Focus Article

Hearing crosstalk: the molecular conversation orchestrating inner ear dorsoventral patterning

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The inner ear is a structurally and functionally complex organ that functions in balance and hearing. It originates during neurulation as a localized thickened region of rostral ectoderm termed the otic placode, which lies adjacent to the developing caudal hindbrain. Shortly after the otic placode forms, it invaginates to delineate the otic cup, which quickly pinches off of the surface ectoderm to form a hollow spherical vesicle called the otocyst; the latter gives rise dorsally to inner ear vestibular components and ventrally to its auditory component. Morphogenesis of the otocyst is regulated by secreted proteins, such as WNTs, BMPs, and SHH, which determine its dorsoventral polarity to define vestibular and cochlear structures and sensory and nonsensory cell fates. In this review, we focus on the crosstalk that occurs among three families of secreted molecules to progressively polarize and pattern the developing otocyst.

How to cite this article:
WIREs Dev Biol 2018, 7:e302. doi: 10.1002/wdev.302

INTRODUCTION

The inner ear is derived from simple ectodermal rudiments that form early in development. In birds and mammals, including humans, the ectoderm becomes specialized during late gastrulation, subdividing into neuroectoderm, which will roll up during neurulation to form the neural tube, the rudiment of the central nervous system; the surface ectoderm, which will form the skin covering the embryo; and a transition zone located between the other two subdivisions called the pre-placodal region, the source of the placodes contributing to the sensory organs and the cranial nerves.[1] In the future otic region, the pre-placodal region adjacent to the newly formed caudal hindbrain thickens to form the otic placode.[2,3] Subsequently, the placode invaginates to form an otic cup, which quickly separates from the adjacent ectoderm to form a closed, single-cell thick, spherical vesicle, the otocyst.[3]

The structurally simple, epithelial otocyst progressively transforms into the complex mature inner ear through a fascinating series of morphogenetic events that ultimately result in the formation of dorsal vestibular and ventral auditory components,[4,5] which contain the sensory organs for balance and hearing, respectively. The vestibular component is presaged by the rapid expansion of the dorsolateral wall of the otocyst to form the primordial canal pouch,[6] the rudiment of the semicircular canals. The pouch soon gives rise to vertical and horizontal outgrowths, with the vertical outgrowth (vertical canal pouch) forming the anterior and posterior semicircular canals, and the horizontal outgrowth (horizontal canal pouch) forming the lateral semicircular canal. To form individual semicircular canals, the two walls of the pouch fuse across the lumen of the pouch in localized regions (two in the vertical canal pouch and one in the horizontal canal pouch), establishing

Additional Supporting Information may be found in the online version of this article.

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Conflict of interest: The authors have declared no conflicts of interest for this article.

fusion plates in which cells are removed through cell death or rearrangement, depending on the species,[7] with the remaining portions of the pouches forming the arch-like loops of the semicircular canals. The utricle and saccule form in concert with the semicircular canals as two localized outpocketings from the 'waist' of the otocyst.

The auditory component of the inner ear consists of a single structure, the cochlea. It originates as an evagination of the ventromedial wall of the otocyst, which rapidly elongates and forms the cochlear duct. The organ of Corti subsequently differentiates within the cochlear duct in a basal-to-apical sequence.

The morphogenetic events that form such diverse dorsal and ventral otocyst structures are regulated by signaling proteins secreted by neighboring embryonic rudiments, such as the neural tube and notochord.[6,8–13] Presumably, as these signals diffuse in the extracellular matrix from their tissue sources of origin, they form concentration gradients that establish the polarity and subsequent patterning of the otocyst, differentially regulating cell behaviors that drive region-specific morphogenesis, and inducing specific cell fates. These regulatory secreted proteins comprise multiple families of growth factors, and it has become clear in recent years that these factors do not regulate morphogenesis independently. Rather, extensive molecular crosstalk occurs among them.[14] Three families of growth factors are known to regulate early dorsoventral (DV) patterning of the otocyst (Figure 1). Two of these are secreted from tissues largely dorsal to the otocyst (WNTs and BMPs) and one is secreted from tissues ventral to the otocyst (SHH). Below, we discuss the molecular crosstalk that occurs among these three families and how it results in the formation of the vestibular and auditory components of the inner ear. However, it should be emphasized here that these factors are unlikely to be the only ones that regulate DV patterning of the otocyst. For example, FGFs play essential roles in otocyst development, including induction of the placodes and formation of the semicircular canals.[15,16] Therefore, further studies are required to define the precise roles of such factors in DV patterning of the otocyst and how they are coordinated with WNT, BMP, and SHH signaling. Finally, although these factors secreted by tissues surrounding the otocyst play roles in its early DV patterning, some of these are expressed within the otocyst at later stages and likely play more specific roles in organ formation and differentiation, discussion of which is beyond the scope of this review.

