

## Study on mechanism of down-regulating *ikca1* molecule affecting the increment of oral squamous cell carcinoma

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**Abstract:** This research aimed to explore the mechanism of mediating the down-regulation of the calcium-activated potassium channel (IKCa1) gene expression in human oral squamous cell carcinoma Tca-8113 cells, thereby affecting cell proliferation and apoptosis. The expression level of IKCa1 in Tca-8113 cell line (oral squamous cell carcinoma) and HOEC cell line (human normal oral epithelial cell) was detected by RT-PCR. Then, after IKCa1 was knocked down in Tca-8113 cell line and HOEC cell line by RNA interference, and then cell proliferation levels were detected by cell counting kit 8 (CCK-8) method. Cell cycle distribution was detected by flow cytometry. Apoptosis was detected by membrane linked protein V-FITC/propidium iodide (PI) double-staining apoptosis detection kit. The protein expression level of IKCa1 was detected by Western Blot method. According to RT-PCR results, IKCa1 was significantly more expressed in Tca-8113 cell line than in HOEC cell line ( $P < 0.01$ ). In addition, the mRNA expression levels in the normal oral epithelium and oral squamous cell carcinoma showed the same trend. After knocking down IKCa1 in Tca-8113 cell line, the IKCa1 siRNA group significantly inhibited cell proliferation compared with the siNC control group. The results of flow cytometry showed that the proportion of apoptotic Tca-8113 cells transfected with IKCa1 siRNA was significantly increased. The ratio of early apoptosis and late apoptosis of Tca-8113 cells increased ( $P < 0.05$ ). To investigate the effect of IKCa1 on apoptosis, we tested the expression levels of apoptosis-related proteins. The results showed that the mRNA level of IKCa1 siRNA group was significantly decreased by 44.41% compared with the control group ( $p < 0.01$ ). Meanwhile, the mRNA level of Bax was significantly increased by 36.0% ( $p < 0.05$ ). Our results showed that knocking down IKCa1 in Tca-8113 cells could induce cell cycle arrest and apoptosis to produce an anti-proliferation effect, thus inhibiting the expression of IKCa1 has an anti-cancer effect in oral squamous cell carcinoma.

**Key words:** Oral squamous cell carcinoma; Tca-8113 cell; IKCa1; Cell proliferation; Cell apoptosis.

### Introduction

Cancer is one of the five leading causes of death in all societies. In the United States, cancer is the second leading cause of death after cardiovascular disease. Despite numerous advances in the treatment of cancer, oral cancer is one of the top ten causes of death due to its late detection, such as asymptomatic early-stage clinical similarities with other lesions and variation in clinical manifestations, it is one of the top ten causes of death. Although this type of cancer is rarer than other types of cancer, it is more important because the oral cavity is a strategic cavity (1-3).

The oral mucosa is pink, smooth and shiny. If everyone checks the entire mouth and mucus once a month, this is the best and most important type of test to prevent oral cancer. Very important structures, such as arteries, veins, nerves pass through the mouth and neck, the brain is very related to the mouth. If the disease develops in the upper jaw, it may also affect the brain and orbital sinuses, or if the patient is late for medical treatment, it may increase the risk of death. The disease has symptoms that everyone should pay attention to during examination, such as white, red or white and red plaques, obvious sores, hard edges, pain, burning or numbness, and obvious fungi or flower clusters (2-4).

There are many reasons for this type of cancer, and both internal and external factors may cause this type of disease. Smoking, tobacco derivatives (chewable and black smoke), alcoholic beverages consumed with tobacco, carcinogens (such as food preservatives, phenol, air pollutants), harmful radiation (such as UV and X-rays), and infections (such as Syphilis, HPV and fungal viruses). External factors affect oral cancer. Iron deficiency anemia, malnutrition (such as vitamin A deficiency), and all systemic diseases that weaken the body's immune system are some internal causes of oral cancer (1-3).

These internal and external factors activate tumor genes and turn off tumor suppressor genes, and provide conditions for cancer. New research is underway to identify tumor genes, tumor suppressor genes and oncogenic pathways that can be used. Control methods, such as gene interaction, molecular therapy, or the injection of cancer intelligence (2-5).

Oral squamous cell carcinoma is a kind of squamous cell carcinoma occurring in oral mucosa, characterized by a high degree of malignancy, the easy occurrence of local invasion and cervical lymph node metastasis, and low survival rate. Oral squamous cell carcinoma is one of the most common oral malignancies, accounting for 2 ~ 4% of all malignancies, which is the main factor lea-

ding to the incidence and death of head and neck cancer patients (1). According to the latest research (2), oral squamous cell carcinoma is eighth cancer in the world, with a 5-year survival rate of less than 60%, posing a serious threat to human life and health. In recent years, although great progress has been made in the treatment of oral squamous cell carcinoma, its high incidence and mortality have not been significantly improved, and the trend is younger. Therefore, it is of great significance to explore the therapeutic drugs and related mechanisms for the treatment of oral squamous cell carcinoma.

IKCa1 channel is the product of the KCNN4 gene, and the formation of the IKCa1 channel subunit is related to calmodulin, making these channels strictly dependent on  $Ca^{2+}$  (3). IKCa1 channel is expressed in many tissues and plays a variety of physiological roles, including regulating intracellular calcium homeostasis (4, 5). In recent years, studies have shown that in prostate cancer, liver cell cancer, endometrial cancer, breast cancer, and glioblastoma several entities such as carcinoma of IKCa1 raised (6-9) channel, and increase in cancer cells IKCa1 mRNA is due to the mechanism of mitogen-activated protein kinase (map) signal activation and the resulting mediated transcription of mRNA mediated by ap-1 (10). Physically, IKCa1 channel adjusts the channel flowing after activation of  $K^+$  and hyperpolarization by the release of  $Ca^{2+}$  stored in cells, IKCa1 channel played a cell membrane hyperpolarization and upstream channel by a positive feedback mechanism, these channels regulate the secretion of the intestinal tract of the moisture content and anionic, original hyperpolarization mediated vasodilation, cell volume, and  $Ca^{2+}$  dynamics (11-13). Some studies have shown that IKCa1 is involved in cell proliferation due to the activation of the  $K^+$  channel, which makes the hyperpolarized cells enhance calcium influx and calcium homeostasis, which is crucial for controlling cell passage through G0/G1 or G1/S (14). Studies have shown that drug blocking or gene lowering of IKCa1 in vitro can increase the expression of p21<sup>Waf1/Cip1</sup> and decrease the expression of cyclin E, thereby inhibiting the proliferation of pancreatic cancer and hepatocellular carcinoma cells (7).

