

## SCO-ping Out the Mechanisms Underlying the Etiology of Hydrocephalus

Michael S. Huh,<sup>1,\*</sup>  
Matthew A. M. Todd,<sup>2,\*</sup>  
and David J. Picketts<sup>2</sup>

<sup>1</sup>Regenerative Medicine Program, Ottawa Health Research Institute, and <sup>2</sup>Department of Biochemistry, Microbiology, and Immunology, University of Ottawa, Ottawa, Ontario, Canada  
dpicketts@ohri.ca

\*M. S. Huh and M. A. M. Todd contributed equally to this manuscript.

The heterogeneous nature of congenital hydrocephalus has hampered our understanding of the molecular basis of this common clinical problem. However, disease gene identification and characterization of multiple transgenic mouse models has highlighted the importance of the subcommissural organ (SCO) and the ventricular ependymal (vel) cells. Here, we review how altered development and function of the SCO and vel cells contributes to hydrocephalus.

The cerebral spinal fluid (CSF) flow tract is a vital lifeline for supplying the brain with essential nutrients and growth factors throughout development and into adulthood. At the same time, the brain exercises constant control to ensure that the flow of CSF is homeostatic. CSF is secreted by the choroid plexus and moves rostrocaudally through the ventricles and into the subarachnoid space before being drained into the venous circulation (80). Failures within this cerebral irrigation network can lead to a buildup of CSF in the ventricular cavities of the brain, a condition known as hydrocephaly, which is fatal without surgical intervention. Hydrocephalus can result from an overproduction of CSF by the choroid plexus, failure to drain the CSF at the subarachnoid space, and the blockage of CSF flow through the narrow Sylvian aqueduct, which is situated between the third and fourth ventricles. Indeed, stenosis of the Sylvian aqueduct is considered the primary cause of congenital hydrocephalus, which is quite frequent, occurring with an incidence of 0.1–0.3% of live births (80). The causes for this disease are quite heterogeneous and have been linked to a number of genetic mutations, both autosomal and X-linked (112). What is intriguing, though, is that many of these mutations impinge on the development or function of the subcommissural organ (SCO) and the ventricular ependymal (vel) cells that collectively facilitate the flow of CSF through the confining canals of the ventricular system (Table 1).

The goal of this review is to summarize the molecular mechanisms that cause 1) the SCO to be absent or disorganized, 2) an inability of the SCO to properly secrete glycoproteins, 3) primary ciliary dyskinesia (PCD) of the ependymal cells, and 4) denudation of the neuroependyma. Although it should be noted that not all SCO/vel defects have been proven to precede the onset of hydrocephaly, indeed it is the aberrant execution of these diverse molecular pathways that can lead to stenosis of the aqueduct and contribute to communicating or noncommunicating hydrocephalus.

### Loss of Developmental Factors Impairs SCO Formation and Function

The SCO is a small secretory gland positioned in the dorsal caudal aspect of the third ventricle underneath the posterior commissure. The SCO is derived from the neuroepithelial cells that line the lumen of the dorsal-caudal aspect of the diencephalon. This anatomical region is also described as the prosomere 1 (P1), demarcated by the pineal gland and the mesencephalon. The primitive epithelial progenitors of the presumptive SCO are driven toward a specialized secretory ependymal cell fate in response to the inductive Wnt and Bmp signals emanating from the roof plate of the dimesencephalon boundary (FIGURE 1A). Indeed, the roof plate acts as an important organizing center for the secretion of these morphogens that are crucial for establishing the dorsal-lateral identity along the central nervous system (CNS) (31, 58, 59). Although malformation of the SCO during gestation leads to ventricular dilation and aqueductal stenosis before birth (FIGURE 1, B AND C) (32, 80), the involvement of specific factors has been slowly elucidated over the past decade through the analysis of mutant mouse models.

One such protein is *Msx1*, a critical factor involved in dorsal neural patterning (3, 84). *Msx1* along with *Msx2* and *Msx3* form a family of homeodomain transcriptional repressors (18, 96, 111). The earliest expression of *Msx1* is observed at the neural fold stage, along the boundary of the neural plate (17, 38). Upon closure of the neural tube, *Msx1* is prominently expressed along the entire length of the dorsal midline of the neural tube. *Msx1* is also expressed in the SCO, choroid plexus (CP), and the habenula in the third ventricle (84). Bach et al. clearly demonstrated that homozygous mutants for *Msx1* were unable to sustain Wnt1 and Bmp6 expression in the dorsal midline of P1, and histological analysis demonstrated the absence of a SCO and a poorly organized posterior commissure (3, 84). Loss of morphogen induction in P1 consequently downregulated cell fate markers such

as Pax6, Pax7, and Lim1, whereas the P2 marker Gbx2 remained unaffected. The *Msx1* mutant mice developed hydrocephaly, and the interactions of *Msx1* with Wnt1 and Pax6 are corroborated by similar hydrocephalic phenotypes in *Wnt1<sup>sw/sw</sup>* and *Pax6<sup>sey/sey</sup>* mice (27, 60).