Crosstalk among secreted factors could upregulate or downregulate specific signaling pathways and could do so either extracellularly or intracellularly. During patterning of the otocyst, examples of both enhanced and suppressed signaling have been identified and will be discussed below. Although extracellular crosstalk among secreted factors cannot be completely ruled out at this time (e.g., the induction and secretion into the extracellular space of agonists or antagonists of one pathway by another pathway), clear evidence has emerged that intracellular crosstalk occurs among at least a subset of these factors that regulate DV patterning of the otocyst.

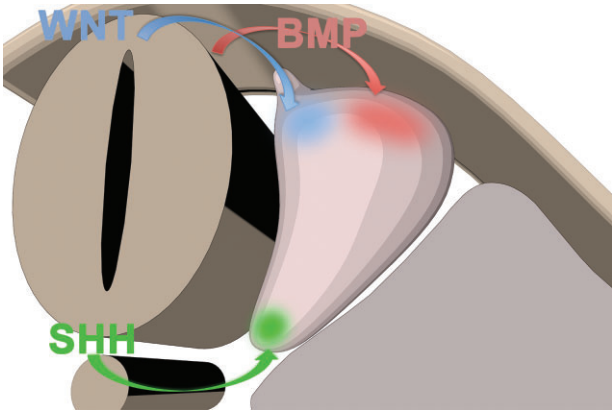

FIGURE 1 | Diagram of the secreted growth factors that pattern the otocyst. Two factors are known to act primarily on the dorsal otocyst during its early patterning. WNTs, secreted by the roof plate of the neural tube, act on the dorsomedial region of the otocyst, and BMPs, similarly secreted by the roof plate, but perhaps also by the cristae of the developing otocyst (not shown), act on the dorsolateral region of the otocyst. By contrast, SHH, secreted by the floor plate of neural tube and the notochord, acts primarily on the ventral otocyst. Crosstalk occurs among these three signaling molecules to orchestrate dorsoventral patterning of the otocyst.

A telltale sign that DV polarity has been established in the otocyst is the regional expression of transcription factors (Figure 2). *Dlx5*, *Gbx2*, and *Hmx3* are all expressed in the dorsal otocyst. By contrast, *Pax2* and *Otx2* are both expressed in the ventral otocyst. As discussed below, the regional expression of these genes is regulated both positively and negatively by the dorsally and ventrally expressed growth factors listed above.

Crosstalk between WNT and SHH Signaling

As just introduced, WNT protein is secreted by the dorsal neural tube and SHH protein is secreted by the ventral neural tube (floor plate) and underlying notochord (Figure 1). WNT signaling positively regulates the expression of two transcription factors in the dorsal otocyst in a dose-dependent manner,[11] *Dlx5* and *Gbx2*

