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MicroRNA15b regulates apoptosis of cutaneous squamous cell carcinoma SCL-1 cell line: a mechanism study

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Abstract. – OBJECTIVE: Cutaneous squamous cell carcinoma is a malignant tumor, which is mostly common in skin epidermis or appendages. microRNA has been proved to regulate growth and survival of cells. Our study was focused on the effect of microRNA15b on cell viability and apoptosis of cutaneous squamous cell carcinoma SCL-1 cell line.

MATERIALS AND METHODS: MicroRNA15b and control microRNA were synthesized and transfected into SCL-1 cells, respectively. Effects of transfection on SCL-1 cells were evaluated by MTT assays and flow cytometry. Western Blot was performed to examine the expression of survivin. MicroRNA15b-transfected SCL-1 cells were further intervened by siRNA targeting survivin or surviving-overexpressing plasmid. Their apoptosis were assessed by flow cytometry.

RESULTS: Compared with control microRNA transfection, microRNA15b transfection significantly reduced cell viability, enhanced apoptosis and decreased protein expression of survivin. Inhibition of survivin expression enhanced microRNA15b-induced apoptosis of SCL-1 cells, while enhancement of survivin expression attenuated the apoptosis-promoting effect of microRNA15b on SCL-1 cells.

CONCLUSIONS: MicroRNA15b reduced the cell viability and promoted the apoptosis of SCL-1 cells via down-regulating the expression of survivin. MicroRNA15b could be a potential therapeutic target for cutaneous squamous cell carcinoma.

Key Words:
microRNA15b, Survivin, Cutaneous squamous cell carcinoma, SCL-1cell line, Apoptosis.

Introduction

Cutaneous squamous cell carcinoma is a malignant tumor in the skin, which is mostly common in skin epidermis or appendages. Cutaneous squamous cell carcinoma is seriously threatening people's health[1]. In China, clinical doctors have constructed some common treatments according to individual characteristics of patients, including radiotherapy, surgery, chemotherapy and combination therapy[2]. Nevertheless, these therapies have several drawbacks and thereby limited treatment efficacy[3,4]. For example, surgical therapy is not suitable for aged patients or patients with serious complications. Chemotherapy may result in severe toxic and side effect. Radiotherapy may also damage normal cells[5-7]. Therefore, novel therapy with better efficacy is warranted for clinical treatment of cutaneous squamous cell carcinoma.

As a hotspot and difficulty of current studies, molecular targeting treatment is a potential therapy for cutaneous squamous cell carcinoma, but its efficacy largely depends on the selection of highly specific molecular target[8]. Moreover, further exploration is needed for molecular targeting treatment for cutaneous squamous cell carcinoma. Survivin has been proved to be a promising molecular target in multiple cancers, such as lung cancer, liver cancer and intestinal cancer[9,10]. Previous studies [11-13] have shown that survivin is mainly expressed in cancer tissue and embryonic tissue, while down-regulation of survivin has anti-tumor effects. What's more, survivin can specifically promote the growth, proliferation and metastasis of cancer[14,15]. We, thereby, hypothesized that survivin might regulate cellular events in SCL-1 cells and thus might be a molecular target for cutaneous squamous cell carcinoma.

MicroRNA is a member of the small RNAs family. MicroRNA is an important regulator with multiple functions, including cell cycle regulation, apoptosis, autophagy and oncogenesis[16-18]. Some studies[19,20] have shown that microRNA15b induces apoptosis of multiple cancers, including lung cancer, colon cancer and liver cancer. However, no investigations have been reported on the

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effect of microRNA15b on cutaneous squamous cell carcinoma. In this study, we investigated the effect and mechanism of microRNA15b on the cell viability and apoptosis of cutaneous squamous cell carcinoma SCL-1 cells.

