Review

Cell junction proteins within the cochlea: A review of recent research

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Abstract

Cell–cell junctions in the cochlea are highly complex and well organized. The role of these junctions is to maintain structural and functional integrity of the cochlea. In this review, we describe classification of cell junction-associated proteins identified within the cochlea and provide a brief overview of the function of these proteins in adherent junctions, gap junctions and tight junctions.
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As a sound sensor in a living organism, the cochlea is a highly sophisticated and complex organ. Its structural integrity at both cellular and tissue levels is critical to ensure normal auditory functions (Slepecky, 1996; Raphael and Altschuler, 2003; Yates, 1995). Cell junctions between sensory cells in the organ of Corti and supporting cells and between supporting cells exist in particular ways to maintain the well-organized structure of the auditory sensor in the three dimensional space, which plays irreplaceable roles in sustaining mechanical connections among cochlear cells, in ensuring the integrity of sensory epithelia and in controlling ion concentrations in the endo- and perilymph (Slepecky, 1996; Müller and Littlewood-Evans, 2001; Tsuprun and Santi, 2002; Edelman, 1986; Schwander et al., 2010; Leonova and Raphael, 1997). There are mainly three types of cell junctions in mammals: i.e. the adherens junction, gap junction and tight junction (Alberts et al., 2008). This review provides a brief summary of functions of these junctions in the cochlea.

1. Adherens junction

Adherens junction refers to the connection to one or multiple lipid-anchored proteins (as well as actins) on the inside of a cell, and with transmembrane adhesion proteins or extracellular matrix of neighboring cells (Alberts et al., 2008). In the cochlea, adherens junction is closely related to physiological processes such as cochlear development, growth of auditory neurons, immune mediation and planar cell alignment.

1.1. Adherens junction and cochlear development

Cadherin is a super family of transmembrane glycoproteins that mediate calcium-dependent intercellular adhesion. It is the main element in mediating adherens junction and is involved in cochlear development. T-cadherin is expressed in the sub-domain in all types of fibroblasts and pillar cells. Expression of E-cadherin opposes that of N-cadherin and both do not overlap T-cadherin expression. E-cadherin expression is positive in all cochlear epithelial cells (including outer hair cells, OHCs) except inner hair cells (IHCs) and the part of the Kolliker's organ in contact with IHCs. During development, E-cadherin is found between OHCs, pillar cells and Deiters cells, responsible for maintaining the distribution and alignment of the reticular lamina (Whitlon, 1993), which is the barrier separating the endo- and perilymph. Cochlear functions depend on its integrity. Expression of N-cadherin is seen in IHCs during development, but never in OHCs. Hensen cells show high levels of E-cadherin expression when differentiating. The β-catenin, which is connected to cadherin, is found in the cell membrane in all epithelial cells, especially in the modiolar extremity of Kolliker's organ, but not in fibroblasts. The polysialylated neuronal cell adhesion molecule (PSA-NCAM) is present around the IHC and may be involved in plasticity of neuron synapses (Simonneau et al., 2003).

The coxsackievirus and adenovirus receptor (CAR) is another type of adhesion proteins and is expressed at high levels in cell junctions of most cochlear cells in newborn mice, but only in supporting cells and stria vascularis cells in adult mice (Excoffon et al., 2006). It is therefore possible that this protein molecule is involved in early stage sensory epithelial differentiation and maturation. Its expression in only the supporting cells and stria vascularis cells in a matured cochlea indicates that these two types of cells may retain potentials of proliferation and differentiation under certain conditions.

1.2. Adherens junction and neuron growth

In the mouse cochlea, the neuronal cell adhesion molecule (NCAM), polysialic acid (PSA), NCAM-L1, E-cadherin, syndecan-1 and tenascin-C are expressed at different levels in different regions and different types of cells, resulting in different attractions among different cells, which lead to variations in micro-environments in different areas in the cochlea that modify the growth of afferent and efferent neurons (Whitlon et al., 1999). In mammal cochleae, a neuronal cell adhesion molecule in the immunoglobulin superfamily, the L1, is capable of regulating the growth of type I spiral ganglion neurons and guides the extension of neuron dendrites toward IHCs and not OHCs (Brand et al., 2013).

