DOI:10.11798/j.issn.1007－1520.202524534

·耳科疾病专栏·

诱导多能干细胞在遗传性耳聋研究中的应用

冯志礼[1,2],陈思源[1,2],马璐[2],蔡岳祥[1,2],敬前程[1,2]

(1.南华大学附属长沙中心医院,耳鼻咽喉头颈外科,湖南 长沙 410018;2.南华大学耳鼻咽喉头颈外科研究所,湖南 长沙 410018)

摘 要:先天性感音神经性耳聋(SNHL)中50%～60%由基因缺陷所致,称为遗传性耳聋。尽管遗传性耳聋的治疗已有所进展,但尚未实现有效防控。诱导多能干细胞(iPSCs)可以从体细胞诱导分化为多能干细胞,通过二维或三维诱导等方式在体外诱导转化为毛细胞、螺旋神经元等内耳感觉上皮细胞的类细胞或类器官,因而受到广泛关注。随着 iPSCs 技术的不断进步,其在遗传性耳聋研究领域的应用日益广泛。本文将从 iPSCs 的优势、诱导策略、应用方式等方面综述其在遗传性耳聋研究中的应用。

关 键 词:遗传性耳聋;诱导多能干细胞;诱导分化;体外模型

中图分类号:R764.43

Application of induced pluripotent stem cells in the study of hereditary deafness

FENG Zhili[1,2], CHENG Siyuan[1,2], MA Lu[2], CAI Yuexiang[1,2], JING Qiancheng[1,2]

(1. *Department of Otolaryngology Head and Neck Surgery, Affiliated Changsha Central Hospital, University of South China, Changsha* 410018, *China*; 2. *Institute of Otolaryngology Head and Neck Surgery, University of South China, Changsha* 410018, *China*)

Abstract: Congenital sensorineural hearing loss (SNHL) is caused by genetic defects in about 50% to 60% of cases, which is often referred to as hereditary deafness. Although progress has been made in treating hereditary deafness, effective prevention and control have not yet been achieved. Induced pluripotent stem cells (iPSCs) are reprogrammed from somatic cells into pluripotent stem cells, which can then differentiate into hair cells, spiral ganglion neurons, and other inner ear sensory epithelial cells or organoids through induction methods of two-dimensional or three-dimensional in vitro. Therefore, it has received widespread attention in the field of regenerative medicine. With the continuous advancement of iPSCs technology, its application in the field of hereditary deafness research is becoming increasingly widespread. This paper reviews the the application of iPSCs in hereditary deafness research, focusing on their advantages, induction strategies, and application methods.

Keywords: Hereditary deafness; Induced pluripotent stem cells; Induced differentiation; In vitro model

耳聋是人类常见的致残性疾病,已成为重要的公共健康问题和社会经济负担[1-2]。耳聋可分为3类:先天性感音神经性耳聋(sensorineural hearing loss,SNHL)、传导性耳聋和混合性耳聋,其中 SNHL 最为常见。根据世界卫生组织预测,SNHL 人数将从2019年的约4.6亿增至2050年的9亿。在先天性 SNHL 患者中,50%～60%是由遗传因素引起的,称为遗传性耳聋[3]。其遗传方式主要包括常染色体显性遗传、常染色体隐性遗传、X 连锁遗传和线粒体遗传。哺乳动物耳蜗的毛细胞和初级传入神经元属于终末分化细胞,受损后无法再生,基因突变可导致结构或功能异常,影响蛋白功能、细胞代谢及凋亡。环境与遗传因素的相互作用可能加剧听力损失[2]。目前耳聋的临床治疗主要依赖助听器和人

基金项目:长沙市自然科学基金项目(kq2208444)。
第一作者简介:冯志礼,男,在读博士研究生,住院医师。
通信作者简介:敬前程,男,博士,副主任医师。

工耳蜗,但二者对耳聋的恢复程度有限[4]。近年来,随着遗传性耳聋基因鉴定和致聋机制研究的发展,基因治疗已经在临床中应用[5-6]。小鼠是目前首选的遗传性耳聋研究模型[7],但由于小鼠耳蜗与人耳差异较大,许多在小鼠实验中有效的治疗方法临床应用受限[8]。因此亟需一种更合适的研究模型,推动基础研究向临床转化。

