ORIGINAL ARTICLES

Inhibitory effect of survivin-shRNA on A431 cutaneous squamous cell carcinoma

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Abstract

Objective: To observe the effect of survivin-shRNA on A431 cutaneous squamous cell carcinoma (SCC) and explore the molecular biological mechanism of nuclear factor κB (NF-κB) in the inhibition of survivin-shRNA on cutaneous SCC.
Methods: Survivin-shRNA adenovirus vector was constructed to screen out the best interference sequence. Nude mice were subcutaneously inoculated with the cultured A431 cell suspension to duplicate A431 transplanted tumor models. These mice were randomly divided into the blank control group, the rAd-EGFP negative control group, the rAd-survivin-shRNA transfection group and the Res positive control group, with 5 mice in each group. The corresponding reagents were injected into the tumors. All the mice were sacrificed on the 20^{th} day. The tumor tissues were isolated, with tumor volume and tumor weight measured, the tumor growth curves were accordingly plotted and the tumor inhibition rate was consequently calculated. Hematoxylin-eosin (HE) staining was used to observe the cellular morphology of the tumors. TUNEL was applied to the detection of cell apoptosis. Western blot was applied to the detection of the expression of Survivin, inhibitor of NF-κB (IκB), P65, P53 and Caspase-3.
Results: As to the transplanted tumors, tumor volume and tumor weight were decreased in nude mice in the rAd-survivin-shRNA transfection group and the Res positive control group, in comparison with the blank control group and the rAd-EGFP negative control group, the differences were of statistical significance ($p < .05$); The results of TUNEL showed that the apoptosis rates were significantly increased in the rAd-survivin-shRNA transfection group and the Res positive control group, in comparison with the blank control group and the rAd-EGFP negative control group, the difference was statistically significant ($p < .05$); In the rAd-survivin-shRNA transfection group and the Res positive control group, the microscopic observation showed a small amount of sparsely-distributed tumor cells, degenerative liquefactive necrosis, cancer cell shrinkage and round-shape, and karyopycnosis; The expressions of Survivin and P65 proteins were decreased in the rAd-survivin-shRNA transfection group and the Res positive control group, in comparison with the blank control group and the rAd-EGFP negative control group, the differences were statistically significant ($p < .05$), while the expressions of IκB, P53 and Caspase-3 proteins were significantly increased, in comparison with the blank control group and the rAd-EGFP negative control group, the differences were statistically significant ($p < .05$).
Conclusions: Survivin-shRNA can inhibit the growth of the transplanted tumors in cutaneous SCC nude mice, one of the mechanisms is to promote the apoptosis of tumor cells and inhibit the growth of SCC transplanted tumors by inhibiting the NF-κB signaling pathway and then activating the tumor suppressor gene P53.

Key Words: Survivin-shRNA, NF-κB, Cutaneous squamous cell carcinoma, Inhibitory effect

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Squamous cell carcinoma (SCC) is a common malignant tumor in terms of dermatology, and the morbidity is increasing year by year.[1] Traditional treatment methods for SCC include surgical resection, radiotherapy, photodynamic therapy, laser therapy, cryotherapy, etc. However, these methods are ineffective for the tumors occurring on exposed or special sites and large-size tumors. Targeting survivin-shRNA has an effect in inhibiting the proliferation of tumor cells and promoting the apoptosis of tumor cells. Nuclear factor κB (NF-κB) is of great significance to the occurrence and development of tumors. The signal transduction pathway can control the apoptosis of tumor cells by regulating various downstream apoptotic genes such as Bax and survivin.[2,3] Survivin is a newly-discovered inhibitor of apoptosis protein (IAP) in recent years. By observing the effect of survivin-shRNA on the growth of human SCC A431 cell strains in nude mice with transplanted tumors, this study was designed to investigate the role of NF-κB signaling pathway in the process where survivin-shRNA induces the apoptosis of SCC transplanted tumors.

1 Data and methods

1.1 Materials and animals

Human cutaneous SCC A431 cell strains, Hela cells, rAd-EGFP and shRNA expression vectors (pYr-1.1) were provided by Changsha Yingrun Biotechnology Co., Ltd., which also constructed rAd-survivin-shRNA adenovirus in addition. Packaging cells HEK 293 were purchased from ATCC (USA), DMEM (High Glucose) medium, fetal bovine serum (FBS), pancreatin and antibiotics were purchased from Invitrogen (USA), KGA Annexin-V-FITC Apoptosis Detection Kits were purchased from Shenzhen Jingmei Biotechnology Co., Ltd., β-acting was purchased from Abgent (USA), and ECL Chemiluminescence Detection Kits were purchased from Pierce (USA). SPF BALB/c-nu mice, 6-8 weeks old, 18-22 g, were purchased from Hunan SJA Laboratory Animal Co., Ltd., Experimental Animal Quality Certification Number: SCXK (Xiang) 2011-0003.

