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Effect of 5-aminolevulinic acid photodynamic therapy on the expression of apoptosis inhibitors Bcl-2 and Survivin in keratinocytes of condyloma acuminatum*

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ABSTRACT

Background: Condyloma acuminatum is a highly infectious disease caused by the human papillomavirus. Previous studies have shown that 5-aminolevulinic acid photodynamic therapy can inhibit proliferation of condyloma acuminatum keratinocytes. However, the effect of 5-aminolevulinic acid photodynamic therapy on condyloma acuminatum tissues has not been systematically studied. Here, we investigated possible molecular mechanisms of 5-aminolevulinic acid photodynamic therapy in the treatment of condyloma acuminatum and its effect on the expression of apoptosis inhibitors Bcl-2 and Survivin.

Methods: Immunohistochemistry streptavidin-peroxidase was used to detect the expression of apoptosis inhibitors Bcl-2 and Survivin in condyloma acuminatum keratinocytes before and after the therapy.

Results: The positive expression rates of Bcl-2 and Survivin in condyloma acuminatum keratinocytes before treatment were 87.50% (42/48) and 79.16% (38/48), respectively. The positive expression rates of Bcl-2 and Survivin in condyloma acuminatum keratinocytes after treatment were 37.50% (18/48) and 41.67% (20/48), respectively. The positive expression intensity of Bcl-2 and Survivin in condyloma acuminatum keratinocytes before 5-aminolevulinic acid photodynamic therapy was mostly ++ to +++, and that after treatment was mostly - to +. There were statistically significant differences in the positive expression rate and the expression intensity of Bcl-2 and Survivin in the two groups before and after 5-aminolevulinic acid photodynamic therapy ($P < 0.001$). There was a positive correlation between the expression of Bcl-2 and Survivin in condyloma acuminatum tissues after 5-aminolevulinic acid photodynamic therapy ($r = 0.480$, $P < 0.05$).

Conclusion: 5-aminolevulinic acid photodynamic therapy may promote apoptosis of condyloma acuminatum cells by reducing the expression of Bcl-2 and Survivin, suggesting that this is potentially one of the molecular mechanisms of 5-aminolevulinic acid photodynamic therapy in the treatment of condyloma acuminatum.

1. Introduction

Condyloma acuminatum, also known as genital warts, is a disease caused by human papilloma virus infection and characterized by verrucous proliferative lesions of skin and mucosa in genital areas. condyloma acuminatum is highly infectious, with rapid growth of warts, and recurrence is common despite treatment, resulting in significant economic and psychological burden to the patients. Currently, there is no medication to directly eliminate human papilloma virus infection. Conventional treatments such as electrocautery, cryotherapy, and topical medications are mainly used to remove exogenous warts, and the recurrence rate is high after treatment. A new method called 5-aminolevulinic acid photodynamic therapy is now used for the treatment of condyloma acuminatum. A large amount of clinical data have shown that 5-aminolevulinic acid photodynamic therapy has advantages in treating condyloma acuminatum, such as minimal invasiveness, improved safety, and high patient tolerance, and it can eliminate subclinical infections, reduce recurrence, and reduce cancer risk. Condyloma acuminatum proliferates rapidly and is prone to recurrence after treatment, which is associated with abnormal cellular proliferation and apoptosis. Previous studies have shown that 5-aminolevulinic acid photodynamic therapy can inhibit the proliferation of condyloma acuminatum keratinocytes and promote their apoptosis [1]. There is no systematic study on the specific mechanism of 5-aminolevulinic acid photodynamic therapy in the treatment of condyloma acuminatum. This study explored possible mechanisms of 5-aminolevulinic acid

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photodynamic therapy in the treatment of condyloma acuminatum through the detection of changes in Bcl-2 and Survivin expression in condyloma acuminatum cells before and after the therapy.

2. Materials and methods

2.1. Materials

Forty-eight tissue specimens were collected from the Dermatology Department of the First Affiliated Hospital of Zhengzhou University from September 2014 to September 2015. All patients met the clinical and laboratory diagnostic criteria for condyloma acuminatum. There were 28 males and 20 females, with ages ranging from 18 to 60 years, and a mean age of 33.25 years. The disease course ranged from 2 weeks to 4 months, with a mean of 53.10 days. Warts in men were mostly located on the penis and perianal areas. Warts in women were mostly located on the labia and the vagina. Clinical manifestations were consistent with clinical features of typical condyloma acuminatum. Acetowhite test was positive. In situ hybridization showed human papilloma virus infection and histopathology showed koilocytes. None of the patients received any treatment prior to consultation. The four common infectious diseases were negative, and other diseases were excluded. All forty-eight patients with condyloma acuminatum met the inclusion criteria and were treated with 5-aminolevulinic acid photodynamic therapy. The condyloma acuminatum tissues before treatment were the control group, and the condyloma acuminatum tissues one week after treatment were the experimental group.

