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Original Article

Diagnostic value of MCM2 immunocytochemical staining in cervical lesions and its relationship with HPV infection

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Abstract: Cervical cancer remains the fourth most common cause of cancer-related deaths in women worldwide, and human papillomavirus infection represents the most important risk factor for the development of cervical cancer. Minichromosome maintenance protein-2 has been previously identified by DNA microarray and transcriptional profiling as genes that is overexpressed in cervical carcinomas. 183 cases were enrolled and tested with thin prep liquid-based cytology test. The expressions of human papillomavirus were detected and minichromosome maintenance protein-2 immuncytochemical test was performed on liquid-based pap smears from the samples. Those results were compared with the cervical histopathology results. The positive expression rates of minichromosome maintenance protein-2 and high-risk type human papillomavirus increased with the severity of cervical lesions. The expression level of MCM2 was positively correlated with high-risk types of human papillomavirus. In cervical carcinoma and precancerous lesions, minichromosome maintenance protein-2 was overexpressed and positively correlated with the high risk types of human papillomavirus. As minichromosome maintenance protein-2 immuncytochemical detection was better than genotyping of human papillomavirus, minichromosome maintenance protein-2 may serve as a useful marker in the screening of cervical carcinoma and precancerous lesions and improve the diagnosis of atypical squamous cell of undetermined significance. The joint application can improve the sensitivity and specificity of diagnosis.

Keywords: Cervical lesions, minichromosome maintenance protein-2, human papillomavirus, immuncytochemical test

Introduction

Cervical cancer (CC) remains the fourth-most common cancer and is also the fourth cause of cancer-related deaths in women around the world, representing 9.8% of all female caners. In 2008, there was an estimation of 530,000 cases the whole year [1, 2]. High-risk regions of CC include Eastern and Western Africa, Southern Africa, South-Central Asia, South America and Middle Africa [2]. Recent clinical, epidemiological, and molecular research have revealed that the main etiological agent involving in the CC development is the persistent human papillomavirus (HPV) Infection [3].

HPV are naturally occurring DNA tumor viruses that induce epithelial cell proliferation during the course of a productive infection. Previous study has shown that infection with specific types of HPV is an essential step in the development of cervical lesion and invasive cancer. HPV DNA can be detected in more than 90% of invasive CC [4], even female who are HPV positive but without cervical lesion are at risk of progression of intraepithelial lesion (SIL) [5]. More than 100 different HPV types have been characterized and epidemiologic studies have divided HPVs into “low risk”, “high risk”, and uncharacterized types. CC is associated with high risk HPV types (mainly 16 and 18) [4].

HPV type detection has been extensively applied in clinic for its potential in the classification of patients having low-grade squamous cytologic abnormalities, which is in order to determine the sensitivity for underlying CC, such as cervical intraepithelial neoplasm (CIN)-

2/3 and squamous cell carcinoma (SCC) [6]). However, HPV detection has yet to be proven for the application for triage of patients with low grade-squamous intraepithelial lesion (LSIL) [7-9]. In the trial of ALTS, it was found that 83% of LSIL causes were positive for HPV type detection, whereas only 25% of the cases were expected to have CIN-2/3 on colposcopic biopsy [7]. Thus, other molecular diagnostic adjuncts are needed in order to improve the diagnosis sensitivity of low-grade cytologic abnormalities along with the HPV type detection.

Minichromsome maintenance protein-2 (MCM2) have been selected as promising candidate in detecting malignancy and premalignancy, which are essential for DNA replication in all eukaryotic cells and for the limiting replication in per cell cycle [10, 11]. Previous studies have revealed the overexpression of MCM-2 in CC by using DNA microarray and transcriptional profiling [12-14]. It was shown that such abnormal expression of MCM-2 can be exploited to improve detection of CIN and SCC [1, 15]. According to these studies, I conducted the test of the sensitivities of HPV type detection and MCM-2 immunocytochemical test in diagnosing the cervical lesions.

Materials and methods

Sample collection

183 patients from the left of thinprep liquid-based cytology test (TCT) were enrolled in the present study from Oct 2011 to Nov 2012 in the affiliated hospital of Inner Mongolia Medical University Hospital. The study was approved by the affiliated hospital of Inner Mongolia Medical University Hospital ethnics committee. The ethics committee approved the relating screening, inspection, and data collection of the patients, and all subjects signed a written informed consent form. All works were undertaken following the provisions of the Declaration of Helsinki. The average age of the patients was 43.2 (range from 19 to 69). The exclusion criteria included hysterectomy, cervical surgery, and pelvic radiotherapy. According to the histology diagnosis, there were 37 cases of SCC, 38 cases of CIN level III, 22 cases of CIN level 22, 29 cases of CIN level I, and 57 cases of chronic inflammation in all the samples according to the; according the diagnosis of cytology, there were 9 cases of squamous carcinoma of cervix, 39 cases of HSIL, 45 cases of LSIL, 7 cases of negative for intraepithelial lesion or malignancy (NILM), and 83 cases of atypical squamous cell of undetermined significance (ASCUS).

