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Effect of hrHPV infection on anti−apoptotic gene and pro−apoptotic gene expression in cervical cancer tissue

Min−Er Tang✉

Department of Clinical Laboratory, the People's Hospital of Nanhai Economic Development Zone Foshan City Guangdong Province, Nanhai District, Foshan City, Guangdong Province, 528222, China

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ABSTRACT

Objective: To study the effect of hrHPV infection on anti-apoptotic gene and pro-apoptotic gene expression in cervical cancer tissue. **Methods:** A total of 56 patients with cervical cancer, 94 cases of patients with cervical intraepithelial neoplasia and 48 cases of patients with chronic cervicitis who were treated in our hospital from May 2013 to December 2015 were selected for study and included in malignant group, precancerous lesion group and benign group respectively. hrHPV infection as well as the expression of anti-apoptotic genes and pro-apoptotic genes in cervical tissue were detected. **Results:** hrHPV infection rate and viral load in cervical tissue of malignant group were significantly higher than those of precancerous lesion group and benign group; P27 and p16 levels in cervical tissue of malignant group were significantly lower than those of precancerous lesion group and benign group, and K-ras, c-myc, Prdx4 and TNFAIP8 levels were significantly higher than those of precancerous lesion group and benign group; the greater the HPV virus load, the lower the p27 and p16 levels and the higher the K-ras, c-myc, Prdx4 and TNFAIP8 levels in cervical tissue. **Conclusions:** hrHPV infection can result in tumor suppressor genes p27 and p16 expression deletion and increase the expression of proto-oncogene and apoptosis-inhibiting genes, and it is associated with the occurrence and development of cervical cancer.

1. Introduction

Cervical cancer is the second most malignant tumor in women, its incidence ranks only second to breast cancer and the incidence is on the rise in recent years. Cervical cancer is mostly developed from cervical intraepithelial neoplasia, the cell proliferation capability is markedly enhanced and apoptosis ability is weakened significantly. Human papillomavirus (HPV) is the important cause of cervical epithelial phenotype changes[1]. High-risk HPV (hrHPV) infection is most closely related to cervical intraepithelial neoplasia and cervical cancer, and the known hrHPV DNA types include thirteen types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68[2−4]. At present, the researches on correlation between hrHPV infections and cervical cancer rely mainly on qualitative assessment of HPV, and researches on HPV virus load and DNA quantitative detection are still sufficient. In the following study, the effect of hrHPV infection on anti-apoptotic gene and pro-apoptotic gene expression in cervical cancer tissue was analyzed.

2. Materials and methods

2.1. Research subjects

Patients with cervical diseases who were treated in our hospital from May 2013 to December 2015 were selected as research subjects, the patients' medical records were reviewed, 56 cases of patients with cervical cancer, 94 cases of patients with cervical intraepithelial neoplasia and 48 cases of patients with chronic

✉Corresponding author: Min-Er Tang, Department of Clinical Laboratory, Xiaotang Hospital Area of the People's Hospital of Nanhai Economic Development Zone, 3rd Ring Road, Xiaotang, Shishan Town, Nanhai District, Foshan City, Guangdong Province, 528222, China.
Tel: 0757-86633929; 13590683999
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cervicitis were screened, and all patients were diagnosed through pathological biopsy, signed informed consent and then were included for study. Cervical cancer patients were included in malignant group, they were 44-65 years old and the average was (56±7) years; patients with cervical intraepithelial neoplasia were included in precancerous lesion group, they were 41-67 years old and average was 58±9 years; patients with chronic cervicitis were included in benign group, they were 40-64 years old and the average was (55±8) years. The comparison of general data among three groups showed no significant difference.

2.2. Research methods

2.2.1. High-risk HPV-DNA assessment

The HC-II kit from Digene Company was used for DNA loads of 13 kinds of high-risk RNA, HPV-DNA 1 pg/mL indicating positive. The following standards were used to divide cervical cancer patients into different subgroups: 0-1 pg/mL group, 1-5 pg/mL group, 5-25 pg/mL group, 25-100 pg/mL group, 100-500 pg/mL group and > 500 pg/mL group.

2.2.2. Apoptosis-related gene expression detection

Biopsy cervical tissue was collected, about 50-80 mg of tissue was weighed, added to PBS liquid and then fully grinded, the grinded fluid was centrifuged in centrifugal machine, the residue after centrifuge was abandoned, supernatant was kept, and enzyme-linked immunosorbent kit was used to determine p27, p16, k-ras, c-myc, Prdx4 and TNFAIP8 content in grinded supernatant.