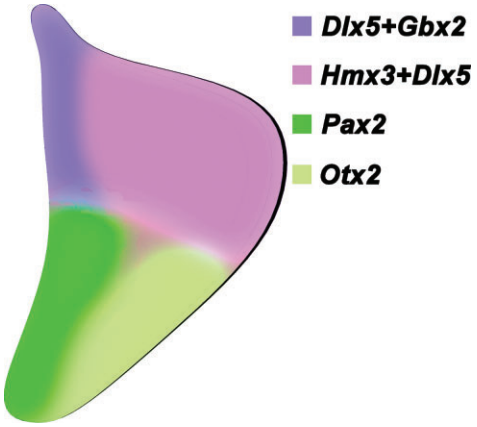

FIGURE 2 | Diagram of the regional expression patterns of transcription factors in dorsal and ventral regions of the early otocyst. *Dlx5* and *Gbx2* are expressed in the dorsomedial otocyst in response to WNT signaling. *Hmx3* is expressed in the dorsolateral otocyst in response to BMP signaling; its expression domain overlaps that of dorsolaterally expressed *Dlx5*, which requires both WNT and BMP signaling. *Pax2* is expressed in the ventromedial otocyst and *Otx2* is expressed in the ventrolateral otocyst, both in response to SHH signaling.

(Figure 2). Both are required for the formation of the semicircular canals.[17,18] However, dorsal polarity, as defined by the expression of these two genes, is actually regulated by the balance between WNT and SHH signaling rather than by just WNT signaling alone. The ventral otocyst receives a high level of SHH signaling due to its close proximity to the floor plate and notochord, the tissue sources of SHH protein. The expression of dorsal-otocyst genes is inhibited when *Shh* is overexpressed in the head mesenchyme adjacent to the otocyst, strongly suggesting that SHH signaling normally prevents their expression in the ventral otocyst during its patterning.[19]

The WNT signaling that regulates the dorsal patterning of the otocyst emanates from a highly restricted craniocaudal level of the neuraxis, namely, the level of the fifth and sixth rhombomeres of the hindbrain. WNT signaling also acts earlier in the development of the inner ear, working in conjunction with similarly restricted FGF signaling from the hindbrain and adjacent head mesoderm, to induce the otic placode.[20,21] In mice harboring a WNT canonical reporter (i.e., TOPGAL mice), WNT-responding cells are initially present in the medial rim of the otic placode, just adjacent to the hindbrain.[11] Subsequently, during invagination of the otic placode to form the otic cup, WNT-responding cells become localized to the dorsomedial part of otic epithelium. Thereafter, as the otic cup forms the otocyst, WNT-responding cells expand to occupy most of the dorsal half of the otocyst, remaining there as DV polarity is established. Intriguingly, the WNT-responding area of the otocyst overlaps with the expression domains of *Dlx5* and *Gbx2,* suggesting that the expression of these transcription factors is dependent on WNT canonical signaling. After administration of the WNT agonist LiCl to TOPGAL embryos, WNT-responding cells now occupy the entire otocyst, and *Dlx5* expression expands accordingly. *Gbx2* expression also expands. However, unlike the expansion of *Dlx5* expression, expansion of *Gbx2* expression is restricted to mainly the dorsomedial region of the otocyst, suggesting that the expression patterns of *Dlx5* and *Gbx2* are regulated in somewhat different ways.

Following the loss of WNT signaling in mutant mice, both *Gbx2* and *Dlx5* expression are diminished, whereas the expression patterns of two other dorsal otocyst genes, *Hmx3* and *Bmp4*, are unaltered, indicating that the dorsal region of the otocyst forms in these mutant embryos even though its patterns of gene expression are altered.[11] Thus, WNT signaling is largely responsible for the expression of *Gbx2* and *Dlx5* during dorsal polarization of the otocyst. In the *Gbx2* mutant mouse, *Dlx5* expression is lost in the medial region of otocyst, which corresponds to the normal domain of *Gbx2* expression, but *Dlx5* expression is maintained in the lateral otocyst,[18] suggesting that WNT signaling induces *Gbx2* expression in the medial region of otocyst, which in turn upregulates *Dlx5* expression.