1.2 Experimental methods

1.2.1 Cell culture

A431 cells were cultured in a high-glucose DMEM medium (containing 10% FBS, 100 u/ml penicillin and 100 u/ml streptomycin) under saturated humidity of 37°C and 5% CO_2. When the cell growth was in the logarithmic phase, the cells were digested with 0.25% trypsin solution and made into single cell suspension, and the hemocytometer was used to count the number of cells, in order to adjust the cell density to 5×10^7 cells/ml, and 4 ml of suspension was taken for standby.

1.2.2 Survivin-shRNA adenovirus vector was constructed to screen out the best interference sequence

According to the selection principle of shRNA target site, 3 survivin-shRNA sequences were optimally chosen from Yingrun shRNA database as target sequences to be screened for follow-up experiments. Target sequence 1 was: GAGGCTGGCTTCATCCACTGC; target sequence 2 was: GAGCCAAGAACAAAATTGC; and target sequence 3 was: GAAAGTGCGCCGTGCCAT. Survivin-shRNA primers were designed on the basis of the above-mentioned target sequences: forward survivin-sh1 was 5'-CACCGAGGCTGGCTTCATCCACTGCCTCGAGGCAGTGGATGAAGCCAGCCTCTTTTTTG-3', and reverse survivin-sh1 was 5'-AGCTCAAAAAAGAGGCTGGCTTCATCCACTGCCTCGAGGCAGTGGATGAAGCCAGCCTC-3'; forward survivin-sh2 was 5'-CACCGAGCCAAGAACAAAATTGCTTCAAGAGAGCAATTTTGTTCTTGGCTCTTTTTTG-3', and reverse survivin-sh2 was 5'-AGCTCAAAAAAGAGCCAAGAACAAAATTGCTCTCTTGAAGCAATTTTGTTCTTGGCTC-3'; forward Survivin-sh3 was 5'-CACCGAAAGTGCGCCGTGCCATCTTCAAGAGAGATGGCACGGCGCACTTTCTTTTTTG-3', and reverse survivin-sh3 was 5'-AGCTCAAAAAAGAAAGTGCGCCGTGCCATCTCTCTTGAAGATGGCACGGCGCACTTTC-3'. The above primers were constructed and synthesized with the help of shRNA expression vectors. The primers were dissolved by the annealed buffer, and then placed in boiling water so that it was naturally annealed. (The formula of the annealed buffer was: 10 mmol Tris-HCl pH 8.0, 50 mmol NaCl and 1 mmol EDTA). There is a Sac I restriction enzyme cutting site (GAGCTC) on the original vector of pYr-1.1. If the foreign shRNA fragment is successfully inserted into this site, it will bring in a new Sac I restriction enzyme cutting site. The fragment length between the two Sac I is approximately 930 bp. Therefore, pYr-1.1-survivin-shRNA can be combined with Sac I to apply to restriction enzyme digestion. The cultured Hela cells were divided into 5 groups: (1) KB group: blank control group; (2) NC group: Hela cells transfected with pYr-1.1-NC plasmids; (3) Survivin-1 group: Hela cells transfected with pYr-1.1-survivin-sh1 plasmids; (4) Survivin-2 group: Hela cells transfected with pYr-1.1-survivin-sh2 plasmids; (5) Survivin-3 group: Hela cells transfected with pYr-1.1-survivin-sh3 plasmids. At the set time point of 48 h after transfection, the cells were collected for quantitative real-time polymerase chain reaction (qRT-PCR) and Western blot detection.

1.2.3 Duplicating A431 transplanted tumor models in nude mice

The cultured A431 cell suspension was extracted with a 1-ml syringe and inoculated into SPF-class BALB/c-nu

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mice at a dosage of 0.2 ml per mouse, and the inoculation quantity was about 1×10^7 cells. The inoculation site was at the subcutaneous part of the right axilla of a nude mouse. Before inoculation, it was required to disinfect the skin with povidone-iodine and observe the living condition of the nude mice. On the 12^{th} day after inoculation, nude mice with a tumor volume of about 100-200 mm^3 were chosen as the experimental models.