Materials and Methods

Cell Line and Reagents

Cutaneous squamous cell carcinoma SCL-1 cell line, Annexin-V FITC and caspase-3 kits were purchased from Shanghai Sangon Biological Engineering Company (Shanghai, China). MTT assays were purchased from Beyotime Institute of Biotechnology (Shanghai, China). Fetal bovine serum (FBS) and cell medium were purchased from Santa Cruz Biotech. (Santa Cruz, CA, USA). Actin monoclonal antibody and survivin antibody (mouse anti-human) were purchased from Sigma-Aldrich (St. Louis, MO, USA). RNA extraction kit and reverse transcription kit were purchased from Dingguo Changsheng Biotech. (Beijing, China). Survivin plasmid was purchased from Unibio Biotech. (Shanghai, China). MicroRNA15b and control microRNA were synthesized by GenePharma Biotech (Shanghai, China). The sequences were as follows: MicroRNA15b, 5'-GTAATAACACAGATGTAAGAATGTCAACA-3' and 5'-TACAGAATTAAGATGTCACAGAGTAAACA-3'; Control microRNA, 5'-CCACACCTCATACTACATTTGGCCACAGA-3' and 5'-CTGCCAGACCTCTACATCAATCACAGCA-3'.

Cell Culture

Cutaneous squamous cell carcinoma SCL-1 cell line was cultured with routine protocol (5% CO_2, 37°C)[9]. Transfection was performed when cell reached a confluence of 80%.

MTT Assays

After 12-h culture, MTT reagents were added into the medium and SCL-1 cells were incubated for an additional 12 h. DMSO (250 μL) was then added to terminate the reaction, and the mixture was incubated for 45 min. Optical density (460 nm) was detected using a microplate reader. Cell growth curve was established as previously described[11].

Intervention of microRNA15b and Survivin

Transfection of microRNA15b and control microRNA was performed with routine protocol[11]. siRNA of survivin was synthesized as previously described[12]. Survivin plasmid (1 μg) was mixed with lipidosome for the intervention of survivin. SiRNA of survivin and survivin plasmid were transfected into SCL-1 cells, respectively. Cell culture was maintained for 72 h.

Flow Cytometry

SCL-1 cells in difference groups were collected by centrifugation and treated with 140 μL of FITC-Annexin V. Flow cytometry was performed after 15 min.

RT-PCR

RNA extraction and RT-PCR were performed using the appropriate kit according to the manufacture's manual[13].

Western Blot

SCL-1 cells in difference groups were collected and lysed on the ice. Electrophoresis was performed with a routine protocol. Transmembrane and seal for 2 h at room temperature. The membrane was incubated with actin monoclonal antibody and survivin antibody for 3 h. The relative expression of survivin was analyzed.

Caspase-3 Activity Assay

SCL-1 cells in difference groups were collected for caspase-3 activity assay. After cell suspension, cell lysis solution was treated with Ac-DEVD-pNA (5 mM) for 20 min at 37°C. Optical density was measured to determine the caspase-3 activity.

Statistical Analysis

Measurement data are expressed as X ± Standard deviation and analyzed using SPSS 17.0 software (IBM SPSS, Chicago, IL, USA). The difference among groups was analyzed by one-way ANOVA followed by Fisher's LSD tests when $p<0.05$ in ANOVA. p-value < 0.05 was considered to be statistically significant.

Results

MicroRNA15b Transfection Inhibited Growth of SCL-1 Cells

The result of MTT assay was showed in Figure 1. Compared with control microRNA (1 μg) transfection and blank control, microRNA15b (1 μg) transfection significantly decreased cell viability of SCL-1 cells ($p=0.0073$).

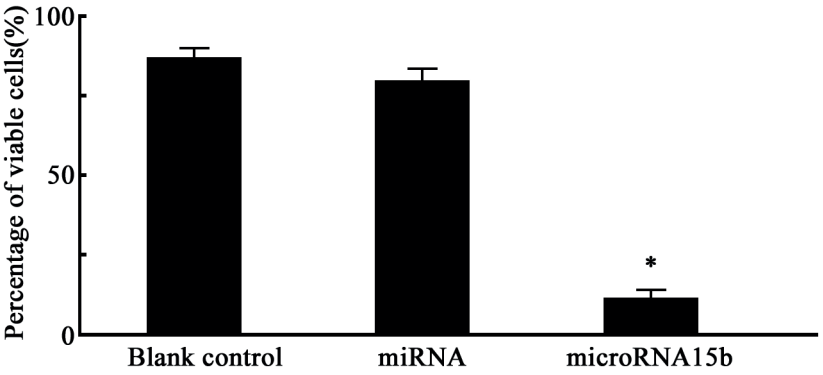

Figure 1. Analysis of the cell viability under different transfection. *p=0.0073.

