The role of epithelial primary cilium in the development of hydrocephalus

PhD short thesis

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Introduction

Cilia are complex organelles that can be either motile or immotile (primary cilia). The importance of motile cilia in processes such as mucus clearance and cerebrospinal fluid movement are well known. In contrast, the primary cilium – that is present on nearly every cell in the mammalian body – was thought to have minimal affect for human health; however, a rapidly expanding number of human disorders have now been attributed to ciliary defects. Importantly, many of these phenotypes are present and can be analyzed using the Oak Ridge Polycystic Kidney (*Tg737*^{orpk}) mouse. The *Tg737*^{orpk} mouse was described as a model for human recessive polycystic kidney disease. The *Tg737*^{orpk} mouse arose through integration of a transgene into an intron of the *Tg737* gene that codes for Polaris protein, resulting in a hypomorphic allele, which mutation impairs intraflagellar transport (IFT), a process required for assembly of both motile and immotile cilia.

The gross phenotype of the *Tg737*^{orpk} mouse is described with a triad of scruffy fur, severe growth retardation, and preaxial polydactyly on all limbs. However, the mouse is best known for its cystic renal phenotype, which resembles that of human autosomal recessive polycystic kidney

Publications related to the thesis

1. Banizs B, Pike MM, Millican CL, Ferguson WB, Komlosi P, Sheetz J, Bell PD, Schwiebert EM, Yoder BK. Dysfunctional cilia lead to altered ependyma and choroid plexus function, and result in the formation of hydrocephalus. *Development.* 2005 Dec;132(23):5329-39.

2. Banizs B, Komlosi P, Bevensee MO, Schwiebert EM, Bell PD, Yoder BK. Altered pH(i) regulation and Na(+)/HCO3(-) transporter activity in choroid plexus of cilia-defective Tg737(orpk) mutant mouse. *Am J Physiol Cell Physiol.* 2007 Apr;292(4):C1409-16. Dec 20.

I dedicate my work to my parents Eva Varga and Dr. Karoly Banizs, to Dr. Peter Komlosi and my children, Gergely and Daniel.

disease. In addition to the above, histological analyses of the *Tg737*^{orpk} mice revealed hepatic and pancreatic ductal abnormalities and cysts, retinal degeneration, further skeletal defects, cerebellar hypoplasia, and hydrocephalus.

Our primary focus in the present work is to explore connection between cilia dysfunction and the development of hydrocephalus in the *Tg737*^{orpk} mutant mice.

Objectives

Objective 1 To characterize the development of hydrocephalus in *Tg737* mutant mice by analyzing the initiation and progression of the pathology using MRI imaging techniques and by assessing the effect of *Tg737orpk* mutation on CSF flow.

Objective 2 To determine the effect of cilial dysfunction on epithelial cells of the comparing the expression and localization of transport proteins involved in ion transport, water movement, and CSF production in mutant and wild type mice.

Objective 3 To determine if choroid plexus cilia dysfunction results in altered ion transport and excess CSF production by analysis of ion transport properties in mutant choroid plexus.

Methods

Fixed brains from wild type and *Tg737*^{orpk} mice were used for morphological and histological analysis (both immunfluorescence and hematoxylin/eosin). Magnetic resonance imaging (MRI) made it possible to follow timely progression of hydrocephalus in mutant animals. Scanning electron microscopy was used to assess changes in cilia morphology of mutant choroid plexus epithelia and ependyma compared to wild type. Videorecording of ependymal cilia gave us information about the function of mutant cilia. Brain ventricular injection of fluorescent DiI allowed us to assess aqueduct patency in wild type and *Tg737*^{orpk} mice at different ages. CSF and choroid plexus tissue from wild type and *Tg737*^{orpk} mice were isolated in order to determine changes in ionic composition and $[cAMP]_i$, respectively. For measurement of pH_i of choroid plexi, two tissue pieces, freshly isolated from similar regions of choroid plexi obtained from a mutant and wild type animal were transferred to a thermo-regulated microscope chamber and were immobilized with glass micropipettes in a position where the epithelium of the two tissue pieces were facing each other. This allowed simultaneous imaging of the

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two preparations. The tissues were then loaded with BCECF, a fluorescent dye, to assess intracellular pH. During the fluorescence microscopical experiment, the bathing solution was exchanged and different agents were added to them. pH_i was measured using a Nikon S Fluor 40x objective and assessed with dual-excitation wavelength fluorescence system.

Results

Our analysis indicates that cilia on cells of the brain ventricles of the *Tg737*^{orpk} mutant mice are severely malformed. On the ependymal cells, these defects lead to disorganized beating and impaired cerebrospinal fluid (CSF) movement. However, the loss of cilia beat and CSF flow is not the initiating factor since the pathology is present prior to the development of motile cilia on these cells and CSF flow is not impaired at early stages of the disease. Rather, our results suggest that loss of cilia leads to altered function of the choroid plexus epithelum as evidenced by elevated CSF chloride and intracellular cAMP levels. This latter one is an intracellular messenger molecule and known to have regulatory effects on ion and fluid movement in many secretory epithelia.

Also, to evaluate whether the hydrocephalus in *Tg737^{orpk}* mutants is associated with defects in ion transport, we compared the steady-state intracellular pH and Na^+-dependent transport activities of isolated choroid plexus epithelial tissues from *Tg737^{orpk}* mutant and wild-type mice. The data indicate that *Tg737^{orpk}* mutant choroid plexus epithelium have lower pH_i and higher Na^+-dependent HCO_3^- transport activity compared to wild-type choroid plexus epithelium. In addition, wild-type choroid plexus epithelium could be converted to a mutant phenotype with regard to the activity of Na^+-dependent HCO_3^- transport by addition of dibutyryl-cAMP and mutant choroid plexus epithelium toward the wild-type phenotype by inhibiting PKA activity with H-89.

Summary

Together, the brain pathology in the *Tg737^{orpk}* mutants appears to be a consequence of several cilia dysfunction-mediated events. The first, and what we believe is an initiating factor, involves altered ion transport across the choroid plexus epithelium and subsequently increased production of CSF. This implicates that cilia have an

important role in regulating normal physiology of choroid plexus epithelium and that ciliary dysfunction in mutants disrupts a signaling pathway leading to elevated intracellular cyclic AMP levels, aberrant regulation of pH_i and ion transport activity. Deformed cilia on choroid plexus cells may alter the function and/or the quantity of proteins involved in ion transport and CSF production, similar to what is occurring in renal epithelia of cystic kidney diseases. The second event is likely the loss of cilia beat on the ependymal cells lining the ducts and ventricles.