As stated above, SHH signaling likely blocks dorsal development in the more ventral otocyst by suppressing dorsal gene expression in this region. *ShhP1* is a transgenic mouse line that ectopically expresses SHH in the dorsal otocyst. In this mutant, *Dlx5* expression is extinguished in the otocyst and WNT-responding cells are absent, suggesting that SHH signaling antagonizes WNT signaling to restrict the *Dlx5* expression domain to the dorsal otocyst.[11] Consistent with this, the *Shh* null mouse shows ventral expansion of *Dlx5* expression in the otocyst, accompanied by the ventral expansion of the WNT-responding area.[11] These findings suggest that *Dlx5* is a target of crosstalk between WNT and SHH signaling that localizes its expression to the dorsal otocyst. However, when *Shh* was overexpressed in dorsal head mesenchymal cells in chick, *Dlx5* expression was not inhibited.[19] These divergent results may reflect species differences in the regulation of *Dlx5* expression, or may be due to differences in how SHH was overexpressed in the two experiments (i.e., in the epithelium of the otocyst using a transgene or in the head mesenchyme by sonoporation). Further studies are required to resolve this issue.

As discussed above, *Gbx2* expression, like *Dlx5* expression, is upregulated by WNT signaling. This raises the question, is *Gbx2* expression suppressed by SHH signaling? The answer to this question is also controversial. Unlike *Dlx5* expression, *Gbx2* expression is downregulated in the *Shh* null mouse. However, the *Gbx2* expression domain largely overlaps the endolymphatic duct, which is lost in the *Shh* mutant.[10] Thus, it is unclear whether SHH signaling maintains *Gbx2* expression, or whether it is required for the formation or maintenance of the endolymphatic duct, which expresses *Gbx2* under the influence of some other factor. This issue needs to be addressed in future research. Interestingly, regarding the regulation of *Gbx2* and *Dlx5* expression, it seems that WNT and SHH signaling are not mediated through a common intracellular pathway, as the otocyst expression of *Gbx2* and *Dlx5* does not change in mice mutant for smoothened (Smo), an essential HH signaling transduction component.[22] This indicates that the antagonistic interaction between WNT and SHH signaling that is responsible for dorsal polarity is not a cell-intrinsic mechanism within the epithelium of the otocyst, but must arise from an interplay between these pathways outside of the otocyst in the extracellular compartment. How such crosstalk occurs between WNT and SHH signaling still remains as a major question to answer.

Another interesting question is, does WNT signaling downregulate SHH signaling during dorsal polarization of the otocyst? The answer to this question also is still unclear. Removal of the dorsal hindbrain, the source of WNT signaling, results in dorsal expansion of the expression of factors downstream of SHH signaling, such as *Gli1* and *Pax2*, which are normally localized to the ventral otocyst[11] and are thought to maintain their expression in a SHH-dependent manner.[10] This result suggests that WNT signaling is a negative regulator of SHH signaling. However, in *Wnt* null embryos, the expression patterns of *Pax2* and *Otx2* are unchanged (the expression of *Otx2* is also thought to be regulated by SHH signaling[11]), suggesting that another factor acts on the dorsal otocyst in cooperation with WNT signaling to antagonize SHH signaling during the formation of dorsal polarity. As discussed in the next section, BMP is likely to be that factor.

Crosstalk between BMP and SHH Signaling

BMP signaling was first implicated in dorsal polarization of the otocyst when it was discovered that BMPs are expressed in the dorsal neural tube (Figure 1), as well as in the otocyst itself (i.e., in the anterior and posterior cristae).[23,24] Recently, it was shown that BMP signaling induces at least two genes essential for dorsal polarity of the otocyst, *Dlx5* and *Hmx3*, and that it does so through distinct molecular pathways, the SMAD pathway and the PKA-GLI3 pathway, respectively.[25] Additionally, this study showed that BMP signaling negatively regulates the expression of *Otx2*,[25] a transcription factor expressed in the ventral otocyst (Figure 2) and upregulated by SHH signaling. This suggests that BMP signaling acts as a negative regulator of SHH signaling and that, conversely, SHH signaling downregulates *Hmx3* expression,[19] thereby acting as a negative regulator of BMP signaling. In other words, BMP and SHH signaling mutually inhibit each other. Thus, these two factors polarize the regions of the otocyst that will form the dorsal and ventral parts of inner ear in both a positive (by inducing gene expression) and negative (by suppressing gene expression) fashion.