MicroRNA15b Transfection Induced Apoptosis of SCL-1 Cells

Results of flow cytometry and caspase-3 activity assay were showed in Figure 2 and Figure 3. Compared with control microRNA (1 μg) transfection, microRNA15b (1 μg) transfection significantly induced apoptosis of SCL-1 cells (p=0.003). Moreover, caspase-3 activity was significantly inhibited after microRNA (1 μg) transfection (Figure 2A and Figure 3).

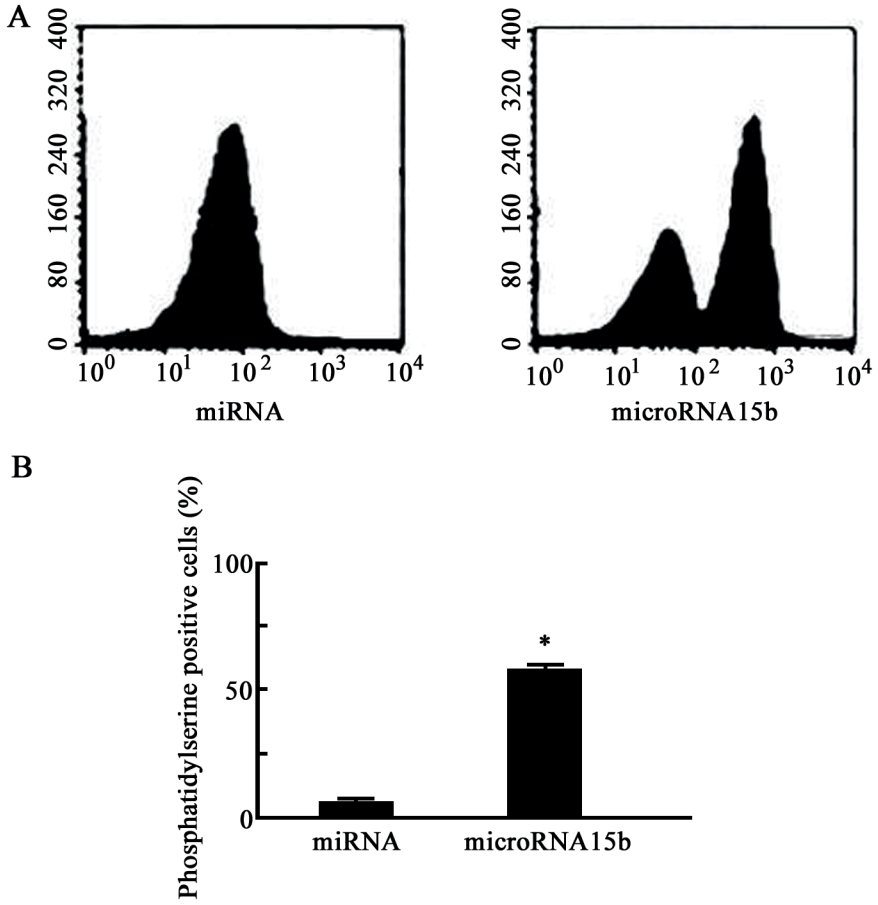

Figure 2. ***(A)*** Caspase-3 activity assay for microRNA (1 μg) transfection and microRNA15b (1 μg) transfection. ***(B)*** Flow cytometry for microRNA (1 μg) transfection and microRNA15b (1 μg) transfection. *p=0.003.

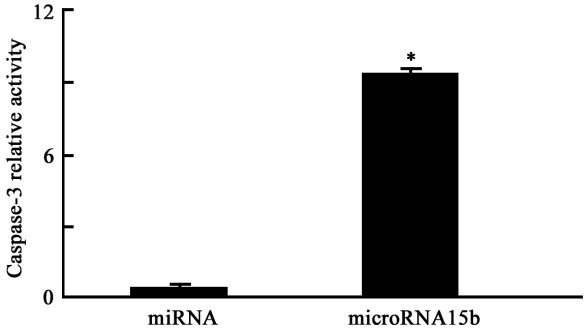

Figure 3. Analysis of caspase-3 relative activity. *p=0.0024.

