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# Primary Cilia are Sensory Hubs for Nitric Oxide Signaling

*Sidney T. Ley and Wissam A. AbouAlaiwi*

## Abstract

Primary cilia are sensory organelles present on the surface of most polarized cells. Primary cilia have been demonstrated to play many sensory cell roles, including mechanosensory and chemosensory functions. We demonstrated previously that primary cilia of vascular endothelial cells will bend in response to fluid shear stress, which leads to the biochemical production and release of nitric oxide. This process is impaired in endothelial cells that lack primary cilia function or structure. In this chapter, we will provide an overview of ciliogenesis and the differences between primary cilia and multicilia, as well as an overview of our published work on primary cilia and nitric oxide, and a brief perspective on their implications in health and disease.

**Keywords:** primary cilia, nitric oxide, signaling, fluid shear stress, mechanosensory transduction

## 1. Introduction

Cilia are found in nearly every cell in the animal body, where they function as highly specialized sensory organelles. Ciliary malfunction, therefore, tends to result in severe abnormalities, which are often multisystemic. These abnormalities are known as ciliopathies, and as our understanding of cilia form and function continues to grow, so too does the list of known ciliopathies. It is now known that mutations in over 40 genes can alter cilia structure or function, and this list continues to grow; over 1000 polypeptides in the ciliary proteome have yet to be researched [1, 2].

The field of cilia research gained interest after the discovery that cilia play a role in the pathogenesis of Polycystic Kidney Disease (PKD) as fluid mechanosensors within the kidney. In addition to renal dysfunction, the cardiovascular system is also affected by PKD, which has prompted further research into the role that primary cilia play within this system. In kidney tubule epithelia, primary cilia activation leads to a calcium influx, and it has been proposed that this may also occur in vascular endothelial cells. In their study, Nauli et al. showed that vascular cilia play a similar function in sensing fluid shear stress, and there was a corresponding increase in calcium levels correlated with nitric oxide (NO) release. This is thought to contribute to blood pressure control directly. Testing this hypothesis, Nauli et al. showed that cilia mutant cell lines had little to no calcium influx, as well as a lack of NO release while under fluid shear stress [1, 3, 4].

Nitric oxide is a signaling molecule that plays many important functional roles in almost every organ system in the body. Various pathologies are associated with wayward NO production and altered bioavailability levels caused by abnormal

signaling cascades, which are often the result of abnormal cilia-regulated signaling pathways. There is a documented connection between cilia and NO in the vasculature, as well as an overlap between signaling pathways in other pathologies. It has been postulated that there is a connection between primary cilia and NO outside of the vasculature, but literature on the subject is scarce [1].

This chapter aims to explain cilia type, structure, and function, as well as ciliogenesis, nitric oxide signaling, and finally the interplay between nitric oxide and primary cilia.

## **2. Cilia type and structure**

To understand what makes primary cilia unique, it is important to understand the differences between cilia form and function. Cilia are dynamic sensory organelles present in nearly every cell in every animal, as well as most protozoa. There are two classes of cilia; motile, which possess the dynein motor complexes needed to move, and nonmotile. Motile and nonmotile cilia both contain a 25  $\mu\text{m}$  diameter cytoskeletal scaffold known as the axoneme [5]. The axoneme is comprised of hundreds of proteins and houses nine peripheral microtubule doublets. These doublets are made up of A and B tubules, and they either surround a central pair of microtubules (9 + 2 pattern), or do not (9 + 0 pattern) [5]. Some motile cilia contain a 9 + 2 pattern and exist in clusters on cells called multiciliated cells (MCCs) [6]. There is also a class of motile cilia that have a 9 + 0 structure and exist as solitary monocilia on cell surfaces. The presence or absence of the central pair leads to significant functional differences in the cilia. The 9 + 2 structure commonly moves in a wave-like motion to move fluid, and an example of this are the ependymal cilia. The 9 + 2 patterned cilia also move cerebral spinal fluid, while the 9 + 0 structured most commonly moves in a rotary or corkscrew motion, as seen in flagella, which is useful for propulsion [7, 8]. There is some debate on whether sperm tail flagella should be classified as motile monocilia; regardless, they also possess a similar axonemal structure [5, 6, 9–11]. Nonmotile cilia, known as primary cilia, have a 9 + 0 structure and exist as monocilia on the surface of cells. As primary cilia can be found on vascular endothelial cells, they will be the focus of this review, but a brief overview of multicilia and their motion will also be covered.

### **2.1 Ciliogenesis**

Cilia formation is known as ciliogenesis. Ciliogenesis is correlated with cell division and occurs at the G1/G0 phase of the cell cycle. Reabsorption or disassembly of the cilium starts after cell cycle re-entry. In the first step of ciliogenesis, the centrosome travels to the cell surface, whereupon a basal body is formed by the mother centriole, and it nucleates the ciliary axoneme at the G1/G0 phase of the cell cycle [12]. This first process is regulated by distal appendage proteins, such as centrosomal protein 164 [13]. During the second step, the cilium elongates; this process is regulated by nuclear distribution gene E homolog 1 (Nde1), up until the cilium is matured [14]. The third step is cilia resorption, followed by axonemal shortening during cell cycle reentry. This third process is controlled by the Aurora A-HDAC6, Nek2-Kif24, and Plk1-Kif2A pathways [15]. In the fourth step, the basal body is released from the cilia, which frees the centrioles that act as microtubule organizing centers (MTOC) or spindle poles for mitosis [12].