It is also possible that *Msx1* may regulate genes that maintain homeostasis in the mature SCO ependymal cells. *Msx1* is already known to influence a diverse set of gene expression programs during neuronal development (85). Moreover, the residual ependymal cells of the SCO were not immunoreactive with anti-Reissner's fiber serum (AFRU), suggesting the absence of glycoprotein secretion (84). Although multiple roles are suggestive, additional studies such as temporal inactivation of *Msx1* will be required to determine whether *Msx1* directly regulates the expression of secreted glycoproteins and cell adhesion molecules in secretory ependymal cells. *Msx1<sup>+/-</sup>* mice are viable, but one-third of them develop hydrocephaly, likely due to the reduction in SCO size (29).

The regulatory factor X (Rfx) family represents a second class of transcription factors expressed in the dorsal neural tube, and two family members, Rfx3 and Rfx4\_v3, are important for the development of the SCO (2, 11). The Rfx proteins are winged-helix transcription

factors that form either homo- or heterodimers to regulate target genes (45, 71, 86) and are known to direct the formation of ciliated cell types in metazoans (1, 12, 26, 35, 52, 97, 101). Both *Rfx3* and *Rfx4\_v3* transcripts are expressed embryonically in the dorsal aspect of the neural tube and are highly expressed in the ependymal cells of the SCO in the embryo and adult (2, 11). Additionally, Rfx3 expression is prominent in the ependymal cells of the CP and is sustained in all ciliated ependymal cells from the moment of birth to adulthood (2). Not surprisingly, *Rfx3<sup>-/-</sup>* mice that come to term invariably develop communicating hydrocephaly (2). In the *Rfx4\_v3* mice, congenital non-communicating hydrocephaly was observed in the heterozygous condition (11). The agenesis of the SCO was a common feature found in both *Rfx3<sup>-/-</sup>* and *Rfx4\_v3<sup>+/-</sup>* mice (2, 11). The absence of a functional SCO was ascertained by the degree of immunoreactivity using anti-SCO-spondin (*Rfx3<sup>-/-</sup>*) and anti-RF (*Rfx4\_v3<sup>+/-</sup>*) in the presumptive SCO region. In both studies, the degree of immunoreactivity was significantly reduced. Although both mouse knockout models resulted in the absence of a functional SCO, an intriguing observation was reported in the case of the Rfx3 phenotype. The *Rfx3<sup>-/-</sup>* mice appeared to have an

**Table 1. Mutations that lead to hydrocephaly in the mouse**

Gene	Description/Function	SCO/Neuroependymal Phenotype	References
<i>Developmental defects</i>			
<i>En-1</i>	Transcription factor	Ectopic expression leads to SCO agenesis, ependymal differentiation defects in CP/SCO	60
<i>Hdh</i>	Unknown	SCO agenesis; ependymal differentiation defects	24
<i>Msx1</i>	Transcription factor	SCO agenesis; neuroependymal denudation	3, 29, 84
<i>Pax6</i>	Transcription factor	SCO agenesis	27
<i>Rfx3</i>	Transcription factor	SCO agenesis; neuroependymal defects	2
<i>Rfx4_v3</i>	Transcription factor	SCO agenesis; severe midline structure defects	11
<i>Wnt1</i>	Secreted morphogen	SCO agenesis	60
<i>Signal transduction defects</i>			
<i>Pac1</i>	G-protein coupled receptor	Overexpression leads to SCO agenesis; ependymal cilia are short and disorganized	50
<i>Socs7</i>	Suppressor of cytokine signaling	SCO cellular structure is disorganized	48
<i>Cilia structure/function defects</i>			
<i>Hydin</i>	Central pair-dynein adaptor	Missing central pair projection; ciliary motility defects	22, 53
<i>Foxj1/Hfh-4</i>	Transcription factor	Ciliogenesis defects	13, 20
<i>Mdnah5</i>	Dynein heavy chain	Outer dynein arms missing; ciliary motility defects	39, 41
<i>Pcdp1</i>	Unknown; may bind to central pair	Ciliary motility defects	55
<i>Polaris/Ift188</i>	IFT particle protein	Ependymal cells are shorter, disorganized, and beat asynchronously; CP defects	6
<i>Pol λ</i>	DNA repair polymerase	Inner dynein arm abnormalities; ciliary motility defects	47
<i>Spag6</i>	Central pair-dynein adaptor	Ciliary motility defects; Spag16 inactivation increases severity of hydrocephalus	93, 116
<i>Cell adhesion defects</i>			
<i>L1-cam</i>	Cell adhesion molecule	Defects in neuronal neurite outgrowth; neuroependyma appears normal	90
<i>Nmhc-IIb</i>	Component of apical cell adhesion complex	Neuroependymal denudation	61, 104
<i>Napa/α-Snap</i>	Apical transporter of cell adhesion molecules	Neuroependymal denudation with mutation that causes reduced expression of protein	19, 36

