Correspondence

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Notch signaling pathway plays a critical role in chemotherapeutic drug-induced vestibular injury

Wen Jiang[1], Jie Yu[2], Maorong Cao[3], Bin Xiao[1], Feifan Li[3], Chengfang Chen[4], Huiming Yang[4], Ziwen Wang[3], Fenye Liu[5,*], Shudong Yu[4,*]

[1] The First Faculty of Clinical Medicine, Shandong University of Traditional Chinese Medicine, Ji'nan, Shandong, China;
[2] Department of Otorhinolaryngology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China;
[3] The Clinical Medical College, Shandong First Medical University (Shandong Academy of Medicine), Ji'nan, Shandong, China;
[4] Department of Otolaryngology, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Ji'nan, Shandong, China;
[5] Department of Traditional Chinese Medicine, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Ji'nan, Shandong, China.

SUMMARY The vestibule of the inner ear is susceptible to certain chemotherapeutic agents in clinical practice. Therefore, it is of great significance to discover molecular pathways and targets that can protect the vestibule from chemotherapeutic drugs. The Notch signaling pathway is closely related to hair cell regeneration in the inner ear. However, the role of Notch signaling in chemotherapeutic drug-induced vestibular injury still remains unclear. The aim of this study was first to evaluate the role of Notch signaling in chemotherapy-induced vestibular injury. Cisplatin-induced vestibular injury of mice was evaluated by the swimming test. Changes of vestibular hair cells and the expression levels of Notch1, Jagged1, and Hes1 in Notch signaling were observed by immunofluorescence. The results showed that Notch signaling was found activated in cisplatin-induced injured vestibular cells, while, DAPT (Notch signaling inhibitor) could reversed this effect. In conclusion, the Notch signaling pathway may play a critical role in chemotherapeutic drug-induced vestibular injury and, therefore, serves as a promising therapeutic target for vestibular injury.

Keywords Vestibular injury, chemotherapeutic drug, Notch signaling pathway

Vestibular injury can be caused by a variety of factors, including ototoxic drugs, excessive noise, and aging (*1*). Indeed, ototoxicity is a major toxic side effect of several beneficial pharmaceutical drugs, including chemotherapy agents such as cisplatin. Cisplatin-induced ototoxicity has a mean rate of 62% (*2*). Chemotherapeutic drugs can cause irreversible damage to the vestibule of the inner ear, leading to oscillopsia, blurry vision, distorted perception of self-orientation, imbalance, gait ataxia, and abnormal posture (*3*). In contrast to the non-regenerative cochlear hair cells of many mammals, vestibular hair cells exhibit spontaneous regeneration. However, the regeneration of vestibular hair cells is very limited, and vestibular function cannot be restored by their spontaneous regeneration (*4*). Therefore, it is very important to protect the vestibular hair cells from damage.

Hair cell production in the adult utricle is regulated by the Notch signaling pathway (*5*). In mammals, the Notch signaling pathway consists of four receptors (Notch1, Notch2, Notch3, and Notch4) and five ligands (Jagged1, Jagged2, DLL1, DLL3, and DLL4). Ligands attach to receptors, and the activated form of Notch protein is cleaved by γ-secretase and released into the cytoplasm (*6*). Subsequently, it may activate the downstream target gene Hes1 (*7*). DAPT, a γ-secretase inhibitor, can block Notch signaling. Therefore, DAPT has been used as a tool drug to inhibit the Notch signaling pathway in a large number of studies. Involvement of the Notch signaling pathway in development of the inner ear has garnered increasing attention in recent years. Notch signaling induces hair cell formation through lateral inhibition and is involved in hair cell proliferation and apoptosis (*8*). Moreover, the activation of Notch signaling has been linked to cochlea hair cells loss (*9*). However, whether the Notch signaling pathway plays a role in chemotherapeutic drug-induced vestibular injury has not been investigated yet.

Therefore, we investigated the role of the Notch signaling in chemotherapeutic drug-induced vestibular injury. To our knowledge, this is the first time this