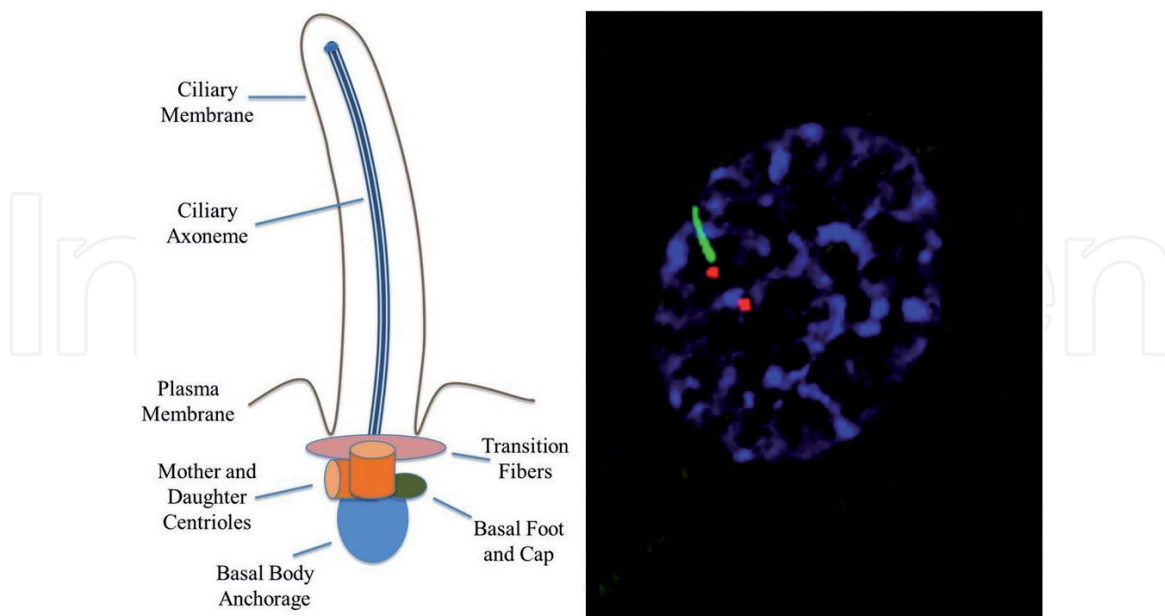
Immotile cilia formation is impacted by the coordination of the assembly and disassembly equilibrium, the IFT system, and membrane trafficking. When the axoneme nucleates from the basal body, it contains a microtubule bundle contained

within the ciliary membrane [16]. Enclosed within are certain signaling molecules and ion channels. Because cilia lack the machinery needed to synthesize ciliary proteins, proteins synthesized by the cell's Golgi apparatus must be transported through a ciliary 'gate' and transition zone near the cilium base [17]. The transition zone, recognizable by a change from triplet to doublet microtubules, is located at the distal end of the basal body (**Figure 1**) [18]. Basal body docking with the plasma membrane can be either permanent, in the case of unicellular organisms, or temporary, in the case of metazoans [5].

Transition fibers, which are present in unicellular organisms, or distal and subdistal appendages, which are present in mammals, are attached to microtubules within the transition zone [19]. Transition fibers function as docking sites for intraflagellar transport (IFT) proteins [20]. IFT transports cargo in a bidirectional manner along the length of cilia, and is mediated by kinesin-2 (anterograde) and cytoplasmic dynein-2 motors (retrograde) attached to multisubunit protein complexes known as IFT particles [21, 22]. Y-linkers exist at the distal end of the transition zone and secure the doublet microtubules to the ciliary membrane in most organisms [19].

## 2.2 Multicilia

In vertebrates, MCCs are present in a wide variety of different tissue types. In mammals, ependymal MCCs line brain ventricles and the airway epithelium. Multicilia are produced by specialized cells for highly specialized functions. MCCs are typically defined as having more than two cilia on their surface, although this occurrence is not well documented or understood. Recently, MCCs have been observed in unicellular eukaryotes and protists, as well as many metazoans, and even in certain plant sperms [23–25]. MCCs result in the production of motile axonemes, with the only notable exception being mammalian olfactory cilia. These olfactory MCCs lack dynein arms and are considered immotile despite having a 9 + 2



**Figure 1.**

*Primary cilia structure. The axonemes of primary cilia are anchored from the basal body and encapsulated within the ciliary membrane. The ciliary membrane is one continuous extension of the plasma membrane. The basal body is composed of the mother and the daughter centrioles, as well as some transition fibers that anchor the basal body to the cell membrane. The ciliary membrane houses specific membrane and protein receptors, all of which facilitate proper cilia signaling (left panel). Primary cilia that are found on vascular endothelial cells are identifiable by an immunofluorescence technique with antibody against acetylated  $\alpha$ -tubulin (green) labeling primary cilia, and pericentrin (red) labeling the centriole or basal body. The nucleus is counterstained with DAPI (blue) to label DNA (right panel). Left panel is adopted from Ref. [1].*



structure. This occurrence is indicative of MCCs being a solution to the need for local fluid flow, possibly due to their ability for hydrodynamic coupling [6, 26, 27].

Multicilia carry out their functions by beating, and the basic machinery and organization of cilia beating seems well conserved between eukaryotes, as well as between single motile cilium and multicilia. Some parameters, such as beat frequency, are under cellular control and are varied among cell types. In addition, only motile cilia and sperm flagella contain the dynein machinery needed to power axonemal beating during ATP hydrolysis [5, 28]. The ciliary beat cycle has two phases: the effective stroke, and the recovery stroke. The effective stroke is the initially bending from its upright position, while the recovery stroke sees it return to its original, unbent position [6, 29]. Ciliary motility is controlled by outer and inner axonemal dynein arms, which slide adjacent doublets in respect to one another. The sliding is mediated by protein bridges between doublets, and by the basal anchoring of the axoneme. As a result, cilia bend [6, 29].

The phenomenon metachrony occurs when cilia are organized in such a way that each cilium, in a two-dimensional array, will beat at the same frequency, but in a phase shifted manner. As a result, a traveling wave of ciliary action moves across the array, which propels fluids in a current. Even if each cilium in an array start off in synch, hydrodynamic forces between each cilium will nudge them back towards metachrony, possibly because in a metachronal array, the work each cilium must do is reduced, and more fluid is displaced. Because of this, multiciliation is thought to be a more evolutionarily efficient way to generate fluid flow [6, 30–32].

### **2.3 Primary cilia**

Nearly all human cells house a single nonmotile cilium on their surface, and these primary cilia serve sensory and signaling purposes. The role of primary cilia function and formation on animal health and pathophysiology has only recently been brought to researcher's attentions and is even now not fully understood. A wealth of new information about the primary cilia has been discovered within the last few decades, shedding some light on the function of the previously thought vestigial organelle [33].

