Transcriptome Comparison of the organ of Corti in chick and zebrafish

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Abstract

Hearing loss is mammalian species is hard to restore, while birds and other species can regenerate hair cells after the damage like noise exposure or old age. Here we try to make a comparison of the transcriptome in the chicken, and zebrafish. We first make a comparison of gene expression at different stages of the key genes that regulate the regeneration of the inner hair cell or other types of hair cells. Then we compared the key genes in different species. We hope the comparison can offer a good view of the gene expression in the regeneration process and shed new light on the potential candidate genes that will further investigate to help the treatment of hearing loss.

Keywords: Transcriptome, Organ of Corti, hair cells

Introduction

Hearing loss affects about 1.1 billion people to some degree at 2013 according to Global Burden of Disease report[1]. It can begin during the childhood and aging will also result in a progressing hearing loss. Noise, chemicals and physical trauma can also lead to hearing loss[2]. Genetic factors make some people more susceptible to hearing loss than others, as their genes make them more predisposed to hearing loss.

Hair cells are the sensory receptors of both the auditory system and the vestibular system in the ears of the vertebrates and lateral line organ of fishes. The inner hair cells transform the sound vibrations in the fluids of the cochlea into electrical signals that are then relayed via the auditory nerve to the auditory brainstem and to the auditory cortex[3].

While in humans and other mammals, the ability to regenerate the inner hair cells is very limited and damage of the hair cells is permanent, unlike birds and fish which keep the power to regenerate the hair cells after damage[4]. Although much is known about how hair cells function in hearing, we still have very limited knowledge of the molecular mechanisms, like gene expression and regulation, that underlie the distinct morphological and functional specializations and the difference among mammals and non-mammals.

Transcriptome analysis of the hair cells or related cells is a nice method to investigate the gene expression pattern either in different developmental stages or after damage, through the comparison we can identify the key genes that have an important function in the hair cell and may also discover potential treatment target[5].

In this paper, we collected the RNA seq data and microarray data of different time points in the developmental stages of chicken and zebrafish. We try to make a comprehensive analysis of the expression trend of key genes. We expect that our study will make a bigger picture of the gene expression of hearing-related cells and hope more and more research will carry out especially on nonmammals like chicken and fish which has a good regenerative ability.

Results

We mainly focus on seven know pathways that are very important for cochlear development and investigate some key genes expression trends during the development period. We try to identify which pattern the key genes have and may indicate new potential treatment markers.

Known pathways in the chicken

As the bird's hair cells can regenerate after the damage, this regeneration can from the support cells, and is investigated to help understand the gene regulatory pathways and hope can be applied to human subjects. While as we searched the paper, still so little published data like RNA seq or microarray data has for the chicken, which surely slows the process of revealing the underlying mechanism.

We look into the relative expression of the known important pathways by the data we got from the public database at E6.5 RNA seq data. At the E6.5 JUN has the highest expression, and TGFBR3 and NFKB1, IGF2, IGF2R have relatively very low expression.

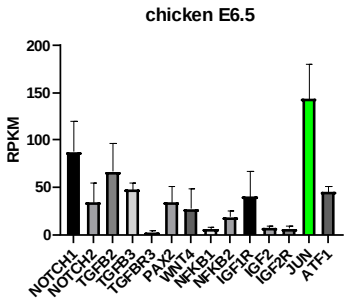

Fig. 1. Key genes expression at E6.5 chicken hair cells

Known pathways in zebrafish

Zebrafish is another very important model organism. Zebrafish can regenerate the hair cells after damage. Understanding the mechanisms that regulate hair cell regeneration in zebrafish may help the finding possible markers that can regenerate hair cells in mammals. If in some way can mimic the zebrafish activation status of a combination of pathways after the hair cell lost either by aging, noise damage, or other drug toxicity, it may gain great success of hair cell regeneration in mammals[6].

From the P4 microarray data, we can see that the key genes are all very active expressed, while the P5 RNA seq data show that JUN has the relatively high expression, while the P4 mouse RNA seq data shows the similar expression pattern of the Zebrafish P5, PA1(Jun, Fos) signaling pathway is very active, while not as the P4 zebrafish microarray data, which may cause as the development difference(Figure 2).