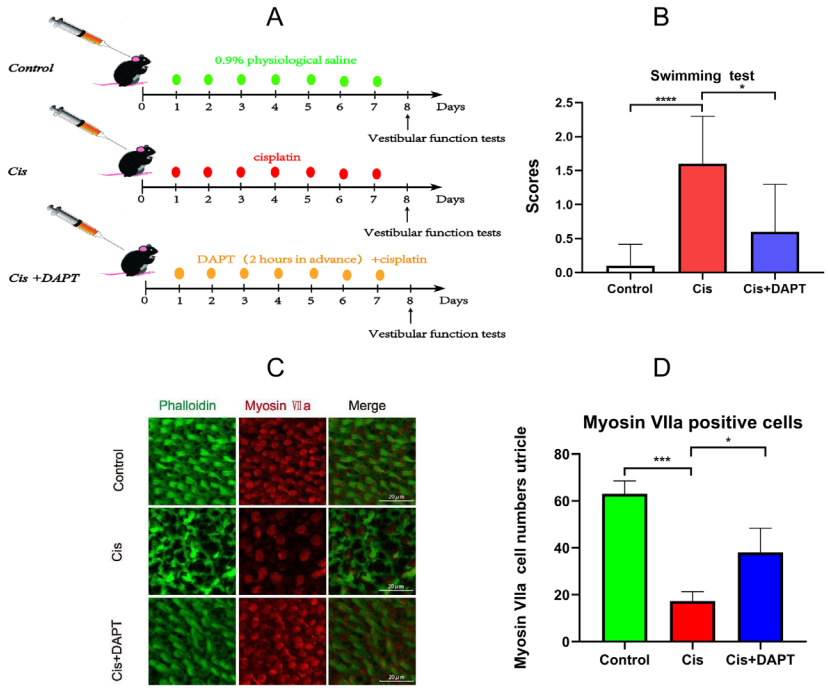

Figure 1. Inhibition of Notch signaling protected against cisplatin-induced vestibular injury. (A) Three groups of C57BL/6 mice received intraperitoneal medication injections as illustrated. **(B)** The scoring criteria swimming test was as follows. Zero points were scored if the mouse could keep its head out of the water and swim straight to the side of the container. One point was scored if the mouse could keep its head out of the water and maintain a slightly circular swimming posture. Two points were scored if the mouse could not keep its head above water and swim slightly in a circular manner. Three points were scored if the mouse could not keep its head above water and swim severely in a circular manner. **(C)** Cuticular plate and hair cell stereociliary bundles are marked with phalloidin (green), while hair cells are labeled with myosin VIIa (red). **(D)** Quantitative analysis of myosin VIIa-positive cell numbers in the utricles.

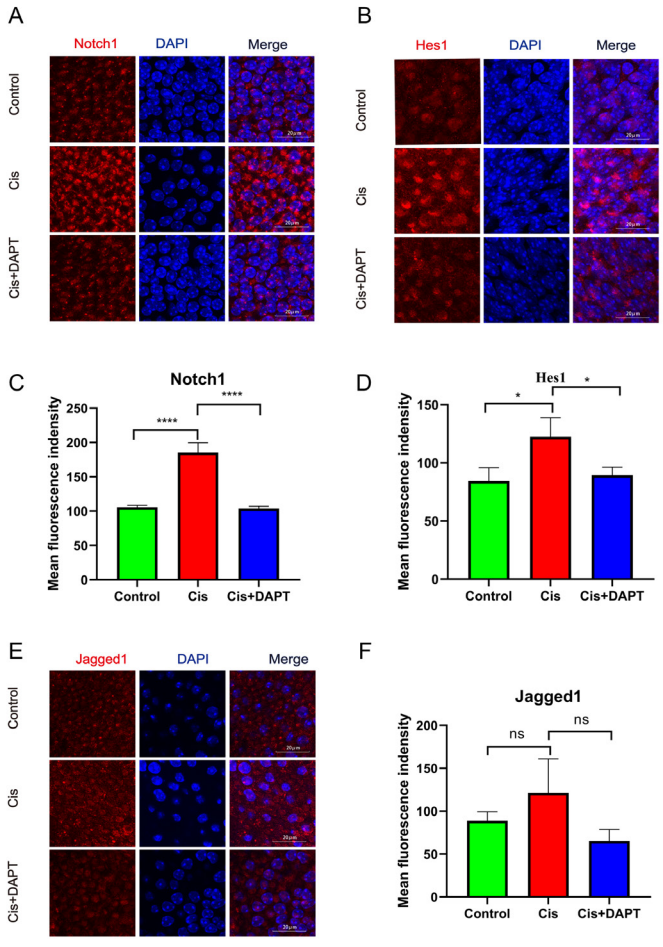

Figure 2. Effects of Notch signaling inhibition on cisplatin-induced vestibular injury in mice. (A) and **(B)** Immunofluorescence with Notch1 and Hes1 (red) antibodies and DAPI (blue) in utricles from control, cisplatin, and cisplatin + DAPT groups. **(C)** and **(D)** Quantitative analysis of Notch1 and Hes1 fluorescence intensity in the utricles. **(E)** Immunofluorescence with Jagged1 (red) antibodies and DAPI (blue) in utricles from control, cisplatin, and cisplatin + DAPT groups. **(F)** Quantitative analysis of Jagged1 fluorescence intensity in the utricles. Control: 0.9% physiological saline, Cis: cisplatin, Cis+DAPT: cisplatin + DAPT. Scale bars = 20 μm. Data were showed as mean ± SD. (Statistical analysis was performed using one-way analysis of variance (ANOVA), ns means no significant difference, $^{*}p < 0.05$, $^{***}p < 0.001$, $^{****}P < 0.0001$).

has been investigated, the results have important implications for the prophylaxis and treatment of vestibular injury.