MicroRNA15b Transfection Decreased Expression of Survivin

RT-PCR and Western blot of survivin were showed in Figure 4 and Figure 5. Compared with control microRNA (1 μg) transfection, microRNA15b (1 μg) transfection not only significantly decreased mRNA expression of survivin in SCL-1 cells, but also inhibited protein expression of survivin in SCL-1 cells.

Down-expression of Survivin Enhanced Apoptosis Induced by microRNA15b

As showed in Figure 6A, siRNA decreased expression of survivin. Moreover, compared microRNA15b transfection, microRNA15b transfection+siRNA further enhanced apoptosis of SCL-1 cell, verified by caspase-3 activity assay in Figure 6B.

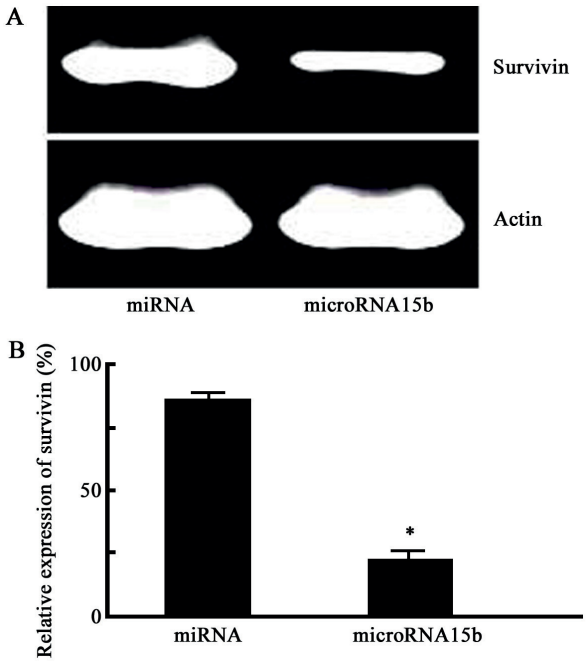

Figure 4. mRNA of survivin in SCL-1 cells under different transfection. ***(A)*** mRNA bands under different transfection. ***(B)*** Analysis of mRNA under different transfection. *p=0.0042.

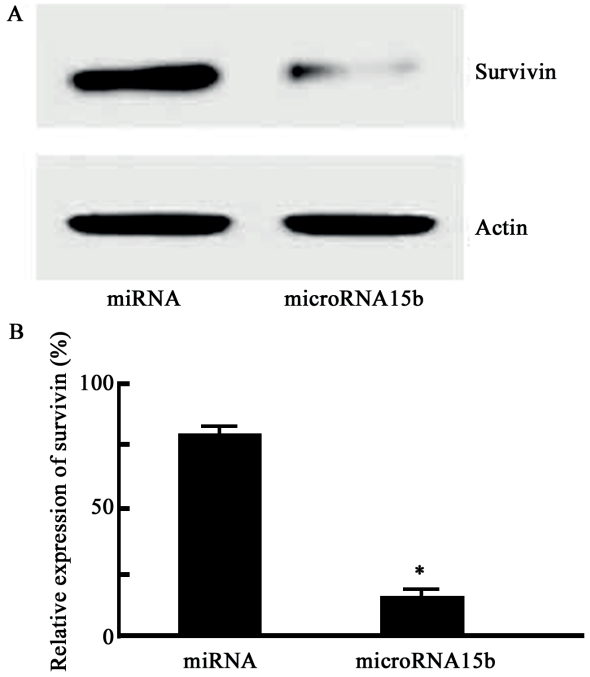

Figure 5. Protein expression of survivin in SCL-1 cells under different transfection. ***(A)*** Protein bands under different transfection. ***(B)*** Analysis of protein under different transfection. *p=0.0031.

