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Inhibitory mechanism of ternary fusion protein MSIK on HPV virus

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Abstract. – OBJECTIVE: We investigated whether the ternary fusion protein (MBP-SERPINA3-IFN-κ, MSIK) can inhibit the proliferation of human papillomavirus (HPV) and the possible mechanism of this inhibitory effect.

MATERIALS AND METHODS: First, the purchased MSIK protein was prepared into MSIK protein solutions of different concentrations, and then epithelial cells and 300 mice were involved in the research. The HSV-1 virus was used as the infection pathogen to explore the mechanism of MSIK reinhibition of HPV virus. The virus content in each sample was detected by PCR technology.

RESULTS: Epithelial cells treated with different concentrations of MSIK had inhibitory effects on the invasion and replication of HSV-1 virus. MSIK protein had a positive effect on wound healing in mice at the same time, and it had an inhibitory effect on the invasion of HSV-1 virus, and the higher concentration of MSIK, the better the effect.

CONCLUSIONS: MSIK can quickly help wound healing and inhibit the replication of HSV-1 virus. It can also effectively prevent the influx of HPV virus and replication has a positive effect on the prevention and treatment of HPV virus.

Key Words:
Ternary fusion protein, Human papilloma disease, Pathogen, Inhibitory effect.

Introduction

According to relevant studies, the number of HPV-infected patients in China is increasing year by year. At the same time, there are more than 130,000 new cervical cancer patients every year, and the mortality rate of this group of people is also increasing[1]. With the development of science and technology, ternary fusion protein (MSIK) includes three proteins: maltose binding protein (MBP), SERPINA3, and INF-k. Among them, MBP can increase the stability and solubility of fusion proteins, SERPINA3 is a chymotrypsin inhibitor that can help the fusion of epithelial cells with minimal wounds, INF-k is an interferon that can effectively activate the antiviral ability of epithelial cells and inhibit virus proliferation[2,3]. Therefore, this study aims to explore the mechanism of MSIK on the replication and proliferation of HPV virus, and to provide a certain reference for the treatment of HPV virus.

Materials and Methods

Experimental Materials

This study purchased MSIK protein cells and experimental mice. Among them, the ternary fusion protein (configured into protein solutions with two concentrations of 10 μg/ml and 50 μg/ml) was used in the study. The experimental strain was HSV-1 virus with a similar life cycle to HPV, as it is difficult to cultivate HPV virus *in vitro*. Keratinocytes were used as cells and 300 BALB/c mice were selected for the study.

Viral RNA Extraction and Detection

All cells to be tested were extracted by the TRIzol method. Then, the total RNA was used for reverse transcription to synthesize cDNA, and finally the reverse transcription was performed for Q-PCR detection. A total of 40 cycles were performed, and then relevant products were detected by a fluorescence signal at the end of the cycle.

To Examine the Effect of Ternary Fusion Protein on HSV-1 in Keratinocytes

In the experiment, a six-well plate was used to culture keratinocytes in a constant temperature incubator (37°C, 5% CO_2) for 18-24 hours. When the confluence of the cultured cells was 70%, the cells were treated with different concentrations of MSIK protein, and the treatment time required more than 2 h, the control group was treated with phosphate-buffered saline (PBS) as a control. Finally, all subjects were infected with HSV -1 virus for 24 hours. Finally, the total RNA of each group

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of keratins was extracted, reverse transcribed, and the expression level of HSV-1 virus was detected by Real-time quantitative PCR. The virus amount was compared to obtain the relative expression amount.

The Effect of the Ternary Fusion Protein on the Back Wound of Mice and the Virus of HSV-1

All mice were anesthetized (4% chloral hydrate) before the experiment, and their back hair was removed with a razor. After disinfection, a hole punch (6 mm in diameter) was used to create wounds on the back of the mice.

After the treatment, 150 mice were selected for grouping, and the mice were divided into three groups (50 mice in each group) by random number method as follows: the control group (treated with PBS solution), the experimental group 1 (using 10 μl concentration of 10 μg/ml MSIK protein solution, which was dripped to the wound), and the experimental group 2 (10 μl of 50 μg/ml MSIK protein solution was used to drip into the wound). The wound healing on the back of the mice was observed every day for 7 days after the mice were treated, and the size of the wound was recorded.

The remaining 150 mice were divided into 3 groups according to the random number method (infection experiment group 1, infection experiment group 2, infection experiment control group with 50 mice in each group), and each group was treated with different concentrations of MSIK solution and treated with PBS solution. Infection experiment control group, HSV-1 infection experiment was performed on mice in each group after 18 hours, 10 μl of HSV-1 virus solution with a concentration of 1×10^9/ml was added dropwise to the wounds of mice in each group of mice, and then the above was repeated. After 3 days of operation, all experimental mice were sacrificed (neck breaking method), and 2 mm of skin tissue around the wound and skin of the mice was extracted for processing. Then, the final amount of virus replication in the mouse tissue was detected. The result was the control group treated with PBS. The virus replication amount was used as the benchmark, and the virus replication amount of the other groups was divided by the control group to obtain the relative virion quality.

Table I. Relative expression of HSV-1 virus in keratinocytes (%).

MSIK protein concentration (μg/ml)	The relative expression of HSV-1 virus
0	2169.2%
10	985.5%
50	421.8%

Statistical Analysis

SPSS 22.0 software (SPSS Inc., Armonk, NY, USA) was selected as the data processing tool, along with the count data expressed as [(%)]. Then, the analysis of variance (ANOVA) or χ^2 test was carried out. $p < 0.05$ was used as the standard to evaluate whether the difference was statistically significant.