BMP family members, particularly *Bmp4*, *5*, and *7*, are expressed in the third and fifth rhombomere levels of the hindbrain during the time of otic placode formation. As the otic placode begins to form the otic cup, both *Bmp4* and *5* are expressed in the posterior margin of the otic cup.[23,24] *Bmp7* expression occurs along almost the entire rim of the otic cup.[24] After the otocyst forms, *Bmp4* expression occurs as two foci, corresponding to the future anterior/lateral and posterior cristae. *Bmp5* expression overlaps *Bmp4* expression in the posterior crista. Unlike *Bmp4* and *5*, *Bmp7* is expressed more broadly, that is, in almost the entire dorsal otocyst.[23,24] Furthermore, phospho-SMAD, a downstream component of BMP signaling, is localized to the dorsolateral otocyst, and its expression persists until DV polarity is fixed. These data indicate that the dorsal otocyst receives BMP signaling. In fact, downregulating BMP signaling by implanting beads coated with Noggin next to the otocyst in chick embryos, or by conditionally knocking out *Bmp4* in mouse, results in partial loss of one or more of the semicircular canals, or even the entire vestibular organ, whereas more ventrally the inner ear, including the cochlear duct, develops normally.[13,26] Thus, BMP signaling is required for the formation of the vestibular organ. Presaging the structural abnormalities in the vestibular organ after attenuation or loss BMP signaling, the expression of two dorsal otocyst genes, *Dlx5* and *Hmx3*, are also dramatically reduced.[25] This suggests that BMP signaling is required for specifying otocyst dorsal polarity by regulating the expression of *Dlx5* and *Hmx3*. Indeed, overexpression of *Bmp4* in the head mesenchyme adjacent to the chick otocyst results in ectopic

expression of both *Dlx5* and *Hmx3* in the ventral otocyst.[25]

How does BMP signaling induce *Dlx5* and *Hmx3* expression in the dorsal otocyst? Recent studies reveal that the canonical BMP pathway, the SMAD pathway, induces *Dlx5* expression,[25] whereas the repressor form of GLI3 (GLI3R), the product formed when GLI3 full-length (GLI3FL) undergoes partial proteolysis, induces *Hmx3* expression in the dorsal otocyst.[25] That is, both canonical and noncanonical BMP signaling coordinate dorsalization of the otocyst, inducing the expression of *Dlx5* and *Hmx3*. Recent studies also showed that crosstalk between BMP and SHH signaling regulates the processing of GLI3 along the DV axis of developing otocyst (Figure 3).

cAMP dependent protein kinase A (PKA) is a key factor involved in this crosstalk. PKA is a kinase that phosphorylates GLI3FL and stimulates proteolytic processing of GLI3FL to GLI3R. In the presence of SHH, PKA is inactivated. Thus, phosphorylation of GLI3FL no longer occurs, or a low level of phosphorylation is maintained. Consistent with this, the ventral otocyst, the region of the otocyst expected to receive the highest level of SHH signaling, shows the lowest level of PKA activity along its DV axis. In contrast, the dorsal otocyst, which is distant from the source of SHH signaling, shows the highest level of PKA activity. Thus, PKA activity forms a DV gradient across otocyst, with low activity ventrally and high activity dorsally (Figure 3). Furthermore, high PKA activity in the dorsal otocyst results not only from being relatively distant from the SHH source, but also from receiving BMP signaling. Thus, overexpression of *Bmp4* increases PKA activity in the chick otocyst.[25]