人类干细胞主要包括两类:人类胚胎干细胞和人诱导多能干细胞(induced pluripotent stem cells, iPSCs)。iPSCs是一类具有自我更新能力的多潜能细胞。可通过体细胞诱导获得,携带个体遗传信息,避免免疫排斥和伦理问题[9]。因此成为疾病建模、药物筛选及细胞治疗的理想研究工具。目前已有成功利用iPSCs技术进行遗传性耳聋、神经退行性疾病[10]、尿素循环障碍[11]等疾病模型的研究报道,为研究遗传性疾病和分子发病机制提供了便利的实验平台[12]。本文将综述利用iPSCs技术在遗传性耳聋研究中的应用进展。

1 iPSCs的诱导策略

iPSCs向内耳细胞分化受到多种信号通路、转录因子、生长因子及表观遗传修饰调控。目前利用iPSCs对内耳的诱导策略主要分为二维诱导和三维诱导,均使用外周血单个核细胞和尿液来源细胞为起始体细胞[13]。二维诱导方法通过应用小分子化合物和/或重组蛋白,调控内耳发育中的关键信号通路传导途径,产生单层分化细胞。可形成内耳感觉上皮祖细胞和毛细胞样细胞;三维诱导技术(类器官培养)可分化出多种细胞类型,部分模拟内耳感觉上皮的生理功能,包含毛细胞、支持细胞和感觉神经元样细胞等一系列高度分化的细胞[13]。Gunewardene等[14]和Matsuoka等[15]利用脑源性神经营养因子、神经营养因子3、骨形态发生蛋白、成纤维生长因子和声波刺猬蛋白,设计了一套类似人类内耳发育轨迹的诱导方案,用于研究基因突变导致耳蜗发育异常的机制。Koehler等[16]通过将成纤维生长因子、骨形态发生蛋白、转化生长因子和分泌型糖蛋白添加到层连蛋白包被的支持细胞单细胞悬液,诱导产生耳蜗细胞团。再使用层连蛋白培养皿,培养发育成内耳类器官。其中感觉区域含有被神经元投射靶向的毛细胞样细胞,用于研究不同调控信号对内耳发育的影响。Chen等[17]利用携带肌动蛋白15A突变的人类耳上皮祖细胞,在经过有丝分裂失活的鸡胚椭圆囊基质细胞上培养,成功培养出了类似毛细胞的结构。这些毛细胞样细胞缺乏天然毛细胞的典型细胞体和毛束形态,但可以表达毛细胞的标志基因。Li等[18]和Kempfle等[19]分别使用神经发生蛋白1、神经性分化1的异位表达和Lin-28同源物A重编程内耳神经胶质细胞,使其诱导发展为螺旋神经节神经元。Mattei等[20]开发了动态三维旋转细胞培养系统成功获得了内耳类器官。其可以体外重现内耳发育的各个阶段并产生大量的毛细胞,而且这些毛细胞具有前庭样的形态和生理表型,类似于发育中的人胎儿内耳毛细胞及耳石样结构。三维内耳类器官既包含感觉上皮细胞(毛细胞和支持细胞),也包含感觉神经元样细胞。该类器官系统为研究内耳感觉神经网络提供了一个有用工具。这些研究对于前庭系统的发育以及探索内耳遗传性疾病的治疗具有重要意义。诱导后的iPSCs需通过特定标志物进行鉴定。Shi等[21]和Matsuoka等[15]通过Ⅱ型外周神经节神经元和POU4F1等特定的标记物鉴定诱导后的细胞类型。Herget等[22]通过AM1-44染料和荧光显微镜,检测毛细胞机械转导功能进行鉴定。然而,当前iPSCs诱导的毛细胞样细胞多表现为前庭毛细胞表型,而非耳蜗毛细胞[23],因此需要更精准的方法优化其分化,使其更接近耳蜗内毛细胞和外毛细胞。

