Original Article
The expressions of DNA methyltransferase 1 (DNMT1) and cyclin A1 (CCNA1) in cervical carcinogenesis

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Abstract: Objectives: The purpose of the present study was to investigate the expressions of DNA methyltransferase 1 (DNMT1) and cyclin A1 (CCNA1) protein and mRNA in the process of cervical carcinogenesis. Methods: The specimens were separated into the following groups: control (n=30), low-grade squamous intraepithelial lesions (LSIL, n=30), high-grade squamous intraepithelial lesions (HSIL) and squamous cell cervical cancers (SCC, n=30). Immunohistochemical examination, Western blot and real-time quantitative PCR were used to investigate the protein and mRNA expressions of DNMT1 and CCNA1 in cervical tissues. Results: We found that the positive expression rate and intensity of DNMT1 mRNA and protein gradually increased in the process of cervical carcinogenesis. However, the increase of the positive expression rate of DNMT1 mRNA and protein form the LSIL group to HSIL group was not significant (*P*>0.05). After cervical intraepithelial neoplasia (CIN), the positive expression rate and intensity of CCNA1 mRNA and protein significantly decreased with the aggravation of cervical lesions (*P*<0.05), and there was no significant difference between the LSIL and control groups (*P*>0.05). Conclusions: With the severity of cervical lesions, the expression of DNMT1 protein and mRNA increased gradually. The expression of CCNA1 protein and mRNA decreased gradually. The DNMT1 and CCNA1 expressions are associated with cervical lesions.

Keywords: DNA methyltransferase 1, cyclin A1, cervical cancer, cervical intraepithelial neoplasia

Introduction

Cervical cancer is a common malignant tumor of the female reproductive system with high morbidity and mortality [1]. Epidemiological studies have shown that the risk factors for cervical cancer include high-risk, human papillomavirus (HPV), HIV infection, premature sexual age, multiple sexual partners, and smoking [2-4]. The pathogenesis of cervical cancer is not caused by a single factor, but a complex process involving multiple genes, factors and steps. In any case, cervical cancer evolves from cervical intraepithelial neoplasia [5, 6]. This pathological process involves gene mutation, oncogene activation, and epigenetic changes in a tumor suppressor gene. Moreover, epigenetic alterations play an important role in all stages of cervical carcinogenesis [7].

DNA methylation is one of the most important epigenetic modification mechanisms and also one of the key mechanisms of tumor suppressor gene inactivation and transcriptional inhibition [8]. DNA methylation is a process by which methyl groups are added to the DNA molecule, which leads to changing the activity of a DNA segment without changing the sequence. DNA methyltransferase (DNMT), including DNMT3a, DNMT3b, and DNMT1, plays an important role in the maintenance and regulation of DNA methylation [8]. DNMT1 is the most important one. Abnormal expression of DNMT1 can cause aberrant methylation of some tumor suppressor genes' CpG islands, further resulting in making the tumor suppressor gene inactive and cell carcinogenesis [9]. Highly expressed DNMT1 has not only been detected in a variety of tumor cells, but has also been found to appear earlier than DNA Methylation [10].

Cyclin A1 (CCNA1) can regulate the synthesis and replication of DNA and promote cells from the interphase to the division phase. The CCNA1 gene is a kind of tumor suppressor gene, which is closely related to tumor occur-

rence, development, invasion and distant metastasis [11]. It is a potential tumor marker. When CCNA1 is abnormally expressed, incompletely synthesized, or mismatched, DNA without repair can enter the cell, resulting in abnormal cell proliferation [12]. CCNA1 is one of the highly conserved cyclin members and possesses obvious tissue specificity. Abnormal expression of CCNA1 is found in breast cancer, acute myeloid leukemia, ovarian cancer, prostate cancer, and esophageal squamous cell carcinoma [13, 14].

With respect to cervical carcinogenesis, an increased expression of DNMT1 protein has been observed during multistage cervical carcinogenesis [15], and high level methylation of the CCNA1 gene has also been reported in cervical cancer [16]. However, studies about the simultaneous expression of DNMT1 and CCNA1 in the process of cervical carcinogenesis are rare. In our study, we detected the mRNA and protein expressions of DNMT1 and CCNA1 in multistage cervical carcinogenesis to explore the relationship between DNMT1/CCNA1 and cervical carcinogenesis.

Materials and methods

Patients

The study was approved and registered by the ethics committee of the Affiliated Hospital of North China University of Science and Technology. In 2016, the ethics committee approved related screening, and data collection of these subjects, and all subjects signed the written informed consent form. All experiments were undertaken following the provisions of the Declaration of Helsinki.