Moreover, TRAM-34, as a special IKCa1 channel blocker, can also inhibit cell growth (15). In conclusion, these studies support IKCa1 as a potential molecular marker for tumor growth and progression, as well as a potential therapeutic target (16, 17). However, as a potential therapeutic target, the specific role and mechanism of IKCa1 in oral squamous cell carcinoma have not been reported. Therefore, the effect of IKCa1 on oral squamous cancer cells proliferation, and its mechanism was studied in this study, the influence of RNA interference on cell proliferation and apoptosis was further observed. Further, it can provide more basis for clinical diagnosis and accurate treatment of IKCa1 of oral squamous cell carcinoma, and more possibilities for developing new therapeutic drugs.

## Materials and Methods

### Cell culture

Tca-8113 cell line of oral squamocellular carcinoma was purchased from Chongqing Medical University and cultured with RPMI-1640 medium containing 10% FBS

(Hyclone, USA) and 1% penicillin-streptomycin (Invitrogen, USA). Human normal oral epithelial HOEC cell lines were purchased from Shenzhen Haodihuatu Biotechnology Co., Ltd., and cultured with DMEM hyperglycaemic medium (Hyclone, USA) containing 10% FBS and 1% penicillin-streptomycin double antibody. The cells were incubated in a 37 °C incubator containing 5%  $CO_2$ . When the degree of cell fusion reached about 90%, passage culture was conducted in the ratio of 1 to 2.

### RNA extraction, Reverse Transcription and PCR experiments

Total RNA in cells was extracted by TRIZOL (Invitrogen) according to the instructions. Reverse transcription and cDNA synthesis were performed according to the instructions of AidTM reverse transcription kit, and then the RT-PCR experiment was performed. Taking GAPDH as the internal reference gene, the sequence of the target gene and internal reference gene were as follows: GAPDH (forward, 5'-ATGCTGGCGCTGAGTACGTC-3', reverse, 5'-GGTCATGAGTCCCTTCCACGATA-3'); IKCa1 (forward, 5'-GTGCGTGCAGGATTTAGGG-3', reverse, 5'-TGCTAAGCAGCTCAGTCAGGG-3'); Bcl-2 (forward, 5'-ATGTGTGTGGAGAGCGTCAA-3', reverse, 5'-TAACTATCCTTGCCCCGAACG-3'); Bax (forward, 5'-CAGGATGCGTCCACCAAGAA-3', reverse, 5'-CGTGTCCACGTCAGCAATCA-3'). The amplification system was as follows: initial denaturation for 4 minutes at 94 °C, denaturation for 30 seconds at 94 °C, denaturation for 45 seconds at 57 °C, denaturation for 1 minute at 72 °C, and delayed termination for 10 minutes at 72 °C. In the results, 2<sup>-DDCT</sup> value relative quantitative method was used to compare the mRNA expression differences of target genes in each group.

### Cell cycle detection

Cells in each group at the logarithmic growth stage were digested with trypsin to prepare cell suspension, and cell count was performed.  $1 \times 10^6$  cells were placed in a 1.5-ml centrifuge tube, and the supernatant was discarded after centrifugation at 1500 r/min for 3 min. The cell suspension was prepared by resuspending cells with 500  $\mu$ l phosphate buffer (PBS) and precooled anhydrous ethanol was added, which was shaken at the same time, and the final concentration of ethanol of fixed cells was (75%) when added to 2 ml, and then incubated overnight at 4 °C. On the second day, the fixed cells were centrifuged at 1500 r/min for 5 min, and the supernatant was thrown out. After adding 1  $\mu$ l PBS, the suspended cells were centrifuged again and the supernatant was discarded. Then, 300  $\mu$ l PI dye solution was added, mixed well, and stained at room temperature for 15 min. The stained cells were filtered and tested by flow cytometry.

### Cell proliferation experiment

Cell proliferation rate was determined by cell counting kit 8 (CCK-8) method. Tca-8113 cells were placed in a 96-well culture plate with a density of 5000 cells per well and cultured overnight. An equal amount of serum-free medium containing 100  $\mu$ l/ml CCK-8 (Dojindo, Japan) was added to each well and incubated at

37°C for 3 h. The absorbance at 450 nm was measured by measuring the absorbance of the cells by means of ELX800 absorbance microplate reader (Biotek Instruments, Winooski, VT).

### Cell transfection

IKCa1siRNA was transfected with liposome 2000 reagent (Invitrogen, USA). SiRNA oligonucleotides were synthesized by Wuhan Cell Biotechnology Co., Ltd. The siRNA sequence was as follows: IKCa1 (sense strand) 5'-CACGCCTGGTTCT AC AAACATTCAA-GACGATGTTTGTAGAATCCAGCTTTTTTTG-3', antisense strand 5'-AAAAGC TG GA TTCTACAAAC ATCGTCTTGAATGTTTGTAGAATCCAGCC-3'), negative control (sense strand 5'-CA CCGCCTGATGACATTGACTTACATTCAAGACGTGTAAGTCAA-TGTCATCA GGCT TTT TTG-3', antisense strand 5'-AGCTCAAAAAAGCCTGATGACATTGACTTACACG TCTTGAATGTA AG TCAATGTC ATCAGGC-3'). The cell transfection experiment was divided into three groups: pGenesil-IKCa1siRNA+Lipo as the experimental group, pGensil-HK+Lipo as the negative control group, and RPMI-1640 +Lipo as the blank control group. Protein levels, mRNA levels, cell proliferation, and apoptosis were detected 48 h after transfection.