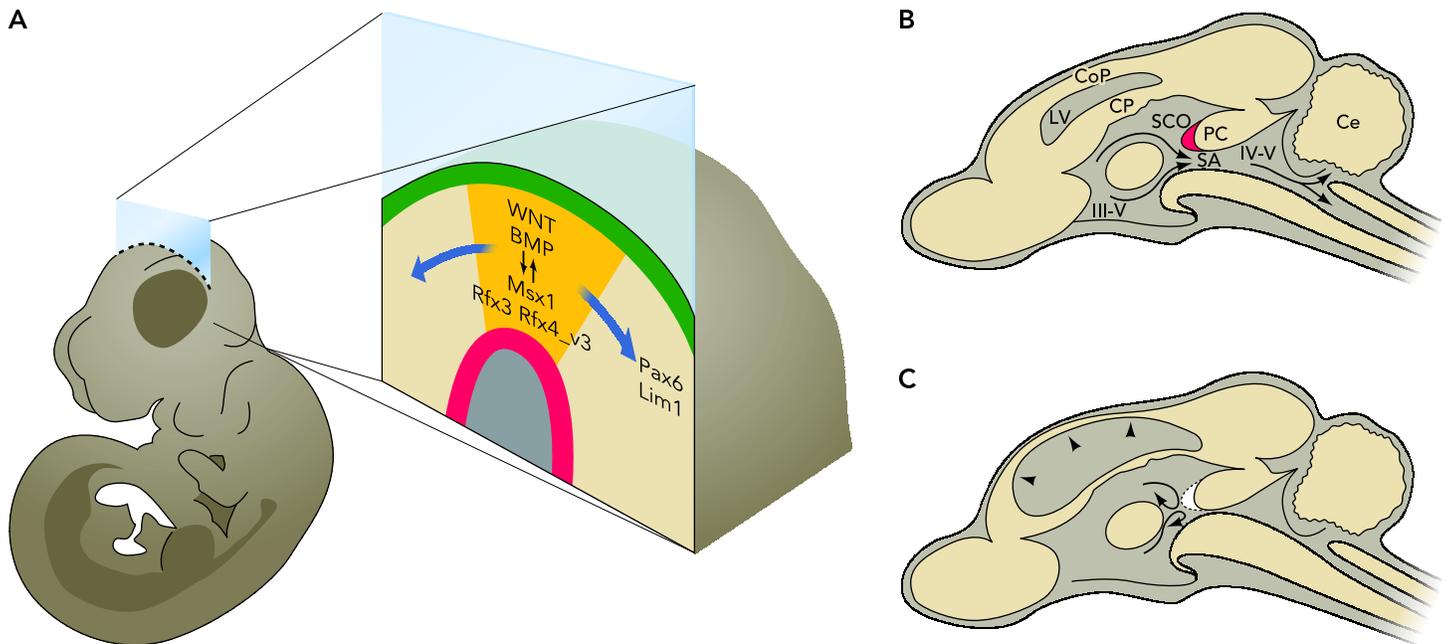
\*Unless otherwise stated, all mutations are inactivating.

overall dysfunction in the development of the ciliated ependymal cells. More specifically, the CP was poorly formed or improperly differentiated, with a lack of cilia present on the apical surface. Furthermore, the cell adhesion properties of the CP ependymal cells were compromised since the interdigitations between cells were poorly formed and the basal membrane was not apposed to the basal lamina. This apparent lack of differentiation was also observed in the SCO where its ependymal cells were more cuboidal with many cilia, appearing similar to the ependyma lining the ventricles (2).

In the case of the *Rfx4\_v3* studies, homozygous knockouts of *Rfx4\_v3* strongly suggest its role as a critical mediator of an earlier dorsal midline patterning decision. *Rfx4\_v3*<sup>-/-</sup> mice were embryonic lethal due to catastrophic midline defects. *Rfx4\_v3*<sup>-/-</sup> telencephalon at E12.5 were characterized by hypoplasia of the dorsal midline and adjacent cerebral cortex. The dorsal midline defect was also evident due to the down regulation of *Wnt7a* and *Wnt7b* in the cortical hem, a midline structure that normally produces these transcripts (11). A recent microarray experiment identified components of the Wnt, Bmp, and retinoic acid signaling pathways to be potential targets of *Rfx4\_v3* in E10.5 brains (110).

From the studies described above, tight regulation along the dorsal midline through Wnt and Bmp signal-

ing pathways is an important regulatory mechanism for the proper development of the differentiated ependymal cells of the SCO and for preventing the onset of hydrocephalus. Indeed, this is also noted in mice that ectopically express *Engrailed-1* (*En-1*), a transcription factor that is important for establishment of the mid-hindbrain boundary, since these animals were presented with hydrocephaly and SCO agenesis in the P1 domain (60). Similarly, a recent report by Dietrich et al. produced a mouse model linking the Huntington's disease gene (*Hdh*) to congenital hydrocephaly (24). In this study, a mid- to hindbrain-specific inactivation of *Hdh* was generated by crossing *Hdh*<sup>lox</sup> mice with animals that express Cre under the control of a *Wnt1* regulatory element. The *Wnt1-Cre:Hdh*<sup>lox</sup> mice came to term but displayed reduced growth and progressive wasting due to the build up of excessive CSF, expansion of the ventricles, and the loss of cortical tissue. Histology revealed expansion of the lateral ventricles by E17.5, indicating the congenital nature of the hydrocephaly. Despite the complete absence of huntingtin (*htt*) in the mutant CP, no gross structural defects were observed using immunohistochemistry for a variety of markers (24). The effect of *htt* loss was more overt in the mutant SCO. Mutant SCO was ~40% the size of the controls. Serial coronal



