Chapter 5

Sensing Fluid-Shear Stress in the Endothelial System with a Special Emphasis on the Primary Cilium

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Additional information is available at the end of the chapter

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Abstract

Fluid shear stress (FSS) is able to generate phenotypic changes in the cells in direct contact with the strain force. In order to detect and transduce FSS into intracellular pathways, biological systems use a specific set of sensors, called mechanosensors. The process involves the conversion of the mechanical force into a chemical or electrical signal. Primary cilium is a non-motile organelle that emanates from the cell surface of most mammalian cell types that act as a mechanosensor. Increasing evidence suggests that primary cilia are key coordinators of signaling pathways in tissue homeostasis and when defective may cause human diseases and developmental disorders. Here, we will describe the endothelial primary cilium as a mechanotransductory organelle sensing FSS. To fulfill this function, primary cilium requires the localization of mechanoproteins, polycystin-1 and -2, in their membrane and the structural gene product, polaris. Physiologically, deflection of primary cilium increases the intracellular calcium concentration triggering a signaling pathway that leads to nitric oxide (NO) formation and vasodilation. Additionally, ciliopathies, such as polycystic kidney disease and atherosclerosis, will also be discussed. We also analyze available information regarding a trio of membrane receptors involved in FSS sensing and transducing such as vascular endothelial growth factor receptors (VEGFRs) and its coreceptor neuropilin (NRP), as well as purinergic receptors (P2Y2). Whether or not they modulate, the primary cilium role in sensing FSS is poorly understood. This chapter highlights the main relevance of primary cilium in sensing blood flow, although exact mechanisms are not fully known yet.

Keywords: shear stress, endothelial dysfunction, primary cilium, nitric oxide, reactive oxygen species, neuropilin, purinergic receptors

1. Introduction

Blood, urine and air are primary examples of biological fluids. Biophysically, fluids can be classified into four basic types: ideal fluid, real fluid, Newtonian fluid and non-Newtonian fluid. Among them, biological fluids are classified only as Newtonian and non-Newtonian. Blood and urine belong to non-Newtonian biofluids since their viscosity is not a constant with respect to the rate of shearing stress; moreover, the removal of the stress causes them to return to their initial viscosity state [1].

In order to regulate blood flow, vascular smooth muscle cells (VSMC) induce changes in blood vessel diameter by contraction and relaxation mechanism. Smooth muscle contraction is regulated by central neuronal as well as by local control mechanisms. In particular, the local control, also termed autoregulation, is an important mechanism of vascular tone regulation, maintaining the immediate control of the amount of blood flow within a specific region. Vessel diameter decreases by a sudden increase of transmural pressure and increases by faster flow or high shear stress [2]. Flow shear stress (FSS) is one of the important blood flow-induced hemodynamic forces (**Table 1**) acting on the blood vessel and is determined by the velocity of blood flow, fluid viscosity and vessel geometry [2–5]. An important determinant of shear stress is the viscosity of blood; shear stress is the energy transferred to the vessel wall due to interaction with a fluid in motion [6]. Shear stress forces are imposed directly to the endothelium and modulate endothelial structure and function through local mechanotransduction mechanisms [5, 7]. FSS is crucial for vascular homeostasis [5].

In a normal homeostatic mechanism and steady laminar shear stress, endothelial cells respond promptly with an increase in the cytosolic calcium (Ca^{2+}), activation of endothelial nitric oxide synthase (eNOS) and nitric oxide (NO) production [4, 8] and with the ultimate gene modulation [3, 5, 8]. However, besides laminar flow, oscillatory and turbulent flow patterns are also imposed to the endothelium, which has then to continuously fine-tune its activities as a response [5].

