Reduced expression of members of the MHC-I antigen processing machinery in ethnic Uighur women with cervical cancer in the Xinjiang region of China

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ABSTRACT

Objective

Cervical cancer is a major cause of mortality in Uighur women compared with Han women in the Xinjiang region of China. Although a reduction in the class I major histocompatibility complex (MHC-I) antigen processing machinery (APM) is associated with the development of cervical cancer, the MHC-I APM has not been studied in this particular group of women, who have the highest incidence rate of cervical cancer in China.

Study Design

We used immunohistochemical staining and polymerase chain reaction amplification of viral DNA from infection with the human papilloma virus (HPV) to study the expression of members of the MHC-I APM in cervical cancer sections collected from Uighur and Han women and in cervicitis samples from age-matched counterparts.

Results

Expression of the molecules of interest was compared between two ethnic groups, and expression of transporter associated with antigen processing 1 and 2, heat shock protein 90, and calnexin were found to be reduced even more significantly in Han women with cervical cancer than in Uighur women with same disease. However, compared with Han women, Uighur women had a higher rate of infection with HPV 16.

Conclusions

The MHC-I APM were reduced in cervical cancer, with heterogeneity in the two ethnic groups. The reduction was more pronounced in Han women, who less frequently had HPV 16 infection, suggesting possible

differences in the roles of members of the MHC-I APM and in the mechanisms of cervical cancer development in these two ethnic groups despite residence in the same region of China.

KEY WORDS

TAP-1, TAP-2, β 2-microglobulin, Hsp70, calnexin, Hsp90, Grp94, HPV 16

1. INTRODUCTION

Cervical cancer is the second most common malignant tumour in women worldwide, with 500 thousand new cases and 275 thousand deaths occurring each year¹. The cervical cancer incidence rate is very high in China, and in particular, the Uighur women who live in southern region of Xinjiang Province, China, have the highest morbidity and mortality from cervical cancer in the country, even when compared with women of other ethnicities from the same geographic area. Cervical cancer is a major cause of death in Uighur women of this region². Furthermore, cervical cancer tends to develop in Uighur women at younger ages. The high rate of cervical cancer development in these ethnic women is probably at least partly attributable to a high prevalence of infection with human papilloma virus (HPV). The overall HPV infection rate is 459 per 100,000, and the high-risk genotype HPV 16 is the most dominant virus type, being shown to be present in 77.6%–82.6% of Uighur women with cervical cancer^{2,3}.

Immunity mediated by T-cells, especially CD8+ cytotoxic T lymphocytes (CTLs), plays a critical role in protecting against the development of cervical cancer. However, tumour cells, including those in cervical cancer, have developed several mechanisms to escape CTL-mediated immune protection⁴. Loss of expression of human leukocyte antigen class I molecules and components of the class I major histocompatibility complex (MHC-I) antigen processing

machinery (APM) is of particular importance, because reductions in those molecules provide a mechanistic pathway for tumour cells to evade CTL-mediated immunity⁵. The APM is responsible for presentation of tumour antigenic peptides by MHC-I molecules. After processing by cytoplasmic immunoproteasomes, tumour antigenic peptides are transported from the cytoplasm to the endoplasmic reticulum by transporter associated with antigen processing (TAP) proteins TAP-1 and TAP-2. The peptides are then cut into segments 8–9 amino acids in length by endoplasmic reticulum aminopeptidase associated with antigen presentation 1, and loaded into empty MHC-I molecules with the help of tapasin and the chaperone molecules calnexin (CNX), calreticulin, and ERp57^{6–9}.

Previous studies demonstrated varying levels of reduction and even complete loss of APM components in human malignancies, including cervical cancer. Reduction of these molecules has been associated with poor prognosis^{10–12}. Infection with HPV is a major risk factor associated with cervical cancer development, and persistent infection with HPV may lead to reduced CTL-mediated immunity against tumour development¹³. However, the exact role of reduced expression of APM molecules in HPV-induced cervical carcinogenesis remains elusive, and the mechanisms related to the high incidence of cervical cancer in Uighur women in Xinjiang remain to be explored. We investigated the expression of major MHC-I-associated APM components such as TAP-1, TAP-2, 70-kDa heat shock protein (Hsp70), β_2 -microglobulin (β_2 M), CNX, heat shock protein 90 (Hsp90), and heat shock protein 90 kDa beta (Grp94) in a large number of cervical cancer specimens collected from Uighur and Han women of the Xinjiang region.

