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Review

Wnt signaling during cochlear development

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ABSTRACT

Wnt signaling is a hallmark of all embryonic development with multiple roles at multiple developmental time points. Wnt signaling is also important in the development of several organs, one of which is the inner ear, where it participates in otic specification, the formation of vestibular structures, and the development of the cochlea. In particular, we focus on Wnt signaling in the auditory organ, the cochlea. Attempting to dissect the multiple Wnt signaling pathways in the mammalian cochlea is a challenging task due to limited expression data, particularly at proliferating stages. To offer predictions about Wnt activity, we compare cochlear development with that of other biological systems such as *Xenopus* retina, brain, cancer cells and osteoblasts. Wnts are likely to regulate development through crosstalk with other signaling pathways, particularly Notch and FGF, leading to changes in the expression of Sox2 and proneural (pro-hair cell) genes. In this review we have consolidated the known signaling pathways in the cochlea with known developmental roles of Wnts from other systems to generate a potential timeline of cochlear development.

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1. Introduction

1.1. Inner ear development

The vertebrate inner ear is a highly complex labyrinth generated through an intimate association of epithelial tissues with the surrounding mesenchyme. The mammalian vestibular system of the inner ear contains five organs responsible for sensing changes in

Abbreviations: bHLH, basic helix-loop-helix; FGF, fibroblast growth factors; PCP, planar cell polarity; OEP, otic-epibranchial placode; Fzd, Frizzled receptor; E, embryonic day.

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angular acceleration or gravity, while the auditory system consists of the coiled cochlea that houses the organ of Corti for detecting sound. Both the vestibular and auditory parts originate from the otic placode, a small patch of surface head ectoderm that acquires otic fate under the influence of surrounding signals during the neurulation stage of embryogenesis. The otic placode invaginates and fuses to form a fluid-filled otic vesicle. Neuroblasts delaminate from the vesicle to take up residence adjacent to the otic ectoderm, eventually becoming the vestibular and cochlear (spiral) cranial ganglia. Meanwhile, the simple epithelium of the otic vesicle is sculpted into a set of chambers and ducts through directed outgrowths, epithelial fusions, and focal hotspots of programmed cell death. The adjacent mesenchymal tissue elaborates an additional fluid compartment surrounding the otic epithelium. These chambers and the associated ganglia are encapsulated and protected by a cartilaginous cover, the otic capsule, which eventually ossifies. This complex morphology optimally stimulates the opening of mechanically gated ion channels located on the sterecociliary bundles protruding from the apical surface of hair cells localized on discrete sensory organs.

Thus far, there are well-established links between members of the Wnt signaling pathways and otic induction, dorsal-ventral axial specification of the otocyst, planar orientation of the stereocilia, and chondrogenesis of the otic capsule. More recently, evidence is emerging for additional roles for Wnts in the establishment and patterning of the cochlear sensory epithelium, and in the proliferative capacity of otic stem cells. This review will focus primarily on Wnt signaling during development of the mammalian cochlea and will draw parallels between the signaling pathways used by this organ and by the retina. The reader is invited to consult several recent reviews that offer additional coverage of Wnt functions in the developing inner ear [1,2].

1.2. Wnt signaling pathways

Wnts are secreted glycoproteins that are important for a wide array of processes throughout embryonic development, including cell fate specification, proliferation, progenitor maintenance, various aspects of planar cell polarity (PCP), and axon guidance. Wnt signaling can be categorized into canonical and non-canonical signaling pathways, although there is increasing evidence for numerous potential extracellular and intracellular intersections of these pathways (Fig. 1) [3]. The canonical Wnt pathway acts through Fzd receptors and Disheveled, culminating in the transcriptional activation of genes regulated by TCF/LEF transcription factors, with β-catenin serving as a major second messenger. Non-canonical Wnt signaling pathways are known to influence cell–cell rearrangements (that occur during convergent–extension movements) or cytoskeletal reorganizations (such as those that take place during stereociliary bundle formation and axon outgrowth). These pathways either use calcium as a second messenger or modulate Jnk kinase [4]. Wnts can also signal through the Ryk receptor tyrosine kinase to repel axons or through Fzd receptors to attract axons in the central nervous system [5].

