Transcriptome Comparison of organ of Corti in mice, chick and zebrafish

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Abstract

Hearing loss is mammalian species is hard to restore, while birds and other species has the capacity to regenerate hair cells after the damage like noise exposure or old age. Here we try to make a comparison of transcriptome in the mice, 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 cell. Then we compared the key genes in different species. We hope the comparison can offer a good view of the gene expression in 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 cell 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 in to electrical signals that are then relayed via auditory nerve to the auditory brainstem and to the auditory cortex[3].

While in humans and other mammals, the ability of regenerate the inner hair cells are 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 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 mice, chicken and zebrafish. We find quite a lot of time points in mice and only very few time points in chicken and zebrafish. So 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 good regenerative ability.

Results

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

Key genes comparison in their regulatory network

Notch regulatory pathway

The Notch signaling pathway is highly conserved cell signaling system present in most animals[6]. Mammals possess four different notch receptors, referred to as Notch1, Notch2, Notch3,Notch4[7]. The notch receptor is a single-pass transmembrane receptor protein. It's a hetero-oligomer composed of a large extracellular portion, which associates in a calcium-dependent, non-covalent interaction with a smaller piece of the notch protein composed of a Short extracellular region, a single transmembrane-pass, and a small intracellular region[8]. Notch signaling promotes proliferative signaling during neurogenesis, and its activity by Numb to promote neural differentiation. It plays a major role in the regulation of embryonic development and it's very important for cell-cell communication.

During the development period, by RNA seq data collected from the public database, we can see the trend of the four different notch receptors expression trend (Figure 1). We can see that at the embryonic period the receptors expression levels are decreased and reach a low level at P28, while it is worth noting that the Notch2 expression is much

higher than the other three receptors. Which may indicate that Notch2 is very important for the development of organ of Corti. We also collect the microarray data at P1, P15, P42 and P70, through the comparison we can see that there is a trend of the decrease to relative low expression level for Notch1, Notch2 and Notch4, Notch3 is still relatively a bit high expression levels, which indicates that Notch3 may play a role in the maintenance function (Figure2).

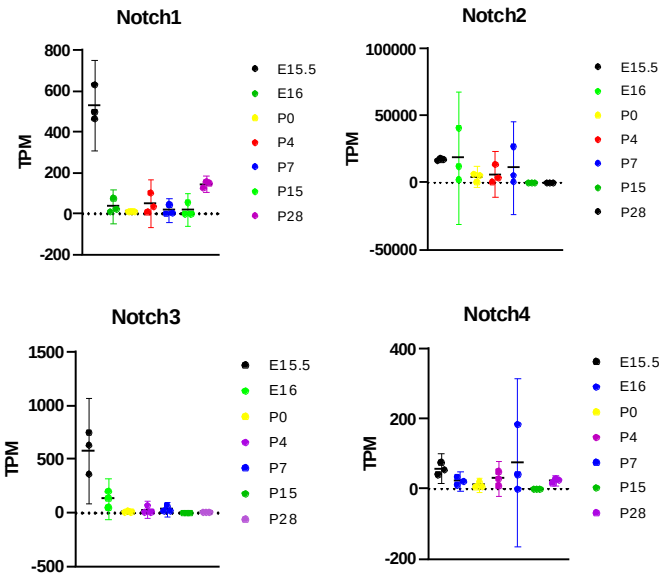
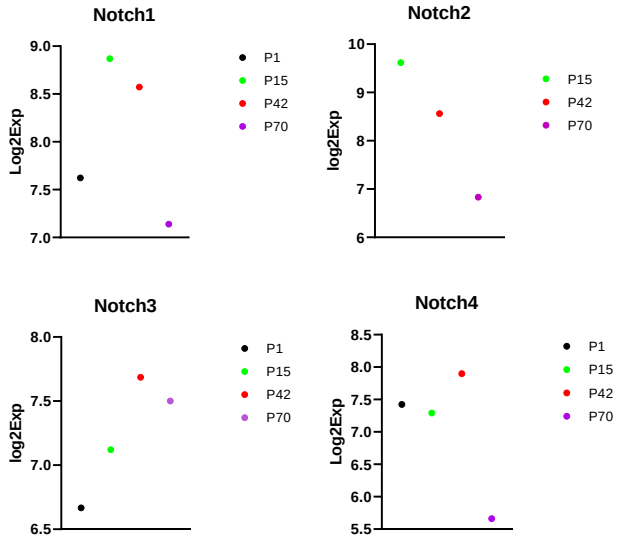


