Review

Cochlear homeostasis and its role in genetic deafness

Zhe Jin[1,2], Inger Uhlen[3], KONG Wei-Jia[4], DUAN Mao-li[1,3,5]

1 Center for Hearing and Communication Research, Department of Clinical Neuroscience, Karolinska Institutet, and the Department of Otolaryngology, Karolinska University Hospital, Stockholm, Sweden

2 Department of Neuroscience, Uppsala University, Uppsala, Sweden

3 Department of Clinical Sciences, Intervention, Department of Audiology, Karolinska Institutet, Stockholm, Sweden

4 Department of Otorhinolaryngology, Union Hospital of Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China.

5 The affiliated hospital of Anhui Medical University, HeFei, China

Abstract Each component of the human ear performs a specific function in hearing. The actual process of sound transduction takes place in the auditory portion of the inner ear, the fluid-filled cochlea. In the cochlea, the sensitivity and efficiency of sensory apparatus to convert mechanical energy into neural activity, largely depends on the fluidic and ionic environment. In the lateral wall of cochlea, the secretory epithelium stria vascularis plays an important role in the maintenance of fluidic and ionic homeostasis. A variety of gene mutations disturbs the cochlear homeostasis and subsequently leads to hearing impairment. The review covers several aspects of cochlear homeostasis, from cochlear fluid and the functional role of stria vascularis, cochlear K^+ recycling and its molecular substrates to genetic deafness with abnormal cochlear homeostasis.

Introduction

Hearing loss is the most common sensory disorders affecting personal communication and daily life. Among them, over 50% deafness cases have genetic components. The majority of genetic deafness targets the hearing organ-cochlea. The mammalian cochlea is a snail-shaped, fluid-filled structure and is divided into three compartments: scala tympani, scala vestibuli and scala media (Fig.1). Scala tympani and scala vestibule are filled with extracellular fluid-like perilymph. Scala media, containing high $[K^+]$ endolymph, houses the mechanoreceptive sensory epithelium- the organ of Corti. Sound wave energy is transmitted through cochlear fluids and converted by the sensory hair cells to nerve pulses. The ionic and fluid homeostasis in the cochlea plays an important role in cochlear physiology. Disruption of cochlear homeostasis due to genetic mutation inevitably compromises normal hearing function. In this review we will focus on the structural basis and molecu-

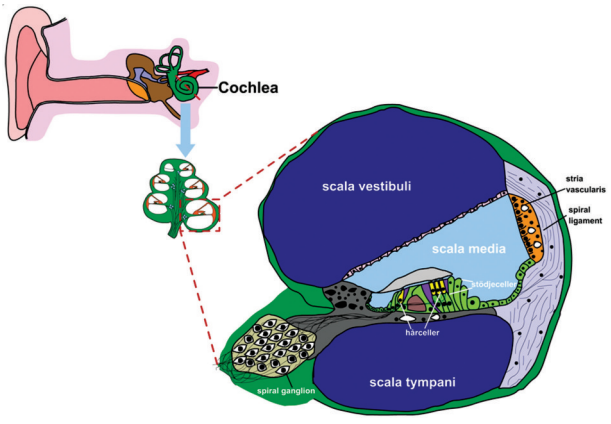

Fig.1 The schematic diagram showing a cross-section view of the cochlea.

Corresponding author: DUAN Maoli, M.D, PhD, Department of Clinical Sciences, Intervention, Department of Audiology, Department of Clinical Neuroscience, Department of Otolaryngology Head and Neck, Karolinska University Hospital, Stockholm, 171 76 Stockholm, Sweden
E mail: maoli.duan@ki.se

lar substrates of cochlear homeostasis and genetic deafness due to abnormal cochlear homeostasis.

Cochlear fluids and strial melanocytes

Cochlear fluids play major roles in cochlear physiology such as transmission of the mechanical stimulus to the hair cells. Three major extracellular fluids have been identified in the cochlea: endolymph, perilymph and intrastrial fluid [1]. The chemical composition varies greatly between the cochlear fluids.

The perilymph is a typical extracellular fluid, and its ionic composition is similar but not identical to that of plasma and cerebrospinal fluid. The dominant cation in the perilymph is sodium. Both scala vestibuli and scala tympani are filled with perilymph which communicates at the cochlear apex via the helicotrema. However, the perilymph of scala vestibuli and scala tympani differs in composition and origin[2]: the perilymph of scala vestibuli originates mainly from plasma across a blood–perilymph barrier, whereas the perilymph of scala tympani is partially formed by cerebrospinal fluid (CSF). The movement of perilymph in response to vibration of oval window membrane causes the motion of basilar membrane and in turn hair cell stimulation.

