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Review

Signaling interplay between primary cilia and nitric oxide: A mini review

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ABSTRACT

New discoveries into the functional role of primary cilia are on the rise. In little more than 20 years, research has shown the once vestigial organelle is a signaling powerhouse involved in a vast number of essential cellular processes. In the same decade that interest in primary cilia was burgeoning, nitric oxide won molecule of the year and a Nobel prize for its role as a near ubiquitous signaling molecule. Although primary cilia and nitric oxide are both involved in signaling, a direct relationship has not been investigated; however, after a quick review of the literature, parallels between their functions can be drawn. This review aims to suggest a possible interplay between primary cilia and nitric oxide signaling especially in the areas of vascular tissue homeostasis and cellular proliferation.

1. Introduction

Understanding the roles of primary cilia in the human body is still in its infancy; however, the last several decades have produced a wealth of new information about the formerly functionless organelle. Cilia can be found in almost every cell of the body, often having a specialized sensory function [1,2]. When cilia malfunction, severe and multi-systemic abnormalities known as ciliopathies occur. The list of ciliopathies is ever expanding as mutations in over 40 genes have been discovered to alter ciliary structure or function with over 1000 polypeptides in the ciliary proteome yet to be fully investigated [3].

Cilia gained notoriety through their involvement in the pathogenesis of Polycystic Kidney Disease (PKD) as fluid mechanosensors in the kidney. Outside of renal abnormalities, the cardiovascular system is also greatly affected by the disease leading researchers to investigate the role of cilia in the fluid filled vascular system. Nauli et al. postulated that primary cilia activation in vascular endothelial cells would lead to a similar calcium influx as observed in kidney tubule epithelia; the group demonstrated that vascular cilia did play a similar role in sensing fluid shear stress and the corresponding increase in calcium levels correlated with nitric oxide (NO) release, thus contributing to blood pressure control. Evidence from cilia mutant cell lines showing little to no calcium influx and no nitric oxide release when subjected to shear stress was also provided in support of this hypothesis [4–6]. Nitric oxide is a ubiquitous signaling molecule with essential functions in almost every organ system [7–9]. A number of pathologies are associated with aberrant NO production or bioavailability due to abnormal signaling cascades. This will occasionally coincide with abnormal cilia-regulated signaling pathways. With a defined connection between cilia and NO in the vasculature and an overlap between signaling pathways in other pathologies, the obvious question becomes “is there a connection between primary cilia and NO?”However, little research is available on the subject and a direct link between NO-related signaling and cilia dysfunction has yet to be demonstrated [10,11]. Thus, this review aims to suggest a critical and complex link between cilia and nitric oxide that extends beyond vasodilation.

2. Primary cilia

Primary cilia are non-motile single cellular extensions that can be found on a majority of mammalian cells including but not limited to endothelia, epithelia, and neurons [12–15]. The cytoskeleton of primary cilia, known as the axoneme, consists of 9 concentric doublet microtubules (9 + 0 [16]. Stemming from the basal body, the cilium is constructed using bi-directional intraflagellar transport (IFT) molecules. The axoneme acts as a scaffold for various protein complexes, such as kinesins and dyneins, that facilitate antero- and retrograde trafficking of cargo proteins along the ciliary shaft [17]. The ciliary membrane is continuous with the cellular membrane; however, it has a distinct composition of receptors and integral proteins due to the ciliary transition zone. The latter is a region between the basal body and the axoneme responsible for the compartmentalization of the cilia while also providing docking sites to enable the transport of molecules in and out of the cilioplasm (Fig. 1) [18–20]. There are several proposed

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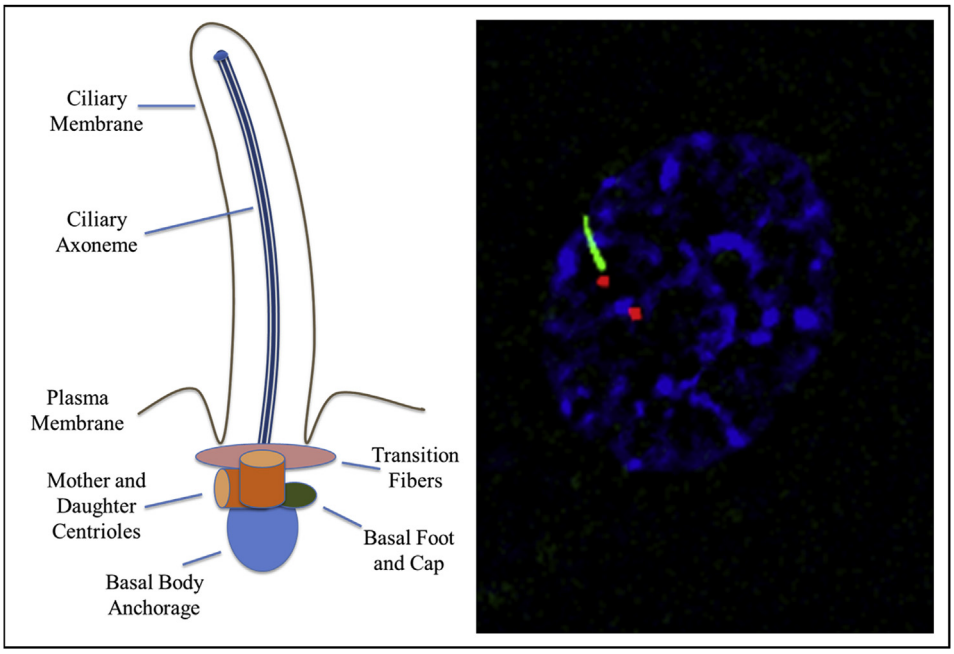

