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Mechano-Sensing and shear stress-shielding by endothelial primary cilia: structure, composition, and function

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Abstract: Primary cilium is an antenna-like and non-motile structure protruding from the apical surface of most mammalian cells including endothelial cells lining the inner side of all the blood vessels in our body. Although it has been over a century since primary cilia were discovered, the investigation about their mechano-sensing and other roles in maintaining normal functions of cardiovascular system has just started in recent years. This focused review aims to give an update about the current literature for the role of endothelial primary cilia in blood flow mechano-sensing and shear stress-shielding. To do this, we first summarized the characteristic features of endothelial primary cilia in terms of structure, dimension, molecular composition, and mechanical properties (e.g., bending rigidity), which are the dominant factors for their functions in mechano-sensing and transduction, as well as vascular protection from the blood flow-induced wall shear stress. We also described the experimental techniques and mathematical models for determining the dimension and mechanical properties of the primary cilium. Then we reviewed the molecular mechanisms underlying mechano-sensing and transduction by endothelial primary cilia and the mathematical model prediction for their roles in redistribution and reduction of wall shear stresses. Finally, we briefly discussed the common cardiovascular diseases, e.g., atherosclerosis, hypertension, and aneurysm, due to defects and malfunction of endothelial primary cilia and suggested potential targets for therapeutic treatments.

Introduction

Primary cilia are microtubule-formed organelles discovered in many types of mammalian cells including epithelial cells (Grisanti *et al.*, 2016; Han *et al.*, 2018), osteocytes (Lee *et al.*, 2015; Spasic and Jacobs, 2017a), chondrocytes (Zhan *et al.*, 2017; Mansini *et al.*, 2018), neurons (Norris and Santoro, 2018), and endothelial cells (Luu *et al.*, 2018; Follain and Goetz, 2018). They were previously thought to be functionless until Praetorius and Spring (2001) observed the influx of Ca^{2+} in response to both micropipette bending and fluid flow towards primary cilia. Since then, primary cilia have been identified to be one of the most vital mechano-sensors and transducers in many tissues and organs. Other roles of endothelial primary cilia in blood vessel development and cardiovascular functions have also been discovered in recent years. Defects in primary cilia could induce various diseases, named ciliopathies, such as mental retardation, polycystic kidney disease, liver disorders, obesity and cardiovascular diseases including hypertension, aneurysm, and atherosclerosis (Luu *et al.*, 2018; Pala *et al.*, 2018; Wang *et al.*, 2021).

Vascular endothelial cells (ECs) lining the inner wall of our blood vessels are continuously exposed to the blood flow. In order to maintain proper functions of the cardiovascular system, ECs should have a variety of mechano-sensors and transducers to sense the blood flow change and adjust the blood perfusion rate (e.g., through vessel diameter) and transport across the vessel wall accordingly. So far, at least a dozen EC mechano-sensors and transducers have been identified either on the EC surface, or at the intra- and trans-EC membrane, or within the EC cytoskeleton. Several prominent ones are endothelial surface glycocalyx, caveolae, integrins, VE-cadherins, PECAM-1, G-protein-coupled receptors and G-proteins, actin filaments, nesprins, and primary cilia (Fu and Tarbell, 2013; Jung *et al.*, 2018; Morikis and Simon, 2018; Liu *et al.*, 2019; Pala *et al.*, 2018). Among these EC mechano-sensors and transducers, the primary cilium is attracting more and

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more attention due to its unique location, one per cell in the middle of the apical surface of ECs, and its long and flexible microtubule-based structure (Fig. 1). It behaves like an antenna, the frontier of the EC that detects the blood flow and transmits the signals to the EC via various signaling pathways along its length. In addition to a sensor and transducer, the primary cilium can serve as a mechanical shield which can reduce and redistribute the wall shear stress acting on the EC surface in pulsatile flows as predicted by a mathematical model (Cui *et al.*, 2020). The aforementioned functions of endothelial primary cilia are determined by their structure, molecular composition, dimension, and mechanical properties (stiffness or bending rigidity), we therefore started the review with these features.

Structure, Molecular Composition, Dimensions and Mechanical Properties

Structure

The antenna-like primary cilium is considered as a cellular organelle, which mainly consists of a membrane, soluble compartment (cilioplasm), axoneme, basal body and ciliary tip. The structure of a general primary cilium is depicted in Fig. 2. It protrudes from a basal body complex with two centrioles. One is called the mother centriole, where the ciliary axoneme is rooted beneath the cell membrane. Another is called the daughter centriole which is perpendicular to the mother centriole. Both centrioles are connected with striated connectors (Hagiwara *et al.*, 2008; Satir *et al.*, 2010). The cilium structure containing the microtubular parts of the cytoskeletal core unit is named the ciliary axoneme. The ciliary axoneme, which is bounded by the ciliary membrane and protrudes from the basal body, consists of nine doublet microtubules distributing evenly along the perimeter (Hoey *et al.*, 2012). Each doublet consists of two tubules named tubule A and tubule B. Tubule A contains 13 protofilaments and tubule B contains 10 protofilaments (Downing and Sui, 2007). Different from the well-known motile cilia, the primary cilia lack two central microtubules and motility (Young *et al.*, 2012). The ciliary membrane is thought to be continuum of cytomembrane, however, the composition of the two are different. Decorated with various proteins, the ciliary membrane seems to coordinate the initiation and transmission of the extracellular stimuli to the interior of the cell. The region between the axoneme and the basal body is the transition zone, which forms a unique subcellular domain and acts as a barrier to prevent free exchange between the cytosol and cilium. At the base of the cilium, there are invaginations of the plasmalemma that abut the ciliary membrane to form the ciliary pocket (Zhang *et al.*, 2019). Cilioplasm, the soluble compartment of the cilium, contains materials that are essential for the proper function of the cilium (AbouAlaiwi *et al.*, 2009a). In the basal body complex, there are structural components termed alar sheet, basal feet, and striated rootlets. Alar sheets are fibrous triangular structures, which originate from the basal body to the transition zone and attach to the cell membrane (Hagiwara *et al.*, 2008). They can regulate protein diffusing into and out of the primary cilium (Hoey *et al.*, 2012). Basal feet are conical structures. Unlike motile cilia, which have only one basal foot, primary cilia have several basal feet protruding from the basal body and connecting with cytoskeleton microtubules to stabilize the basal body (Hagiwara *et al.*, 2008). Striated rootlets are coiled filamentous structures, which extend from the basal body of the cilium towards the cell nucleus and connecting with the membrane structures (Hagiwara *et al.*, 2008). Wheatley (2008) proposed that striated rootlets act like muscle contraction, which pulls the primary cilium towards the cell surface in response to specific stimuli.