The endolymph is a unique extracellular fluid with unusually high $[K^+]$ but low $[Na^+]$ and $[Ca^{2+}]$. Enclosed in scala media, the endolymph has direct contact with several different epithelial cell types including sensory hair cells. It is well known that the endolymph originates from the perilymph through the labyrinthine epithelium, rather than from blood plasma [3, 4]. Interestingly, the endolymph in scala media possesses a large positive transepithelial potential with respect to perilymph and plasma, designated as the endocochlear potential (EP). The EP was first recorded by von Békésy [5] and its magnitude is around 80–100mV in the mammalian cochleas studied. However, no such high potential has been detected in the vestibular endolymphatic labyrinth or any other mammalian organ. The EP in the cochlea is generally considered to be generated by stria vascularis [5, 6] and to serve as a major driving force for sensory transduction. The spiral ligament might also contribute to the generation or maintenance of EP as evidenced by a dramatic reduction of EP in Pou3f4–deficient mice and Tbx18–deficient mice with defect of spiral ligament fibrocytes [7, 8]. Several electrophysiological models have been proposed to explain the mechanism underlying the EP generation [9–11]. Nevertheless, the molecular substrate that produces EP has only been identified until very recently. The EP is essentially generated by the potassium channel subunit KCNJ10 (Kir 4.1) located in the intermediate cells of stria vascularis [12, 13].

The intrastrial fluid fills the narrow intrastrial compartment, which is isolated from perilymph in the spiral ligament and endolymph in the scala media by basal and marginal cell layers, respectively. The ionic composition of the intrastrial fluid resembles that of the perilymph containing low $[K^+]$ but relatively high $[Na^+]$. Notably, the intrastrial fluid also maintains a positive voltage potential of ~100 mV relative to the perilymph. It is referred to as intrastrial potential [11] and has been assumed as a source of EP.

The stria vascularis is a highly vascularized epithelium with high secretory and metabolic activity, which is mainly composed of marginal, intermediate and basal cells together with capillary endothelial cells (Fig.2). The characteristic cellular architecture of the mature stria vascularis appears quite similar across different mammalian species, including mouse, cat, guinea pig, and human. The luminal side of stria vascularis facing the scala media is composed of ectoderm–derived marginal cells, which possess extensive basolateral membrane infoldings and a rich population of mitochondria.

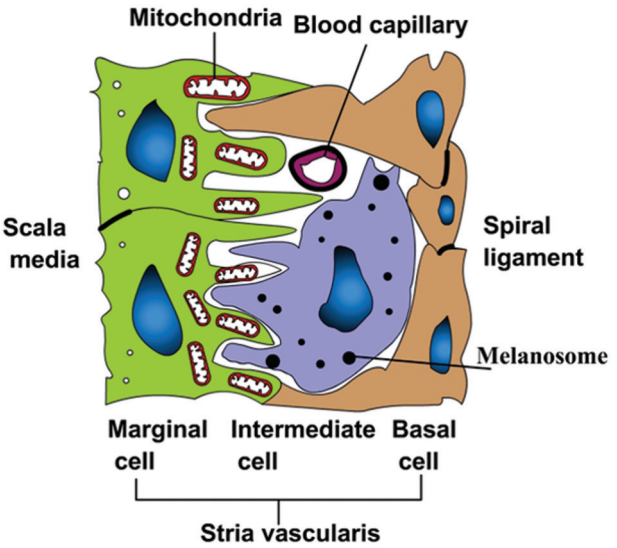

Fig.2 The schematic structure of the stria vascularis

On the other side of stria vascularis, the mesoderm-derived basal cells are spindle shaped, arranged tangentially and form a continuous barrier layer separating the stria vascularis from the adjacent spiral ligament. The intermediate cells are neural crest-derived melanocytes, which can easily be identified by their melanin pigments. The intermediate cells as well as blood capillaries are sandwiched between the marginal and basal cell layers. The migration of melanocytes from the neural crest to the stria vascularis is essential for the development and interaction of other strial cells[14, 15]. Between the adjacent marginal cells and basal cells are tight junctions, which insulate the stria vascularis as a unique compartment.

