ГИГИЕНА И ПРОФИЛАКТИЧЕСКАЯ МЕДИЦИНА

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CONSTRUCTION AND VALIDATION OF PRESTIN OVEREXPRESSION ADENO - ASSOCIATED VIRUS VECTOR IN OUTER HAIR CELLS

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Annotation . OBJECTIVE: By constructing cochlear hair cell recombinant Prestin adeno-associated virus-2 (AAV-2) vector, it provides a tool for the study of cochlear Prestin protein regulation. METHODS: The guinea pig Prestin gene was used as a template for PCR amplification and extraction. The AAV-2-Prestin vector was constructed by genetic engineering. The expression level of Prestin was detected by Western blot on the 1st, 3rd and 5th day after transfection of cultured cochlear hair cells. RESULTS: After transfecting HEK-293T cells with pAAV-EGFP-Prestin proviral plasmid, the expression of weak green fluorescent protein was observed on the 1st day, and the expression of green fluorescent protein was peaked on the 3rd day. Conclusion: The recombinant adeno-associated virus-2-Prestin vector was successfully constructed and has good transfection efficiency and persistence.

Key word: Outer hair cells ; Adeno-associated virus ; Prestin

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Introduction

Noise-induced hearing loss (NIHL) is a sensory deafness caused by prolonged exposure to noisy environments. It is one of the most common occupational diseases. There is no effective treatment for NIHL. The root cause is that the outer hair cells (OHCs) of the cochlea are irreversibly damaged by noise[1]. In the previous study, it was found that Prestin protein is an important sensory function protein unique to cochlear OHCs, which is the molecular basis of OHCs electrokinetics and cochlear amplification effect. Prestin protein compensated for the loss of OHCs to compensate for hearing loss [2]. Prestin protein belongs to the 5th member of the anion transporter family (SLC26A5)[3]. In 2000, a kind of Outer Hair Cells (OHCs)was isolated for the first time. The new gene, the protein it expresses is the Prestin protein, and Prestin is a transmembrane motor protein uniquely expressed in the inner ear OHCs [4]. Based on the study of the regulation mechanism of OHCs Prestin protein in the cochlea, it can be speculated that Prestin protein not only plays an important role in the mechanism of sensorineural deafness, but also may have certain

potential value for the repair of sensorineural deafness, but there is no related studies to verified this.

Purpose of the study

This study aims to construct a guinea pig cochlear Prestin overexpressing virus vector to provide a tool for the regulation of OHCs Prestin protein regulation. Due to the tropism of cochlear OHCs virus transfection, Adeno-associated virus (AAV) was selected as a vector for transfection of OHCs gene, and it is expected to have high transfection efficiency, stable expression and targeting.

1 Materials and methods of research

1.1 Materials

HEI-OC1 cells (House Institute, USA), HEK-293T cells (赛哲生物, China), DMEM medium (Gibco, USA), fetal bovine serum (Hangzhou Sijiqing, China), pAAV vector (赛业Biotech Co., Ltd., China), Penicillin-Streptomycin (Gibco, USA), trypsin TrypLETM Express Enzyme (Gibco, USA), DPBS (HyClone, USA), GAPDH antibody (Proteintech, USA), Prestin antibody (Santa Cruz, US), Western Cell Lysate (Proteinsimple, USA), Anti-Rabbit Detection Module for Wes Kit (Proteinsimple, USA), 12-230kDa Wes Separation Module Kit (Proteinsimple, USA), Protease Inhibitor (Biyuntian Biological Research Institute, China), BCA Protein Concentration Assay Kit (Biyuntian Biological Research Institute, China), Primer Design Synthesis (Saizhe Biotech, China), PCR Instrument (Bio-RAD, USA), Cell Culture Incubator (SANTYO, Japan)), Wes type automatic Western blot analyzer (Proteinsimple, USA), fluorescence microscope (OLYMPUS, Japan) .

1.2 Methods

1.2.1 Amplification and extraction of guinea pig Prestin gene

The guinea pig Slc26a5 gene (Prestin) sequence (ID: XM_003469805.1) was searched in the MedGen database of NCBI, and the primer sequence was designed as a template for PCR amplification(upstream:GCCGAATTCCGATGGATCATGCTGAAG,downstream:TAACTCGAGGGCCTCGGGTGTGGC), and the amplified Prestin gene (2232 bp) was extracted and purified.

1.2.2 Construction of Prestin Adeno-associated Virus (pAAV) Plasmid

The pAAV vector proviral plasmid consists of the promoter CAG-regulated green fluorescent protein (EGFP) gene (reporter gene) and the AAV terminal inverted repeat sequence. The guinea pig Prestin gene fragment was cloned into the plasmid to form the pAAV-Prestin provirus plasmid (pAAV- EGFP-Prestin). The pAAV-Prestin proviral plasmid was transfected into HEK-293T cells, and the expression of green fluorescent protein was observed under fluorescence microscope at 1 d, 3 d, and 5 d after infection, and photographed.

1.2.3 Preparation of AAV-2-Prestin Vector

The pAAV-Prestin plasmid was prepared by assembly using the AAV-2 packaging plasmid (pAAV2 RepCap), and the plasmid was purified by QIAGEN Plasmid Purification Kit (provided by Guangzhou Saiye Biotechnology Co., Ltd.).

The titer of the prepared virus was 2.68×1012 GC/ml, and was sent to Yunzhou Biotechnology (Guangzhou) Co., Ltd. for sequencing verification.

1.2.4 AAV-2-Prestin vector transfection to culture HEI-OC1 cells

AAV-2-Prestin vector was transfected into cultured HEI-OC1 cells (33 ° C, 10%), 6-well cultured cells were collected at 1 d, 3 d, 5 d after transfection, and non-transfected HEI- OC1 cells were used as blank control. Protein was extracted, and the expression level of Prestin in HEI-OC1 cells was detected by Western blot (WB).