1.2.4 Experimental grouping and treatment

20 mice with neoplasia were randomly divided into the blank control group, the rAd-EGFP negative control group, the rAd-survivin-shRNA transfection group and the Res positive control group, with 5 mice in each group. The corresponding reagents (0.15 ml) were injected into the tumors, 1 time/2 days, 6 times in total. After mice with tumors were treated, it was required to record the condition of tumor growth every 4 days and sacrifice all the mice by means of cervical dislocation on the 20^{th} day after appropriate medication. The tumor tissues were taken for follow-up experiments. (1) Blank control group: simple injection of DMEM culture medium with no serums and antibiotics; (2) rAd-EGFP negative control group: rAd-EGFP; (3) rAd-survivin-shRNA transfection group: rAd-survivin-shRNA; (4) Res positive control group: 50 mg/kg Res was injected.

1.2.5 Measurement of tumor volume and weight and calculation of tumor inhibition rate

Tumor volume was measured with digital vernier caliper. After BALB/c-nu mice were subcutaneously inoculated with A431 cells, it was observed that tumors began to grow on the 8th day after inoculation. Since then, tumors in each group had been grown day by day. Tumor volume basically reached 100 mm^3 or more on the 12^{th} day. The tumor growth curves were plotted on the basis of tumor volume and inoculation time. On the 20^{th} day after appropriate medication, the tumors were separated from the nude mice that were sacrificed for the measurement of tumor weight and the calculation of tumor inhibition rate. Inhibition rate of tumor weight (%) = (tumor weight of rAd-EGFP negative control group - tumor weight of the experimental group)/tumor weight of rAd-EGFP negative control group × 100%; inhibition rate of tumor volume (%) = (tumor volume of rAd-EGFP negative control group - tumor volume of the experimental group)/tumor volume of rAd-EGFP negative control group × 100%. The tumor volume was calculated by use of the following formula: $V = L \times S^2/2$, whereas, V was the tumor volume (mm^3), L was the long diameter of the tumor (mm), and S was the short diameter of the tumor (mm). As to the tumors with irregular shape: L was considered as the longest diameter, and S was considered as the intermediate value of the short diameter perpendicular to the longest diameter.

1.2.6 Hematoxylin-eosin (HE) staining

After the tumor tissues were dewaxed and hydrated, these tissues were stained conventionally with HE, added by neutral balsam and mounted with a cover slip. The cellular morphology was observed under the microscope.

1.2.7 The application of TUNEL to the detection of cell apoptosis

Fluorescein-marked dUTP can be added to the 3'-OH terminus of broken DNA molecules in apoptosis cells under the action of deoxynucleotide TdT, which can be detected with the fluorescence microscope. Since normal or proliferating cells have almost no DNA breakage, no 3'-OH is formed and rarely marked. TUNEL results were interpreted as follows: apoptotic nuclei showed green. 5 fields of view were selected for each section at high magnification (400 ×), with apoptosis cells counted among 100 cells in each field of view, 5 fields of view were chosen in total (> 500 cells). The positive expression (the percentage of positive nuclei in total nuclei in each field of view) was detected and considered as cell apoptosis index (AI).

1.2.8 The application of Western blot to the detection of the expression levels of survivin, P53, IκB, P65 and Caspase-3 proteins

Discarded the medium in the culture bottle, into which an appropriate amount of normal saline was added. Shaked the bottle gently to wash the cells and discarded the scrubbing solution. According to the number of samples, BCA working solution was prepared by mixing Solution A with Solution B under the volume ratio of 50:1. After SDS-PAGE electrophoresis and transfer membrane, the membrane was moved to the plate with blocking buffer containing 5% skim milk (dissolved with TBST). Blocked it for 1 h on a decolorization shaker at room temperature. Substrate A was mixed with an equal volume of substrate B in EP tube. After 1 min, membrane proteins were placed into the Seal-A-Meal with its face up. In the Seal-A-Meal, the mixture of substrates A and B was added onto the membrane, with the remnant removed and the membrane wrapped. Placed it in an X-ray film holder, scanned or photographed the film. Gray analysis was performed by use of Gel Documentation System.

1.3 Statistical methods

SPSS 19.0 statistical software was applied to the statistical analysis. The measurement data were represented by mean ± standard deviation ($\bar{x} \pm s$). One-way ANOVA was used for the comparison among groups, SNK-q test was used for the comparison between two groups, and χ^2 test was used for categorical data. The difference ($p < .05$) was statistically significant.

