

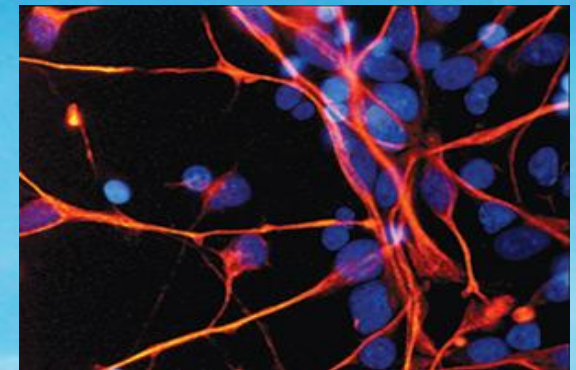
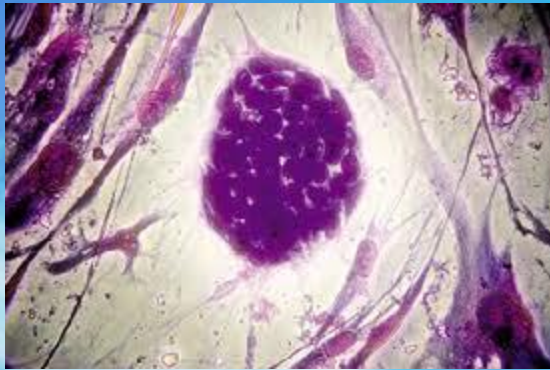


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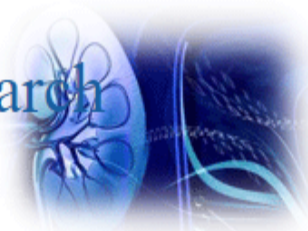
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The Research Progress of Stem Cell Based Therapies for Regenerating Inner Ear Hair Cells

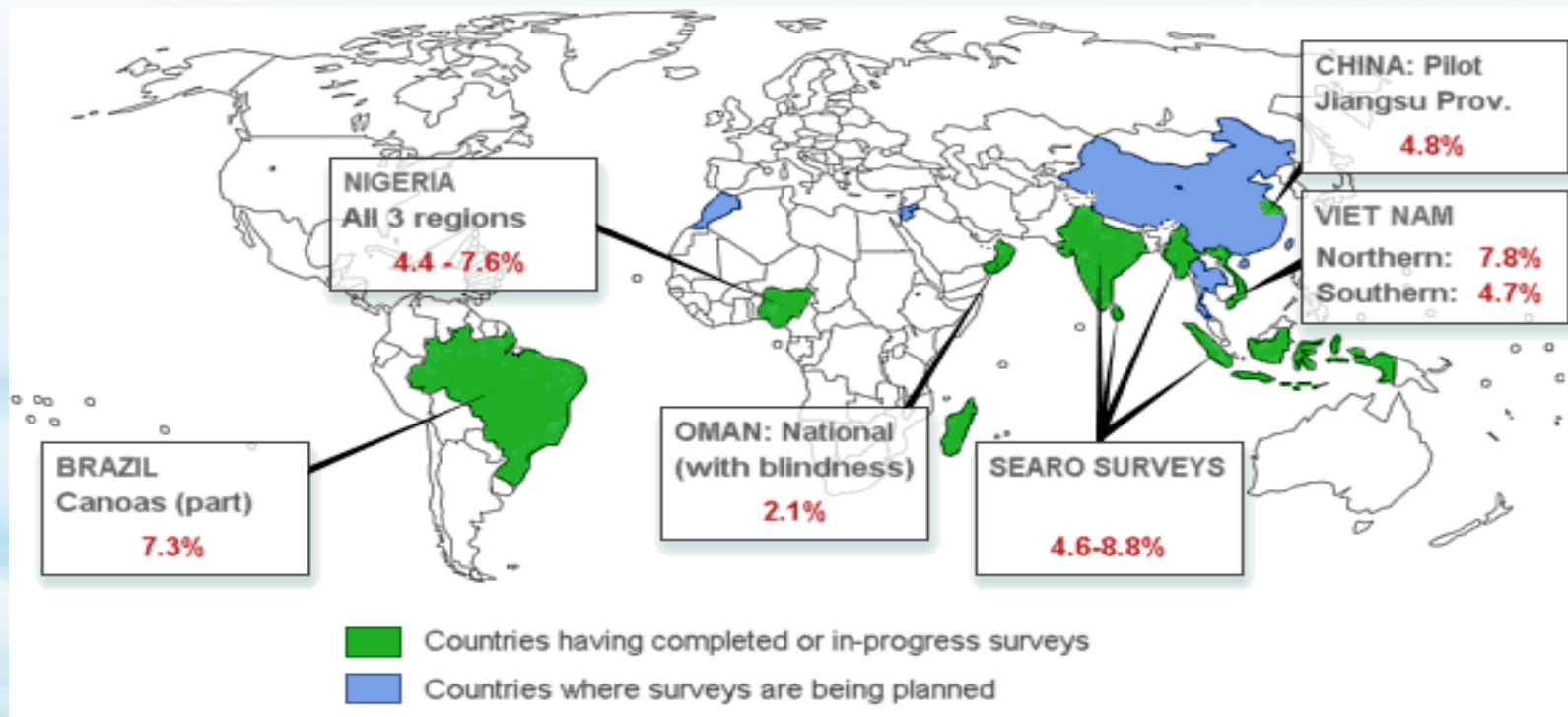


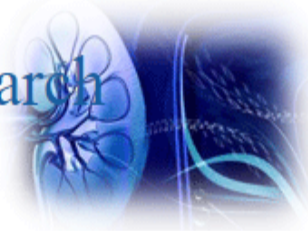
Professor Wang Jinfu
College of Life Sciences, Zhejiang University
Institute of Cell and Developmental Biology



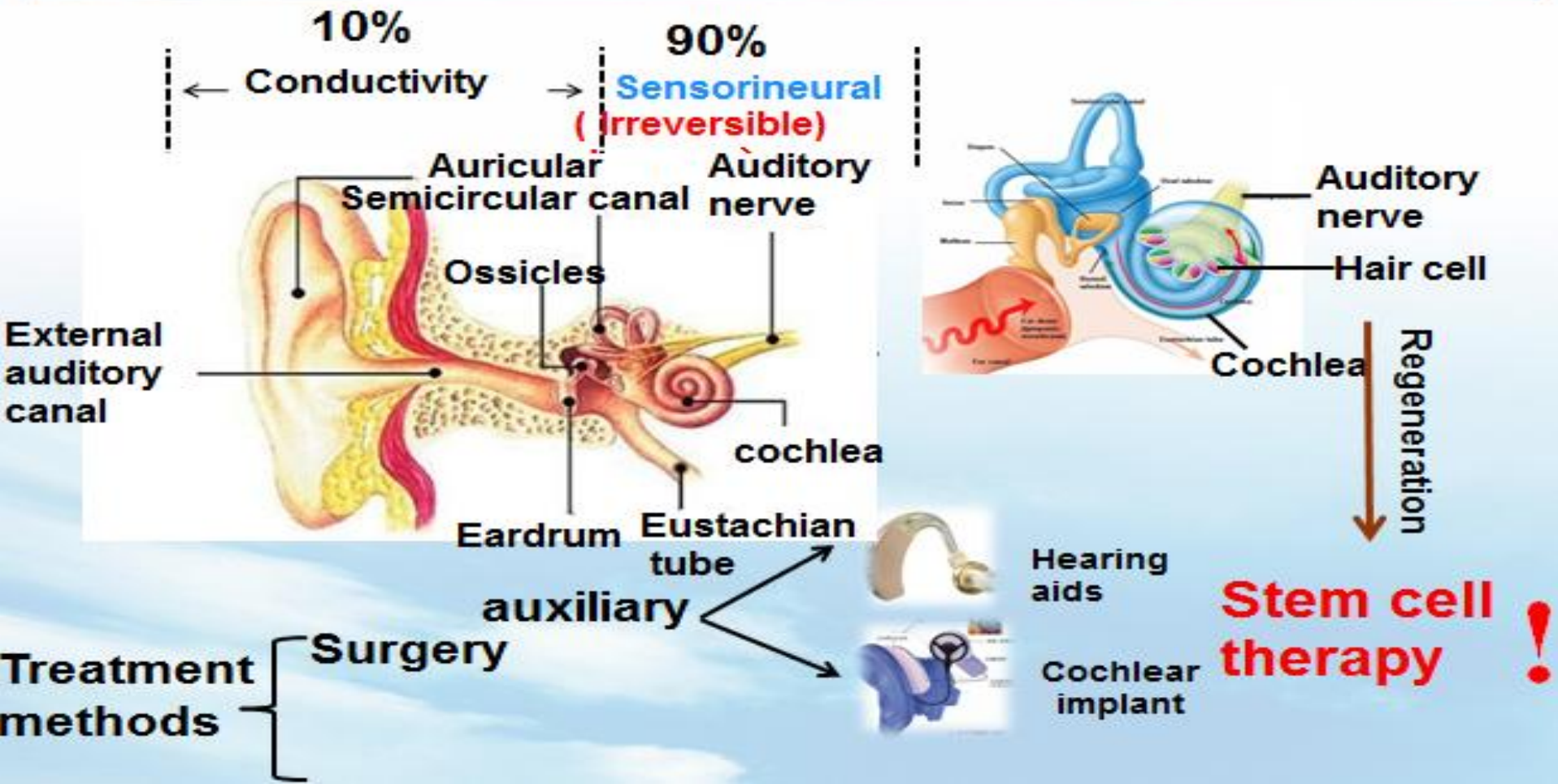
Deafness Harm to Human Health

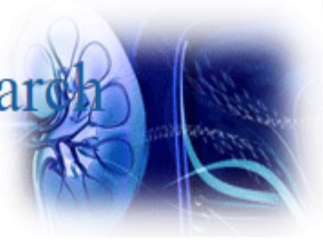
- The global deaf population is about 280 million
- The deaf population in our country is about 30 million





Lack of Effective Methods to Treat Sensorineural Deafness





1

Inner ear stem cells

2

Embryonic stem cells

3

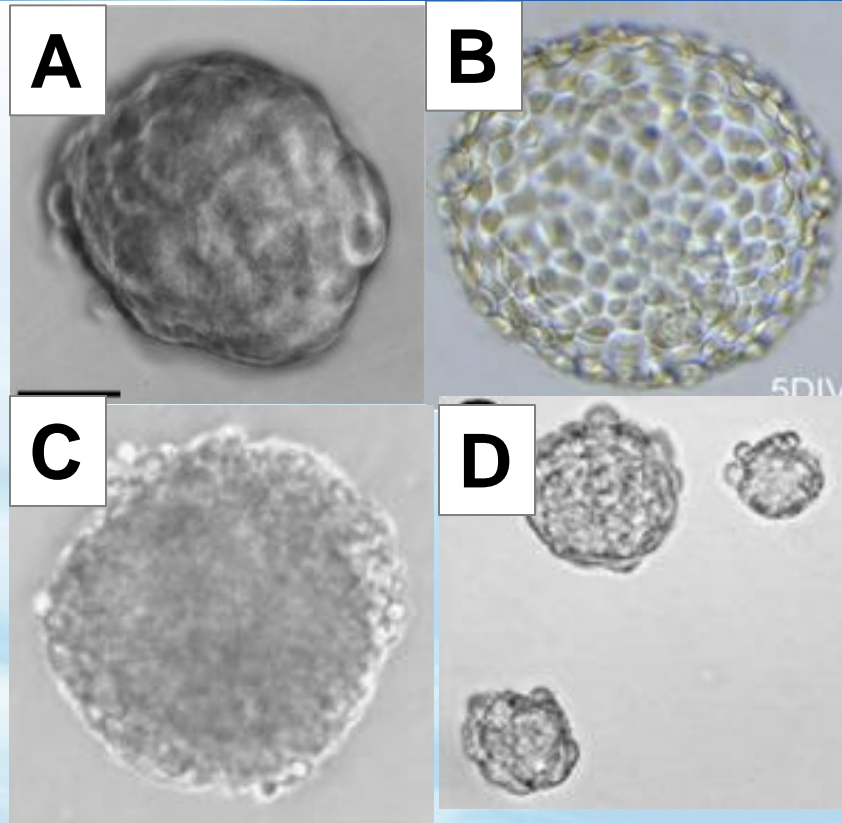
Mesenchymal stem cells

4

Induced pluripotent stem cells

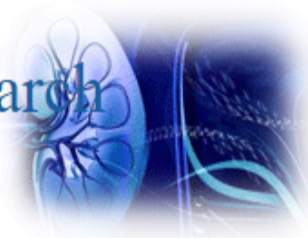


Isolation and Culture of Inner Ear Stem Cells



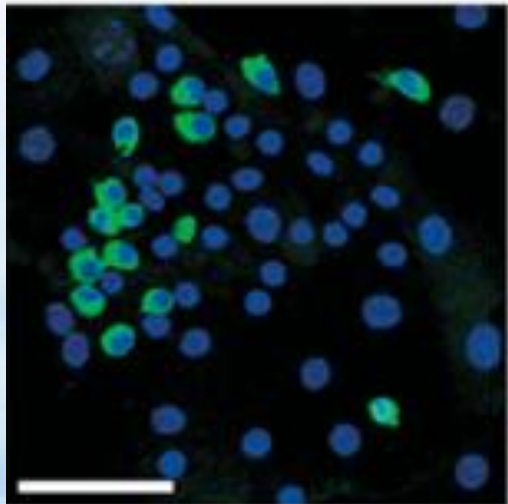
Oshima, K et al. *JARO*. 2007.