From November 2016 to October 2017, 30 cases of squamous cell cervical cancers (SCC), 30 cases of high-grade squamous intraepithelial lesions (HSIL), and 30 cases of low-grade squamous intraepithelial lesions (LSIL) were recruited as case groups. 30 cases without SCC, HSIL, and LSIL, who underwent a colposcopy biopsy or a hysterectomy for a non-malignant condition, were recruited as a control group. None of the cases had any liver, kidney, or immune system diseases, nor had any received radiotherapy and chemotherapy. The cervical tissue was obtained from operations and stored at -80°C for further study.

Immunohistochemistry analysis

Briefly, the sections (3 mm × 3 mm × 3 mm) of formalin-fixed and paraffin-embedded tissue specimens were deparaffinized and dehydrated. For antigen retrieval, the sections were heated for 5 min at 95°C. Non-specific reactions were blocked by incubating the samples with 2% normal swine serum for 20 minutes at room temperature. All sections were incubated with a rabbit anti human DNMT1 antibody (Affinity BioReagent, Golden, CO, USA; dilution 1:50) or a rabbit anti human CCNA1 antibody (Affinity BioReagent, Golden, CO, USA; dilution 1:100) at 4°C overnight, followed by incubation with biotinylated goat anti-rabbit IgG secondary antibodies (ZSGB-BIO, Beijing, China) at room temperature for 30 min. The staining was performed with a DAB Staining Kit (ZSGB-BIO, Beijing, China) according to the protocol of the manufacturer, and visualized by using a TS100 inverted phase contrast microscope (Nikon, Kanagawa, Japan). The DNMT1 and CCNA1 positive tissues were analyzed by Image-Pro-Plus 4.5 software (Media Cybernetics, Silver Spring, USA).

The immunohistochemical assessment was performed following a previous study with minor revision [15]. The standard for the interpretation of immunohistochemical results is that the DNMT1 and CCNA1 proteins were positive for brown and yellow granules in the nucleus. In each sample, the intensity of the DNMT1 (or CCNA1) nuclear immunoreactivity was graded as 0 (no color), 1 (pale yellow), 2 (brown) and 3 (brown yellow), and at least 500 cells were randomly counted. The incidence of DNMT1 (or CCNA1) nuclear immunoreactivity was graded as 0 (less than 5% of the counted cells), 1 (5% or more, and less than 25%), 2 (25% or more, and less than 50%), 3 (50% or more, and less than 75%) and 4 (75% or more). The protein expression score (0-12) was determined as the product of the intensity grade (0, 1, 2, or 3) and the incidence grade (0, 1, 2, 3, or 4). The scoring method is 0 (negative, -), 1-4 (weak positive, +), 5-8 (moderate positive, ++) and 9-12 (strong positive, +++).

Western blot analysis

The nuclear protein was extracted from cervical tissue using a Nuclear and Cytoplasmic Protein Extraction Kit (Wanleibio, Shenyang, China), and quantified using a BCA Protein Assay Kit (ThermoFisher Scientific, Rockford, IL, USA).
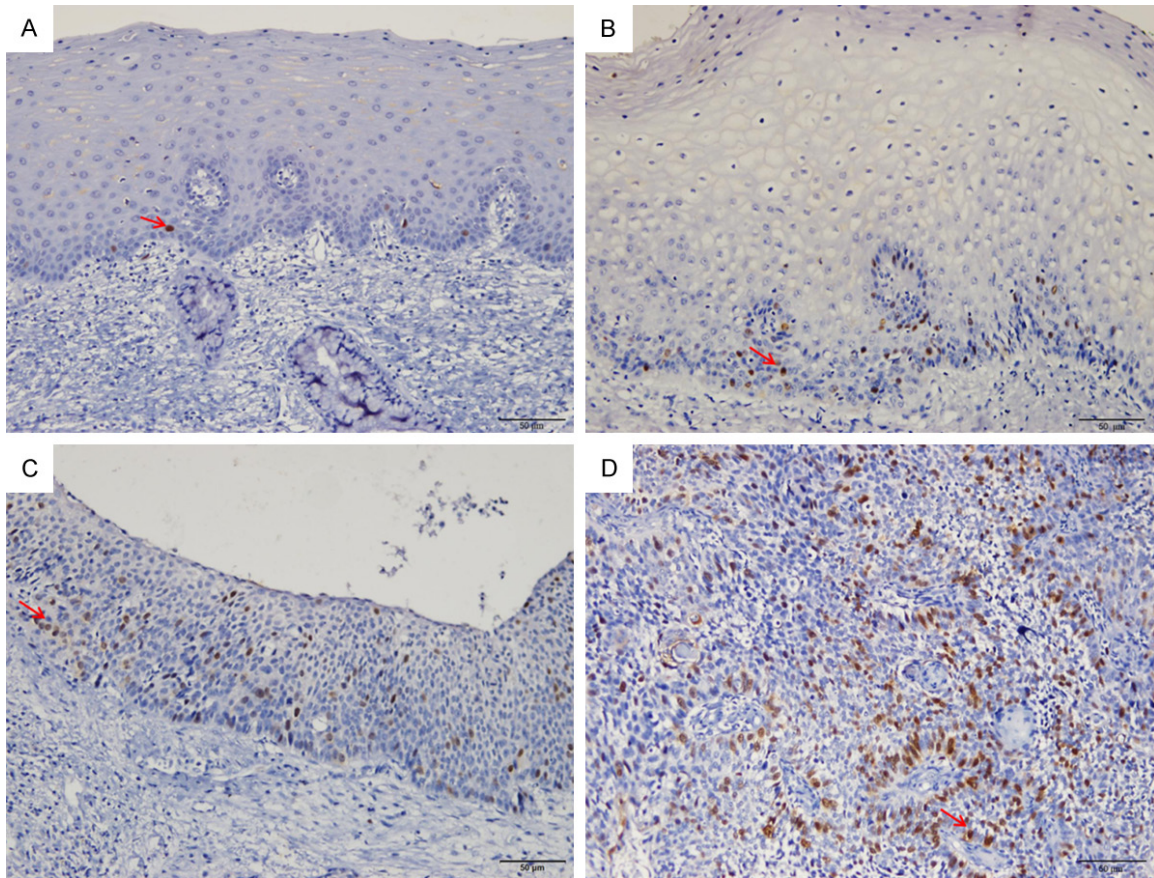
Figure 1. Immunohistochemical examination of the DNMT1 protein in the control group (A), LSIL group (B), HSIL group (C), and SCC group (D). The DNMT1 protein was positive for brown and yellow granules in the nucleus (red arrows, 200 ×).