Over-expression of Survivin Attenuated Apoptosis Induced by microRNA15b

As showed in Figure 7A, survivin plasmid increased expression of survivin. Moreover, compared microRNA15b transfection, microRNA15b transfection+siRNA apoptosis attenuated the apoptosis-promoting effect of microRNA15b on SCL-1 cell line, verified by caspase-3 activity assay in Figure 7B.

Discussion

Cutaneous squamous cell carcinoma is a malignant tumor with poor prognosis[21]. Studies[22] have shown that its mortality is gradually increasing in recent years. Accordingly, promoting-apoptosis studies on about cutaneous squamous cell carcinoma have both theoretical and practical significance. Researches[23] have proven the potential efficacy of molecular target therapy for cancers.

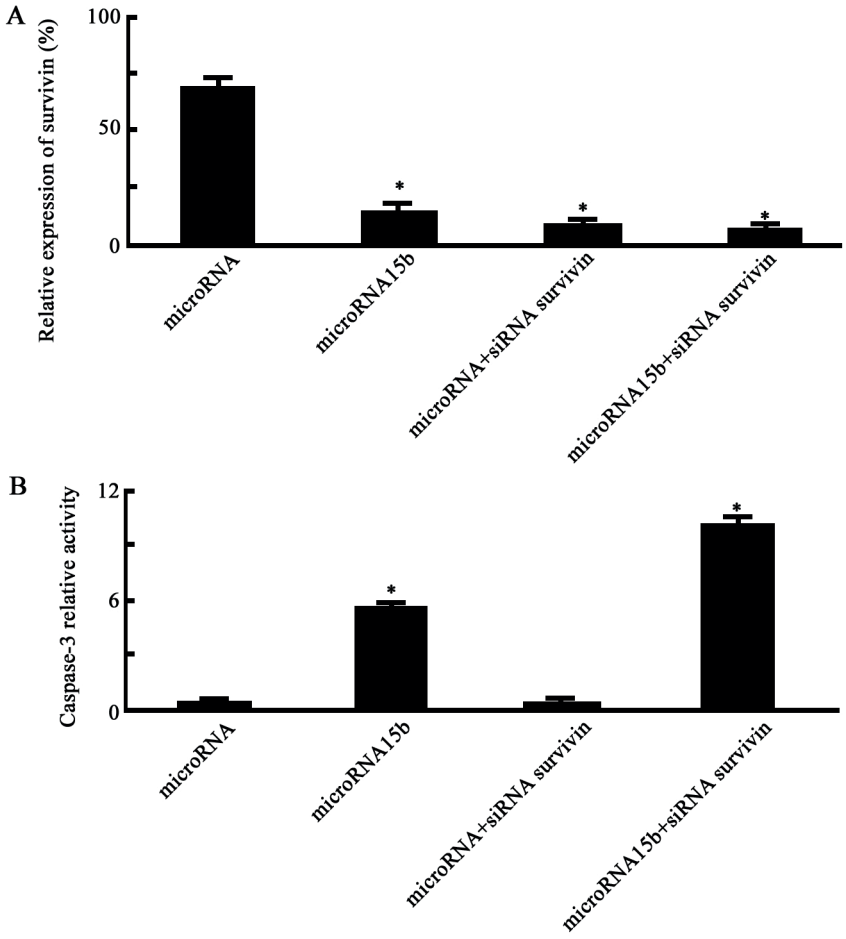

Figure 6. ***(A)*** Analysis of expression of survivin under different transfection. $^{*}p<0.05$, compared with control microRNA transfection. ***(B)*** Caspase-3 activity assay under different transfection. $^{*}p<0.05$, compared with control microRNA transfection.

However, no specific molecular target was detected for cutaneous squamous cell carcinoma[24].

MicroRNA15b is involved in apoptosis and cell cycle of multiple cancers[25,26], but it is unclear whether microRNA15b influences cutaneous squamous cell carcinoma or not. Our work demonstrated the effect of microRNA15b on cutaneous squamous cell carcinoma. Our findings showed that microRNA15b inhibited cell growth of SCL-1 cells, and enhanced apoptosis of SCL-1 cells, suggesting that microRNA15b alleviated cutaneous squamous cell carcinoma.