Primary cilia are part of various signaling pathways in vertebrates, and usually lack a microtubule central pair (9 + 0 axoneme), as well as the structures associated with the central doublet pair, such as the inner and outer dynein arms required for ciliary movement [34, 35]. Primary cilia can be found in, but are not limited to; endothelia, epithelia, and neurons. Although found in nearly every mammalian cell, notable exceptions include the intercalated cells of the kidney collecting ducts, red blood cells, hepatocytes, and MCCs [34].

Primary cilia function mainly as sensory hubs and are host to many different groups of mechanosensory proteins, chemosensory receptors, and ion channels. These translate extracellular stimuli into an intracellular biochemical signal, which cause different cellular responses. There are two current models that attempt to explain how primary cilia function; the compartment model, and the scaffold model. The compartment model states that the ciliary structure is essential for proper signaling, while the scaffold model states that IFT molecules must play a part in either signaling itself, or the acquisition of outside transduction intermediates [36, 37]. As stated in the ciliogenesis section, primary cilia form through IFT, which transports proteins along the microtubules of the axoneme. The axoneme acts like a scaffold for certain protein complexes, including kinesins and dyneins, which facilitate back-and-forth trafficking of cargo proteins along the length of the cilium. Along with their creation, IFT is also required for maintenance of the primary cilium, and possibly even their core functions [1, 10].

The ciliary membrane is an extension of the cellular membrane where a host of proteins and receptors are housed due to the ciliary transition zone, which provides docking sites for molecular transport into and out of the cilioplasm (**Figure 1**) [38–40]. While there is no confirmed mechanism by which molecules enter and exit the cilia, several mechanisms have been proposed. One such mechanism is the active transport of vesicles from the Golgi apparatus to docking sites in the transition zone [41]. The vesicles are thought to interact with exocyst complexes, where they experience soluble N-ethylmaleimide-sensitive factor (NSF) attachment protein receptor (SNARE) mediated diffusion across the cilioplasm/cytoplasm barrier [37]. The BBSome, which resides in the basal body and is an octameric protein complex, is involved in the movement of transmembrane proteins to the ciliary membrane. BBSomes are known to recognize ciliary targeting sequences and will readily interact with molecules that are upstream of Rab8 activation. BBSomes are not thought to be required for any aspect of ciliogenesis; nevertheless, if BBSomes fail to deliver certain vital proteins to the cilia, the cilia may lose functionality [42–45]. Another proposed mechanism of molecule trafficking is the action of transmembrane proteins, of which some are associated with specific protein sequences that target cilia localization, such as the N-terminal RVxP sequence on polycystin-2 (PC-2) [42, 46].

Primary cilia serve many mechanosensory functions within the body. For example, proper kidney function depends on regulated fluid flow through the nephrons and collecting ducts, which controls the glomerular filtration rate [47]. This flow is sensed by the epithelial primary cilia present in the kidney. Fluid redirection by the primary cilia causes an increase in intracellular calcium. This calcium influx is also mediated by the PC1/2 ion channel complex, and both the mechanosensitive polycystin-1 (PC-1) membrane protein and the PC-2 channel localize to the primary cilia [48–50]. In renal cells, defects in this ion channel complex, along with complete disruption of cilia formation, is known to result in PKD [51].

### **3. Nitric oxide**

Nitric oxide is a signaling molecule involved in a wide array of cellular pathways; mainly, NO contributes to the normal functions of a variety of organ systems [52]. NO is highly reactive, and readily diffuses across cellular membranes. As a result, NO is found in many paracrine signaling pathways. NO is mainly synthesized from l-arginine, oxygen, and NADPH in a redox reaction, catalyzed by nitric oxide synthase (NOS) [53]. NOS has three isoforms, but only endothelial NOS (eNOS) and neuronal NOS (nNOS) are constantly and consistently expressed in cells. Both eNOS and nNOS are calcium dependent, while the other NOS isoform, cytokine inducible NOS (iNOS), is expressed by pro-inflammatory cytokines on an as needed basis [54]. iNOS and nNOS are both soluble enzymes that exist within the cytosol. eNOS, however, is found to localize to the plasma or Golgi body membranes. Because of its unique and wide cellular and subcellular distribution, NOS has many diverse functions throughout the entirety of the body [1, 54, 55].

### **4. Cilia and nitric oxide interplay**

Although primary cilia and NO have various independent roles within the body, especially in the vasculature, their functions often intersect and cooperate with each other. Most research on the interaction between endothelial primary cilia and nitric oxide focuses on vascular homeostasis, but their interactions extend into other areas [1]. However, this chapter will focus on signaling cascades that lead

to NO biosynthesis or increased NO bioavailability. The following discussion of the interactions between primary cilia and nitric oxide will focus on vasodilation, wound healing, dopamine signaling, and cellular proliferation.

#### 4.1 Vasodilation

Primary cilia and NO independently effect the vasculature in different ways, but recent studies suggest a direct relationship may exist between the two. Vascular endothelial cells are present in the blood vessel wall and are in continuous contact with blood flow-generated fluid shear stress. Endothelial cells are known mechanotransducers of fluid shear stress, which causes the biosynthesis of NO. This helps regulate vascular tone; NO will diffuse into the surrounding smooth muscle, producing vasorelaxation [56].

Evidence supports primary cilia as the main sensor in this mechanosensitive pathway. As stated previously, PC-1, a mechanosensory protein that malfunctions in polycystic kidney disease, localizes to vascular endothelial primary cilia. In an *in vitro* study performed by Nauli et al., which investigated PC-1's fluid shear mechanosensory properties, it was found that in contrast to wildtype endothelial cells, the PC-1 knockout cells failed to produce an increase in cytosolic calcium and the corresponding NO flux in response to fluid shear stress. The authors, to demonstrate that calcium and NO signals are induced in response to ciliary PC-1 activation, used *Tg737<sup>orpk/orpk</sup>* endothelial cells that lack ciliary ultrastructure but have functional PC-1. The results showed that neither calcium nor NO signals were present at flow rates up to 50 dyne/cm<sup>2</sup> [3]. These results suggest that PC-1 is responsible for proper cilia mechanosensory function, and that ciliary PC-1 specifically elicits NO production.