1.3. Adherens junction and immune mediation

Glycosylation-dependent cell adhesion molecule-1 (GlyCAM-1) is found in the lateral wall, tectorial membrane, modiolus, organ of Corti and modiolar veins in the cochlea. It recognizes leukocyte L-selectin and participates in inflammatory responses in epithelial tissues (Kanoh et al., 1999). As a signal receptor, T-Cadherin may be involved in responses to hair cell injuries. Its mRNA expression is higher in the spiral ganglion (SG) and stria vascularis (SV) than in the organ of Corti (OG) (Listyo et al., 2011).

1.4. Adherens junction and planar cell alignment

Adhesion proteins can influence alignment of sensory epithelial cells. The p120-catenin can interact with cellular skeletons to form a molecular spring of structural mechanical significance (Garcia-Anoveros and Duggan, 2007). When the gene for p120-catenin is conditionally knocked out, the dynamic distribution of E-cadherin and N-cadherin will change and planar cell polarity (PCP) will be affected, seen as significant changes in cellular contact and geometry (Chacon-Heszele et al., 2012). Nectin-1 and Nectin-3 are also capable of influencing cochlear cells alignment. They are immunoglobulin-like adhesion molecules expressed in hair cells and supporting cells in mice. Their interaction mediates attachment between these two types of cells, leading to alignment of hair cells and supporting cells in a checkerboard pattern (Togashi et al., 2011).

Also, the Wnt/β-catenin signal pathway regulates the proliferation and differentiation of cochlear IHCs (Jacques et al., 2012). During early stages of cochlear development, inhibitory

Wnt/β-catenin signals can suppress hair cell differentiation to form the organ of Corti (Shi et al., 2014), while excitatory signals promote hair cells differentiation and increase the number of precursor sensory cells.

1.5. Adherens junction and susceptibility to noise damage

The edh23 gene is an important member in the cadherin gene family. The protein coded by edh23 is closely related to the structure and alignment of hair cell sterocilia and ciliary bundles and participates in the formation of tip links, which are involved in OHC active mechanisms. Up-regulation of edh23 can lead to breast cancer, while its absence can result in early onset presbycusis and susceptibility to noise-induced hearing damage. C57BL/6J mice with edh23 mutations show age-related degenerative disorders at a very young age when the cochlea has barely matured, and experience inner ear hair cell sterocilia damage when exposed to loud noise with hearing loss more severe than normal mice (Kane et al., 2012; Zheng et al., 2009).

2. Functions of gap junction proteins in cochlea

Gap junction proteins are specific connexins in the membrane of neighboring cells, responsible for exchanges of electric and chemical signals between cells (Alberts et al., 2008). In the cochlea, gap junctions make signal transmission and small molecules transportation among cells possible. Mutations of the gap junction protein family is an important cause of hereditary deafness (Zhang et al., 2013; Hou et al., 2012; Dai et al., 2014; Wang et al., 2013).

There are two independent groups of gap junctions in mammal cochleae, i.e. epithelial gap junctions and connective tissue gap junctions. The former are seen in non-sensory epithelial cells, inner sulcus cells, supporting cells in the organ of Corti and outer sulcus cells, and the latter in basal and intermediate cells of the SV, fibrocyte types of the spiral ligament and mesenchymal cells (Kikuchi et al., 1995). Gap junction proteins are composed mainly of the connexin family. Functional gap junction channels of connexins are a necessary condition for cochlear functions. Common connexins include connexin26, connexin30, connexin31, connexin43, etc (Kikuchi et al., 2000a). Connexin26 and connexin30 are widely expressed in human cochlea. There are channels comprising heteromeric Cx26/Cx30 connexons in the lateral wall, and both homomeric/homotypic pattern and hybrid pattern (heteromeric or heterotypic) are seen in the areas of Claudius cells and Deiter cells in the OC. Connexin26, connexin30 and connexin36 are co-expressed in type I SG neurons, of which connexin36 is unique to gap junctions among neurons (Liu et al., 2009).