### Apoptosis detection

Apoptosis was detected using membrane protein V-FITC/propidium iodide (PI) double staining assay kit (Kgi, China). Cells were collected and stained based on manufacturer's instructions. Apoptosis data were collected and analyzed through flow cytometry.

### Western blot test

Tca-8113 cells from the three transfection groups were collected and centrifuged at 14000×g for 10 min to prepare lysates. Total protein concentrations were quantified using BCA (Kgi, China). Equivalent amounts of protein were loaded and separated using SDS-PAGE and then transferred to the nitrocellulose membrane. Sealed with 10% skim milk, and incubated in rabbit anti-human polyclonal antibody IKCa1 (Abcam, UK, ab215990) (1:500) overnight at 4 °C, washed with PBS for 3 times the next day, and incubated with horseradish peroxidase combined with secondary antibody (Zhong-

han Jinqiao, China, zb-2306) (1:2000) at 37 °C for 1 h. The expression of IKCa1 was quantitatively analyzed by the Odyssey infrared fluorescence scanning system (LICOR) and Odyssey v1.2 software. The relative expression of the IKCa1 protein was determined by the optical density ratio of IKCa1 to  $\beta$ -actin.

### Statistical analysis

SPSS13.0 was used for statistical analysis, t-test was applied to compare the samples of the two groups, and Anova was used to compare the samples of multiple groups. The statistical data were expressed as mean  $\pm$  standard deviation, and  $P < 0.05$  was considered as a statistically significant difference.

## Results

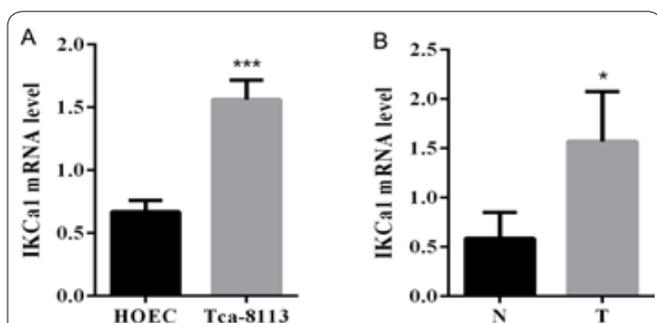
### Expression of IKCa1 in Oral squamous cell carcinoma

To investigate whether the high expression of IKCa1 in oral squamous cell carcinoma affects cell growth, we conducted the following experiment. The expression of IKCa1 in the Tca-8113 cell line (oral squamous cell carcinoma) and HOEC cell line (human normal oral epithelial cell) was detected by RT-PCR. According to the results, compared with the HOEC cell line, IKCa1 was highly expressed in the Tca-8113 cell line, with a significant difference ( $P < 0.001$ ). The mRNA expression level of IKCa1 was detected in tissues. Rt-PCR in 15 pairs of normal oral squamous epithelial tissues and oral squamous cell carcinoma tissues showed that the mRNA level of IKCa1 was also highly expressed in oral squamous cell carcinoma tissues, with significant differences ( $P < 0.001$ ). This suggests that IKCa1 is significantly up-regulated in oral squamous cell carcinoma. See Figure 1.

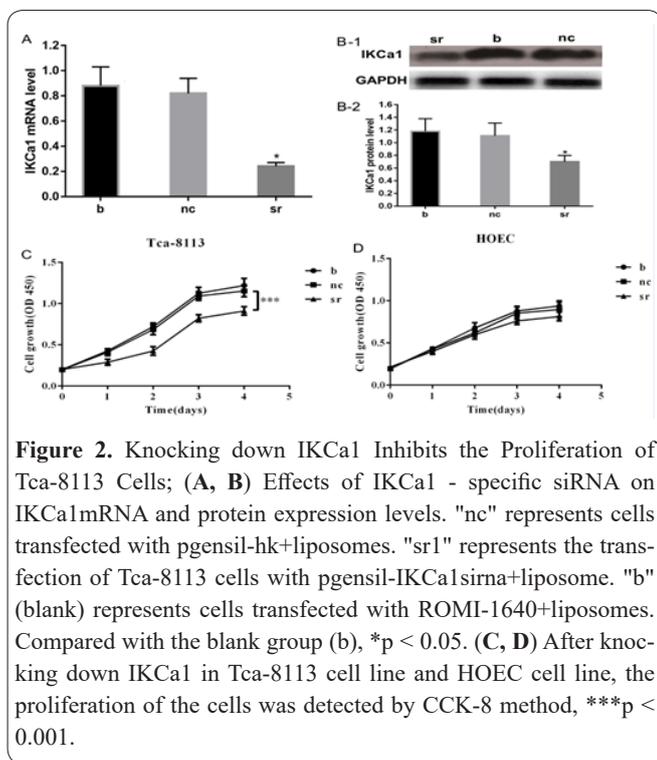
### Effects of IKCa1 on cell proliferation

In order to investigate whether the up-regulated IKCa1 in Tca-8113 cell line may affect the growth of oral squamous cell carcinoma, the following experiment was conducted. The proliferation of Tca-8113 and HOEC cells was detected by knocking down IKCa1, respectively. First, the knockdown effect of IKCa1siRNA was verified. IKCa1siRNA was transfected into the Tca-8113 cell line with transfection reagent lipo2000, and the expression of IKCa1 was detected by RT-PCR 48 hours after transfection. The results showed that IKCa1siRNA significantly reduced the mRNA expression level by 72.7% (Figure 2a,  $p < 0.05$ ). Meanwhile, the expression level of IKCa1 protein was also significantly reduced by 40.7% (Figure 2b,  $p < 0.05$ ). There was no significant difference in IKCa1 mRNA and protein expression between the blank control group and the negative control group (Figures 2a and 2b,  $p > 0.05$ ). It proves that IKCa1siRNA can play an effective role and significantly reduce the expression of IKCa1 in cells.