2 Results

2.1 Restriction enzyme digestion, sequencing and screening results of the best interference sequences

The restriction enzyme digestion of pYr-1.1-survivin-shRNA Sac I was shown in Figure 1. From the result, the cloning of pYr-1.1-survivin-sh1, pYr-1.1-survivin-sh2 and pYr-1.1-survivin-sh3 proved to be correct. The above-mentioned pYr-1.1-survivin-sh1, pYr-1.1-survivin-sh2 and pYr-1.1-survivin-sh3 clones were sent for sequencing. Sequencing primer was: 5'-GACTATCATATGCTTACCGT-3'.

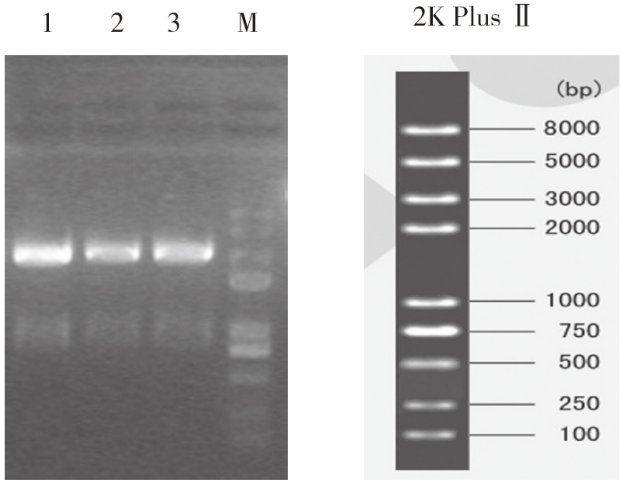

Figure 1: pYr-1.1-survivin-shRNA restriction enzyme digestion
1: pYr-1.1-survivin-sh1 was digested with Sac I;
2: pYr-1.1-survivin-sh2 was digested with Sac I;
3: pYr-1.1-survivin-sh3 was digested with Sac I;
M: 2K Plus II

Figure 2: The sequencing result of pYr-1.1-survivin-sh1

The sequencing result of pYr-1.1-survivin-sh1 was shown in Figure 2. Sequence 0: to acquire survivin-shRNA-1 sequence, Sequence 1: the sequence of pYr1.1-survivin-sh1.ab1 sequencing, sequence alignment tool: DNAssist.

The sequencing result of pYr-1.1-survivin-sh2 was shown in Figure 3. Sequence 2: to acquire survivin-shRNA-2 sequence, Sequence 3: the sequence of pYr1.1-survivin-sh2.ab1 sequencing, sequence alignment tool: DNAssist.

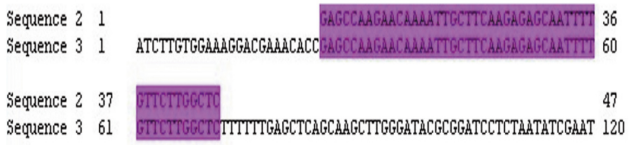

Figure 3: The sequencing result of pYr-1.1-survivin-sh2

The sequencing result of pYr-1.1-survivin-sh3 was shown in Figure 4. Sequence 7: to acquire survivin-shRNA-3 sequence, Sequence 9: the sequence of pYr1.1-survivin-sh3.ab1 seqeuncing, sequence alignment tool: DNAssist.

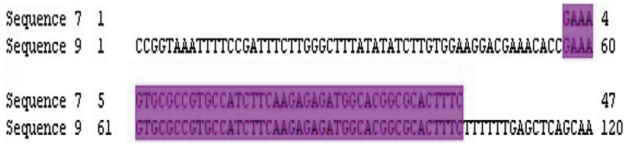

Figure 4: The sequencing result of pYr-1.1-survivin-sh3

From the above results, pYr-1.1-survivin-sh1, pYr-1.1-survivin-sh2 and pYr-1.1-survivin-sh3 were constructed successfully. After qRT-PCR and Western-blot detections, survivin-sh2 showed obvious interference effects and were chosen as the best interference sequence. qRT-PCR was used to detect the expression of survivin mRNA (see Figure 5). Western blot was used to detect the expression of survivin proteins (see Figure 6).