Pannexins are another type of newly found junction proteins, of which Panx1 and Panx2 are expressed in the cochlea. Panx1 is seen in inner and outer sulcus cells and in Claudius cells, while Panx1 and Panx2 are co-expressed in SG and Scarpa neurons (Tang et al., 2008). Additionally, there are other small molecule transportation proteins.

2.1. Gap junction and signal transmission and small molecules transportation

Connexin26 is seen in gap junctions of all cells in the cochlea. Its function is to participate in the circulation of potassium ions from hair cells to marginal cells of the SV. Disruption of this circulation leads to hearing loss (Kikuchi et al., 2000b). Functions of connexin26 may be replaced by connexin32. In mice, expression of connexin32 can compensate for the hearing loss caused of missing connexin26 (Degen et al., 2011). Another gap junction protein, glucose transporter 1 (GLUT1) is capable of transporting glucose (Suzuki et al., 2009). Additionally, vesicular glutamate transporter (VGLUT) plays an important role in transportation of vesicles in synapses between IHCs and SG neurons. In VGLUT3 knockout mice, hearing loss occurs as a result of failure of glutamate transportation with progressive loss of neurons in the SG (Peng et al., 2013).

2.2. Gap junction and hereditary deafness

A major portion (>50%) of non-syndromic hereditary deafness is caused by connexin26 gene (GJB2) mutations. Products of this gene participate in the maintenance of cellular homeostasis and rapid electric current communications among cells, which are vital to normal operations of the cochlea. Loss of connexin26 results in congenital hearing loss and cochlear development failure, as well as dysplasia of hair cells. In mice with conditional connexin26 knockout (cCx26ko), transmission electron microscopy shows little change in cochlear ultrastructure at early stage, but cell death with only residual Hensen cells on P10 and microglia-like cells on P180. Missing connexin26 also leads to reduced endocochlear potential (EP) and decreased signal amplification by OHC microphonics. In another study with connexin26 knockout mice, it was found that when the knockout took place before P5, congenital hearing loss ensued with typical cochlear development failure including lack of formation of Corti's tunnel, but cochlear development was not affected with normal Corti's tunnel if knockout was carried out after P5, suggesting that timing of connexin26 knockout may be critical to cochlear development (Chen et al., 2014; Xu and Nicholson, 2013; Anniko and Bagger-Sjoback, 1982).

3. Tight junction proteins

Tight junctions are located on the lateral side of epithelial cells, surrounding the lateral cellular wall to seal spaces between cells and prevent free movement of substances across the cellular wall. This forms the structural basis of selective cellular permeability (Alberts et al., 2008). Tight junctions are important in maintaining ion concentration gradient between the endo- and perilymph. Mutations of tight junction proteins are also a cause of hereditary deafness.

3.1. Tight junction and maintenance of endo- and perilymphion concentrations

Before differentiation of inner ear secretory epithelial cells, tight junctions form a loose network of 0—4 tight chains. Following differentiation of inner ear secretory epithelial cells, the number of tight chains increases and mature tight junctions are formed within a few days after birth. With intact tight junctions, high potassium concentrations develop in the endolymph (Kitajiri et al., 2004a), which is the main drive for electric current within the auditory sensor. An EP inside the endolymphatic space of 80—120 mV is critical for maintaining normal hearing.

Variable combinations of tight junctions are necessary for formation of different kinds of lymph. Immunofluorescent microscopy of the inner ear shows that claudin-1, 2, 3, 9, 10, 12, 14 and 18 are expressed in the OC, while claudin-1, 2, 3, 8, 9, 10, 12, 14 and 18 are expressed in marginal cells of the SV but only claudin-11 is expressed in SV basal cells, indicating complex claudin expression patterns in the inner ear with variable barrier functions across different areas (Jeon et al., 2011).