Then, IKCa1 was knocked down in Tca-8113 cell line and HOEC cell line, respectively, and the proliferation of the cells was detected by CCK-8 method. According to the experimental results, IKCa1siRNA group can significantly inhibit cell proliferation compared with siNC control group after IKCa1 knockdown in Tca-8113 cell line (Figure 2c). Moreover, compared



**Figure 1.** mRNA Expression Levels of IKCa1 in Squamous Cell Lines and Tissues of the Mouth; (A) mRNA expression of IKCa1 in HOEC cell line and Tca-8113 cell line. Compared with HOEC, \*\*\* $p < 0.001$ . (B) The mRNA expression of IKCa1 in squamous epithelial tissues of normal human mouth and squamous cell carcinoma tissues of human mouth ( $n=15$ ), \* $p < 0.05$ .



**Figure 2.** Knocking down IKCa1 Inhibits the Proliferation of Tca-8113 Cells; (A, B) Effects of IKCa1 - specific siRNA on IKCa1mRNA and protein expression levels. "nc" represents cells transfected with pgenil-hk+lipoosomes. "sr1" represents the transfection of Tca-8113 cells with pgenil-IKCa1siRNA+lipoosome. "b" (blank) represents cells transfected with ROMI-1640+lipoosomes. Compared with the blank group (b), \*p < 0.05. (C, D) After knocking down IKCa1 in Tca-8113 cell line and HOEC cell line, the proliferation of the cells was detected by CCK-8 method, \*\*\*p < 0.001.

with HOEC cell line, Tca-8113 cell line showed more significant changes in proliferation after knocking down IKCa1 (Figures 2c and 2d). It can be concluded that inhibiting the expression of IKCa1 can specifically inhibit the proliferation of Tca-8113 cells.

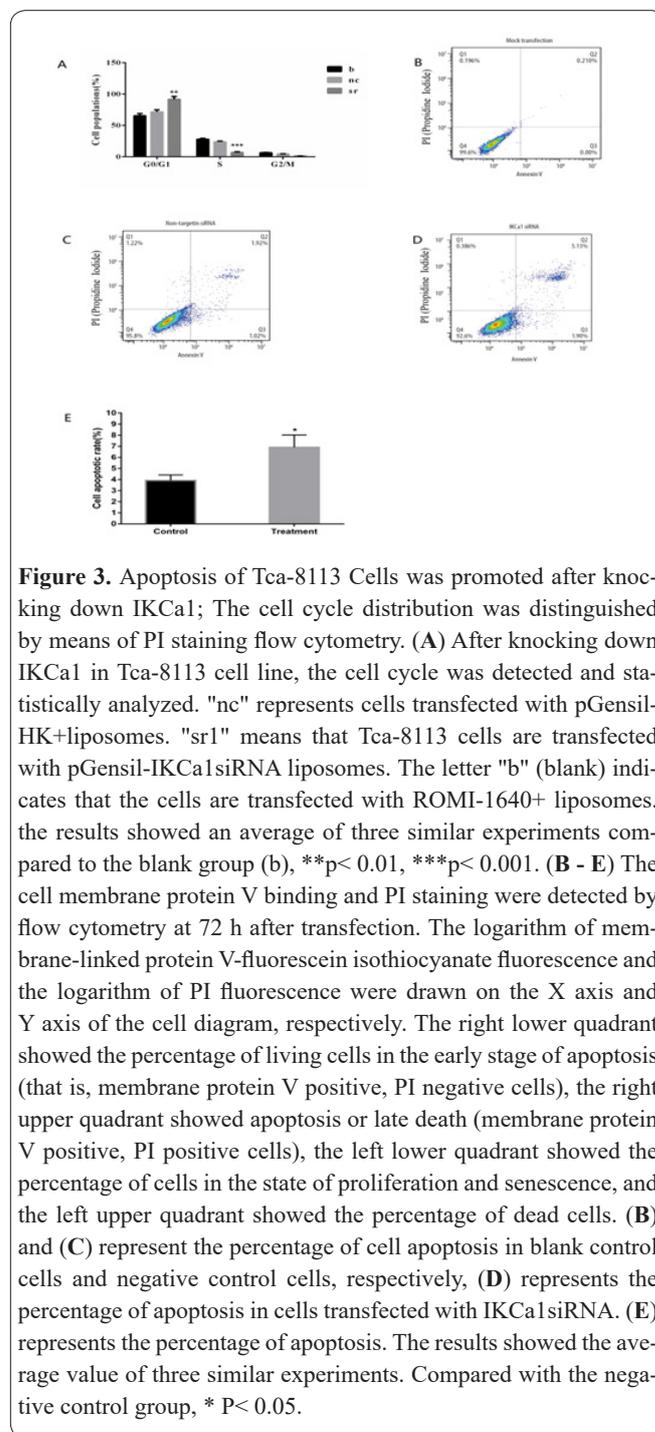
**Effects of IKCa1 on cell cycle and apoptosis**

In order to investigate whether the inhibition of cell proliferation after knockdown of IKCa1 was caused by affecting the process of cell cycle, flow cytometry was applied to analyze the cell cycle distribution. The results showed that compared with the control group, the Tca-8113 cells in G0/G1 phase increased from 71.6 ± 3.4% to 91.7 ± 4.6% (P < 0.001), and the cells in S phase decreased from 23.8 ± 1.1% to 7.1 ± 0.8% (P < 0.01). Therefore, after knocking down IKCa1, the cells were blocked in G0/G1 phase.