2. METHODS

2.1 Patients and Tissue Samples

We collected cervical tissue samples from 142 cervical cancer patients (99 of Uighur ethnicity, 43 of Han ethnicity). As a control, we also collected cervical tissue sections from 87 patients with cervicitis (36 of Uighur ethnicity, 51 of Han ethnicity) during clinic visits between 2002 and 2007 to the First Affiliated Hospital of Xinjiang Medical University, Urumqi, and the First People's Hospital of Kashi District, Xinjiang, China. All patients were residents of the region and had not received preoperative radiotherapy or chemotherapy at the time of sample collection.

Mean age of the Uighur women was 47 years (range: 23–70 years), and mean age of the Han women was 38 years (range: 28–65 years). The tissues were formalin-fixed and paraffin-embedded, and the diagnosis was histologically confirmed by experienced pathologists from the departments of pathology at the respective hospitals. The use of clinical materials for this study was approved by the Institutional Review

Board of Xinjiang Medical University, following the guidelines of the Chinese Federation of Medical Research Associations.

2.2 Reagents

The rabbit polyclonal antibodies used in this study were anti- β_2 M (DakoCytomation, Glostrup, Denmark), anti-CNXX (Proteintech Group, Chicago, IL), anti-TAP-1 (Abgent, San Diego, CA, U.S.A.), anti-Hsp70 and anti-Hsp90 (Cell Signaling Technology, Danvers, MA, U.S.A.), anti-Grp94 (Lab Vision Corporation, Fremont, CA, U.S.A.), and anti-TAP-2 (Atlas Antibodies AB, Stockholm, Sweden). Histo-stain-Plus kits were obtained from Jingmei Biotech Corporation (Shenzhen, China).

2.3 Immunohistochemical Staining

Immunohistochemical staining was performed as previously described¹⁴. Briefly, 4- μ m sections were dewaxed in xylol and methanol, and endogenous peroxidases were blocked for 15 minutes in methanol with 3% H₂O₂. Antigenic epitopes were unmasked by microwave heating at 98.5°C for 15 minutes in 0.01 mol/L citrate-buffered solution (pH 6.0), and cooled to room temperature in the same solution for 30 minutes. The slides were rinsed in phosphate-buffered saline (PBS) with 0.05% polysorbate 20 surfactant for 20 minutes. For immunohistochemical detection of proteins, the slides were pre-incubated with 10% normal goat serum in PBS and then incubated with the respective antibodies diluted in PBS with 1% goat serum to an optimal concentration based on pilot experiments. After 30 minutes' incubation at room temperature, the slides were washed with PBS containing polysorbate 20 surfactant and then incubated with biotinylated goat anti-rabbit antibody. Incubation with streptavidin-labelled horseradish peroxidase followed. Reactivity was visualized with diaminobenzidine (all chemicals from Jingmei Biotech Corporation) for 5 minutes at room temperature. The slides were then counterstained with hematoxylin and mounted in Permount Mounting Medium (Thermo Fisher Scientific, Waltham, MA, U.S.A.). Slides with known positive tissue sections were used as positive controls, and slides stained with isotype-matched immunoglobulin molecules were used as negative controls.

2.4 Evaluation of Immunohistochemistry

Because TAP-1, TAP-2, Hsp70, β_2 M, CNX, Hsp90, and Grp94 are expressed in the cervical epithelial cell cytoplasm, the appearance under the microscope of brown immunohistochemical staining in the cytoplasm of cells was determined to be positive for the target protein. Positive samples were further graded into four levels based on the area occupied by cells

positive for the target antigen: less than 5% was graded as negative; 5%–25%, as weakly positive (+); 26%–50%, as positive (++); 51%–75%, as strongly positive (+++); and more than 75%, as very strongly positive (++++). Two pathologists read the slides back-to-back in a blind manner.

2.5 DNA Extraction

Depending on the size of the tissue samples, 5–20 tissue sections 10- μm^2 in size were collected in 1.5 mL Eppendorf tubes, and DNA was extracted using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's directions. The quality and quantity of extracted DNA samples were determined by optical density measurements at 260 nm and 280 nm.