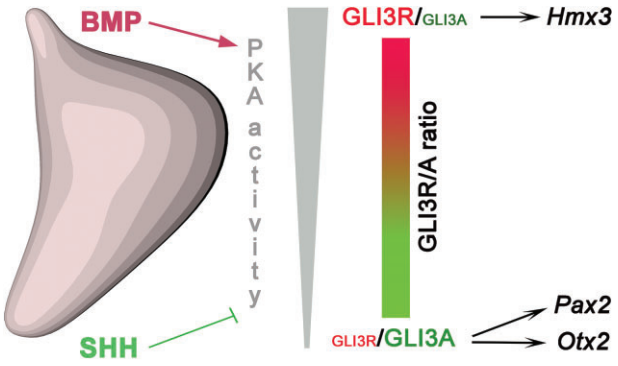

FIGURE 3 | Diagram showing how integrated BMP and SHH signaling contributes to dorsoventral (DV) patterning of the otocyst. Owing to their sites of expression in the embryo and their limited diffusion in the head mesenchyme, BMP ligands act mainly on the dorsal otocyst and SHH ligands act mainly on the ventral otocyst. BMP signaling activates PKA in the dorsal otocyst, while SHH inhibits it in the ventral otocyst, establishing a dorsal-high to ventral-low gradient of PKA activity. Activated PKA phosphorylates GLI3FL, partially processing it to form GLI3R; in contrast, in the absence of activated PKA, GLI3A is not proteolytically processed. Thus, a dorsal-high to ventral-low gradient in the ratio of GLI3R/GLI3A spans the DV axis of the otocyst. Dorsally, GLI3R activates transcription of the BMP target gene, *Hmx3*. Ventrally, GLI3A activates transcription of the SHH target genes, *Pax2* and *Otx2*.

The dorsal-high to ventral-low gradient of PKA activity in the otocyst resulting from crosstalk between BMP and SHH signaling is responsible for establishing a dorsal-high to ventral-low gradient in the otocyst of the ratio of GLI3R to GLI3FL (Figure 3). As described above, PKA controls the proteolytic processing of GLI3FL to GLI3R. Considering the importance of PKA in GLI3FL processing, high GLI3FL processing in the dorsal otocyst correlates with high PKA activity, whereas low GLI3FL processing in the ventral otocyst correlates with low PKA activity. In the *Gli3* null mutant, in which the level of GLI3 repressor would be expected to be low in the dorsal otocyst because GLI3 is absent and cannot be proteolytically processed to GLI3R, the semicircular canals are disrupted, whereas in the *Gli3Δ699* mutant, which expresses only the repressor form of GLI3 and is, therefore, expected to have low GLI activator in the ventral otocyst, the cochlear duct is truncated.[27] These results suggest that the ratio of GLI3R to GLI3FL plays an important role in establishing otocyst DV polarity. Moreover, western blotting of dorsal, middle, and ventral otocyst fragments showed that the ratio of GLI3R and GLI3FL is distributed in a dorsal-high to ventral-low gradient across the otocyst.[19] Furthermore, attenuation of PKA activity with PKIα, a naturally occurring inhibitor of PKA, significantly decreased the proteolysis of GLI3FL, suggesting that PKA activity is required to establish the dorsal-high to ventral-low gradient of the GLI3R/GLIFL ratio in the otocyst.[19] Gain of GLI3R function in the ventral otocyst induced ectopic *Hmx3* expression, whereas gain of GLI3A function (using GLI3FL, which presumably functions as the GLI3 activator, GLI3A) in the dorsal otocyst abolishes *Hmx3* expression, suggesting that the ratio of GLI3R/GLI3FL directly affects the level of *Hmx3* expression. PKA activity, adjusted to the proper level along the DV axis of the otocyst through crosstalk between BMP and SHH signaling, in turn results in a dorsal-high to ventral-low gradient in the ratio of GLI3R/GLI3FL, which determines the level of *Hmx3* expression along the DV axis of the otocyst.

As just discussed, the intracellular concentration of cAMP along the DV axis of the otocyst seems to be crucial in determining the level of PKA across

the otocyst, which in turn generates a dorsal-high to ventral-low gradient of GLI3R/GLI3FL. BMP signaling is required to induce PKA activity in the dorsal otocyst. However, the molecular pathway by which this occurs still remains to be established. Presumably, a currently unknown G-protein coupled receptor (GPCR) interacts with BMP signaling to increase cAMP production.[28,29] Parathyroid hormone (PTH)/PTH-related protein (PTHrP) pathway is one potential candidate, as components of this pathway are present in the rodent otocyst, and PTH receptor, which is coupled with a $G_{\alpha s}$ subunit, is able to activate adenylate cyclase.[30] Future studies are needed to define the molecular pathway by which BMP signaling regulates cAMP production through a GPCR. A recent study revealed a novel SHH signaling component, Gpr161, which is a GPCR that activates $G_{\alpha s}$ in the absence of SHH ligands. SHH signaling blocks its activity, in turn, reducing the level of cAMP.[31] But how Gpr161 regulates SHH signaling during DV patterning of the otocyst remains to be elucidated.