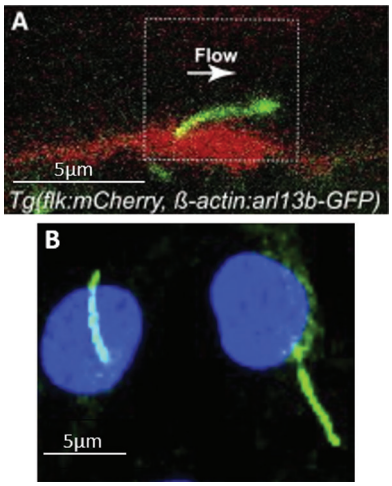

FIGURE 1. Appearance of endothelial primary cilia. (A) High-speed confocal image of a primary cilium in the central region of the dorsal aorta in a live zebrafish embryo. Endothelial cells are marked by flk: mCherry (red) and the primary cilium by β-actin: Arl13b-GFP (green) (Adapted with permission from Goetz *et al.* (2014) https://www.cell.com/cell-reports/fulltext/S2211-1247(14)00066-7). (B) Confocal images of primary cilia marked by acetylated α-tubulin (green), which are extending from the apical surface of human umbilical vein endothelial cells (HUVECs, nuclei stained with DAPI). (Adapted with permission from Russellpuleri *et al.* (2017) https://journals.physiology.org/doi/full/10.1152/ajpheart.00035.2016).

Molecular composition

Various molecules residing on the primary cilia contribute to the maintenance of the proper ciliary structure and function. Any alteration in the fluid pressure and shear stress can be detected by the sensory proteins localized on the cilia and these alterations are transduced into the interior of the cell via various signaling pathways. The signaling pathways organized by primary cilia are rather diverse and depend on the cell type. Here we only focus on several proteins that are widely described on endothelial primary cilia, which are illustrated in Fig. 2.

Intraflagellar transport (IFT) particles, including intraflagellar transport protein 88 (IFT88), are essential for building primary cilia, or ciliogenesis. During ciliogenesis, the centrosome migrates towards the cell surface when a cell enters G_0 (resting state) and the mother centriole attaches to a Golgi-derived vesicle (Satir *et al.*, 2010). Then ciliary axoneme projects from the mother centriole into the vesicle lumen. The axonemal microtubules polymerize at the tip of the projection where the cargo is delivered by IFT. The

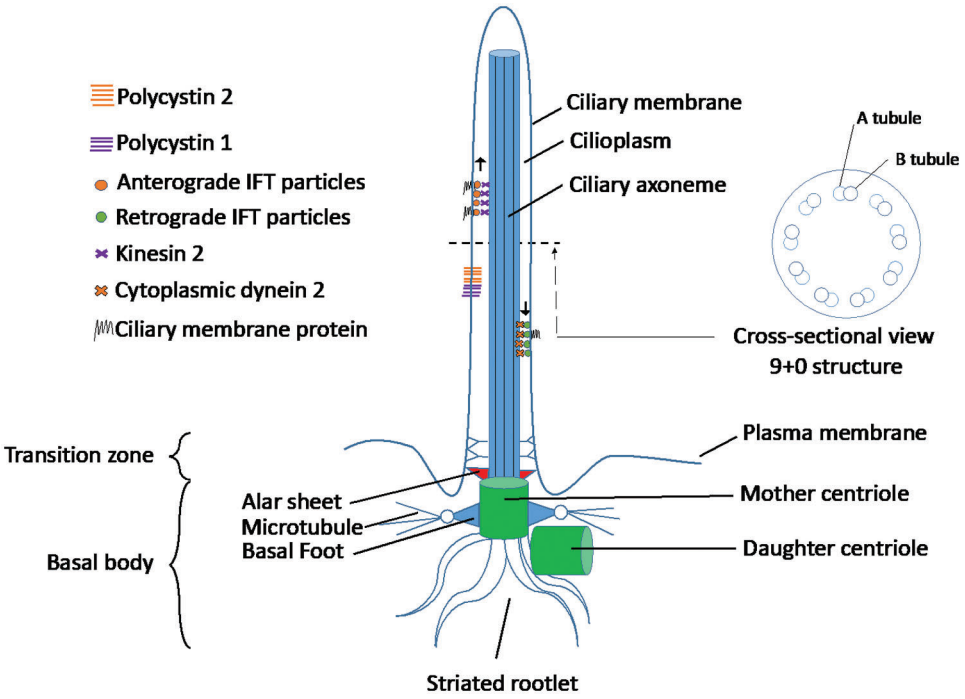

FIGURE 2. Schematic for the structure and molecular composition of a primary cilium. A primary cilium consists of the ciliary membrane, cilioplasm, ciliary axoneme, transition zone with ciliary pocket, and the basal body complex including the mother centriole, daughter centriole, alar sheet, basal feet, and straited rootlets. Basal feet connect with the cell cytoskeleton microtubules to stabilize the primary cilium. The ciliary axoneme consists of nine doublet microtubules evenly distributed along the perimeter of a cilium. In the cilioplasm, IFT particles cooperate with kinesin 2, cytoplasmic dynein 2 and ciliary membrane proteins to assemble and maintain the structure of the primary cilium. Transmembrane proteins, polycystin-1 and polycystin-2, form a complex at the ciliary membrane to mediate calcium influx. (The schematic is drawn based on the information described in Hoey *et al.* (2012); Satir *et al.* (2010); Mohieldin *et al.* (2016)).

vesicle is ultimately exocytosed to expose the primary cilium at the cell surface, where the ciliary growth continues to form the mature cilium (Satir *et al.*, 2010). IFT particles, along with several other molecules (kinesin 2, cytoplasmic dynein 2 and ciliary membrane proteins), contribute to delivering cargos to assemble and maintain the axoneme of the primary cilia, including kinesin-based anterograde and dynein-powered retrograde transport (Satir *et al.*, 2010). Knockdown of IFT88 causes deficiency of primary cilia. This approach has been widely used to investigate the role of primary cilia. Tg737 is a mouse homologue gene of IFT88 and cells with mutation in Tg737 exhibit no or shorter cilia (Pazour *et al.*, 2000; Yoder *et al.*, 2002).

