016	0.177
Grade (EECs)				
Low	1.08 ± 0.93	-	-2.39 ± 0.46	-
High	0.10 ± 0.55	-	-2.86 ± 0.43	-
p	0.371		0.470	
Myometrial invasion				
< 1/2	-0.39 ± 0.57	3.90 ± 1.20	-2.32 ± 0.35	-2.43 ± 1.18
≥ 1/2	-0.88 ± 0.75	1.11 ± 0.90	-3.41 ± 0.66	-3.56 ± 0.96
p	0.617	0.099	0.129	0.464
Lymph node metastasis				
No	-0.36 ± 0.63	2.46 ± 0.90	-2.15 ± 0.58	-2.16 ± 1.03
Yes	-0.80 ± 0.47	-0.56	-2.82 ± 0.79	-4.97 ± 1.90
p	0.588	0.337	0.567	0.239
Vessel invasion				
No	-0.58 ± 0.54	2.11 ± 1.27	-2.45 ± 0.36	-2.81 ± 1.14
Yes	0.30 ± 1.30	1.95 ± 0.90	-3.40 ± 0.62	-2.22 ± 0.54
p	0.547	0.924	0.260	0.648
Stage				
I & II	-0.43 ± 0.54	1.47 ± 1.03	-2.45 ± 0.34	-1.75 ± 1.11
III & IV	-0.80 ± 0.47	2.84 ± 1.30	-3.64 ± 0.77	-5.07 ± 1.31
p	0.617	0.422	0.204	0.104

[a]Compared with normal controls. EEC: endometrioid carcinoma; NEEC: non-endometrioid carcinoma.

nal factors related to lower occurrence of endometrial cancer in Uygur women compared with Han women. However, only the times of pregnancy and childbirth between Uygur and Han women with endometrial cancer were compared, thus lacking further efforts to compare with normal Uygur and Han women. Thus, this is the limitation of the present study. Obviously, it is easier for us to access to endometrial cancer patients than to normal control people, in particular in cancer hospital.

More interestingly, miRNA signatures might have meaningful insight into the basic biological differences between the two ethnic groups. Further analysis of 62 Uygur and 74 Han endometrial cancer tissues showed that the expression patterns of miR-145 and miR-143 were distinct between the two ethnic groups. Studies have indicated that miR-145 and miR-143 are present at high levels in germline and mesoderm-derived tissues, including uterus, ovary and testis, and are downregulated in various malignancies [6, 7, 15]. Thus, miR-145 and miR-143 are considered to act as broad tumor suppressors. In our previous study we found that miR-145/143 decreased in Han women with endometrial cancer and was more often associated with EECs compared with NEECs. Furthermore, there was a trend that EECs with worse clinicopathological factors had lower expression levels of miR-145/143 [8]. In the present study we reconfirmed such observation. However, we found that these two miRNAs had quite different expression patterns in Uygur women. They increased in Uygur group and the expression levels of these two miRNAs were significantly upregulated in NEECs than EECs. Moreover, there was a trend in the Uygur group that NEECs with favorable clinicopathological factors including superficial myometrial invasion, no lymph node metastasis and no vessel invasion exhibited higher miR-145/143 expression. Together, these findings suggest that miR-145 and miR-143 expression might be associated with ethnic factor and tumor subtype in Xinjiang women with endometrial cancer.

Additionally, these data also confirm a tumor suppressor role of the two miRNA in endometrial cancer with Uygur patients benefitting more. First, patients with miR-145 or miR-143 upregulation had a significantly prolonged survival. Second, as mentioned, the two miRNA increased in Uygur women but decreased in Han women. Third, in this cohort, Uygur patients had a significant lower lymph node metastasis rate and favorable survival compared with Han