In order to further study whether IKCa1 can affect apoptosis, flow cytometry was carried out. The results showed that the proportion of apoptotic cells in Tca-8113 cells transfected with IKCa1siRNA was significantly increased (3.9 ± 0.52% VS 6.9 ± 1.12%) (Figure 3). The proportion of early apoptosis and late apoptosis of Tca-8113 cells increased significantly (P < 0.05) (The percentage of apoptosis was determined via the sum of early and late apoptosis).

**Effect of IKCa1 on the expression of apoptotic protein**

To explore the mechanism of IKCa1 affecting apoptosis, we detected the expression of apoptosis-related proteins. The mRNA expression of anti-apoptosis protein Bcl-2 and apoptosis-promoting protein Bax was detected by RT-PCR after IKCa1siRNA was transferred into Tca-8113 cells. The results showed that the mRNA level of Bcl-2 in IKCa1siRNA was significantly decreased by 44.41% compared with the control group (Figure 4a, p < 0.01). At the same time, the mRNA level of Bax was significantly increased by 36.0% (Figure 4b,



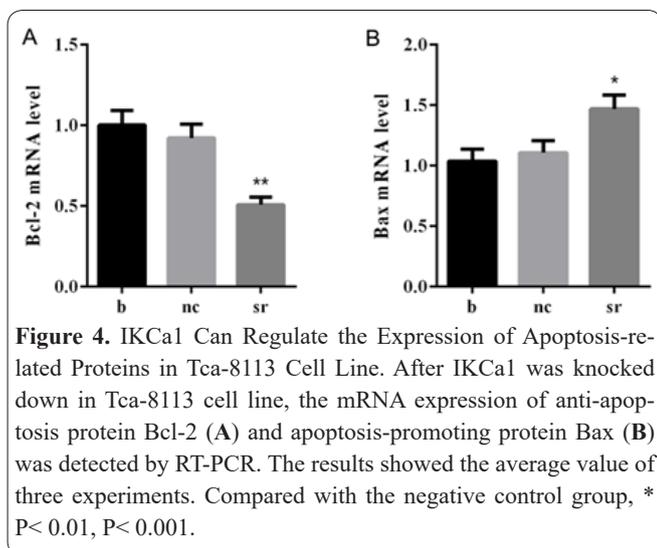
**Figure 3.** Apoptosis of Tca-8113 Cells was promoted after knocking down IKCa1; The cell cycle distribution was distinguished by means of PI staining flow cytometry. (A) After knocking down IKCa1 in Tca-8113 cell line, the cell cycle was detected and statistically analyzed. "nc" represents cells transfected with pGensil-HK+lipoosomes. "sr1" means that Tca-8113 cells are transfected with pGensil-IKCa1siRNA lipoosomes. The letter "b" (blank) indicates that the cells are transfected with ROMI-1640+ lipoosomes. The results showed an average of three similar experiments compared to the blank group (b), \*\*p < 0.01, \*\*\*p < 0.001. (B - E) The cell membrane protein V binding and PI staining were detected by flow cytometry at 72 h after transfection. The logarithm of membrane-linked protein V-fluorescein isothiocyanate fluorescence and the logarithm of PI fluorescence were drawn on the X axis and Y axis of the cell diagram, respectively. The right lower quadrant showed the percentage of living cells in the early stage of apoptosis (that is, membrane protein V positive, PI negative cells), the right upper quadrant showed apoptosis or late death (membrane protein V positive, PI positive cells), the left lower quadrant showed the percentage of cells in the state of proliferation and senescence, and the left upper quadrant showed the percentage of dead cells. (B) and (C) represent the percentage of cell apoptosis in blank control cells and negative control cells, respectively, (D) represents the percentage of apoptosis in cells transfected with IKCa1siRNA. (E) represents the percentage of apoptosis. The results showed the average value of three similar experiments. Compared with the negative control group, \* P < 0.05.

p < 0.05). Therefore, IKCa1 may affect tumor growth through the apoptosis-related pathway.

**Discussion**

Oral squamous cell carcinoma of the mouth is the most common type of squamous cell carcinoma of the head and neck, which is one of the top ten most common cancers worldwide. Despite routine treatment regimens, patients with oral squamous cell carcinoma have poor prognosis and short survival. The incidence of oral squamous cell carcinoma is increasing year by year, but the molecular mechanism of tumor proliferation is still unclear. Therefore, it is necessary to identify new molecular markers and study effective therapeutic drugs to inhibit the growth of oral squamous cell cells (18, 19).

A large number of studies have confirmed that ion channels are one of the structural units that regulate the



**Figure 4.** IKCa1 Can Regulate the Expression of Apoptosis-related Proteins in Tca-8113 Cell Line. After IKCa1 was knocked down in Tca-8113 cell line, the mRNA expression of anti-apoptosis protein Bcl-2 (A) and apoptosis-promoting protein Bax (B) was detected by RT-PCR. The results showed the average value of three experiments. Compared with the negative control group, \*  $P < 0.01$ , \*\*  $P < 0.001$ .

physiological functions of cells, such as growth, differentiation and proliferation. During cell proliferation,  $Cl^-$ ,  $K^+$ , aquaporin and  $Ca^{2+}$  channels are activated (20, 21), and these and other ion channels are considered to be important factors in the proliferation of normal cells and cancer cells (22). The  $K^+$  channel helps to set up membrane potential and ACTS as a driving force for  $Ca^{2+}$  inflow, which is also crucial for the regulation of cell growth and plays a role in carcinogenesis (23-25).  $Ca^{2+}$  dependent potassium channels (KCa) also regulate cell cycle progression, attracting increasing attention as a potential therapeutic target. Previous studies have found that IKCa1 is highly expressed in many malignant tumors (6-9). IKCa1 is one of the  $K^+$  channels regulating the proliferation and migration of cancer cells (26-28), mainly controlling the late G1 phase, G1/S transition period and G2/M phase. We investigated the role of IKCa1 in the development of oral squamous cell carcinoma at the molecular level using RNA interference techniques. In this study, we demonstrated that decreased expression of KCa1 had a strong anticancer effect in human oral squamous cell lines. In addition, the inhibitory effect of KCa1 expression on cell proliferation was associated with cell cycle arrest and apoptosis induction in oral squamous cell carcinoma.