2.2. Experimental reagents

Rabbit anti-human Bcl-2 polyclonal antibody was purchased from Beijing Bioss Biotechnology Co., Ltd., and rabbit anti-human Survivin polyclonal antibody and the streptavidin-peroxidase kit were purchased from Wuhan Boster Biotechnology Co., Ltd. DAB color reagent kit was purchased from Beijing Solarbio Technology Co. Ltd.

2.3. Methods

5-Aminolevulinic acid (Fudan Zhangjiang Biomedical Co., Ltd.) was fully dissolved in 0.5 mL of sterilized water for injection. The solution was formulated to a concentration of 20%, protected from light, dripped on sterile cotton ball to make it fully wet, which was used to cover the wart tissue and the surrounding skin surfaces. Volatilization was reduced by covering the application area with a plastic film. The application was repeated once every 30 min to ensure that the area irradiated was covered with the drug for no less than 3 h devoid of light. The skin lesions were irradiated by 635 nm He-Ne laser with an energy of 100 mW/cm^2 for 30 min. Immediately after biopsy, the tissue sample was put into a fixation solution for 24 h. After paraffin wax embedding, the block was thinly sliced. The thickness of the slices was 4–6 microns. The slices were placed and covered on slides. One slice was selected for hematoxylin-eosin staining, and the others were used for examinations.

The expression of Bcl-2 and Survivin in condyloma acuminatum tissues was detected by immunohistochemical SP method. All operations were carried out according to the kit instructions. Known positive sections were used as positive control, and PBS was used as negative control instead of primary antibody incubation.

2.4. Result interpretation

The positive expression of Bcl-2 was localized in the cytoplasm, while the positive expression of Survivin was mainly localized in the cytoplasm and nucleus, both of which showed pale yellow to brown granular substances. Referring to relevant literature [2], semi-quantitative grading of staining intensity and percentage of positive cells was carried out. The grading criteria of staining intensity were 0, 1, 2, and 3 points, corresponding to unstained, light yellow, yellow, and brown, respectively. The total percentages of positive cells based on scores 0, 1, 2, 3, and 4 were ≤10%, 11%–25%, 26%–50%, 51%–75%, and ≥76%, respectively. The total score was 0 = negative (−), 1–4 = weakly positive (+), 6–8 = positive (++), and 9–12 = strongly positive (+++).

2.5. Statistical analysis

The statistical software SPSS21.0 was used to analyze the data. χ^2 test, rank-sum test, and Spearman rank correlation analysis were used with the standard $P < 0.05$ indicating statistically significant difference.

3. Results

3.1. Expression of Bcl-2 in condyloma acuminatum tissue cells before and after 5-aminolevulinic acid photodynamic therapy

Before 5-aminolevulinic acid photodynamic therapy, the tissues of 42 out of 48 condyloma acuminatum cases were positive for Bcl-2, the positive expression rate was 87.50% (42/48), and the expression intensity was ++ to +++ (Fig. 1). After treatment, the tissues of 18 out of 48 condyloma acuminatum cases were positive for Bcl-2, the positive expression rate was 37.50% (18/48), and the expression intensity was + (Fig. 2). The positive expression rates of Bcl-2 before and after treatment were statistically significant ($\chi^2 = 25.60$, $P < 0.001$) as shown in Table 1, and the expression intensity was also significantly different ($H = 38.40$, $P < 0.001$) as shown in Table 2.

3.2. Expression of Survivin in condyloma acuminatum tissue cells before and after 5-aminolevulinic acid photodynamic therapy

Before 5-aminolevulinic acid photodynamic therapy, the tissues of 38 out of 48 condyloma acuminatum cases were positive for Survivin, the positive expression rate was 79.16% (38/48), and the expression intensity was + to +++ (Fig. 3). After treatment, the tissues of 20 out of 48 condyloma acuminatum cases were positive for Survivin, the positive expression rate was 41.67% (20/48), and the expression intensity was mostly + (Fig. 4). The positive expression rate of Survivin before and after treatment was significantly different ($\chi^2 = 14.11$, $P < 0.001$) as shown in Table 3, and the expression intensity was significantly different ($H = 19.72$, $P < 0.001$) as shown in Table 4.

