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Original Article

Study on the role of PI3Kδ/AKT in regulating glucocorticoid resistance in sudden sensorineural hearing loss

Lingyun Lv[1,2], Jie Hou[1,3], Ziwen Gao[1], Hui Qi[3], Wenyan Zhu[1,2], Zhibiao Liu[1,2], Yanhong Dai[1,3], Wandong She[1,3]

[1]Department of Otolaryngology-Head and Neck Surgery, Nanjing Drum Tower Hospital Clinical College of Nanjing Medical University, Nanjing, Jiangsu Province, China; [2]Department of Otorhinolaryngology-Head and Neck Surgery, The Affiliated Huaian No. 1 People's Hospital of Nanjing Medical University, Huaian, Jiangsu Province, China; [3]Department of Otolaryngology-Head and Neck Surgery, Jiangsu Provincial Key Medical Discipline, Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School, Nanjing, Jiangsu Province, China

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Abstract: Objective: To investigate the role of PI3Kδ/AKT in regulating glucocorticoid resistance in Sudden Sensorineural Hearing Loss (SSNHL). Methods: From March 2017 to February 2018, 27 patients with SSHL were enrolled in this study and divided into the efficacy group and inefficacy group according to the conditions of glucocorticoid resistance. Peripheral blood mononuclear cells (PBMCs) were collected from both groups. HDAC2 mRNA and protein expression levels were detected by qPCR and Western blot. p-AKT/AKT protein expression was detected by Western blot. HDAC2 and p-AKT/AKT expression in PBMCs were compared between both groups. The H_2O_2-induced oxidative stress model of human histiocytic lymphoma cells (U937) was established. U937 cells were divided into the H_2O_2 group, H_2O_2+Lenti-EGFP group, and H_2O_2+lenti-PI3Kδ siRNA group according to the cell transfection. U937 cells were divided into the H_2O_2 group and H_2O_2+IC-87114 group according to whether cells were treated with PI3Kδ specific inhibitor. HDAC2 and p-AKT/AKT expression were compared among the above groups. Results: After treatment, HDAC2 mRNA and protein expression of PBMCs in the efficacy group were significantly higher than those in the inefficacy group (all $P < 0.05$) while p-AKT/AKT protein expression in PBMCs from the efficacy group was obviously lower than that in the inefficacy group ($P < 0.001$). Compared with the H_2O_2 group and H_2O_2+Lenti-EGFP group, the HDAC2 mRNA and protein expression in the H_2O_2+lenti-PI3Kδ siRNA group were markedly higher and p-AKT/AKT protein expression was obvious lower (all $P < 0.05$). In contrast to the H_2O_2 group, HDAC2 mRNA and protein expression in the H_2O_2+IC-87114 group was markedly higher and the p-AKT/AKT protein expression was significantly lower (all $P < 0.05$). Conclusions: For glucocorticoid resistance in patients with SSHL, HDAC2 expression was significantly decreased and p-AKT/AKT protein expression was obviously increased. The activation of the PI3Kδ/AKT signaling pathway could lead to glucocorticoid resistance in SSHL.

Keywords: Sudden sensorineural hearing loss, glucocorticoid resistance, PI3Kδ/AKT signal pathway, histone deacetylase-2

Introduction

Sudden sensorineural hearing loss (SSNHL) is defined as hearing impairment over 30 dB in three adjacent frequencies, and it is a common otorhinolaryngological disease [1, 2]. The incidence of SSNHL has become increasingly high. The etiology of SSNHL, which is not clear, may involve many factors such as virus infection, vascular deficiencies, autoimmunity and metabolic disorders [3]. Glucocorticoids (GC) are the main therapy option for SSNHL. However, a large number of patients are insensitive and resistant to GC therapy [4]. Therefore, it is important to identify the mechanism of GC resistance so that it may help to improve the prognosis of patients with SSNHL.

It was reported that the expression of histone deacetylase-2 (HDAC2) could enhance the sensibility of GC therapy by affecting the deacetylation of glucocorticoid receptors (GR) [5]. Reduced expression of HDAC2 is considered an important cause of GC resistance in SSNHL.

Many studies have reported that oxidative stress induced by reactive oxygen species may lead to SSNHL [6]. The activity of HDAC2 is decreased through oxidative stress-induced posttranscriptional modifications, such as nitrification of tyrosine residues, phosphorylation of serine residues causing GC resistance in patients with SSNHL [7]. Previous studies have reported that in the development of GC resistance in respiratory inflammation, phosphatidylinositol-3-kinase delta/protein kinase B (PI3Kδ/AKT) is activated by oxidative stress and leads to reduced expression of HDAC2 [8]. In addition, To et al. reported that amionophylline had antioxidant effects and low-dose amionophylline could decrease the expression of PI3Kδ and then increase the activation of HDAC2 in the development of chronic obstructive pulmonary disease [9]. It was reported that amionophylline could increase HDAC2 expression and improve the effect of GC in LPS-induced hearing loss in guinea pigs [4]. However, it is still unclear whether oxidative stress can inhibit HDAC2 expression and lead to GC resistance through PI3Kδ/AKT signal pathway.

In this study, we observed the expression levels of HDAC2 and p-AKT/AKT in SSNHL patients with GC resistance. The H_2O_2-induced oxidative stress model of human histiocytic lymphoma cells (U937) was established to explore the role of PI3Kδ/AKT in regulating HDAC2 expression in GC resistance. The results of this study may provide new ideas for clinical treatment of SSNHL patients with GC resistance.