Several structures and processes have been implicated in FSS mechanotransduction into specific biochemical signals, intracellular signaling pathways and gene modulation [5]. Among those structures implicated, the primary cilium emerges as a key sensor of FSS under physiological conditions [9]. Nevertheless, in vascular injury occurring as a result of hypertension for example, normal homeostatic mechanisms are disturbed and vessel wall becomes dysfunctional associated with impaired formation and/or function of primary cilium [5, 10]. Moreover,

Hemodynamic forces	Generated by	Force name
Distention force	Surrounding muscle	Stretch force
Contractile force	Differential pressure along the vascular system	Compression force
Pulsatile force	Turbulent flow of blood	Cyclic strain
Systolic force on intima surface (endothelial cells)	Blood flow	Pressure force
Drag force on intima surface (endothelial cells)	Blood flow	Shear stress

Table 1. Various types of hemodynamic forces acting on the blood vessel wall.

ciliopathies or ciliary dysfunctions can lead to a series of disorders such as PKD, hypertension and atherosclerotic lesions [9].

Physiologically, the primary cilium, a solitary non-motile microtubule-based organelle, protrudes from the surface of mammalian cells [11] into the surrounding tube (vessel/tubule) lumen. Primary cilia work as a chemo- and mechanosensors responding to diverse stimuli, including FSS [12, 13].

Since the importance of the primary cilium as a sensor of FSS has been described mainly in the kidney and in the blood vessels, it is worth to describe in brief some aspects of the renal system.

During the process of urine formation, the flow of the ultrafiltrate through the proximal tubule (PT) is pulsatile, with variable oscillations due to the heart rate and to tubuloglomerular feedback mechanism mediated by the *macula densa*. This ultrafiltration mechanism exposes kidney epithelial cells to a constant FSS in a similar way that mimics vascular endothelial cells [14]. Changes in urinary flow through the nephron depend on short-term variations in glomerular filtration rate, tubuloglomerular feedback and fluid absorption along the nephron as well as on long-term factors such as high salt or high protein diet, hypertension and early stages of diabetes [15]. Variations in luminal urinary flow alter the mechanical forces (shear stress, stretch and pressure), which in turn affect epithelial cells in the nephron. Thus, kidney epithelial cells exhibit a highly differentiated brush border composed by microvilli, glycocalyx and primary cilium in order to sense apical shear stress [14]. Tubular flow acts as potent modulator of epithelial kidney cell phenotype by affecting the organization of the cytoskeleton and the brush border, changing cell polarity and modifying various cellular functions such as solute reabsorption and extracellular matrix remodeling.

Recently, several reports showed that an alteration of primary cilia length and function is associated with acute and chronic kidney disease [12, 13, 16–21]. However, the underlying mechanisms behind these associations are still unclear. The main scope of this chapter focuses on the role of primary cilium as one of the multiple mechanotransduction machineries in sensing FSS in the endothelial vascular and epithelial renal system.

In the blood vessels, endothelial cells exhibit cilia that have been involved in blood vessel autoregulation [9], as well as in the pathogenesis of hypertension [9] and atherosclerotic lesions [22, 23].

In this chapter, we will present the physiological role of the endothelial primary cilium as a sensor of FSS. We will make a short review about potential implication of reactive oxygen species (ROS), vascular endothelial growth factor (VEGF) and purinergic signaling as modulators of the function of primary cilium. Finally, implication of the primary cilium dysfunction in the kidney and atherosclerotic lesions will be overviewed.

2. Structure of the primary cilium

Primary cilia differ from motile cilia in both structure and function and are usually classified as non-motile organelles, which were first described in 1867 by Alexander Kowalesky in

vertebrate cells [24]. Motile cilia contain microtubules (MT) arranged in a (9 + 2) manner consisting of a nine doublets MT ring surrounding a central pair of MT and presenting protein spokes and dynein inner and outer arms necessary for movement. In contrast the primary cilium shows (9 + 0) organization with nine pairs of MT at the periphery lacking the central pair of MTs, as well as the protein spoke and the dynein arms (**Figure 1**). In both cases, MT extend from a basal body originating from "mother" centriole of the centrosome [25]. The structure and maintenance of the primary cilium are regulated by intraflagellar transport (IFT) particles [26].