Inner ear stem cells have been isolated from **Vestibular utricle (Fig A)**, **Cochlea corti (Fig B)**, **Spiral ganglion (Fig C)** and **Stria vascularis (Fig D)**. These cells form typical cell mass under culture in vitro. It is one of the most typical growth characteristics of inner ear stem cells.

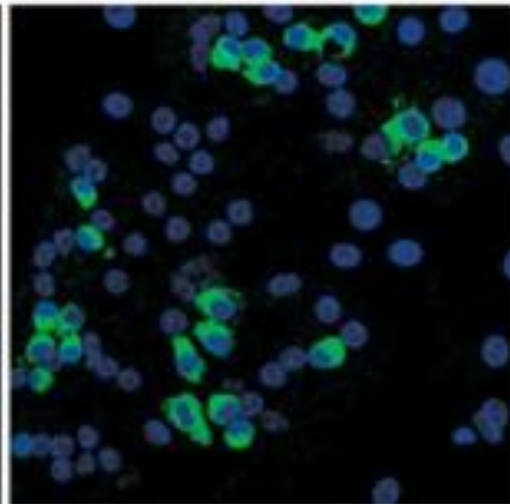


Inner Ear Stem Cells of Utricle Induced for Differentiation

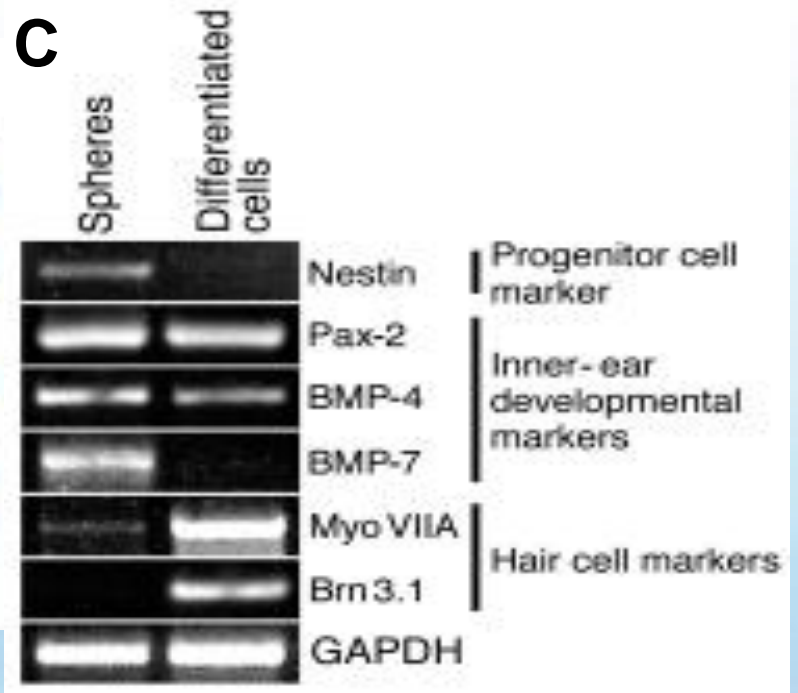
A Myosin VIIA



B espin

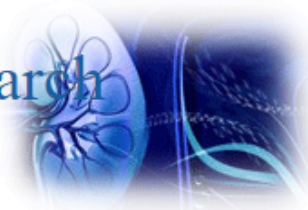


C

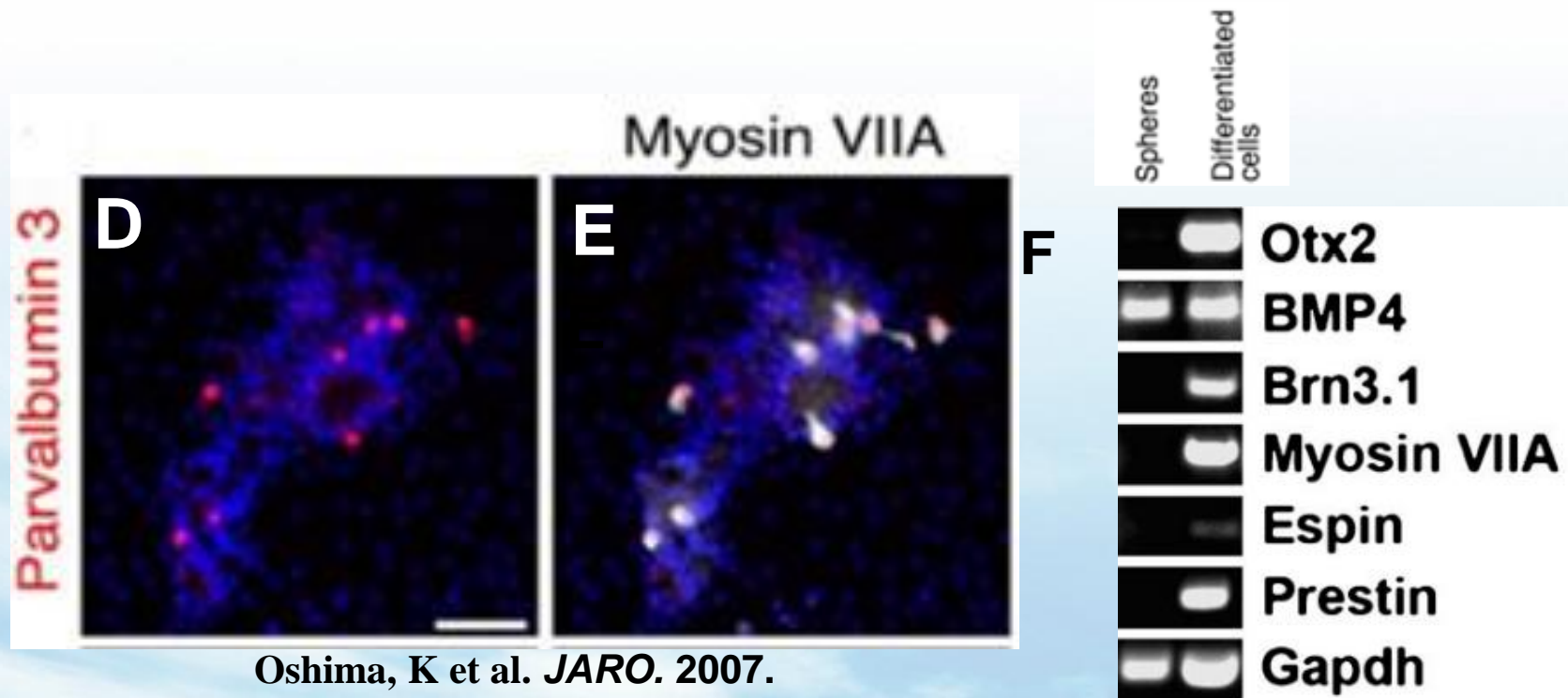


Li, W et al. *Nature medicine*. 2003.

The stem cells from utricle could differentiated into hair cells in vitro. Induced cells expressed specific markers of hair cells, such as myosin VIIA、espin、parvalbumin 3 and Brn3.1.

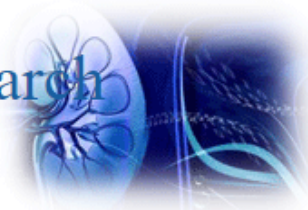


Inner Ear Stem Cells of Corti Induced for Differentiation

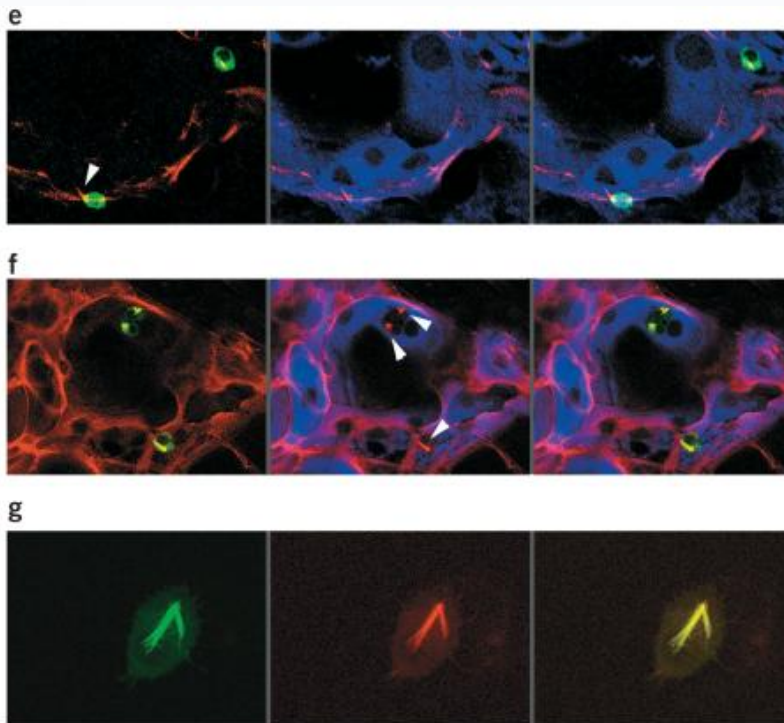


Oshima, K et al. *JARO*. 2007.

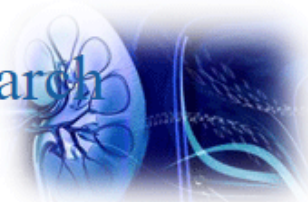
The stem cells from Cochlea corti could also differentiate into hair cells in vitro. Induced cells expressed the specific marker genes such as myosin VIIA, Espin, Parvalbumin3 and prestin.



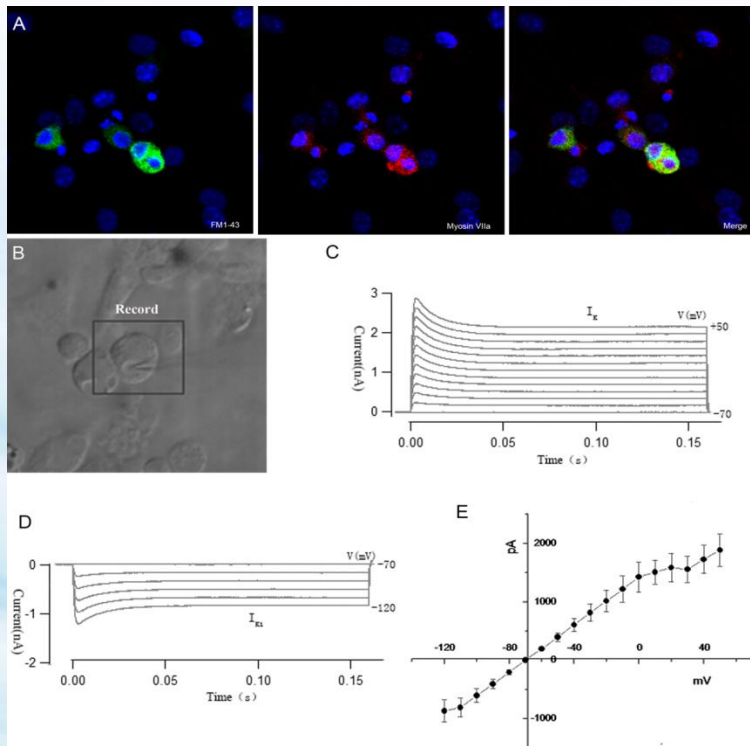
The Hair Cell-Like Cells Have Stereociliary Bundle-Like Protrusions



The inner ear stem cells could be induced into hair cell-like cells in vitro, and express the specific marker genes such as myosin VIIA (Fig e) and espin (Fig f). The protrude stereociliary bundle-like protrusions on the surface of these hair cell-like cells.

