Molecular Characterization and Structural Assembly of Voltage-gated Sodium Channels in mouse Cochlea

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In this research, all subtypes of VGSCs were identified in cochlear basilar membrane. Sequence analysis of these cochlea-specific VGSCs indicated that some post-transcriptional events, e.g. alternative splicing, RNA-editing and scattered point mutations were frequently observed in the highly-organized assembly of the VGSCs motif. Functional characterization of these distinct mutants showed that single site mutation could lead to significant alterations in gating properties. This study provide the first-handed evidences VGSCs in cochlear basilar membrane could play an unique role in shaping spontaneous action potential in hearing system.

Key words: Cochlea; Basilar membrane; Voltage-gated sodium channel; Alternative splicing; RNA editing.

Introduction:

The role of the cochlea in mature mammalian auditory organ is to relay sound information into the central neural system, thereby form the basis for the sense of hearing. Among all the organelles in cochlea, hair cells are responsible for the transformation external sound stimuli into the electrical neural signals. According to the mode of actions and morphological characters, hair cells can be classified into inner hair cells (IHCs) and outer hair cells (OHCs). The IHCs are mainly targeted by auditory afferent nerve fibres and working for sending information to the brain, while the OHCs are mainly innervated by inhibitory afferent fibres, which influence the sensitivity and selectivity for different sound stimulus and also function as signal amplifiers in the cochlea. The precise regulation and transmission of sound signals in cochlea rely largely on the synergetic and coordinated manipulations by both IHCs and OHCs. Due to the difficulties in dissecting the OHCs from the basilar membrane, a supportive structure for both IHCs and OHCs in cochlea, functional characterizations for the hair cells could only be achieved through IHCs.

Before the onset of hearing at postnatal day 12 (P12), IHCs in rodent animals produce spontaneous spike and evoke action potentials. These Ca^{2+}-dependent spikes could trigger neurotransmitter release onto the auditory afferent fibers and thus driving the dynamic regulations in response to the sound stimulus. Unlike other excitable cells in neuronal cells, the action potentials in IHCs of premature cochlea are mainly contributed by an inward Ca^{2+} current and a Ca^{2+}-activated K^+ current (I_{KCa}), a slow activating and delayed rectifier potassium current which governs the resting phase of action potentials in IHCs (1). However, as the dominent molecule acting in action potentials in most of excitable cells, the voltage-gated sodium channels (VGSCs) were deemed to be playing a minor role in the sponontaneous spike and action potentials of IHCs (ref). The Na^+ currents, which are possibly generated by VGSCs, shorten the time to reach the action-potential threshold, whereas the Ca^{2+} currents are mainly involved, together with the K^+ currents, in determining the speed and size of the spikes (1). Unfortunately, little was known about the tissue- or developmental expression and the

subtype information of VGSCs in IHCs, which restricts the better understanding of their physiological role in the whole cochlea.

The VGSC is composed of an ion-conducting α-subunit with one or more auxiliary β subunits [7]. Generally, the α-subunit comprises four repeated domains (DI–DIV) cross linked by two cytoplasmic loops, each containing six transmembrane α-helixes (S1–S6) and a hairpin-like pore loop lining between S5 and S6. The highly conserved structure of VGSC protein indicates unique properties critical for its dynamic neuronal activities. For exemple, the S4 segment within each transmembrane domain are voltage-sensors associated with voltage-dependent activation and inactivation, the four extracellular loops between S5-S6 form the ion-pemeable selective filters. Specifically, the IFM motif locating at DIII-IV intracellular loop has been investigated to be hinged lid for fast inactivation.

Since the groundbreaking finding of VGSC gene by Noda and Noma in jellyfish electrogenic organs, at least nine VGSC subtypes from various tissues of mammals have been identified and functionally characterized, including $Na_v1.1$-1.3 (brain), $Na_v1.4$ (skeletal muscle), $Na_v1.5$ (heart), $Na_v1.6$ (CNS, PNS) and $Na_v1.7$-1.9 (PNS), each of which exhibit distinct tissue-specific distribution and gating properties caused by subtle differences in amino acicd sequence. Recent researches have been found that I_{Na} contributing to the action potentials in cochlea is likely to be carried by the TTX-sensitive channel subunits $Na_v1.1$ and $Na_V1.6$ in both apical and basal IHCs (plos one, dev and func). Eventhough, with regard to the divergent characteristics of Na^+ currents in premature hair cells compared to those expressed in other tissues, direct evidence from molecular level, such as whether there may exist novel VGSC subtypes in IHCs, and if the general structures could be comparable to the known VGSCs, what about their distinct assembly pattern of each mechanical parts mentioned above have yet to be worked out.

The structure of VGSCs may undergo many subtle but substantial modifications in

adapt to tissue or spatial-temporal specific informatic transmissions in neuronal system. Such structural modifications in conservative regions in VGSCs are thought to induce significant shifts in voltage-dependent properties and channel activities, confering genetically or physiologically distinct functions to the VGSCs. Indeed, significant modifications of functional properties of VGSCs by alternative splicing or RNA-editing have been demonstrated. A recent study reported that alternative splicing of $Na_v1.7$ exon 5 affects ramp current properties, which might impact the functional consequences of inherited erythromelalgia (IEM) or paroxysmal extreme pain disorder (PEPD). The apparent sensitivity of the infant brain to the alternative splicing state of the $Na_v1.2$ adult form (L1563V) may mediate a transcriptionally mediated pathological mechanism that can increase seizure susceptibility in human infants. In addition to alternative splicing, RNA editing is another post-translational modification pattern that could increase sodium channel functional plasticity. Several tssue-specific RNA-editing variants from cockroach have been shown to induce a broad range of changes on voltage-dependent properties. However, none of RNA-editing event has been detected in mammalian VGSCs yet.

To explore the molecular basis for the VGSCs in hearing system, we chose the study the structural features of VGSCs existing in cochlear HCs of premature mouse., Several questions might be well addressed: 1. By fishing the conserved structures of all the known VGSCs identified in other tissues, whether novel VGSCs subtype that distinct to other VGSCs could be identified. 2. Some structural variations due to the post-translational modification in cochear VGSCs might exist, what's the underlying structure-function relationship in associating with cochlea-specific charactics? 3. Through a global examination on expression level of all the known VGSCs, which VGSCs subtype play dominant role among others that contributing to the action potentials in HCs.

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