In physiological conditions, nearly all quiescent differentiated mammalian cells exhibit a primary cilium, which emanates from the surface as a single long hair-shaped projection [27]. Therefore, primary cilia are found in a large number of mammalian cells including stem cells, epithelial and endothelial cells [19]. Their presence was demonstrated in adult vascular system (reviewed in [2]), developing chicken endocardium [4], embryonic mouse aortic endothelium [9], cultured human umbilical vein endothelial cells (HUVECs) [28, 29] and epithelial cells including *macula densa* [30] or tubular epithelial cells [20]. Nevertheless, alteration in the number, length and structural features has been implicated in pathological conditions such as polycystic kidney disease, atherosclerosis and hypertension, among others [18, 23, 31].

Depending on structural and functional features, five distinct domains were described in the primary cilium [2] (**Figure 1**):

1. The ciliary membrane housing many sensory receptors and channels supporting sensory function of cilia.
2. The soluble compartment or cilioplasm constituting the fluid between the ciliary membrane and the axoneme and where IFT machinery is located to assemble and maintain the cilia.
3. The axoneme composed of tubulin that supports ciliary transport. It is composed of nine pairs of MTs.
4. The ciliary tip is the distal part of the axoneme where specialized proteins localize whose function is still unclear.
5. The basal body, the network foundation from which the primary cilium emanates.

3. Primary cilium sensing fluid-shear depends on mechanoproteins polycystins and structural polaris

3.1. Intraflagellar transport

IFT is required for assembly and maintenance of cilia. Briefly, ciliogenesis is initiated in the apical cytoplasm at the basal body. Proteins involved in cilium formation concentrate and assemble into complexes that migrate along the cilia axonemal microtubules through a process called IFT. The anterograde movement of particles from the cell body to the tip of the flagella/cilia is driven by

