RESEARCH ARTICLE

Folate Deficiency and FHIT Hypermethylation and HPV 16 Infection Promote Cervical Cancerization

Li-Xia Bai[1], Jin-Tao Wang[2]*, Ling Ding[2], Shi-Wen Jiang[3], Hui-Jie Kang[2], Chen-Fei Gao[2], Xiao Chen[2], Chen Chen[2], Qin Zhou[2]

Abstract

Fragile histidine triad (FHIT) is a suppressor gene related to cervical cancer through CpG island hypermethylation. Folate is a water-soluble B-vitamin and an important cofactor in one-carbon metabolism. It may play an essential role in cervical lesions through effects on DNA methylation. The purpose of this study was to observe effects of folate and FHIT methylation and HPV 16 on cervical cancer progression. In this study, DNA methylation of FHIT, serum folate level and HPV16 status were measured using methylation-specific polymerase chain reaction (MSP), radioimmunoassay (RIA) and polymerase chain reaction (PCR), respectively, in 310 women with a diagnosis of normal cervix (NC, n=109), cervical intraepithelial neoplasia (CIN, n=101) and squamous cell carcinoma of the cervix (SCC, n=101). There were significant differences in HPV16 status (χ^2=36.64, P<0.001), CpG island methylation of FHIT (χ^2=71.31, P<0.001) and serum folate level (F=4.57, P=0.011) across the cervical histologic groups. Interaction analysis showed that the ORs only with FHIT methylation (OR=11.47) or only with HPV 16 positive (OR=4.63) or with serum folate level lower than 3.19ng/ml (OR=1.68) in SCC group were all higher than the control status of HPV 16 negative and FHIT unmethylation and serum folate level more than 3.19ng/ml (OR=1). The ORs only with HPV 16 positive (OR=2.58) or with serum folate level lower than 3.19ng/ml (OR=1.28) in CIN group were all higher than the control status, but the OR only with FHIT methylation (OR=0.53) in CIN group was lower than the control status. HPV 16 positivity was associated with a 7.60-fold increased risk of SCC with folate deficiency and with a 1.84-fold increased risk of CIN. The patients with FHIT methylation and folate deficiency or with FHIT methylation and HPV 16 positive were SCC or CIN, and the patients with HPV 16 positive and FHIT methylation and folate deficiency were all SCC. In conclusion, HPV 16 infection, FHIT methylation and folate deficiency might promote cervical cancer progression. This suggests that FHIT may be an effective target for prevention and treatment of cervical cancer.

Keywords: Folate - FHIT - methylation - cervical cancerization - prevention

Asian Pac J Cancer Prev, **15 (21)**, 9313-9317

Introduction

Cervical cancer is one of the most common cancers in women globally and is responsible for the large percentage of women's death in developing countries. Epidemiological and laboratory data have shown that persistent infection with oncogenic human papillomavirus (HPV), particularly HPV-16 and HPV-18, is the main risk factor, but not solely responsible for the development of cervical cancer. Other cofactors including genetic and epigenetic factors, may facilitate the progression of cervical cancer as well.

Previous studies have reported that DNA methylation of genes is a frequent epigenetic factor in cervical cancer (Piyathilake et al., 2010). Aberrant DNA methylation in the CpG islands at the promoter region is an important epigenetic mechanism underlying the inactivation of tumor suppressor genes (Abouzeid et al., 2011). The fragile histidine triad (FHIT) gene is a candidate tumor suppressor gene located at chromosome 3p14.2 encompassing the FRA3B site, which is the most active fragile site in the human genome. The reduced FHIT expression was associated with the high CpG island methylation (Al-Temaimi et al., 2013). The promoter methylation status of the FHIT gene was observed in a number of cancers, such as breast cancer (Jeong et al., 2013), lung cancer (Tan et al., 2013), non-small cell lung carcinomas (Haroun et al., 2014; Li et al., 2014), bladder cancer (Han et al., 2011) and Nasopharyngeal Carcinoma (Chen et al., 2013), and others as well. In cervical cancer tissues, the methylation rate of the FHIT gene promoter was also significantly higher than in cervical intraepithelial neoplasia and normal cervical tissues (66.0 *vs*. 59.1 *vs*. 25.0 %, P=0.0033) (Banzai et al., 2014).