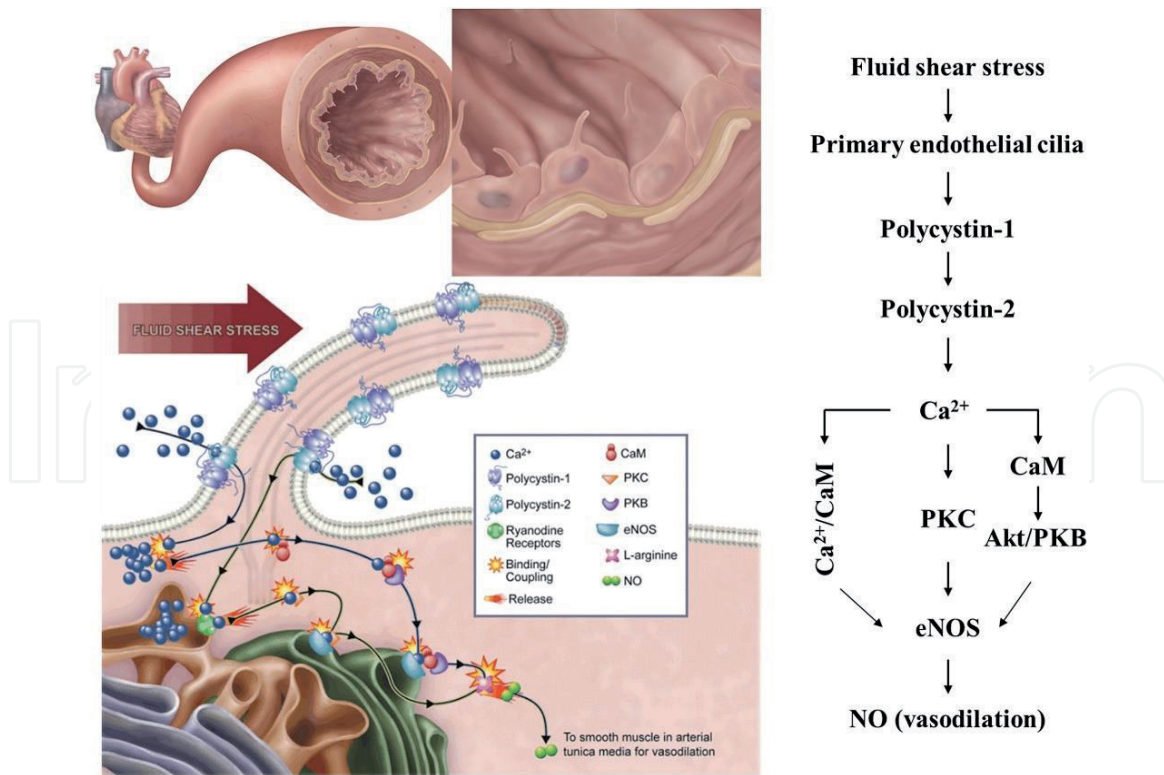
Follow-up studies by AbouAlaiwi et al. Showed that PC-2, which, as stated previously, is a calcium permeable cation channel that forms a complex with PC-1, is also important for mechanotransduction. Studies using a PC-2 knockdown line of cells showed a reduction in calcium and NO flux under shear stress when compared to control cells. This was further validated in *ex vivo* studies, where endothelial cells isolated from *pkd2<sup>-/-</sup>* mice arteries failed to respond to fluid shear stress [4]. These results indicate both PC-1 and PC-2 are needed for cilia mechanosensation, and further suggest that activation of the PC-1/PC-2 complex will start the signaling cascade needed for calcium-dependent NO biosynthesis. In addition, the results show that the increase in intracellular calcium is caused by an increase in intra-ciliary calcium. However, other researchers have proposed that calcium moves bidirectionally between the cilia and the cytosol [57–59]. Regardless, the increase in intracellular calcium triggers the calcium/calmodulin complex, which activates constitutive NOS, such as eNOS, by binding to its target site on the enzyme [60].

The calcium/calmodulin complex can also indirectly activate eNOS through activation of the AKT/PKB pathway, which stimulates AMPK (Figure 2) [61]. eNOS activation is mainly calcium dependent, but some studies have shown that a calcium independent pathway exists, via heat shock protein 90 (HSP90). HSP90 is also known to localize to cilia axonemes, and may act as a signal transducer that interacts with eNOS in the vasculature [62, 63]. HSP90 activation can lead to an increase in eNOS activity while calcium levels are high, and can also lead to more eNOS activity at low calcium levels due to its ability to directly bind to eNOS and increase the binding affinity for calmodulin [64, 65].

#### 4.2 Wound healing

An under researched aspect of NO and primary cilia is their interaction in the vascular smooth muscle cell (VSMC) layer. Depending on blood vessel type, all





**Figure 2.** Primary cilia activation via fluid shear stress and NO signaling in the vascular endothelia. The left panel shows primary cilia bending while under fluid shear stress, with the resultant production and release of nitric oxide (NO). The production and release of NO is dependent on the activation of endothelial primary cilia within the vasculature. The bending of cilia via fluid-shear stress activates the mechanosensory polycystins complex, which initiates the synthesis and the release of NO. This biochemical cascade, shown in the right panel, involves an extracellular calcium influx ( $Ca^{2+}$ ), followed by the activation of multiple calcium-dependent proteins, including calmodulin (CaM), protein kinase C (PKC) and Akt/PKB. Figure is adopted from Ref. [1].

three isoforms of NOS may exist within the VSMC layers [66]. During normal function, the cilia on the VSMC layer extend towards the extracellular matrix. Under abnormal conditions, such as a scratch wound, VSMC primary cilia will migrate to the wound edge. A recent study showed that VSMC cilia express polycystins, as well as  $\alpha 3$ - and  $\beta 1$ -integrins. When the researchers blocked integrin function, the percent of cilia migrating to the wound edge dropped from about 88% to around 30% [67]. This drop suggests that VSMC primary cilia may be involved in integrin-mediated wound healing.

In further support of the purported role primary cilia play in wound healing, results from experiments using VSMC that lacked cilia showed a slower scratch-wound healing time than the ciliated control cells displayed [67]. While the cilia in the wounded area are directly exposed to constant fluid shear stress from blood flow, activation of the mechanosensory ciliary polycystin complex could occur, resulting in an increase in intracellular calcium [1]. This could potentially trigger vasoconstriction in VSMC, resulting in the isolation of the wounded area to allow the platelets to begin clot formation. As soon as the calcium amount reached a certain level, the calcium/calmodulin complex would form and activate eNOS and nNOS, leading to vasodilation and the next step in the wound healing process.