HPV type detection

The whole genome DNA was extracted using HPV GenoArray Diagnostic Kit (Hybribio, Guangzhou, China) according to the manufacture's protocol. The PCR process of the template DNA was carried out in PE9600 PCR system (MBI, USA). The HPV types were determined after flow-through hybridization (Hybribio, Guangzhou, China). According to the protocol of the Kit, every array could detect 13 "high risk" HPV types, including 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68.

Cell sampling and immunocytochemistry

Cells were prepared as monolayer samples by ThinPrep 2000 system. The immunocytochemistry process was conducted using SP (Streptavidin-Peroxidase) method on DAB (3, 3'-diaminobenzidine) stainer according to the manufacture's protocol of the kit (MXB, Fuzhou, China). I used a mixture of purified mouse monoclonal primary antibodies against MCM2. Assessment criteria were derived from preliminary immunocytochemical investigations, a sample was scored as MCM-2 expression positive if yellow (score 1), brownish yellow (score 2), or brown (score 3) particles were present in the cell nucleus. And the number of positive cells was recorded, and levels were defined as followings: ≤ 25% (score 1), 25-50% (score 2), 51-75% (score 3), > 75% (score 4). Two scores of the investigations were multiplied to determine the assessment level: < 3 (-), 3-4 (+), 4-5 (++), > 5 (+++).

Statistical analysis

All the data were expressed in the form of mean ± SD and Chi-square test were conducted using SPSS version 16.0 (IBM, Armonk, NY, USA) with significant level of 0.05.

Results

Expression of MCM2 in different cervical lesions

The expressions of MCM2 in different types of cervical lesion were shown in Supplementary Figure 1, including NILM, low-grade squamous

Table 1. Expression of MCM2 protein and high-risk HPV in different diagnosis of cytology and histology

Diagnosis type	Case (n)	MCM2 expression level					Detection of "High-risk" HPV type		
		-	+	++	+++	Positive rate (%)	-	+	Positive rate (%)
Cytology									
SCC	9	0	1	1	7	100a	0	9	100a
HSIL	39	2	6	6	25	94.87a	4	35	89.74a
SII	45	6	12	9	18	86.67a	2	43	95.5a
ASCUS	83	37	29	9	8	55.42b	36	47	56.63b
NILM	7	5	2	0	0	28.57b	7	0	0c
Histology									
SCC	37	1	3	4	29	97.30a	4	33	89.19ab
CIN III	38	1	9	11	17	97.37a	1	37	97.37a
CIN II	22	4	6	7	5	81.82b	6	16	72.73bc
CIN I	29	12	15	0	2	58.62bc	9	20	68.97c
Chronic Inflammation	57	32	17	3	5	43.86c	29	28	49.12c

Note: Positive rate (%) followed by the same footnote symbol(s) for each group are not significantly different at $P < 0.05$.

Table 2. Relationship between MCM2 and high-risk HPV

"High-risk" HPV	Case (n)	MCM2 expression level			
		-	+	++	+++
+	134	26	35	23	50
-	49	24	15	2	8
Total	183	50	50	25	58

Note: $\chi^2 = 15.28$, $P < 0.05$.

intraepithelial lesion (LSIL), ASCUS, high-grade squamous intraepithelial lesion (HSIL), and squamous cell carcinomas (SCC).

Expression of MCM2 and detection of HPV types in different diagnosis of cytology or histology

The top five frequently detected types of HPV in all the samples were 16, 58, 31, 85, and 33. The sensitivity of HPV genotyping and immunocytochemistry of MCM2 varied significantly among different classifications according to the diagnosis of cytology or histology (**Table 1**). In addition, the positive rate between HPV type detecting was in positive relationship with MCM2 (**Table 2**).

Concatenated application of HPV type detecting and immunocytochemistry of MCM2 in diagnosis of cervical lesions

The positive rate of the total samples using the concatenated application of the two methods was similar to that of immunocytochemistry of MCM2 but significantly higher than that of HPV type detecting (**Table 3**). Regarding special classifications, such as 84 cases of ASCUS, the positive rate of immunocytochemistry of MCM2, HPV type detecting, and concatenated application were 72.09%, 62.79%, and 86.05%, respectively, exhibiting the same pattern as for total samples.