2.6 Detection of Viral Infection

The quality of DNA samples was validated by amplification of a 110-bp fragment of the housekeeping *HBB* gene. Infection with HPV was examined by polymerase chain reaction (PCR) amplification of HPV-specific DNA sequences in the samples extracted from the paraffin-embedded cervical tissues. This procedure used a universal HPV primer pair ("consensus primer") and the primer pairs specific for high-risk HPV 16 and HPV 18.

The sequences of primer sets for HPV 16 (with a 96-bp product size) and HPV 18 (with a 115-bp product size) were these:

- Forward 5'-GGTCGGTGGACCGGTCGATG-3' and reverse 5'-GCAATGTAGGTGTATCTC-CA-3' (HPV 16)
- Forward 5'-CCTTGGACGTAAATTTTGG-3' and reverse 5'-CACGCACACGCTTGGCAG-GT-3' (HPV 18)

The PCR was performed in a 50- μL reaction volume containing 500 ng DNA, 1.5 U TaKaRa Ex Taq Polymerase (Millipore Corporation, Billerica, MA, U.S.A.), dNTP mix (Life Technologies, Beijing, PR China) at 100 $\mu\text{mol/L}$, and 2 μL of each primer (20 pmol/mL). The cycling conditions were denaturation at 95°C for 3 minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds, primer annealing at 59°C (HPV 18) or 60°C (HPV 16) for 20 seconds, and extension at 72°C for 25 seconds, with a final extension at 72°C for 7 minutes¹⁵. The cycling protocol for the Gp consensus primers (Gp5 and Gp6) was 40 cycles, which included denaturation at 94°C for 30 seconds, primer annealing at 45°C for 30 seconds, and extension at 72°C for 30 seconds¹⁶. All reagents were obtained from Takara Biotechnology (Dalian, China).

For analysis of PCR results, 5 μL of each PCR reaction product was resolved by electrophoresis in

1.5% agarose gel (Agarose B: BBI-Biotech, Berlin, Germany), and the images were recorded using a Gel Doc 2000 system (BioRad, Hercules, CA, U.S.A.).

2.7 Statistical Analysis

The statistical analysis was performed using the SPSS software application (version 13.0: SPSS, Chicago, IL, U.S.A.). Data were evaluated using the log-rank and chi-square tests. The level of significance was set at $p < 0.05$. Correlations between the categorized biomarkers were examined. A chi-square test was performed for each ethnic group and was used to compare protein expression in the presence or absence of cervical lesions.

3. RESULTS

Figure 1 shows a representative result for all members of the APM associated with MHC-I antigen presentation and processing. The immunohistochemical streptavidin–peroxidase method successfully visualized the presence of target proteins in the tissue sections

FIGURE 1 Expression of components of the class I major histocompatibility complex (MHC-I) antigen processing machinery (APM) in cervical tissues from patients with cancer and cervicitis (200 $\times$ original magnification).

from both cervicitis and cervical cancer patients. The positive cells in the cervicitis and cervical cancer samples were stained brown in cytoplasm, and the cervical epithelial cells were stained at the base or diffusely. Most cells were stained in the cytoplasm; a few cells were also stained in the cell nuclei.

Because of sample losses during the staining process, results were obtained for 111 of 142 cervical cancer tissue sections and for 68 of 87 cervicitis lesions (Table 1). Overall, our results demonstrated that, regardless of ethnicity, positive expression of TAP-1, TAP-2, β 2M, and Hsp90 were significantly reduced in cervical tissue sections from patients with cervical cancer compared with sections from patients with cervicitis. The expression of the remaining antimicrobial peptides (Hsp70, CNX, and Grp94) were slightly more reduced in cervical cancer lesions than in cervicitis, but the differences were nonsignificant. The reduction in TAP-1, TAP-2, Hsp90, and CNX were even more pronounced in cervical cancer sections from Han women than in those from their Uighur counterparts. However, β 2M was more significantly reduced in Uighur women with cervical cancer than in their Han counterparts, suggesting differences in the pattern of change to those molecules between the two ethnic groups.

We observed positive correlations between the expressions of TAP-2 and β 2M in Han women with squamous cell cervical cancer ($r = 0.433$, $p < 0.05$) and cervicitis ($r = 0.687$, $p < 0.05$). Other positive correlations were observed between the expressions of Hsp70 and CNX in Han women with squamous cell cervical cancer ($r = 0.419$, $p < 0.05$) and between the expressions of TAP-1 and β 2M in Uighur women with squamous cell cervical cancer ($r = 0.339$, $p < 0.05$).