Fig. 1. Notch receptors expression trend at E15.5, E16, P0, P4,P7, P15, P20



TGFB regulatory pathway

Fig. 2. Notch receptors expression trend at P1, P15, P42, P70

TGFB is involved in many cellular processes in both the adult organism and the developing embryo including cell growth, cell differentiation, apoptosis, cellular homeostasis and other cellular functions. TGFB superfamily ligands bind to type II receptor, which recruits and phosphorylates a type I receptor, the type I receptor then phosphorylates SMADs and regulate target gene expression.

TGFB family include TGFB1, TGFB2,TGFB3, and TGFBR3 is the most abundant of the TGFB receptors yet[9]. TGFB3 has no known signaling domain, it however may serve to enhance the binding of TGFB ligands to TGFB type II receptors by binding TGFB and presenting it to TGFBR2. Here we show the expression trend of the four genes both use the collected RNA seq data (Figure3) and microarray data (Figure4).

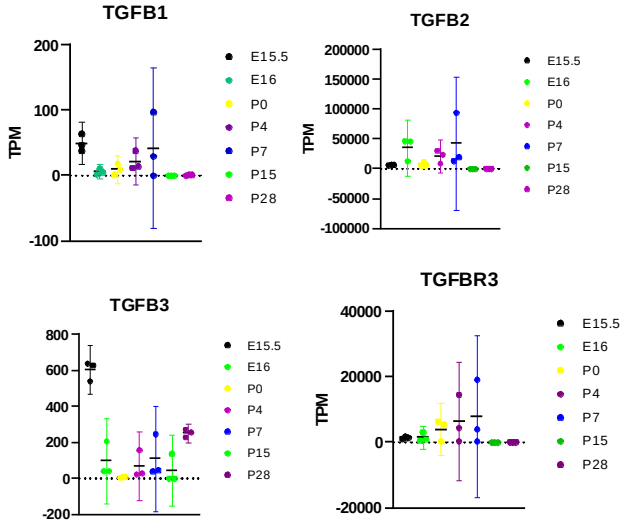
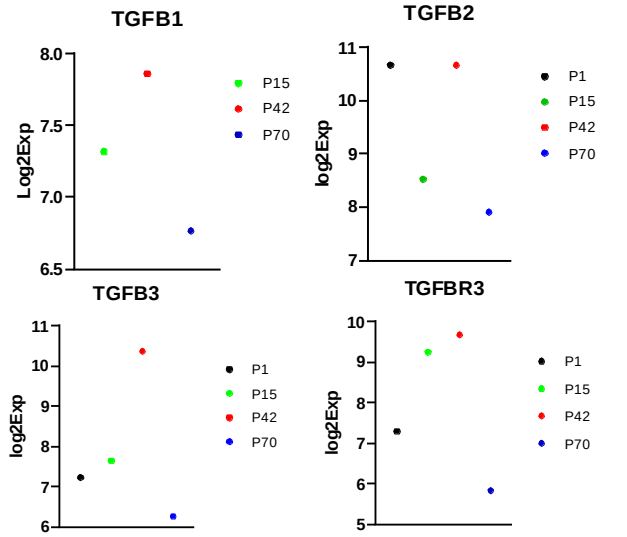


Fig. 3. TGFB1, TGFB2, TGFB3 and TGFBR3 expression trend at E15.5, E16, P0, P4, P7, P15, P20



TGFB1, TGFB3 both has high expression at P7, P15 and decrease at

Fig. 4. TGFB1, TGFB2, TGFB3 and TGFBR3 expression trend at P1, P15, P42, P70

adult time (P28, P100). TGFB2 has a relatively high expression at the early postnatal stage (near P1) indicates it has more function in the early postnatal stages which may contribute to the mature of the embryo. TGFBR3 as expected, follows the similar expression pattern as the TGFB1, TGFB2 and TGFB3, indicates the active functions follows the active expression.