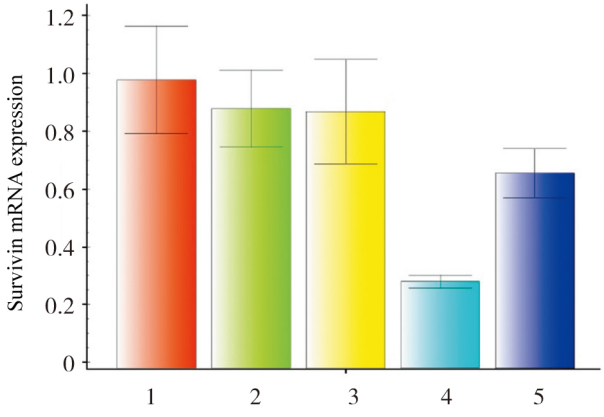

Figure 5: The expression of mRNA in each group of cells
1: KB group; 2: NC group; 3: Survivin-1 group;
4: Survivin-2 group; 5: Survivin-3 group

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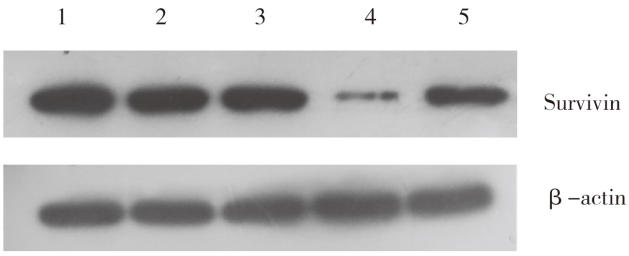

Figure 6: The expression of survivin protein in each group of cells
1: KB group; 2: NC group; 3: Survivin-1 group;
4: Survivin-2 group; 5: Survivin-3 group

2.2 Comparison of tumor growth curve, tumor volume, tumor weight and tumor inhibition rate between groups

None of the animals in each group died before the end of the experiment, and all of them were involved in the measurement of indicators. As can be seen from the growth curve of tumors in each group (see Figure 7), in comparison with the blank control group and the rAd-EGFP negative control group, the growth of tumors was inhibited more significantly in the rAd-survivin-shRNA transfection group and the Res positive control group after corresponding intervention. At the end of the intervention, the differences in tumor volume and tumor weight between the rAd-survivin-shRNA transfection group & the Res positive control group and the blank control group & the rAd-EGFP negative control group were statistically significant ($p < .05$). Besides, the rAd-survivin-shRNA transfection group and the Res positive control group both showed higher inhibition rates, which were 45.39% and 56.55% (inhibition rate of tumor volume) respectively, and 41.25% and 56.25% (inhibition rate of tumor weight) respectively (see Table 1).

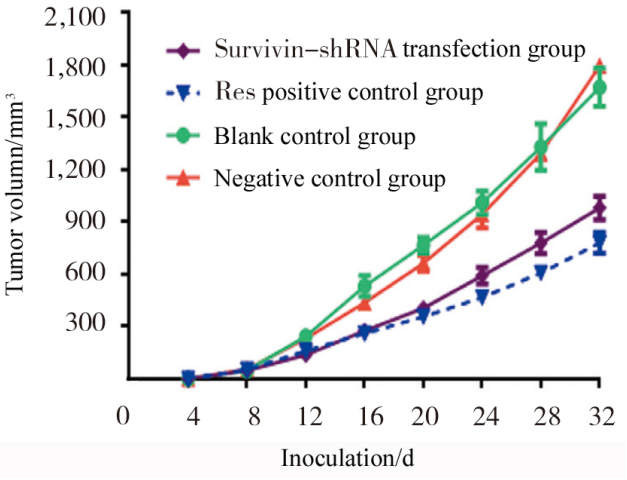

Figure 7: Tumor growth curve schema of transplanted tumors in each group of nude mice

Table 1: Comparison of inhibition rates of tumor volume and tumor weight between groups ($\bar{x} \pm s$)

Group	Tumor Volume/mm^3	Inhibition Rate of Tumor Volume/%	Tumor Weight/g	Inhibition Rate of Tumor Weight
Blank Control Group	1,668.98 ±242.56	-	1.06 ±0.23	-
rAd-EGFP Negative Control Group	1,792.36 ±55.10	-	1.60 ±0.29	-
rAd-survivin-shRNA Transfection Group	978.86 ±150.61*	45.39	0.94 ±0.32$^{*\#}$	41.25
Res Positive Control Group	778.73 ±131.80$^{*\#}$	56.55	0.7 ±0.23$^{*\#}$	56.25
F value	49.273	-	9.791	-
p value	.000	-	.010	-

Note. * In comparison with the rAd-EGFP negative control group, $p < .05$; $^{\#}$ in comparison with the blank control group, $p < .05$

2.3 HE staining results of transplanted tumor tissues in each group

HE staining sections were observed under the light microscope, showing that there was necrosis in the tumor tissues of each group, and the boundaries between necrotic lesions and tumor tissues were clear. In the blank control group and the rAd-EGFP negative control group, there was only a little necrosis in each section, most of which were tumor tissues, clustered densely and grew vigorously, with a large amount of polygonal cells of large and deep nuclei. The rAd-survivin-shRNA transfection group and the Res positive control group had more necrotic cells, a small amount of sparsely-distributed tumor tissues, degenerative liquefactive necrosis, cancer cell shrinkage and round-shape, and karyopycnosis (see Figure 8).

