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Emerging role of single-cell RNA sequencing in studies of cochlear aging

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Aging-related hearing loss (ARHL), also known as presbycusis, is a multifactorial disorder resulting from aging of the auditory system, particularly the cochlea. It is characterized by progressive, bilateral, and symmetrical hearing loss, which is most pronounced at high frequencies (Jafari et al. 2019; Wu and Liberman 2022). ARHL is the most common chronic sensory deficit in the elderly population; cases double every decade from 20 years of age such that approximately half of those over 70 years old and more than 80% of those over 80 years old are affected by ARHL, with a higher incidence in men than in women (Wang and Puel 2018; Schubert et al. 2022). Given that the aging global population is increasing, the number of people affected by ARHL is expected to increase annually.

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ARHL can hinder normal communication and lead to social isolation. As a result, ARHL is associated with several comorbidities such as frailty, falls, and late-onset depression (Forster et al. 2022; Rivas-Chacon 2022; Paciello et al. 2023; Cominetti et al. 2023). Furthermore, there is mounting evidence that ARHL is linked with cognitive decline and an increased risk of dementia among the elderly population, which negatively impacts on quality of life (Cominetti et al. 2023; Martin et al. 2022; Fefer et al. 2022). Apart from the health burden, ARHL also results in substantial economic costs. Stucky et al. estimated that direct medical costs and costs for lost productivity of hearing loss in people aged 65 years and older amounts to billions of dollars per year in the United States (Stucky et al. 2010). Consequently, health, social, and economic costs of ARHL are substantial and are projected to continue to rise.

ARHL primarily results from aging of the cochlea, a complex structure with intricate physiology (Sun et al. 2023; Wang et al. 2022; Yang et al. 2022). The cochlea, which is shaped like a snail's shell, comprises a hard bony outer shell and a longitudinal compartment along the cochlear axis with similar sections, each of which encompasses the three chambers present in the cochlea: the scala vestibuli, the scala media, and the scala tympani (Zdebik et al. 2009; Ashmore and Gale 2000; OJ ON et al. 2023). The scala vestibuli and the scala tympani contain perilymph, while the scala media is filled with endolymph. The basal cochlear cells detect high-frequency sounds, whereas the apical cochlear cells detect low-frequency sounds. The cochlea is composed of different anatomical regions, including the lateral wall, the organ of Corti, and the modiolus (Zdebik et al. 2009; Ashmore and Gale 2000). The lateral wall contains cells including intermediate cells (ICs), basal cells (BCs), marginal cells

(MCs), capillary endothelial cells (CECs), perivascular resident macrophage-like melanocytes (PVM_Ms), fibroblast (FBs), fibrocytes (FCs), smooth muscle cells (SMCs). The organ of Corti primarily comprises hair cells (HCs), deiter cells and pillar cells (DCs_PCs), inner phalangeal cells / inner border cells (IPhCs_IBCs), and tympanic border cells (TBCs). Spiral ganglion neurons (SGNs), satellite glial cells (SGCs), schwann cells (SCs), chondrocytes (CCs), and osteoblasts (OBs) are present in the modiolus (Sun et al. 2018; Shrestha et al. 2018; Burns et al. 2015; Jean et al. 2023; Grandi et al. 2020; Li et al. 2020; Milon et al. 2021; Kolla et al. 2020; Ranum et al. 2019; Hoa et al. 2020; Yamashita et al. 2018; Petitpre et al. 2018). Immune cells including macrophage (M), T cell (T), B cell (B), and granulocytes/neutrophils (Neu) may infiltrate different anatomical regions (Milon et al. 2021). Based on the different pathological features of cochlear aging, ARHL can be classified as neural ARHL (loss of the SGNs), sensory ARHL (degeneration of the inner and outer HCs), strial ARHL (also known as metabolic ARHL, metabolic and vascular changes within cochlea), conductive ARHL (changes in the conduction or resonance of the cochlear duct) and mixed ARHL (a combination of the above) (Jafari et al. 2019). However, because of its intricate structure and the diversity of its cellular composition, decoding the cellular and molecular mechanisms of ARHL has been challenging. Unlike bulk RNA-seq, emerging single-cell RNA-seq enables a more precise analysis of the cellular and molecular basis underlying cochlear aging at the single-cell level.