Materials and methods

Subjects

From March 2017 to February 2018, 27 SSNHL patients who were admitted to the Department of Otolaryngology in Nanjing Drum Tower Hospital were enrolled in this study. The inclusion criteria was as follows: pure tone audiometry (PTA) (0.25~8 kHz) was more than 60 dB and patients met the diagnostic criteria of SSNHL published in 2019 [10]; patients were more than 18-years old and newly diagnosed; related drugs such as glucocorticoid and amionophylline that affect experimental results were used in recent three months; patients with high compliance finished the prescribed course of treatment; and the clinical data of patients was complete. The exclusion criteria were as follows: patients with history of acoustic neuroma, family history of sensorineural deafness, use of ototoxic drugs, history of systemic infectious disease, history of autoimmune disease, and history of hematonosis disease, and patients who were loss to follow-up and dropped out of this study. All patients gave their informed consent and this study was approved by the hospital ethic committee.

SSNHL patients underwent conventional therapy based on glucocorticoid as follows: after admission, methylprednisolone (Pfizer Inc, USA) was given at 80 mg per day through an intravenous drip for 4 days. Then, methylprednisolone was reduced to 40 mg per day for 3 days. Finally, methylprednisolone was reduced to 20 mg per day for 3 days. Through an intravenous drip, patients also received Extract of Ginkgo biloba (Dr. Willmar Schwabe GmbH & Co. KG, German) 105 mg per day and Sodium Monosialotetrahexosylganglioside injection (Qilu Pharmaceutical Co. Ltd, China) 40 mg per day. The treatment time was 10 days. After that, patients were switched to oral administration of Ginkgo leaf tablets and Mecobalamin Tablets for two months. All patients were followed up for three months. Patients were divided into the efficacy group (N = 17) and inefficacy group (N = 10) according to whether the improvement degree of PTA was more than 15 dB [11].

Separation of PBMCs

PBMCs were isolated using the method of density gradient centrifugation. Fifteen mL of peripheral venous blood was drawn from patients in each group and equal amounts of PBS solutions was added. The above mixture was slowly added to the liquid surface of Lymphocytes Separation Medium and a distinct interphase was kept in this process. Then, it was centrifuged for 20 min at the speed of 2000 rpm/min. The contents in centrifuge tube were divided into four layers. PBMCs were located in the second layer. This layer was extracted using a micropipettor and kept in a new centrifuge tube. After centrifugation for 10 min at the speed of 1500 rpm/min, the supernatant was discarded. Then after washing with PBS solutions, PBMCs was used for further experiments.

Table 1. Primer sequence

Primers	Sequences
HDAC2-F	ATGGCG TACAGTCAAGGAGG
HDAC2-R	TGCGGATTCTATGAGGCTTCAA
β-actin-F	GTCCACCGCAAATGCTTCTA
β-actin-R	TGCTGTCACCTTCACCGTTC

Cell culture and grouping

U-937 cells (Cell bank of Chinese academy of sciences, China) were cultured in RPMI1640 (Gibco, USA) containing 10% fetal bovine serum (FBS) (Gibco, USA) and 100 μg/mL streptomycin and 100 μg/mL penicillin. The cells were incubated in the conditions of 5% CO_2 and 37°C. The oxidative stress model of cells was established following treatment with 0.2 mmol/L of H_2O_2 for 20 min. The transfection of cells with PI3Kδ siRNA (Genechem, China) was done using lentivirus, they were divided into three groups: H_2O_2 group, H_2O_2+Lenti-EGFP group, and H_2O_2+lenti-PI3Kδ siRNA group. According to whether application of IC-87114 (PI3Kδ specific inhibitor), the cells were divided into two groups: H_2O_2 group and H_2O_2+IC-87114 group.

Real-time PCR assay

Total RNA in each group was extracted with Trizol Reagent (Invitrogen, USA) according to the operation instructions. TaqMan MicroRNA reverse transcription Kit (Promega, USA) was applied to synthesize the above RNA into cDNA. β-actin served as normalization. Real-time PCR was performed with SYBRH Premix Ex TaqTM (TaKaRa, Japan). The primer design is shown in **Table 1**. PCR amplification was performed by the following conditions: predenaturation 5 min in 95°C, denaturation 25 s in 95°C and annealing/extension 50 s at 60°C, a total of 35 cycles. The relative expression amount of HDAC2 mRNA was calculated according to the 2-ΔΔCt method through ABI 7300 System software.

Western blot assay

Total protein in each group was extracted with RIPA protein lysis buffer following the operation instructions. BCA method was used to detect the concentration of protein. Proteins were separated using SDS-PAGE gel electrophoresis and subsequently transferred onto a PVDF membrane. Then at room temperature the membrane was incubated with TBST containing 5% non-fat milk powder and blocked for 1 h. The primary antibodies; p-AKT (dilution 1:500, Santa Cruz, USA), AKT (dilution 1:800, Santa Cruz, USA) and HDAC2 (dilution 1:1000, Santa Cruz, USA) were incubated with the membranes overnight. Next, HRP-conjugated secondary antibody (dilution 1:1000, Santa Cruz, USA) was incubated with a PVDF membrane for 30 min. Finally, ECL solution was applied to evaluate the expression of the targeted proteins. Bio-Rad image software was applied to quantify the target band intensities. β-actin served as an internal control.

Statistical analysis

SPSS 22.0 software was used for the statistical analysis of data in this study. The measurement data are expressed as mean ± standard deviation (SD). The comparison between both groups was performed by t test. The comparison between groups was performed by One-way ANOVA. Enumeration data was presented as cases or percentage and the comparison among groups was conducted through χ^2 test. $P < 0.05$ indicated significant statistical differences.