PAX regulatory pathway

There are four well defined groups of Pax genes including 9 Pax genes (Pax1, Pax9, Pax2, Pax5, Pax8, Pax3, Pax7, Pax4, Pax6), and we focus on Pax1, Pax2, Pax3 and Pax4.

Pax proteins are important in early animal development for the specification of specific tissues, as well as during epimorphic limb regeneration in animals capable of such. This indicates it may also has very important roles in the hearing regeneration.

Pax1 has high expression at P0 and low expression at other time points, Pax2 has high expression at E15.5 and low expression at other time points, while Pax3 has high expression at E16.5 not E15, indicates it's differentiation from Pax1, Pax4 has high expression at P4 (Figure5). For the postnatal stage, Pax1, Pax2 and Pax3 has similar expression pattern which may indicate a synergic function. While Pax4 increased expression as the development (Figure 6).

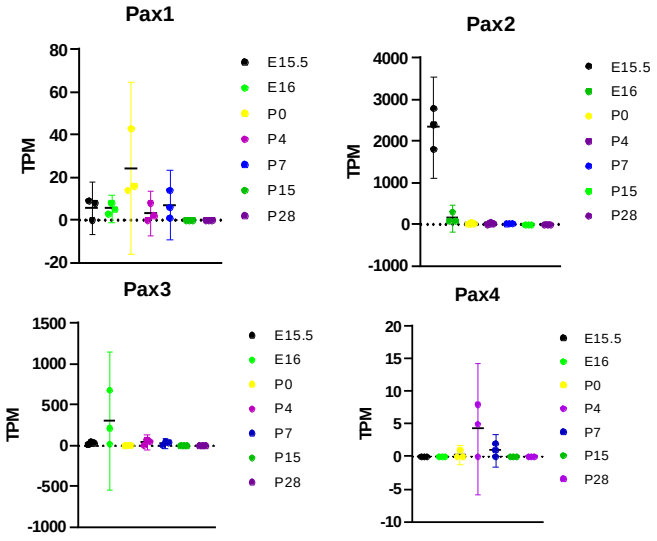


Fig. 5. Pax1, Pax2, Pax3 and Pax4 expression trend at E15.5, E16, P0, P4, P7, P15, P20

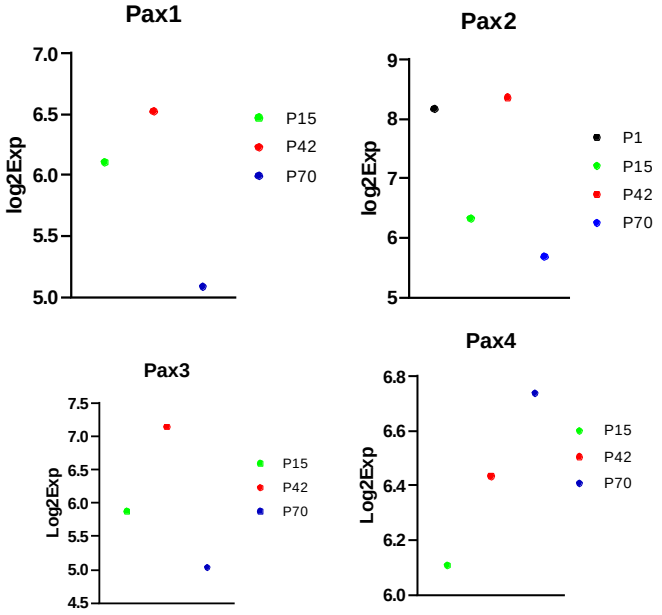


Fig. 6. Pax1, Pax2, Pax3 and Pax4 expression trend at P1, P15, P42, P70

Wnt regulatory pathway

Wnt signaling pathways are a group of signal transduction pathways which begin with proteins that pass signals into a cell through cell surface receptors. Wnt pathways use either nearby cell-cell communication(paracrine) or same-cell communication(autocrine). They are highly evolutionarily conserved in animals, across animal species from fruit to humans[10].