Results

Relative Expression of HSV-1 Virus in Keratinocytes

It can be seen from the results that under the same conditions, the higher the MSIK protein content, the lower the HSV-1 virus expression level in the keratinocytes (Table I).

The Back Healing of the Three Groups of Mice

The results showed that the wounds healed better after the mice were treated with MSIK protein on the back, and the higher the concentration of MSIK, the better the healing effect (Table II).

The Effect of MSIK on HSV-1 Virus in Mouse Wounds

According to the research results, MSIK protein has an inhibitory effect on HSV-1 virus at the end of the third day of the experiment, and at the same time, the inhibitory ability of the virus at different concentrations is different. The higher the concentration of MSIK, the stronger the inhibitory ability (Table III).

Discussion

Cervical cancer is a common malignant tumor in gynecology. Although many advances have been made in treatment, conventional treatments mostly involve hysterectomy. For women who still need pregnancy, it is necessary to meet the patient's fertility needs to the greatest extent under the premise of ensuring safety[5-7]. These patients need fertility-sparing surgical treatments and it is hard to suggest them to preserve, before surgical treatments, their fertility by an ovarian stimula-

Table II. Comparison of wound healing in each group of mice (%).

Group	1d	2d	3d	4d	5d	6d	7d
Control group	6.65±0.42 mm	6.08±0.39 mm	5.97±0.41 mm	5.84±0.42 mm	5.43±0.41 mm	4.78±0.37 mm	4.27±0.42 mm
Experiment 1 group	6.57±0.39 mm	5.94±0.44 mm	5.56±0.38 mm	5.33±0.43 mm	4.72±0.45 mm	4.21±0.46 mm	3.82±0.47 mm
Experiment 2 group	6.55±0.46 mm	5.87±0.39 mm	5.25±0.49 mm	4.96±0.41 mm	4.33±0.42 mm	3.89±0.40 mm	3.37±0.39 mm
F	0.802	3.445	35.405	55.317	85.293	59.951	55.288
p	0.4504	0.0345	<0.001	<0.001	<0.001	<0.001	<0.001

tion by antagonist protocol and freeze all their gametes by vitrification for future pregnancy[8-10].

HPV virus is a small double-stranded DNA virus with many genotypes, which can cause proliferative lesions of the skin and mucous membranes and is divided into high-risk and low-risk types according to its carcinogenicity[4]. In recent years, studies have found that the incidence of cervical cancer has a gradual upward trend, and there is a trend of younger people, which seriously threatens the life safety of women, and high-risk HPV virus is one of the high-risk factors leading to cervical cancer. Therefore, the prevention and treatment of HPV virus have always been an important research content. The study by Pett et al[11] found that high risk human papillomavirus (hr-HPV) integration is a critical genetic event in cervical carcinogenesis. The integration rate is highly associated with the severity of cervical intraepithelial neoplasia, making it potential marker for carcinogenesis and progression of cervical cancer.

Since HPV virus needs to enter the human body through a minimally invasive opening of epithelial cells to complete the infection, there are many factors that lead to the occurrence of minimally invasive openings and easy to occur, and the recovery of the human body from the minimally invasive wound is relatively slow, which makes it difficult for the human body to carry out an effective immune response to HPV infection[12]. In addition, scholars[13] have shown that the immune system of female cervical epithelial cells is not active, which makes the body often unable to effectively deal with HPV infection.

In this study, three proteins, maltose binding protein (MBP), SERPINA3, and INF-k, were selected to make a ternary fusion protein, and the mechanism of their intervention on HPV virus was explored. It can be seen from the results that MSIK has inhibitory effects on HSV-1 virus in mouse wounds and epithelial cells. At the same time, MSIK protein also has a positive effect on wound healing in mice. There may be several reasons for this. In the study, the wounds on the back of mice instilled with MSIK healed faster than the wounds of untreated mice. The HSV-1 virus was difficult to penetrate at the healing site, which ultimately led to lower HSV-1 virus content in the wound and periwound of the MSIK mice. On the other hand, the MSIK fusion protein is fused with IFN-κ interferon, but the E6 gene in the HPV virus can inhibit the expression of IFN-κ interferon. In epithelial cell experiments, the decreased intracellular viral load of HSV-1 may be related to the increased content of IFN-κ interferon. However, in practice, IFN-κ interferon has a strong hydrophobicity. Then, MBP is added in the fusion as it has strong hydrophilicity, which can increase the stability of MSIK expression on the cell surface, and it provides help for the dissolution of ternary fusion protein.

Conclusions

The inhibitory effect of MISK protein on HPV virus is mainly reflected in shielding virus invasion and inhibiting virus replication. On the one hand, it is related to the promotion of wound fusion by SERPINA3; on the other, it is related to the inhibition of virus replication by IFN-κ. At the

Table III. Analysis of the effect of MSIK on HSV-11 virus in mouse wounds (%).

Group	MSIK protein concentration (μg/ml)	Relative viral load
Infection experimental control group	0	1
Infection experiment group 1	10	0.58
Infection experiment group 2	50	0.34

same time, MBP protein increases the solubility of MSIK and provides help for the expression of the two active ingredients, finally making MSIK effective to inhibit the replication of HPV virus, which provides certain support for the subsequent use of MSIK to prevent and treat HPV virus.

Conflict of Interest

The authors declare that they have no conflict of interests.

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None.

Availability of Data and Materials

The data used to support the findings of this study are available from the corresponding author upon request. No additional unpublished data are available.

Ethics Approval

This study reporting animals was approved by Medical Institutional Review of Hunan Environment Biological Polytechnic.

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