Further studies by Schneider et al. revealed that, once cellular growth was halted, platelet derived growth factor receptor alpha (PDGFR $\alpha$ ), a tyrosine kinase with a prominent function in cellular proliferation, was found to localize to fibroblast primary cilia. Furthermore, ligand activation of PDGFR $\alpha$  will lead to the activation of the AKT and MEK/ERK proliferative pathways. Because AKT and ERK1/2 regulate eNOS activity in endothelial cells, PDGFR $\alpha$  activation could indirectly lead to eNOS



activation [68]. Moreover, data from recent studies on endothelial progenitor cells indicate that platelet-derived growth factor AA (PDGF-AA) might contribute a vital role in wound healing, possibly by its effects on angiogenesis through the PI3K/Akt/eNOS signaling pathway [69]. Bone morphological protein (BMP) receptor II (BMPRII), which is highly expressed on endothelial cells in lung vasculature, as well as moderately expressed in smooth muscle, is also involved in cell wound migration. In this pathway, migration is triggered by the ligands BMP2 and BMP4, which result in eNOS being phosphorylated [70]. While not conclusive, this evidence, when taken all together, suggests that primary cilia may have a significant part to play in the wound healing process.

When tissues begin to repair themselves after a wound, clots must be dissolved to maintain proper blood flow. This is known as clot retraction and platelet inhibition. NO is known to inhibit platelet aggregation, secretion, adhesion, and fibrinogen binding; all through activation of guanylyl cyclase and cGMP, alongside the inhibition of thromboxane A2. By this mechanism, platelet aggregation and accumulation are reduced, enabling the clot to dissolve, and the wound to heal fully [71–73]. Given the evidence, it is possible that an interaction between primary cilia and NO could be important in the wound healing and repair processes.

### 4.3 Dopamine signaling

Hypertension present in polycystic kidney disease (PKD) patients in the later stages of the disease is made worse by increased kidney volume. However, hypertension can also be seen in children, as well as the early stages of PKD, long before renal function starts to deteriorate. Some evidence suggests that an increase in sympathetic activation occurs in these patients, independently of their kidney function. Dopamine, an endogenous neuronal hormone that acts within the sympathetic nervous system, is confirmed to be involved in the regulation of blood pressure. Abnormal dopamine signaling can lead to hypertensive states in humans. Dopamine receptor 1 (D1) and dopamine receptor 5 (D5) receptors have been found to localize to primary cilia [39, 74–76]. While there are no current therapies that target D1 or D5, some studies using dopamine 1-like receptor subtypes showed vasodilatory effects in peripheral arteries [77].

The D5 receptor is thought to have both a chemosensory and mechanosensory role within primary cilia. Subjecting endothelial ciliary knockout cells *pkd1*<sup>-/-</sup> (lacking PC-1), and *Tg737<sup>orpk/orpk</sup>* cells that have no cilia, to dopamine under static conditions revealed a significantly subdued calcium influx when compared to the control cells. The researchers attributed this to the presence of underdeveloped cilia in the knockout cells, which would have less D5 receptors on them due to their smaller size. Under flow conditions with added dopamine, the mechanosensory function of the cilia knockout cells was restored, in comparison to the untreated knockout cells. Because calcium influx in these cell lines is associated with eNOS activation, the results of this study suggest a potential restoration of lost vasodilatory responses caused by a failed ciliary induction of NO biosynthesis [74]. There is additional evidence that suggests dopamine receptor 2 (D2) may also localize, or possibly get transported to, the primary cilia [78]. In one study, cerebral vasospasms were reversed with dopamine treatment; but when haloperidol, a D2 selective antagonist drug, was administered, the vasorelaxation failed to occur. It was also reported that, after administration of dopamine, a large increase in eNOS and iNOS expression was seen, and administration of haloperidol also blocked this effect [79].

D2 is also possibly transported to the primary cilia under specific conditions to mediate NOS activity within cells. Evidence supporting the role of ciliary dopamine receptors in the mediation of NO can be found in Autosomal Dominant Polycystic

Kidney Disease (ADPKD) patient clinical trials. ADPKD patients experience extra-renal maladies that mainly affect their cardiovascular system, such as hypertension. The hypertensive state could be brought on, in part, by the inability of primary endothelial cilia to respond to alterations in blood pressure. This would cause a failure to synthesize NO. In a study conducted by Lorthioir et al., flow-mediated dilation of normotensive ADPKD patients was compared to that of adults without ADPKD. It was shown that ADPKD patients had significantly less vasodilation during sustained flow increases, as well as a total loss of NO release when compared to those without ADPKD. When ADPKD patients were administered brachial infusions of 0.25–0.5 µg/kg/min of dopamine, there was an increase in flow-mediated dilation, and a statistically significant increase in dilatory response at the highest dose [80]. According to these results, dopamine receptors may facilitate a connection between primary cilia, NO, and blood pressure regulation in ADPKD patients [1].

#### **4.4 Cell proliferation**

Primary cilia also help regulate cell proliferation. As stated in the ciliogenesis section, the cilia extends from the basal body, which is composed of mother and daughter centrioles, and cilia are reabsorbed after cell cycle re-entry [12]. In cancerous cell clusters, cilia are missing from the more prolific dividers, which suggests that despite not playing a major role in cell division, primary cilia are important for starting and stopping cell mitosis [81–84].

NO possibly plays a role in cell proliferation as well, in conjunction with primary cilia. NO has been proven to halt the cell cycle by preventing the transition from G1 to S phase, in a dose dependent manner. The spike in NO is caused by an increase in free l-arginine, which is mediated by various cytokines. PC-1 is a known mediator of the JAK/STAT pathway by activating STAT3; when the cytosolic tail of PC-1 is cleaved upon once luminal flow halts, it can coactivate STAT-1, -3, and -6, along with JAK2. The PC-1 tail causes the cells to sensitize to cytokines and growth factor signaling, which then causes an exaggerated cellular response, which could potentially lead to an increase in l-arginine [85, 86]. Through this mechanism, overly prolific cell division would be arrested.