Two transcription factors are expressed early in the ventral otocyst, *Pax2* and *Otx2* (Figure 2), and the expression patterns of both of these genes are likely positively regulated by SHH signaling, and negatively regulated by BMP signaling. The expression domains of both *Pax2* and *Otx2* expand into the dorsal otocyst after overexpression of *Shh* in the head mesenchyme adjacent to the otocyst, whereas the expression of *Otx2* but not that of *Pax2* was reduced after overexpression of *Bmp4*.[19] These results suggest that the expression of *Otx2* (and perhaps that of *Pax2*) in the ventral otocyst is regulated by crosstalk between SHH and BMP signaling. Inhibition of the SHH pathway in the ventral otocyst dramatically reduced *Otx2* expression in transfected cells.[25] However, activation of the SHH pathway in the dorsal otocyst did not induce *Otx2* expression.[19] This finding indicates that *Otx2* expression likely occurs in the region where there is a low level of GLI3R, such as in the ventral otocyst, which receives high SHH signaling that reduces the proteolysis of GLI3FL.

Crosstalk between WNTs and BMPs

Crosstalk between WNT and BMP signaling in the dorsal otocyst is still poorly understood. *Dlx5* expression, which occurs throughout the entire dorsal half of the otocyst and is indispensable for forming the vestibular organ, requires both signaling pathways for its full expression. It is likely that canonical WNT signaling regulates *Dlx5* expression in the portion of the otocyst closest to the hindbrain (i.e., its dorsomedial portion), and canonical BMP signaling induces *Dlx5* expression in the more dorsolateral otocyst. Therefore, both WNT and BMP signaling are responsible for the establishing dorsal polarity, as evidenced by *Dlx5* expression (Figure 2).

Intriguingly, deletion of *Bmp4* in the otic epithelium resulted in the loss of *Dlx5* expression in the dorsolateral otocyst, but *Dlx5* expression still occurred in the endolymphatic duct.[13] This finding suggests that the main *Dlx5* expression domain in the otocyst, but not that in the endolymphatic duct, is regulated by BMP signaling. Consistent with this, *Noggin* overexpression in chick resulted in loss of *Dlx5* expression in the dorsolateral otocyst, whereas *Dlx5* in the endolymphatic duct still persisted.[25] In addition, inhibition of the BMP canonical pathway using the inhibitory SMAD, *Smad6*, diminished *Dlx5* expression in transfected cells of the dorsolateral otocyst, but *Dlx5* expression in transfected cells of the endolymphatic duct was not altered.[25] These findings