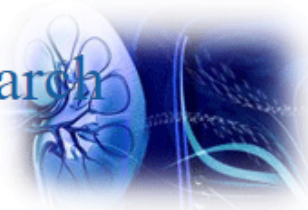


Mechanical Responses Elicited from Inner Ear Stem Cells-Derived Bundle-Bearing Cells

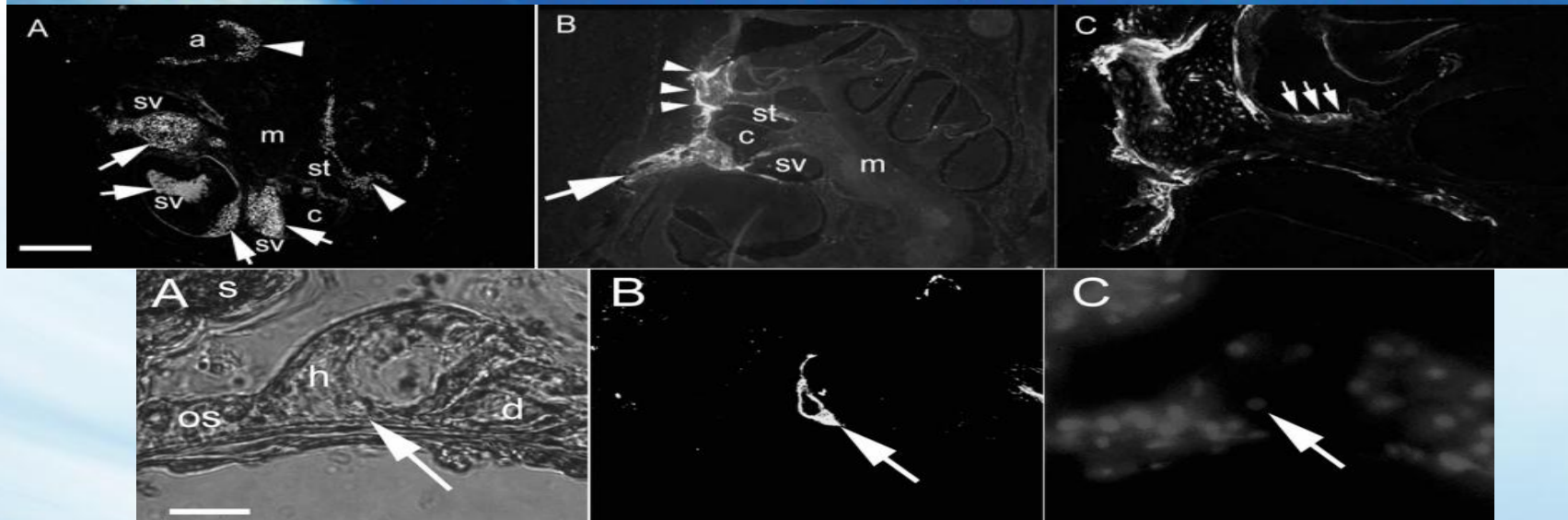


Voltage-dependent currents elicited from inner-ear stem-cell-derived FM1-43 positive cells. (A) FM1-43-positive cells (green, left) co-expressed the hair cell marker myosin VIIa (red, center). (B) White light image of a recording electrode on an FM1-43-positive cell. (C) Outward K⁺ currents recorded from hair-cell-like cells. (D) Inward K⁺ currents recorded from hair-cell-like cells. (E) Average current-voltage (I-V) curve for the outward and inward K⁺ current measured at the steady-state level (100–160 ms).

Liu, QW et al. *unpublished*.

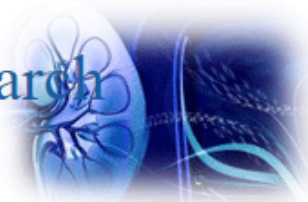


Inner Ear Stem Cells Transplantation In Vivo

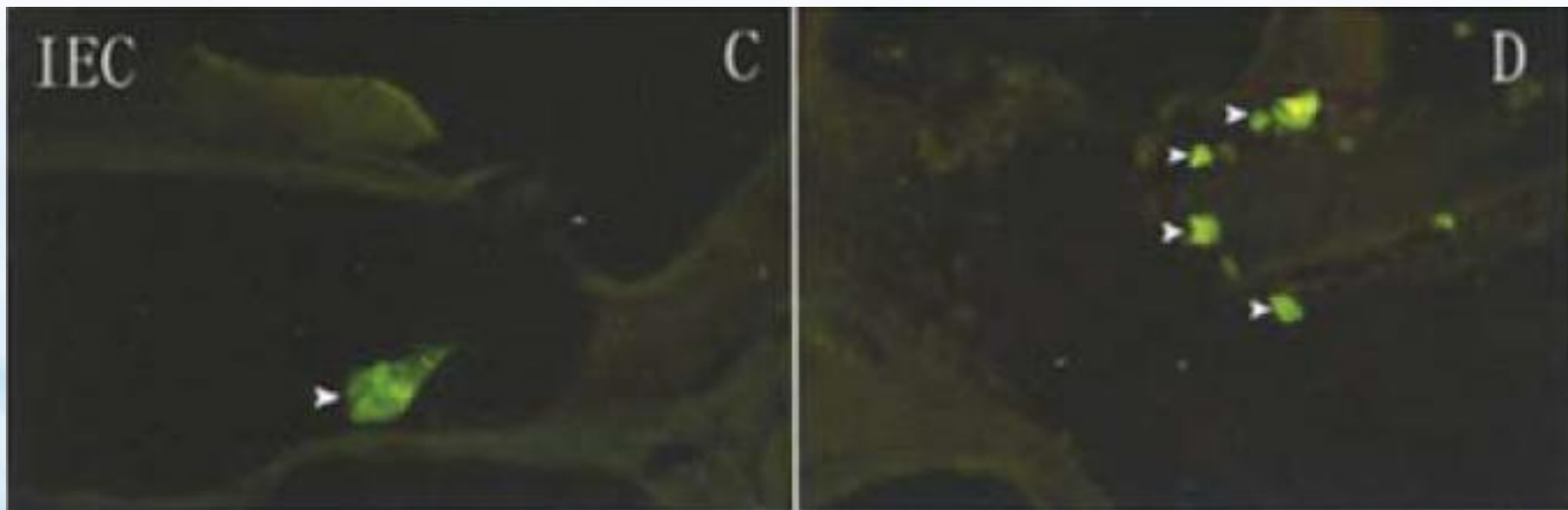


Kojima, K et al. *Acta Otolaryngol.* 2004.

Kojima et al. injected the inner ear stem cells from cochlea into the mouse model of hearing loss. They found that the transplanted cells could survive in the inner ear, and could migrated into the cochlea epidermis.

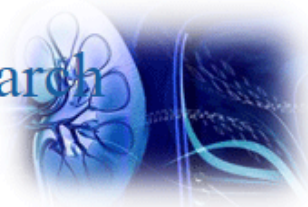


Inner Ear Stem Cells Transplantation In Vivo



Lou, X. The Doctoral Dissertation of east china normal university, 2007

Lou et al. injected the inner ear stem cells into the inner ears of the normal (Fig C) or deaf (Fig D) guinea pig . They found that the transplanted cells not only survived in the inner ear, but also could migrate to the basement membrane and corti.



1

Inner ear stem cells

2

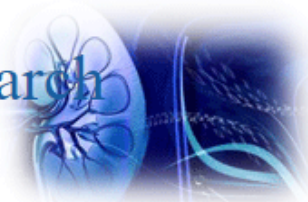
Embryonic stem cells

3

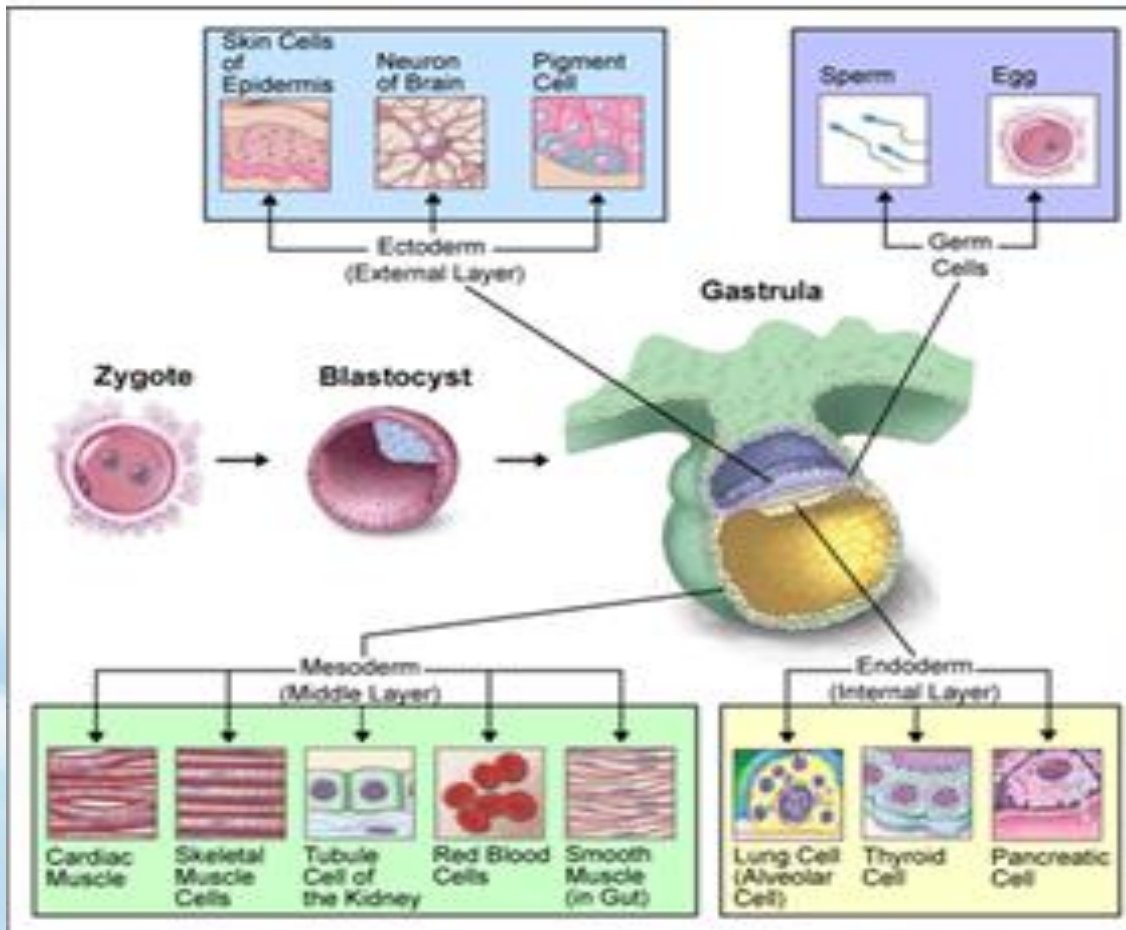
Mesenchymal stem cells

4

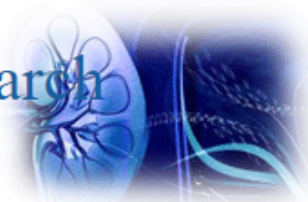
Induced pluripotent stem cells



Embryonic Stem Cells (ESCs)

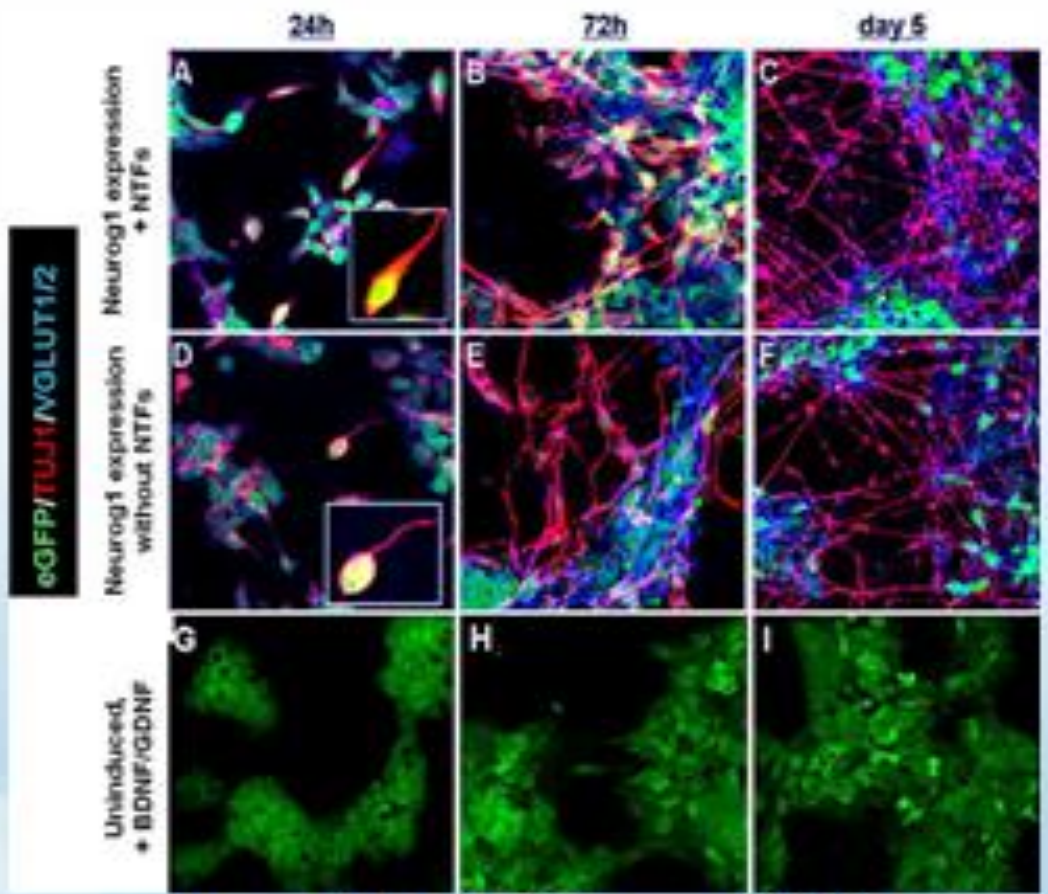


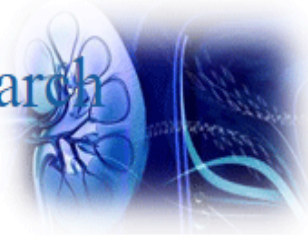
ES cells are totipotent cells. They can differentiate into all types of cells under certain conditions, such as the hematopoietic cells, dopaminergic neurons, cardiac cells, germ cells and so on.