Melanocytes have been identified in various sites of the inner ear including the stria vascularis, the cochlear modiolus as well as specific locations of the vestibular end organs[16]. Generally, otic melanocytes originate from the embryonic neural crest[17]. Their precursors, melanoblasts, migrate from neural crest to the inner ear during early development and ultimately differentiate into melanocytes. The melanoblast is an unpigmented cell, but it can be identified with a marker dopachrome tautomerase (Dct)[18]. In the stria vascularis, the melanocytes, also known as intermediate cells, contain melanin pigments and extend dendritic processes to interdigitate with adjacent marginal and basal cells. It has been showed that there are two forms of intermediate cells present in the mouse stria vascularis: light and dark intermediate cells[19]. The light intermediate cells which are present from birth contain large amount of organelles but very few melanin pigments, whereas the dark intermediate cells which are only observed in the adult stria vascularis, are more heavily pigmented and exhibit pynotic nuclei and contain few organelles. These dark intermediate cells are presumed to be a degenerate form of the light ones.

What is the exact role of strial melanocytes in cochlear function? Although it is still not fully understood, the putative roles of strial melanocytes include 1) normal structural development of stria vascularis. The migration of melanocytes into the stria vascularis is important for the differentiation of marginal cells, the interdigitation between the marginal and basal cells as well as the sustainment of strial capillary network, as evidenced by the findings in the stria vascularis of melanocyte-deficient mutant animals[14, 20]. 2) generation and maintenance of EP. In the dominant white spotting (*W*) mutant mice which did not contain strial melanocytes, no EP was generated[21]. In addition, the presence of strial melanocytes is constantly correlated with a measurable EP[22]. In mutant mice displaying a progressive degeneration of strial melanocytes (e.g. B^{lt} light mutant mice), the EP gradually decreased with age[23]. It indicates that the continued presence of strial melanocytes is required for the maintenance of EP. Interestingly, the EP is independent of the pigment production ability of strial melanocytes since albino animals still have a normal EP despite of the presence of amelanotic melanocytes in the stria vascularis.

A complex molecular network composed of various genes is participating in the different aspects of strial melanocytes development (proliferation, migration, survival and differentiation)[24, 25]. The reciprocal interaction between these regulatory genes has been extensively studied. Proto-oncogene Kit and its ligand Kitl are necessary for the survival and/or migration of melanoblasts[26]. Dominant white spotting (*W*) and Steel (*Sl*) mutant mice, which have the mutation in the Kit and Kitl genes respectively, lack strial melanocytes and thus cause hearing impairment[18, 22, 27, 28]. Endothelin 3 (*Edn3*) and its receptor *Ednrb*, transcription factors *Pax3, Sox10*, and *Mitf* have also been considered indispensable for the migration, differentiation, and proliferation of melanoblasts and melanocytes. Among these genes, *Mitf* plays a central role in the development and function of melanocytes[24, 25]. Mutations in the human *EDN3*, *EDNRB*, *PAX3*, *SOX10* and *MITF* genes result in the hearing loss of several subtypes of Waardenburg (auditory-pigmentary) syndrome[29, 30].

Cochlear potassium recycling and its molecular correlates

In the cochlea, K^+ ions provide major charge carriers for hair cell transduction as well as for the EP production. Therefore, cochlear K^+ homeostasis is essential for

maintaining high sensitivity of hair cells and thus for normal hearing function. It has been shown by radioactive tracer experiment that K^+ ions in the endolymph are derived from perilymph rather than from blood plasma [3]. The different epithelial cells lining scala media are coupled by tight junctions, which limit extracellular diffusion of K^+ ions between endolymph and perilymph. An ensuing question arises as to how K^+ ions are recycled from perilymph back to endolymph. Several putative routes for K^+ recirculation in the cochlea have been proposed so far(Fig.3)[31-34]. Driven by the high endocochlear potential, K^+ ions in the endolymph pass through the apical mechanotransduction channels into the sensory hair cells, and then exit the hair cells via K^+ channels(e.g. Kcnq4, Kcnn2 and Kcnma1) along the their basolateral membranes[35]. The released K^+ ions are subsequently taken up by surrounding supporting cells via potassium channels and transporters (e.g. K-Cl cotransporters Kcc3 and Kcc4). With aid of gap junction systems, the K^+ ions are further transported either medially toward the spiral limbus and back to endolymph[36], or laterally toward the spiral ligament[37]. Alternatively, K^+ ions can flow through the perilymph above or below the scala media, or through outer sulcus cells toward the spiral ligament[38, 39]. The epithelial cell system and the connective tissue cell system are two independent gap junction networks in the cochlea, which are mainly composed of GJB2, GJB3 and GJB6. It has been thought that both gap junction networks are participating in cochlear K^+ recirculation pathway by providing intercellular transport routes[40]. Through gap junction networks, K^+ ions are delivered to the stria vascularis and released from the intermediate cells via the KCNJ10 channel into the intrastrial compartment. From there K^+ ions were taken up by the basolateral Na-K-Cl cotransporter(Slc12a2) and Na-K-ATPase (Atp1a1/Atp1b2) and secreted back into endolymph by the apical Kcnq1/Kcne1 potassium channel of the marginal cells.