Folate is a water-soluble B-vitamin and an important cofactor in one-carbon metabolism in which folate

*[1]Maternal and Children Health care of Shanxi Province affiliated to Shanxi Medical University, [2]Department of Epidemiology, School of Public Health, Shanxi Medical University, Taiyuan China, [3]Department of Biomedical Science, Mercer University School of Medicine, USA *For correspondence: wangjt59@163.com*

participates in nucleotide synthesis and methylation reactions (Wang et al., 2011; Pathak et al., 2012). The relationship between folate deficiency and cervical cancer has not been consistent. Some evidence has suggested that folate deficiency may increase the risk of cancers and may have other negative effects on human health (Tomaszewski et al., 2011). However, in an India study, there was no statistically significant association between folate levels and cervical carcinogenesis. This was observed in 136 control subjects, 92 low-grade squamous intraepithelial lesions (LSIL) subjects, and 94 invasive cervical cancer cases (ICC) in Kerala, South India (Ragasudha et al., 2012). The previous findings of our team indicated that the low level of serum folate was significantly associated with cervical carcinogenesis (Jin-Tao et al., 2014), and the cell proliferation decreased and the apoptosis increased with the concentration of folate increasing in human cervical cancer cell lines (Ding et al., 2013).

Above all, high FHIT methylation and low serum folate and HPV infection may be risk factors for cervical cancer independently. However, few studies reported how they work together in cervical cancer progression. Hence, the present study was conducted to study their interactions by examining serum folate and DNA methylation of tumour suppressor gene FHIT and HPV 16 in women ranging from normal cervix to cervical intraepithelial neoplasia to squamous cell carcinoma of the cervix.

Materials and Methods

In this study, after been approved by the institutional Review Board (IRB) of the appropriate hospitals where the participants came from and informed consent was obtained from the patients, the author obtained blood and tissues of participants.

Blood collection

Prior to surgery or other treatments, a 3 ml blood sample was drawn in the morning after an overnight fasting (12 hours) and centrifuged at 4000 rpm for 15 minutes at room temperature within 10 hours of collection. Plasma and serum were separated, and serum was drawn into an EP tube and stored at -80°C until analysis. Blood folate (folic acid) levels were determined by radioimmunoassay (RIA) (Dongya Institute of Biological Medicine, Beijing, China).

Cervical tissues collection

Samples of squamous cell carcinoma of the cervix tissues were derived from patients who experienced primary surgery for cervical diseases at the Department of Gynaecology and Obstetrics in Tumor hospital of Shanxi Province (Taiyuan, China), and cervical intraepithelial neoplasia tissues and normal cervix underwent vaginoscope inspection from the Second Affiliated Hospital of Shanxi Medical University (Taiyuan, China) from June 2008 to August 2009. All of the selected cervical tissues met the criteria including no history of any other type of malignant tumor, without neoadjuvant therapy prior to surgery and without using vitamin B in the past three months. All the cervical tissues were put into the nitrogen canister immediately and then storaged in a refrigerator at -80°C.

Polymerase chain reaction (PCR)

Polymerase Chain Reaction (PCR) primers for HPV16 E2 and E6 were purchased from Integrated DNA Technologies (Shanghai, China). All primers are shown in Table 1.

PCR was performed in 50μl reaction volumes containing 10×PCR buffer. DNA was amplified using the following protocol: 95ºC for 5 minutes followed by 30 cycles of 95ºC for 60 seconds, 55ºC for 60 seconds, 72ºC for 60 seconds, and finally of 72ºC for 10 minutes.

Methylation-specific polymerase chain reaction

Methylation-specific polymerase chain reaction (MSP) primers for FHIT (Gene ID: 2272) were purchased from Integrated DNA Technologies (Shanghai, China). All primers are also shown in Table 1.

MSP was performed in 50μl reaction volumes containing 10×PCR buffer. DNA was amplified using the following protocol: 95ºC for 5 minutes followed by 40 cycles of 94ºC for 30 seconds, 56ºC for 30 seconds, 72ºC for 30 seconds, and finally of 72ºC for 7 minutes.

Statistical analysis

A comparison of categorical variables among histology groups was examined by χ^2 test; continuous variables were tested by ANOVA analysis and if a significant difference was detected, further Post-Hoc test was used.