**FIGURE 1. Genetic determinants of congenital hydrocephaly and subcommissural organ agenesis**

A: molecular regulation of dorsal midline patterning in prosomere1 (P1) in an E10.5 mouse embryo. A coronal section of the neural tube through the di-mesencephalic boundary depicts the roof plate in yellow and the underlying dorsal primitive neuroepithelium in red that gives rise to the subcommissural organ (SCO). *Msx1* expression in the midline activates the expression of Wnts and Bmps. Wnt and Bmp signaling from the roof plate induces the expression of *Pax6* and *Lim1* in a dorsal-lateral gradient. *Rfx3* and *Rfx4\_v3* are also expressed at this stage in the dorsal-medial region. B: sagittal section of a normal E18.5 mouse brain depicting the cerebral spinal fluid (CSF) flow. The CSF flows unimpeded, circulating from the lateral (LV) and third ventricles (III-V) and in through the Sylvian aqueduct (SA). The SCO (red) is positioned immediately anteriorly to SA and is believed to facilitate the flow of the CSF by the secretion of glycoproteins and the formation of Reissner's fibers (not represented in this figure). Other structures in this panel are cortical plate (CoP), choroid plexus (CP), fourth ventricle (IV-V), posterior commissure (PC), and cerebellum (Ce). C: sagittal section depicting a hydrocephalic E18.5 mouse brain. Mutations in genes that regulate the tightly controlled patterning in the dorsal midline of P1 lead to SCO malformation and congenital hydrocephaly. *Wnt1*, *Msx1*, *Rfx3*, *Rfx4\_v3*, *Pax6*, and *Engrailed* have been demonstrated to be critical players in this process. Misregulation of these genes frequently leads to agenesis of the SCO (dotted area) and the stenosis of the SA. The closure of the SA causes an accumulation of the CSF resulting in dilatation of the III-V and LV. The enlargement of the ventricles results in a secondary loss of cortical tissue and a characteristic doming of the cortex.

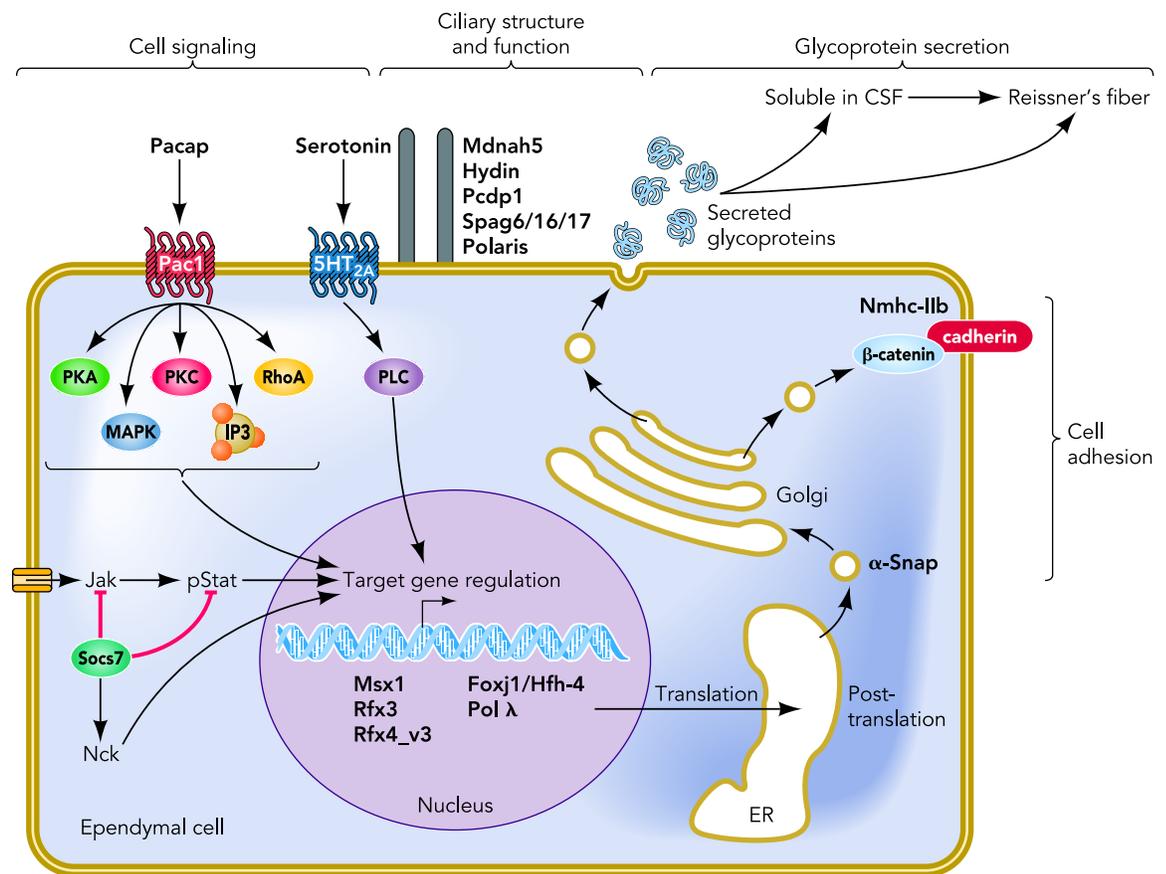
sections revealed that only the rostral portion of the SCO appeared properly differentiated as detected by SCO-spondin staining. Moreover, caudal to the SCO domain, ectopic SCO-spondin expression was detected in the ependymal lining of the Sylvian aqueduct (24). Further characterization of these mice is needed to determine whether the defect lies in inappropriate secretion of glycoproteins from the SCO or altered function of the vel cells lining the aqueduct. Nonetheless, these findings suggest that *htt* has a role in regulating the function of specialized ependymal cells.