Results

Our study has three main results: 1) microRNA15b repressed cutaneous squamous cell carcinoma, meanwhile, microRNA15b decreased expression of survivin; 2) Down-expression of survivin enhanced apoptosis induced by microRNA15b; 3) Over-expression of survivin abrogated apoptosis-promoting effect of microRNA15b. These results indicated that microRNA15b repressed via reducing the expression of survivin.

As an important member of apoptosis-related proteins, survivin plays a regulator role in cancers, including lung cancer, liver cancer and colon cancer. Survivin promotes the infiltration and metastasis of cancers. Our study showed that microRNA15b transfection resulted in a decreased expression of survivin, suggesting that survivin was also a risk factor for cutaneous squamous cell carcinoma.

There are some limitations in our paper. No clinical specimen or animal models were collected to validate our findings. Further experiment should be focused on the effect of microRNA15b

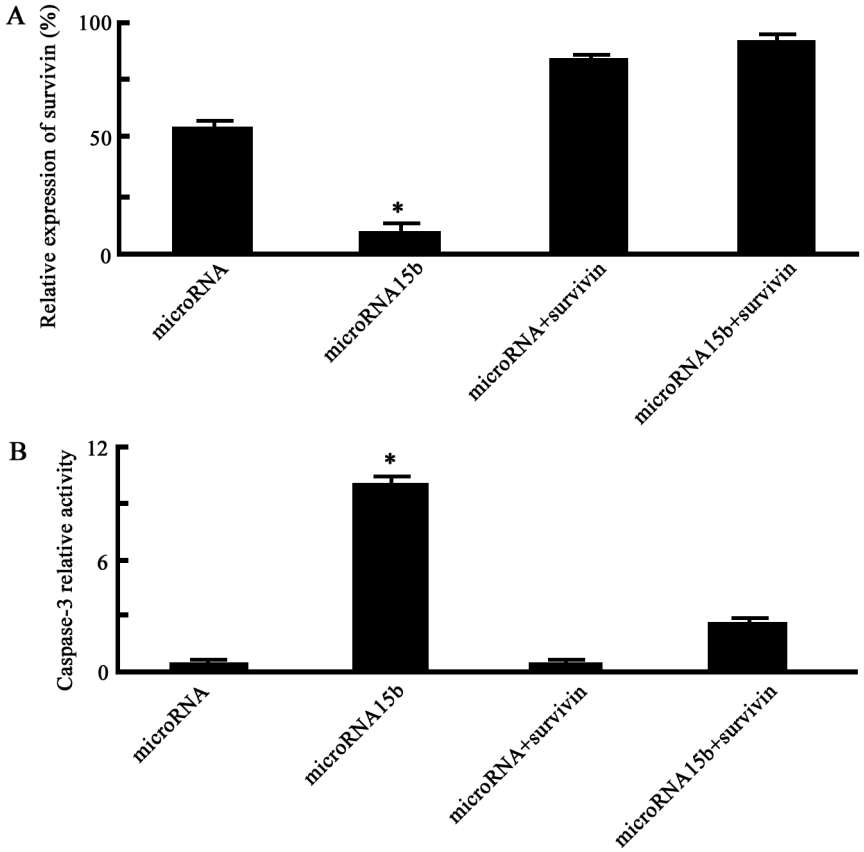

Figure 7. ***(A)*** Analysis of expression of survivin under different transfection. $^{*}p<0.05$, compared with control microRNA transfection. ***(B)*** Caspase-3 activity assay under different transfection. $^{*}p<0.05$, compared with control microRNA transfection.

on cutaneous squamous cell carcinoma *in vivo*. Moreover, although our study has observed the inhibitory effect of microRNA15b on the expression of survivin, the underlying mechanism is not yet clarified.

Conclusions

microRNA15b can alleviate cutaneous squamous cell carcinoma line via repressing the expression of survivin. MicroRNA15b is a promising molecular target for the treatment of cutaneous squamous cell carcinoma.

Acknowledgments

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Conflict of interest

The authors declare no conflicts of interest.

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