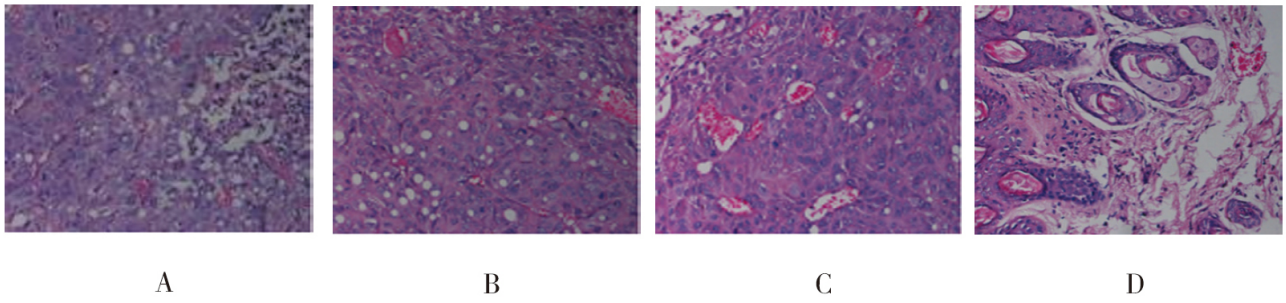

Figure 8: Histopathological changes of transplanted tumors in each group under the light microscope (× 200)
A: Blank control group; B: rAd-EGFP negative control group; C: rAd-survivin-shRNA transfection group; D: Res positive control group

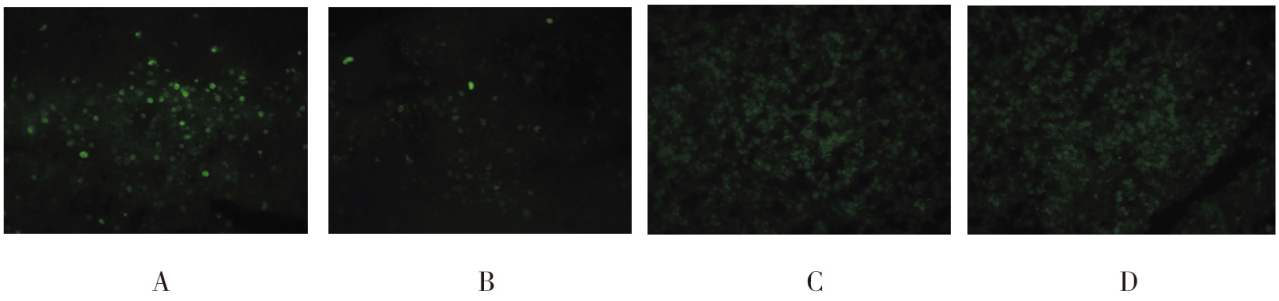

Figure 9: The positive expression of apoptotic cells in each group under the fluorescence microscope (× 400)
A: Blank control group; B: rAd-EGFP negative control group; C: rAd-survivin-shRNA transfection group; D: Res positive control group

2.4 TUNEL detection results of transplanted tumor tissues in each group

TUNEL was used to detect the positive expression level of cell apoptosis, in which there was a difference between groups, and the difference was statistically significant (F = 22.177, p = .000) after one-way ANOVA. In comparison with the Res positive control group, the difference in the positive expression level of cell apoptosis between the rAd-survivin-shRNA transfection group and the Res positive control group was not statistically significant (t = 1.569, p = .169). Compared with the blank control group and the rAd-EGFP negative control group, the rAd-survivin-shRNA transfection group showed a more increased positive expression level of cell apoptosis, and the difference was statistically significant (t = 4.769 and 6.289, p = .001 and .000). The positive expression of apoptotic cells under the fluorescence microscope can be seen in Figure 9.