As shown in Figure 2, we successfully amplified HPV 16 and HPV 18 genes from cervical lesions. The results of that experiment, shown in Table 1, demonstrate

that HPV 16 was present in 35 of 76 cervical cancer samples (46.05%) from Uighur women. In contrast, only 3 of 35 cervical cancer samples (8.57%) from Han women were positive for HPV 16. Thus, in the same geographic region, Uighur women with cervical cancer were infected with HPV 16 significantly more frequently than were their Han counterparts. The same imbalance was true for the HPV 16 infection rate: cervical samples from Uighur women with cervicitis showed a HPV 16 infection rate that was higher by a factor of 10 than that in Han women with cervicitis. Overall, the HPV 16 infection rate was significantly higher in women with cervical cancer than in those with cervicitis. However, the HPV 18 infection rate was very low: just 7 of 68 cervical cancer tissue samples were positive for HPV 18.

We also analyzed the relationship between changes in the expression of APM components and infection with HPV 16 in cervical cancer samples. In

FIGURE 2 Polymerase chain reaction (PCR) amplification of human papilloma virus (HPV) 16 and HPV 18 from DNA samples isolated from cervical cancer lesions. From left to right, the PCR bands show marker and samples that are negative, positive for HPV 16, and positive for HPV 18.

TABLE 1 Expression of class I major histocompatibility complex antigen processing machinery components in cervicitis and cervical cancer sections from Uighur and Han women

Pathologic type	Pts (n)	Patients [n (%)] positive for							
		β 2M	Tap1	Tap2	Hsp70	Hsp90	Cnx	Grp94	HPV 16
Cervicitis	68	64 (94.12)	55 (80.88)	66 (97.06)	50 (73.53)	67 (98.53)	64 (94.12)	64 (94.12)	8 (11.76)
Han	37	33 (89.19)	27 (72.97)	35 (94.59)	26 (70.27)	36 (97.30)	33 (89.19)	33 (89.20)	1 (2.70)
Uighur	31	31 (100)	28 (90.32)	31 (100)	24 (77.42)	31 (100)	31 (100)	31 (100)	7 (22.58)
Cervical cancer	111	69 (62.16) ^a	56 (50.45) ^a	68 (61.62) ^a	86 (77.48)	65 (58.56) ^a	96 (86.49)	91 (81.98)	38 (34.23) ^a
Han	35	29 (82.86) ^b	11 (31.43) ^b	15 (42.86) ^b	23 (65.71)	12 (34.29) ^b	24 (68.57) ^b	27 (77.14) ^a	3 (8.57) ^b
Uighur	76	40 (52.63)	45 (59.21)	53 (69.74)	63 (82.89)	53 (69.74)	72 (94.74)	64 (84.21)	35 (46.05)

^a $p < 0.05$, cervical cancer versus cervicitis.

^b $p < 0.05$, Han versus Uighur women with cervical cancer.

β 2M = β 2-microglobulin; Tap1 = transporter associated with antigen processing 1; Tap2 = transporter associated with antigen processing 2; Hsp70 = heat-shock protein 70; Hsp90 = heat-shock protein 90; Cnx = calnexin; Grp94 = glucose regulated protein 94; HPV = human papilloma virus.

Uighur women with cervical cancer, we observed a positive correlation between the expressions of β 2M and HPV 16 ($p = 0.021$, $r = 0.162$), between Hsp90 and HPV 16 ($p = 0.000$, $r = 0.483$), and between Grp94 and HPV 16 ($p = 0.009$, $r = 0.765$). To our surprise, although Han women with cervical cancer were infected with either HPV 16 or HPV 18 less frequently than were their Uighur counterparts, expressions of TAP-1, TAP-2, Hsp90, and CNX were significantly lower in those women than in Uighur women.

4. DISCUSSION

The results from our analysis of APM components of MHC-I antigen presentation in a relatively large sample of cervical cancer and cervicitis patients demonstrated that the expressions of MHC-I-associated APM components were lower in cervical tissues from cervical cancer patients than in samples from cervicitis patients and that there were significant differences in the levels of expression of TAP-1 and TAP-2 between women of different ethnicities from the same geographic region. In both cervicitis and cervical cancer, HPV 16 was the dominant type of HPV infecting Uighur women, whose infection rate was significantly higher than that of Han women with same disease.