Recent single-cell RNA sequencing (scRNA-seq) analysis of the cochlea have primarily focused on mouse cochlear development and impact of noise on the cochlea in mice. Burns et al. conducted a study using 301 single cells from the utricular and cochlear sensory epithelia of newborn mice, identifying major sensory epithelial cell types and demonstrating differences in cell types between the cochlear and vestibular sensory epithelia (Burns et al. 2015). Other studies reported subtypes of type I SGNs identified by scRNA-seq and that inner HC-driven activities are necessary for SGN diversification (Sun et al. 2018; Shrestha et al. 2018). ScRNA-seq analysis has also been applied to resolve the cellular heterogeneity of adult mouse cochlear supporting cells and stria vascularis (Hoa et al. 2020; Korrapati et al. 2019). In addition, Yamashita et al. conducted scRNA-seq analysis on mouse cochlear organ of Corti harvested at multiple time points after conditional overexpression of *Atoh1*, identifying 51 reprogramming transcription factors, including *Isl1*, that were important for the efficiency of HCs conversion (Yamashita et al. 2018). It was also found that *Tgfβr1* is essential for the developmental maturation of outer HCs in a study analyzing the transcriptome of approximately 30,000 cells from mouse cochlear sensory epithelium at four developmental time points (Kolla et al. 2020). Finally, Milon et al. revealed cochlear cell type-specific transcriptional changes upon noise exposure using single-cell transcriptomics (Milon et al. 2021). These studies have advanced our understanding of cochlear development and responses to noise at the single-cell level.

In the context of aging, several studies have contributed to advance our understanding of cochlear aging at the single-cell level. For instance, Shrestha et al. analyzed the changes in the proportion of three subtypes of typeI SGNs with aging and found that type IC SGNs are selectively vulnerable to aging (Shrestha et al. 2018). In related work, Liu et al. discovered that aging-associated upregulated genes in HCs are mainly associated with oxidative stress (*Kdm6b*, *FOXO3*, *Sod1*), DNA damage (*Marf1, Rad9b, Actr2*) and autophagy (*Pikfyve*, *Gsk3b*, *Yod1*), based on their analysis of inner and outer HCs, identified by morphology, from the young and old mouse cochlea (Liu et al. 2022). Although these pioneering studies unveiled age-related changes in specific cochlear cell types, a complete scRNA-seq analyses of all cochlear cell aging remained outstanding. Notably, a study published in 2023 constructed the first high-resolution single-cell transcriptome atlas of mice cochlear aging across multiple time points (including 1, 2, 5, 12, and 15 months old mice) to systematically reveal the cellular and molecular mechanisms of mouse cochlear aging (Sun et al. 2023). In this study, multiple cochlear cell types (HCs, DCs_PCs, IPhCs_IBCs, TBCs, ECs, SGNs, SGCs, SCs, CCs, OBs, RMCs, ICs, MCs, BCs, CECs, PVM_Ms, FBs, FCs, SMCs, M, T, B, Neu) and especially ICs, SCs, HCs, SGNs were found to have aging-associated elevated immune inflammatory responses (*S100a8, S100a9, Ifi35*), and increased levels of oxidative stress (*Apoe*, *Sod2*, *Gpx4*), apoptosis (*Atf4*, *Ddit3*, *Casp3*), and endoplasmic reticulum stress (*Hsp90aa1*, *Calr*, *Hspa5*) (Sun et al. 2023). Most strikingly, *Hsp90aa1* was found to be a potential target for delaying aging of cochlear ICs. This work provides a rich resource for uncovering the cellular and molecular mechanisms of cochlear aging, and provides a foundation for advancing the development of diagnostic and therapeutic intervention strategies for ARHL (Fig. 1) (Sun et al. 2023).

The fact that cells in the cochlear basal and apical turns perceive sound at different frequencies suggests that the same cell type have different properties depending on its location in the cochlea. Furthermore, it has been reported that cells in different cochlear locations are affected by aging to varying degrees, for example, the cells in the basal turn of the human cochlea are more susceptible to aging, leading to high-frequency hearing loss (Wang et al. 2020; Gates et al. 2005; Fu et al. 2018; Tian et al. 2020). Therefore, a more in-depth analysis of the