Equal amounts of proteins (100 μg per lane) were separated by 10% SDS-PAGE gels, and transferred to polyvinylidene fluoride (PVDF) membrane by a semi-dry transfer apparatus (Bio-Rad, CA, USA). The PVDF membrane was blocked by 5% skim milk for 1 h at room temperature. Primary rabbit anti DNMT1 antibody (dilution 1:1000) (or rabbit anti CCNA1 antibody, dilution 1:1000; or rabbit anti GAPDH antibody, dilution 1:2000) was added to blocking buffer at 4°C overnight. Next, the PVDF membrane was washed three times in 1 × TTBS (Tween/Tris-buffered salt solution) for 10 min on a shaker. The blot was then incubated with goat anti-rabbit IgG-HRP secondary antibodies (dilution 1:5000, ZSGB-BIO, Beijing, China) for 1 h at room temperature, and was washed in 1 × TTBS for three times. Finally, the proteins were visualized by Super Signal ECL (Applygen Technologic Inc, Beijing, China), and the signals were quantified using Image Lab5.0 (Bio-Rad, CA, USA). The protein levels of GAPDH were assayed as an internal in all samples.

RNA isolation and real-time quantitative PCR (qPCR)

RNA was isolated form cervical tissue using a TRIzol reagent (ThermoFisher Scientific, Rockford IL, USA). The RNA was reversely transcribed to cDNA using a RevertAid First Strand cDNA Synthesis Kit (ThermoFisher Scientific). In all samples, the RNA levels of GAPDH were assayed as an internal. The following primers were used: DNMT1-forward: 5'-GGA GGG CTA CCT GGC TAA AG-3'; DNMT1-reverse: 5'-CTC TCC ATC GGA CTT GCT CC-3'; CCNA1-forward: 5'-GCA CTT GCC AGT TGT TCC G-3'; CCNA1-reverse: 5'-TCC AGG GTA CAT GAT TGC GG-3'; GAPDH-forward: 5'-GGA AAT GAA TGG GCA GCC GT-3'; GAPDH-reverse: 5'-AGG AGA AAT CGG GCC AGC TA-3'.