Wnt signaling was first identified for its role in carcinogenesis, then for its function in embryonic development. It functions including the body patterning, cell fate specification, cell proliferation and cell migration, which is very important for proper formation of important tissues including bone, heart and muscle. Here we mainly focus on Wnt4 as previous report show that Wnt4 is very important for hearing regeneration[11].

Wnt4 shows relatively high expression at P15 (Figure7 left), and increase at P42, and decrease at P70 (Figure7 right). It's interesting that wnt4 is required for sex development, which near P15 to P42 overlaps the sex maturation. This may also indicate that maybe there is some connection between the hearing regeneration and sex maturation.

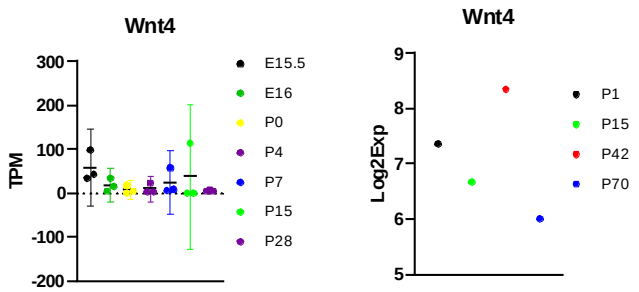


Fig. 7. Wnt4 expression trend at E15.5, E16, P0, P4, P7, P15, P28(left) and P1, P15, P42, P70(right)

Nfkb regulatory pathway

Nfkb is a protein complex that controls transcription of DNA, cytokine production and cell survival. Nfkb is found in almost all animal cell types and is involved in cellular responses to stimuli such as stress, cytokines, free radicals, heavy metals, ultraviolet irradiation, and bacterial or viral antigens[12]. Nfkb plays a key role in regulating the immune response to infection, cancer, inflammatory and immune development, also been implicated in processes of synaptic plasticity and memory[13].

Nfkb1, Nfkb2, Rela and Relb are the members of the Mfkb family. They have different expression pattern. Nfkb1 and Relb has relatively high expression at P7, and decrease at the later stage, Nfkb2 and Rela has active expression and increase at P28, which indicates the function at later stages (Figure8). At later stages by the microarray data, all the four members has active expression and decrease at P70 except Nfkb1, which suggest Nfkb1 still functions at the adult cochlear (Figure9).

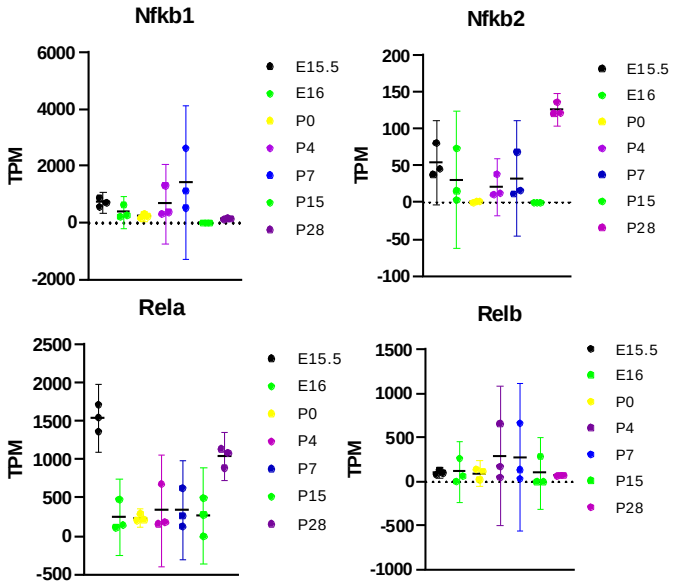
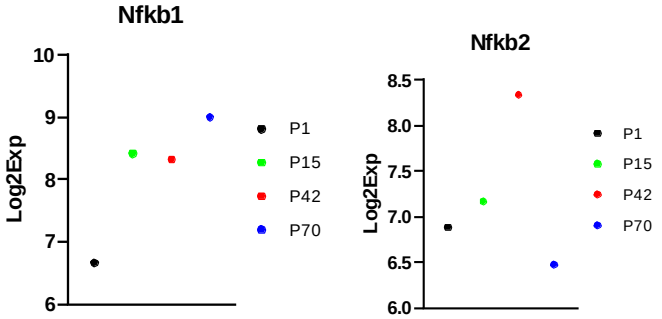