In this study, according to RT-PCR results, IKCa1 was significantly more highly expressed in Tca-8113 cell lines than in normal oral epithelial HOEC cell lines ( $P < 0.001$ ) (Figure 1a), and the expression level of IKCa1 in oral squamous cell carcinoma was higher than that in normal oral epithelial tissues according to RT-PCR results. After knocking down IKCa1 in the Tca-8113 cell line, IKCa1siRNA significantly inhibited cell proliferation compared with siNC in the control group (Figure 2c). Moreover, compared with the HOEC cell line, the Tca-8113 cell line showed more significant changes in proliferation after knocking down IKCa1 (Figures 2c, 2d). Therefore, inhibiting IKCa1 expression can specifically inhibit the proliferation of Tca-8113 cells. It can be seen that IKCa1 can be used as a marker of malignant tumor in the oral squamous cell system, providing a new molecular marker for accurate detection and treatment in clinical practice. Compared with the control group, Tca-8113 cells with IKCa1 knockdown increased from  $71.6 \pm 3.4\%$  to  $91.7 \pm 4.6\%$  ( $P < 0.01$ ) in G0/G1 phase, and decreased from  $23.8 \pm 1.1\%$  to  $7.1 \pm 0.8\%$  ( $P < 0.001$ ) in

S phase. Flow cytometry showed that the proportion of apoptotic tca-8113 cells transfected with IKCa1siRNA was significantly increased ( $3.9 \pm 0.52\%$  VS  $6.9 \pm 1.12\%$ ). The ratio of early apoptosis and late apoptosis of Tca-8113 cells increased ( $P < 0.05$ ). (Figure 3) Therefore, it can be seen that the effect of IKCa1 on cell proliferation may be caused by apoptosis. To investigate the effect of IKCa1 on apoptosis, we tested the expression levels of apoptosis-related proteins. The results showed that the mRNA level of IKCa1siRNA was significantly decreased by 44.41% compared with the control group (Figure 4a,  $p < 0.01$ ). Meanwhile, Bax mRNA level was significantly increased by 36.0% (Figure 4b,  $p < 0.05$ ). It is well known that Bcl-2 family members play a decisive role in regulating apoptosis and determining cell fate by regulating the balance between proapoptotic (Bax, BAK, and BH3) and anti-apoptotic (Bcl-2 and its homology) proteins. When the ratio of Bax to Bcl-2 increases, the mitochondrial permeability conversion hole opens, and then releases apoptotic mitochondrial protein to activate caspase and induce apoptosis. However, previous studies have not linked IKCa1's anticancer effects to this apoptotic pathway. Thus, to our knowledge, this is the first study to investigate the involvement of the Bcl2 family in IKCa1-mediated apoptosis of human oral squamous cell carcinoma cells. However, further confirmation that the activation of the Bcl2 family regulated apoptotic pathway is Kca1 inhibiting effect and the specific mechanism of action needs to be further confirmed by more experiments.

The most recent studies have focused on the malignant cell behavior of KCa1 in cancer (29-34). This study also demonstrated its effect on cell proliferation in oral squamous cell carcinoma. Therefore, we can effectively inhibit the expression of KCa1 through genetic and pharmacological means according to its characteristics, thus significantly reducing the proliferation of tumor cells and changing the susceptibility of tumors to establish cancer therapies, thus increasing the efficacy of existing therapies. In this paper, the effects of IKCa1 on the proliferation of oral squamous cell carcinoma cell lines and their mechanisms were studied to further observe the effects of RNA interference on cell cycle and apoptosis, to provide more evidences for clinical diagnosis and accurate treatment of oral squamous cell carcinoma cell lines and provide more possibilities for the development of new therapeutic drugs (35-51).

In conclusion, our results show that IKCa1 is significantly up-regulated in oral squamous cell carcinoma. Knocking down IKCa1 in Tca-8113 cells can induce cell cycle arrest and apoptosis to produce an anti-proliferation effect, so inhibiting IKCa1 expression has a strong anticancer effect in oral squamous cell carcinoma. This study can provide more evidence for the research and development of clinical therapeutic drugs and the research of drug resistance mechanisms.

## References

1. Abram MH, van Heerden WF, Rheeder P, Girdler-Brown BV, van Zyl AW. Epidemiology of oral squamous cell carcinoma. *SADJ* 2012; 67: 550-553.
2. Blatt S, Krüger M, Ziebart T. Biomarkers in diagnosis and therapy of oral squamous cell carcinoma: A review of the literature. *J*