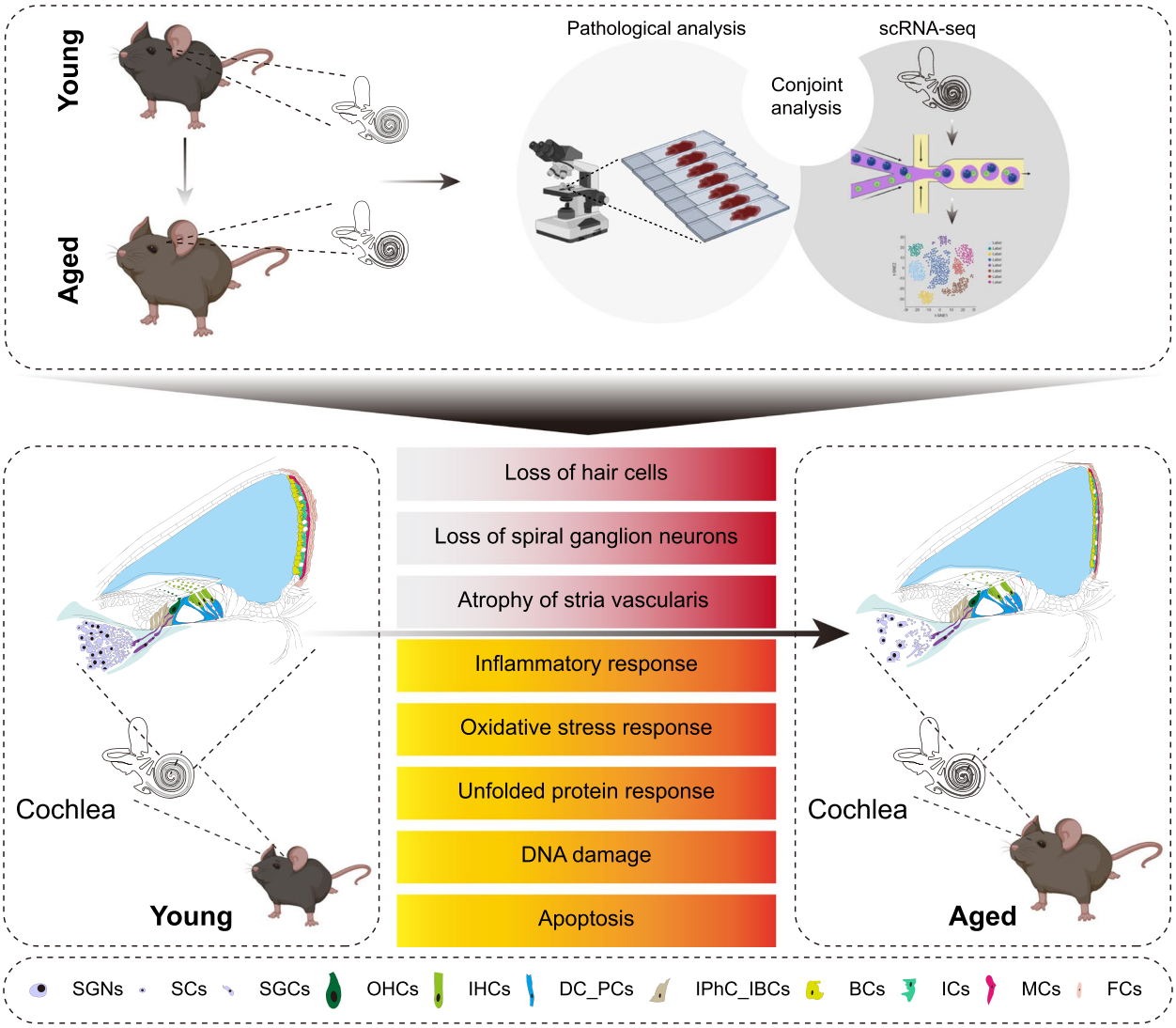

Fig. 1 Schematic diagram showing the mechanism of cochlear aging revealed by scRNA-seq. Top, young and aged mouse cochlear tissues were subjected to histopathological analysis and single-cell transcriptomic sequencing. Bottom, cellular and molecular alterations associated with age in the mouse cochlea. Spiral ganglion neurons (SGNs), schwann cells (SCs), satellite glial cells (SGCs), outer hair cells (OHCs), inner hair cells (IHCs), deiter cells and pillar cells (DCs_PCs), inner phalangeal cells / inner border cells (IPhCs_IBCs), intermediate cells (ICs), basal cells (BCs), marginal cells (MCs), fibrocytes (FCs)

molecular mechanisms of cochlear aging at the spatial level is needed to better understand the effects of aging on different regions of the cochlea.

Lastly, the cochlear structure of non-human primates is more similar to that of humans than that of the rodents. For instance, the cochleae of both non-human primates and human have 2.5–3.5 turns, whereas mice only have 1.5–2.5 turns. As a result, non-human primates can perceive sounds within a frequency range comparable to humans (Recanzone et al. 2011; Sun et al. 2021; Engle et al. 2013; Ayala et al. 2017). Thus, the non-human primate cochlea is a superior model for gaining a more comprehensive understanding of the cellular and molecular mechanisms underlying cochlear aging in higher vertebrates, and ultimately therefore more likely to inform identification of new targets that can be targeted therapeutically to mitigate human cochlear aging and ARHL.

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G.H.L., S.W., and J. C. I. B. designed the work. G. -Q. S. and S.W. wrote the initial draft of this paper. All the authors participated in editing both form and content of this paper and approved the final version.

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