Results

Comparison of HDAC2 mRNA in PBMCs between both groups

Before treatment, there was no significant difference in HDAC2 mRNA of PBMCs between the efficacy group and inefficacy group (0.252±0.019 vs 0.253±0.021). After treatment, HDAC2 mRNA of PBMCs in the efficacy group was significantly higher than that in the inefficacy group, and there was clear statistical differences (0.300±0.010 vs 0.289±0.011, $t = 2.661$, $P = 0.013$), as shown in **Figure 1**.

Comparison of HDAC2 protein expression in PBMCs between both groups

There was no remarkably difference for HDAC2 protein expression in PBMCs between the efficacy group and the inefficacy group before treatment (0.509±0.011 vs 0.507±0.018). HDAC2 protein in PBMCs after treatment in the efficacy group was remarkably higher than

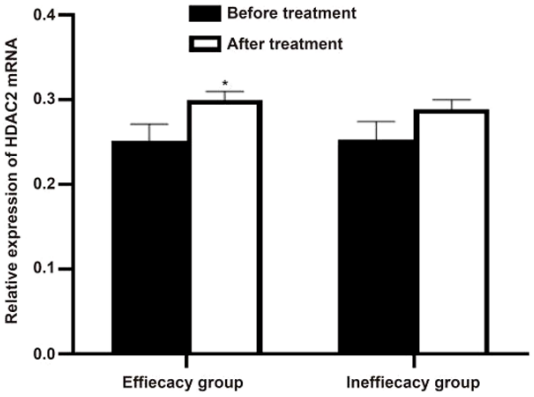


Figure 1. Comparison of HDAC2 mRNA in PBMCs between the efficacy group and inefficacy group. Compared with the inefficacy group after treatment, *P < 0.05.

that in the inefficacy group, and there was a remarkable statistical difference (0.607±0.018 vs 0.510±0.017, t = 13.790, P < 0.001), as seen in **Figure 2**.

Comparison of p-AKT/AKT expression in PBMCs between both groups

There was no obvious difference for p-AKT/AKT expression in PBMCs between the efficacy group and inefficacy group before treatment (0.203±0.048 vs 0.193±0.046). p-AKT/AKT expression in PBMCs after treatment in the efficacy group was obviously lower than that in the inefficacy group, and there was a remarkable statistical difference (0.121±0.032 vs 0.188±0.053, t = 4.118, P < 0.001), as seen in **Figure 3**.

The effect of PI3Kδ siRNA on HDAC2 expression in the H_2O_2-induced oxidative stress model of U937 cells

The efficacy of transfection with recombinant lentivirus in U937 cells was 70%, as shown in **Figure 4**. As shown in **Figure 5**, compared with those in the H_2O_2 group (0.337±0.051), and H_2O_2+Lenti-EGFP group (0.387±0.072), HDAC2 mRNA expression in the H_2O_2+lenti-PI3Kδ siRNA group (0.837±0.090) was significantly higher, and there were significant statistical differences (all P < 0.001). There was no significant difference for HDAC2 mRNA expression between the H_2O_2 group and the H_2O_2+lenti-EGFP group. In contrast to those in the H_2O_2 group (0.117±0.025), and the H_2O_2+lenti-EGFP group (0.127±0.036), the HDAC2 protein expression in the H_2O_2+lenti-PI3Kδ siRNA group (0.702±0.090) was markedly higher, and there were significant statistical differences (all P < 0.05). There was no significant difference for HDAC2 protein expression between the H_2O_2 group and H_2O_2+lenti-EGFP group.

The effect of PI3Kδ siRNA on p-AKT/AKT expression in the H_2O_2-induced oxidative stress model of U937 cells

As shown in **Figure 6**, p-AKT/AKT expression in the H_2O_2+lenti-PI3Kδ siRNA group (0.076± 0.017) was significantly lower than that in the H_2O_2 group (0.796±0.037) or the H_2O_2+lenti-EGFP group (0.757±0,081), and there were significant statistical differences (all P < 0.05). There was no significant statistical difference for p-AKT/AKT expression between the H_2O_2 group and H_2O_2+lenti-EGFP group.

The effect of PI3Kδ inhibitor IC-87114 on HDAC2 expression in the H_2O_2-induced oxidative stress model of U937 cells

As shown in **Figure 7**, HDAC2 mRNA expression in the H_2O_2+IC-87114 group was significantly higher than that in the H_2O_2 group, and there were significant statistical differences between both groups (0.537±0.023 vs 0.692± 0.026, t = 9.984, P < 0.001). HDAC2 protein expression in the H_2O_2+IC-87114 group was also obvious higher, compared with that in the H_2O_2 group, and a significant difference could be found between both groups (0.317±0.052 vs 0.452±0.050, t = 4.185, P = 0.003).

The effect of IC-87114 on p-AKT/AKT expression in the H_2O_2-induced oxidative stress model of U937 cells

p-AKT/AKT expression in the H_2O_2+IC-87114 group was remarkably lower than that in the H_2O_2 group, and a significant difference was found between both groups (0.632±0.041 vs 0.452±0.049, t = 6.300, P < 0.001), as shown in **Figure 8**.