Polycystin-1 and -2 (PC1/2) have been thought to form a heteromeric ion channel complex on the primary cilia, regulating Ca^{2+} influx into the cell (AbouAlaiwi *et al.*, 2009b; Theodorakopoulou *et al.*, 2019). PC1, coded by PKD1, is an 11-transmembrane protein with a long extracellular N-terminus that contains multiple cell adhesion domains (Hughes *et al.*, 1995; AbouAlaiwi *et al.*, 2009b). PC2, coded by PKD2, belongs to a superfamily of transient receptor potential (TRP) ion channel with 6-transmembrane domains (AbouAlaiwi *et al.*, 2009b). Although the interaction of PC1 and PC2 may be controversial (Liu *et al.*, 2018; Ha *et al.*, 2020; Ta *et al.*, 2020), it has been widely accepted that this complex is necessary for normal vascular development and is required for endothelial cilia to sense the blood flow induced forces and promote normal intracellular signaling (AbouAlaiwi *et al.*, 2009b; Diagbouga *et al.*, 2018; Theodorakopoulou *et al.*, 2019). AbouAlaiwi *et al.* (2009b) also demonstrated that in response to fluid shear stress, mouse endothelial cells with knockdown or knockout of PKD2 lose the ability to generate nitric oxide (NO). They thus proposed a new role for PC2 in transmitting extracellular shear stress to intracellular NO synthesis. In addition to PC1 and PC2, there are other Ca^{2+} flux mediating channels including transient receptor potential vanilloid 4 (TRPV4) and PIEZO1/2 (Spasic and Jacobs, 2017b; Lee *et al.*, 2015; Praetorius, 2015).

The TRP channels (e.g., TRPV4, TRPC1, and TRPP2) also exist at the EC membrane and can be activated by mechanical stimuli such as stretch and flow induced-shear stress to regulate endothelial [Ca^{2+}] and cell membrane potential (Kohler *et al.*, 2006; Kwan *et al.*, 2007). These stimuli can either directly activate the TRP channels on the EC membrane or through other mechano-sensors located at the EC surface such as endothelial glycocalyx (Dragovich *et al.*, 2016). In contrast, the TRP channels on the primary cilium might only be activated by bending of the cilium through a force perpendicular to the cilium, similar to that for activating the PC1 channel (Nauli *et al.*, 2008). In addition, the TRP channels on the cilium only regulate the Ca^{2+} influx, not the EC membrane potential.

Dimensions

Methods for determining the dimensions of primary cilia

To investigate dimensions (e.g., length and diameter) of primary cilia, various techniques have been employed including transmission electron microscopy, scanning electron microscopy (Collin and Collin, 2000; Van der Heiden *et al.*, 2006;

Geerts *et al*., 2011; Egorova *et al*., 2012) and fluorescent confocal microscopy (Van der Heiden *et al*., 2006; Geerts *et al*., 2011; Russellpuleri *et al*., 2017; Nauli *et al*., 2008; Antal *et al*., 2017; Vo and Bols, 2016; Iomini *et al*., 2004; Ki *et al*., 2020; Dummer *et al*., 2018; Goetz *et al*., 2014). Transmission electron microscopy can provide the detailed ultrastructure information of the cilium and scanning electron microscopy can provide information regarding cell surface morphology, cilia density and the contact of the cilia with the cell surface. But electron microscopy is expensive, time-consuming and may induce artifacts due to dehydration and other processes during sample preparation. Fluorescent confocal microscopy is chosen when the function and molecular mechanism of cilia are investigated in either live samples or fixed samples without dehydration. But its spatial resolution is not as good as that of electron microscopy.

Due to its convenience, easiness in sample preparation and capability in identifying molecular composition, fluorescent confocal microscopy is the most commonly used technique in determining the ciliary length (Dummer *et al*., 2016). Protruding from the apical surface of cells, primary cilia may appear in random orientations with some cilia standing perpendicular to or tilted (angled) to the cell surface while others lying in the cell surface (focus plane),which are named flat cilia (Dummer *et al*., 2016). Therefore, imaging only one focus plane may underestimate the ciliary length. The application of confocal Z-stacks of many imaging planes can better reconstruct the cilium. From the confocal Z-stacks, three methods have been used to determine the ciliary length: the maximum intensity projection (MIP), the Pythagorean theorem (PyT), and 3D alternative angled slicing (DAAS) (Dummer *et al*., 2016). MIP is the projection of the fluorescently labeled ciliary components with the maximum intensity from each slice of the collected confocal Z-stacks. PyT calculates the actual ciliary length c by using $c^2 = a^2 + b^2$. Here a and b represent the length determined by the MIP and the height of the cilium provided by the number and thickness of Z-slices, respectively. In DAAS, the 3D reconstruction of the cilium from the collected Z-slices is separated by a bilinear interpolation to several sections and each section is measured. The total length of a cilium is the summation of all the section lengths. Each method has its advantages and disadvantages. In general, MIP is the easiest approach that can provide an accurate result for flat cilia but underestimates the length of angled cilia, whereas PyT is able to accurately determine both flat and angled straight cilia (Dummer *et al*., 2016). For irregularly shaped cilia, DAAS is the best, but it is time-consuming (Dummer *et al*., 2016). In addition to the measuring methods, the results may also be influenced by the artifact in the fixation and/or immune-staining procedure, and by the choice to label specific components of the cilia. The common antibodies used to label endothelial primary cilia are monoclonal acetylated-tubulin antibody (Russellpuleri *et al*., 2017; Dummer *et al*., 2018; Ki *et al*., 2020), anti–polycystin-1 antibody (Nauli *et al*., 2008), antibody to polaris18 (Nauli *et al*., 2008), monoclonal antibody IFT-71 ab (Iomini *et al*., 2004), and anti-ARL13b antibody (Ki *et al*., 2020).

Lengths of endothelial primary cilia

Endothelial primary cilia have been investigated in different species and in different types of ECs located at aortas, arteries, arterioles, veins, and microvessels, as well as under different physiological and pathological conditions. Although their lengths vary from 0.5 to over 15 μm, endothelial primary cilia have a constant diameter of ~0.2 μm (Jin *et al*., 2014). The lengths of primary cilia of ECs in various vessel types and in various species are summarized in Tab. 1. They are either measured from the images presented in the literature (Russellpuleri *et al*., 2017; Nauli *et al*., 2008; Antal *et al*., 2017; Vo and Bols, 2016; Iomini *et al*., 2004; Ki *et al*., 2020; Dummer *et al*., 2018; Goetz *et al*., 2014) or directly cited from the literature (Abdul-Majeed and Nauli, 2011; Lim *et al*., 2015b).