2.3. Statistical methods

SPSS20.0 software was used to input data, measurement data was tested by homogeneity of variance at first, normal distribution data according with homogeneity of variance was in terms of average ± standard deviation and variance analysis was used to compare the differences between groups, partial distribution data not according with homogeneity of variance was in terms of median and inter-quartile range and nonparametric rank-sum test was used to compare the differences between groups. Differences were considered to be statistically significant at a level of $P< 0.05$.

3. Results

3.1. hrHPV infection in cervical tissue of different groups

hrHPV infection rate and viral load were different in cervical tissue of benign group, precancerous lesion group and malignant group; hrHPV infection rate and viral load in cervical tissue of malignant group and precancerous lesion group were significantly higher than those of benign group, and hrHPV infection rate and viral load in cervical tissue of malignant group were significantly higher than those of precancerous lesion group, shown in Table 1.

3.2. Apoptosis-related gene expression in cervical tissue of different groups

p27, p16, K-ras, c-myc, Prdx4 and TNFAIP8 levels were different in cervical tissue of benign group, precancerous lesion group and malignant group; p27 and p16 levels in cervical tissue of malignant group and precancerous lesion group were significantly lower than those of benign group, and K-ras, c-myc, Prdx4 and TNFAIP8 levels were significantly higher than those of benign group; p27 and p16 levels in cervical tissue of malignant group were significantly lower than those of precancerous lesion group, and K-ras, c-myc,

Table 1
hrHPV infection rate and viral load in cervical tissue of different groups.

Groups	Case No.	hrHPV infection rate [n(%)]	hrHPV virus load	
			Median	Inter-quartile range
Control group	48	25(52.08%)	0.74	0.55-5.49
Precancerous lesion group	94	71(75.53%)◇	58.41	2.96-252.67◇
Malignant group	56	53(94.64) ◇◆	148.62	32.96-576.81◇◆

◇: compared with control group, $P<0.05$; ◆: compared with precancerous lesion group, $P<0.05$.

Table 2
Comparison of apoptosis-related gene expression in cervical tissue of different groups.

Groups	Tumor suppressor gene/pro-apoptosis gene		Pro-proliferation gene/anti-apoptosis gene			
	P27 (μg/L)	P16 (μg/L)	K-ras (ng/L)	C-myc (μg/L)	Prdx4 (ng/L)	TNFAIP8 (ng/L)
Control group	42.8±6.3	68.2±9.5	14.6±2.4	5.9±0.8	31.5±6.2	47.6±7.5
Precancerous lesion group	13.6±1.9◇	23.6±4.6◇	48.4±6.8◇	12.6±1.8◇	103.5±16.5◇	152.6±21.8◇
Malignant group	9.4±1.1◇◆	10.4±2.4◇◆	62.3±9.7◇◆	19.4±2.7◇◆	154.1±24.2◇◆	228.2±31.9◇◆

◇: compared with control group, $P<0.05$; ◆: compared with precancerous lesion group, $P<0.05$.

Prdx4 and TNFAIP8 levels were significantly higher than those of precancerous lesion group, shown in Table 2.

3.3. Apoptosis-related gene expression in cervical tissue with different hs-HPV virus loads

p27, p16, K-ras, c-myc, Prdx4 and TNFAIP8 levels were different in cervical tissue with different hs-HPV virus loads; the greater the HPV virus load, the lower the p27 and p16 levels and the higher the K-ras, c-myc, Prdx4 and TNFAIP8 levels in cervical tissue, shown in Table 3.

4. Discussion

Cervical cancer is one of the common gynecologic malignant tumors, and hsHPV infection is the main cause of cervical cancer[5,6]. Analysis in the study also confirmed that hrHPV infection rate was different in cervical tissue of benign group, precancerous lesion group and malignant group, and hrHPV infection rate of malignant group was significantly higher than that of benign group and precancerous lesion group. In spite of this, qualitative hrHPV examination alone is not enough to provide sufficient basis for cervical cancer screening, and is also not conducive to early detection and diagnosis. HPV-DNA quantitative detection methods have continuously developed and been increasingly used in clinical practice, but studies are still insufficient on quantitative HPV virus load inspection for cervical cancer evaluation. In the research, the kit was used to determine HPV-DNA in cervical tissue in order to reflect hrHPV virus load, and the analysis results indicated that hrHPV virus load was different in cervical tissue of benign group, precancerous lesion group and malignant group, and hrHPV virus load of malignant group was significantly higher than that of benign group and precancerous lesion group. This means that hrHPV infection and viral load increase are associated with the occurrence and development of cervical intraepithelial neoplasia and cervical cancer.