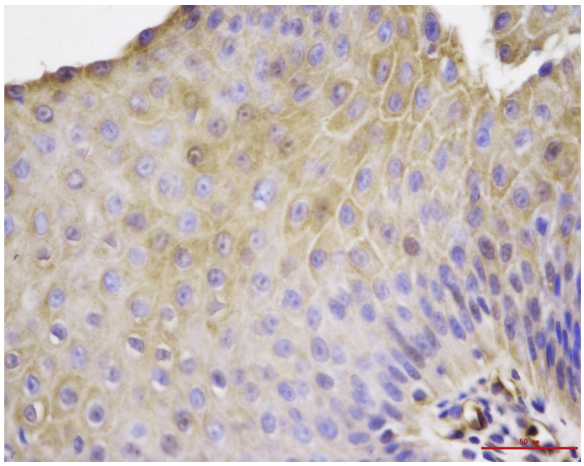

Fig. 1. Expression of Bcl-2 before 5-aminolevulinic acid photodynamic therapy. (SP × 400).

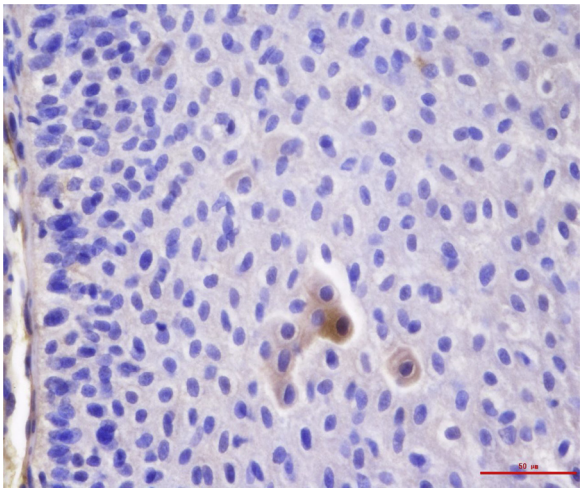

Fig. 2. Expression of Bcl-2 after 5-aminolevulinic acid photodynamic therapy. (SP × 400).

Table 1
Expression rate of Bcl-2 in condyloma acuminatum tissues before and after 5-aminolevulinic acid photodynamic therapy.

Group	Cases	Positive	Negative	Positive rate	χ^2	*P*
Before	48	42	6	87.50%	25.60	< 0.001
After	48	18	30	37.50%		

Table 2
Expression strength of Bcl-2 in condyloma acuminatum tissues before and after 5-aminolevulinic acid photodynamic therapy.

Group	N(cases)	Expression strength				*H*	*P*
		−	+	++	+++		
Before	48	4	2	22	20	38.40	< 0.001
After	48	22	16	8	2		

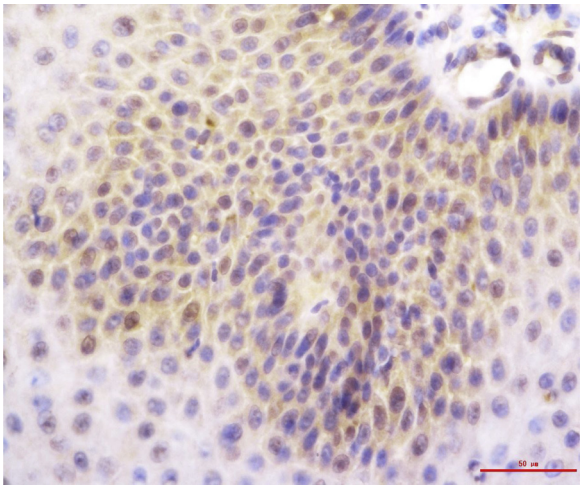

Fig. 3. Expression of Survivin before 5-aminolevulinic acid photodynamic therapy.
(SP × 400).

3.3. Correlation between Bcl-2 and Survivin in condyloma acuminatum tissue cells before and after 5-aminolevulinic acid photodynamic therapy

After 5-aminolevulinic acid photodynamic therapy, the tissues of 18 condyloma acuminatum cases were positive for Bcl-2, and the tissues of 30 condyloma acuminatum cases were negative for Bcl-2; the tissues of 20 condyloma acuminatum cases were positive for Survivin and the tissues of 28 condyloma acuminatum cases were negative for Bcl-2. Spearman rank correlation analysis showed that the expression of Bcl-2 and Survivin was positively correlated in all 48 condyloma acuminatum tissues after 5-aminolevulinic acid photodynamic therapy ($r = 0.48$, $P < 0.05$) (Table 5).