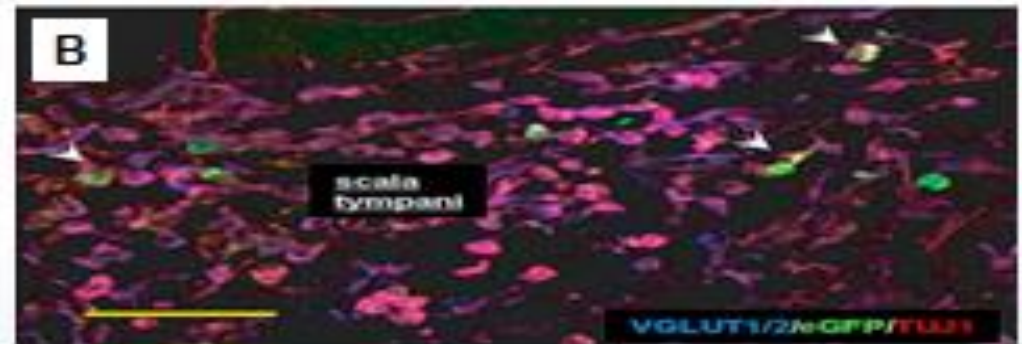
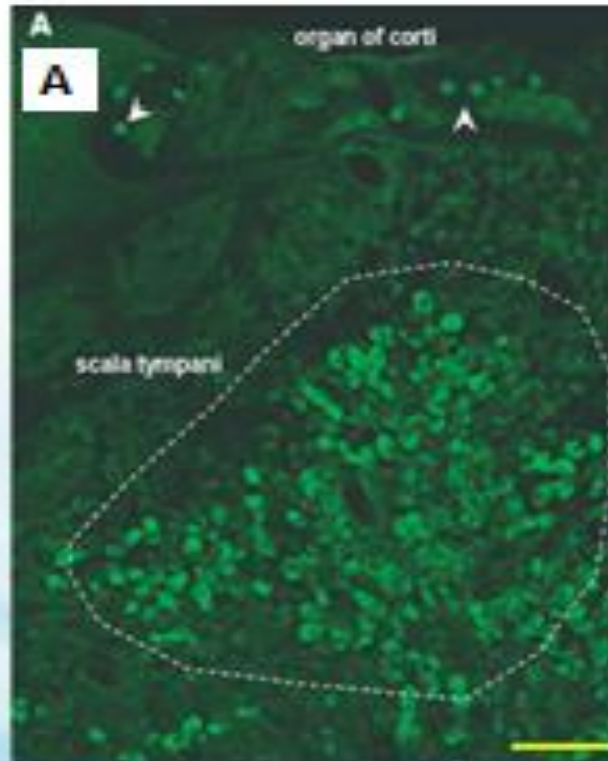
The Embryo Stem Cells Differentiate into Glutamatergic Neurons in Vitro

Reyes et al. transduced Neurog1 gene into mouse ES cells, and induced the ES cells to express Neurog1 by doxycycline (Dox) treatment. These ES cells could differentiated into neurons that express TUJ1 and VGLUT1 / 2 glutamate under treatment with BDNF and GDNF.



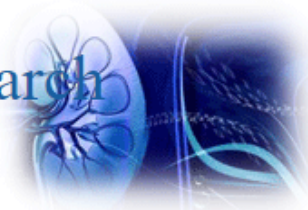


The Embryo Stem Cells Differentiate into Glutamatergic Neurons in Vitro

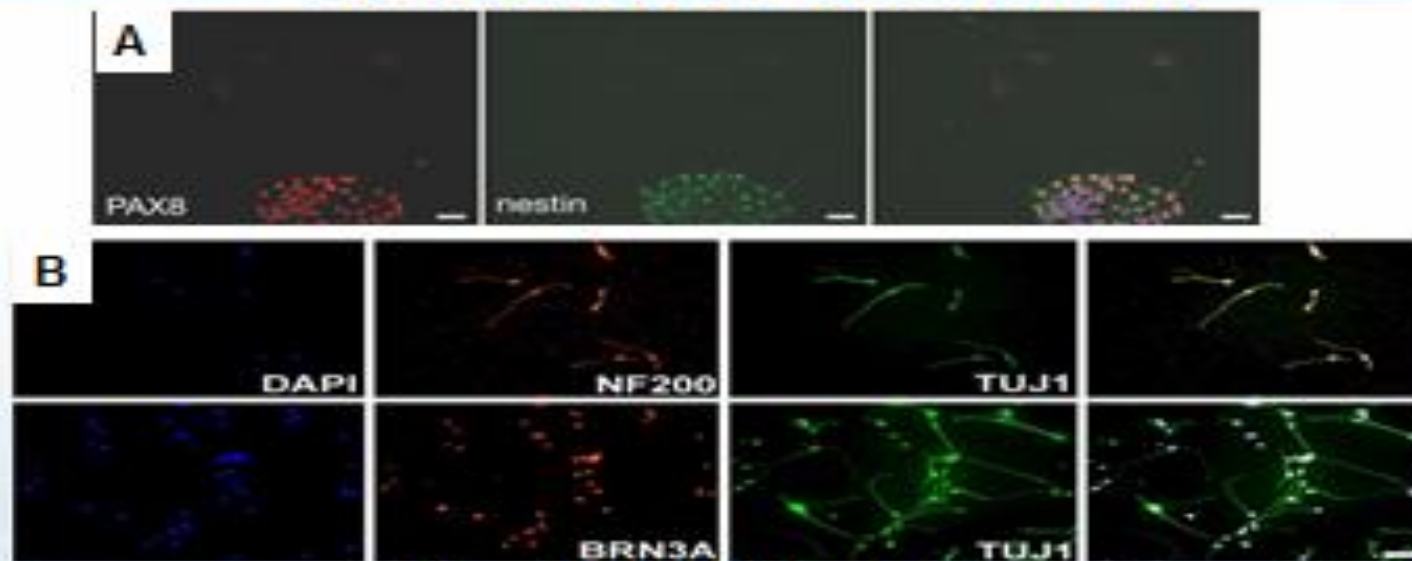


Reyes et al. transplanted mouse ES cells into the inner ear of guinea pig, and treated the guinea pig with Dox, BDNF and GDNF. Then they found that these cells were survival in the body (Fig A) and differentiated into glutamatergic neurons (Fig B).

Reyes, JH et al. *J Neurosci.* 2008.

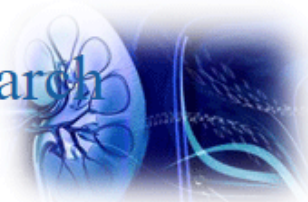


Human Embryonic Stem Cells Differentiate into Inner Ear Sensory Nerve Cells in Vitro

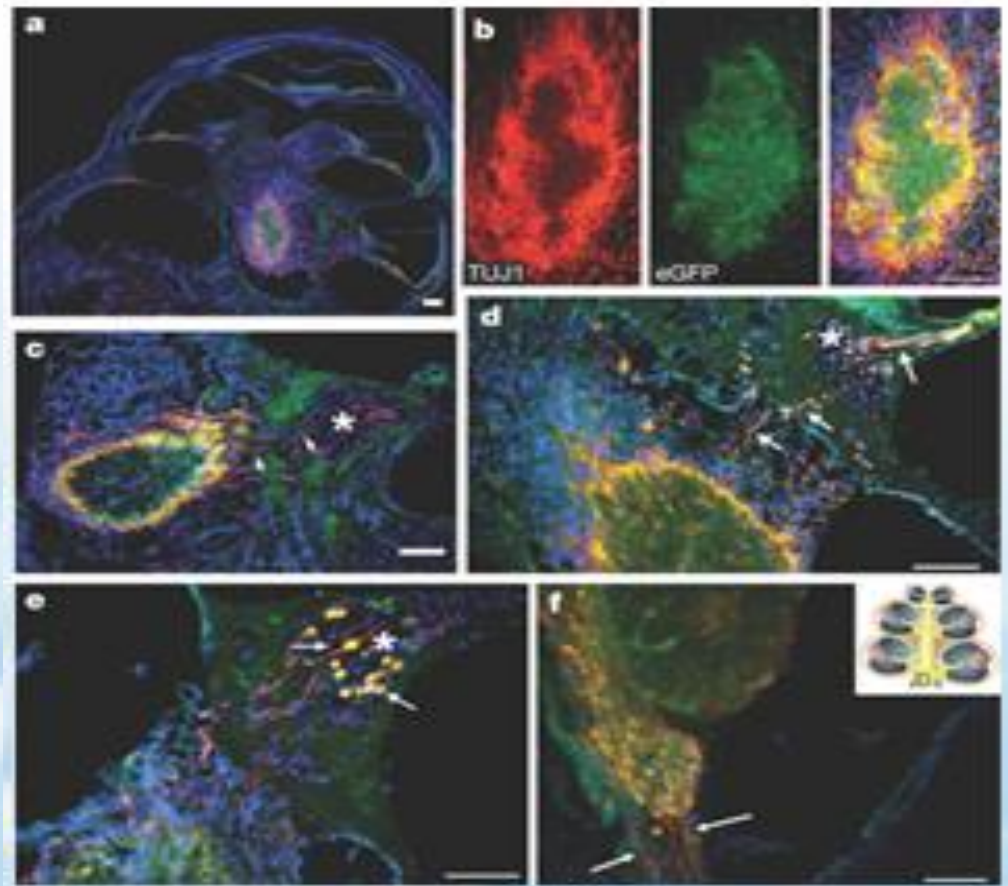


Chen, W et al. *Nature*. 2012.

Chen et al. treated human ES cells with FGF3 and FGF10, and found that these cells expressed the markers of inner ear neural progenitor cells (Fig A). Then, the progenitor cells differentiated into inner ear sensory nerve cells that expression NF200 and TUJ1 BRN3A by treatment with bFGF, shh, NTF and BDNF (Fig B).

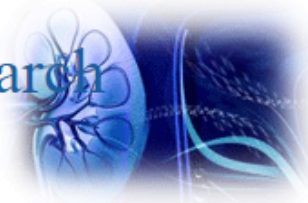


Inner Ear Neural Progenitor Cells Derived from ES in Vivo Transplantation

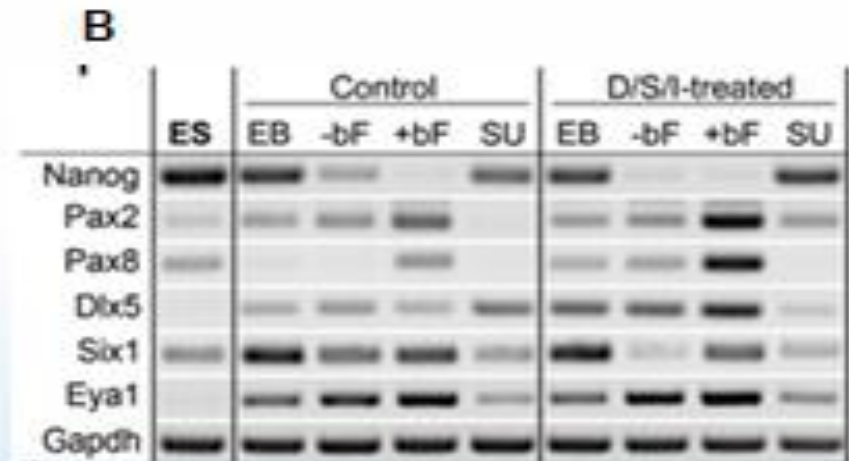
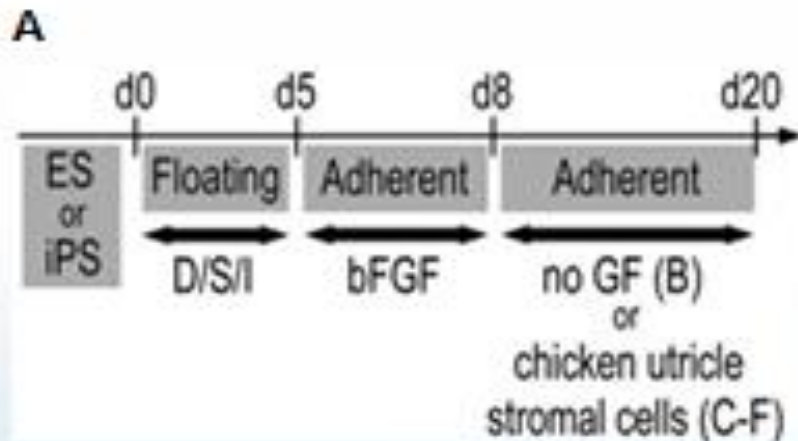


Chen et al. transplanted the inner ear neural progenitor cells induced from ES cells into the mouse model. These cells could survive and form ectopic spiral ganglion in the cochlea. Some progenitor cells migrated into other parts of the cochlea and differentiated into neurons (Fig e).