Fig. 1. Primary cilia structure. The axoneme of primary cilia is mainly anchored from the basal body and enclosed within the ciliary membrane which is continuous with the plasma membrane. The basal body is composed of the mother and the daughter centrioles and some transition fibers to anchor the basal body to the cell membrane. The ciliary membrane hosts specific membrane and protein receptors that facilitate proper cilia signaling (Left panel). Primary cilia found on the surface of vascular endothelial cells can be identified by a simple immunofluorescence technique utilizing antibody against acetylated α-tubulin (green) to label primary cilia and pericentrin (red) to label centriole or basal body. The nucleus is counterstained with DAPI to label DNA (Right panel). Left panel is adopted from Ref. [73].

mechanisms for the trafficking of molecules to the cilia. Briefly, transmembrane proteins are often associated with a specific protein sequence that targets ciliary localization, one example being the N-terminal RVxP sequence on polycystin-2 [21,22]. Active transport of vesicles from the golgi apparatus to specific docking sites at the transition zone is another proposed mechanism [23]. Similar to how importins and exportins function at nuclear pores, the vesicles are believed to interact with exocyst complexes and undergo SNARE (Soluble N-ethylmaleimide-sensitive factor (NSF) attachment protein receptor) mediated diffusion across the barrier separating the cilioplasm from the cellular cytoplasm [24]. The BBSome, an octameric protein complex and a component of the basal body and is involved in trafficking transmembrane proteins to the ciliary membrane through a combination of the mechanisms listed above. BBSomes are capable of recognizing ciliary targeting sequences and interacting with several molecules upstream of Rab8 activation. Although they are not required for ciliogenesis, the failure of BBSomes to deliver specific proteins to the cilia can lead to loss of ciliary function [22,25–27].

Primary cilia function as sensory hubs, housing a variety of mechanosensory proteins, chemosensory receptors, and ion channels to translate the extracellular stimuli into an intracellular biochemical signal triggering a cellular response. Due to their extensive sensory role, much research has been dedicated to understanding how cilia organize signaling cascades. Currently, there are two main models: the compartment model and the scaffold model. The compartment model suggests that the ciliary ultrastructure itself is essential for proper signaling whereas the scaffold model suggests that after stimuli, the diffusion of signaling molecules and secondary messengers is not sufficient on its own, thus requiring IFT molecules to scaffold signaling components or import specific transduction intermediates into the cilia (28 or 29). A more detailed explanation of these topics can be found in (28 or 29 could be up through 37) as the complex mechanisms of these models are beyond the scope of this review, thus we will only focus on the relevant signaling cascades the lead to NO production. The inferred interplay between primary cilia and nitric oxide will mainly be discussed in the context of vasodilation, wound healing, dopamine signaling, and cellular proliferation.

3. Nitric oxide

Nitric oxide is a gaseous signaling molecule involved in a variety of cellular pathways contributing to the normal functions in a majority of organ systems [38]. It is a highly reactive and readily diffuses across cellular membranes making it an ideal paracrine signaling molecule. NO is mainly synthesized from L-arginine, oxygen, and NADPH in a redox reaction catalyzed by nitric oxide synthase (NOS) [39]. Of the three NOS isoforms, endothelial NOS (eNOS or NOS3) and neuronal NOS (nNOS or NOS1) are constitutively expressed in cells and are calcium dependent. Cytokine inducible NOS (iNOS or NOS2) is expressed by macrophages and other pro-inflammatory cytokines as needed [11]. iNOS and nNOS are both soluble enzymes existing within the cytosol; whereas eNOS is largely membrane associated, specifically localizing to the plasma or the golgi body membranes. The unique cellular and subcellular distribution of NOS may contribute to its diverse functions throughout the body [40].

4. Cilia and NO interplay

4.1. Vasodilation

The majority of research on the connection between cilia and NO centers around the vasculature. Both primary cilia and NO have well defined independent roles within the vascular system. However, recent studies have suggested a direct relationship between the two. Vascular endothelial cells line the blood vessel wall and are in continuous contact with fluid shear stress generated by blood flow. It has long been established that endothelial cells are mechanotransducers of shear stress which triggers the biosynthesis of NO, aiding in the regulation of vascular tone as NO readily diffuses into the surrounding vascular smooth muscle cells producing vasorelaxation [41]. Although a number of mechanosensitive and stretch sensitive receptors can be found on the cell membrane, evidence has supported primary cilia as the chief sensor in this pathway. Polycystin-1 (PC-1), a mechanosensory protein found dysfunctional in PKD, has been shown to localize to vascular endothelial primary cilia. *In vitro* studies investigating the role of PC-1 as a fluid shear mechanosensor, reported that in contrast to wildtype endothelial cells, PC-1 knockout cells did not produce an increase in cytosolic calcium or a corresponding NO flux in response to fluid shear