Results

Demographic characteristics

In the present study, there were three groups of participants. Specifically, 100 women were aged 31-75 years (median±SD: 49.00±10.75 y) in squamous cell carcinoma of the cervix group (SCC), 101 women were aged 29-67 years (48.00±12.00 y) in cervical intraepithelial neoplasia group (CIN), and 109 women were aged 24-81 years (47.00±13.50 y) in normal cervix

Table 1. The Sequences and Length of HPV16 E2 and E6 and FHIT

Product name	Primer sequences	Length
HPV16 E2	P1: AAG GGC GTA ACC GAA ATC GGT	351bp
	P2: CAT ATA CCT CAC GTC GCA G	
HPV16 E6	P1: CTT GGG CAC CGA AGA AAC AC	208bp
	P2: TTG GTC ACG TTG CCA TTC AC	
FHIT(M)	P1: 5'-TTG GGG CGC GGG TTT GGG TTT TTA CGC-3'	74 bp
	P2: 5'-CGT AAA CGA CGC CGA CCC CAC TA-3'	
FHIT(U)	P1: 5'-TTG GGG TGT GGG TTT GGG TTT TTA TG-3'	74 bp
	P2: 5'-CAT AAA CAA CAC CAA CCC CAC TA-3'	

Table 2. Correlation between HPV-16 Status and Cervical Histologic Groups

Group	HPV 16 positive, n(%)	HPV 16 negative, n(%)	χ^2	P
NC	22 (20.2)	87 (79.8)	36.64	<0.001[a]
CIN	38 (37.6)	63 (62.4)	36.29	<0.001[b]
SCC	61 (61.0)	39 (39.0)	7.34	0.005[c]
total	122 (39.0)	188 (61.0)		

[a]comparison between three groups; [b], comparison between CIN and NC groups; [c]comparison between SCC and NC groups

(NC) group (control group). There were no significant differences in age, nationality, birthplace, city of residence, and marital status among the SCC, CIN and NC groups.

Detection of HPV16

Table 2 shows the HPV 16 status of the women with different cervical histology diagnoses. HPV 16 was detected in 39.4% of the study participants. There was a significant difference in HPV16 status across the cervical histologic groups (total χ^2=36.64, P<0.001). The Post-Hoc analysis showed that women with SCC were more likely to be HPV 16 positive than women with normal cervix (χ^2=36.29, P<0.001), and that women with CIN also had an increased risk of HPV 16 infection (χ^2=7.34, P=0.005).

Detection of CpG island methylation of FHIT

Table 3 shows a statistically significant difference in the proportion of CpG island methylation of tumor suppressor gene FHIT across the cervical histologic grades (χ^2=71.31; P<0.001). The rate of CpG island methylation of FHIT in the SCC group was significantly higher than that in the NC group (χ^2=42.67; P<0.001), but the significant difference was not observed between the CIN and NC groups (Fhisher χ^2, P=1.000).

Serum folate levels

The level of serum folate among the three groups was significantly different which was tested by the ANOVA (Table 4). Women with SCC had a significantly lower serum folate level than women with normal cervical histology (P<0.0167, Adjusted test $\alpha=\alpha/3=0.0167$), but there was no significant difference between SCC and CIN groups (P>0.0167) and between CIN and NC groups (P>0.0167).

Interactions between HPV infection and FHIT methylation and serum folate levels in SCC and CIN groups

Table 5 shows the three factors for interactions between HPV infection and FHIT methylation and serum folate levels in SCC and CIN groups respectively. The odds ratios (OR) in different conditions were calculated compared with the "control status" of HPV 16 negative and FHIT unmethylation and serum folate level more than 3.19ng/ml. It was observed that women only with FHIT methylation (OR=11.47) or only with HPV 16 positive (OR=4.63) or with a serum folate level lower than 3.19ng/ml (OR=1.68) were more likely to be SCC compared with the "control status" (OR=1), and women only with HPV 16 positive (OR=2.58) or with a serum folate level lower than 3.19ng/ml (OR=1.28) were more likely to be CIN compared with the "control status" , but women only with FHIT methylation (OR=0.53) in CIN group were lower than the control status. HPV 16 positive was associated with a 7.60-fold increased risk of SCC with folate deficiency and with a 1.84-fold increased risk of CIN. The patients with FHIT methylation and folate deficiency or with FHIT methylation and HPV 16 positive were all SCC or CIN, and the patients with HPV 16 positive, FHIT methylation and folate deficiency were all SCC.