Taken together, the proper development of the SCO is essential for CSF circulation through the narrow Sylvian aqueduct, and other factors that regulate dorsal patterning at or near the mid-hindbrain boundary are potential candidates for genetic causes of congenital hydrocephaly. In this regard, a protein like *Snf2l*, which modulates chromatin structure and expression of *En-1*, may potentially have dire consequences for hydrocephaly (8). Although we are only beginning to unravel the multitude of factors that control the cell fate decisions in this region and the

terminal differentiation of the specialized cells of the SCO and vel, it is apparent that there is extreme heterogeneity underlying hydrocephalus. It should also be mentioned that other transcription factors, such as *Otx2*, are known to be associated with hydrocephalus when inactivated in mice; however, such a phenotype is unlikely to be SCO-specific and likely constitutes a more general defect in brain development (62). Although development seems to have a significant role in the etiology of hydrocephalus, aberrant function of the SCO and vel cells has also been implicated in this disease.

### Signal Transduction and the Regulation of SCO Secretion

The primary function of the SCO is to secrete high molecular weight glycoproteins that facilitate CSF flow and, in rodents, contribute to the formation of Reissner's fiber (RF), which extends along the length of the CSF tract to the ampulla caudalis (89). The major secreted glycoprotein and constituent of RF is SCO-spondin, a highly conserved protein containing



**FIGURE 2. Molecular pathways in ependymal cells implicated in congenital hydrocephalus**

Schematic representation of a SCO/vel ependymal cell highlighting the numerous molecular pathways underlying congenital hydrocephalus. Neuropeptides (Pacap, serotonin) induce cell signaling pathways that alter target gene regulation, presumably genes encoding secreted glycoproteins and proteins involved in ciliary function. The *Socs7* protein is a regulator of the Jak/Stat signaling pathway. Ablation of several transcription factors (*Msx1*, *Rfx3*, *Rfx4\_v3*) alters the terminal differentiation or cell fate of the ependymal cells. Numerous proteins (*Mdnah5*, *Hydin*, *Pcdp1*, *Spag6*, *Polaris*) and transcriptional regulators (*Foxj1/Hfh-4*, *Pol λ*) involved in the formation and function of cilia are implicated in hydrocephalus and are shown at the top of the diagram. The  $\alpha$ -Snap protein is involved in regulating the transport of cell adhesion molecules (*Nmhc-IIb*, *cadherin*) to the apical cell surface and presumably also for proper glycoprotein secretion.

many protein-protein interaction domains that have fostered its numerous putative functions, including axonal pathfinding, promoting neuronal survival and neurite extension, and a detoxifying activity by removing monoamines from the CSF (67, 68). Regardless of its precise function, alterations in SCO secretions have been shown to precede diagnoses of hydrocephalus (99). Although transcription factors that promote terminal differentiation are important for regulating the expression of secreted proteins (e.g., *Msx1*) from the SCO, increasing evidence implicates signal transduction mechanisms in the processes regulating SCO secretion as a cause of hydrocephalus (FIGURE 2). The importance of signaling pathways downstream of G-protein-coupled receptors (GPCR) (73) in particular may modulate SCO glycoprotein secretion through the induction of transcriptional regulators, such as CREB, and perhaps also through the induction of intracellular calcium levels (10, 50, 74, 81, 94, 95). Recently developed hydrocephalic transgenic mouse models (*Pac1*, *Ro1*) seem to indicate that GPCR concentrations are rigidly controlled (50, 100).