Previous studies showed that the expressions of TAP-1 and TAP-2 were lower in 72% of patients (21 of 29) with cervical cancer¹⁷. The rate of TAP-1 disappearance in cervical cancer is about 48.64%¹⁸, and decreased TAP protein expression was correlated with lesser differentiation of cancer cells. In accord with previous reports, our study also demonstrated that expressions of TAP-1 and TAP-2 proteins were lower in cervical cancer than in cervicitis in women of both ethnicities, and that expressions of β 2M, Hsp90, and CNX were also reduced in cervical cancer tissues compared with cervicitis tissues. The reductions in these proteins were significantly more pronounced in Han women with cervical cancer than in Uighur women with same disease, suggesting that a decrease in TAP proteins might be a common feature in cervical cancer patients regardless of ethnicity. The decline in TAP proteins might possibly lead to decreased expression of class I MHC molecules on cancer cells, which might in turn serve as an effective way for cancer cells to escape immune surveillance.

We observed a positive correlation between the expressions of TAP-2 and β 2M in Han women with squamous cell cervical cancer ($r = 0.433$, $p < 0.05$). However, in Uighur women, we observed a positive correlation between the expressions of β 2M and TAP-1 in squamous cell cervical cancer ($r = 0.339$, $p < 0.05$), suggesting a similar pattern of decline of β 2M and TAP proteins in cancer patients. Those findings might imply that a decline in TAP proteins in cancer cells leads to reduced antigenic peptide loading to MHC-I molecules and therefore to lesser expression

of β 2M. Hence, it is reasonable to speculate that the reduction in β 2M may be secondary to the reduction in TAP proteins in cervical cancer patients.

An essential part of the MHC-I molecule, β 2M is indispensable for stable expression and efficient antigenic peptide presentation to CTL cells^{19,20}. It can facilitate the binding of antigenic peptides to cell-surface MHC-I^{21,22} and is therefore required for initiation of a normal immune response. The occurrence and development of tumours is tightly associated with β 2M, and the combination of β 2M with other tumour markers might increase the sensitivity of cancer diagnosis²³. Studies from Western countries have demonstrated reduced β 2M in patients with pre-malignant cervical lesions^{24,25}. Another study also demonstrated that the expressions of TAP-1, TAP-2, CNX, and β 2M were reduced in cancer lesions²⁶. In the present study, the positive expression rate of β 2M in cervical cancer (62.16%) was significantly lower than that in cervicitis (94.12%, $p < 0.05$), although β 2M was not reduced in Han women with cervical cancer compared with Han women having cervicitis. However, the expression of β 2M in Uighur patients with cervical cancer was lower—or even lost—compared with such expression in cervicitis, suggesting a disease-specific reduction of β 2M, at least in Uighur women.

The heat shock proteins are involved in MHC-I and MHC-II restricted antigen presentation and stimulation of CD8⁺ CTLs^{27,28}, and those proteins might be able to be used as vaccines for adjuvant cancer therapy. The heat shock proteins are widely associated with cancer cell cycle regulation, proliferation, apoptosis, differentiation, multidrug resistance, tumour immunity, and immunity against viral infection^{29,30}. Chen *et al.*³¹ showed that the expression of Hsp70 was higher in cervical cancer patients than in normal control subjects. Studies by Guo *et al.*³² indicated that the expression of Hsp70 was associated with pathologic grade, clinical stage, and metastasis to lymph nodes in cervical cancer. Other studies have also demonstrated a gradually elevated expression of Hsp70 in cervical cancer cells with high, moderate, and poor differentiation, suggesting that elevated expression of Hsp70 is associated with active tumour growth, poor differentiation, and greater malignancy. However, we did not see any differences in the expression of Hsp70 in cervical cancer lesions compared with cervicitis in either ethnic group. That observation might be a result of differences in the reagents and clinical samples between previous studies and our study. An investigation by Zhao *et al.*³³ indicated that the expression of Hsp90 was significantly higher in invasive carcinoma of cervix and cervical intraepithelial neoplasia than it was in cervicitis and normal cervix. The increase in the expression of Hsp90 in cervical cancer might be attributable to increased demand for heat shock protein in cancer cells to regulate