The length of endothelial primary cilia varies among various types of ECs and at the different locations of the vascular system. As proposed by Resnick and Hopfer (2007), the longer the cilium, the more sensitive the cell becomes to flow changes. A longer cilium increases the torque, which is the cross product of the lever-arm vector and the force vector, leading to a renewed force equilibrium. The longer the cilium is, the less force is needed to bend the cilium and activate the Ca^{2+} influx (Dummer *et al*., 2016). In addition, with the length increase, the volume of the cilium increases, leading to potential concentration adaptations of ions, proteins, and signaling molecules, and resulting in biological responses. Increased length of the primary cilium results in higher strains on both the cilium structure and the intracellular components (Khayyeri *et al*., 2015). With the cilium elongation, the ciliary membrane also increases in its surface area. Since the composition of the ciliary membrane is highly regulated and contains a distinct population of receptors, increased membrane area might affect the number of available receptors for signaling. Taken together, differences in cilium length might regulate and fine-tune signaling in the cilium and in the cell.

The length of cilia can change during injury and inflammation. For example, the length of cilia increases when exposed to pro-inflammatory cytokines (Wann and Knight, 2012). This is consistent with the finding that the length of cilia in ECs of patients with pulmonary arterial hypertension (PAH) is usually longer than that of healthy ECs (Dummer *et al*., 2018). The EC seems to elongate its cilium to increase its mechano-sensitivity under low biophysical stimuli and to shorten its cilium to reduce the strains under the high stimuli (Khayyeri *et al*., 2015). High shear stresses may lead to endothelial cilia glutamylation and deciliation (Ki *et al*., 2020). The ciliary length varies in different types of ECs and also depends on the cultural conditions. Lim *et al*. (2015b) found that the length of the primary cilia on human umbilical vein endothelial cells (HUVECs) and that on human microvascular endothelial cells (HMECs) are distinct. Even on the same type of cells, the ciliary length varies when the culture media have different serum concentrations and under different culture periods.

Mechanical properties of primary cilia

Methods for determining mechanical properties of primary cilia

Besides structure, molecular composition and dimension, mechanical properties, e.g., stiffness or bending rigidity, are essential for the primary cilium to serve as a blood flow

TABLE 1

Length of endothelial primary cilium

Cell type	Length (μm)	References
Bovine aortic endothelial cells	0.7–6.1	Russellpuleri *et al.* (2017)
Mouse embryonic aorta endothelial cells	2.1–6.2	Nauli *et al.* (2008)
Porcine coronary artery endothelial cells	0.5–2.3	Antal *et al.* (2017)
Mouse femoral artery endothelial cell	0.5–0.8	Abdul-Majeed and Nauli (2011)
Endothelial-like cell from the bulbus arteriosus	1.1–3.4	Vo and Bols (2016)
Human umbilical vein endothelial cells	1.8–11.1	Lim *et al.* (2015b)
	0.5–5.3	Iomini *et al.* (2004)
	1.4–5.3	Ki *et al.* (2020)
Human microvascular endothelial cell	1.1–16.5	Lim *et al.* (2015b)
Human pulmonary microvascular endothelial cells	1.2–3.5	Dummer *et al.* (2018)
Mouse embryonic endothelial cells	1.7–4.1	Dummer *et al.* (2018)
Zebrafish embryonic endothelial cells	3.4–3.9	Goetz *et al.* (2014)

sensor and transducer. The mechanical properties of primary cilia can be determined by combining the bending behavior of the cilium recorded by the image-based techniques and the mathematical models for the force-deflection of cilia (Lim *et al.*, 2015a). Briefly, imaging technology is used to quantify the deflection of primary cilia in response to the known flow. Then the presumed mathematical model for the cilia-fluid flow is employed to simulate the flow-deflection process and the mechanical properties of primary cilia (e.g., bending rigidity) can be determined by iteratively updating during each calculation in the model until the predicted bending shape matches the experimentally observed deflection (Lim *et al.*, 2015a). Imaging technologies range from the application of light microscopes (Schwartz *et al.*, 1997) to that of laser scanning confocal microscopes (Rydholm *et al.*, 2010; Young *et al.*, 2012; Downs *et al.*, 2014), enabling the ciliary bending behavior to be observed from 2 to 3 dimensions. In addition to the *in vitro* sample, high-speed confocal microscopy has been applied to record the bending of the endothelial primary cilia under flow in the vascular system of zebrafish embryo *in vivo* (Goetz *et al.*, 2014; Boselli *et al.*, 2015). In their study, fluorescent transgenic zebrafish lines are used to provide simultaneous imaging of both the endothelial cilia and EC surface. The mechanical property, e.g., the bending rigidity of the cilium, was determined by matching the prediction of a mathematical model for the bending cilium with the observation by confocal microscopy (Goetz *et al.*, 2014; Boselli *et al.*, 2015).

The bending rigidity of the cilium can also be obtained by using an optical trap with a magnetic bead affixed to the primary cilium (Resnick, 2015). The optical trap can apply a compressive (buckling) force to the cilium, which relies on fewer parameters than the application of a shearing force (Resnick, 2016). Resnick (2015) measured the mechanical properties of a primary cilium by using an optical trap to induce resonant oscillation of the structure. Their data indicated that the primary cilium is not a simple cantilevered beam. Instead, the base of the cilium may be modeled as a nonlinear rotatory spring. Interestingly, their data implied that the ciliary base may be an essential regulator of mechano-transduction signaling. Compared to the static method, measuring dynamic responses of the cilium provides more information about the mechanical properties of the cilium as well as the cilium-fluid interaction. It also eliminates the need for the detailed shape fitting, which requires either side-on views of the cilium or insertion of fluorescent transmembrane proteins (Resnick, 2015).

The mathematical models for the cilium-fluid flow (or other applied forces) are necessary in determining the mechanical properties of the cilia. Schwartz *et al.* (1997) first employed the Euler-Bernoulli beam formulation and laminar fluid velocity to simulate the primary cilium under fluid flows. Later, Liu *et al.* (2005) considered a more precise fluid flow profile and allowed a small rotation at the base of the cilium near the EC surface. Downs *et al.* (2014) modeled the primary cilium based on the Euler-Bernoulli beam theory but further included novel features such as the ciliary cap, initial base rotation, and initial cilium bending profile. Young *et al.* (2012) studied the dynamics of the cilium under the Stokes flow using slender-body theory, with the cilium base modeled as a nonlinear rotational spring. Resnick (2015) later determined the mechanical properties of the primary cilium by employing an optical trap to induce resonant oscillations of the structure. Their results revealed that the primary cilium is not a simple cantilevered beam, its base rather behaves like a nonlinear rotator spring. The linear and nonlinear spring constants determined for the base of the primary cilium of mCCD (a mouse cell line derived from the cortical collecting duct) are listed in Tab. 2. Resnick (2016) also employed the optical trap method and found that hypoxia-inducible factor (HIF) stabilization by a pharmaceutical agent significantly weakens the primary cilia on MDCK cells. Khayyeri *et al.* (2015) established a finite-element model for the cell-cilium system, which is composed of nucleus, cytoplasm, cortex, microtubules, and actin bundles. Their model suggested that the length and stiffness of the primary cilia are responsible