Discussion

Application of HPV detection for cervical lesions has successfully reduced the morbidity and mortality of CC and HPV detection has been taken as a screening for CIN level of the cervical lesions, and in particular, where the strong association between the "high risk" HPV types can be used to improve the management of women with low-grade cytological abnormalities [5]. However, HPV is the etiologic agent of squamous and glandular carcinoma, "high risk" HPV testing has relatively limited specificity for underlying significant disease [16, 17]. These shortcomings in the test performance of HPV detection has required other alternative methods based on molecular markers, which are likely to offer better specificity than HPV detection. In the present study, I selected immunocytochemistry of MCM2 as a concatenated technique with HPV detection to improve the diagnostic sensitivity of different types of cervical lesions.

MCM2 are useful markers of cell-cycle entry, which are rich in the nucleus throughout the whole cell cycle and lost on cycle ending, with

Table 3. Comparison of CIN 2 and worse lesion screening

	Case (n)	MCM2 expression			Expression of "High risk" HPV type			MCM2 expression and "High risk" HPV type		
		+	-	Positive rate (%)	+	-	Positive rate (%)	+	-	Positive rate (%)
CIN II or Higher grade	97	91	6	93.81*	86	11	88.66#	95	2	97.94△
CIN I and chronic inflammation	86	42	44	48.84	48	38	55.81	64	22	74.42
Total	183	133	50	72.68	134	49	73.22	159	24	86.89

Note: *vs. #$\chi^2 = 1.61$, $P > 0.05$; *vs. △$\chi^2 = 2.09$, $P > 0.05$; #vs. △$\chi^2 = 6.68$, $P < 0.05$.

rapid loss from differentiating [11, 18, 19]. Immunocytochemical staining for MCMs allows discrimination between immunopositive abnormal cells and their immunonegative normal counterparts. In the present study, MCM2 was mainly detected in nucleus. With the increase in the severity of cervical lesions based on the classifications of cytodiagnosis, the immunocytochemistry of MCM2 strengthened (**Table 1**). The results were similar to that of previous study [20], which confirmed the consistency between immunocytochemistry of MCM2 and cytodiagnosis. Moreover, greater expression levels of MCM2 were detected in more serious cervical lesion types based on diagnosis of histology (**Table 1**), which was identical with the results of Ge et al [21]. Our results also revealed a similar pattern between the expression of MCM2 and detection of "high risk" HPV types (**Table 2**). It was reported that the replacement of the transcription factor E2F by HPV would lead to the abnormal expression of MCM2, which might result the high level of MCM2 in CC and CIN [22].

To maximize the probability of detecting individuals with cervical lesions, HPV type detection and immunocytochemistry of MCM2 were integrated used as a novel method for the diagnosis of cervical lesions. Previous finding has already indicated the high sensitivity using MCM2 as a marker in detecting CIN higher than II [23]. In the present study, the sensitivities of the three methods in detecting CIN higher than II were 93.81%, 88.66%, and 97.94% with the specificities of 8%, 77.55%, and 91.67%. Significantly greater sensitivity of immunocytochemistry of MCM2 compared with HPV type detection was noted. Although the difference between immunocytochemistry of MCM2 and the concatenated method was not significant, the latter one had the highest sensitivity and specificity among the three methods. The results showed that the exclusive application of HPV type detection or immunocytochemistry of MCM2 would both lead to the overlook of some cervical lesions (**Table 3**) while the integrated application of the two methods would give the best diagnosis.

In conclusion, great sensitivity of immunocytochemistry of MCM2 in detect cervical lesions was detected in the present study. However, either immunocytochemistry of MCM2 or HPV type detection could not cover all the patients with cervical lesions. Our results showed that in the detection of cervical lesion, MCM-2 immunocytochemical test was more effective than HPV type detection. And the concatenated application of the two techniques could significantly improve the sensitivity and specificity of the diagnosis. I expected our study could provide an effective and economic method in detecting cervical lesion for developing countries, in which CC is more popular. However, investigation should be further conducted in the future to improve the procedure of the concatenated method and the application in clinic.

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Disclosure of conflict of interest

None.

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References

[1] Santin AD, Zhan FH, Bignotti E, Siegel ER, Cané S, Bellone S, Palmieri M, Anfossi S, Thomas M, Burnett A, Kay HH, Roman JJ, O' Brien TJ, Tian E, Cannon MJ, Shaughnessy J Jr, Pecorelli S. Gene expression profiles of primary HPV16- and HPV18-infected early stage cervical cancers and normal cervical epithelium: identification of novel candidate molecular markers for cervical cancer diagnosis and therapy. Virology 2005; 331: 269-91.