1.3 Statistical analysis

The data were analyzed by SPSS 20.0 software. The measurement data were described by means of mean±standard deviation. The average value of the relative expression levels of Prestin protein in each group after transfection was analyzed by one-way ANOVA test. The test level α=0. 05.

2 Results of the study and discussion

2.1 pAAV- Prestin proviral plasmid verification

After transfection of pAAV-Prestin proviral plasmid into HEK-293T cells, the expression of green fluorescent protein was observed under fluorescence microscope. The weak fluorescence was observed on the 1st day, the fluorescence expression reached the peak on the 3rd day, and the fluorescence intensity on the 5th day was weaker than the 3d day (Fig. 1).

Figure 1. Comparison of green fluorescent protein expression at different transfection times

2.2 AAV-2-Prestin transfection of HEI-OC1 cells Prestin expression results

AAV-2-Prestin was transfected into HEI-OC1 cells. The expression levels of Prestin in each group are shown in Figure 2. The relative gray value of Prestin is 0.045±0.002 in the control group, and 0.052±0.001 in the 1d. The relative gray value of Prestin is 0.076±0.002 in the 3d, and 0.061±0.002 in the 5d. Compared with the control group, the expression levels of Prestin protein in each group were significantly increased at different transfection times ($F=414.755$, $P< 0.001$), the expression of Prestin protein in the 3d and 5d groups was significantly higher than 1d ($P<0.001$), and the expression of Prestin protein in the 3d group was significantly higher than that in the 5d group ($P<0.001$).

Figure 2 Western-blot detection of Prestin expression in HEI-OC1 cells

2.3 Discussion

The expression of Prestin protein is an important factor affecting the audio signal of OHCs. Noise, radiation, hypoxia and drug toxicity may lead to changes in the expression level of Prestin of OHCs. One of the molecular mechanism is the decline of Prestin expression, but some studies have found that noise-induced hearing loss leads to compensatory increase in Prestin protein expression levels, indicating that Prestin expression is closely related to multiple sensorineural hearing loss diseases. It can be confirmed that the expression level of Prestin has a great correlation with the hearing level. This correlation provides an imaginary space for the potential repairment of the hearing loss, and the Prestin protein overexpression model for constructing OHCs contributes to the study of multiple hearing disorders.

Viral vectors have certain targeting effects on gene transfection of inner ear cells. Viral vectors commonly used for transfection of inner ear cells include adenovirus, adeno-associated virus, lentivirus, herpes simplex virus and vaccinia virus vector. Adeno-Associated Virus (AAV) is a potent tool for transducing OHCs in the inner ear. AAV is a type of single-stranded linear DNA-deficient virus, according to the AAV viral capsid protein. The differences have identified 12 serotypes (AAV-1 to AAV-12) [5]. Different serotype AAV vectors transfected into different target cells in inner ear, of which AAV-2 [6] is currently the most effective for cochlear OHCs. The serotype has the advantages of high safety, low immunogenicity, high targeting transfection of outer hair cells of the cochlea and high transfection efficiency. Therefore, the selection of AAV-2 as a vector is the primary option for efficient transfection of cochlear OHCs Prestin gene.

Mature hair cells can not be subcultured in vitro so HEI-OC1 cell line is isolated from the immortalized mouse cochlear Corti apparatus by American House Research Institute, and can stably subculture the hair cell strain in vitro. Transfection of HEI-OC1 cell line with recombinant AAV-2 virus can maximize the tropism of the virus on hair cell transfection. The results showed that the expression level of Prestin protein in transfected hair cells was significantly increased. The peak was reached 3 days after transfection, indicating the recombinant Prestin overexpressing adeno-associated virus vector was successfully constructed. It has the targeting and stability of hair cell transfection, and the transfection rate is good.

Conclusion

At present, the use of recombinant adeno-associated vector to construct over-expressing Prestin has not been reported. This study successfully constructed the expression vector of guinea pig Prestin gene, and confirmed that Prestin gene can be successfully expressed in hair cells cultured in vitro. This provides a good tool for the expression regulation of Prestin.

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ГИГИЕНИЧЕСКАЯ ОЦЕНКА ПИТАНИЯ СТУДЕНТОВ

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HYGIENIC ASSESSMENT OF THE NUTRITION OF STUDENTS

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Аннотация. В статье рассмотрены особенности питания студентов УГМУ в сравнении со студентами немедицинских ВУЗов.

Annotation. The article discusses the nutritional habits students of the Ural State Medical University in comparison with students of non-medical universities.

Ключевые слова: питание, студенты, физическая активность, образ жизни.

Key words: nutrition, students, physical activity, lifestyle.

Введение

Питание как фактор, связанный с образом жизни во многом определяет состояние здоровья. Многие неинфекционные алиментарно-зависимые заболевания связаны с нерациональным питанием [4]. Сбалансированное питание является одним из ключевых факторов гармоничного развития молодого организма. Однако особенности деятельности студентов (нарушение режима труда и отдыха, интенсивные информационные нагрузки, транспортные переезды между клиническими базами, стрессогенные ситуации: зачеты и экзамены, влияние факторов внутрибольничной среды) ведут к нарушению режима питания и его качественного состава [2]. Как отмечают отечественные исследователи [1,3], это приводит к развитию хронических заболеваний, снижению трудоспособности студента как в процессе обучения, так и в дальнейшей профессиональной деятельности.

Цель исследования – гигиеническая оценка питания студентов 3 курса УГМУ и студентов высших учебных заведений другого профиля методом анкетирования.