TABLE 2

Mechanical properties of primary cilia

Cell type	Parameter	Value	References
mCCD 1296 (d)	Linear spring constant	$(4.6 \pm 0.62) \times 10^{-12}$ N/rad	Resnick (2015)
mCCD 1296 (d)	Nonlinear spring constant	$(-1 \pm 0.34) \times 10^{-10}$ N/rad^2	Resnick (2015)
mCCD 1296 (d)	Bending rigidity	$(1–2) \times 10^{-23}$ Nm^2	Resnick (2015)
IMCD	Bending rigidity	$(1–5) \times 10^{-23}$ Nm^2	Young *et al.* (2012)
MDCK	Bending rigidity	$(0.9–1.96) \times 10^{-23}$ Nm^2	Resnick (2016)
EC	Bending rigidity	$(0.5–1) \times 10^{-23}$ Nm^2	Goetz *et al.* (2014)

Note: Abbreviations: mCCD 1296 (d): a mouse cell line derived from the cortical collecting duct; IMCD: inner medullary collecting-duct kidney epithelial cells; MDCK: Madin-Darby canine kidney cells; EC: endothelial cells from the dorsa aorta of zebrafish.

for the transmission of mechanical stimuli to the cytoskeleton. Most recently, Flaherty *et al.* (2020) modeled the axonemal microtubule as an anisotropic elastic shell with actomyosin-driven stochastic basal body motion and showed that the longer cilia are stiffer than the shorter ones, indicating a length-dependent persistence length for primary cilia.

Bending rigidity and spring constants of primary cilia

So far, almost all the studies investigating the mechanical properties of the cilia that we can find are on the epithelial cells or other types of cells except one on the endothelial cells. As described above, the bending rigidity and spring constants are the parameters charactering the mechanical properties of primary cilia, we thus list in Tab. 2 the values of these parameters for the endothelial cells and for the epithelial cells. From Tab. 2, we can see that the bending rigidity of primary cilia is in the similar range, $0.5–5 \times 10^{-23}$ Nm^2, for the endothelial and epithelial cells.

Primary Cilium as a Mechano-Sensor and Transducer to Blood Flows

Protruding from the middle of the apical surface of an EC into the vessel lumen, the endothelial primary cilium has the best position to sense the blood flow. It has thus been widely recognized as one of the prominent mechano-sensors and mechano-transducers, which could sense and respond to the blood flow and convert the blood flow induced mechanical stimuli into biochemical signaling through its transmembrane proteins and other accessories. In fact, primary cilia are linked to the EC cytoskeleton via the microtubule-organizing center. As a result, the torque exerted on the primary cilium by the blood flow can be transmitted throughout the cell. Meanwhile, there are many other mechano-sensors located on the EC, which can be affected and activated by the alteration of cytoskeletons caused by bending of primary cilia. Although the exact molecular mechanism by which primary cilia act as a blood flow sensor and transducer is still under investigation, the proper mechanical properties (reviewed in the section above) and adequate mechanical strength of the primary cilium is essential for this duty.

Primary cilia in flow sensing

It has been found that endothelial primary cilia participate in regulating actin organization, focal adhesion formation, directional migration, vascular development, and cell permeability via flow sensing (Jones *et al.*, 2012; Goetz *et al.*, 2014; Vion *et al.*, 2018; Chen *et al.*, 2017; Ma and Zhou, 2020). Human induced pluripotent stem cell (hiPSC)-derived ECs lacking cilia do not align to shear stress and have aberrant calcium influx upon shear exposure (Smith *et al.*, 2018). The flow sensing of primary cilia varies with flow magnitudes and patterns. In zebrafish development, through primary cilia, ECs can sense extraordinarily low flow forces and discriminate between the very subtle variations in flow regimes generated along the developing vascular network (Goetz *et al.*, 2014). It is also suggested that endothelial cilia have a critical role in vascular maturation by transducing low-magnitude mechanical stimuli into the molecular signals (Vion *et al.*, 2018; Chen *et al.*, 2017).

In contrast, high shear stresses (HSS) may cause disassembly of primary cilia both *in vitro* and *in vivo*. Ki *et al.* (2020) found that HSS-exposed cilia show stronger glutamylation intensity, which would contribute to the disassembly of EC primary cilia. They also found that HUVECs exposed to a low shear stress (3.96 dyn/cm^2) have similar number of cilia to those of static control cells, but cells exposed to a high shear stress (19 dyn/cm^2) have fewer number of cilia (Ki *et al.*, 2020). Vion *et al.* (2018) showed that in confluent monolayers of HUVECs, about 15% of cells demonstrate an apical primary cilium under static conditions. This percentage decreases with increasing shear stress levels, dropping to 5% under HSS (20 dyn/cm^2). In an *in vivo* study using zebrafish, Goetz *et al.* (2014) found that endothelial cilia are almost completely abrogated in zebrafish trunk and caudal vasculature, concomitant with increases in flow velocity and shear stress levels.

The generation of primary cilia, or ciliation, is highly dependent on the flow pattern rather than the flow magnitude. Egorova *et al.* (2012) discovered that only oscillatory flows could cause ECs to regain their cilia upon deciliation under high shear stress. Venous ECs or atheroprone regions within the vasculature, which are exposed to oscillatory low shear forces, are highly ciliated *in vivo* (Smith *et al.*, 2018). Primary cilia are not uniformly distributed in aortic and arterial vessels. They prefer to locate at the vascular bifurcation and branching points, and curved and arch-shaped sites, where the flow patterns are

disturbed and the flow levels are relatively low (van der Heiden *et al.* 2008; Dinsmore and Reiter, 2016; Eisa-Beygi *et al.*, 2018; Zhang *et al.*, 2019).

Molecular mechanism of flow sensing and transducing by primary cilia

As expected, many molecules should be coordinated for the primary cilia to perform their functions. By flow sensing, endothelial primary cilia can regulate actin organization, focal adhesion formation, directional migration, and cell permeability via heat-shock protein 27 (hsp27) pathway (Jones *et al.*, 2012). Histone deacetylase 6 (HDAC6), a post-transcriptional regulator, can deacetylate α-tubulin and actin-remodeling protein cortactin (Kaluza *et al.*, 2011). Inhibition of HDAC6 activity in human induced pluripotent stem cell (hiPSC)-ECs lacking cilia rescues cilia formation and restores mechanical sensing (Smith *et al.*, 2018).