Chen, W et al. *Nature*, 2012.

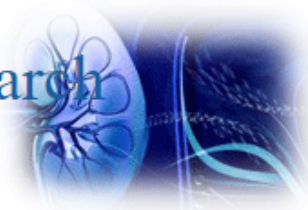


Mouse Embryonic Stem Cells Differentiate into Hair Cell in Vivo

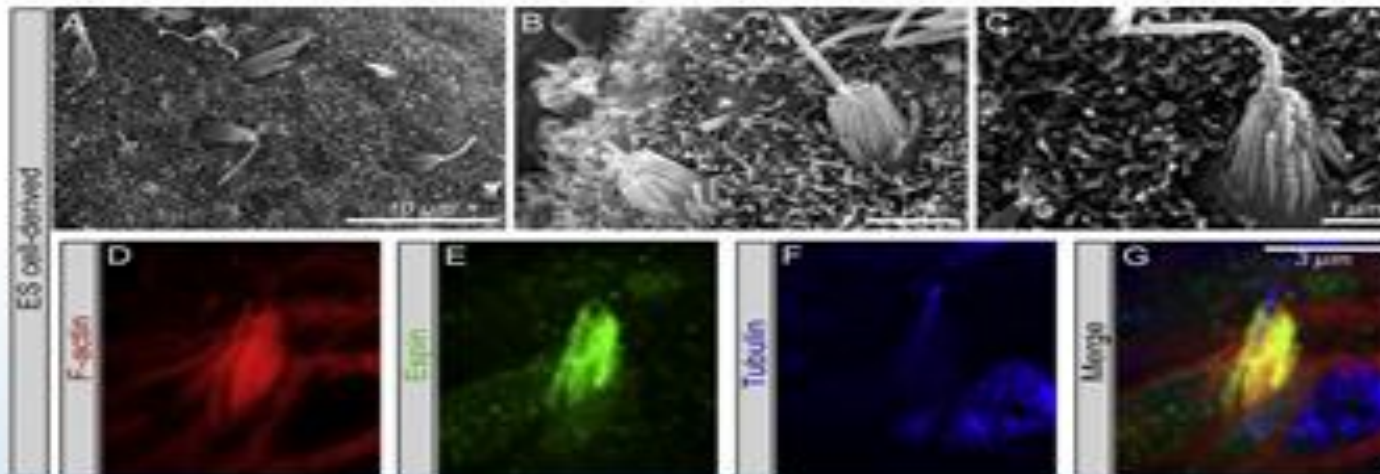


Oshima, K et al. *Cell*. 2010.

Oshima et al. inhibited the meso- and endodermal differentiation by adding DKK1, SIS3, and IGF-1 during ES cells forming embryoid bodies, and promote the differentiation of ectoderm. Then, they added bFGF in the adherent culture process, these cells finally differentiated into inner ear progenitor cells which express Pax2 and Pax8.

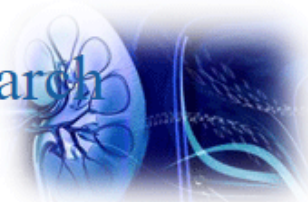


Mouse Embryonic Stem Cells Differentiate into Hair Cell in Vivo



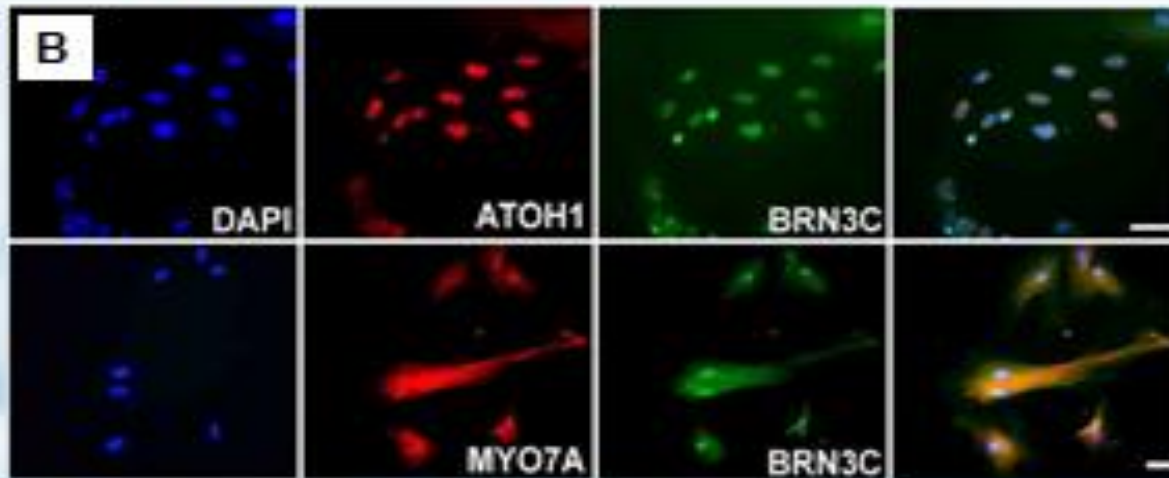
Oshima, K et al. *Cell*. 2010.

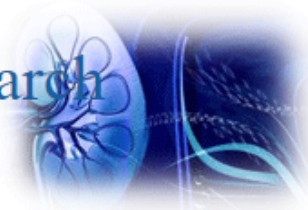
Oshima et al. co-cultured the ESC-derived progenitors with chicken utricle stromal cells, finally these progenitors differentiated into the hair cell-like cells. These hair cell-like cells have typical stereociliary hair bundles and expressed some proteins, such as F-actin, espn and tubulin.



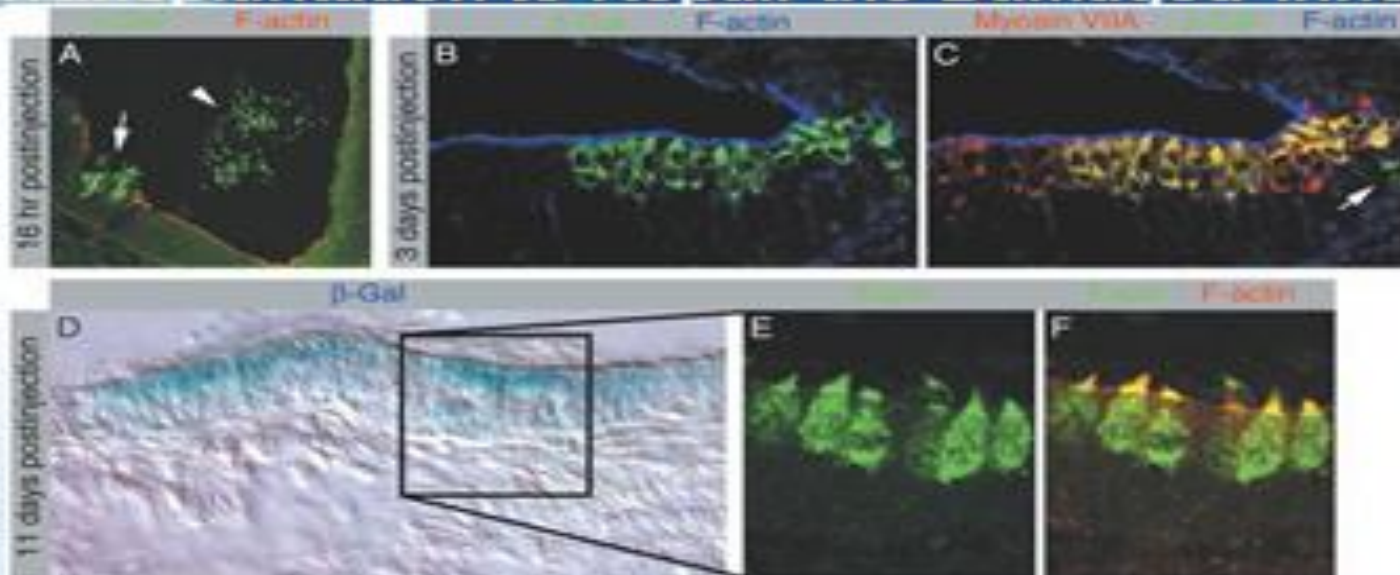
Human Embryonic Stem Cells Differentiate into Hair Cell in Vitro

Chen et al. induced ES cells to differentiate into inner ear epithelium progenitor cells with FGF3 and FGF10 (OEP cells in Fig A). Then, they induced these progenitor cells to differentiate into hair-cell-like cells which express ATOH1, BRN3C and MYO7A with all trans retinoic acid and EGF (Fig B).



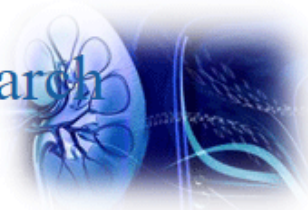


Human Embryonic Stem Cells in Vivo Transplantation to Repair the Damaged Inner Ear

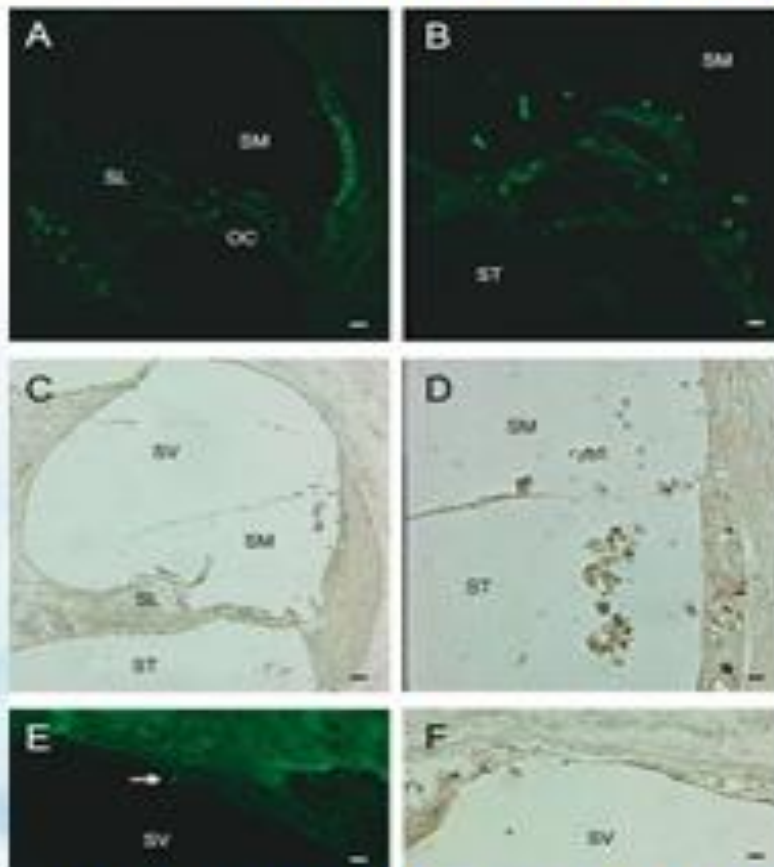


Li, H et al. *PNAS*. 2003.

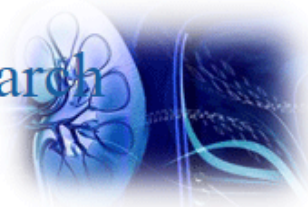
Li et al. induce ES cells to differentiate into inner ear progenitor cells by using the EGF, IGF, bFGF, and then injected these cells into chicken embryo inner ear. These cells could migrate into the epithelium around the acoustic vesicles (Fig A, B) and differentiate into the cells which express hair cell marker proteins—Myosin VIIA (Fig C) and Stereocilia beam protein Espin (Fig E).