Tight junctions in the SV epithelium are closely linked to the generation of endolymph and EP. In mice missing claudin-11, no tight junctions are found in the basal area of the SV and EP is down to 30 mV despite near normal concentrations of potassium in the endolymph. This suggests that claudin-11 in basal cells of the SV may be involved in the generation and maintenance of EP (Kitajiri et al., 2004b).

3.2. Tight junction and hereditary deafness

Studies have shown that ethylnitrosourea (ENU) can induce phenotype of recessive inherited deafness in mice with mutations (nmf329) with massive loss of sensory cells after P14. Forced cloning reveals that this is caused by mutation of the gene coding the tight junction protein claudin-9. In nmf329 mice, elevated potassium concentrations in perilymph indicates disrupted tight junctions in the cochlea, i.e. mutation of claudin-9 leads to failure of tight junctions at the lateral base of hair cells and loss of ion barrier functions (Nakano et al., 2009). Mutation of another tight junction protein, tricellulin, can also lead to non-syndromic deafness. This is a membrane protein forming tight junctions among three cells. Its mutation can cause increase of potassium on the surface near the lateral base of hair cells or increase of ATP near ciliary bundles, and subsequently dysfunction and dysplasia of these cells (Higashi et al., 2013).

4. Summary and future

Cell junctions are specialized structures for contacts among cells in predetermined fashions in all tissues. The orderly and indispensable cell junctions seen in the cochlea are determined by the diversity, complexity and functional precision of various types of cells in this highly specialized auditory sensory organ. Loss of any type of cell junctions can lead to compromised structural and/or functional integrity of the cochlea. In recent years, cell junctions and attachment have found to be important targets of acoustic injuries. Sound stimulation can induce complex responses at the site of junctions or attachment that involve numerous signal transmission pathways of relevant adhesion proteins (Cai et al., 2012). All these findings point to participation in and regulation of cochlear responses to noise by cell junction proteins, which await further research in the future.

Conflict of interest

No conflict of interest declared.

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References

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., Walter, P., 2008. Molecular Biology of the Cell. Garland Science, New York, p. 1.

Anniko, M., Bagger-Sjoback, D., 1982. Maturation of junctional complexes during embryonic and early postnatal development of inner ear secretory epithelia. Am. J. Otolaryngol. 3 (4), 242—253.

Brand, Y., Sung, M., Chavez, E., Wei, E., Pak, K.K., Housley, G.D., et al., 2013. Neural cell adhesion molecule L1 modulates type I but not type II inner ear spiral ganglion neurite outgrowth in an in vitro alternate choice assay. J. Mol. Neurosci. MN 51 (3), 663—670.

Cai, Q., Patel, M., Coling, D., Hu, B.H., 2012. Transcriptional changes in adhesion-related genes are site-specific during noise-induced cochlear pathogenesis. Neurobiol. Dis. 45 (2), 723—732.

Chacon-Heszele, M.F., Ren, D., Reynolds, A.B., Chi, F., Chen, P., 2012. Regulation of cochlear convergent extension by the vertebrate planar cell polarity pathway is dependent on p120-catenin. Development (Cambridge, England) 139 (5), 968—978.

Chen, J., Chen, J., Zhu, Y., Liang, C., Zhao, H.B., 2014. Deafness induced by Connexin 26 (GJB2) deficiency is not determined by endocochlear potential (EP) reduction but is associated with cochlear developmental disorders. Biochem. Biophys. Res. Commun. 448 (1), 28—32.

Dai, Z., Sun, B., Huang, S., et al., 2014. Audiology phenotype of GJB2 and its relations to GJB2 genotypes. Chin. J. Otol. 12 (1), 34—36.

Degen, J., Schutz, M., Dicke, N., Strenzke, N., Jokwitz, M., Moser, T., et al., 2011. Connexin32 can restore hearing in connexin26 deficient mice. Eur. J. Cell Biol. 90 (10), 817—824.

Edelman, G.M., 1986. Cell adhesion molecules in the regulation of animal form and tissue pattern. Annu. Rev. Cell Biol. 2 (1), 81—116.