The PCR reaction and amplification systems were done using a PCR kit (Invitrigen, USA). The PCR conditions were: denature the DNA at 95°C for 2 min, followed by denaturing at 95°C
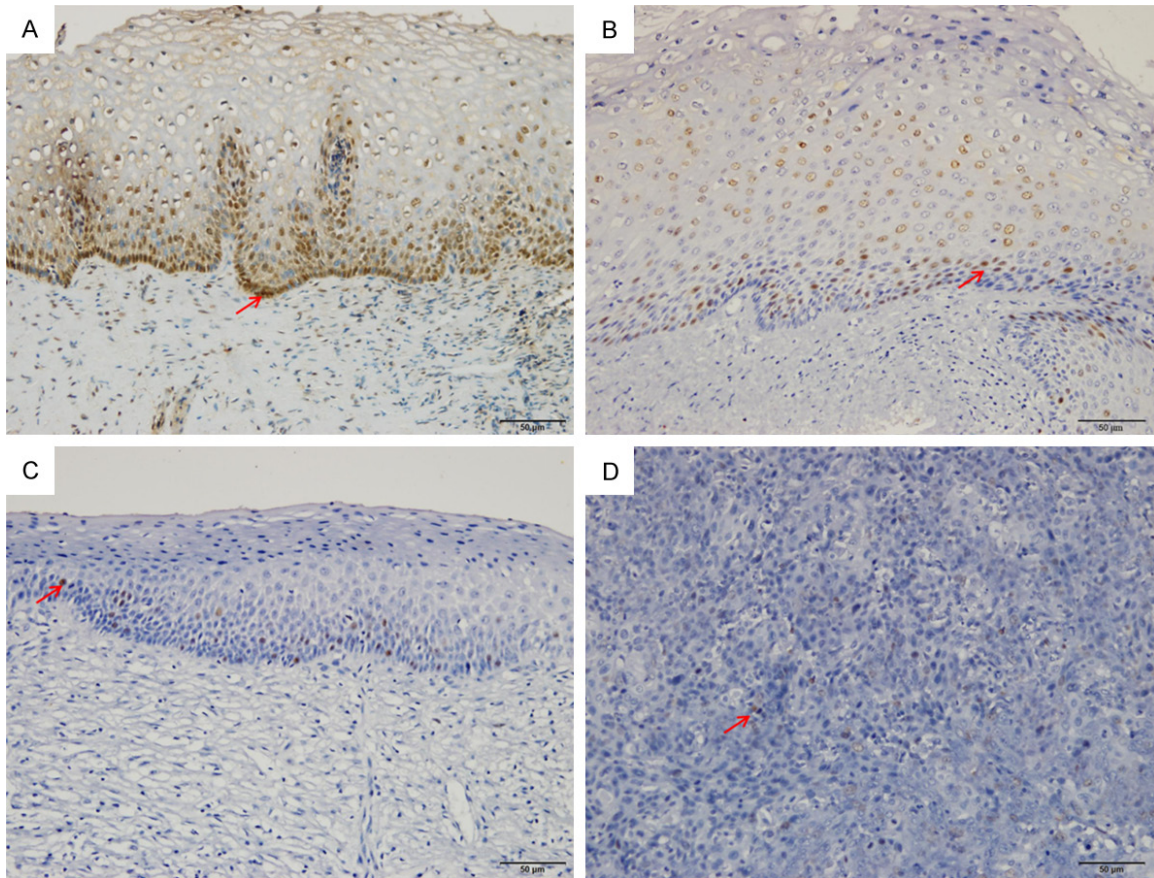
Figure 2. Immunohistochemical examination of the CCNA1 protein in the control group (A), LSIL group (B), HSIL group (C), and SCC group (D). The CCNA1 protein was positive for brown and yellow granules in the nucleus (red arrows, 200 ×).

Table 1. Immunohistochemical examination for the DNMT1 protein in cervical tissues of the control, LSIL, HSIL, and SCC groups

Groups	n	Number of specimens				P^e
		-	+	++	+++	
Control	30	23	5	2	0	
LSIL	30	14	6	7	3	0.008[a,b]
						0.025[b,c]
HSIL	30	7	6	8	9	0.000[a,c]
						0.045[c,d]
SCC	30	1	9	3	17	0.000[a,d]
						0.000[b,d]

Note: a, control; b, LSIL; c, HSIL; d, SCC; e, nonparametric test, $P<0.05$.

Table 2. Immunohistochemical examination for CCNA1 protein in cervical tissues of the control, LSIL, HSIL and SCC groups

Groups	n	Number of specimens				P^e
		-	+	++	+++	
Control	30	0	0	4	26	
LSIL	30	3	1	5	21	0.088[a,b]
						0.000[b,c]
HSIL	30	10	4	5	11	0.000[a,c]
						0.001[c,d]
SCC	30	22	3	3	2	0.000[a,d]
						0.000[b,d]

Note: a, control; b, LSIL; c, HSIL; d, SCC; e, nonparametric test, $P<0.05$.

for 15 s, then annealing at 60°C for 30 s, and extension at 95°C for 30 s with 40 cycles using only DNMT1 or GAPDH primers, then with another 45 cycles after the addition of CCNA1 primers.