The superfamily of TGF-β signaling provides a fascinating system of cellular crosstalk, in which the effects of the same ligand can be unique depending on the cell type and the physiological conditions. This family is composed of more than 30 different ligand types of the TGF-β-activin-Nodal BMP subfamilies that can activate receptor serine/threonine kinases of types I and II (TGFβRI/II and BMP-RI/II, respectively). Ciliopathies widely overlap with phenotypes associated with aberrant TGF-β/BMP signaling. Prominent examples include structural heart defects associated with congenital heart disease (CHD) [87], suggesting that cardiac primary cilia may contribute to cellular events regulated by TGF-β/BMP signaling events during heart development. Moreover, different components of the TGF-β signalosome, including TGF-βRI, TGF-βRII, SMAD2/3, SMAD4, and SMAD7 are present at the cilia-centrosome axis. In a recent study, Feng et al. concluded that high salt (HS)-induced endothelial dysfunction and the development of salt-dependent increases in blood pressure (BP) were related to endothelial TGF-β signaling. Specifically, TGF-β-dependent ALK5 signaling increases endothelial NADPH oxidase-4 (NOX4), an enzyme that produces hydrogen peroxide, which limits NO bioavailability and ultimately promotes increased BP [88]. BMPRII contributes to cell proliferation through its interactions with primary cilia, eNOS, and NO. Using pulmonary artery endothelial cells, studies have shown that stimulation of BMPRII results in eNOS activation. BMPRII ligands BMP2 and BMP4 stimulate eNOS phosphorylation at a regulatory site via activation by protein kinase A. This eNOS

stimulation results in increased NO bioavailability; loss of BMPRII function, therefore, is proposed to contribute to endothelial dysfunction [70].

## **5. Conclusion**

Primary cilia and nitric oxide are both essential for normal tissue functions. While they can operate independently of one another, often their roles and cellular pathways are complementary. While this chapter only touched on a small portion of their possible connections, scattered research suggests a more complex linkage between the two in many more organ systems and cellular pathways. However, current research on the direct links between primary cilia and NO are scarce. The aim of this chapter was to discuss the more well-known links between primary cilia and NO, and to initiate discussion leading to further examination of the topics covered. Revealing the connections between cilia and NO would provide insight into various ciliopathies and could reveal new targets for therapies.

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### **Author details**

Sidney T. Ley and Wissam A. AbouAlaiwi\*  
Department of Pharmacology and Experimental Therapeutics, The University of Toledo, USA

\*Address all correspondence to: [wissam.abou-alaiwi@utoledo.edu](mailto:wissam.abou-alaiwi@utoledo.edu)

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## References

- [1] Saternos HC, AbouAlaiwi WA. Signaling interplay between primary cilia and nitric oxide: A mini review. *Nitric Oxide-Biology and Chemistry*. 2018;**80**:108-112
- [2] Waters AM, Beales PL. Ciliopathies: An expanding disease spectrum. *Pediatric Nephrology*. 2011;**26**(7):1039-1056
- [3] Nauli SM et al. Endothelial cilia are fluid shear sensors that regulate calcium signaling and nitric oxide production through polycystin-1. *Circulation*. 2008;**117**(9):1161-1171
- [4] AbouAlaiwi WA et al. Ciliary polycystin-2 is a mechanosensitive calcium channel involved in nitric oxide signaling cascades. *Circulation Research*. 2009;**104**(7):860-869
- [5] Mitchison HM, Valente EM. Motile and non-motile cilia in human pathology: From function to phenotypes. *The Journal of Pathology*. 2017;**241**(2):294-309
- [6] Brooks ER, Wallingford JB. Multiciliated cells. *Current Biology: CB*. 2014;**24**(19):R973-R982
- [7] Omran AJA et al. Alcohol consumption impairs the ependymal cilia motility in the brain ventricles. *Scientific Reports*. 2017;**7**(1):13652-13652
- [8] Nonaka S et al. Determination of left-right patterning of the mouse embryo by artificial nodal flow. *Nature*. 2002;**418**(6893):96
- [9] Veland IR et al. Primary cilia and signaling pathways in mammalian development, health and disease. *Nephron. Physiology*. 2009;**111**(3):p39-p53
- [10] Arnaiz O et al. Remodeling Cildb, a popular database for cilia and links for ciliopathies. *Cilia*. 2014;**3**(1):9
- [11] Pazour GJ et al. Proteomic analysis of a eukaryotic cilium. *The Journal of Cell Biology*. 2005;**170**(1):103-113
- [12] Avasthi P, Marshall WF. Stages of ciliogenesis and regulation of ciliary length. *Differentiation*. 2012;**83**(2):S30-S42
- [13] Graser S et al. Cep164, a novel centriole appendage protein required for primary cilium formation. *The Journal of Cell Biology*. 2007;**179**(2):321-330
- [14] Kim S et al. Nde1-mediated inhibition of ciliogenesis affects cell cycle re-entry. *Nature Cell Biology*. 2011;**13**(4):351
- [15] Korobeynikov V, Deneka AY, Golemis EA. Mechanisms for nonmitotic activation of Aurora-A at cilia. *Biochemical Society Transactions*. 2017;**45**(1):37-49
- [16] Goetz SC, Anderson KV. The primary cilium: A signalling centre during vertebrate development. *Nature Reviews Genetics*. 2010;**11**(5):331
- [17] Reiter JF, Blacque OE, Leroux MR. The base of the cilium: Roles for transition fibres and the transition zone in ciliary formation, maintenance and compartmentalization. *EMBO Reports*. 2012;**13**(7):608-618
- [18] Lin H, Guo S, Dutcher SK. RPGRIP1L helps to establish the ciliary gate for entry of proteins. *Journal of Cell Science*. 2018;**131**(20):jcs220905
- [19] Fisch C, Dupuis-Williams P. Ultrastructure of cilia and flagella—back to the future! *Biology of the Cell*. 2011;**103**(6):249-270
- [20] Deane JA et al. Localization of intraflagellar transport protein IFT52 identifies basal body transitional



fibers as the docking site for IFT particles. *Current Biology*. 2001;**11**(20):1586-1590

[21] Pedersen LB, Rosenbaum JL. Chapter two intraflagellar transport (IFT): Role in ciliary assembly, resorption and signalling. *Current Topics in Developmental Biology*. 2008;**85**:23-61