Table 3. Comparison of FHIT Methylation Rates between Cervical Histologic Groups

Group	FHIT methylation, n(%)	FHIT unmethylation, n(%)	χ^2	P
NC	3(2.8)	106(97.2)	71.31	<0.001[a]
CIN	3(3.0)	98(97.0)	-	1.000[b]
SCC	39(39.0)	61(61.0)	42.67	<0.001[c]
total	45(14.5)	265(85.5)		

[a]comparison between three groups; [b]comparison between CIN and NC groups, Fhisher χ^2; [c] comparison between SCC and NC groups

Table 4. Comparison of Serum Folate Levels between Cervical Histologic Groups(ng/ml)

Group	n	serum folate	F	P
NC	109	3.31±1.73	4.57	0.01
CIN	101	3.06±1.86[a]		
SCC	100	2.59±1.60[b, c]		

[a]comparison between CIN and NC groups, P>0.0167; [b]comparison between SCC and NC groups, P<0.0167; [c]comparison between SCC and CIN groups, P>0.0167

Table 5. Interaction between HPV Infection and FHIT Methylation and Serum Folate Levels in SCC and CIN Groups

HPV 16	FHIT methylation	serum folate level (ng/ml)	NC	SCC	OR	CIN	OR
-	-	≥3.19	43	10	1	27	1
-	-	<3.19	41	16	1.68	33	1.28
-	+	≥3.19	3	8	11.47	1	0.53
-	+	<3.19	0	5	-	2	-
+	-	≥3.19	8	7	4.63	13	2.58
+	-	<3.19	14	28	8.6	25	2.84
+	+	≥3.19	0	3	-	0	-
+	+	<3.19	0	23	-	0	-

Discussion

The key finding in this study is that the serum folate level, CpG island hypermethylation of FHIT and HPV 16 positive are significantly associated with the progression of cervical lesions. Serum folate levels significant differed between the SCC and NC groups, but no significant difference was observed between SCC and CIN groups. FHIT showed increased methylation with increasing severity of cervical neoplastic changes. Interaction analysis showed the odds ratios of HPV 16 infection and FHIT methylation and folate deficiency existed at the same time or when two of them existed were higher than one of them existed, the ORs of which were all higher than the control status of HPV 16 negative and FHIT unmethylation and serum folate level higher than 3.19ng/ml in SCC and CIN.

Folate is important for normal cell division. Folate deficiency has many negative effects on health and can be one of the risk factors of cancers which has been widely documented. This study showed that folate level in SCC group was statistically lower than that in the NC group, but no significant difference was found between the SCC and CIN groups and between the CIN and NC groups. It implied that folate deficiency had more influence on SCC than CIN and NC. Previous studies reported that folate status was associated with the natural history of HR-HPV infection. An Indian study with 742 women found that women with higher serum folate levels (>6ng/ml) were at a lower risk of HR-HPV positivity compared to those

with serum folate levels ≤6ng/ml (Piyathilake et al., 2010). In a prospective study including 345 subjects, the results showed that women with higher folate status had a higher risk of becoming HR-HPV-positive (Piyathilake et al., 2004). A research study in Turkey including 122 women also indicated that the means of serum folate level in HSIL or LSIL or ASCUS group were lower than that of the control group ($P<0.05$). Another report showed that in all cervical dysplasia groups, folate levels were lower in HPV-positive patients than in HPV-negative patients ($P<0.05$) (Abike et al., 2011). A multicenter case control study that involved 927 Korean women (440 controls, 165 patients with CIN1, 167 with CIN2/3, and 155 with cervical cancer) reported that patients with cervical cancer had significantly lower median serum folate levels than controls and the linear trend test showed significant associations between higher serum folate levels and lower cancer risks (P for linear trend=0.0058) (Tong et al., 2011). However, a recent study including 322 women (136 control subjects, 92 low-grade squamous intraepithelial lesions (LSIL), 94 invasive cervical cancer cases(ICC)) in South India did not find significant associations between folate levels and cervical carcinogenesis (Ragasudha et al., 2012).

FHIT is a suppressor gene related to cervical cancer through CpG island hypermethylation. In this study, the SCC group had a significantly higher rate of CpG island methylation than the NC group, although no statistical difference was observed between the CIN and NC groups. A positive correlation was observed between methylation rates and progression of cervical cancer. The results showed that the vital role of FHIT methylation in cervical carcinogenesis, which was supported by previous studies. A case-control study found that FHIT methylation was observed in 28.3% (17/60) of cervical cancer cases, while FHIT was not methylated in any of the 23 healthy NCs (Neyaz et al., 2008). Another case-control study in China indicated that the rate of 5'CpG island methylation of the FHIT gene was 40.0% (16/40) in cervical cancer groups, while the rate of methylation in normal cervical tissue was 0% (0/10). The FHIT methylation rate in CIN1 (14.3%, 2/14) was statistically lower than its rate in CIN2 (56.3%, 13/23) ($P<0.05$) (Shi et al., 2005).