Statistical analysis

SPSS 22.0 statistical software (IBM, Chicago, IL) was used to analyze all the data. A nonparametric test was used to analyze the results of
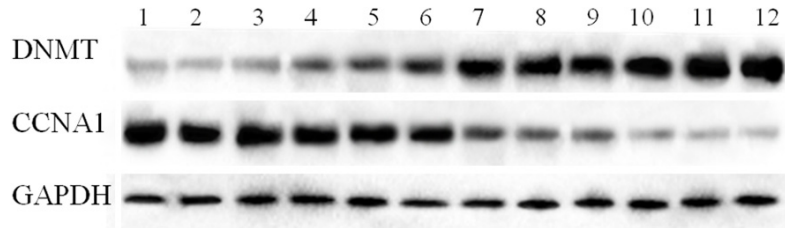
Figure 3. The expression of the DNMT1 and CCNA1 proteins in cervical tissues in the control group (1, 2, 3), LSIL group (4, 5, 6), HSIL group (7, 8, 9), and SCC group (10, 11, 12).

Table 3. The positive expression rate of DNMT1 protein in cervical tissues of the control, LSIL, HSIL, and SCC groups

Groups	n	Positive rate	P^e
Control	30	4 (13.33%)	
LSIL	30	11 (36.67%)	$0.037^{a,b}$
			$0.121^{b,c}$
HSIL	30	17 (56.67%)	$0.000^{a,c}$
			$0.426^{c,d}$
SCC	30	20 (66.67%)	$0.000^{a,d}$
			$0.020^{b,d}$
Amount	120	52 (43.33%)	$0.000^{a,b,c,d}$

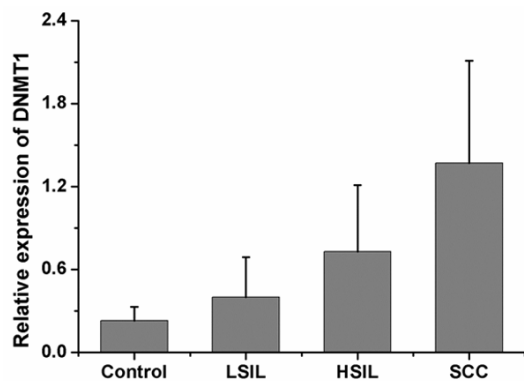Note: a, control; b, LSIL; c, HSIL; d, SCC; e, chi-square test, $P<0.05$.

Figure 4. The relative expression of the DNMT1 protein (ratio of optical density of DNMT1 to GAPDH) in the control, LSIL, HSIL, and SCC groups. Control group, n=4; LSIL group, n=11; HSIL group, n=17; SCC group, n=20. Student-Newman-Keuls (SNK) test: compared with control and LSIL groups, $P<0.05$; compared with LSIL and HSIL groups, $P<0.05$; compared with HSIL and SCC groups, $P<0.05$.

the immunohistochemistry. The relative expression quantity of protein and mRNA are shown as the mean ± SD. The chi-square and Student-Newman-Keuls (SNK) tests were used for statistical analysis.

Result

The expressions of the DNMT1 and CCNA1 proteins in the process of cervical carcinogenesis

The immunohistochemical staining for the DNMT1 and CCNA1 proteins in the tissue samples are shown in **Figures 1** and **2**, respectively. The intensity and incidence of the DNMT1 protein increased with the process of cervical carcinogenesis, but those of the CCNA1 protein decreased. According to a nonparametric test, the positive expressions of the DNMT1 and CCNA1 proteins in each sample group are also listed in **Tables 1** and **2**, respectively. The number of positive DNMT1 protein expressions increased progressively from the control group to the LSIL, HSIL, and SCC groups (**Table 1**). After cervical intraepithelial neoplasia (CIN), the number of positive DNMT1 protein expressions increased and peaked in the SCC group. There was a significant increase in the amount of positive DNMT1 protein expression from the control group to the LSIL group ($P=0.008$), the LSIL group to the HSIL group ($P=0.025$), and the HSIL group to the SCC group ($P=0.045$). Conversely, the amount of positive CCNA1 protein expression was reduced in process of cervical carcinogenesis (**Table 2**). Notably, there was no significant decrease in the amount of positive CCNA1 protein expression from the control group to the LSIL group ($P=0.088$), and a significant decrease from the LSIL group to the HSIL group ($P=0.000$), and the HSIL group to the SCC group ($P=0.001$).