The PACAP type I (*Pac1*) receptor is one example of a GPCR that contributes to hydrocephaly (50). Murine *Pac1* is highly expressed in neural progenitor cells, and transgenic *Pac1* was also found to be highly expressed in the differentiated SCO and ventricular ependyma (50, 106). Pituitary adenylate cyclase-activating polypeptide (PACAP) is the primary *Pac1* ligand, and its interactions with *Pac1* have been shown to activate or inhibit a variety of downstream signaling pathways, including PKA, PKC, MAPK, RhoA, and  $IP_3$  (FIGURE 2) (66). Transgenic mice that overexpressed *Pac1* developed communicating hydrocephalus with an enlarged aqueduct, which was associated with apoptosis-related SCO agenesis and shortened disorganized ventricular cilia (50). The mutant phenotype was correlated with an activation of both PKA and PKC signaling; however, the impact on SCO secretion was not assessed.

Neurotransmitting hormones are some of the most well characterized ligands of GPCRs, and the SCO is well innervated by serotonergic, GABAergic, dopaminergic, and noradrenergic fibers (42). Serotonin and GABA have also been previously characterized as regulators of SCO secretion; however, the molecular mechanisms have not been well explored (56, 70, 92). The most recent molecular studies concerning the effect of neurotransmitters on SCO secretion demonstrate a significant amount of serotonin and dopamine receptors on the apical surface of the mammalian SCO ependyma, implicating the CSF as the primary source of their respective ligands (87, 103). Although dopamine signaling did not have any significant effect on SCO secretion, serotonin signaling through the  $5HT_{2A}$  receptor, which primarily stimulates the phospholipase C (PLC) pathway, was shown to inhibit the transcription of SCO-spondin. To date, defects in serotonin signaling have not been identified

as a primary cause of hydrocephalus. However, a significant reduction in the rate of serotonin turnover has been observed in the CSF of human infants suffering from hydrocephalus (34), which may account for secondary defects in SCO secretion that could contribute to a worsening of the disease.

*“Recent molecular evidence in humans indicates that the direction of cell adhesion molecules to the proper surface is vital.”*

Abnormal cytokine and growth factor signaling pathways have also been linked with hydrocephaly (reviewed in Ref. 112). One study has shown that suppressor of cytokine signaling 7 (*Socs7*) mutant mice develop late-onset communicating hydrocephalus, where the only observable physiological abnormality is a disorganized SCO (48). Abnormalities in SCO secretion and RF formation were not examined. SOCS proteins are traditionally considered to suppress cytokine signaling by binding to JAK receptors to prevent STAT transduction into the nucleus (51). Although this may be the role of *Socs7* in SCO cells, the protein has also been implicated in several nontraditional transduction roles, including its binding to phosphorylated STATs (63), binding to other SOCS proteins (82), binding to the actin cytoskeleton through interactions with septins or vinexin (46, 49, 64), or contributing to the regulation of the actin cytoskeleton and cell cycle mechanisms by shuttling NCK to the nucleus (46, 49). At this time, the molecular significance of *SOCS7* with respect to the SCO remains to be characterized.

Taken together, these studies highlight the possibility that constituents of the CSF feedback on the SCO to regulate glycoprotein production and/or secretion. Although disruptions in specific signaling pathways have not yet been proven to be a primary cause of hydrocephaly, it represents an exciting area for future studies that would provide an additional layer of complexity to our understanding of the causal mechanisms of congenital hydrocephalus and SCO function.

### The Contribution of Ventricular Cilia to CSF Homeostasis

The ventricular ependyma shares complementary functions with the SCO in regulating CSF homeostasis and preventing the onset of hydrocephalus (81). Besides the requirement for SCO glycoproteins and RF to prevent stenosis of the Sylvian aqueduct, a unidirectional laminar flow of CSF generated by the ciliated ependyma is essential (41).

Hydrocephalus in humans arises in several ciliopathies, disorders that have been attributed to defects in cilia structure and function (4, 30, 40, 109). In the ventricular ependyma, the ciliary core, or axoneme, consists of an arrangement of a central pair of microtubules surrounded by nine peripheral microtubule doublets. Radial spokes project from the central pair and interact with the peripheral doublets, presumably to regulate interactions with dyneins, which are motor proteins that are responsible for generating ciliary beat (91).

Mouse axonemal dynein heavy chain (*Mdnah5*) was identified as a protein that is important for laminar CSF flow through the Sylvian aqueduct (41). *Mdnah5*-null mice have immotile cilia due to the absence of outer dynein arms in the axoneme (39). Its human counterpart, DNAH5, has been linked to outer dynein arm defects that lead to PCD, although only a small percentage of human patients carrying *DNAH5* mutations develop hydrocephalus (37, 75).

The molecular characterization of the hydrocephalus-3 (*hy3*) mouse, a commonly studied model of hydrocephalus, resulted in the identification of the *Hydin* gene (22). *Hydin* is a putative microtubule binding protein (83) required for the formation of a specific projection emanating from one of the central pair microtubules (23, 53, 54). Lechtreck et al. believe that *Hydin* is a dynein adaptor that is required to regulate the transition between the ciliary effective and recovery strokes (53, 54). This proposal is consistent with observations that the ependymal cilia of *hy3* mice have difficulty bending, leading to ciliary beats with reduced frequency and impaired synchronicity (53). At the same time, these cilia do not appear to have any structural defects other than the lack of this single central pair projection. The authors note that the phenotype is similar to that of PCD patients who suffer defects in the inner dynein arms or the radial spokes (21, 53).