2.5 The expression of FAS proteins in each group

The expressions of survivin, P65, P53, IκB and Caspase-3 proteins in each group were detected by use of Western blot, there were differences in the expressions of survivin, P65, P53, IκB and Caspase-3 between groups, and the differences were statistically significant (p < .05). Compared with the Res positive control group, the differences in the expressions of survivin, P65 and IκB between the Res positive control group and the rAd-survivin-shRNA transfection group were not statistically significant (t = 0.045, 1.451 and 0.369; p = .965, .185 and .722). The rAd-survivin-shRNA transfection group showed lower expressions of P53 and Caspase-3 proteins, and the differences were statistically significant (t = 3.476 and 3.041; p = .020 and .023). Compared with the blank control group and the rAd-EGFP negative control group, the rAd-survivin-shRNA transfection group showed lower expressions of survivin and P65 proteins, and the differences were statistically significant ($t_{survivin}$ = 16.974 and 19.594, t_{P65} = 9.808 and 8.385, all p = .000), with higher expressions of P53, IκB and Caspase-3 proteins, and the differences were statistically significant (t_{P53} = 14.645 and 15.236, all p_{P53} = .000; $t_{I\kappa B}$ = 2.795 and 2.807, all $p_{I\kappa B}$ = .023; $t_{Caspase-3}$ = 6.206 and 6.731, all $p_{Caspase-3}$ = .000, see Table 2). The detection results of the expression of FAS proteins in each group can be seen in Figure 10).

Table 2: Comparison in the expression of FAS proteins between groups ($\bar{x} \pm s$)

Group	Survivin	P65	P53	IκB	Caspase-3
Blank Control Group	0.95 ±0.06	0.92 ±0.09	0.34 ±0.07	0.45 ±0.22	0.41 ±0.08
rAd-EGFP Negative Control Group	0.99 ±0.05	0.97 ±0.93	0.36 ±0.06	0.43 ±0.23	0.39 ±0.12
rAd-survivin-shRNA Transfection Group	0.25 ±0.07*#	0.34 ±0.10*#	0.94 ±0.06*#	0.86 ±0.24*#	0.81 ±0.12*#
Res Positive Control Group	0.25 ±0.07*#	0.72 ±0.03*#	1.25 ±0.02*#	0.92 ±0.30*#	1.18 ±0.24*#
F value	218.075	72.588	75.904	5.362	33.298
p value	.000	.000	.000	.010	.000

Note. * In comparison with the rAd-EGFP negative control group, $p < .05$; # in comparison with the blank control group, $p < .05$

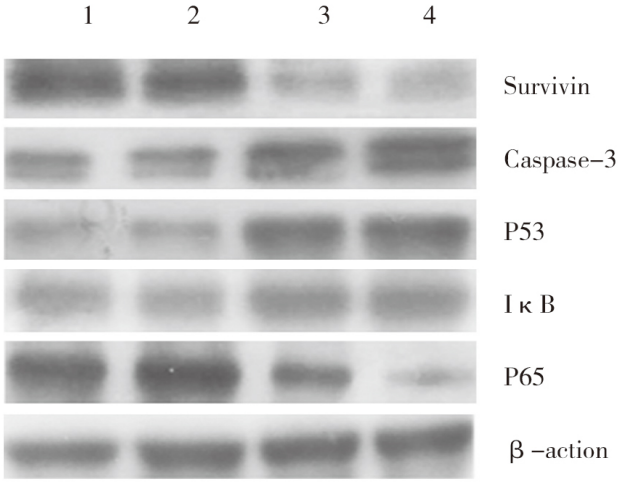

Figure 10: The expression of FAS proteins in each group 1: Blank control group; 2: rAd-EGFP negative control group; 3: rAd-survivin-shRNA transfection group; 4: Res positive control group

3 Discussion

Survivin is one of factors in IAP family, with the lowest molecular weight and the strongest effectiveness. Differing from the other members in IAP family, survivin is highly expressed in a variety of human malignant tumors, but not expressed or lowly expressed in differentiated and mature tissues. This characteristic makes it a marker protein in the study of tumor markers and anti-tumor treatment.[4]

Tumors result from the regulation of multiple gene interactions, RNA interference (RNAi) technology is to silence the gene after transcription by use of small interfering RNA (siRNA) that causes specific degradation of target messenger RNA (mRNA). RNA interference technology can silence multiple tumor-associated genes simultaneously. Hence, it has become a research focus in the area of tumor treatment. Vector-mediated shRNA expression technology can inhibit the expression of target genes in a long-term and stable manner, and can be used to construct an ideal experimental cell model. Therefore, shRNA expression vector technology has become a powerful tool for the tumor-targeted gene therapy.[5]