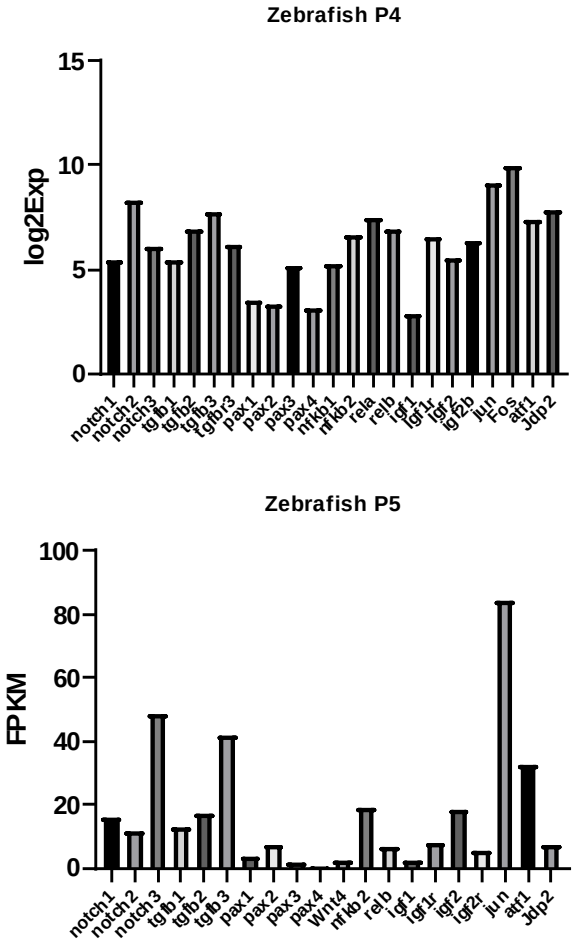

Fig. 2. Key genes expression at P4 and P5 zebrafish hair cells

Discussion

Through the transcriptome analysis and microarray data analysis of the key genes pathways that participate in the regeneration of inner hair cells, we show that there are differences between the species and within the gene family, indicates the specific function of each gene and the pathways.

Chicken only has E6.5 and P10 data by far through search the literature, and zebrafish only has P4 and P5 data. The lack of sequencing data on species that can regenerate hair cells certainly hampered the revealing mechanisms of hair cell regeneration, as if more we illuminate the mechanisms underlying the regeneration, we can better mimic the gene expression patterns in the mammals and may have great success in restore hearing loss.

Also, the structure of the auditory organ is different among the different species, mouse cochlea corresponding to the chicken basilar papilla, and the zebrafish lateral line system. For the vertebrates, the vestibular portion of the ear is relatively conserved, fish like zebrafish have a specialization that increases their sensitivity to sound, the specialization consists of a series of bones[7]. In fish, more function endowed the lateral line organ as it also detects water movements, allowing aquatic animals to have a sense of 'distant touch' of their local environment[8].

We briefly investigated the several known pathways that have import function during the hair cells regeneration, and show the different expression pattern within the gene families and among species, as the lack of the chicken and zebrafish data, we just make a simple relatively comparison, and hope more research can conduct on these species, and also several other species that can help identify the potential treatment markers.

Materials and Methods

RNA seq analysis

All RNA seq raw data were downloaded from the geo dataset website. For the analysis, first, the low-quality reads and adaptors were removed from the raw data by Trimmomatic (V0.39), and then the clean reads were mapped to the mm10 reference genome by STAR (2.7.1a), then we use featureCounts to extract the raw counts from the aligned bam file. Raw counts files were combined and use custom R scripts to transform to TPM value. GraphPad Prism (8.4.1) was used to display the TPM values of the key gene signaling pathways.

Microarray data analysis

We download the raw microarray data (CEL file) from the GEO datasets website and use Transcriptome Analysis Console (TAC) software from the Thermofisher website. We follow the TAC manual to

analyze the data with the default parameters, the array library files download by the software according to the specific file used for the sample.

End Matter

Author Contributions and Notes

Sen Guo collects the raw data and analyzed data, and Sen Guo wrote the paper.

The authors declare no conflict of interest.

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The paper is under big modification(current version is truncated version, full revised version will be update later), and far from perfect, please send comments and suggestions to senguo@wayne.edu , we appreciate your ideas.

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