Discussion

In this study, we explored the role of PI3Kδ/AKT in glucocorticoid resistance using an H_2O_2-induced oxidative stress model of human histiocytic lymphoma cells and PBMCs from SSNHL patients. We demonstrated for the first

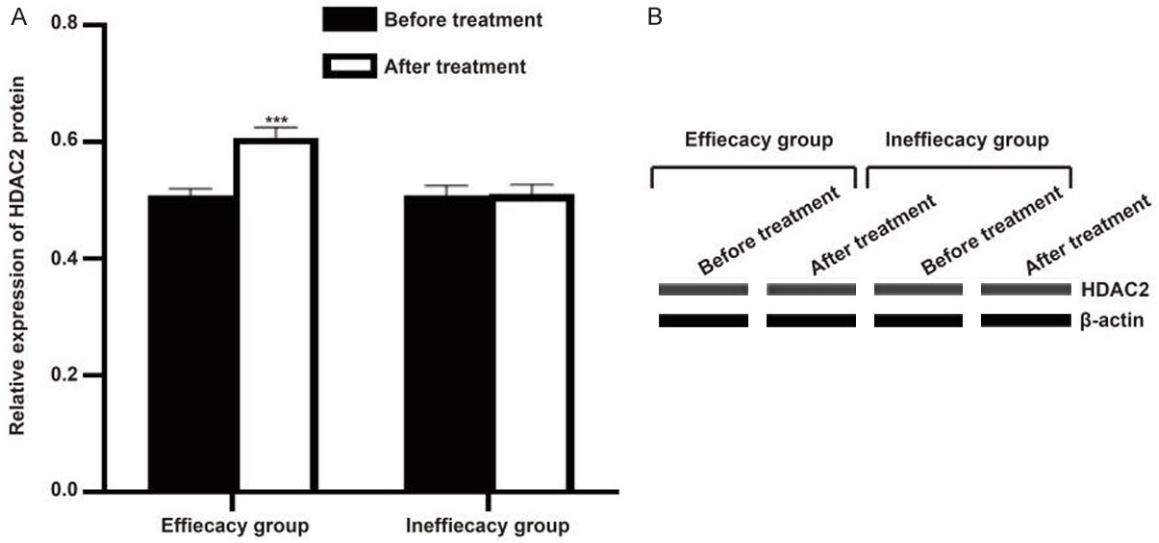


Figure 2. Comparison of HDAC2 protein expression in PBMCs between the efficacy group and inefficacy group. A. Quantitative analysis of HDAC2 protein expression; B. Western blot of HDAC2 protein. Compared with the inefficacy group after treatment, ***P < 0.001.

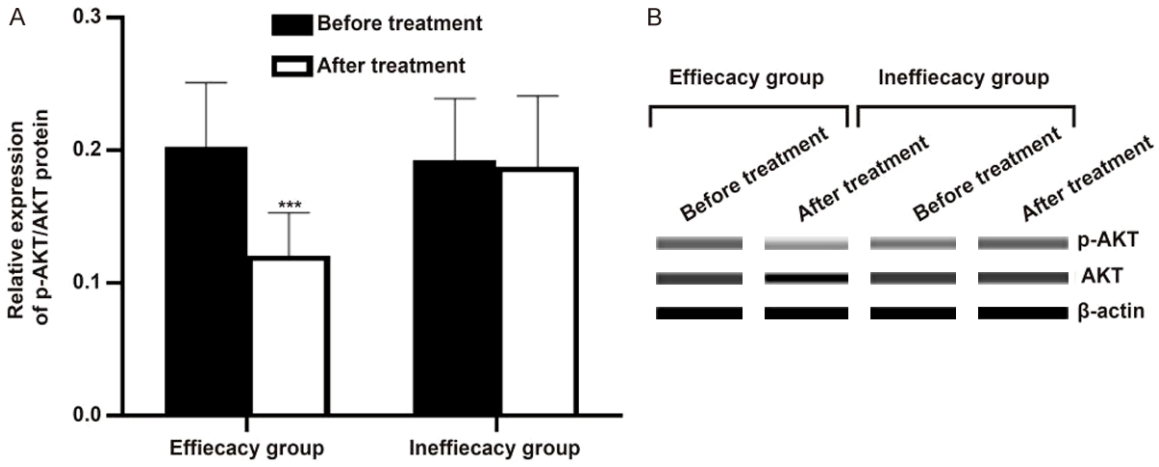


Figure 3. Comparison of p-AKT/AKT protein expression in PBMCs between the efficacy group and inefficacy group. A. Quantitative analysis of p-AKT/AKT protein expression; B. Western blot of p-AKT/AKT protein. Compared with the inefficacy group after treatment, ***P < 0.001.