Sperm-associated antigen 6 (*Spag6*) is another factor that associates with a specific central pair projection and, along with its interacting partners *Spag16* and *Spag17*, is required for ciliary motility (93, 113–115). Like *Hydin*, *Spag6*<sup>-/-</sup> mutants suffer from hydrocephalus. The extent of the phenotype is made more severe when both *Spag6* and *Spag16* are knocked out, although *Spag16*<sup>-/-</sup> mice are not hydrocephalic (116). The ultrastructure of the ependymal cells in these animals appears normal, suggesting a molecular pathology that is similar to *hy3* mice. Moreover, *Spag6* has been described as a candidate gene for hydrocephalus in H-Tx rats, another commonly studied hydrocephalus model (43).

PCD protein 1 (*Pcdp1*) is another putative central pair-binding protein that, when ablated in mice, results in hydrocephalus (55). As with *hy3* and *Spag6*<sup>-/-</sup> mice, the tracheal cilia of the *Pcdp1*<sup>-/-</sup> mice

have a reduced beat frequency but do not display any obvious ultrastructural axonemal defects. The ultrastructure and ciliary beat frequency of ependymal cilia were not reported, although *Pcdp1* was identified throughout the cilia of human brain ependymal cells.

In the *Tg737<sup>orpk</sup>* mouse (57), mutations in the intraflagellar transport protein (91) *Polaris/Ift188* also led to hydrocephalus. Although ependymal cilia are shorter, disorganized, and beat asynchronously, the primary cause of hydrocephaly in these mice appears to be the regulation of intracellular and secretory mechanisms of the choroid plexus in response to the defects in the structure and function of its cilia (5, 6). IFT components appear to be well conserved and many await characterization, so it remains to be seen whether mutations in additional IFT factors also result in hydrocephalus.

Defects in the expression of transcriptional regulators that mediate ciliogenesis can also lead to hydrocephalus. For example, *Foxj1/Hfh-4* is an important transcriptional activator of numerous genes required for the development of motile cilia and has been associated with left-right asymmetry developmental defects and associated hydrocephaly (13, 20, 108). Similarly, mice that lack DNA polymerase  $\lambda$  (*Pol*  $\lambda$ ) also develop hydrocephalus (47). Although *Pol*  $\lambda$  is generally considered to be a DNA repair enzyme, it is required for the proper expression of the axonemal inner dynein arms whose dysfunction likely contributes to the hydrocephaly in these animals (33).

In this section, we have highlighted recent advances characterizing defects in ciliary structure and function that result in hydrocephalus. It is likely that additional proteins that interact with or comprise the cilia ultrastructure are potential candidates to be implicated in hydrocephalus in the near future.

## Cell Adhesion Molecules and Hydrocephalus

The ependymal cells of the SCO possess tight junctions and zonulae adherens, which contribute to the formation of a blood-brain barrier between the vasculature and the ventricular cavities (89). Adding to the complexity of its functions is accumulating evidence that the SCO is able to act as a mechanosensor, altering the way it secretes glycoproteins in response to signals sent from circulating blood, as shown in hypertensive rats (65). In this regard, it is intriguing that hypertension was also observed in both *Pac1*-null and -overexpressing transgenic mice (50). Regardless, the presence of such a blood-brain barrier necessitates a functional complement of cell adhesion molecules.

Recent molecular evidence in humans indicates that the direction of cell adhesion molecules to the proper surface is vital. Mutations in the vesicular

transport proteins FILAMIN A and ARFGEF2 lead to periventricular heterotopia, which is associated with a reduction in cell adhesion and a loss of the neuroependyma, and is sometimes accompanied by hydrocephalus (28, 77, 98). In the hydrocephalus with hop gait (*hyh*) mouse, hydrocephalus is accompanied by a denudation of the ependymal cell layer (14, 79, 105). Ependymal denudation of the SCO has been identified in cases of human hydrocephaly (25).

A mutation in the soluble NSF attachment protein  $\alpha$  ( $\alpha$ -Snap), which directs secretory vesicles to the apical cell surface, was found to be responsible for the *hyh* phenotype (19, 36).  $\alpha$ -Snap is known to direct cell adhesion molecules, such as E-cadherin and  $\beta$ -catenin, to the apical surface of neural progenitors (9, 19, 76). In vel cells, non-muscle myosin II-B heavy chain (Nmhc-IIb) forms a mesh-like structure with N-cadherin and  $\beta$ -catenin at the apical surface, thus it is possible that  $\alpha$ -Snap is involved with the localization of one or more of these structural components (FIGURE 2) (61). Similar to the *hyh* phenotype, the loss of Nmhc-IIb results in a loss of integrity in the neuroepithelium, leading to hydrocephalus and defects in neuronal cell migration (61, 104). This phenotype can be somewhat rescued by replacing the expression of Nmhc-IIb with Nmhc-IIa (7).