[2] Pedroza-Torres A, López-Urrutia E, García-Castillo V, Jacobo-Herrera N, Herrera LA, Peralta-Zaragoza O, López-Camarillo C, Cantú De Leon D, Fernández-Retana J, Cerna-Cortés JF, Pérez-Plasencia C. MicroRNAs in cervical cancer: evidences for a miRNA profile deregulated by HPV and its impact on radio-resistance. Molecules 2014; 19: 6263-81.

[3] Hausen ZH. Papillomaviruses and cancer: From basic studies to clinical application. Nat Rev Cancer 2002; 2: 342-50.

[4] Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, Snijders PJ, Peto J, Meijer CJ, Muñoz N. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 1999; 89: 12-9.

[5] Hwang TS, Jeong JK, Park M, Han HS, Choi HK, Park TS. Detection and typing of HPV genotypes in various cervical lesions by HPV oligonucleotide microarray. Gynecol Oncol 2003; 90: 51-6.

[6] Shroyer KR, Homer P, Heinz D, Singh M. Validation of a novel immunocytochemical assay for topoisomerase II-a and minichromosome maintenance protein 2 expression in cervical cytology. Cancer Cytopathol 2006; 108: 324-330.

[7] Human papillomavirus testing for triage of women with cytologic evidence of low-grade squamous intraepithelial lesions: baseline data from a randomized trial. The Atypical Squamous Cells of Undetermined Significance/Low-Grade Squamous Intraepithelial Lesions Triage Study (ALTS) Group. J Natl Cancer Inst 2000; 92: 397-402.

[8] Herbst AL, Pickett KE, Folleen M, Noller KL. The management of ASCUS cervical cytologic abnormalities and HPV testing: a cautionary note. Obstet Gynecol 2001; 98: 849-51.

[9] Solomon D, Schiffman M, Tarone R; ALTS Study Group. Comparison of three management strategies for patients with atypical squamous cells of undetermined significance: baseline results from a randomized trial. J Natl Cancer Inst 2001; 93: 293-9.

[10] Kearsey SE, Labib K. MCM proteins: evolution, properties, and role in DNA replication. Biochim Biophys Acta 1998; 1398: 113-36.

[11] Gonzalez MA, Tachibana KE, Laskey RA, Coleman N. Control of DNA replication and its potential clinical exploitation. Nat Rev Cancer 2005; 5: 135-41.

[12] Murphy N, Ring M, Heffron CCBB, King B, Killaea AG, Hughes C, Martin CM, McGuinness E, Sheils O, O'Leary JJ. p16INK4a, CDC6, and MCM5: predictive biomarkers in cervical preinvasive neoplasia and cervical cancer. J Clin Pathol 2005; 58: 525-34.

[13] Chen Y, Miller C, Mosher R, Zhao XM, Deeds J, Morrissey M, Bryant B, Yang D, Meyer R, Cronin F, Gostout BS, Smith-McCune K, Schlegel R. Identification of cervical cancer biomarkers by cDNA and tissue microarrays. Cancer Res 2003; 63: 1927-35.

[14] Williams GH, Romanowski P, Morris L, Madine M, Millis AD, Stoeber K, Marr J, Laskey RA, Coleman N. Improved cervical smear assessment using antibodies against proteins that regulate DNA replication. Proc Natl Acad Sci U S A 1998; 95: 14932-7.

[15] Mukherjee G, Muralidhar B, Bafna UD, Laskey RA, Coleman N. MCM immunocytochemistry as a first line cervical screening test in developing countries: a prospective cohort study in a regional cancer centre in India. Br J Cancer 2007; 96: 1107-1111.

[16] Arbyn M, Paraskevaidis E, Martin-Hirsch P, Prendiville W, Dillner J. Clinical utility of HPV-DNA detection: triage of minor cervical lesions, follow-up of women treated for high-grade CIN: an update of pooled evidence. Gynecol Oncol 2005; 99: S7-S11.

[17] Andersson S, Dillner L, Elfgren K, Mints M, Persson M, Rylander E. A comparison of the human papillomavirus test and Papanicolaou smear as a second screening method for women with minor cytological abnormalities. Acta Obstet Gynecol Scand 2005; 84: 996-1000.

[18] Kang YH, Galal WC, Farina A, Tappin I, Hurwitz J. Properties of the human Cdc45/Mcm2-7/GINS helicase complex and its action with DNA polymerase epsilon in rolling circle DNA synthesis. Proc Natl Acad Sci USA 2012; 109: 6042-7.