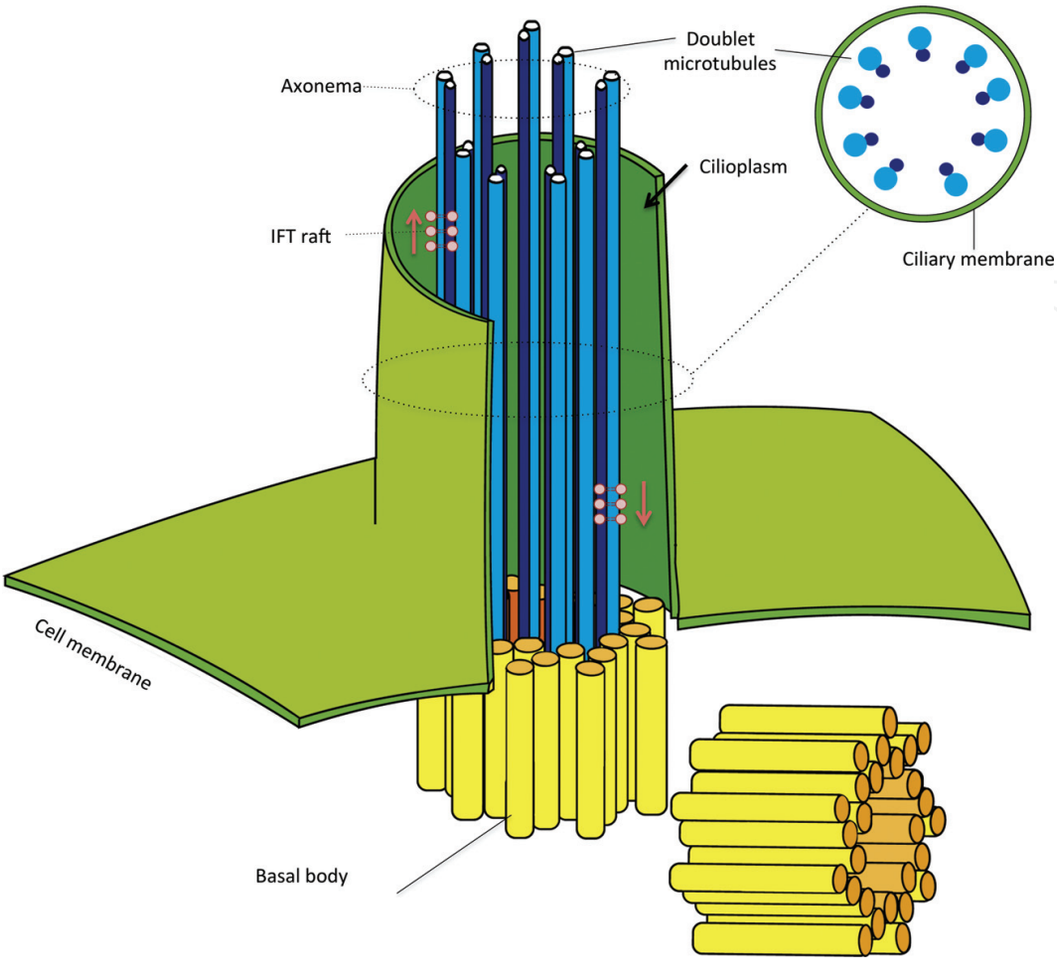

Figure 1. Scheme of the primary cilium. Longitudinal section showing the axoneme with the nine doublets of microtubules originating from the basal body. The right part of the figure shows transversal sections of motile and non-motile primary cilia. Note the absence of the central pair of microtubules and dynein arms in the primary cilium. Figure adapted with permission of [90].

kinesin II [26], whereas the retrograde movement from the tip back to the cell body is driven by cytoplasmic dynein [32]. The protein polaris is the gene product of the IFT particle 88 (*ift88*) that in mammalis is homologous to the gene *Tg737*. This protein is localized to the basal body [26, 33] and is required for ciliogenesis.

3.2. Polycystin-1, polycystin-2 and polaris

Among sensory molecules housing into the primary cilium, both polycystin-1 (PKD1) and polycystin-2 (PKD2) have been described. These are membrane integral proteins. Experimental data show that they are highly expressed in human endothelial and epithelial cells and are required for normal physiological cilia function (reviewed in [2]). The importance of these proteins has been highlighted due to the finding that mutations in *pkd1* or *pkd2* genes result in polycystic kidney disease, hence their name [9].

PKD1 is a 3327 amino acids long transmembrane protein with 11 membrane-spanning domains. Its long extracellular N-terminus has a mechanosensory function, while its short intracellular

C-terminus is involved in intracellular signaling and interaction with PKD2 [34, 35]. PKD1 has been shown to mediate fluid-shear sensing in epithelial and endothelial cells [9, 36].

PKD2, a 968 amino acids long protein, is a non-selective Ca^{2+} permeable transient receptor potential (TRP) channel consisting of six membrane-spanning domains and intracellular C- and N-terminal domains [37]. The sensory function of PKD2 depends on PKD1 and has to be localized to endothelial primary cilia [38]. Accordingly, PKD2 functioning as a Ca^{2+} channel [29] allows extracellular Ca^{2+} influx into the cilioplasm in response to FSS [39]. Thus, mechanistically, PKD1 and PKD2 interact through their C-terminus [29, 34, 35] and localized to the ciliary membrane; they are able to detect extracellular FSS and to increase cytosolic Ca^{2+}. This turns on a signaling cascade leading to the production of NO [9, 38, 40].

A series of mutation and deletion experiments demonstrated that besides PKD1 and PKD2, the protein polaris also orchestrates FSS sensing. The physiological Ca^{2+} and NO increase in response to FSS is abolished when the *pkd1*, *pkd2* and *polaris* genes are mutated or knocked out [29]. Interestingly, mutations or deletion of *polaris* seem to affect the structural integrity of cilia through the PKD1 and PKD2 mislocalization, which remain concentrated at the basal body [9, 29, 32, 41]. Together these findings evidence that polaris mediates the PKD1 and PKD2 primary cilium localization, implying a polaris cilium sensory function regulation. In order to achieve a proper fluid-shear sensing by endothelial cells and an adequate response, all three components, PKD1, PKD2 and polaris, are thus indispensable.

3.3. Molecular cascade involved in shear stress-induced calcium and NO signaling

FSS leads to cilia bending leading to PKD2-mediated increase of intracellular Ca^{2+} that leads to activation of ryanodine receptors (RyR) and inositol 1,4,5-triphosphate receptor (InsP3R) present in the endoplasmic reticulum, which then releases its stores of Ca^{2+} enhancing the intracellular levels of Ca^{2+} [42, 43]. Subsequently, Ca^{2+} activates several intracellular signaling pathways, including the activation of the eNOS-bound calmodulin, thus increasing the production of NO that diffuses from endothelial cells to neighboring VSMC inducing vasodilatation [2, 29]. This particular pathway is summarized in **Figure 2**.