### Mouse-Human SCO Differences and the Relationship to the Human Condition

CNS patterning is a highly conserved process underpinned by fundamental molecular pathways and interactions in all vertebrates. Moreover, select classes of transcription factors such as the bHLH, homeobox, and paired box family are key regulators of CNS development in metazoans. Taken together, it suggests that mouse models represent a powerful system in which to understand the etiology of hydrocephaly in humans. Indeed, the presence of the SCO/vel in even the earliest of vertebrates suggests a high degree of conservation in the expression of factors that regulate the development and functionality of these tissues, and one might predict that most of the factors identified in mice (e.g. Rfx3, Rfx4\_v3) would be prime candidates to cause human hydrocephaly. Unfortunately, studies that implicate the SCO-related developmental transcriptional regulators as a direct cause for human hydrocephaly have not been forthcoming as of yet.

Despite the similarities in SCO development and function, there are controversies that surface when drawing parallels between human and mouse that arise from SCO morphological differences. Unlike most other vertebrates, in which the SCO remains fully differentiated and active throughout adulthood, the human SCO reaches its functional peak of glycoprotein secretion during embryonic development (88). During later embryonic stages and postnatally, the SCO begins to regress to the point where, by late child-

hood, secretion is limited to only a few remaining islets. Additionally, the glycoproteins secreted by the human SCO do not aggregate into RF nor do they immunoreact to antibodies raised against RF-glycoproteins in other mammals, indicative of some differences in the secretory role of the human SCO vs. that of the mouse (88). Indeed a pessimist would argue that these interspecies differences supersede the benefits of studying hydrocephaly in models where the SCO is implicated. Although the species-specific roles of the SCO present challenges to the field, the adult vel still contributes to hydrocephalus in both species. In this regard, shared vel signaling pathways or factors involved in ciliary structure and function should allow for meaningful comparisons to be made; however, these comparisons are rare and tenuous at best. For example, the gene for the human PAC1 receptor is located at 7p15. Although a link could be made to communicating hydrocephalus, only a single patient with a duplication of this region has been described (69). The gene for its ligand, PACAP, is located at 18p11, and congenital hydrocephalus is one of the symptoms experienced by patients with tetrasomy 18p (102). Similarly, HYDIN, located at 16q22, is a candidate for one case of human hydrocephaly (16). A large-scale genetic screen has also recently revealed that mutations in a human-specific HYDIN paralog located at 1q21 may contribute to a number of behavioral and congenital brain disorders, although hydrocephalus was present in only a small number of these patients (15). Additional comparisons between human and mouse are summarized in a recent review of the genetics of human hydrocephaly (112).

Although we have highlighted a role for many factors in the etiology of hydrocephaly in the mouse, human studies identifying causative genes are lagging far behind. One reason for this delay may evolve from the general nature of hydrocephaly itself. It is a very heterogeneous disorder and without the availability of large families for genetic studies it is difficult to group patients together for successful linkage analyses. Another consideration is that many of the animal studies describe the ablation of specific genes that may be embryonic or early postnatal lethal in humans. Alternatively, viable human mutations in the gene may result in subtler phenotypes with little or no penetrance of hydrocephaly. On the other hand, the identification of human genes should be studied in mice to further characterize the similarities and differences between the two species in disease development. In this regard, L1-CAM, an X-linked cell adhesion molecule important for neurite outgrowth, is the best characterized determinant of congenital human hydrocephaly, and mouse models have been shown to accurately recapitulate the human phenotype (44, 72, 90, 107). Overall, a combination of animal and human studies remains the best approach for disease gene identification.

## Concluding Remarks

Collectively, these studies demonstrate that defects in multiple aspects of SCO cell function are implicated in the development of congenital hydrocephalus. As such, the new frontier for SCO research is clearly toward a further understanding of the molecular mechanisms governing SCO development and function. The plethora of genetic mouse models available is phenomenal and should continue to be extremely valuable for researchers in this field and in the hunt for causes of human hydrocephaly.

Developmental studies will continue to elucidate the hierarchical transcription factor organization that dictates the stage-specific differentiation of these cells and underlies the critical points of functional regulation. Defining these pathways will present researchers with candidate disease genes and opportunities to augment differentiation of progenitors toward a SCO fate to generate cell culture models. Similarly, a complete understanding of the signaling mechanisms between the CSF and SCO/vel cells should identify ligands that could be administered to promote glycoprotein secretion and restore CSF homeostasis. Finally, the use of high throughput approaches will enhance the knowledge of the important protein-protein interactions. For example, proteomic approaches have been used in the initial characterization of the secreted glycoproteins from the SCO and, more generally, for delineating the ciliary proteome (78). Interestingly, this proteomic study identified more than 600 proteins, of which over 200 were conserved between humans and *Chlamydomonas*, including a surprising number of proteins involved in signal transduction mechanisms (78). All of these approaches will increase our knowledge of SCO function and help elucidate where similarities to human hydrocephaly exist, thereby facilitating the characterization of genetic causes of congenital hydrocephalus. ■

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