2 利用iPSCs进行内耳疾病致病机制研究

iPSCs在遗传性耳聋研究中提供了重要的体外模型。相比小鼠模型,患者来源的iPSCs能更准确模拟人类突变基因的作用机制,避免物种间差异导致的误差[24-25]。iPSCs模型已被用于研究毛束形态发生、离子稳态、细胞外基质组成及转录因子功能[17,26]。同时避免了使用人类胚胎干细胞可能引发的伦理问题。携带患者遗传背景的iPSCs细胞株,结合CRISPR/Cas9靶向编辑技术[27],还可用于评估基因治疗对遗传性耳聋的效果。Tang等[28]通过三维培养的类器官系统,建立了模拟跨膜丝氨酸蛋白酶3基因缺陷与人类听力损失相关的iPSCs细胞系。进一步发现跨膜丝氨酸蛋白酶3基因缺陷导致钙钾通道数量减少和编码钙离子结合蛋白的基因表达降低,细胞内Ca^{2+}的平衡破坏,最终导致了内耳细胞的凋亡。Tang等[29]利用携带肌动蛋白*7A*基因突变的患者家系的体细胞,建立了两组携带突变的iPSCs细胞系以及一组正常细胞系。通过比对

不同组别的 iPSCs 细胞系形态,发现了毛细胞样细胞立体纤毛样突起形态的差异。随后,运用 CRISPR/Cas9 技术纠正这些突变,使携带肌动蛋白 *7A* 突变的 iPSCs 毛细胞样细胞的形态学和功能得以恢复,揭示了肌动蛋白 *7A* 基因在立体纤毛束组装中的重要作用。Chen 等[17]使用类似的方法,建立了携带肌动蛋白 *15A* 突变的 iPSCs 细胞系,进一步揭示了肌动蛋白 *15A* 对内耳毛细胞立体纤毛的伸长至关重要。Chen 等[30]利用 1 例患有肌阵挛性癫痫伴破碎红纤维症突变患者的成纤维细胞制作了 iPSCs 细胞株。他们通过引入转录因子无调同源物 1(atonal homolog 1,Atoh1)、调节因子 X1、调节因子 X3 进行诱导,成功增强了细胞中肌动蛋白 *7A* 基因的表达,并观察到细胞表现出更强的分化能力。在使用该方案驱动的内耳细胞分化过程中,产生的内耳样细胞中立体纤毛的长度与正常毛细胞纤毛束的长度相当。Koehler 等[16]通过 CRISPR/Cas9 技术在 *Atoh1* 基因的终止密码子处插入 2A-eGFP,构建 Atoh1-2A-eGFP 细胞系。跟踪毛细胞发育,再用毛细胞标志物如 POU4 类同源盒 3 和埃斯宾蛋白基因对成熟的毛细胞进行免疫染色,从而证明了这种类器官的特异性。研究还发现新生的毛细胞展现出与正常毛细胞相似的电生理特性,并且诱导形成的类器官有类似于内耳迷路的结构特征,包括蜿蜒、曲折和腔室外观等。通过分阶段的染色实验,研究团队完整地跟踪了 *Atoh1* 基因在毛细胞发育不同阶段的表达情况,为揭示人类内耳发育机制和测试治疗策略提供了工具。

3 利用 iPSCs 进行药物测试

目前,小鼠模型是耳聋药物研究的首选研究平台[31-32]。但因物种差异[33],其结果普适性仍需要其他哺乳动物模型验证[34-35]。同时临床动物模型也难以预测药物在人体的不良反应[36]。iPSCs 衍生的内耳细胞或类器官可作为人源化药物测试平台,评估潜在药物的疗效和副作用,提高实验准确性[37]。Kurihara 等[38]利用 iPSCs 诱导分化出耳蜗螺旋神经节样细胞的内耳类器官,其具有模拟药物诱导神经病变方面的潜力。并测试耳毒性药物瓦巴因和顺铂,证明其可用于药物筛选。Daniszewski 等[39]和 Fang 等[40]开发 iPSCs 自动培养系统,实现大规模细胞库构建,结合 AI 技术优化药物筛选[41]。三维培养更能模拟体内细胞环境[16,33],但当前类器官模型仍面临缺乏血管组织的问题[42]。Vatine 等[43]采用微流控技术构建 iPSCs 衍生的血脑屏障模型,在血管化类器官中实现氧气、营养和代谢物交换,改善细胞培养环境。Chadly 等[44]设计微流体装置,用于人类胚胎干细胞向听觉神经元样细胞的分化,提高培养物均一性。目前微流控设备已经在其他 iPSCs 来源的上皮组织(如肠、肺、肾)成功测试[45],也开始在内耳类器官培养领域逐步发展。这些新技术有望缩短药物筛选时间,提高实验效率[46]。