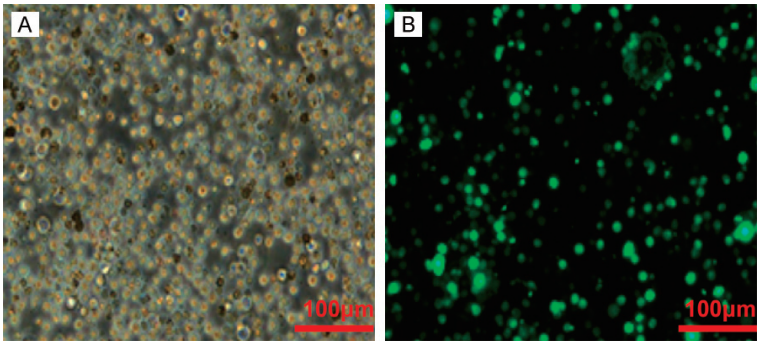
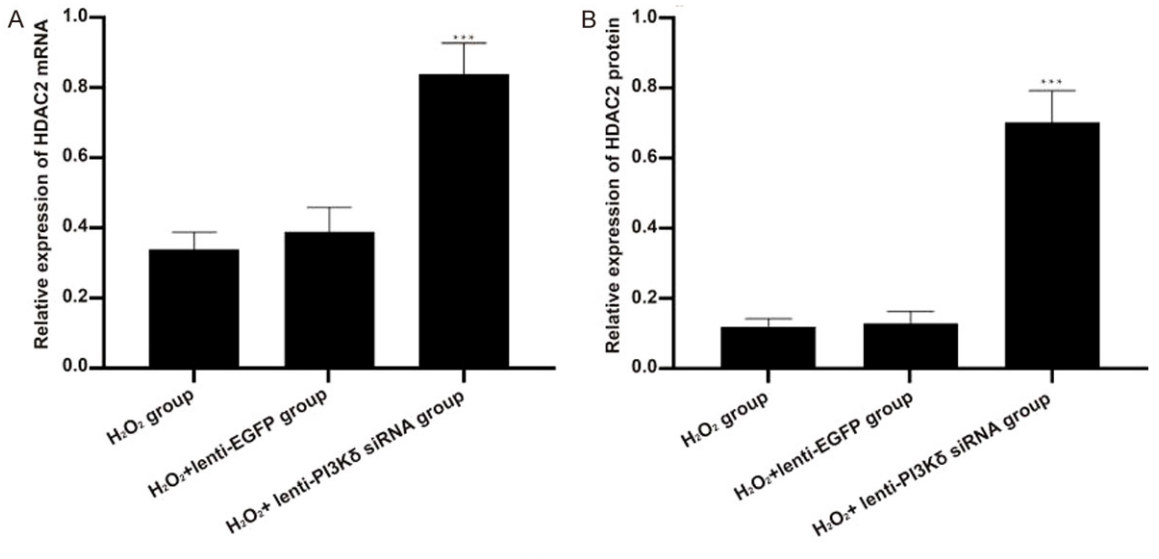


Figure 4. U937 cells were transfected with PI3Kδ siRNA using recombinant lentivirus. A. Observation under white light. B. Observation under fluorescence light.

time that the PI3Kδ/AKT signaling pathway plays an important role in glucocorticoid resistance induced by oxidative stress in SSNHL. Our results also indicated that inhibiting the activation of PI3Kδ/AKT could reduce glucocorticoid resistance.

At present, glucocorticoids are widely clinically used for SSNHL, but a lot of patients show resistance to glucocorti-



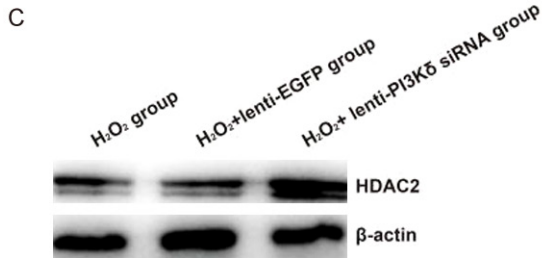


Figure 5. The effect of PI3Kδ siRNA on HDAC2 expression in the H_2O_2-induced oxidative stress model of U937 cells. A. Quantitative analysis of HDAC2 mRNA expression; B. Quantitative analysis of HDAC2 protein expression; C. Western blot of HDAC2 protein. Compared with H_2O_2 group or H_2O_2-lenti-EGFP group, ***P < 0.001.

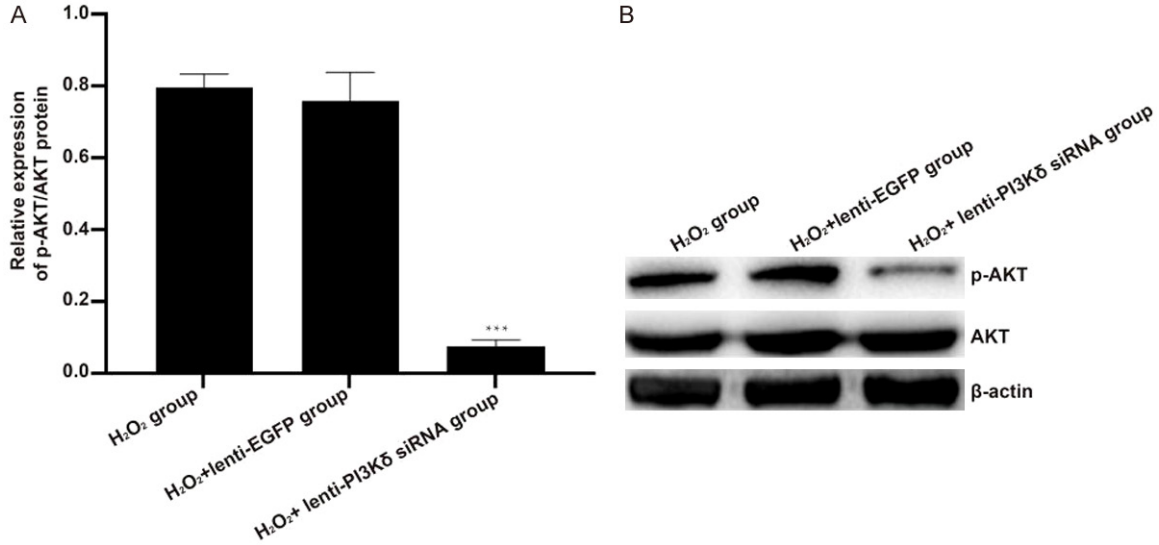


Figure 6. The effect of PI3Kδ siRNA on p-AKT/AKT expression in the H_2O_2-induced oxidative stress model of U937 cells. A. Quantitative analysis of p-AKT/AKT protein expression; B. Western blot of p-AKT/AKT protein expression. Compared with the H_2O_2 group or H_2O_2-lenti-EGFP group, ***P < 0.001.