Excoffon, K.J., Avenarius, M.R., Hansen, M.R., Kimberling, W.J., Najmabadi, H., Smith, R.J., et al., 2006. The coxsackievirus and adenovirus receptor: a new adhesion protein in cochlear development. Hear. Res. 215 (1—2), 1—9.

Garcia-Anoveros, J., Duggan, A., 2007. TRPA1 in Auditory and Nociceptive Organs.

Higashi, T., Lenz, D.R., Furuse, M., Avraham, K.B., 2013. A "Tric" to tighten cell-cell junctions in the cochlea for hearing. J. Clin. Investig. 123 (9), 3712—3715.

Hou, B., Guo, W., Yang, S., et al., 2012. Development of cochlear stria vascularis and deafness related to its abnormalities. Chin. J. Otol. 10 (4), 528—532.

Jacques, B.E., Puligilla, C., Weichert, R.M., Ferrer-Vaquer, A., Hadjantonakis, A.K., Kelley, M.W., et al., 2012. A dual function for canonical Wnt/beta-catenin signaling in the developing mammalian cochlea. Development (Cambridge, England) 139 (23), 4395−4404.

Jeon, J.H., Park, J.W., Lee, J.W., Jeong, S.W., Yeo, S.W., Kim, I.B., 2011. Expression and immunohistochemical localization of TMEM16A/anoctamin 1, acalcium-activated chloride channel in the mouse cochlea. Cell Tissue Res. 345 (2), 223−230.

Kane, K.L., Longo-Guess, C.M., Gagnon, L.H., Ding, D., Salvi, R.J., Johnson, K.R., 2012. Genetic background effects on age-related hearing loss associated with Cdh23 variants in mice. Hear. Res. 283 (1−2), 80−88.

Kanoh, N., Dai, C.F., Tanaka, T., Izawa, D., Li, Y.F., Kawashima, H., et al., 1999. Constitutive expression of GlyCAM-1 core protein in the rat cochlea. Cell Adhes. Commun. 7 (3), 259−266.

Kikuchi, T., Kimura, R.S., Paul, D.L., Adams, J.C., 1995. Gap junctions in the rat cochlea: immunohistochemical and ultrastructural analysis. Anat. and Embryol. 191 (2), 101−118.

Kikuchi, T., Adams, J.C., Miyabe, Y., So, E., Kobayashi, T., 2000. Potassium ion recycling pathway via gap junction systems in the mammalian cochlea and its interruption in hereditary nonsyndromic deafness. Med. Electron Microsc. Off. J. Clin. Electron Microsc. Soc. Jpn. 33 (2), 51−56.

Kikuchi, T., Kimura, R.S., Paul, D.L., Takasaka, T., Adams, J.C., 2000. Gap junction systems in the mammalian cochlea. Brain Res. Brain Res. Rev. 32 (1), 163−166.

Kitajiri, S.I., Furuse, M., Morita, K., Saishin-Kiuchi, Y., Kido, H., Ito, J., et al., 2004. Expression patterns of claudins, tight junction adhesion molecules, in the inner ear. Hear. Res. 187 (1−2), 25−34.

Kitajiri, S., Miyamoto, T., Mineharu, A., Sonoda, N., Furuse, K., Hata, M., et al., 2004. Compartmentalization established by claudin-11-based tight junctions in stria vascularis is required for hearing through generation of endocochlear potential. J. Cell Sci. 117 (Pt 21), 5087−5096.

Leonova, E.V., Raphael, Y., 1997. Organization of cell junctions and cytoskeleton in the reticular lamina in normal and ototoxically damaged organ of Corti. Hear. Res. 113 (1), 14−28.

Listyo, A., Brand, Y., Setz, C., Radojevic, V., Resink, T., Levano, S., et al., 2011. T-cadherin in the mammalian cochlea. The Laryngoscope 121 (10), 2228−2233.

Liu, W., Bostrom, M., Kinnefors, A., Rask-Andersen, H., 2009. Unique expression of connexins in the human cochlea. Hear. Res. 250 (1−2), 55−62.