4 展望

iPSCs 技术为遗传性耳聋研究提供了重要的模型平台,有助于基础研究向临床转化[40,47],通过 iPSCs 技术,能更深入理解遗传性耳聋的病理机制[12],并用于评估基因治疗的有效性。然而,目前 iPSCs 研究仍面临诸多挑战,包括如何优化分化方案,使其更精准地模拟耳蜗结构和功能,以及如何改进递送策略,提高治疗效率等。此外,标准化流程和降低研究成本也是未来发展的关键方向。未来,个体化治疗有望成为现实。通过 iPSCs 技术,我们可以针对患者特定突变建立疾病模型,筛选有效治疗策略,并最终应用于临床。随着研究深入,iPSCs 技术有望推动耳聋治疗新方案的开发,提高患者生活质量。

参考文献:

[1] Huddle MG, Goman AM, Kernizan FC, et al. The economic impact of adult hearing loss: A systematic review[J]. JAMA Otolaryngol Head Neck Surg, 2017, 143(10): 1040-1048.

[2] Jacob S, Johansson C, Fridberger A. Noise-induced alterations in cochlear mechanics, electromotility, and cochlear amplification [J]. Pflug Arch Eur J Phy, 2013, 465(6): 907-917.

[3] 高儒真, 樊悦, 杨腾裕,等. 北京协和医院新生儿耳聋基因筛查 10 年数据分析[J]. 协和医学杂志, 2022, 13(6): 1020-1027.

[4] Lesica NA. Why do hearing aids fail to restore normal auditory perception? [J]. Trends Neurosci, 2018, 41(4): 174-185.

[5] Qi J, TaN F, Zhang L, et al. AAV-Mediated gene therapy restores hearing in patients with DFNB9 deafness[J]. Adv Sci, 2024, 11(11): e2306788.

[6] Lv J, Wang H, Cheng X, et al. AAV1-hOTOF gene therapy for autosomal recessive deafness 9: A single-arm trial[J]. Lancet, 2024, 403(10441): 2317-2325.

[7] Ohlemiller KK, Jones SM, Johnson KR. Application of mouse models to research in hearing and balance[J]. Jaro, 2016, 17