An array of functional proteins including ion channels, cotransporters, ATPases, and intercellular junctions are actively participating the cochlear K^+ recycling pathway(Fig.3). The dysfunction of these key regulators is associated with deafness in humans and mouse mutants.

Kcnq1 and *Kcne1* encode the α- and β-subunits of

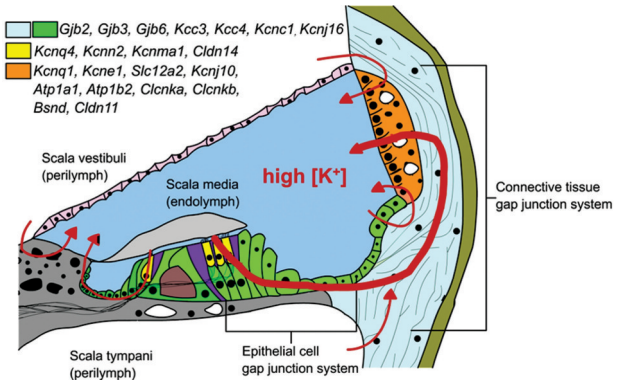

Fig.3 The schematic illustration of cochlear K^+ recycling pathway and the distribution of its key molecular players in the cochlea.

K^+ channel, respectively. These protein subunits co-assemble to form functional channels and have been detected in the apical membrane of strial marginal cells and vestibular dark cells[41-43]. Additionally, Kcnq1/Kcne1 K^+ currents were also recorded in both cell types. Kcnq1/Kcne1 K^+ channel is by far the sole functional element to secret K^+ ion across the apical membrane of marginal cells. The essential role of Kcnq1/Kcne1 K^+ channel for K^+ secretion in the cochlea was clearly illustrated in the mouse mutants with a targeted deletion of either *Kcnq1* or *Kcne1* gene[44, 45]. Genetic mutations affecting either *KCNQ1* or *KCNE1* are responsible for cardioauditory syndrome (Jervell and Lange-Nielsen syndrome)[42, 46].

Slc12a2 encodes a Na-K-Cl cotransporter which is primarily expressed in the basolateral membrane of strial marginal cells and vestibular dark cells[47]. In the cochlea, Slc12a2 is important for effective uptake of K^+ from the intrastrial compartment. K^+ secretion from the stria vascularis was abolished in mice with *Slc12a2* mutations[48, 49]. However, no human deafness related to mutations in *SLC12A2* gene has been yet identified.

Kcnj10(Kir4.1), an inward rectifier K^+ channel subunit is specifically expressed in the strial intermediate cells[50]. The time course of its developmental expression was closely correlated to the elevation of EP[51]. Loss of EP and partial reduction of cochlear endolymphatic volume and $[K^+]$ in the *Kcnj10*-null mice[13], indicate that Kcnj10 channel is essential for EP generation and play a major role in the cochlear K^+ recycling pathway.

Claudin 11 encoded by *Cldn11* gene, a member of the claudin family, is an integral membrane protein and one component of tight junction strands. It is exclusively localized between strial basal cells in the cochlea[52]. Claudin 11 co-assembling with other tight junction components connects strial basal cells and forms a continuous barrier separating intrastrial fluid from the perilymph in the spiral ligament. The important role of Claudin 11 in the maintenance of the electrically isolated intrastrial compartment is evidenced by depression of EP in the *Cldn11*-null mice [53, 54].

Connexin 26 (Gjb2), one of major gap junction proteins, is abundantly distributed in both the epithelial cell and connective tissue gap junction systems in the cochlea. Gap junctions, especially connexin 26, provide an intercellular passage for cochlear K^+ ions and therefore are essential for cochlear function. Mutations in the GJB2 gene account for about 50% autosomal recessive non-syndromic deafness (DNFB1)[55]. Two independent transgenic mouse mutants with the mutated *Gjb2* gene [56, 57] display hearing loss, but the EP as well as the endolymphatic $[K^+]$ and volume is not altered. It suggests that Gjb2 might not be essential for EP production and cochlear K^+ recirculation.