The semi-quantitative Western blot method was also used to test the expressions of the DNMT1 and CCNA1 proteins in the process of cervical carcinogenesis (**Figure 3**). A chi-square test was used to compare the positive expression rate of the DNMT1 protein in the different groups (**Table 3**). The positive expression rate of DNMT1 in the SCC, HSIL, LSIL, and control groups was 66.67%, 56.67%, 36.67%, and 13.33%, respectively. The positive expression rate in the case (SCC, HSIL and LSIL) groups was significantly higher than it was in the control group ($P<0.05$), and the expression in the SCC group was significantly higher than it was

Table 4. The positive expression rates of CCNA1 in cervical tissues of the control, LSIL, HSIL, and SCC groups

Groups	n	Positive rate	P^e
Control	30	30 (100.00%)	
LSIL	30	27 (90.00%)	0.236[a,b]
			0.015[b,c]
HSIL	30	19 (63.33%)	0.000[a,c]
			0.001[c,d]
SCC	30	6 (20.00%)	0.000[a,d]
			0.000[b,d]
Amount	120	82 (68.33%)	0.000[a,b,c,d]

Note: a, control; b, LSIL; c, HSIL; d, SCC; e, chi-square test, $P<0.05$.

Table 5. The positive expression rates of DNMT1 mRNA in cervical tissues of the control, LSIL, HSIL, and SCC groups

Groups	n	Positive rate	P^e
Control	30	5 (16.67%)	
LSIL	30	13 (43.33%)	0.024[a,b]
			0.121[b,c]
HSIL	30	19 (63.33%)	0.000[a,c]
			0.405[c,d]
SCC	30	22 (73.33%)	0.000[a,d]
			0.018[b,d]
Amount	120	59 (49.17%)	0.000[a,b,c,d]

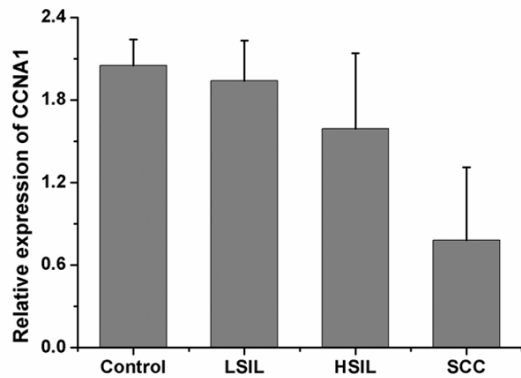Note: a, control; b, LSIL; c, HSIL; d, SCC; e, chi-square test, $P<0.05$.

Figure 5. The relative expression of the CCNA1 protein (ratio of optical density of CCNA1 to GAPDH) in the control, LSIL, HSIL and SCC groups. Control group, n=30; LSIL group, n=27; HSIL group, n=19; SCC group, n=6. Student-Newman-Keuls (SNK) test: compared with the control and LSIL groups, P=0.35; compared with LSIL and HSIL groups, P<0.05; compared with HSIL and SCC groups, P<0.05.
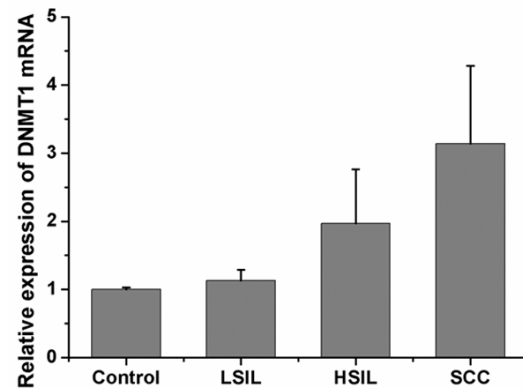

Figure 7. The relative expression of DNMT1 mRNA in the control, LSIL, HSIL, and SCC groups. Control group, n=5; LSIL group, n=13; HSIL group, n=19; SCC group, n=22. The relative expression of DNMT1 mRNA in the control group was 1, and the relative expression of DNMT1 mRNA in other group was the ratio of Ct of the DNMT1 gene to Ct of GAPDH gene. Student-Newman-Keuls (SNK) test: compared with the control and LSIL groups, P<0.05; compared with the LSIL and HSIL groups, P<0.05; compared with the HSIL and SCC groups, P<0.05.
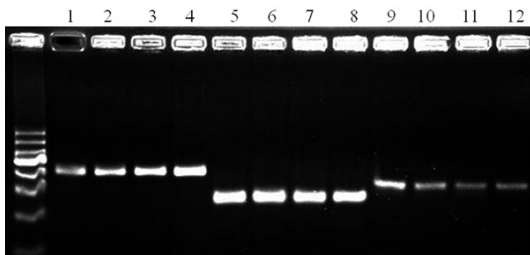

Figure 6. Identification of qPCR products by agarose gel electrophoresis. Target gene: DNMT1 (1, 2, 3, 4), GAPDH (5, 6, 7, 8) and CCNA1 (9, 10, 11, 12); Groups: control (1, 5, 9), LSIL (2, 6, 10), HSIL (3, 7, 11) and SCC (4, 8, 12).

in the LSIL group (P<0.05). This result indicates that the risk of cervical lesions gradually increases with an increasing positive expression of the DNMT1 protein. The relative expression level of the DNMT1 protein was the strongest in the SCC (1.37±0.74) group, followed by the HSIL (0.73±0.48), LSIL (0.40±0.29), and control (0.23±0.10) groups, as shown in **Figure 4**. With the aggravation of cervical lesions, the expression of the DNMT1 protein gradually increased, showing a significant upward trend (P<0.05).