(6):493-523.
[8] Fujioka M, Akiyama T, Hosoya M, et al. A phase I/IIa double blind single institute trial of low dose sirolimus for Pendred syndrome/DFNB4[J]. Med, 2020, 99(19): e19763.
[9] Bragança J, Lopes JA, Mendes-Silva L, et al. Induced pluripotent stem cells, a giant leap for mankind therapeutic applications[J]. World J Stem Cells, 2019, 11(7): 421-430.
[10] Bhargava A, Sandoval Castellanos AM, Shah S, et al. An insight into the iPSCs-derived two-dimensional culture and three-dimensional organoid models for neurodegenerative disorders[J]. Interface Focus, 2022, 12(5): 20220040.
[11] Duff C, Baruteau J. Modelling urea cycle disorders using iPSCs [J]. NPJ Regen Med, 2022, 7(1): 56.
[12] Marei HE, Khan MUA, Hasan A. Potential use of iPSCs for disease modeling, drug screening, and cell-based therapy for Alzheimer's disease[J]. Cell Mol Biol Lett, 2023, 28(1): 98.
[13] Ray A, Joshi JM, Sundaravadivelu PK, et al. An overview on promising somatic cell sources utilized for the efficient generation of induced pluripotent stem cells[J]. Stem Cell Rev Rep, 2021, 17(6): 1954-1974.
[14] Gunewardene N, Bergen NV, Crombie D, et al. Directing human induced pluripotent stem cells into a neurosensory lineage for auditory neuron replacement[J]. BioResearch open access, 2014, 3(4): 162-175.
[15] Matsuoka AJ, Morrissey ZD, Zhang C, et al. Directed differentiation of human embryonic stem cells toward placode-derived spiral ganglion-like sensory neurons[J]. Stem Cells Transl Med, 2017, 6(3): 923-936.
[16] Koehler KR, Nie J, Longworth-Mills E, et al. Generation of inner ear organoids containing functional hair cells from human pluripotent stem cells[J]. Nat. Biotechnol, 2017, 35(6): 583-589.
[17] Chen JR, Tang ZH, Zheng J, et al. Effects of genetic correction on the differentiation of hair cell-like cells from iPSCs with MYO15A mutation[J]. Cell Death Differ, 2016, 23(8): 1347-1357.
[18] Li X, Bi Z, Sun Y, et al. In vivo ectopic Ngn1 and Neurod1 convert neonatal cochlear glial cells into spiral ganglion neurons[J]. FASEB J, 2020, 34(3): 4764-4782.
[19] Kempfle JS, Luu NC, Petrillo M, et al. Lin28 reprograms inner ear glia to a neuronal fate[J]. Stem Cells, 2020, 38(7): 890-903.
[20] Mattei C, Lim R, Drury H, et al. Generation of vestibular tissue-like organoids from human pluripotent stem cells using the rotary cell culture system[J]. Front Cell Dev Biol, 2019, 7: 25.
[21] Shi F, Corrales CE, Liberman MC, et al. BMP4 induction of sensory neurons from human embryonic stem cells and reinnervation of sensory epithelium[J]. Eur J Neurosci, 2007, 26(11): 3016-3023.
[22] Herget M, Scheibinger M, Guo Z, et al. A simple method for purification of vestibular hair cells and non-sensory cells, and application for proteomic analysis[J]. PloS One, 2013, 8(6): e66026.
[23] Moore ST, Nakamura T, Nie J, et al. Generating high-fidelity cochlear organoids from human pluripotent stem cells[J]. Cell Stem Cell, 2023, 30(7): 950-961. e7.
[24] Dong Y, Peng T, Wu W, et al. Efficient introduction of an isogenic homozygous mutation to induced pluripotent stem cells from a hereditary hearing loss family using CRISPR/Cas9 and single-stranded donor oligonucleotides[J]. J Int Med Res, 2019, 47(4): 1717-1730.
[25] Menendez L, Trecek T, Gopalakrishnan S, et al. Generation of inner ear hair cells by direct lineage conversion of primary somatic cells[J]. eLife, 2020, 9: e55249.
[26] Cai CY, Meng FL, Rao L, et al. [Induced pluripotent stem cell technology and its application in disease research][J]. Hereditas, 2020, 42(11): 1042-1061.
[27] 刘学忠, 牛志杰, 冯永. CRISPR/Cas 靶向编辑技术在遗传性耳聋研究中的应用前景[J]. 中国耳鼻咽喉颅底外科杂志, 2016,22(3): 179-182.
[28] Tang PC, Alex AL, Nie J, et al. Defective tmprss3-associated hair cell degeneration in inner ear organoids[J]. Stem cell reports, 2019, 13(1): 147-162.
[29] Tang ZH, Chen JR, Zheng J, et al. Genetic correction of induced pluripotent stem cells from a deaf patient with MYO7A mutation results in morphologic and functional recovery of the derived hair cell-like cells[J]. Stem Cells Transl Med, 2016, 5(5): 561-571.
[30] Chen YC, Tsai CL, Wei YH, et al. ATOH1/RFX1/RFX3 transcription factors facilitate the differentiation and characterisation of inner ear hair cell-like cells from patient-specific induced pluripotent stem cells harbouring A8344G mutation of mitochondrial DNA [J]. Cell Death Dis, 2018, 9(4): 437.
[31] Ingham NJ, Pearson SA, Vancollie VE, et al. Mouse screen reveals multiple new genes underlying mouse and human hearing loss [J]. PLoS Biol, 2019, 17(4): e3000194.
[32] Choo DI, Tawfik KO, Martin DM, et al. Inner ear manifestations in CHARGE: Abnormalities, treatments, animal models, and progress toward treatments in auditory and vestibular structures[J]. Am J Med Genet C, 2017, 175(4): 439-449.
[33] Romano DR, Hashino E, Nelson RF. Deafness-in-a-dish: Modeling hereditary deafness with inner ear organoids[J]. J Hum Genet, 2022, 141(3-4): 347-362.
[34] Chowdhury S, Owens KN, Herr RJ, et al. Phenotypic optimization of urea-thiophene carboxamides to yield potent, well tolerated, and orally active protective agents against aminoglycoside-induced hearing loss[J]. J Med Chem, 2018, 61(1): 84-97.
[35] Bergman JE, Davies C, Denton AJ, et al. Advancements in stem cell technology and organoids for the restoration of sensorineural hearing loss[J]. J Am Acad Audiol, 2021, 32(10): 636-645.
[36] Atkins JT, George GC, Hess K, et al. Pre-clinical animal models are poor predictors of human toxicities in phase 1 oncology clinical trials[J]. BJC, 2020, 123(10): 1496-1501.
[37] Stojkovic M, Han D, Jeong M, et al. Human induced pluripotent stem cells and CRISPR/Cas-mediated targeted genome editing:

Platforms to tackle sensorineural hearing loss[J]. Stem Cells, 2021, 39(6): 673-696.

[38] Kurihara S, Fujioka M, Hirabayashi M, et al. Otic Organoids containing spiral ganglion neuron-like cells derived from human-induced pluripotent stem cells as a model of drug-induced neuropathy[J]. Stem Cells Transl Med, 2022, 11(3): 282-296.

[39] Daniszewski M, Crombie DE, Henderson R, et al. Automated cell culture systems and their applications to human pluripotent stem cell studies[J]. SLAS Technol, 2018, 23(4): 315-325.

[40] Fang Y, Eglen RM. Three-dimensional cell cultures in drug discovery and development[J]. SLAS Discovery, 2017, 22(5): 456-472.

[41] Kusumoto D, Yuasa S, Fukuda K. Induced pluripotent stem cell-based drug screening by use of artificial intelligence[J]. Pharm Rev, 2022, 15(5): 562.

[42] Lancaster MA, Knoblich JA. Organogenesis in a dish: Modeling development and disease using organoid technologies[J]. Science, 2014, 345(6194): 1247125.

[43] Vatine GD, Barrile R, Workman MJ, et al. Human iPSC-derived blood-brain barrier chips enable disease modeling and personalized medicine applications[J]. Cell Stem Cell, 2019, 24(6): 995-1005.e6.

[44] Chadly DM, Oleksijew AM, Coots KS, et al. Full factorial microfluidic designs and devices for parallelizing human pluripotent stem cell differentiation[J]. SLAS technology, 2019, 24(1): 41-54.

[45] Schutgens F, Clevers H. Human Organoids: Tools for understanding biology and treating diseases[J]. Annu Rev Pathol, 2020, 15: 211-234.

[46] Kim JA, Hong S, Rhee WJ. Microfluidic three-dimensional cell culture of stem cells for high-throughput analysis[J]. World J Stem Cells, 2019, 11(10): 803-816.

[47] Nishiga Y, Drainas AP, Baron M, et al. Radiotherapy in combination with CD47 blockade elicits a macrophage-mediated abscopal effect[J]. Nat Cancer, 2022, 3(11): 1351-1366.

（收稿日期:2024-12-17）

本文引用格式:冯志礼,陈思源,马璐,等.诱导多能干细胞在遗传性耳聋研究中的应用[J].中国耳鼻咽喉颅底外科杂志, 2025,31(6):42-46. DOI:10.11798/j.issn.1007-1520.202524534

Cite this article as:FENG Zhili, CHENG Siyuan, MA Lu,et al. Application of induced pluripotent stem cells in the study of hereditary deafness[J]. Chin J Otorhinolaryngol Skull Base Surg, 2025,31(6): 42-46. DOI:10.11798/j.issn.1007-1520.202524534