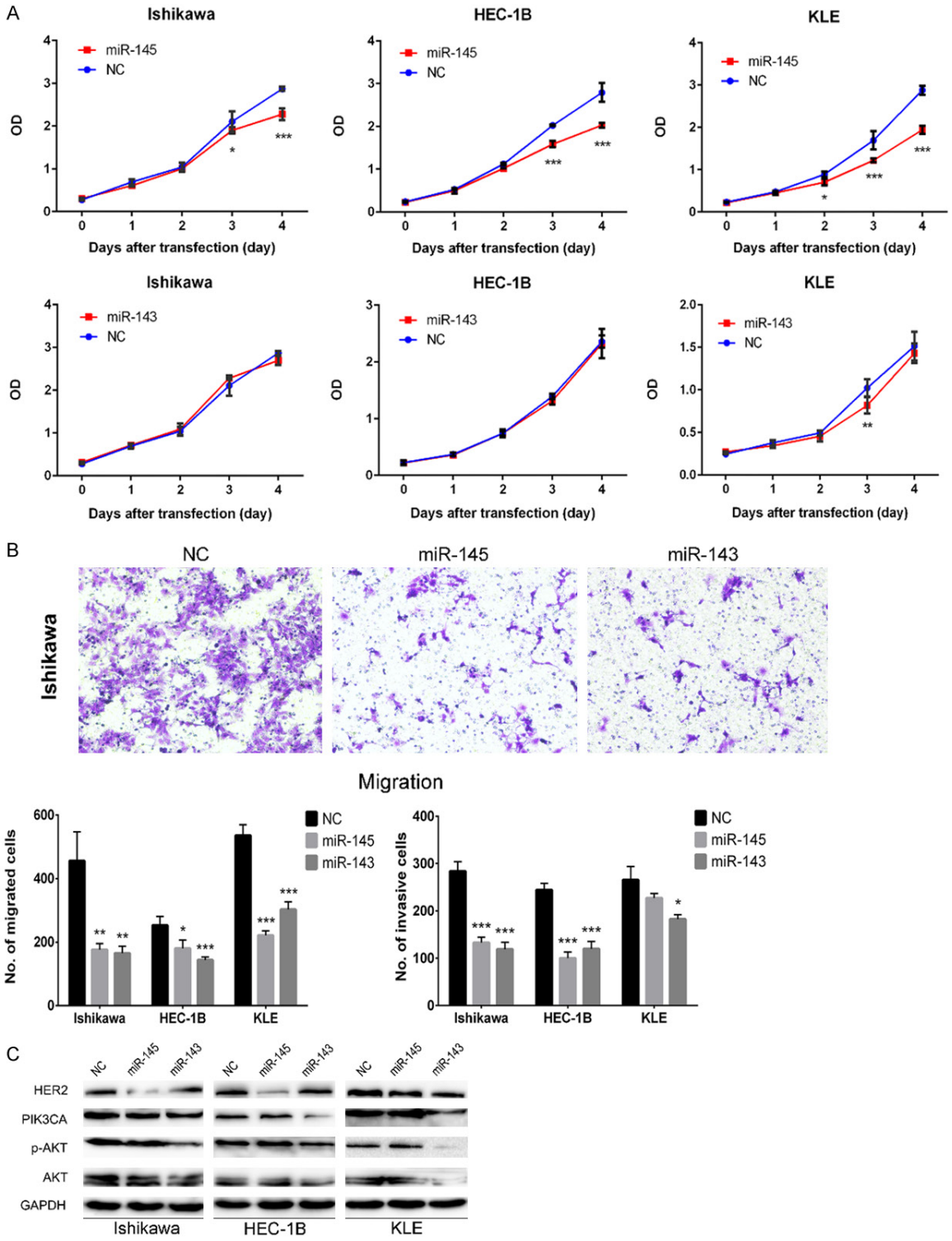

Figure 3. Effect of transient transfection of miR-145 or miR-143 mimics on endometrial cancer cells. A. The proliferation of endometrial cancer cells was inhibited after transfection with miR-145 mimics, whereas the phenomena were nearly not observed with miR-143 mimics. B. Ectopic expression of miR-145 or miR-143 suppressed the motility and invasion abilities in all the three cell lines. C. Effect of miR-145 or miR-143 on HER2-PI3K signaling was examined by Western blotting. Endometrial cancer cells were transiently-transfected with miR-145 or miR-143 for 48 h. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with controls.

patients. Together, all these findings may imply that upregulation of miR-145/143 might possess favorable prognostic impacts and this might be related to the less occurrence and mild behavior of endometrial cancer in Uygur women compared with Han women.

Furthermore, we found a vital link between miR-143 and PI3K/AKT pathway, which is frequently activated in endometrial cancer, might exist. First, using the algorithms of target prediction, PIK3CA and AKT, the PI3K pathway key elements, were identified as potential targets of miR-143. In addition, HER2, situated in the upstream of PI3K/AKT pathway, was identified as potential target of miR-145. Second, enforced miR-143 expression could reduce PIK3CA, AKT and p-AKT in all the three cell lines. Enforced miR-145 expression significantly downregulated HER2 protein levels in Ishikawa and HEC-1B cells, and moderately in KLE cells, but did not affect the PI3K pathway. Third, miR-143 only affected the migration and invasion properties whereas miR-145 inhibited proliferation, migration and invasion in all the three cell lines. Together, these findings suggest that miR-145 and miR-143 may inhibit endometrial cancer cell growth or invasiveness through targeting different genes. Although PI3K/AKT pathway is in the downstream of HER2, miR-145 seemed to just target HER2 but have no relationship to PI3K/AKT pathway activity.

In summary, these findings suggest that miR-145 and miR-143 expression in endometrial cancer might be associated with ethnic factors and tumor subtype in Xinjiang women. Upregulation of these two miRNAs in Uygur women might have favorable prognostic impacts whereas downregulation of them in Han women may cause negative effects. Additionally, this might be related to the mild behavior of endometrial cancer in Uygur women compared with Han women. Furthermore, in the cell lines, miR-145 and miR-143 may function as tumor suppressors through targeting different molecular pathways. Our findings thus provide insight into the basic biological differences between Uygur and Han women with endometrial cancer. Thus, advances in the understanding of the distinct molecular mechanisms will lead to the development of novel and individualized anticancer therapeutic strategies.

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Disclosure of conflict of interest

None.

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