Fig. 3 demonstrates the major signaling pathway by which a primary cilium senses and transduces the blood flow induced forces (drag and shear) into ECs. Bending of a primary cilium by the drag and shear forces triggers the stretch-activated channels polycystin-1/polycystin-2 (PC1/2), inducing increase in [Ca^{2+}] in EC cytoplasm, via Ca^{2+} influx and release from the internal store (AbouAlaiwi *et al.*, 2009a; Besschetnova *et al.*, 2010; Pala *et al.*, 2018). Cytosolic Ca^{2+} activates calcium-dependent protein kinase (PKC) and forms complex with calmodulin (CaM) (Pala *et al.*, 2018). The Ca^{2+}-CaM complex can activate endothelial nitric oxide synthase (eNOS) through serine-threonine kinase/protein kinase B (Akt/PKB) signaling (Pala *et al.*, 2018). The activated eNOS induces upregulation of nitric oxide (NO) (AbouAlaiwi *et al.*, 2009a; Pala *et al.*, 2018). NO derived from eNOS is an important vasodilator, which can regulate vascular tone and blood flow (Yen *et al.*, 2015; Zhang *et al.*, 2019). NO can also regulate endothelial permeability, control vascular smooth muscle proliferation, inhibit platelet aggression and adhesion, and inhibit leukocyte adhesion and vascular inflammation (Ma and Zhou, 2020; Yen *et al.*, 2015; Zhang *et al.*, 2016; Zhang *et al.*, 2019). Using the ECs from the aorta of a mouse embryo in an *in vitro* experiment, Nauli *et al.* (2008) demonstrated that the wild type ECs responded to the shear stress with the increase in [Ca^{2+}] and NO, whereas the ECs mutant in PKD1/PKD2 and Tg737 did not have the response (Nauli *et al.*, 2008; AbouAlaiwi *et al.*, 2009b). It is notable that increasing in [Ca^{2+}] by tangential forces (drag and shear) is a cilia-specific mechanism, which is evidenced by the observation that cilia-genetic-deficient ECs still show increases in cytosolic Ca^{2+} in response to other types of mechanical forces (circumferential stretching force and manipulator touching of the EC apical surface), as well as pharmacological stimuli (acetylcholine treatment) (Nauli *et al.*, 2008).

In addition to increasing cytosolic [Ca^{2+}] acutely upon the flow, the antenna-like cilium is thought to amplify the cytoskeletal strain and exert a prolonged effect on the expression of shear responsive transcription factors, including Krüppel–like factor 2 (Klf2) and Krüppel–like factor 4 (Klf4) (Egorova *et al.*, 2011; Egorova *et al.*, 2012; Ten Dijke *et al.*, 2012). In general, Klfs are induced by the HSS, and they coordinate a major part of the phenotypic response of ECs to shear forces (Egorova *et al.*, 2011). It has been proposed that under HSS, the regulation of EC function and the establishment of a quiescent, anti-inflammatory, and anti-thrombotic phenotype are largely rendered through the family of Klfs (Shaaban and Duerinckx, 2000). Expression of Klfs is downregulated in areas where flows are low and disturbed (Egorova *et al.*, 2012; Zhang *et al.*, 2019), but the ciliated ECs exhibit more expression of Klfs than non-ciliated ECs (AbouAlaiwi *et al.*, 2009a), indicating a potential role of endothelial cilia in the quiescent state of the healthy vessel.

Endothelial primary cilia also play a role in maintaining normal morphology of ECs lining vascular walls. Under fluid shear stresses (FSS), the wild-type ciliated ECs retain cobblestone morphology, while the non-ciliated ECs undergo endothelial-mesenchymal transition (EndoMT) and become spindle-shaped (Ten Dijke *et al.*, 2012). It was found that Klf4 overexpression prevents flow-induced EndoMT in non-ciliated cells by maintaining the cobblestone morphology of ECs (Ten Dijke *et al.*, 2012).

Besides morphological maintenance of ECs under blood flows, endothelial primary cilia contribute to EC directional migration and vascular stability. Vion *et al.* (2018) found that primary cilia strongly enhance EC sensitivity to bone morphogenic protein 9 (BMP9), particularly under low flows. Activin receptor-like kinase 1 (ALK1) is the receptor of BMP, which is essential for the EC polarization and migration against blood flows (Rochon *et al.*, 2016). The BMP–ALK1–SMAD pathway is important for EC quiescence and the vascular stability (Rochon *et al.*, 2016; Vion *et al.*, 2018). ECs lacking primary cilia display reduced

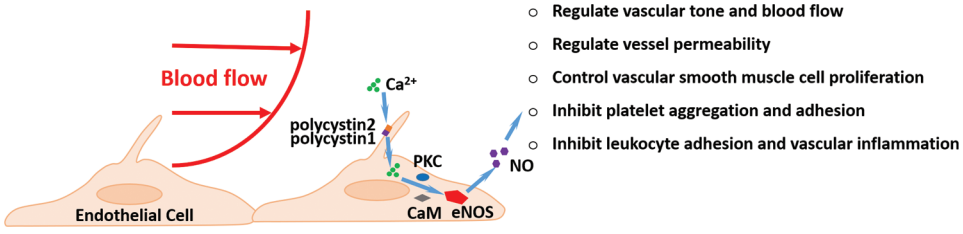

FIGURE 3. The role of endothelial primary cilia in mechano-sensing and transduction under the blood flow. Bending of a primary cilium by the blood flow-induced drag and shear forces triggers the stretch-activated channels polycystin-1/polycystin-2, inducing increase in [Ca^{2+}] in EC cytoplasm, via Ca^{2+} influx and release from the internal store. Cytosolic Ca^{2+} activates calcium-dependent protein kinase (PKC) and forms complex with calmodulin (CaM). The Ca^{2+}-CaM complex activates endothelial nitric oxide synthase (eNOS) to release nitric oxide (NO). NO can dilate the vessel and regulate vessel permeability. NO can also inhibit oxidative stress, inflammation, leukocyte adhesion and platelet aggregation.

polarization against blood flows, especially at low and intermediate flows, and demonstrate a stronger migratory behavior and random regression of vessels (Vion *et al.*, 2018).

Endothelial primary cilia also mediate Wnt, Sonic Hedgehog (SHH) activity and Notch signaling pathways during EC development and function (Guo and Wang, 2009). By detecting the expression and localization of several core proteins of the Wnt/β-catenin and Wnt/planar cell polarity (PCP) signaling pathways, Sheng *et al.* (2018) elucidated the relationship between the Wnt signaling pathways and ciliogenesis. By detecting the expression of PTCH1 and GLI1, reference genes for activating the Sonic Hedgehog (SHH) pathway, Vion *et al.* (2018) discovered that silencing either IFT88 or KIF3a leads to decrease in SHH activity. Chen *et al.* (2017) found that during vascular myogenesis in zebrafish, the primary cilia are responsible for recruiting vascular mural cell (vMC) through a foxc1b mediated Notch pathway in the arterial vessels of zebrafish embryos. Recently, Liu *et al.* (2019) demonstrated that via Notch signaling, the cilia modulate hematopoietic stem and progenitor cells (HSPC) development, especially in hemogenic endothelium (HE) specification in zebrafish embryos. HSPCs are capable of producing all mature blood lineages, as well as maintaining the self-renewal ability throughout life (Liu *et al.*, 2019).