The works of AbouAlaiwi et al. [29] have helped to elucidate this last mechanism. In order to prove that FSS-dependent primary cilia bending induces extracellular Ca^{2+} influx, they used Ca^{2+} chelator EGTA (ethylene glycol-bis (β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid). In these experiments, EGTA abolished both Ca^{2+} and NO increases. In addition, the inhibitor of the eNOS, N^G-nitro-L-arginine methyl ester (L-NAME) blocked the FSS-induced NO release without affecting Ca^{2+} increase. The same effect was shown after blocking calcium-dependent mechanisms of NO production using calphostin C as an inhibitor of protein kinase C (PKC) or W7 as antagonist of calmodulin. Similarly, inhibiting protein kinase B (PKB)/Akt abolished NO release without altering Ca^{2+} increase. Inhibiting IP_3 kinase using LY-294002 did not alter neither Ca^{2+} nor NO increase. These findings indicate that calmodulin, PKC and Akt/PKB are downstream of the calcium pathway and that they are necessary for NO release during primary cilium-mediated FSS signaling [29] (**Figure 2**).

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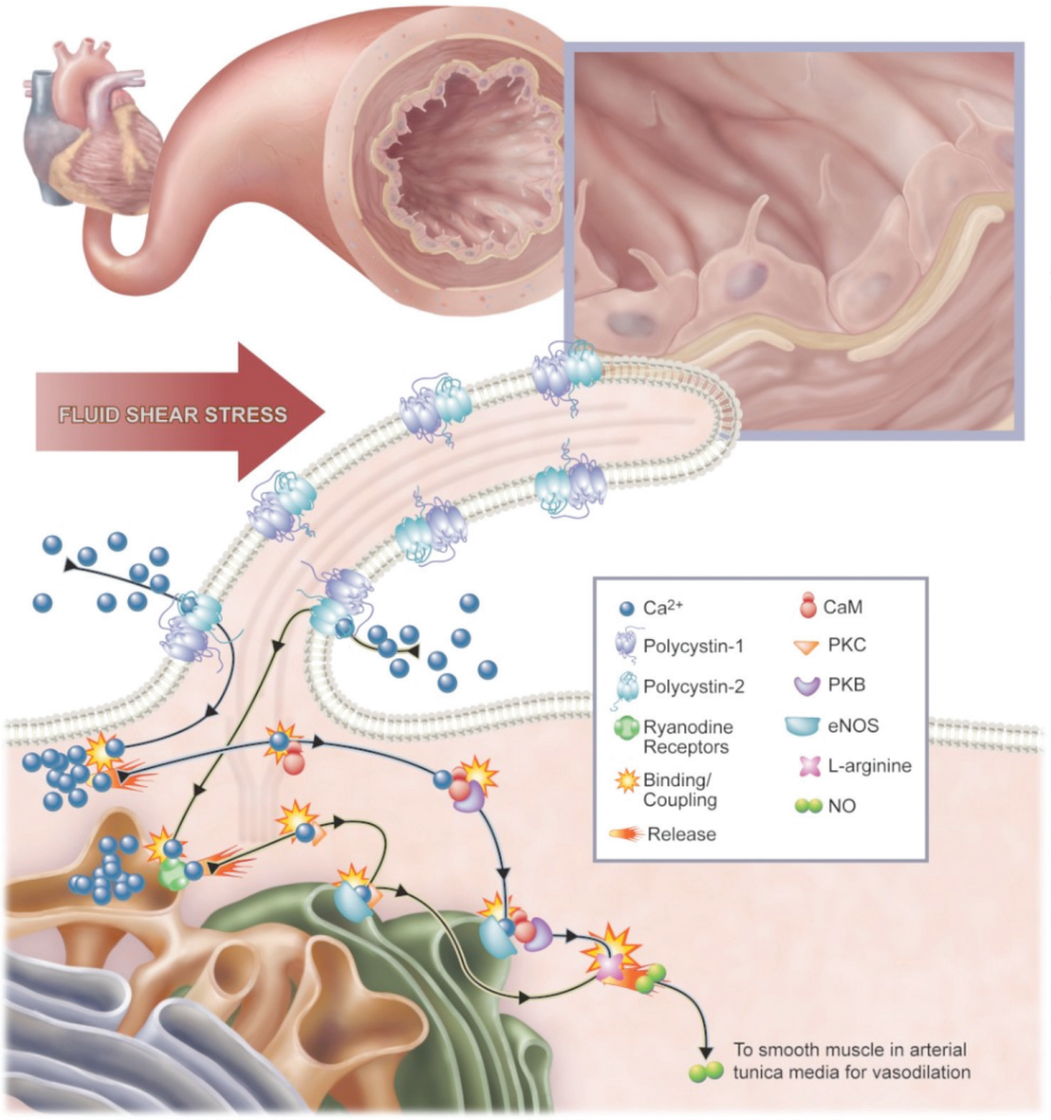