Genetic deafness with abnormal cochlear homeostasis

Disturbance of cochlear homeostasis leads to many forms of genetic hearing loss including the most common syndromic and non-syndromic deafness (Table 1). The inner ear pathology of these forms of genetic deafness varies widely. A specific type of human inner ear defect also known as Scheibe's dysplasia (cochleosaccular defect) was first described in congenitally deaf patients[58]. This is caused by the underdevelopment of the inferior part of the membranous labyrinth which forms the cochlear duct and saccule, although the bony labyrinth is fully developed[59]. Despite the presence of some variability, the typical cochlear pathology in Scheibe's dysplasia/cochleosaccular defect includes a primary stria vascularis defect, reduction/absence of cochlear duct, and variable loss of sensory hair cells and spiral ganglion neurons, indicative of abnormal endolymph homeostasis. Scheibe's dysplasia is the most common form of inner ear aplasia associated with congenital deafness in humans[60]. It is more frequently observed in temporal bones of patients with syndromic deafness, such as Waardenburg (auditory-pigmentory), Usher (auditory-visual), and Jervell and Lange-Nielsen (cardioauditory) syndromes. The molecular correlates for several types of syndromic deafness have been disclosed recently[61]. However, cochleosaccular defect is rarely associated with non-syndromic hearing loss. Another type of human inner ear defect is characterized by enlargement of the endolymphatic compartment, which is commonly observed in patients with Pendred syndrome (hearing loss and goiter). Mutations in *SLC26A4* gene accounts for about 50% cases of Pendred syndrome.

Deaf animal mutants provide excellent models to study the mechanism of human genetic deafness with abnormal cochlear homeostasis (Table.1). One group deaf animal mutants carries mutated or eliminated version of target genes which are known to be essential for cochle-

Table.1 Human genetic deafness and animal mutants with abnormal cochlear homeostasis

Human genetic deafness	Gene name	Animal mutants
Jervell and Lange-Nielsen syndrome	KCNQ11	Kcnq1$^{-/-}$ mice
	KCNE1	Kcne1$^{-/-}$ mice
		Punk rocker
Waardenburg syndrome	MITF	VGA-9 mice
		Microphthalmia mice
		JF1 mice
	EDNRB	WS4 mice
		Piebald mice
	EDN3	Lethal spotting mice
		Edn3$^{-/-}$ mice
	PAX3	Splotch mice
DNFB1, DFNA3	GJB2	Gjb2R75 W mice
		Gjb2OtogCre mice
	GJB6	Gjb6$^{-/-}$ mice
SeSAME syndrome	KCNJ10	Kcnj10$^{-/-}$ mice
Pendred syndrome	SLC26A4	Slc26a4$^{-/-}$ mice

ar K^+ recirculation, such as *Kcnq1*, *Kcne1*, *Slc12a2* and *Kcnj10*. In these postnatal mouse mutants, the endocochlear potential (EP) was reduced or abolished; the cochlear duct was diminished to a variable degree following the collapse of Reissner's membrane. However, it is not necessary that all the mouse mutants with targeted disruption of genes involved in cochlear ionic homeostasis display the same cochlear pathology. As an example, no obvious morphological malformation was detected in the cochlea of tight junction gene Cldn11-null mouse despite of the reduced EP[53, 54].

The other group of deaf animal mutants is associated with melanocyte defects in the stria vascularis as well as in the skin. Therefore, they usually have abnormal coat color, such as white spots or patches. Deaf white cats, Dalmatian dogs, white spotting (*W*) rats and Steel (*Sl*) mice are well-known examples of animals with pigment-associated deafness. Interestingly, the absence of strial melanocytes, the lack of EP and the resulting hearing loss can be highly variable between and within each individual animal: some cochleas have no strial melanocyte and no detectable EP, while in other cochleas strial melanocytes are present only in the pigmented portion of the stria vascularis and a reduced EP can be measured [22]. Another deaf animal mutant, German waltzing guinea pig, has pigmented eyes as well as normal coat color, but the strial melanoctyes are abnormal in the malformed stria vascularis [62, 63]. Expression of several key players in the endolymph homeostasis also reduced in the inner ear of German waltzing guinea pig [64].

Summary

Cochlear homeostasis plays a critical role in cochlear physiology and normal hearing function. Mutations in a wide range of genes can disturb the cochlear homeostasis and consequently cause hearing impairment. Deaf animal mutants provide a useful tool to understand the underlying molecular mechanism of genetic deafness with abnormal cochlear homeostasis, and to develop novel therapeutic strategies to cure this form of genetic deafness.

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