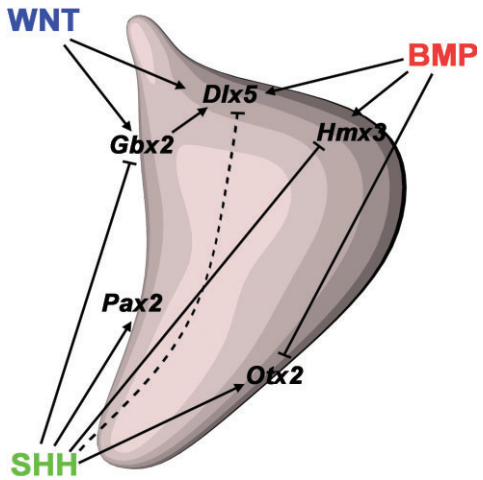

FIGURE 4 | Summary of the known otocyst transcription factors regulated by crosstalk among three families of secreted proteins during dorsoventral (DV) patterning. DV patterning of the otocyst is orchestrated through crosstalk among three families of secreted proteins that regulate the expression of transcription factors in a temporospatial manner. BMP signaling, which is required for the development of the dorsal (vestibular) component of the inner ear, upregulates the expression of two transcription factors, *Dlx5* and *Hmx3*, both of which are essential for vestibular development. WNT and BMP signaling partner to induce *Dlx5* expression in the dorsolateral wall of the otocyst, whereas WNT signaling acts alone in the dorsomedial wall to regulate the expression of *Gbx2*, which in turn seems to be required for *Dlx5* expression. SHH signaling, which is required for the development of the ventral (cochlear) component of the inner ear, upregulates the expression of two transcription factors, *Pax2* and *Otx2*, both of which are essential for cochlear development. The expression of transcription factors in the dorsal and ventral otocyst also is inhibited by secreted factors, with BMP signaling downregulating *Otx2* expression and SHH signaling downregulating *Hmx3* expression and arguably downregulating *Dlx5* expression.

strongly suggest that *Dlx5* expression in the dorsolateral otocyst is largely dependent on BMP canonical signaling, and that another signal regulates *Dlx5* expression in the endolymphatic duct. Moreover, in the *Gbx2* null mutant, *Dlx5* expression is absent in the dorsomedial otocyst, but is still present in the dorsolateral otocyst.[18] As described above (See '*Crosstalk between WNT and SHH signaling*'), *Gbx2* is normally expressed in the dorsomedial otocyst, that is, the region receiving high WNT signaling, and its expression seems to be dependent on WNT signaling.[11] Thus, it is likely that *Dlx5* expression in the dorsomedial otocyst is mediated through *Gbx2* expression induced by WNT signaling. Taken together, crosstalk between WNT and BMP signaling is required to induce the full range of gene expression throughout the dorsal otocyst.

CONCLUSIONS

In this review, we discussed the crosstalk that occurs among secreted signaling factors to polarize and pattern the otocyst and to direct its further development to form the vestibular and auditory components of the inner ear. Because multiple families of growth factors act coordinately to pattern the DV axis of the otocyst, crosstalk among the factors is vital for ensuring correct patterning along the axis. For BMP and SHH signaling, crosstalk occurs at the level of activation/inactivation of PKA, which in turn results in enhanced or diminished proteolytic processing of full-length GLI3 and a dorsal-high to ventral-low GLI3R/A ratio in the otocyst. For WNT and SHH or WNT and BMP signaling, crosstalk occurs at the regulation of expression of the genes that specify dorsal or ventral identity, rather than at the signaling level.

Figure 4 summarizes our discussion and shows the link between secreted growth factors and the regionally expressed transcription factors essentially for DV patterning of the otocyst. Signaling factors coordinate the patterning and morphogenesis of inner ear structures in a temporal and spatial manner. For example, WNT and BMP signaling cooperate to establish dorsal polarity of the otocyst, as evidenced by the expression patterns of *Gbx2*, *Dlx5*, and *Hmx3*. By contrast, SHH signaling is required to establish its ventral polarity, as evidenced by the expression patterns of *Pax2* and *Otx2*. Inhibitory interactions occur as well to patterning the otocyst dorsoventrally, with BMP signaling inhibiting *Otx2* expression and SHH signaling inhibiting *Gbx2* expression, and perhaps *Dlx5* expression.

Despite these recent advances in our understanding of DV patterning of the otocyst, many questions remain for future studies. In addition to fully elucidating the signaling pathways involved and the details of their molecular crosstalk, a major question remaining is, how is the expression of patterning genes, and in particular that of transcription factors, translated into coordinated cell behaviors that lead to differential growth and regional morphogenesis of the otocyst? Answering this question will close a loop and reveal in all its cellular and molecular complexity the secrets underlying how a simple epithelial vesicle transforms into the elaborate and beautiful structure that becomes the inner ear.

ACKNOWLEDGMENTS

The research reported herein from the Schoenwolf laboratory was supported by the National Institute on Deafness and Other Communication Disorders of the National Institutes of Health (R01 DC011819). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. We thank Drs. Suzanne L. Mansour and Doris K. Wu for insightful input.

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