coid treatment. According to the epidemiological survey, SSNHL patients with glucocorticoid resistance usually have a poor prognosis [12]. This is often contributed to adverse effects resulting from the long-term use of glucocorticoids, besides primary disease itself. Compared with SSNHL patients with glucocorticoids sensitivity, these patients have much higher rates of hospitalization [13]. Therefore, glucocorticoid resistance not only affects the physical

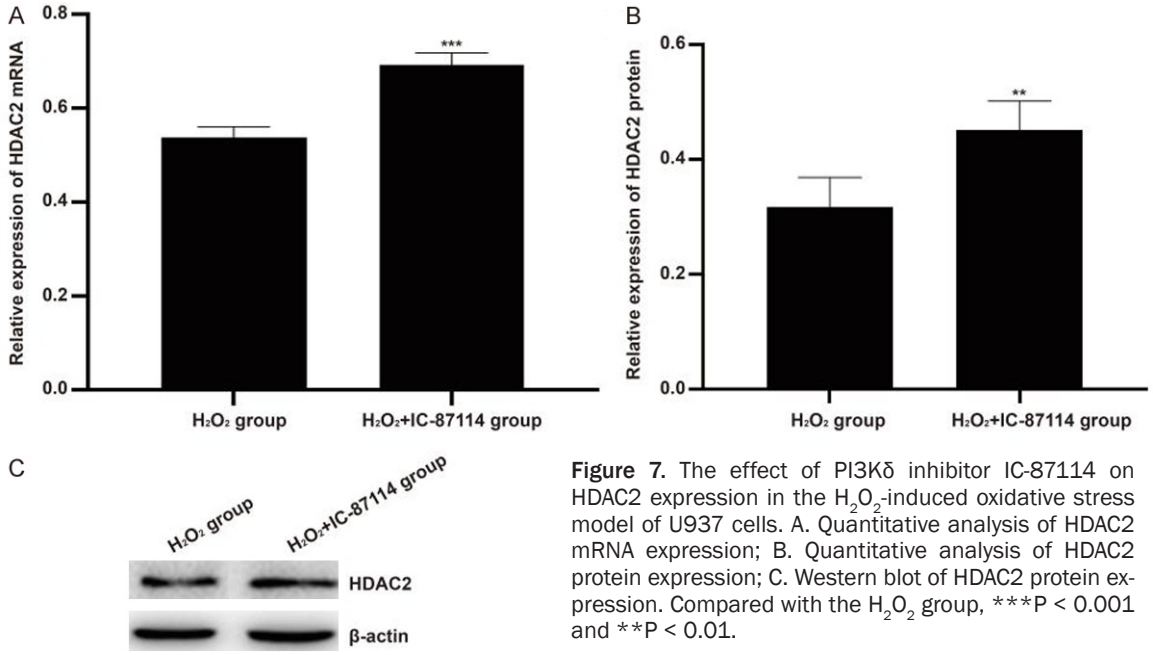


Figure 7. The effect of PI3Kδ inhibitor IC-87114 on HDAC2 expression in the H_2O_2-induced oxidative stress model of U937 cells. A. Quantitative analysis of HDAC2 mRNA expression; B. Quantitative analysis of HDAC2 protein expression; C. Western blot of HDAC2 protein expression. Compared with the H_2O_2 group, ***P < 0.001 and **P < 0.01.

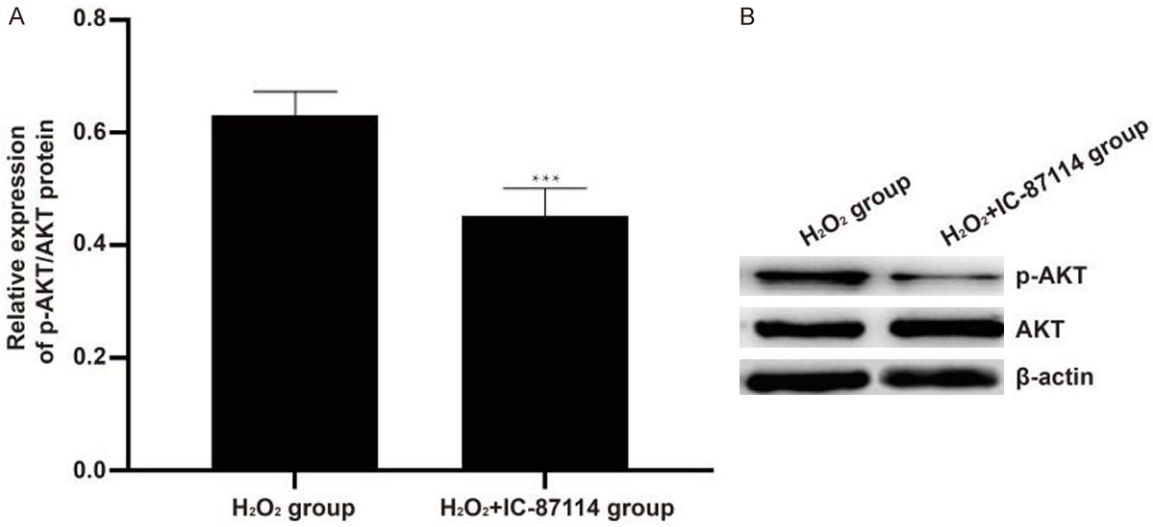


Figure 8. The effect of IC-87114 on p-AKT/AKT expression in the H_2O_2-induced oxidative stress model of U937 cells. A. Quantitative analysis of p-AKT/AKT protein expression; B. Western blot of p-AKT/AKT protein expression. Compared with the H_2O_2 group, ***P < 0.001.

and psychological health, but also exerts an economic burden for these SSNHL patients.