The present study shows in the beginning that there was a significant interaction between HPV 16 infection and serum folate levels and FHIT methylation in cervical tissue. The factors for HPV 16 infection and FHIT methylation and folate deficiency existed at the same time or two of them existed have higher risks than that of one of them existed or all absent. It is generally known that HPV infection is the primary cause of SCC. Aberrant DNA methylation is associated with cervical pathogenesis (Flatley et al., 2012; Pathak et al., 2012). The study demonstrated that women with a lower serum folate level and HPV 16 infection had a greater risk of SCC than those with a higher serum folate level and HPV negative, which was also revealed in a previous study (Piyathilake et al., 2007).

The study also found that folate deficiency dramatically increased the risk of cervical cancer patients with high FHIT methylation for SCC. This could be due to low serum folate levels which lead to decreased DNA methyltransferase 1 (DNMT1) expression, which plays a significant role in maintaining DNA methylation status and regulating expression of tumor suppressor genes in cervical cancer cells (Zhang et al., 2011). Chromosomal fragile site FRA3B at 3p14.2 located in the FHIT gene may be particularly prone to forming gaps or breaks on metaphase chromosomes under the conditions that inhibit DNA replication or repair, such as folate deficiency, which is an essential cofactor in de novo synthesis of purines and thymidylate-nucleotides necessary for DNA repair. The interaction between FHIT and HPV 16 would be caused by the fact that FRA3B fragile site of FHIT gene is a candidate region for HPV 16 interaction (Wilke et al., 1996).

The study has several strengths. Firstly, it observed interactions between serum folate levels and HPV status and FHIT methylation rates for the first time. Second, serum and tissue samples originated from different stages of cervical cancer progression, including normal cervix, CIN and cervical cancer, which document natural cervical cancer progression. However, this study also has limitation. We only showed the relationships between folate and FHIT and HPV in cervical cancer progression, but did not indicate mechanisms of action even though other studies have indicated that folate may play an essential role in cervical lesions through impacting on DNA methylation (Flatley et al., 2009; Piyathilake et al., 2011). Future studies should avoid these limitations.

Acknowledgements

This study was supported by National Natural Science Foundation of China (Grant No. 30872166, No. 81273157) and the Natural Science Foundation of Shanxi Province, China (Grant No. 2008011075-1).

References

Abike F, Engin AB, Dunder I, et al (2011). Human papilloma virus persistence and neopterin, folate and homocysteine levels in cervical dysplasias. *Arch Gynecol Obstet*, **284**, 209-14.

Abouzeid HE, Kassem AM, Abdel Wahab AH, et al (2011). Promoter hypermethylation of RASSF1A, MGMT, and HIC-1 genes in benign and malignant colorectal tumors. *Tumour Biol*, **32**, 845-52.

Al-Temaimi RA, Jacob S, Al-Ali W, et al (2013). Reduced FHIT expression is associated with mismatch repair deficient and high CpG island methylator phenotype colorectal cancer. *J Histochem Cytochem*, **61**, 627-38.

Banzai C, Nishino K, Quan J, et al (2014). Promoter methylation of DAPK1, FHIT, MGMT, and CDKN2A genes in cervical carcinoma. *Int J Clin Oncol*, **19**, 127-32.

Chen X, Li P, Yang Z, Mo WN (2013). Expression of fragile histidine triad (FHIT) and WW-domain oxidoreductase gene (WWOX) in nasopharyngealcarcinoma. *Asian Pac J Cancer Prev*, **14**, 165-71.

Ding L, Ma J, Zhou Q, Wang J (2013). Effect of folate on the proliferation of human cervical cancer cell and relationship with HPV16. *Wei Sheng Yan Jiu*, **42**, 748-53.

Flatley JE, McNeir K, Balasubramani L, et al (2009). Folate Status and Aberrant DNA Methylation Are Associated With

HPV Infection and Cervical Pathogenesis. *Cancer Epidemiol Biomarkers Prev*, **18**, 2782-9.

Han Y, Zhang Z, Zhang GJ, et al (2011). Aberrant FHIT expression is linked to bladder carcinogenesis and apoptosis. *Asian Pac J Cancer Prev*, **12**, 2915-20.