[22] Taschner M, Lorentzen E. The intraflagellar transport machinery. *Cold Spring Harbor Perspectives in Biology*. 2016;**8**(10):a028092

[23] Mizukami I, Gall J. Centriole replication: II. Sperm formation in the fern, *Marsilea*, and the cycad, *Zamia*. *The Journal of Cell Biology*. 1966;**29**(1):97-111

[24] Hodges ME et al. The evolution of land plant cilia. *New Phytologist*. 2012;**195**(3):526-540

[25] Nielsen C. Structure and function of metazoan ciliary bands and their phylogenetic significance. *Acta Zoologica*. 1987;**68**(4):205-262

[26] Lidow MS, Menco BPM. Observations on axonemes and membranes of olfactory and respiratory cilia in frogs and rats using tannic acid-supplemented fixation and photographic rotation. *Journal of Ultrastructure Research*. 1984;**86**(1):18-30

[27] Satir P, Christensen ST. Overview of structure and function of mammalian cilia. *Annual Review of Physiology*. 2007;**69**:377-400

[28] King SM. Axonemal dynein arms. *Cold Spring Harbor Perspectives in Biology*. 2016;**8**(11):a028100

[29] Golestanian R, Yeomans JM, Uchida N. Hydrodynamic synchronization at low Reynolds number. *Soft Matter*. 2011;**7**(7):3074-3082

[30] Machemer H. Ciliary activity and the origin of metachrony in *Paramecium*: Effects of increased viscosity. *Journal of Experimental Biology*. 1972;**57**(1):239-259

[31] Satir P, Sleight MA. The physiology of cilia and mucociliary interactions. *Annual Review of Physiology*. 1990;**52**(1):137-155

[32] Elgeti J, Gompper G. Emergence of metachronal waves in cilia arrays. *Proceedings of the National Academy of Sciences*. 2013;**110**(12):4470-4475

[33] Davenport JR, Yoder BK. An incredible decade for the primary cilium: A look at a once-forgotten organelle. *American Journal of Physiology. Renal Physiology*. 2005;**289**(6):F1159-F1169

[34] Chen JC, Jacobs CR. Cellular and molecular mechanotransduction in bone. In: *Osteoporosis: Fourth Edition*. Amsterdam: Elsevier Inc.; 2013. pp. 453-475

[35] Gonçalves J, Pelletier L. The ciliary transition zone: Finding the pieces and assembling the gate. *Molecules and Cells*. 2017;**40**(4):243-253

[36] Lehtreck KF. IFT-cargo interactions and protein transport in cilia. *Trends in Biochemical Sciences*. 2015;**40**(12):765-778

[37] Hu Q, Nelson WJ. Ciliary diffusion barrier: The gatekeeper for the primary cilium compartment. *Cytoskeleton*. 2011;**68**(6):313-324

[38] Malicki J, Avidor-Reiss T. From the cytoplasm into the cilium: Bon voyage. *Organogenesis*. 2014;**10**(1):138-157

[39] Leaf A, Von Zastrow M. Dopamine receptors reveal an essential role of IFT-B, KIF17, and Rab23 in delivering specific receptors to primary cilia. *eLife*. 2015;**4**:e06996

- [40] Mukhopadhyay S et al. Trafficking to the primary cilium membrane. *Molecular Biology of the Cell*. 2017;**28**(2):233-239
- [41] Kim H et al. Ciliary membrane proteins traffic through the Golgi via a Rabep1/GGA1/Arl3-dependent mechanism. *Nature Communications*. 2014;**5**:5482
- [42] Szymanska K, Johnson CA. The transition zone: An essential functional compartment of cilia. *Cilia*. 2012;**1**(1):10
- [43] Klink BU et al. A recombinant BBSome core complex and how it interacts with ciliary cargo. *eLife*. 2017;**6**:e27434
- [44] Zhang Q et al. BBS7 is required for BBSome formation and its absence in mice results in Bardet-Biedl syndrome phenotypes and selective abnormalities in membrane protein trafficking. *Journal of Cell Science*. 2013;**126**(11):2372-2380
- [45] Jensen VL et al. Whole-organism developmental expression profiling identifies RAB-28 as a novel ciliary GTPase associated with the BBSome and intraflagellar transport. *PLoS Genetics*. 2016;**12**(12):e1006469
- [46] Geng L et al. Polycystin-2 traffics to cilia independently of polycystin-1 by using an N-terminal RVxP motif. *Journal of Cell Science*. 2006;**119**(7):1383-1395
- [47] Leyssac PP. Changes in single nephron renin release are mediated by tubular fluid flow rate. *Kidney International*. 1986;**30**(3):332-339
- [48] Praetorius HA, Spring KR. The renal cell primary cilium functions as a flow sensor. *Current Opinion in Nephrology and Hypertension*. 2003;**12**(5):517-520
- [49] Praetorius H, Spring KR. Bending the MDCK cell primary cilium increases intracellular calcium. *The Journal of Membrane Biology*. 2001;**184**(1):71-79
- [50] Piperi C, Basdra EK. Polycystins and mechanotransduction: From physiology to disease. *World Journal of Experimental Medicine*. 2015;**5**(4):200
- [51] Yoder BK, Hou X, Guay-Woodford LM. The polycystic kidney disease proteins, polycystin-1, polycystin-2, polaris, and cystin, are co-localized in renal cilia. *Journal of the American Society of Nephrology*. 2002;**13**(10):2508-2516
- [52] Trouillon R. Biological applications of the electrochemical sensing of nitric oxide: Fundamentals and recent developments. *Biological Chemistry*. 2013;**394**(1):17-33
- [53] Wu G, Morris SM Jr. Arginine metabolism: Nitric oxide and beyond. *The Biochemical Journal*. 1998;**336** (Pt 1):1-17
- [54] Luiking YC, Engelen MP, Deutz NE. Regulation of nitric oxide production in health and disease. *Current Opinion in Clinical Nutrition and Metabolic Care*. 2010;**13**(1):97-104
- [55] Forstermann U, Sessa WC. Nitric oxide synthases: Regulation and function. *European Heart Journal*. 2012;**33**(7):829-837
- [56] Boo YC, Jo H. Flow-dependent regulation of endothelial nitric oxide synthase: Role of protein kinases. *American Journal of Physiology-Cell Physiology*. 2003;**285**(3):C499-C508
- [57] Su S et al. Genetically encoded calcium indicator illuminates calcium dynamics in primary cilia. *Nature Methods*. 2013;**10**(11):1105
- [58] Nauli SM, Pala R, Kleene SJ. Calcium channels in primary cilia. *Current Opinion in Nephrology and Hypertension*. 2016;**25**(5):452