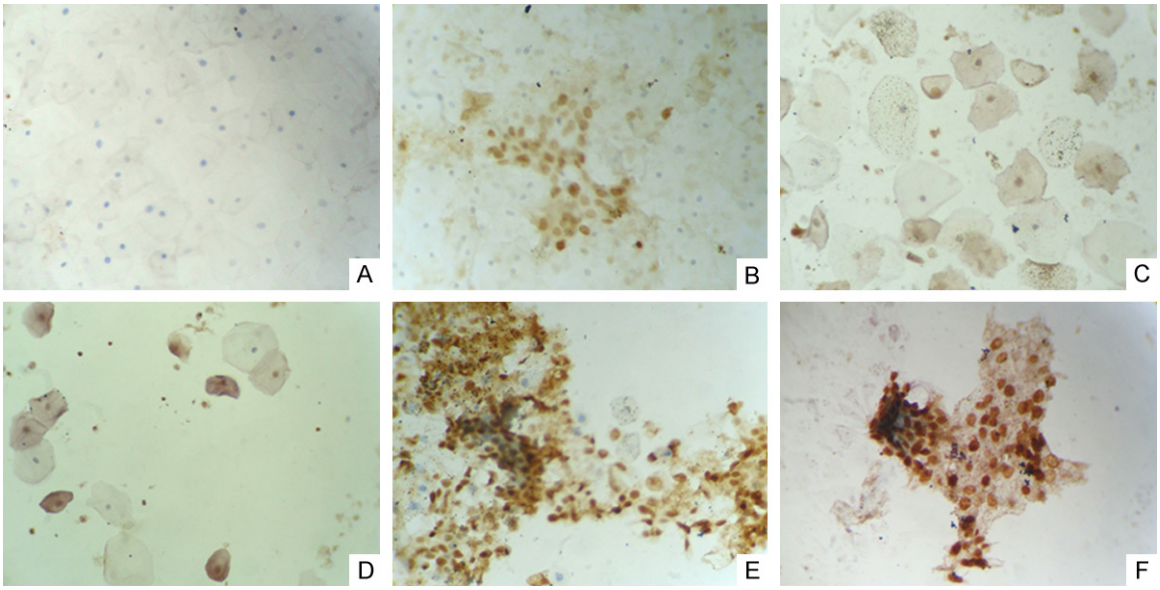
[19] Rusiniak ME, Kunnev D, Freeland A, Cady GK, Pruitt SC. Mcm2 deficiency results in short deletions allowing high resolution identification of genes contributing to lymphoblasticlymphoma. Oncogene 2012; 31: 4034-44.

[20] Conesa-Zamora P, Doménech-Peris A, Orantes-Casado FJ, Ortiz-Reina S, Sahuquillo-Frías L, Acosta-Ortega J, García-Solano J, Pérez-Guillermo M. Effect of human papillomavirus on cell cycle-related proteins p16, Ki-67, Cyclin D1, p53, and ProEx C in precursor lesions of cervical carcinoma: a tissue microarray study. Am J Clin Pathol 2009; 132: 378-90.

[21] Ge Y, Mody DR, Smith D, Anton R. p16(INK4a) and ProEx C immunostains facilitate differential diagnosis of hyperchromatic crowded groups in liquid-based Papanicolaou tests with menstrual contamination. Acta Cytol 2012; 56: 55-61.

[22] Halloush RA, Akpolat I, Zhai QJ, Schwartz MR, Mody DR. Comparison of ProEx C with p16INK4a and Ki-67 immunohistochemical staining of cell blocks prepared from residual liquid-based cervicovaginal material: a pilot study. Cancer 2008; 14: 474-80.

[23] Kelly D, Kincaid E, Fansler Z, Rosenthal DL, Clark DP. Detection of cervical high-grade squamous intraepithelial lesions from cytologic samples using a novel immunocytochemical assay (ProEx C). Cancer 2006; 108: 494-500.



Supplementary Figure 1. MCM2 protein immunocytochemical staining in different cervical lesion cells (SP ×200), positive staining in the nucleus: A: MCMC expression in control with pathology of chronic inflammation (negative); B: MCM2 expression in LSIL with pathology of chronic inflammation (positive); C: MCM2 expression in ASCUS with pathology of CIN I (positive); D: MCM2 expression ASCUS with pathology of CIN II (positive); E: MCM2 expression in HSIL with pathology of CIN III (positive); F: MCM2 expression in squamous carcinoma of cervix with pathology of SCC (positive).