In recent years, the mechanism of glucocorticoid resistance in SSNHL has been a hot area of research. Many factors are involved in the molecular mechanism of glucocorticoid resistance [14]. Oxidative stress has been confirmed as one of the mechanisms in glucocorticoids resistance [15]. It has been found that HDAC2 expression was associated with glucocorticoid resistance [16]. Kazuhiro, et al. reported that in patients with COPD, oxidative stress could induce glucocorticoid insensitivity by impaired HDAC2 expression and that overexpression of HDAC2 in glucocorticoid-insensitive alveolar macrophages was able to restore the sensitivity of glucocorticoid [17]. Adenuga, et al. [18] reported that abnormal oxidant burden was correlated with obviously reduced expression of HDAC2 and glucocorticoid resistance, and that HDAC2 deficiency could lead to glucocorti-

coid resistance in inhibiting the lung inflammatory response due to an oxidant/antioxidant imbalance. Another study reported that in pediatric B-cell acute lymphoblastic leukemia (B-ALL), PI3K inhibitor could sensitize B-ALL cells to dexamethasone by restoring translocation of the GC receptor and counteract stroma-induced dexamethasone-resistance, suggesting that PI3K inhibition may be a promising strategy for treating B-ALL patients by overcoming GC-resistance [19]. To et al. found that theophylline was a potent selective inhibitor of oxidant-activated PI3K-δ, which was up-regulated in peripheral lung tissue of patients with COPD, and knock-down of PI3K-δ failed to induce GC resistance with oxidative stress [9]. However, the roles of PI3K in regulating HDAC2 in glucocorticoid resistance for SSNHL patients are still unknown.

Increasing evidence shows that oxidative stress plays a crucial role in GC resistance in SSNHL. In this study, it was demonstrated that compared with the inefficacy group p-AKT/AKT expression in PBMCs from the efficacy group was significantly increased while HDAC2 expression was obviously decreased. Previous studies have reported that p-AKT/AKT is a direct target of PI3K and a surrogate marker for PI3K activation in different cell systems. These results indicated that an increased level of p-AKT/AKT expression may be associated with low expression of HDAC2, which may lead to GC resistance in SSNHL. This was in accordance with the results reported by Yuan et al. [20]. In addition, the H_2O_2-induced oxidative stress model of human histiocytic lymphoma cells was applied in this study. The results showed that the reduction of HDAC2 activity was reversed using Lenti-PI3Kδ siRNA and IC87114, a selective inhibitor. PI3Kδ is one of the isoforms of the catalytic subunit in Class I-PI3K. It was reported that PI3Kδ can regulate neutrophil activation, trafficking, and directional movement [21]. It was found that PI3Kδ can inhibit neutrophil accumulation with theophylline [22]. Other studies have shown that the PI3Kδ isoform is involved in GC insensitivity in the smoking murine model [23]. Marwick, et al. [24] reported that PI3Kδ knockout resulted in protection against GC resistance and reduced HDAC2 activity after exposure to oxidative stress and this agrees with the results shown here.

In summary, in SSNHL patients with glucocorticoid resistance, p-AKT/AKT expression was obviously increased and HDAC2 expression was markedly decreased. In the H_2O_2-induced oxidative stress model of human histiocytic lymphoma cells, high expression of p-AKT/AKT and low expression HDAC2 were revealed, and inhibition of PI3Kδ/AKT can manifest the reversion of the above results, indicating improvement of glucocorticoid sensibility. However, there are some limitations in this research. First, the H_2O_2-induced oxidative stress model of human histiocytic lymphoma cells dose not completely represent the clinical pathogenesis of SSNHL. More studies can be conducted using cell models more suitable for SSNHL studies. Secondly, this is the first study to use a PI3Kδ/AKT inhibitor for the treatment of an inner ear disease, the functions of PI3Kδ/AKT are very complex, and there are many unclear aspects that remain to be investigated. The role of PI3Kδ/AKT in an animal model of SSNHL may also be explored in future experiments.

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Disclosure of conflict of interest

None.

Address correspondence to: Wandong She, Department of Otolaryngology-Head and Neck Surgery, Nanjing Drum Tower Hospital Clinical College of Nanjing Medical University, No. 321 Zhongshan Road, Nanjing 210008, Jiangsu Province, China. Tel: +86-025-68182139; Fax: +86-025-83317016; E-mail: shewangdong@sina.com

References

[1] Chandrasekhar SS, Tsai Do BS, Schwartz SR, Bontempo LJ, Faucett EA, Finestone SA, Hollingsworth DB, Kelley DM, Kmucha ST, Moonis G, Poling GL, Roberts JK, Stachler RJ, Zeitler DM, Corrigan MD, Nnacheta LC and Satterfield L. Clinical practice guideline: sudden hearing