Fig. 8. Nfkb expression trend at E15.5, E16,P0,P4,P7,P15,P28



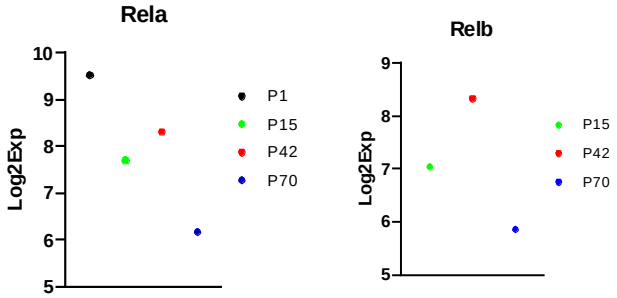


Fig. 9. Nfkb expression trend at P1, P15, P42, P70

IGF regulatory pathway

The insulin-like growth factor (IGF) signaling pathway functions in mediating cell proliferation, apoptosis, differentiation, survival, metabolism, and migration. These interactions provide a potent system that regulates many other processes such as cardiovascular, muscle, bone, and brain development. The IGF system comprises of IGF-1 and IGF-2 and their respective receptors, IGF-1 receptor and IGF-2 receptor, as well as six binding proteins, IGFBP1-6. The IGF signaling pathway has been implicated in aging, since it has important roles in many of the functions necessary for growth, metabolism and fertility[14]. It has also been implicated in neurodegeneration, diabetes, and cancer progression[15].

IGF1, IGF1R and IGF2R both has relatively similar expression pattern, while IGF1R and IGF2R has more expression at the embryonic period, which may indicate other upstream regulators may also included in the cochlear development (Figure 10). Later stages show IGF1 and IGF1R has very similar patterns, first increase and then decrease at P70, while IGF2 has high expression at P15 and decrease as development, IGF2R still has high expression at the adult stages and do not has a tendency to decrease its expression (Figrue11).

