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The inhibition of resveratrol to human skin squamous cell carcinoma A431 xenografts in nude mice

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ABSTRACT

Squamous cell carcinoma (SCC) is one of the commonest dermatological malignancies. Resveratrol (Res) is one type of polyphenolic compound which was first identified from the roots of *Veratrum grandinorum* in 1940. The previous studies found that Res can promote apoptosis of a variety of tumor cell, especially SCC cells. However it is rare to study the inhibition mechanism of Res in the animal model. In this study, through the establishment of human cutaneous SCC A431 xenografts in nude mice, we observed Res inhibition effect and investigated the inhibition mechanism by checking the expression of apoptosis-related factors, p53, ERK and survivin. The results showed that the xenograft volume and weight of Res groups were less than those of the control groups ($P<0.05$), but the net body mass of nude mice of Res groups was not significantly different from the control groups ($P>0.05$). The apoptotic index of Res groups were significantly higher than the control groups ($P<0.05$). The protein and mRNA expression of p53 and ERK were statistically positively correlated ($P<0.05$) and significantly increased in Res high- and medium-dose groups compared with the control groups ($P<0.05$). Moreover, the protein and mRNA expression of SVV were negatively correlated with p53 ($P<0.05$) and lower than the control groups ($P<0.05$). The results demonstrate Res inhibitory effect and indicate that the inhibition mechanism of Res is to upgrade the protein and mRNA expression of p53 and to downgrade the protein and mRNA expression of SVV, thus inducing the apoptosis of tumor cells.

1. Introduction

Squamous cell carcinoma (SCC) is a common dermatological malignancy which keeps increasing year by year. In the global scale, skin cancer accounts for 30% of newly diagnosed cancers every year [1], however the therapeutic method for SCC is currently quite limited. Surgical resection associated with chemotherapy is the most preferred treatment, but it would usually damage the patient's appearance. Moreover the postoperative chemotherapy drug would inevitably cause a series of side effects, not to mention the extremely high cost of treatment, so it is naturally of significant clinical and social importance to search for a new and effective treatment for squamous cell carcinoma by natural medicine.

Resveratrol, 3,4′,5-trihydroxy-trans-stilbene, is a non-flavonoid polyphenol compound containing stilbene structure similar to that of estrogen diethylstilbestrol. It can be widely found in some natural plants or fruits, e.g., grape, pine tree, polygonum, cassia, and peanut, which is a phytoalexin produced by many plants when they are subject to biotic or abiotic stresses, such as fungous infections, ultraviolet radiation, etc. In addition to the improvement of plant disease resistance, Res shows many other biological activity and pharmacological

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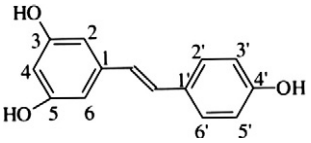
effects such as anti-tumor, anti-cardiovascular disease, anti-inflammatory, antioxidant, liver protection, nervous system protection, etc. It has become a highly important natural active ingredient with great medicinal value and market prospects. As for its anti-tumor effect, Res has become the research focus in cancer chemoprevention and chemotherapy thanks to Jang et al. who published a series of studies about the inhibition effect of Res on the origin, enhancement and development of cancer in *Science* in 1997 [2–8].



In recent years, the relative studies have shown that Res can inhibit a variety of tumor cell growth in vitro [9], and inhibit the human cutaneous squamous cell carcinoma A431 cell proliferation and induce apoptosis [10]. Previously, the inhibitory effect of Res has been reported in skin cancer in nude mice induced either by UVB irradiation or by 7,12-DMBA [1,11], but by now there is no in-vivo study about the inhibition mechanism of Res on the relevant animal model of cutaneous squamous cell carcinoma. This study was to investigate the inhibition mechanism of Res to human skin squamous cell carcinoma A431 xenograft tumor through the establishment of human skin squamous cell carcinoma A431 xenograft model in nude mice, observing the inhibition of Res on the xenograft tumor growth, and observing the impact of Res on the apoptosis-related factors survivin, p53 expressions by Western-blot and real-time PCR assay. We believe that this study would provide the valuable information of further identifying the drug target site.