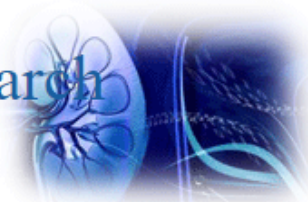
Human Embryonic Stem Cells in Vivo Transplantation to Repair the Damaged Inner Ear



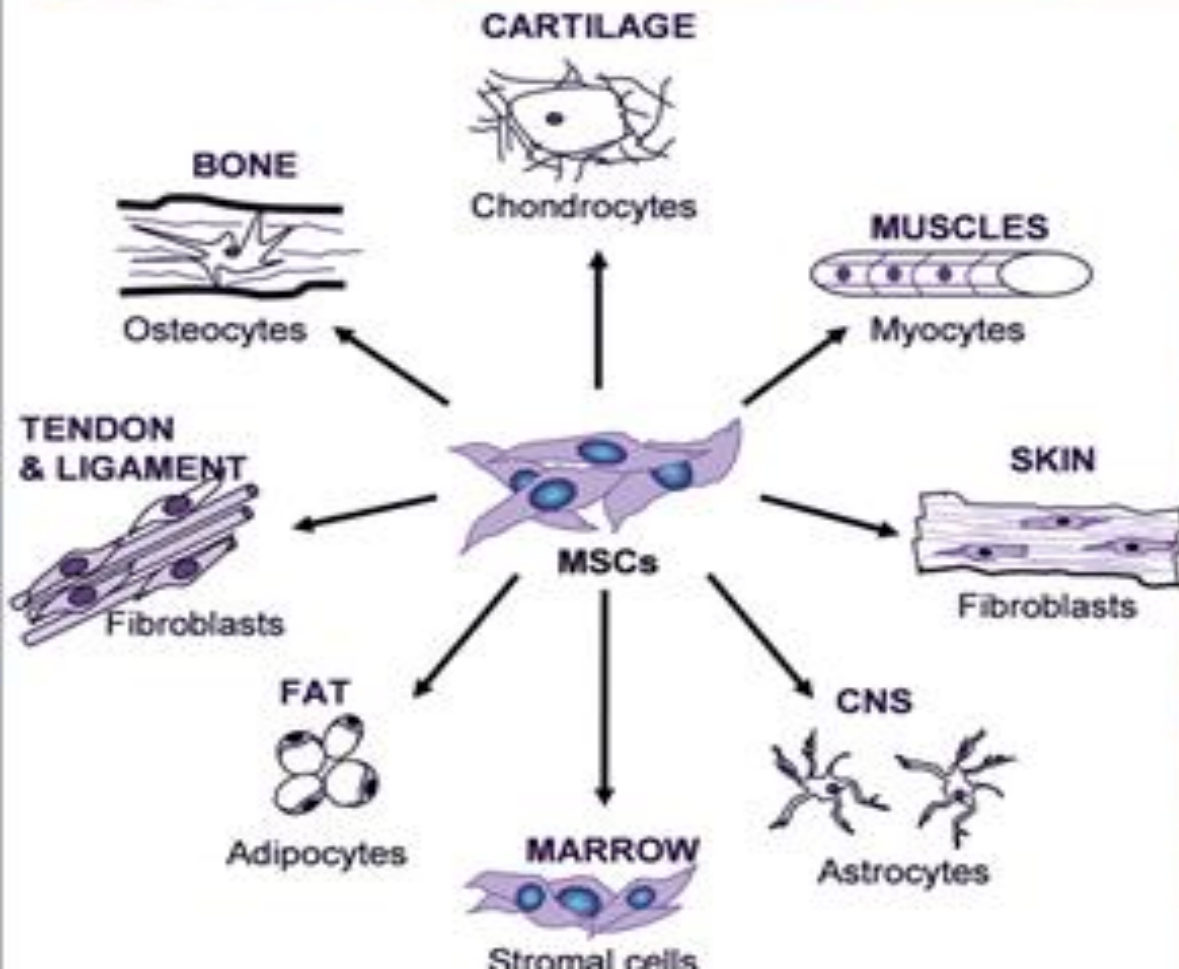
Hildebrand et al. induced mouse ES cells to differentiate into neural ectoderm-like embryoid bodies in vitro, then transplanted it into the cala media of deaf guinea pig. 9 weeks later, exogenous cells in the body was not only survival, but also moved to vestibular canal (E, F).



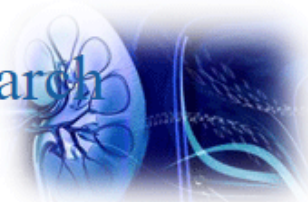
- 1 Inner ear stem cells**
- 2 Embryonic stem cells**
- 3 Mesenchymal stem cells**
- 4 Induced pluripotent stem cells**



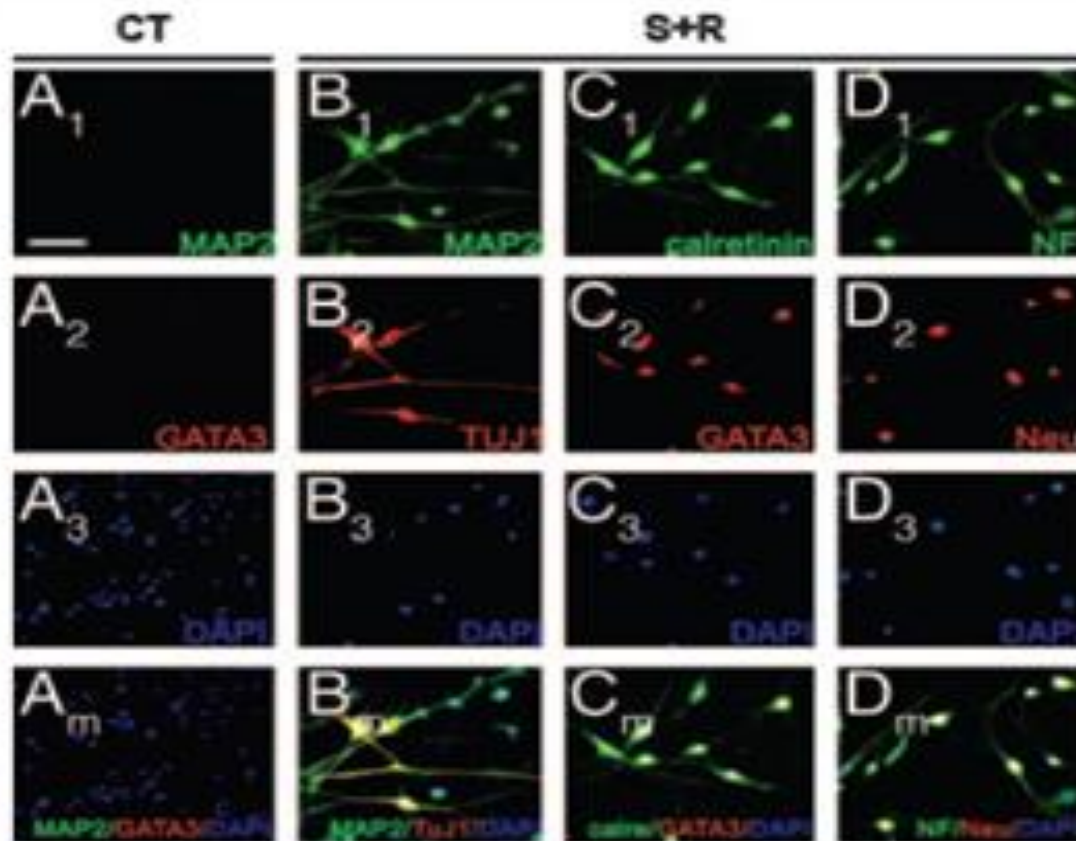
Mesenchymal Stem Cells



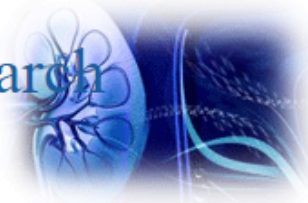
Mesenchymal stem cells are pluri-potent adult stem cells, which contribute to the regeneration of many tissues such as bone, cartilage, fat, muscle and neurons.



MSCs Can Differentiate into Neurons Precusors in Vitro

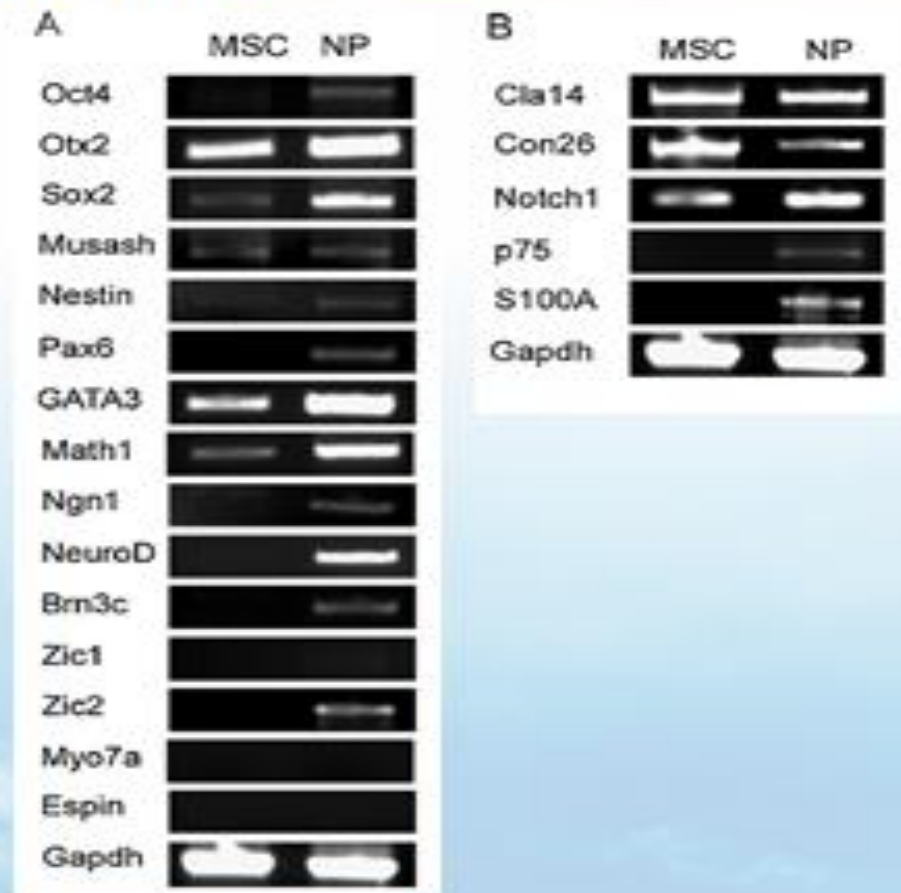


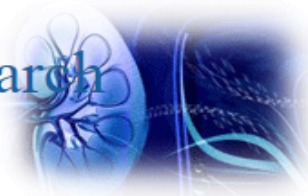
Kondo et al. found that mouse MSCs could be induced to neuronal-restricted precursor cells and expressed several marker proteins of nerve cell by adding the retinoic acid and hedgehog protein.



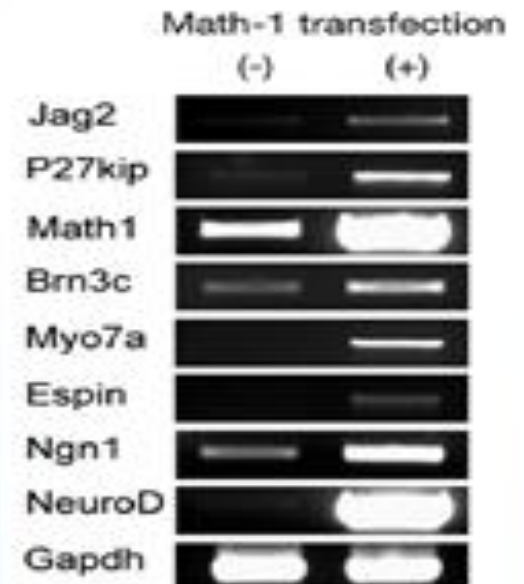
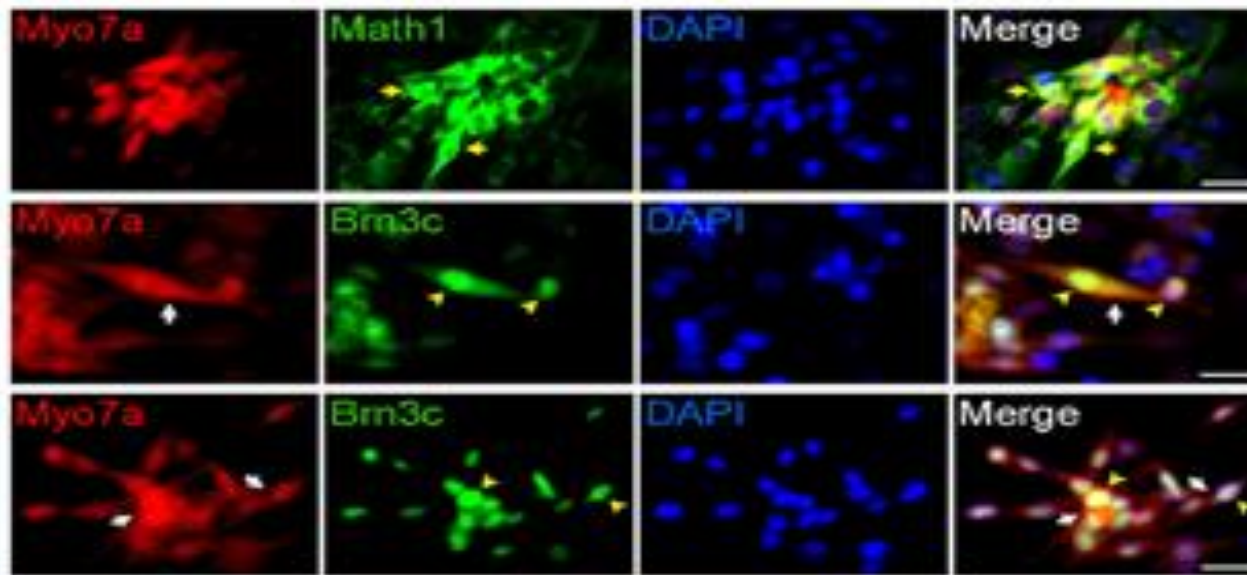
MSCs Can Be Induced to Differentiate into Hair Cell Precursors

Jeon et al. imitated the embryonic microenvironment during the development of ear sensory epithelium. By addition of EGF, IGF-1, bFGF, BDNF and NT-3, the mouse MSCs were induced to the cell group showing the expression pattern similar to hair cell precursors .