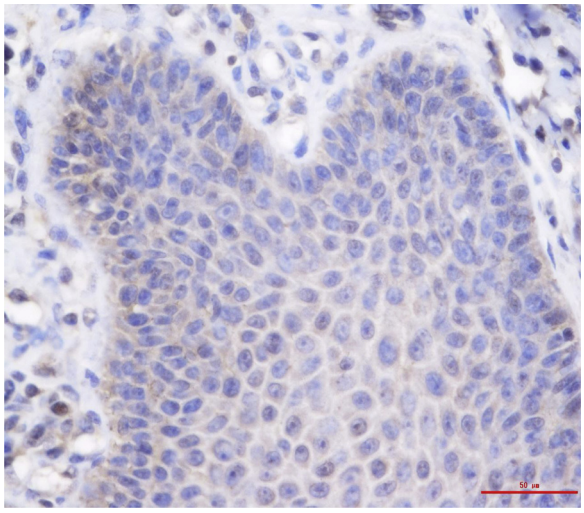

Fig. 4. Expression of Survivin after 5-aminolevulinic acid photodynamic therapy.
(SP × 400).

Table 3
Expression rate of Survivin in condyloma acuminatum tissues before and after 5-aminolevulinic acid photodynamic therapy.

Group	Cases	Positive	Negative	Positive rate	χ^2	*P*
Before	48	38	10	79.16%	14.11	< 0.001
After	48	20	28	41.67%		

Table 4
Expression strength of Survivin in condyloma acuminatum tissues before and after 5-aminolevulinic acid photodynamic therapy.

Group	N(cases)	Expression strength				*H*	*P*
		−	+	++	+++		
Before	48	10	8	16	14	17.92	< 0.001
After	48	28	11	6	3		

4. Discussion

Condyloma acuminatum is a disease mainly transmitted through sexual contact, with a high incidence rate worldwide [3]. Studies have shown that condyloma acuminatum is associated with human

Table 5
Correlation analysis of Bcl-2 and Survivin expression in condyloma acuminatum tissue after 5-aminolevulinic acid photodynamic therapy.

Bcl-2	Survivin	
	+	−
+	13	5
−	7	23

papilloma virus infection, and the rate of low-risk strains human papilloma virus 6 and human papilloma virus 11 detected in condyloma acuminatum is high [4]. Chesson et al. noted that more than 80% of the population would have an human papilloma virus infection before the age of 45 years [5]. Approximately, 70%–90% of the patients infected with human papilloma virus can eliminate the virus with their own immune system within 1–2 years, and about 10%–15% of patients continue to have the infection, as the virus evades the monitoring of the body's immune system. The current theories to explain the immune evasion by human papilloma virus are mainly related to "low expression of viral genes" and "viral regulation of host cell apoptosis" [6,7]. The former refers to the fact that after human papilloma virus infection, the virus can only survive in specific tissue cells that lack secretion of immune effectors. Because of the limited expression, the virus cannot be effectively eliminated by the immune system. The latter refers to the fact that human papilloma virus can escape host immunity by regulating apoptosis of host cells. In addition, human papilloma virus can evade host immunity by altering the functions of immune cells such as natural killer cells and cytotoxic T lymphocytes, as well as cytokines, such as interferon, interleukin, and tumor necrosis factor.

Laser therapy, cryotherapy, electrocautery, and topical cytotoxic drugs are commonly used in the treatment of condyloma acuminatum, but the therapeutic effect varies from person to person, and the recurrence rate is high, especially at the urethra, anal canal, cervix, and other locations difficult for clinicians to treat. 5-Aminolevulinic acid photodynamic therapy is a new method to treat condyloma acuminatum, as it can induce apoptosis, autophagy or necrosis of abnormal proliferative cells without damaging normal cells. Compared with conventional methods, 5-aminolevulinic acid photodynamic therapy has the advantages of a high cure rate with few side effects. A large number of clinical data confirm that 5-aminolevulinic acid photodynamic therapy has unique advantages in the treatment of condyloma acuminatum, but the specific mechanism of 5-aminolevulinic acid photodynamic therapy in the treatment of condyloma acuminatum is still unclear. Our previous studies have shown that 5-aminolevulinic acid photodynamic therapy can promote apoptosis of keratinocytes in condyloma acuminatum tissues. However, the mechanism by which 5-aminolevulinic acid photodynamic therapy promotes apoptosis remains unclear, though we hypothesized that the mechanism may be related to the changes in the expression of apoptosis-related genes in condyloma acuminatum tissues before and after the therapy. Therefore, we measured the expression of Bcl-2 and Survivin in condyloma acuminatum tissues before and after 5-aminolevulinic acid photodynamic therapy in this study.