- Craniomaxillofac Surg 2017; 45: 722-730.
3. Ng JH, Iyer NG, Tan MH, Edgren G. Changing epidemiology of oral squamous
  4. cell carcinoma of the tongue: A global study. *Head Neck* 2016; 39: 297-304.
  5. Bi D, Toyama K, Lemaître V, The Intermediate Conductance Calcium-activated Potassium Channel *KCa3.1* Regulates Vascular Smooth Muscle Cell Proliferation via Controlling Calcium-dependent Signaling. *J Biol Chem* 2013; 288: 15843-15853.
  6. Pedarzani P, Stocker M. Molecular and cellular basis of small- and intermediate-conductance, calcium-activated potassium channel function in the brain. *Cell Mol Life Sci* 2008; 65: 3196-3217.
  7. Sharma P, Saxena S, Aggarwal P. Trends in the epidemiology of oral squamous cell carcinoma in Western UP: an institutional study. *Indian J Dent Res* 2010; 21: 316-319.
  8. Boeve K, Melchers LJ, Schuurings E, Addition of tumour infiltration depth and extranodal extension improves the prognostic value of the pathological TNM classification for early-stage oral squamous cell carcinoma. *Histopathology* 2019; 75: 329-337.
  9. Hutchings CJ, Colussi P, Clark TG. Ion channels as therapeutic antibody targets. *MAbs* 2018; 11: 265-296.
  10. Roy JW, Cowley EA, Blay J, Linsdell P. The intermediate conductance  $Ca^{2+}$ -activated  $K^{+}$  channel inhibitor TRAM-34 stimulates proliferation of breast cancer cells via activation of oestrogen receptors. *Br J Pharmacol* 2009; 159: 650-658.
  11. Wulff H, Kolski-Andreaco A, Sankaranarayanan A, Sabatier JM, Shakkottai V. Modulators of Small- and Intermediate-Conductance Calcium-Activated Potassium Channels and their Therapeutic Indications. *Curr Med Chem* 2007; 14: 1437-1457.
  12. McFerrin MB, Turner KL, Cuddapah VA, Sontheimer H. Differential Role of IK and BK Potassium Channels as Mediators of Intrinsic and Extrinsic Apoptotic Cell Death. *Am J Physiol Cell Physiol* 2012; 303: C1070- C1078.
  13. Hua X, Deuse T, Chen YJ, The Potassium Channel *KCa3.1* as New Therapeutic Target for the Prevention of Obliterative Airway Disease. *Transplantation* 2013; 95: 285-292.
  14. Qian X, Francis M, Kohler R, Solodushko V, Lin M, Taylor MS. Positive Feedback Regulation of Agonist-Stimulated Endothelial  $Ca^{2+}$  Dynamics by *KCa3.1* Channels in Mouse Mesenteric Arteries. *Arterioscler Thromb Vasc Biol* 2013; 34: 127-135.
  15. Zhang Y, Xie ZY, Guo XT, Xiao XH, Xiong LX. Notch and breast cancer metastasis: Current knowledge, new sights and targeted therapy. *Oncol Lett* 2019; 18: 2743-2755.
  16. Bharadwaj R, Sahu BP, Haloi J. Combinatorial therapeutic approach for treatment of oral squamous cell carcinoma. *Artif Cells Nanomed Biotechnol* 2019; 47: 572-585.
  17. Katase N, Gunduz M, Beder LB, Gunduz E, Sheikh Ali MA, Tamamura R, Yaykasli KO, Yamanaka N, Shimizu K, Nagatsuka H. Frequent allelic loss of *Dkk-1* locus (10q11.2) is related with low distant metastasis and better prognosis in head and neck squamous cell carcinomas. *Cancer Invest* 2010; 28(1):103-110.
  18. Beder LB, Gunduz M, Hotomi M, Fujihara K, Shimada J, Tamura S, Gunduz E, Fukushima K, Yaykasli KO, Grenman R, Shimizu K, Yamanaka N. T-lymphocyte maturation-associated protein gene as a candidate metastasis suppressor for head and neck squamous cell carcinomas. *Cancer Sci* 2009; 100(5):873-880.
  19. Francis M, Waldrup JR, Qian X, Solodushko V, Meriwether J, Taylor MS. Functional Tuning of Intrinsic Endothelial  $Ca^{2+}$  Dynamics in Swine Coronary Arteries. *Circ Res* 2016; 118: 1078-1090.
  20. Morishita K, Watanabe K, Ichijo H. Cell volume regulation in cancer cell
  21. migration driven by osmotic water flow. *Cancer Sci* 2019; 110: 2337-2347.
  22. Lang F, Föllner M, Lang K. Cell Volume Regulatory Ion Channels in Cell Proliferation and Cell Death. *Methods Enzymol* 2007; 428: 209-225.
  23. Pollaers K, Massingham I, Friedland PL, Farah CS. The economic burden of oral squamous cell carcinoma in Australia. *J Oral Pathol Med* 2019; 48: 588-594.
  24. Gackière F, Warnier M, Katsogiannou M, Functional coupling between large-conductance potassium channels and *Cav3.2* voltage-dependent calcium channels participates in prostate cancer cell growth. *Biol Open* 2013; 2: 941-951.
  25. Lallet-Dahe H, Roudbaraki M, Bavencoffe A, Intermediate-conductance  $Ca^{2+}$ -activated  $K^{+}$  channels (*IKCa1*) regulate human prostate cancer cell proliferation through a close control of calcium entry. *Oncogene* 2009; 28: 1792-1806.
  26. Faouzi M, Valérie Chopin, Ahidouch A, Ouadid-Ahidouch H. Intermediate  $Ca^{2+}$ -Sensitive  $K^{+}$  Channels are Necessary for Prolactin-Induced Proliferation in Breast Cancer Cells. *J Membr Biol* 2010; 234: 47-56.
  27. Alaeddini M, Etemad-Moghadam S. Are Ski and SnoN Involved in the Tumorigenesis of Oral Squamous Cell Carcinoma Through Smad4? *Appl Immunohistochem Mol Morphol* 2019; 27: 626-630.
  28. Chandy KG, Wulff H, Beeton C, Pennington M, Gutman GA, Cahalan MD.  $K^{+}$  channels as targets for specific immunomodulation. *Trends Pharmacol Sci* 2004; 25: 280-289.
  29. Saira, Ahmed R, Malik S, Khan MF, Khattak MR. Epidemiological and clinical correlates of oral squamous cell carcinoma in patients from north-west Pakistan. *J Pak Med Assoc* 2019; 69: 1074-1078.
  30. Tharp DL, Wamhoff BR, Wulff H, Raman G, Cheong A, Bowles DK. Local Delivery of the *KCa3.1* Blocker, TRAM-34, Prevents Acute Angioplasty-Induced Coronary Smooth Muscle Phenotypic Modulation and Limits Stenosis. *Arterioscler Thromb Vasc Biol* 2008; 28: 1084-1089.
  31. Cheong A, Bingham AJ, Li J Downregulated REST transcription factor is a switch enabling critical potassium channel expression and cell proliferation. *Mol Cell* 2005; 20: 45-52.
  32. Migueláñez-Medrán BC, Pozo-Kreiling JJ, Cebrián-Carrettero JL, Martínez-García MA, López-Sánchez AF. Oral squamous cell carcinoma of tongue: Histological risk assessment. A pilot study. *Med Oral Patol Oral Cir Bucal* 2019; 24: e603-e609.
  33. Saito T, Fujiwara Y, Fujiwara R Role Of Augmented Expression Of Intermediate-Conductance  $Ca^{2+}$ -Activated  $K^{+}$  Channels In Postischaemic Heart. *Clinical and Experimental Pharmacology & Physiology* 2002; 29: 324-329.
  34. McDonald OG, Wamhoff BR, Hoofnagle MH, Owens GK. Control of SRF binding to *CaRg* box chromatin regulates smooth muscle gene expression in vivo. *J Clin Invest* 2006; 116: 36-48.
  35. Chen HX, Huang L, Yang L, Chen YT, Huang JM. Model-based method with nonlinear ultrasonic system identification for mechanical structural health assessment. *Trans Emerge Telecom Technol* 2020; 1-15.
  36. Chen X, Xu Y, Meng L, Chen X, Yuan L, Cai Q, Shi W, Huang G. Non-parametric partial least squares-discriminant analysis model based on sum of ranking difference algorithm for tea grade identification using electronic tongue data. *Sens Actuators B Chem* 2020; 311:127924-127931.
  37. Guo T, Lin Q, Li X, Nie Y, Wang L, Shi L, Xu W, Hu T, Guo T, Luo F. Octacosanol Attenuates Inflammation in Both RAW264.7 Macrophages and a Mouse Model of Colitis. *J Agri Food Chem* 2017; 65(18): 3647-3658.
  38. Jiang X, Zhu B, Chevallier J, Xie R. Allocating provincial CO2 quotas for the Chinese national carbon program. *Australian J Agri Res Econ* 2018; 62(3): 457-479.
  39. Li W, Jia MX, Wang JH, Lu JL, Deng J, Tang JX, Liu, C. As-