P53 is a nuclear transcription factor that can be activated by a series of activations of stress factors such as DNA damage, hypoxia and oncogenes. One of the key roles of P53 is to maintain genetic stability by participating in the regulation of cell cycle checkpoints. As a tumor suppressor gene, P53 also plays a key role in the prevention of tumorigenesis. Its regulation in survivin also has a cell cycle dependence. Survivin, as an IAP specifically expressed in G2/M phase, is involved in the regulation of cell cycle and apoptosis. As an apoptosis-activated gene, P53 plays its biological function by inhibiting the activity of survivin at cell cycle checkpoints, while survivin can regulate P53 at the post-transcriptional level. The two factors participate in the regulation of tumorigenesis by regulating cell cycle and apoptosis.[6,7]

NF-κB is a key transcription factor present in the occurrence and development of tumors, and it participates in a variety of cellular biological reactions. It can enter into the nucleus after activation and regulate the expressions of encoding cytokines, growth factors, cell adhesion molecules and FAS proteins. NF-κB family includes 5 members: NF-κB1 (P50 and its precursor P105), NF-κB2 (P52 and its precursor P100), RelA (P65), RelB and c-Rel, which can form different homodimers and heterodimers. Different signaling pathways result in NF-κB activation of different dimers, leading to different biological results.[8] Of which, RelA (P65) is mainly involved in cell proliferation and effective immune responses.

IκB is the inhibitory protein of NF-κB. In unstimulated cells, the binding of NF-κB and IκB is isolated from the cytoplasm to prevent NF-κB from binding to DNA, consequentially inhibiting the biological function of NF-κB. The activation of NF-κB signal is enabled by extracellular stimulations. They are recognized by receptors and trans-

duced to cells, leading to the activation of IκB kinase (IKK), which results in phosphorylation and ubiquitination of IκB. In the end, IκB is degraded so that NF-κB protein that is released is transported to the nucleus and bound to the corresponding target sequence to activate genes and initiate transcription.[9] As to B-cell lymphoma, colorectal cancer and T-cell leukemia, it has been reported that NF-κB can bind to survivin promoter region and result in the enhancement of transcription.[10]

Studies by Cui et al.[11] showed that in bladder cancer, NF-κB can facilitate cell cycle progression, and the activated NF-κB signaling pathway can help to up-regulate the expression of survivin and then reduce cell apoptosis, thereby increasing cell proliferation. Zeng et al.[10] found in the study of esophageal cancer that up-regulating the expression of survivin can increase the upstream gene IKK of P65 at the transcription level and promote the increase in the expression of P65 protein. On the contrary, it can inhibit the expression of P65 protein, and survivin can bind to IKK promoter region. Over-expressed survivin can activate the activity of P65, inhibit cell apoptosis and promote cell proliferation. Bao JX et al.[12] found that silencing P65 gene could reduce the gene expression of survivin in colon cancer.

The experimental results showed that targeting survivin-shRNA could inhibit the increase in tumor volume and weight of transplanted tumors in nude mice. Under the microscope, the rAd-survivin-shRNA transfection group had fewer sparsely-distributed tumor cells, degenerative liquefactive necrosis, cancer cell shrinkage and round-shape, and karyopycnosis. According to TUNEL detection, the apoptosis rate was increased in the rAd-survivin-shRNA transfection group. Targeting survivin-shRNA can inhibit the growth of transplanted tumors of SCC nude mice and promote the apoptosis of tumor cells, which once again has proven that it has an inhibitory effect on tumors. The expressions of survivin, P53, IκB, P65 and Caspase-3 proteins were detected by use of Western blot, and the results showed that, in the rAd-survivin-shRNA transfection group, the expressions of P53, IκB and Caspase-3 proteins were increased, while the expressions of survivin and P65 proteins were decreased, which was consistent with the results in related literatures.[10] Therefore, it is speculated that one of the mechanisms of survivin-shRNA to inhibit the growth of transplanted tumors of SCC nude mice may be that, targeting survivin-shRNA inhibits the expression of survivin, and then inhibits the expression of the upstream gene IKK of P65 to promote the binding of IκB to P65, inhibit the expression of P65 and further promote the apoptosis of tumor cells. Ultimately, it leads to the inhibition in the growth of transplanted tumors of SCC. Secondly, targeting survivin-shRNA reduces the expression of survivin and activates Caspase-3 to promote cell apoptosis. In addition, the apoptotic protein that is formed can further activate P53 to intensely expand the apoptotic effect.

Conflicts of Interest Disclosure

The authors have no conflicts of interest related to this article.

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