2. Material and methods

2.1. Reagents

The resveratrol (Res) was a commercial product from Xi'an Oceanside (98%, HPLC); the fetal calf serum was a commercial product from Hangzhou Evergreen Biological Engineering & Materials Co., Ltd.; the DMEM high glucose culture medium was a commercial product from Thermo Fisher Biochemical Products (Beijing) Co., Ltd.; the rabbit anti-human/mouse p53 (BA0521), survivin (BA1420), P-ERK antibody and TUNEL apoptosis detection kit (MK1020) were from Wuhan Boster Biological Engineering Co., Ltd.; ECL chemiluminescence kit and BCA kit were from Amersham Life Sciences; SYBR Premix Ex Taq kit was from Invitrogen; TRIzol kit was from TaKaRa bio Dalian Baosheng; M-MLV reverse transcriptase kit was from Promega; the up- and down-stream primers of the relative genes and β-actin internal reference calibration gene were delegated to be designed and synthesized by TaKaRa Bio company, with the following detailed sequences: p53 (115 bp) up-stream primer 5′-TCTGACTGTACCACCATCCACTA-3′ and down-stream primer 5′-CAAACACGCACCTCAAAGC-3′, survivin (456 bp) up-stream primer 5′-CTTTCTCAAGGACCACCG-3′ and down-stream primer 5′-GCACTTTCTCCGCAGTTT-3′, ERK1 (567 bp) up-stream primer 5′-TCAACACCACCTGCGACCTT-3′ and down-stream primer 5′-GCTCCTTCAGCCGCTCCTTA-3′, β-actin (111 bp) up-stream primer 5′- CGGGAAATCGTGCGTGACAT-3′ and down-stream primer 5′-GAAGGAAGGCTGGAAGAGTG-3′.

2.2. The experimental animals and tumor strain

60 Balb/c (Nu/Nu) nude mice of SPF grade, evenly distributed in male and female, 6–8 weeks old, 18–22 g, purchased from Beijing Vital River Laboratory Animal Technology Co. Ltd. (the qualified animal license number: SCXK (Beijing) 2006–0009). The human skin squamous cell carcinoma A431 cells were purchased from Nantong Bai Ao Maike (Biomics) Biotechnology Co., Ltd., Batch No.: CRL-1555, from ATCC.

2.3. Cell culture

Human skin squamous cell carcinoma cell line A431 was adherent cultured in DMEM (high glucose type) complete medium (containing 10% fetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin), in the incubator under the condition of 37 °C, saturated humidity and 5% CO_2. The modeling phase was prepared by using the logarithmic growth of the cell.

2.4. The model preparation of human skin squamous cell carcinoma xenograft in nude mice

The nude mice were raised in clean biological laminar flow rack which had been with UV disinfection on a regular basis and maintained at a constant temperature (25 ± 2 °C) and humidity (45% to 50%). The cages and water were sterilized by high-pressure steam, and experimental operations were conducted in a sterile hood. We took the logarithmic phase of A431 cells, after digestion centrifuge-washed with serum-free culture medium twice, then adjusted cell suspension concentration to $4–6 \times 10^7$ unit/ml by serum-free culture medium. Under the sterile conditions, 0.2 ml of suspension was inoculated subcutaneously into the left armpit of each Nu/Nu nude mouse. 7–8 days after planting, the mice with about 1000 mm^3 tumor volume were selected as the experimental model.

2.5. In-vivo inhibition experiment

2.5.1. Experimental groups and drug intervention

The selected nude mice were stochastically divided into six groups: ① saline negative control group: 30 μg/g.d; ② Res low-dosage group: 10 μg/g.d; ③ Res medium-dosage group: 20 μg/g.d; ④ Res high-dosage group: 40 μg/g.d; ⑤ CTX positive control group: 20 μg/g.d; ⑥ blank control group. Each group had been exploiting the intervention of celiac injection for 14 days.

2.5.2. The tumor volume change curve and the inhibition rate calculation

After intervention, the tumor long diameter (a) and maximum perpendicular diameter (b) were measured every 4 days until the day prior to sacrificing the mice. The tumor volume was calculated according to $V = 1/2 \cdot a \cdot b^2$, and the averaged value of each group was recorded to obtain the implanted tumor growth