and stabilize abnormal growth because of uncontrolled cell growth and increased cell metabolism. Another possibility is that increased expression of Hsp90 may act as cell-surface marker for escaping from immune surveillance, thus facilitating cancer development or affecting the function of cancer killers, such as natural killer cells³⁴. However, our study did not support that notion: The expression of Hsp90 was lower in cervical cancer samples than in cervicitis in both ethnic groups, and we could not make any reasonable speculation about the discrepancies between earlier reports and our findings. However, we did observe a positive correlation in the expression levels of Hsp90 and Hsp70 in cervical cancers from Uighur women ($r = 0.265$, $p < 0.05$), an observation that might be a result of a similar regulatory mechanism in cancer cells, because those two proteins belong to the same family.

Calcium binding protein in the endoplasmic reticulum, CNX, is a molecular chaperone that helps with the correct folding of MHC class I molecules to form a functional dimer^{35,36}. Reduced expression or absence of CNX may therefore lead to downregulation of MHC-I expression^{37,38}. A study by Ritz *et al.*²⁶ showed a significant reduction of CNX in tissues from cervical cancer patients compared with normal cervical tissues. Our results confirmed that finding, with reduced expression of CNX in cervical cancer than in cervicitis in women of both ethnicities. Expression of CNX in Uighur women was higher than that in Han women in both disease groups, and the difference was statistically significant ($p < 0.05$). Pronounced reduction of CNX in cervical cancer in Han women compared with their Uighur counterparts might play a role, at least in part, in the more pronounced reduction of other components of the APM in Han women with cervical cancer.

The molecular chaperone Grp94 (“glucose-regulated protein”) is a more abundant protein component of the normal cellular endoplasmic reticulum. It helps with correct folding and assembly of proteins in the cell. The literature documents the initiation of a stress reaction in glucose metabolism in cancer cells with the start of tumour growth^{39,40}, and therefore Grp94 is associated with tumorigenesis and tumour growth. In studies that mimicked cutting of the blood supply in solid tumours to reduce glucose levels in cancer tissues, the expression of Grp94 increased by a factor of 9 from the expression level found in normal breast epithelial cells. However, in our study, expression of Grp94 did not increase in cervical cancer compared with cervicitis; however, expression levels were reduced more significantly in Han women with cervical cancer than in Uighur women with the same disease. Expression of Grp94 might be associated with growth retardation and differentiation of tumour cells⁴¹.

The different changes of $\beta 2M$ and Grp94 in the two ethnic groups of women with cervical cancer

suggest that expression of some of the members of the MHC-I APM in cervicitis and cervical cancer may differ depending on ethnicity.

According to previous reports, the rate of infection with HPV 16 in cervical cancer patients in other countries is 54.6%⁴²; it is 60.0%⁴³ and 63.4%⁴⁴ in cervical cancer patients from Inner Mongolia in China; and in Uighur women with cervical cancer, it ranges from 61.6% to 84.61%^{45,46}. In accord with those findings, our study showed that the rate of infection with HPV 16 in Uighur women with cervical cancer in Xinjiang was 58%–62% and that their rate is significantly higher than the rate in Han women with cervical cancer from the same region. The difference in rates of HPV 16 infection in these two ethnic groups might be a result of differences in ethnic culture and lifestyle.

Only 7 of our 68 Uighur cervical cancer patients were positive for HPV 18 infection, and none of our 35 Han cervical cancer patients was positive for HPV 18. That finding might be related to the fact that all the cervical cancer specimens collected were squamous cell carcinoma⁴⁷. The frequency of concurrent infection with both HPV 16 and HPV 18 was higher in Uighur women with cervical cancer⁴⁸.

5. CONCLUSIONS

Our study demonstrated reduced expression of MHC-I antigen presentation-associated molecules in cervical cancer lesions. The level of the reduction was heterogeneous and, for some molecules, showed ethnic specificity. The mechanisms underlying the reduced expression of these proteins, with differences between ethnic groups, and the exceptionally high HPV 16 infection rate in Uighur women with cervical cancer (with less pronounced reduction of APM members) and their association with cervical cancer need to be addressed in future studies.

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7. CONFLICT OF INTEREST DISCLOSURES

The authors declare that they have no financial conflicts of interest.

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