- [59] Delling M et al. Primary cilia are not calcium-responsive mechanosensors. *Nature*. 2016;**531**(7596):656
- [60] Leonard PM et al. Crystal structure of the Lrp-like transcriptional regulator from the archaeon *Pyrococcus furiosus*. *The EMBO Journal*. 2001;**20**(5):990-997
- [61] Stahmann N et al. Activation of AMP-activated protein kinase by vascular endothelial growth factor mediates endothelial angiogenesis independently of nitric-oxide synthase. *Journal of Biological Chemistry*. 2010;**285**(14):10638-10652
- [62] Chen Y et al. Differential effects of heat shock protein 90 and serine 1179 phosphorylation on endothelial nitric oxide synthase activity and on its cofactors. *PLoS One*. 2017;**12**(6):e0179978
- [63] Chen K, Pittman RN, Popel AS. Nitric oxide in the vasculature: Where does it come from and where does it go? A quantitative perspective. *Antioxidants & Redox Signaling*. 2008;**10**(7):1185-1198
- [64] Takahashi S, Mendelsohn ME. Synergistic activation of endothelial nitric-oxide synthase (eNOS) by HSP90 and Akt calcium-independent eNOS activation involves formation of an HSP90-Akt-CaM-bound eNOS complex. *Journal of Biological Chemistry*. 2003;**278**(33):30821-30827
- [65] Takahashi S, Mendelsohn ME. Calmodulin-dependent and-independent activation of endothelial nitric-oxide synthase by heat shock protein 90. *Journal of Biological Chemistry*. 2003;**278**(11):9339-9344
- [66] Buchwalow IB et al. Vascular smooth muscle and nitric oxide synthase. *The FASEB Journal*. 2002;**16**(6):500-508
- [67] Lu C et al. Non-random distribution and sensory functions of primary cilia in vascular smooth muscle cells. *Kidney and Blood Pressure Research*. 2008;**31**(3):171-184
- [68] Schneider L et al. PDGFR $\alpha$  signaling is regulated through the primary cilium in fibroblasts. *Current Biology*. 2005;**15**(20):1861-1866
- [69] Wu LW et al. Platelet-derived growth factor-AA is a substantial factor in the ability of adipose-derived stem cells and endothelial progenitor cells to enhance wound healing. *The FASEB Journal*. 2019;**33**(2):2388-2395
- [70] Gangopahyay A et al. Bone morphogenetic protein receptor II is a novel mediator of endothelial nitric-oxide synthase activation. *The Journal of Biological Chemistry*. 2011;**286**(38):33134-33140
- [71] Wang G-R et al. Mechanism of platelet inhibition by nitric oxide: in vivo phosphorylation of thromboxane receptor by cyclic GMP-dependent protein kinase. *Proceedings of the National Academy of Sciences*. 1998;**95**(9):4888-4893
- [72] Du X. A new mechanism for nitric oxide-and cGMP-mediated platelet inhibition. *Blood*. 2007;**109**(2):392-393
- [73] Riddell DR, Owen JS. Nitric oxide and platelet aggregation. In: *Vitamins & Hormones*. Amsterdam: Elsevier; 1997. pp. 25-48
- [74] Abdul-Majeed S, Nauli SM. Dopamine receptor type 5 in the primary cilia has dual chemo-and mechano-sensory roles. *Hypertension*. 2011;**58**(2):325-331
- [75] Upadhyay V et al. Roles of dopamine receptor on chemosensory and mechanosensory primary cilia in renal epithelial cells. *Frontiers in Physiology*. 2014;**5**:72

- [76] Marley A, von Zastrow M. DISC1 regulates primary cilia that display specific dopamine receptors. *PLoS One*. 2010;5(5):e10902
- [77] Asghar M et al. Potential dopamine-1 receptor stimulation in hypertension management. *Current Hypertension Reports*. 2011;13(4):294-302
- [78] Omori Y et al. Identification of G protein-coupled receptors (GPCRs) in primary cilia and their possible involvement in body weight control. *PLoS One*. 2015;10(6):e0128422
- [79] Pyne-Geithman GJ et al. Dopamine D 2-receptor-mediated increase in vascular and endothelial NOS activity ameliorates cerebral vasospasm after subarachnoid hemorrhage in vitro. *Neurocritical Care*. 2009;10(2):225
- [80] Lorthioir A et al. Polycystin deficiency induces dopamine-reversible alterations in flow-mediated dilatation and vascular nitric oxide release in humans. *Kidney International*. 2015;87(2):465-472
- [81] Goto H, Inoko A, Inagaki M. Cell cycle progression by the repression of primary cilia formation in proliferating cells. *Cellular and Molecular Life Sciences*. 2013;70(20):3893-3905
- [82] Ke Y-N, Yang W-X. Primary cilium: An elaborate structure that blocks cell division? *Gene*. 2014;547(2):175-185
- [83] Plotnikova OV, Golemis EA, Pugacheva EN. Cell cycle-dependent ciliogenesis and cancer. *Cancer Research*. 2008;68(7):2058-2061
- [84] Cao M, Zhong Q. Cilia in autophagy and cancer. *Cilia*. 2015;5(1):4
- [85] Talbot JJ et al. Polycystin-1 regulates STAT activity by a dual mechanism. *Proceedings of the National Academy of Sciences*. 2011;108(19):7985-7990
- [86] Weimbs T, Olsan EE, Talbot JJ. Regulation of STATs by polycystin-1 and their role in polycystic kidney disease. *Jak-Stat*. 2013;2(2):e23650
- [87] Koefoed K et al. Cilia and coordination of signaling networks during heart development. *Organogenesis*. 2014;10(1):108-125
- [88] Feng W et al. Transforming growth factor-beta mediates endothelial dysfunction in rats during high salt intake. *American Journal of Physiology. Renal Physiology*. 2015;309(12):F1018-F1025