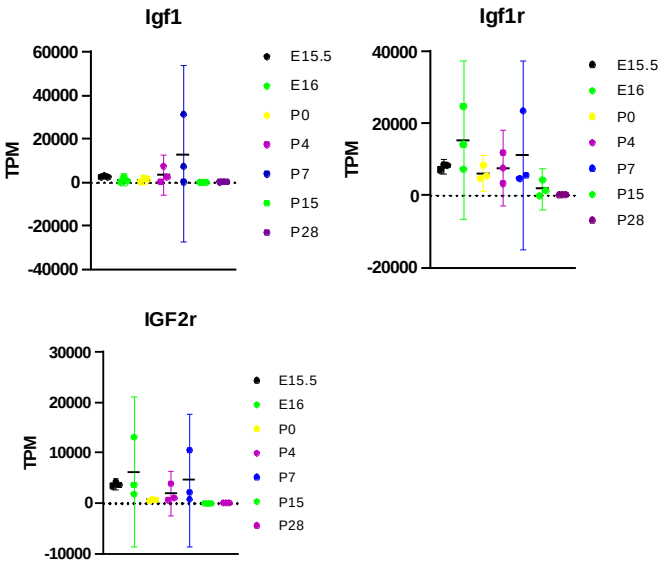


Fig. 10. IGF expression trend at E15.5, E16, P0, P4,P7,P15,P28

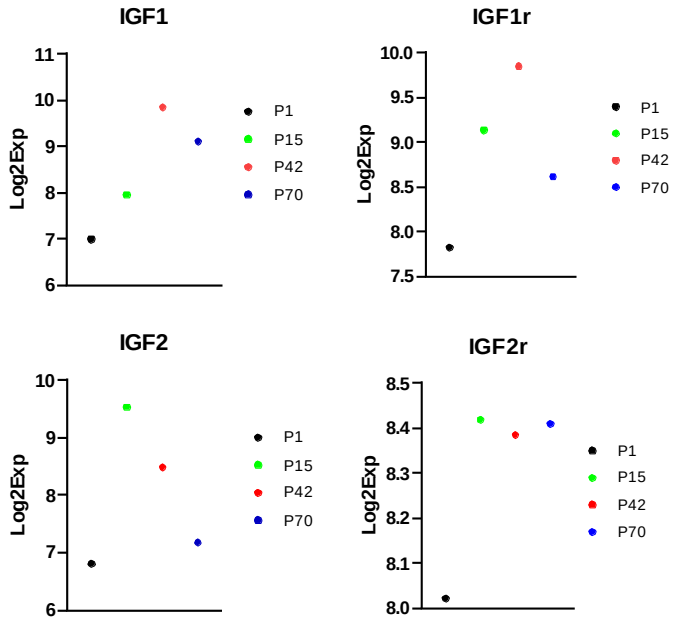
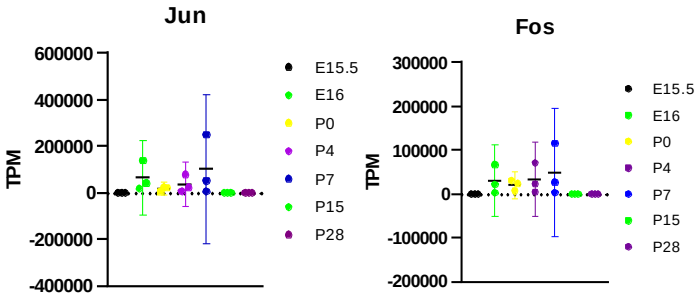


Fig. 11. IGF expression trend at P1, P15, P42, P70

AP1 regulatory pathway

AP1 is a transcription factor that regulates gene expression in response to a variety of stimuli, including cytokines, growth factors, stress, and bacterial and viral infections, and mainly composed of Jun, Fos, ATF and JDP protein dimers[16]. AP1 transcription factors are also associated with numerous physiological functions especially in determination of organisms' life span and tissue regeneration.

Jun, Fos, Atf1 and Jdp2 has very similar expression pattern from E15.5 to P28, especially at P7, all reach at peek expression, P7 is also a time point some key genes like Atho1 down regulated (Figure 12). At later stages, we can see the expression pattern is similar, both has relatively high expression at the early adult stages and decrease at P70, which may be connected with the sex maturation (Figure 13).