Inhibition of Notch signaling protected against cisplatin-induced vestibular injury

In this study, thirty C57BL/6 mice (aged 7 weeks) were randomly divided into control, cisplatin, and cisplatin + DAPT groups, with 10 mice in each group. Mice in the control group were intraperitoneally injected with 0.9% physiological saline (0.6 mL/100g). Mice in the cisplatin group were intraperitoneally injected with cisplatin (3 mg/kg; Sigma, St. Louis, MO, USA). Mice in the cisplatin + DAPT group were first intraperitoneally injected with DAPT (10 mg/kg; Sigma), and 2 h later were injected intraperitoneally with cisplatin (3 mg/kg). All the above operations were performed continuously for 7 days (Figure 1A).

Vestibular function was assessed by observing the swimming posture of mice. It was showed that the vestibular injury was less severe in the cisplatin + DAPT group than in the cisplatin group. Mice in the cisplatin group displayed a severe circular swimming posture and were unable to keep their heads above water. In addition, mice in the cisplatin group showed abnormal vestibular function compared with mice in the control group. Mice in the cisplatin + DAPT group had a steady swimming posture and kept their heads above water, indicating that their vestibular function was not significantly impaired. Indeed, swimming test scores were significantly improved in the cisplatin + DAPT group compared with the cisplatin group (Figure 1B).

Changes of vestibular hair cells were observed by immunofluorescence. In the cisplatin group, the number of hair bundles and hair cells in the utricles was significantly lower, and were disorganised and spread out. In comparison, hair bundles and hair cells were effectively preserved, morphologically normal, and tightly arranged in the cisplatin + DAPT group (Figure 1C). The results of quantitative hair cell analysis were consistent with the swimming test (Figure 1D). It is therefore likely that inhibition of Notch signaling protected against cisplatin-induced vestibular injury.

Notch-related expression in the mouse utricle after Notch signaling pathway inhibition

Immunofluorescence were used to observe protein expression levels of Notch signaling pathway molecules in the utricles. As shown in Figures 2A and 2B, Notch1 and Hes1 protein levels were elevated in the cisplatin group compared with the control group. In addition, the cisplatin + DAPT group showed decreased protein levels of Notch1 and Hes1 in the utricles compared with the cisplatin group (Figures 2C and 2D). However, there was no significant difference in Jagged1 protein expression levels between cisplatin and cisplatin + DAPT groups (Figures 2E and 2F).

The relationship between Jagged1, Notch1, and Hes1 expression remains unclear in the inner ear. After cochlear hair cell injury, Jagged1 expression levels in cochlea hair cells did not change (*10*). Other studies have shown that Jagged1 is mainly expressed in support cells (*11*). However, Notch1 can interact with Jagged2, DLL1, DLL3, and DLL4 to activate the Notch signaling pathway. In addition, other signaling pathways (such as Fgf, Bmp, or Wnt) can regulate the Notch signaling pathway (*12,13*). In our study, we found that the Notch signaling pathway was activated after vestibular injury. Ligands or signaling pathways that interact with Notch1 in the vestibular system require further study.

In conclusion, our study revealed that the Notch signaling pathway may play a critical role in chemotherapeutic drug-induced vestibular injury. These results lay the foundation for preventing the side effects of chemotherapy. It should be pointed out that this study is a preliminary exploration of the role of the Notch signaling pathway in the vestibule. Accordingly, future research is still needed to explore the molecular mechanism by which Notch signaling reduces chemotherapeutic drug-induced vestibular injury. In addition, DAPT is a γ-secretase blocker, and it is necessary to explore whether other Notch signaling blockers or approaches have the above-mentioned effects.

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Conflict of Interest: The authors have no conflicts of interest to disclose.

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**Address correspondence to:*
Fenye Liu, Department of Traditional Chinese Medicine, Shandong Provincial Hospital Affiliated to Shandong First Medical University, 324 Jingwuweiqi Road, Ji'nan, Shandong 250021, China.
E-mail: drzhang1978@163.com

Shudong Yu, Department of Otolaryngology, Shandong Provincial Hospital Affiliated to Shandong First Medical University, 324 Jingwuweiqi Road, Ji'nan, Shandong 250021, China.
E-mail: yushudong@126.com

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