sociation of MMP9-1562C/T and MMP13-77A/G Polymorphisms with Non-Small Cell Lung Cancer in Southern Chinese Population. *Biomolecules* 2019; 9(3): 107-119.

40. Liang Y, Lin Q, Huang P, Wang Y, Li J, Zhang L, Cao J. Rice Bioactive Peptide Binding with TLR4 To Overcome H<sub>2</sub>O<sub>2</sub>-Induced Injury in Human Umbilical Vein Endothelial Cells through NF- $\kappa$ B Signaling. *J Agri Food Chem* 2018; 66(2): 440-448.

41. Lou Y, Guo D, Zhang H, Song L. Effectiveness of mesenchymal stems cells cultured by hanging drop vs. conventional culturing on the repair of hypoxic-ischemic-damaged mouse brains, measured by stemness gene expression. *Open Life Sci* 2016; 11(1): 519-523.

42. Lou Y, Shi J, Guo D, Qureshi AK, Song L. Function of PD-L1 in antitumor immunity of glioma cells. *Saudi J Biol Sci* 2017; 24(4): 803-807.

43. Lou Y, Yang J, Wang L, Chen X, Xin X, Liu Y. The clinical efficacy study of treatment to Chiari malformation type I with syringomyelia under the minimally invasive surgery of resection of Submeningeal cerebellar Tonsillar Herniation and reconstruction of Cisterna magna. *Saudi J Biol Sci* 2019; 26(8): 1927-1931.

44. Nie Y, Luo F, Lin Q. Dietary nutrition and gut microflora: A promising target for treating diseases. *Trends Food Sci Technol* 2018; 75: 72-80.

45. Nie Y, Luo F, Wang L, Yang T, Shi L, Li X, Shen J, Xu W, Guo T, Lin Q. Anti-hyperlipidemic effect of rice bran polysaccharide

and its potential mechanism in high-fat diet mice. *Food Func* 2017; 8(11): 4028-4041.

46. Ren Y, Jiao X, Zhang L. Expression level of fibroblast growth factor 5 (FGF5) in the peripheral blood of primary hypertension and its clinical significance. *Saudi J Biol Sci* 2018; 25(3): 469-473.

47. Wang L, Lin Q, Yang T, Liang Y, Nie Y, Luo Y, Shen J, Fu X, Tang Y, Luo F. Oryzanol Modifies High Fat Diet-Induced Obesity, Liver Gene Expression Profile, and Inflammation Response in Mice. *J Agri Food Chem* 2017; 65(38): 8374-8385.

48. Zhang T, Wu X, Shaheen SM, Zhao Q, Liu X, Rinklebe J, Ren H. Ammonium nitrogen recovery from digestate by hydrothermal pretreatment followed by activated hydrochar sorption. *Chem Eng J* 2020; 379: 1-54.

49. Zhu B, Pang R, Chevallier J, Wei YM, Vo DT. Including intangible costs into the cost-of-illness approach: a method refinement illustrated based on the PM<sub>2.5</sub> economic burden in China. *Europ J Health Econ* 2019; 20(4): 501-511.

50. Bordbar M, Darvishzadeh R, Pazhouhandeh M, Kahrizi D. An overview of genome editing methods based on endonucleases. *Modern Genetics J* 2020; 15(2): 75-92.

51. Chen H, Chen Y, Yang L. Intelligent early structural health prognosis with nonlinear system identification for RFID signal analysis. *Comput Commun* 2020; 157: 150-161.