loss (Update). Otolaryngol Head Neck Surg 2019; 161: S1-S45.
[2] Herrera M, Garcia Berrocal JR, Garcia Arumi A, Lavilla MJ and Plaza G; Grupo de Trabajo de la Comision de Audiologia de la SEORL. Update on consensus on diagnosis and treatment of idiopathic sudden sensorineural hearing loss. Acta Otorrinolaringol Esp 2019; 70: 290-300.
[3] Li G, You D, Ma J, Li W, Li H and Sun S. The role of autoimmunity in the pathogenesis of sudden sensorineural hearing loss. Neural Plast 2018; 2018: 7691473.
[4] Zhou QQ, Dai YH, Du XP, Hou J, Qi H and She WD. Aminophylline restores glucocorticoid sensitivity in a guinea pig model of sudden sensorineural hearing loss induced by lipopolysaccharide. Sci Rep 2017; 7: 2736.
[5] Hou J, She W, Du X, Dai Y, Xie L and Zhou Q. Histone deacetylase 2 in sudden sensorineural hearing loss patients in response to intratympanic methylprednisolone perfusion. Otolaryngol Head Neck Surg 2016; 154: 164-170.
[6] Gul F, Muderris T, Yalciner G, Sevil E, Bercin S, Ergin M, Bababemez MA and Kiris M. A comprehensive study of oxidative stress in sudden hearing loss. Eur Arch Otorhinolaryngol 2017; 274: 1301-1308.
[7] Barnes PJ. Glucocorticosteroids. Handb Exp Pharmacol 2017; 237: 93-115.
[8] Mercado N, To Y, Ito K and Barnes PJ. Nortriptyline reverses corticosteroid insensitivity by inhibition of phosphoinositide-3-kinase-delta. J Pharmacol Exp Ther 2011; 337: 465-470.
[9] To Y, Ito K, Kizawa Y, Failla M, Ito M, Kusama T, Elliott WM, Hogg JC, Adcock IM and Barnes PJ. Targeting phosphoinositide-3-kinase-delta with theophylline reverses corticosteroid insensitivity in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2010; 182: 897-904.
[10] Lechner M, Sutton L, Ferguson M, Abbas Y, Sandhu J and Shaida A. Intratympanic steroid use for sudden sensorineural hearing loss: current otolaryngology practice. Ann Otol Rhinol Laryngol 2019; 128: 490-502.
[11] Almosnino G, Holm JR, Schwartz SR and Zeitler DM. The role of hyperbaric oxygen as salvage therapy for sudden sensorineural hearing loss. Ann Otol Rhinol Laryngol 2018; 127: 672-676.
[12] Leung MA, Flaherty A, Zhang JA, Hara J, Barber W and Burgess L. Sudden sensorineural hearing loss: primary care update. Hawaii J Med Public Health 2016; 75: 172-174.
[13] Wei FQ, Wen L, Chen K, Liu M and Wu X. Different prognoses in patients with profound sudden sensorineural hearing loss. Acta Otolaryngol 2019; 139: 598-603.
[14] Vandewalle J, Luypaert A, De Bosscher K and Libert C. Therapeutic mechanisms of glucocorticoids. Trends Endocrinol Metab 2018; 29: 42-54.
[15] Maslov LN, Naryzhnaya NV, Boshchenko AA, Popov SV, Ivanov VV and Oeltgen PR. Is oxidative stress of adipocytes a cause or a consequence of the metabolic syndrome? J Clin Transl Endocrinol 2019; 15: 1-5.
[16] Guan FJ, Peng QQ, Wang LL, Yan XB, Dong C and Jiang XH. Histone deacetylase-2 expression and activity in children with nephrotic syndrome with different glucocorticoid response. Pediatr Nephrol 2018; 33: 269-276.
[17] Ito K, Yamamura S, Essilfie-Quaye S, Cosio B, Ito M, Barnes PJ and Adcock IM. Histone deacetylase 2-mediated deacetylation of the glucocorticoid receptor enables NF-kappaB suppression. J Exp Med 2006; 203: 7-13.
[18] Adenuga D, Caito S, Yao H, Sundar IK, Hwang JW, Chung S and Rahman I. Nrf2 deficiency influences susceptibility to steroid resistance via HDAC2 reduction. Biochem Biophys Res Commun 2010; 403: 452-456.
[19] Evangelisti C, Cappellini A, Oliveira M, Fragoso R, Barata JT, Bertaina A, Locatelli F, Simioni C, Neri LM, Chiarini F, Lonetti A, Buontempo F, Orsini E, Pession A, Manzoli L, Martelli AM and Evangelisti C. Phosphatidylinositol 3-kinase inhibition potentiates glucocorticoid response in B-cell acute lymphoblastic leukemia. J Cell Physiol 2018; 233: 1796-1811.
[20] Yuan L, Liu C, Wan Y, Yan H and Li T. Effect of HDAC2/Inpp5f on neuropathic pain and cognitive function through regulating PI3K/Akt/GSK-3beta signal pathway in rats with neuropathic pain. Exp Ther Med 2019; 18: 678-684.
[21] Sadhu C, Masinovsky B, Dick K, Sowell CG and Staunton DE. Essential role of phosphoinositide 3-kinase delta in neutrophil directional movement. J Immunol 2003; 170: 2647-2654.
[22] Ueda K, Nishimoto Y, Kimura G, Masuko T, Barnes PJ, Ito K and Kizawa Y. Repeated lipopolysaccharide exposure causes corticosteroid insensitive airway inflammation via activation of phosphoinositide-3-kinase delta pathway. Biochem Biophys Rep 2016; 7: 367-373.
[23] Xie M, Yang A, Ma J, Wu M, Xu H, Wu K, Jin Y and Xie Y. Akt2 mediates glucocorticoid resistance in lymphoid malignancies through FoxO3a/Bim axis and serves as a direct target for resistance reversal. Cell Death Dis 2019; 9: 1013.
[24] Marwick JA, Caramori G, Stevenson CS, Casolari P, Jazrawi E, Barnes PJ, Ito K, Adcock IM, Kirkham PA and Papi A. Inhibition of PI3Kdelta restores glucocorticoid function in smoking-induced airway inflammation in mice. Am J Respir Crit Care Med 2009; 179: 542-548.