Bcl-2 gene is the earliest-discovered apoptotic suppressor gene. It is located on the outer membrane of mitochondria, rough endoplasmic reticulum, and nuclear membrane. Bcl-2 proteins are dimeric and can inhibit apoptosis induced by various factors, thereby contributing to cell immortalization. Previous studies [8] reported that Bcl-2 was highly expressed in condyloma acuminatum tissues. Bcl-2 may play a role in the occurrence and development of condyloma acuminatum by inhibiting apoptosis and promoting the proliferation of condyloma acuminatum cells. In this study, we found that the expression rate and the expression intensity of the apoptosis inhibitor Bcl-2 in condyloma acuminatum cells after 5-aminolevulinic acid photodynamic therapy were significantly reduced. It is speculated that 5-aminolevulinic acid photodynamic therapy may promote condyloma acuminatum cell apoptosis by reducing the expression of Bcl-2, which may be one of the mechanisms by which 5-aminolevulinic acid photodynamic therapy promotes condyloma acuminatum cell apoptosis.

Survivin is a new member of the apoptosis suppressor protein family. It is located on human chromosome 17q25 and consists of 142 amino acids. Survivin can inhibit apoptosis, regulate cell cycle, and participate in the development and progression of malignant tumors [9]. It has an unparalleled anti-apoptotic ability compared with other members. Survivin expression is highly tissue-specific. It is not expressed in normal differentiated tissues, but only in embryonic tissues, multiple tumor tissues, and some proliferative lesions. The degree of expression is related to tissue origin, pathological type, and the degree and the prognosis of the malignancy. Previous studies have found that Survivin, an apoptosis inhibitor, is highly expressed in condyloma acuminatum tissues [10]. Survivin may promote the development and progression of condyloma acuminatum by inhibiting the apoptosis of condyloma acuminatum cells. This study found that the expression rate and the expression intensity of Survivin in condyloma acuminatum tissue cells after 5-aminolevulinic acid photodynamic therapy were significantly reduced. Thus, our results suggest that 5-aminolevulinic acid photodynamic therapy might inhibit the proliferation of warts by reducing the expression of Survivin, promoting the apoptosis of condyloma acuminatum cells, and inhibiting the proliferation of condyloma acuminatum cells, which may also be another mechanism of 5-aminolevulinic acid photodynamic therapy-mediated promotion of condyloma acuminatum cell apoptosis.

In addition, Survivin and Bcl-2 may have a common pathway in regulating apoptosis [11]. The Survivin gene and the Bcl-2 gene are regulated by GC-rich TATA-less promoters, and both of them enhance cell proliferation after transcriptional activation. It is speculated that the Survivin gene and the Bcl-2 gene may have a common transcriptional activation mechanism, and their synergistic effect plays an anti-apoptotic role [11,12]. This study found that the positive expression rate and the expression intensity of Survivin and Bcl-2 in condyloma acuminatum tissue cells were significantly decreased after 5-aminolevulinic acid photodynamic therapy. Spearman rank correlation analysis showed that the decreased expression of Survivin and Bcl-2 was positively correlated, suggesting that 5-aminolevulinic acid photodynamic therapy could synergistically promote the apoptosis of condyloma acuminatum cells, inhibit their proliferation, and promote the remission of warts by reducing the expression of Survivin and Bcl-2.

5. Conclusion

5-Aminolevulinic acid photodynamic therapy may promote the apoptosis of keratinocytes in condyloma acuminatum by reducing the expression of Survivin and Bcl-2, and we infer that this may be one of the mechanisms by which 5-aminolevulinic acid photodynamic therapy exerts its therapeutic effect during treatment of condyloma acuminatum.

Prospect

We have conducted a preliminary study on the mechanism by which 5-aminolevulinic acid photodynamic therapy affects the apoptosis of condyloma acuminatum keratinocytes, although the molecular mechanism requires further investigation. In the future, we aim to explore the exact mechanism of photodynamic effect on apoptosis through extensive research to enrich the theories on 5-aminolevulinic acid photodynamic therapy for condyloma acuminatum.

Disclosure

All authors report no conflicts of interest relevant to this article.

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