As shown in **Table 4**, the positive rate of the CCNA1 protein in the SCC (20.00%) group was also obviously lower than in the HSIL (63.33%),

Table 6. The positive expression rates of CCNA1 mRNA in cervical tissues of the control, LSIL, HSIL, and SCC groups

Groups	n	Positive rate	P^e
Control	30	30 (100.00%)	
LSIL	30	28 (93.33%)	$0.472^{a,b}$
			$0.005^{b,c}$
HSIL	30	19 (63.33%)	$0.000^{a,c}$
			$0.002^{c,d}$
SCC	30	7 (23.33%)	$0.000^{a,d}$
			$0.000^{b,d}$
Amount	120	84 (70.00%)	$0.000^{a,b,c,d}$

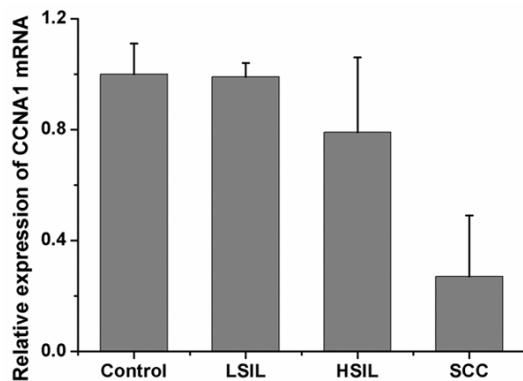Note: a, control; b, LSIL; c, HSIL; d, SCC; e, chi-square test, $P<0.05$.

Figure 8. The relative expression of CCNA1 mRNA in the control, LSIL, HSIL and SCC groups. Control group, n=30; LSIL group, n=28; HSIL group, n=19; SCC group, n=7. The relative expression of CCNA1 mRNA in the control group was 1, and the relative expression of CCNA1 mRNA in the other groups was the ratio of the Ct of the CCNA1 gene to the Ct of the GAPDH gene. Student-Newman-Keuls (SNK) test: compared with the control and LSIL groups, $P=0.65$; compared with the LSIL and HSIL groups, $P<0.05$; compared with the HSIL and SCC groups, $P<0.05$.

LSIL (90.00%), and control (100.00%) groups ($P<0.05$). Similarly, there was no significant difference in the positive rate of CCNA1 protein between the LSIL and control groups ($P=0.236$). Similar to the immunohistochemical result, the relative expression level of the CCNA1 protein gradually decreased with the aggravation of cervical lesions (**Figure 5**). The relative expression of the CCNA1 protein in the SCC (0.78±0.53) group was obviously lower than HSIL (1.59±0.55), LSIL (1.94±0.29), and control (2.05±0.19) groups ($P<0.05$). However, there was no significant difference in the relative expression of the CCNA1 protein between the LSIL and control groups ($P=0.35$).

The expressions of DNMT1 and CCNA1 mRNA in the process of cervical carcinogenesis

The expressions of DNMT1 and CCNA1 mRNA in the process of cervical carcinogenesis was measured by qPCR (**Figure 6**). The amplification product was single and had a strong specificity. As shown in **Table 5**, the positive rates of DNMT1 mRNA in the SCC, HSIL, and LSIL groups were 73.33%, 63.33% and 43.33%, respectively, which were significantly higher than they were in the control group (16.67%) ($P<0.05$). The DNMT1 mRNA in the SCC group was obviously higher than it was in the LSIL group ($P=0.018$), but not significantly higher than it was in the HSIL group ($P=0.405$). As shown in **Figure 7**, the relative expression of DNMT1 mRNA significantly increased with the aggravation of cervical lesions ($P<0.05$). This result agreed with the immunohistochemistry and Western blot results of the DNMT1 protein.

On the contrary, the positive expression rates of the CCNA1 mRNA were 23.33%, 63.33%, 93.33%, and 100.00% in the SCC, HSIL, LSIL, and control groups (**Table 6**), respectively. The CCNA1 mRNA in the SCC and HSIL groups was significantly lower than it was in the LSIL and control groups ($P<0.05$). The relative expression of CCNA1 mRNA was reduced in the process of cervical carcinogenesis (**Figure 8**). There was no significant decrease in the relative expression of CCNA1 mRNA from the control group to the LSIL group ($P=0.65$), and a significant decrease from the LSIL group to the HSIL group ($P<0.05$), and the HSIL group to the SCC group ($P<0.05$).