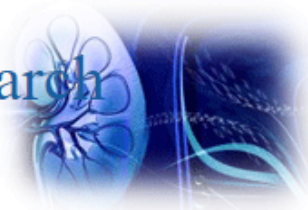


The Overexpression of Math1 Promotes the Differentiation of MSCS into Hair Cells

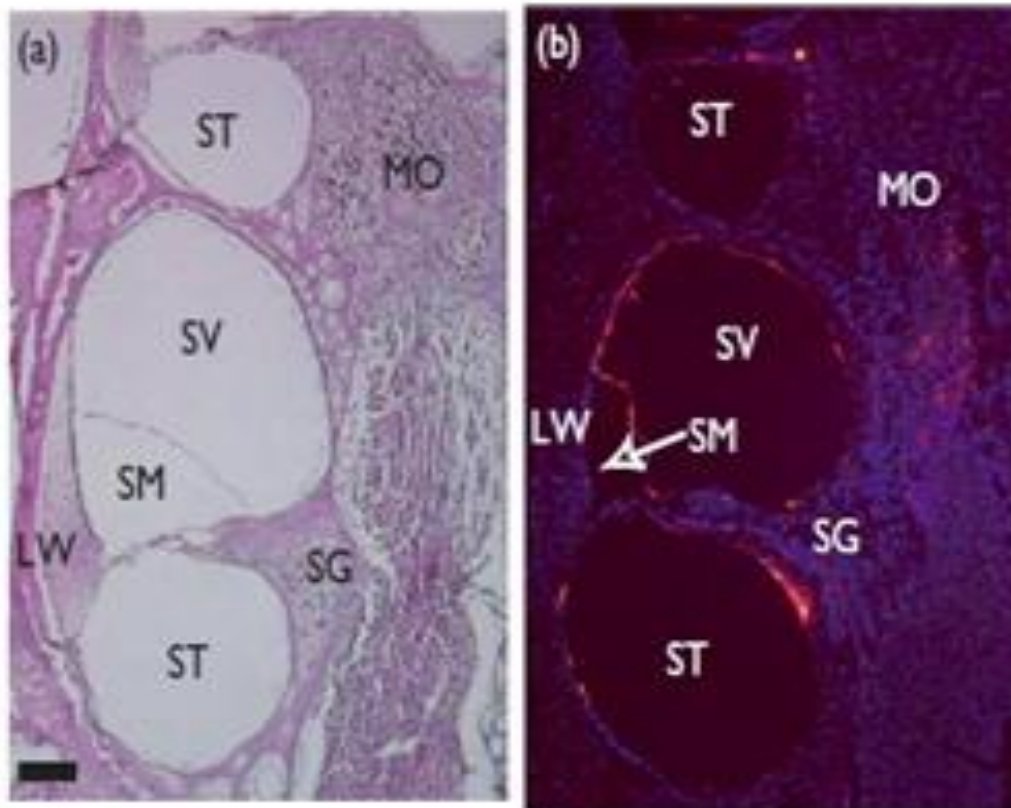


Jeon, SJ et al. *Mol Cell Neurosci.* 2007.

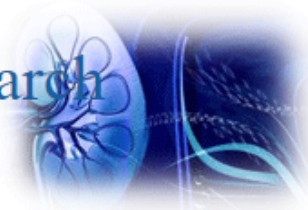
Mouse MSCs could be induced to differentiate into hair-cell-like cells through the transfection expression of Math1. The hair-cell-like cells expressed many marker proteins found in the mature hair cells, including the Myo7A, Brn3c and so on.



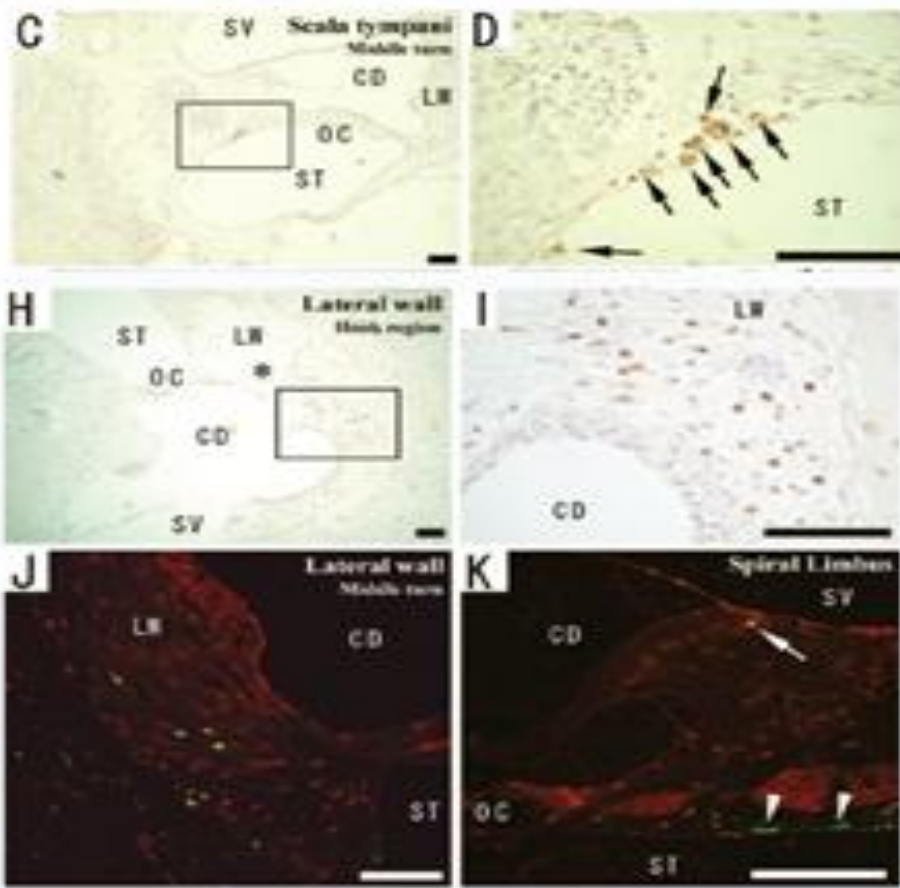
MSCs Repair the Damaged Inner Ear in Vivo



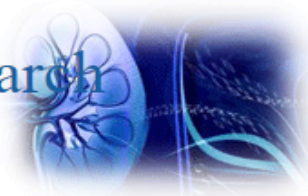
Natio et al. transplanted the Chestnut rat bone MSCs into the inner ear of deaf rat. Three weeks later, they found the transplanted MSCs could survive in the inner ear, migrate to modiolus, vestibular canal, tympanic canal, spiral ganglion and other sites.



MSCs Repair the Damaged Inner Ear in Vivo



Kamiya et al. used the mitochondrial virus to induce the acute apoptosis of rat cochlea, then they transplanted the MSCs into the damaged inner ear semicircular canal. Eleven days later, the MSCs migrated into inner ear, especially enriched in the damaged area.



MSCs Repair the Damaged Inner Ear in Vivo

Fig 1

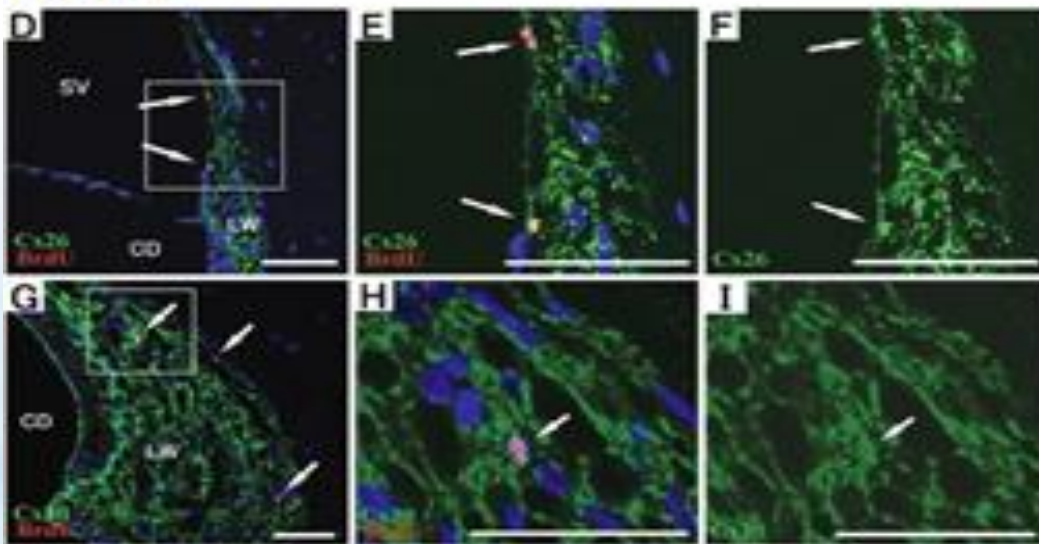
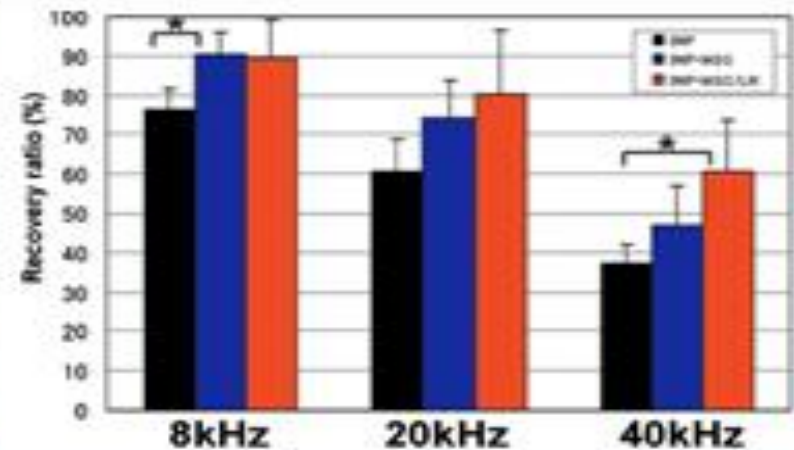


Fig 2

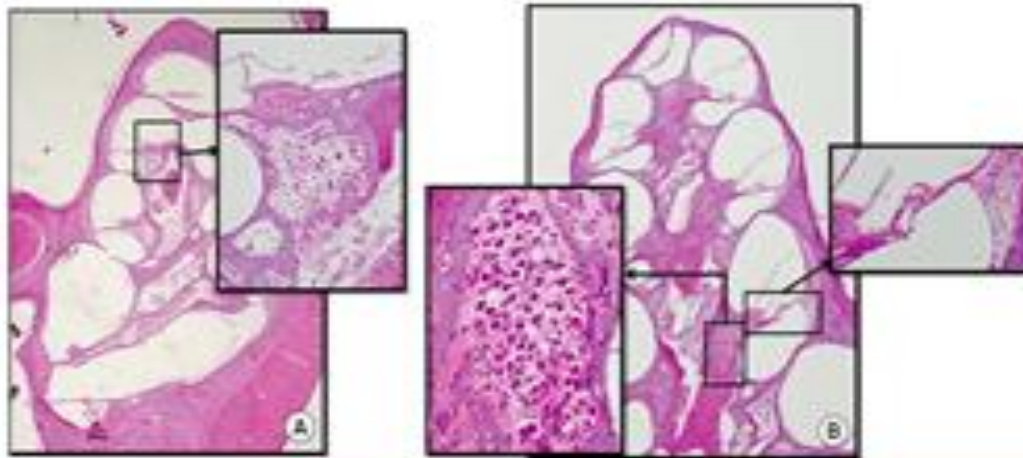
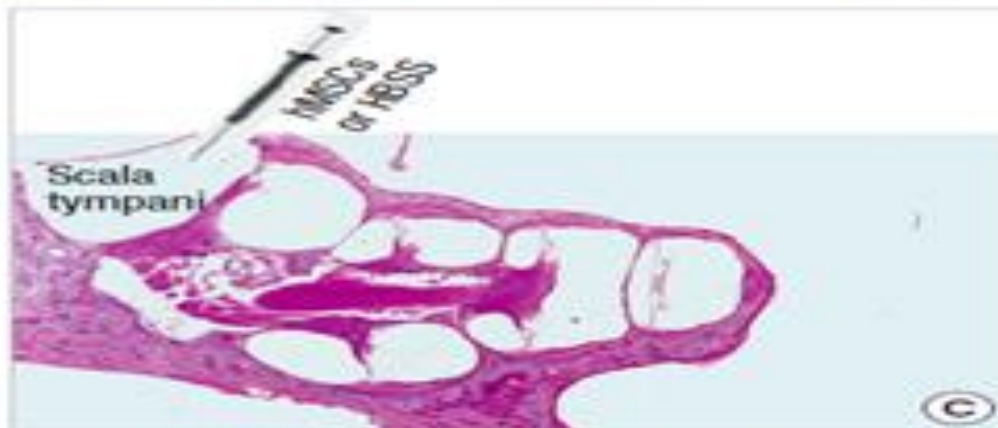


Kamiya, K et al. *AJP*. 2007.