Haroun RA, Zakhary NI, Mohamed MR, et al (2014). Assessment of the prognostic value of methylation status and expression levels of FHIT, GSTP1 and p16 in non-small cell lung cancer in Egyptian patients. *Asian Pac J Cancer Prev*, **15**, 4281-7.

Jeong YJ, Jeong HY, Lee SM, et al (2013). Promoter methylation status of the FHIT gene and Fhit expression: association with HER2/neu status in breast cancer patients. *Oncol Rep*, **30**, 2270-8.

Jin-Tao W, Ling D, Shi-Wen J, et al (2014). Folate Deficiency and Aberrant Expression of DNA Methyltransferase 1 were Associated with Cervical Cancerization. *Curr Pharm Des*, **20**, 1639-46.

Li W, Deng J, Tang JX (2014). Combined effects methylation of FHIT, RASSF1A and RARβ genes on non-small cell lung cancer in the Chinese population. *Asian Pac J Cancer Prev*, **15**, 5233-7.

Neyaz MK, Kumar RS, Hussain S, et al (2008). Effect of aberrant promoter methylation of FHIT and RASSF1A genes on susceptibility to cervical cancer in a North Indian population. *Biomarkers*, **13**, 597-606.

Pathak S, Bhatla N, Singh N (2012). Cervical cancer pathogenesis is associated with one-carbon metabolism. *Mol Cell Biochem*, **369**, 1-7.

Piyathilake CJ, Henao OL, Macaluso M, et al (2004). Folate is associated with the natural history of high-risk human papillomaviruses. *Cancer Res*, **64**, 8788-93.

Piyathilake CJ, Macaluso M, Brill I, et al (2007). Lower red blood cell folate enhances the HPV-16-associated risk of cervical intraepithelial neoplasia. *Nutrition*, **23**, 203-10.

Piyathilake CJ, Badiga S, Paul P, et al (2010). Indian women with higher serum concentrations of folate and vitamin B12 are significantly less likely to be infected with carcinogenic or high-risk (HR) types of human papillomaviruses (HPVs). *Int J Womens Health*, **2**, 7-12.

Piyathilake CJ, Macaluso M, Celedonio JE, et al (2010). Mandatory fortification with folic acid in the United States appears to have adverse effects on histone methylation in women with pre-cancer but not in women free of pre-cancer. *Int J Womens Health*, **9**, 131-7.

Piyathilake CJ, Macaluso M, Alvarez RD, et al (2011). A higher degree of LINE-1 methylation in peripheral blood mononuclear cells, a one-carbon nutrient related epigenetic alteration, is associated with a lower risk of developing cervical intraepithelial neoplasia. *Nutrition*, **27**, 513-9.

Ragasudha PN, Thulaseedharan JV, Wesley R, et al (2012). A case-control nutrigenomic study on the synergistic activity of folate and vitamin B12 in cervical cancer progression. *Nutr Cancer*, **64**, 550-8.

Shi HR, Wu QH, Suo ZH, Nesland JM (2005). Correlation between methylation of 5'-CpG islands and inactivation of FHIT gene in cervical cancer. *Ai Zheng*, **24**, 7-11.

Tan S, Sun C, Wei X, et al (2013). Quantitative assessment of lung cancer associated with genes methylation in the peripheral blood. *Exp Lung Res*, **39**, 182-90.

Tomaszewski JJ, Cummings JL, Parwani AV, et al (2011). Increased cancer cell proliferation in prostate cancer patients with high levels of serum folate. *Prostate*, **71**, 1287-93.

Tong SY, Kim MK, Lee JK, et al (2011). Common polymorphisms in methylenetetrahydrofolate reductase gene are associated with risks of cervical intraepithelial neoplasia and cervical cancer in women with low serum folate and vitamin B12. *Cancer Causes Control*, **22**, 63-72.

Wang JT, Huo XX, Ding L, et al (2011). Effect of folic acid and DNA methyltransferase 1 on cervical cancer and its precancerous lesion. *Zhonghua Liu Xing Bing Xue Za Zhi*, **32**, 617-21.

Wilke CM, Hall BK, Hoge A, et al (1996). FRA3B extends over a broad region and contains a spontaneous HPV16 integration site: direct evidence for the coincidence of viral integration sites and fragile sites. *Hum Mol Genet*, **5**, 187-95.

Zhang Y, Chen FQ, Sun YH, et al (2011). Effects of DNMT1 silencing on malignant phenotype and methylated gene expression in cervical cancer cells. *J Exp Clin Cancer Res*, **17**, 98.