Discussion

DNA methylation is catalyzed by DNMT, which adds methyl to the 5'-position of cytosine within the CpG dinucleotide and regulates gene expression in cells [17, 18]. Abnormal methylation of the promoter islands of the tumor suppressors and carcinogenic genes is an important cause of tumorigenesis. Abnormal DNA methylation is ubiquitous in tumor cells, which can participate in the occurrence, development, and metastasis of tumors and play an important role in regulating gene expression, gene imprinting, gene mutation, tumor suppressor gene inactivation, cell proliferation, and differentiation [19]. Epigenetic changes in tumor suppressor genes are necessary in the progression of CIN to cervical cancer [7], and

widespread genomic DNA methylation is present in cervical cancer [20]. Human papillomavirus DNA methylation has been used as a biomarker for cervical precancer [21]. DNMT1 is the major human DNMT.

In a previous study [15], the DNMT1 protein expression score was increased in low-grade CIN in comparison with a normal squamous epithelium; it was further increased in higher-grade CIN compared to low-grade CIN; it remained at a plateau in microinvasive carcinoma (Stage IA) compared to higher-grade CIN, then it decreased with cancer invasion compared to Stage IA. In addition, the expression of the DNMT1 protein in cervical cancer was significantly higher than it was in a normal cervical epithelium, and the high positive expression rate and intensity score for the DNMT1 protein were significantly associated with a poor survival outcome [22]. In the present study, the positive expression rate and intensity of the DNMT1 mRNA and protein gradually increased in the process of cervical carcinogenesis. But, the increase of the positive expression rate of the DNMT1 mRNA and protein form the LSIL group to the HSIL group was not significant. This result demonstrated that the high expression of DNMT1 was related to the occurrence of cervical cancer, especially in the early stages of cervical precancerous lesions. Recently, it has been found that the use of the histone deacetylase inhibitor apicidin can down-regulate the expression of DNMT1 in cervical cancer cells, inhibit the cell cycle, and promote cell apoptosis [23]. Therefore, the degree of DNMT1 expression can be considered a potential target for the treatment of cervical cancer.

CCNA1 plays an important role in enabling cells to pass the S and G2/M phases successfully, and is a potential epithelial tumor suppressor gene [12]. Methylation of CCNA1 may result in cell proliferation. As one of the highly conserved cyclins, CCNA1 exhibits a high expression in breast cancer, acute myeloid leukemia, ovarian cancer, prostate cancer, and esophageal squamous carcinoma [13, 14, 24]. Methylation of the CCNA1 gene is prevalent in cervical cancer [16]. When the methylation of the CCNA1 gene occurs, the expression of the CCNA1 protein reduces and further throws the cell cycle regulation out of control, resulting in excessive cell proliferation or even a tumor. The methylation rates of the CCNA1 gene for HSIL,

microinvasive, and invasive cancers were 33.6%, 60.0%, and 93.3%, respectively; no methylation of the CCNA1 gene was detected in LSIL and the normal cervix [25]. Methylation of the CCNA1 promoter also proved to be a marker for differentiating LSIL and HSIL [26].

In this study, after CIN, the positive expression rates and intensity of CCNA1 mRNA and protein gradually decreased with the aggravation of cervical lesions, and there was no significant difference between the LSIL and control groups. This result indicates that the methylation rate of the CCNA1 gene increased with the aggravation of cervical lesions. In the LSIL group, a negative expression of CCNA1 mRNA indicated the possibility of methylation of the CCNA1 gene, a different result than was found in a previous study [25]. This is perhaps induced by race and sample size differences. The tissue research result was consistent with the cell research result [25], suggesting that the low expression of CCNA1 was related to the occurrence of cervical cancer. In our study, we also found the expression level of CCNA1 in the HSIL and LSIL groups was significantly different. Hence, the degree of CCNA1 expression can be considered a potential molecular marker for the detection of HSIL and SCC of the cervix.

In conclusion, a high expression of DNMT1 emerges earlier than inhibition of CCNA1, so the former is an early molecular event in cervical cancer. This suggests that DNMT1 can regulate the methylation of the CCNA1 promoter, reduce CCNA1 mRNA and protein expression, and throw the cell cycle out of control, thus causing excessive cell proliferation and promoting the development of cervical precancerous lesions and cervical cancer. DNMT1 and CCNA1 are expected to become new targets for tumor detection and treatment, providing a new way to explore the treatment of CIN and cervical cancer.

Disclosure of conflict of interest

None.

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