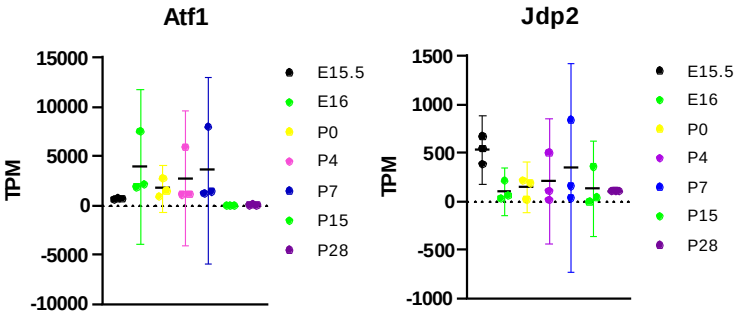


Fig. 12. AP1 gene family expression trend at E15.5, E16,P0,P4,P7,P15,P28

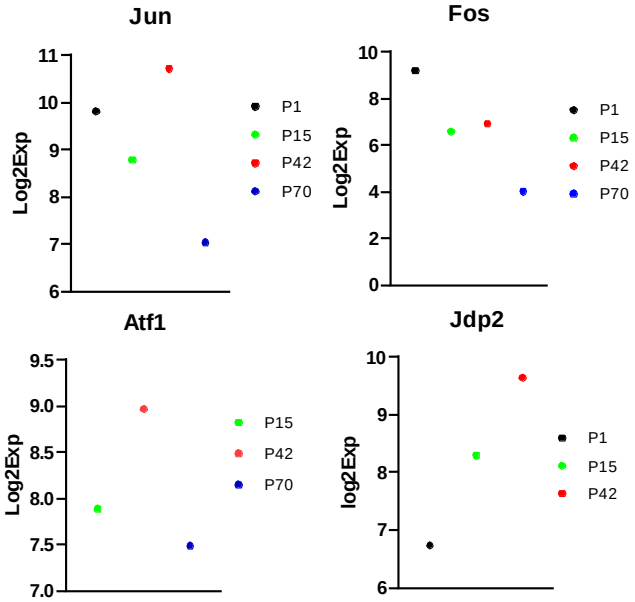


Fig. 13. AP1 gene family expression trend at P1, P15, P42, P70

Known pathways in 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 regulate 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 slow 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 and P10 microarray data. At the E6.5 JUN has the highest expression, and TGFBR3 and NFKB1, IGF2, IGF2R has relatively very low expression,

and at P10 we can still see that JUN has relatively high expression, which may indicate that JUN has import function for hair cell regeneration.

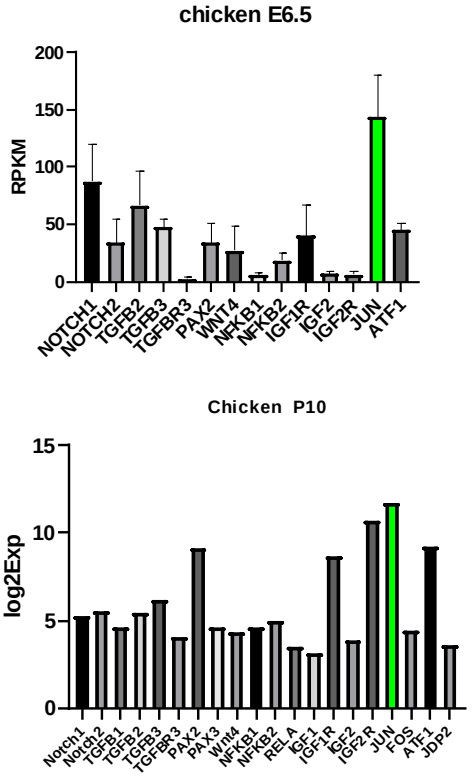
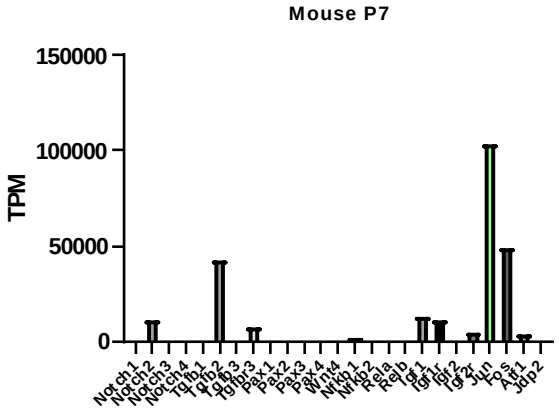


Fig. 14. Key genes expression at E6.5 and P10 chicken hair cells

As we could not found the P10 data in the public data base on mice, we check the near time points to see if there are some similarity between mice and chicken. Jun expression is also relatively very high at mouse P7, which is very similar between the mice and chicken. Jun expression decrease to very low level at P15, and at this time point, IGF1R has relatively high expression level, which may indicate its necessary function in this time point (Figure 15).