After infecting host cells, hs-HPV can cause cell phenotype and biological behavior changes, and that genes express early protein E6/E7 integrate into the host cell genome DNA is an important way to cause changes in cell biology behavior[7]. E6 protein and E7 protein can be combined with a variety of tumor suppressor genes and make them inactivate, causing that cells cannot enter into apoptosis process, abnormally proliferated cells cannot be cleared and cells eventually become cancerous[8]. The greater the hs-HPV viral loads in cervical tissue, the more the viral DNA integrating into the host cells and the stronger the inhibiting effect on tumor suppressor gene expression. Currently known tumor suppressor genes closely related to cervical cancer are *p27* and *p16*. p27 and p16 can be combined with cyclin-CDK to suppress the downstream Rb protein phosphorylation and block the cell cycle G_1/S phase transition[9-11]. In the research, analysis of the p27 and p16 protein levels in cervical tissue showed that p27 and p16 levels in cervical tissue of malignant group were significantly lower than those of precancerous lesion group and benign group. This means that there are different levels of p27 and p16 expression deletion in cervical cancer tissue. Further analysis of the effect of different hsHPV virus loads on p27 and p16 expression showed that the greater the HPV virus load, the lower the p27 and p16 levels in cervical tissue. Thus it confirms that hsHPV infection can cause p27 and p16 expression deletion and the greater the HPV virus load, the more significant the inhibition of p27 and p16 expression.

hsHPV infection and tumor suppressor gene expression deletion in cervical tissue will indirectly result in up-regulated expression of pro-proliferation genes and anti-apoptotic genes. K-ras and c-myc are two classes of proto-oncogenes closely related to cervical cancer, and they can promote cell proliferation[12]; Prdx4 is a kind of peroxiredoxin that can clear the killing effect of ROS on cells and can also inhibit apoptosis mediated by TRAIL[13,14]; TNFAIP8 is a kind of apoptosis-regulatory factor, its upstream regulating signal is NF-κB, and it can inhibit cascade apoptosis response mediated by Caspase to promote cell survival[15]. In the research, analysis of K-ras, c-myc, Prdx4 and TNFAIP8 protein content in cervical tissue showed that K-ras, c-myc, Prdx4 and TNFAIP8 levels in

Table 3
Comparison of apoptosis-related gene expression in cervical tissue with different hs-HPV virus load.

HsHPV Virus load (pg/mL)	Tumor suppressor gene/pro-apoptosis gene		Pro-proliferation gene/anti-apoptosis gene			
	P27 (μg/L)	P16 (μg/L)	K-ras (ng/L)	C-myc (μg/L)	Prdx4 (ng/L)	TNFAIP8 (ng/L)
0-1	23.4±5.2	41.8±6.2	20.2±3.8	7.6±0.9	42.9±6.6	84.9±11.2
1-5	14.8±2.6①	29.2±5.3①	33.1±5.2①	8.9±1.2①	61.7±8.2①	110.3±17.6①
5-25	7.5±0.9①②	11.6±2.5①②	52.8±7.1①②	11.8±1.6①②	89.3±11.5①②	132.7±17.8①②
25-100	5.4±0.8①②③	7.6±1.0①②③	77.4±10.2①②③	16.4±2.2①②③	126.7±18.4①②③	173.4±21.4①②③
100-500	4.1±0.6①②③④	4.2±0.7①②③④	89.2±12.6①②③④	22.1±5.2①②③④	174.1±21.6①②③④	247.6±40.3①②③④
>500	2.8±0.4①②③④⑤	3.4±0.5①②③④⑤	125.4±19.3①②③④⑤	40.3±7.4①②③④⑤	287.2±36.2①②③④⑤	413.4±64.4①②③④⑤

①: compared with 0-1 pg/mL hs-HPV group, $P<0.05$; ②: compared with 1-5 pg/mL hs-HPV group, $P<0.05$; ③: compared with 5-25 pg/mL hs-HPV group, $P<0.05$; ④: compared with 25-100 pg/mL hs-HPV group, $P<0.05$; ⑤: compared with 100-500 pg/mL hs-HPV group, $P<0.05$.

cervical tissue of malignant group were significantly higher than those of precancerous lesion group and benign group. This means that there is different degree of K-ras, c-myc, Prdx4 and TNFAIP8 over-expression in cervical cancer tissue. Further analysis of different hsHPV virus loads on K-ras, c-myc, Prdx4 and TNFAIP8 expression showed that the greater the HPV virus load, the higher the K-ras, c-myc, Prdx4 and TNFAIP8 levels in cervical tissue. Thus it confirms that hsHPV infection can cause increased expression of K-ras, c-myc, Prdx4 and TNFAIP8 and the greater the HPV virus load, the more significant the increase of the above gene expression.

To sum up, hrHPV infection can result in tumor suppressor genes p27 and p16 expression deletion and increase the expression of proto-oncogene and apoptosis-inhibiting genes, and it is associated with the occurrence and development of cervical cancer.

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