Müller, U., Littlewood-Evans, A., 2001. Mechanisms that regulate mechanosensory hair cell differentiation. Trends Cell Biol. 11 (8), 334−342.

Nakano, Y., Kim, S.H., Kim, H.M., Sanneman, J.D., Zhang, Y., Smith, R.J., et al., 2009. A claudin-9-based ion permeability barrier is essential for hearing. PLoS Genet. 5 (8), e1000610.

Peng, Z., Wang, Guo-Peng, Zeng, Rong, et al., 2013. Temporospatial expression and cellular localization of VGLUT3 in the rat cochlea. Brain Res. 1537, 100−110.

Raphael, Y., Altschuler, R.A., 2003. Structure and innervation of the cochlea. Brain Res. Bull. 60 (5), 397−422.

Schwander, M., Kachar, B., Müller, U., 2010. The cell biology of hearing. J. Cell Biol. 190 (1), 9−20.

Shi, F., Hu, L., Jacques, B.E., Mulvaney, J.F., Dabdoub, A., Edge, A.S., 2014. beta-Catenin is required for hair-cell differentiation in the cochlea. J. Neurosci. Off. J. Soc. Neurosci. 34 (19), 6470−6479.

Simonneau, L., Gallego, M., Pujol, R., 2003. Comparative expression patterns of T-, N-, E-cadherins, beta-catenin, and polysialic acid neural cell adhesion molecule in rat cochlea during development: implications for the nature of Kolliker's organ. J. Comp. Neurol. 459 (2), 113−126.

Slepecky, N.B., 1996. Structure of the mammalian cochlea. In: The Cochlea. Springer, pp. 44−129.

Suzuki, T., Matsunami, T., Hisa, Y., Takata, K., Takamatsu, T., Oyamada, M., 2009. Roles of gap junctions in glucose transport from glucose transporter 1-positive to -negative cells in the lateral wall of the rat cochlea. Histochem. Cell Biol. 131 (1), 89−102.

Tang, W., Ahmad, S., Shestopalov, V.I., Lin, X., 2008. Pannexins are new molecular candidates for assembling gap junctions in the cochlea. Neuroreport 19 (13), 1253−1257.

Togashi, H., Kominami, K., Waseda, M., Komura, H., Miyoshi, J., TakeichiM, et al., 2011. Nectins establish a checkerboard-like cellular pattern in the auditory epithelium. Science (New York, NY) 333 (6046), 1144−1147.

Tsuprun, V., Santi, P., 2002. Structure of outer hair cell stereocilia side and attachment links in the chinchilla cochlea. J. Histochem. Cytochem. 50 (4), 493−502.

Wang, H., Yu, F., Dai, P., et al., 2013. GJA1 mutations in non-syndromic deafness patients carrying simple GJB2 heterozygous mutations. Chin. J. Otol. 11 (1), 100−103.

Whitlon, D.S., 1993. E-cadherin in the mature and developing organ of Corti of the mouse. J. Neurocytol. 22 (12), 1030−1038.

Whitlon, D.S., Zhang, X., Pecelunas, K., Greiner, M.A., 1999. A temporospatial map of adhesive molecules in the organ of Corti of the mouse cochlea. J. Neurocytol. 28 (10−11), 955−968.

Xu, J., Nicholson, B.J., 2013. The role of connexins in ear and skin physiology - functional insights from disease-associated mutations. Biochim. Biophys. Acta 1828 (1), 167−178.

Yates, G.K., 1995. Cochlear structure and function. Hearing 41−74.

Zhang, P., Lin, X., Qu, Y., 2013. Connexin and deafness. Chin. J. Otol. 11 (1), 156−159.

Zheng, Q.Y., Ding, D., Yu, H., Salvi, R.J., Johnson, K.R., 2009. A locus on distalchromosome 10 (ahl4) affecting age-related hearing loss in A/Jmice. Neurobiol. Aging 30 (10), 1693−1705.