Redistribution and Reduction of WSS by Primary Cilia

Independent of mechano-sensing and transduction, due to its unique location, dimension, structure and mechanical properties, the primary cilium has recently proposed to redistribute and reduce the WSS under real physiological pulsatile flows, and thus play a protective role for the ECs lining the blood vessel walls. Cui *et al.* (2020) modeled the EC primary cilium base as a nonlinear rotational spring and applied an explicit immersed boundary-lattice Boltzmann method to numerically investigate the dynamics of primary cilium in pulsatile blood flows with two-way fluid–structure interaction. Their study predicted that due to the obstruction of the primary cilium, the WSS distribution on the cell surface no longer remains uniform as in the absence of cilia. The presence of the cilium reduces the overall level of the WSS, especially at the region near the cilium anchor point. Fig. 4 demonstrates this role of endothelial primary cilia in WSS shielding. In addition, primary cilia can do a periodic flapping during each cardiac cycle, which depends on the peak Reynolds numbers (Re_{peak}) and Womersley numbers (Wo) (Cui *et al.*, 2020). When subject to fluid flows with higher Re_{peak} or lower Wo, the flapping amplitude, tip angular speed, basal rotation and maximum tensile stress all increase in cilia (Cui *et al.*, 2020). Under lower Re_{peak} and higher Wo, the flapping pattern in primary cilia transfers from two-side into one-side (Cui *et al.*, 2020). They also predicted that under pulsatile flow conditions, the maximum tensile stress in the cilium can propagate periodically within a certain distance to the base instead of at the base all the time (Cui *et al.*, 2020). Their findings show the potential protective role of cilia in vascular ECs by redistributing and reducing the blood-flow induced forces. The mild disturbance by the interaction between the cilia and fluid flow may also be good for mass transport near the vessel wall to bring the nutrients and remove the metabolic wastes.

The Role of Primary Cilia in Cardiovascular Diseases

Dysfunction of primary cilia induces many diseases including cardiovascular diseases. These diseases are collectively named as ciliopathies. Specifically, malfunction of endothelial primary cilia in shear forces (or tangential forces) sensing causes atherosclerosis, hypertension, and aneurysm formation. This section briefly summarizes the role of endothelial primary cilia in these diseases. Extensive reviews can be found in (Luu *et al.*, 2018; Pala *et al.*, 2018; Wang *et al.*, 2021).

Atherosclerosis plaques preferentially occur at bifurcations, branch points and inner surfaces of arched arteries with relatively low and disturbed blood flows, where primary cilia are usually enriched (Hoi *et al.*, 2011). Van der Heiden *et al.* (2006) investigated the correlation between the location and the frequency of endothelial primary cilia and atherogenesis. They found that primary cilia are located at the atherosclerotic predilection sites, where the flow is disturbed, in wild type mice and they occur on and around atherosclerotic lesions in apolipoprotein-E-deficient mice, which have significantly more primary cilia in the aortic arch than wild type mice. The reason for why there are more cilia in atherosclerosis-prone regions is unclear. But the lack of endothelial cilia in vascular branches results in significant upregulation of the expression of the genes encoding the pro-inflammatory cytokines, the inflammatory adhesion molecules, the macrophage marker, and the lymphocyte marker (Dinsmore and Reiter, 2016). These are the key factors causing atherosclerosis. They also reported that when the mice with genetic constitutive deletion of endothelial primary cilia were fed with a high-fat and high-cholesterol diet, compared to the wild type mice, they exhibited larger atherosclerotic plaques, accompanying with decreased levels of eNOS and Klfs but increased levels of pro-inflammatory cytokines. Some studies also show a protective role of primary cilia in atherosclerosis by attenuating vascular calcification in mice (Egorova *et al.*, 2012).

Autosomal dominant polycystic kidney disease (ADPKD) is caused by mutations in PKD1 or PKD2 genes encoding the proteins polycystin-1 or polycystin-2. The patients with ADPKD also show many cardiovascular manifestations such as hypertension, cardiac valveabnormalities, and pericardial effusions (Luciano and Dahl, 2014; Lai *et al.*, 2016; Diagbouga *et al.*, 2018). Even with the transplanted kidney, the patients with ADPKD still suffer from cardiovascular complications (Luu *et al.*, 2018), suggesting the essential role of primary cilia in maintaining normal cardiovascular functions. Impaired polycystins lead to abnormal intracellular signal transmission in ECs, resulting in reduced NO production and increased oxidative stress and asymmetric dimethylarginine (ADMA) release, followed by systemic vasoconstriction and hypertension. It also causes regional vasoconstriction, impaired renal blood flow, vascular remodeling, and renal hypoxia. These