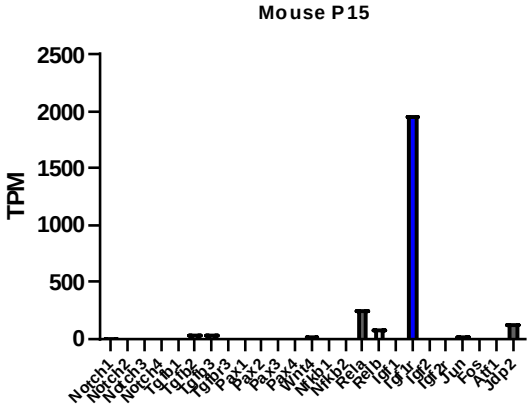
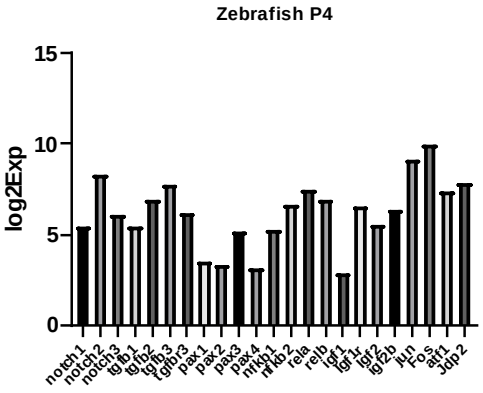


Fig. 15. Key genes expression at P7 and P15 mice hair cells

Known pathways in zebrafish

Zebrafish is another very import important model organism. Zebrafish has the ability to 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 cell 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[17].

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 16).



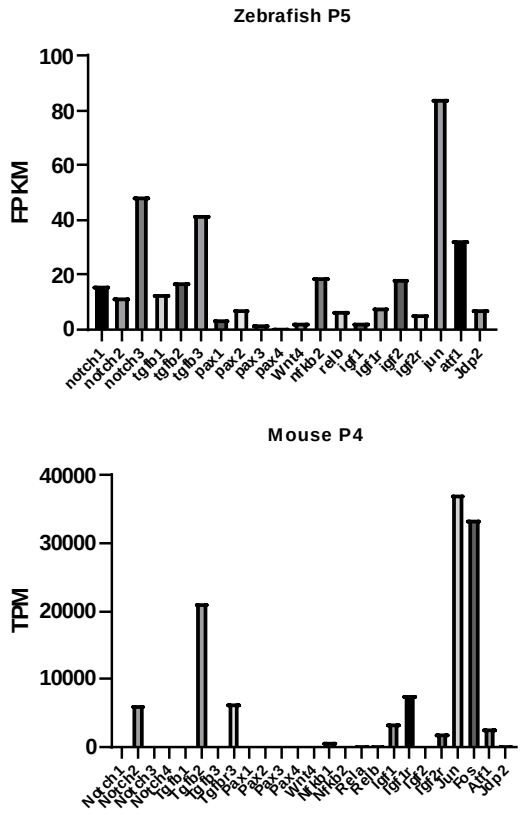


Fig. 16. Key genes expression at P4 and P5 zebrafish and P4 mice hair cells

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.

Mouse has the most abundant data, from the embryo stages to near one month, 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 hairs 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 a great success in restore hearing loss.

Also, 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[18]. In fish, more function endowed the lateral line organ as it also detect water movements, allowing aquatic animals to have a sense of 'distant touch' of their local environment[19].

We briefly investigated the several known pathways that has 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 download from 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 analysis 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

Professor Zhengqing Hu designed research, Sen Guo collect the raw data and analyzed data; and Sen Guo wrote the paper.

The authors declare no conflict of interest.

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