1.3. Wnt signaling during otic induction and axial polarity

During early embryonic development, Wnts are known to be important for establishing the anteroposterior body axis. Wnt inhibition anteriorizes while Wnt activation posteriorizes the embryo [6]. At the anterior (head) end of the embryo, the dorsal ectoderm acquires an anterior neural fate (the future brain), which then sends signals to the adjacent ectoderm to become a continuous field of pre-placodal cells. Various signaling molecules emanate from the germ layers of the head to influence the segregation of pre-placodal ectoderm into its component parts- olfactory, lens, trigeminal, otic and epibranchial placodes. For example, in the development of the inner ear, the initial formation of the otic-epibranchial placode (OEP) relies on fibroblast growth factors (FGFs) secreted from the endoderm, mesoderm and/or neural ectoderm to specify Pax2-positive ectoderm [7–9]. Next, high Wnt signaling to the OEP from adjacent hindbrain induces otic fate medially, whereas low Wnt signaling coupled with high FGF signaling restricts epibranchial fate to the lateral OEP territory [2,10–12]. Thus, while Wnt inhibition was initially required to form the anterior half of the embryo, Wnt activation later becomes important to direct development of the pre-placodal field.

As the otic placode invaginates to form an otic vesicle, Wnt signaling plays a prominent role in establishing the dorsoventral axis. Wnt signaling from the dorsal hindbrain regulates the expression of Wnt target genes such as *Dlx5/6, Hmx2/3* and *Gbx2* in the dorsal portion of the otocyst [13–16]. These genes are preeminent in the formation of the endolymphatic duct and semicircular canals in the vestibular system. On the other hand, Shh signaling from the notochord or floor plate (in chick) becomes important in specifying the ventral structures of the otocyst such as the saccule and the cochlea [17,18].

2. Sensory organ development

2.1. The emergence of cell types in the mouse cochlea

While there is overwhelming evidence that Wnts play a dominant role in the formation of the dorsal structures of the inner ear, their importance in the development of the cochlea and the organ of Corti remains an active area of investigation. We will extend our discussion beyond experimental evidence to speculate how Wnt signaling may intersect with other known signaling pathways to regulate cochlear cell fate and patterning, taking clues from other model systems. In the mouse otocyst at embryonic day (E) 10.5–11, cochlear duct formation initiates as an evagination of the ventral portion of the otic vesicle. As the cochlear duct elongates, the cells at the apical end are the first to exit the cell cycle, on E12.5, but are the last to differentiate. The differentiation of sensory cells initiates at the mid-base on about E14.5 and progresses outward toward both the extreme base and the apex over a period of 1–3 days [19,20]. The "neural" cells of the organ of Corti are the mechanosensory hair cells that relay electrical signals to the spiral ganglion. The "non-neural" cells of the organ of Corti, the supporting cells, provide structural and trophic support for hair cells and thus, are important for long-term hair cell survival.

Cochlear development involves cell state transitions from progenitor to prosensory to proneural to a fully differentiated state (Fig. 2). The transcription factor, Sox2, marks precursor cells in all states up to, but excluding, their specification into hair cells. Sox2 is a member of the SoxB1 HMG box family of transcription factors and is frequently referred to as a stem cell marker. In the organ of Corti, only cells that assume the alternative fate of a supporting cell will retain Sox2 expression as they differentiate. The association of Sox2 with the progenitor state, and a requirement for its down-regulation to initiate neuronal differentiation, is well known throughout the nervous system. Here we wish to draw attention to a parallel between the progressive development of mammalian cochlear cells and those of the *Xenopus* retina. The retinal progenitors go through a comparable sequence of state changes, terminating in both Sox2-negative (neuronal) and Sox2-positive (glial) fates. The Sox2-positive cells in the retina become Müller glia, which, like the supporting cells of the organ of Corti, serve a supportive function for retinal neurons (including the photoreceptors). Temporal changes in the responsiveness of retinal cells to perturbations in