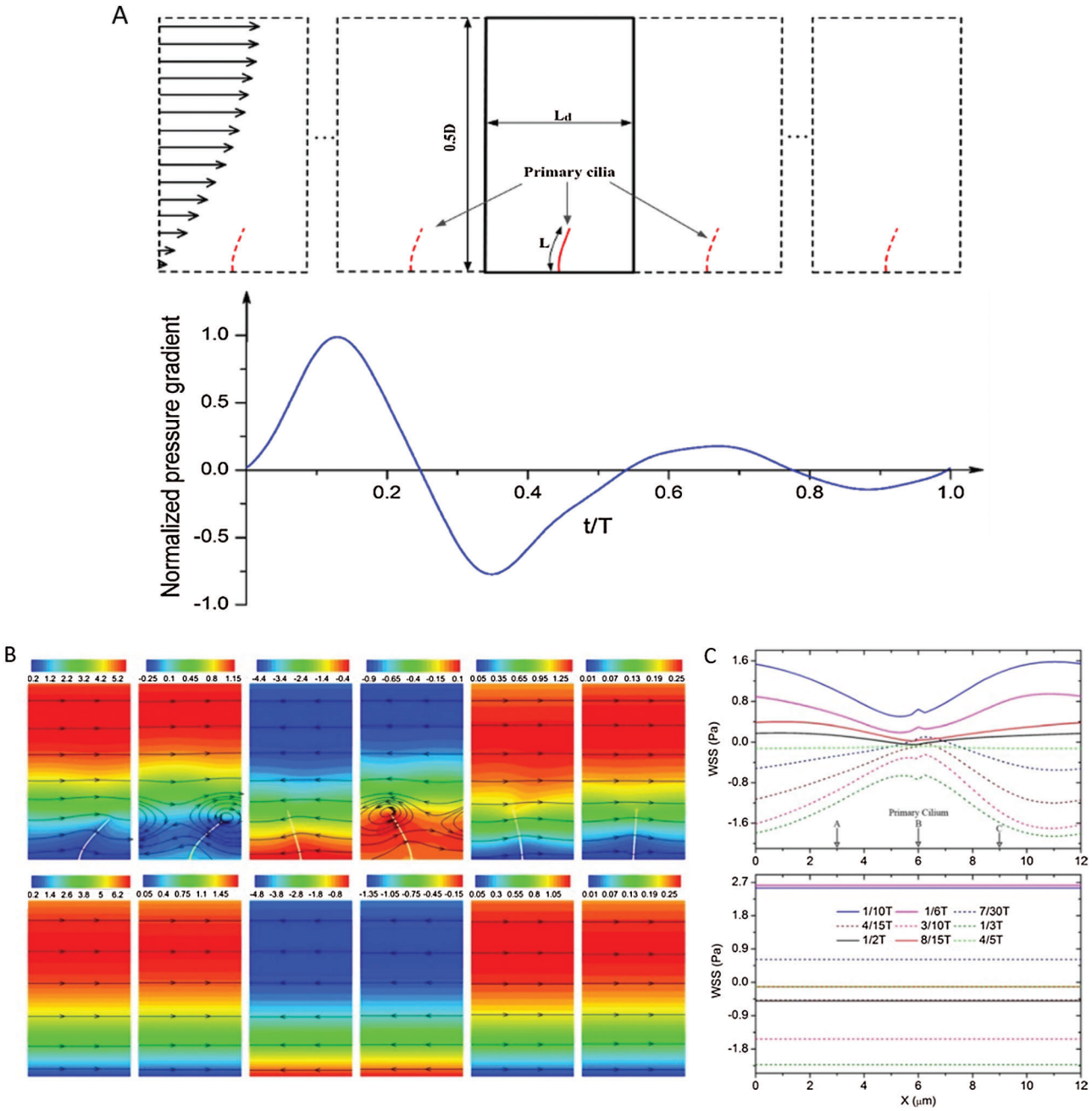

FIGURE 4. The role of endothelial primary cilia in wall shear stress shielding. (A) Schematic view of the primary cilia periodically located at the center of individual endothelial cells lining the blood vessel wall (upper) and pressure waveform (bottom); (B–C) Comparison of the velocity contours and streamlines near the vessel wall (B) and the wall shear stress (WSS) distributions (C) in the presence (top panel) and absence (bottom panel) of primary cilia (Reprinted with the permission from Cui *et al.* (2020), https://link.springer.com/article/10.1007/s10237-019-01192-8).

disorders increase the levels of hypoxia-induced factor 1α (HIF1α) and other angiogenetic factors and lead to increased apoptosis and fibrosis in the renal tissue in patients with ADPKD (Theodorakopoulou *et al.*, 2019). Primary cilia further play a role in pulmonary arterial hypertension (PAH). Inflammatory cytokines could lead to a significant elongation of healthy endothelial cilia and anti-inflammatory cytokines, such as IL10, could block and reverse the cilia elongation (Dummer *et al.*, 2018). However, compared to the healthy controls, primary cilia of ECs from the PAH patients are significantly longer. But they do not elongate further upon pro-inflammatory stimulation nor shorten under anti-inflammatory treatments (Dummer *et al.*, 2018). Loss of ciliary length regulation upon cytokine stimulation is one of the endothelial dysfunctions in PAH (Dummer *et al.*, 2018).

An aneurysm is a formation of a swelling in an area of a blood vessel that can rupture, leading to bleeding and possibly to death if occurs in vital organs such as brain. Aneurysm formation and rupture are one of the major complications associated with ADPKD because polycystin-1 and polycystin-2 at endothelial primary cilia are required in blood vessels for proper flow sensing and transduction. AbouAlaiwi *et al.* (2014) showed that abnormal function of

mechano-sensory cilia leads to survivin downregulation, which is associated with abnormal ploidy formation and eventually contributes to vascular aneurysm phenotypes. Liu *et al.* (2018) found that in the mice model of intracranial aneurysm (IA), the number of primary cilia significantly decreases in the IA region.

Conclusions and Perspectives

The unique location, structure, mechanical properties and molecular compositions, all of them contribute to the function of endothelial primary cilia in maintaining normal cardiovascular system. As an antenna, the primary cilium can sense the changes in external blood flows and transduce them into ECs via various signaling pathways along the ciliary membrane, cilioplasm, axoneme, and at the basal body complex. Even without the signaling function, the endothelial cilium can still behave like a structural component to redistribute and reduce the flow generated wall shear stresses to protect the vessel wall. The disturbance near the vessel wall by the interaction between the cilium and fluid flow also favors the mass transport at the EC surface to bring nutrients from the bulk flow and remove the metabolic wastes. Lack of primary cilia or malfunction of cilia cause many cardiovascular diseases including atherosclerosis, hypertension, and aneurysm formation.

Although the molecular mechanism by which endothelial primary cilia perform their flow sensing and transducing has been partially elucidated, further investigation is highly expected. With the development of super high resolution optical microscopy, more detailed information for the structural (molecular) components of EC primary cilia can be revealed, especially when the ECs are alive, and the forces are known. As the force and stress distribution on a cilium and other structural and mechanical factors are beyond the capability of current experimental approaches due to the nanometer scales in its structure and the pulsatile nature of the real physiological flows, more sophisticated mathematical models and numerical approaches should be developed. By combining the model predictions with the experimental observations, the more detailed molecular mechanism underlying the cilium sensing-transducing can be elucidated under realistic physiological conditions. In addition, the interactions of primary cilia with other endothelial mechano-sensors and transducers also deserve investigation to give a more comprehensive understanding for how the ECs sense and transduce the external stimuli into internal signals for their functions.

The understanding for how the primary cilia function will also help to design proper therapies for cardiovascular diseases due to lack of cilia or malfunction of cilia. For example, targeted gene therapy for PKD1/PKD2 may reverse the ADPKD and related cardiovascular diseases such as hypertension and aneurysm. Pharmacological agents can be invented to either help in generating primary cilia or in informing alternative flow sensors and transducers on ECs in the absence of cilia. Since pulsatile flows at certain levels can help regenerate lost primary cilia, proper exercises can be designed for this purpose.

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