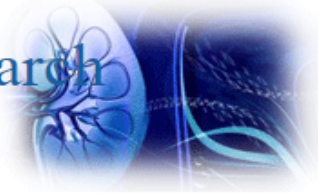
Fig 2 showed that the rat recovered its hearing after the transplantation of MSCs. MSCs could be detected in the damaged area (the red in Fig 1 is BrdU positive), and some cells expressed the gap junction protein Connexin26 (Cx26) and Cx30 (the green in Fig 1).



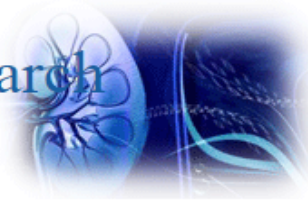
MSCs Repair the Damaged Inner Ear of Cavy in Vivo



Yong-Bum et al. injected the MSCs into the deaf guinea pig (Fig C), and then they found that the number of spiral neurons (SGN) cells and sensory hair cells in the pig ear increased significantly. In addition, the transplanted hMSCs resulted in repairing of hearing function.

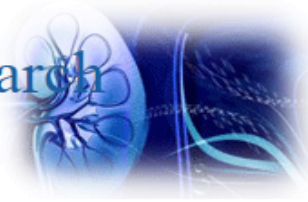


- 1 Inner ear stem cells**
- 2 Embryonic stem cells**
- 3 Mesenchymal stem cells**
- 4 Induced pluripotent stem cells**

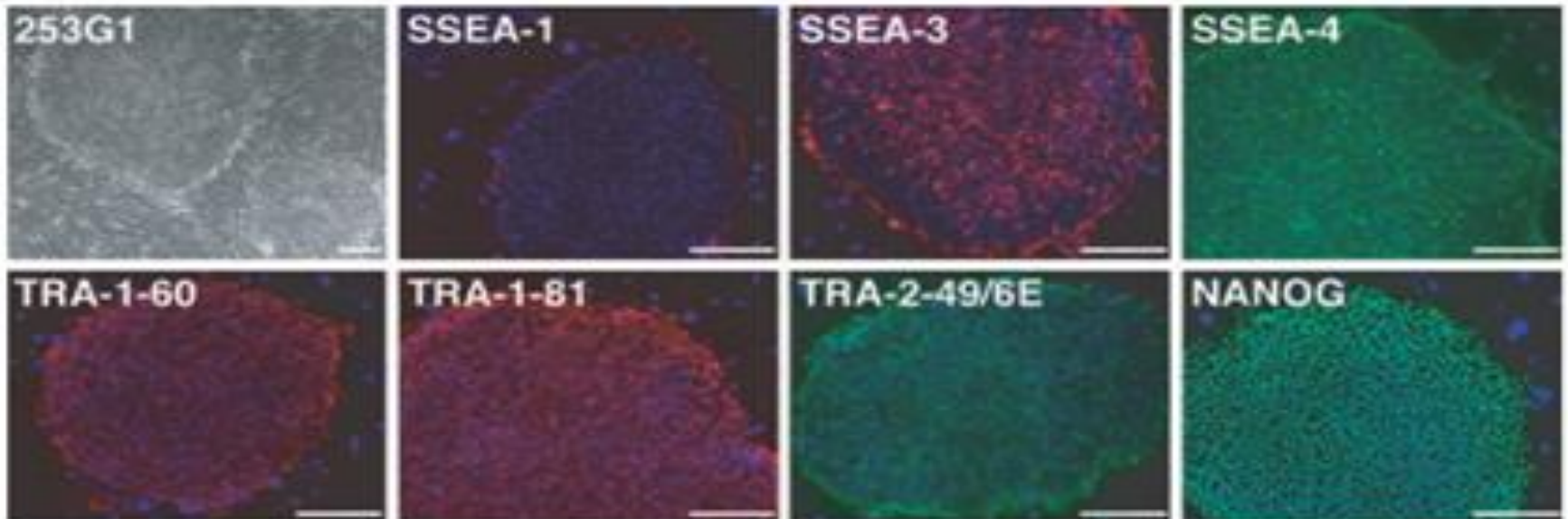


Induced Pluripotent Stem Cells

- In 2006, *Oct3/4*, *Sox2*, *Klf4*, and *c-Myc* were transduced into mouse fibroblasts using retrovirus by Takahashi and Yamanaka. Subsequently, cells with remarkably similarity with stem cells were obtained which were called induced pluripotent stem (iPS) cells.
- Recently, there are continuous modification about iPS induction. Currently, iPS cells can be obtained with various kinds of methods and somatic cells.



Similarity between iPS Cells and ES Cells

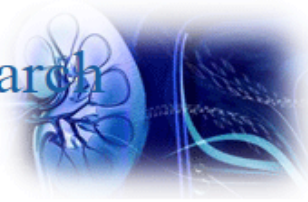


Nakagawa, M et al. *Nat Biotechnol.* 2008.

iPS cells share great similarity with ES cells in pluripotency as well as in morphology, surface marker, expression of key genes. To some extent, iPS cells are equivalent with ES cells.



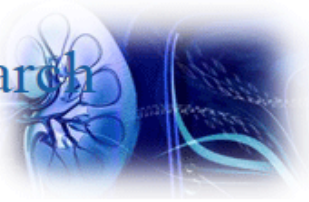
ISSN: 2161-0991



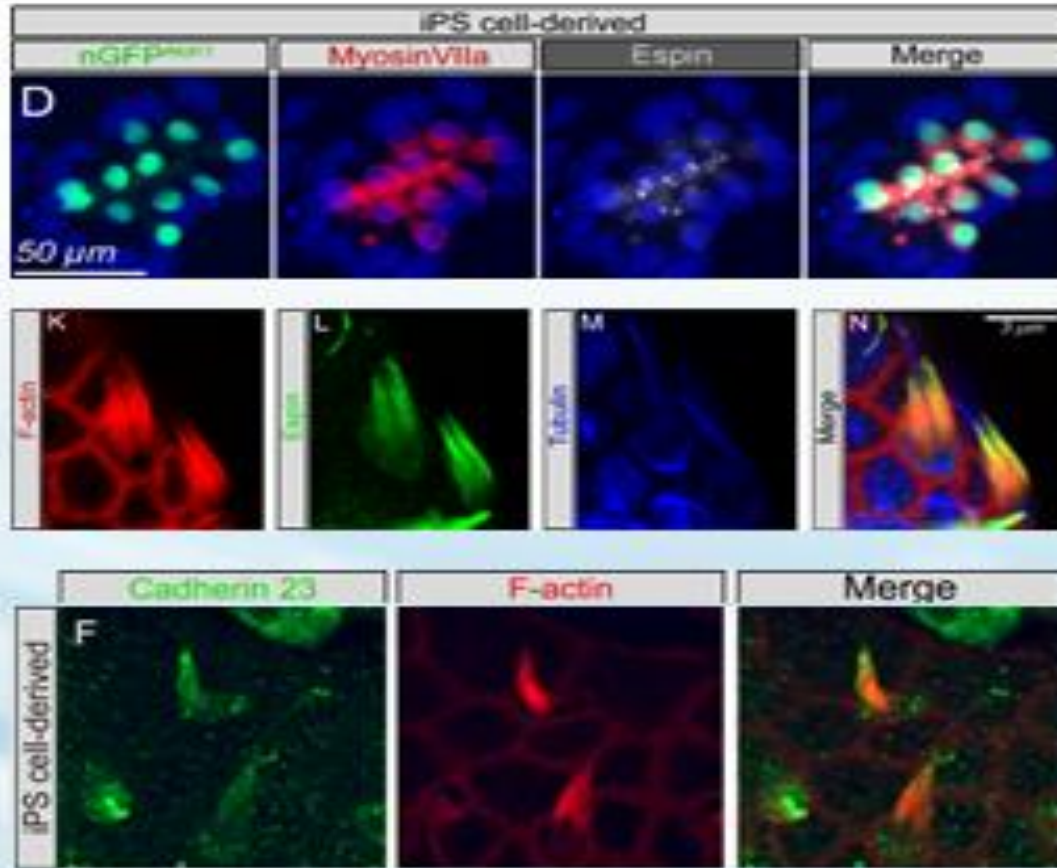
浙江大学
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iPS and Hair Cell Regeneration

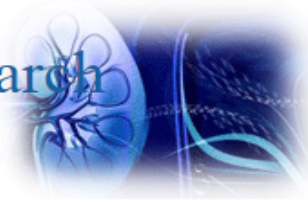
- In 2010, Oshima et al. induced fibroblast of **Math1/GFP** mouse to become **iPS** cells, and then successfully induced **iPS** cells to differentiate into hair cell-like cells with stereocilium. This is the first report that **iPS** cells are able to regenerate hair cells.



iPS and Hair Cell Regeneration

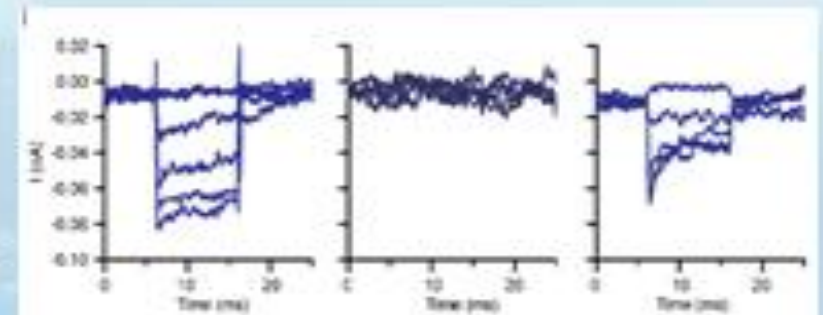
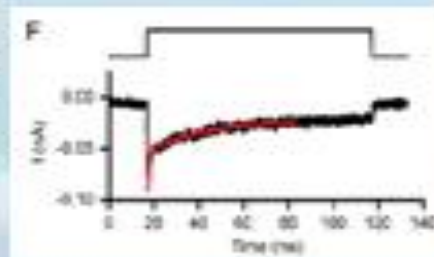
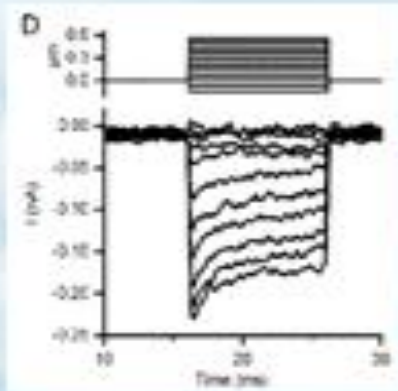
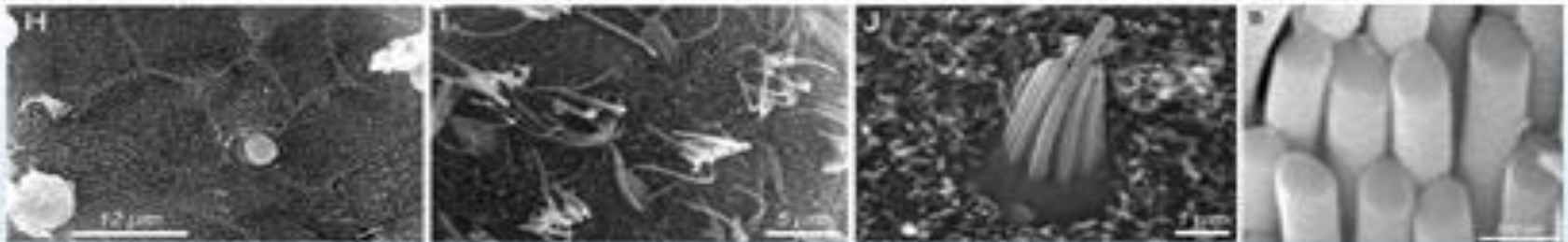


- Induced hair cell-like cells expressed hair cell-specific proteins, such as Myosin VIIA, epsin and cadherin23.



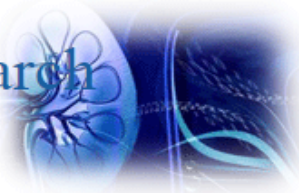
iPS and Hair Cell Regeneration

- Hair cell-like cells from iPS cells are very similar to hair cells from morphological features to electrophysiological reaction.





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Conclusion

The regeneration of inner ear hair cells is a topic that is largely unexplored, particularly in mammals. The *in vitro*-generated hair cell-like cells with mechanosensitivity from different stem cells demonstrated that generation of replacement hair cells from stem cells is feasible and promising the development of stem cell-based treatment strategies for hearing and balance disorders.

Transplantation Technologies & Research Related Journals

- Hair: Therapy & Transplantation
- Liver: Disease & Transplantation
- Surgery: Current Research



Transplantation Technologies & Research

Related Conferences

- 4th International Conference on Surgery and Anesthesia
- World Summit on Pediatric Cardiology and Cardiac Surgery