Figure 2. Mechanotransduction of FSS at the endothelial primary cilium. Extracellular FSS leads to cilia bending and activation of polycycstin-1/-2 complex, conducing to extracellular calcium influx. Calcium binds to ryanodine receptors and an efflux of intracellular organelle calcium. This is followed by activation of calmodulin (CaM), protein kinase C (PKC), protein kinase B (PKB/Akt), eNOs and NO production. Figure reproduced with permission [38].

4. The regulation of ciliary function

Changes in fluid patterns generate differential biomechanical forces, which lead to alteration of cilia function or structure [2]. Indeed, almost all blood vessels possess cilia [4, 23]. Particularly, arteries with low FSS or high fluid turbulence have cilia [2]. A prolonged exposure of endothelial cells to high FSS induces the disassembly of cilia [28] and inactivation of PKD1 by proteolytic cleavage [9], suggesting that primary cilia may not be required only to sense high shear stress [2].

The process of disassembly observed here involves the termination of IFT and the inability of the oldest centriole to maintain or initiate the assembly of primary cilia under laminar shear stress [28]. The disassembly of cilia parallels a major rearrangement of the cytoskeleton and an increase of acetylation of MT [18, 44].

In the renal system, tubular flow and ROS act as potent modulators of epithelial kidney cell phenotype also by affecting the organization of the cytoskeleton and the brush border, changing cell polarity and modifying various cellular functions such as solute reabsorption and extracellular matrix remodeling [17]. Under oxidative stress, ROS directly induce the breakdown of the cell cytoskeleton, activate various cell death-associated signals and regulate elongation, shortening and release of cilia [45]. The mechanism and implications of this regulation are still unclear.

5. Reactive oxygen species, shear stress and cilia function

ROS and NO have been implicated in sensing FSS in both vascular homeostasis and diseases [46]. ROS include collective oxygen (O_2) radicals such as superoxide, $O_2^{\cdot -}$ and hydroxyl radical, OH, and non-radical derivatives of O_2, including hydrogen peroxide (H_2O_2) and ozone (O_3). Several sources of ROS have been extensively described in the literature, in which the nicotidamine adenine dinucleotide phosphate (NADP) oxidase (Nox) has been described as one of the main cellular sources of ROS generation in endothelial cells under FSS [47].

Flow patterns and the magnitude of shear determine the amount of ROS produced by endothelial cells, usually an irregular flow pattern (disturbed or oscillatory) producing higher levels of ROS than a regular flow pattern (steady laminar or pulsatile) [48]. In addition to flow pattern, endothelial cells exposed to a prolonged laminar shear stress for more than 24 h display a reduced $O_2^{\cdot -}$ formation and ROS levels [49]. ROS production is closely linked to NO generation: elevated levels of ROS lead to low NO bioavailability, as is often observed in endothelial cells exposed to irregular flow patterns [48]. The low NO bioavailability is partially provoked by ROS reaction with NO to form peroxynitrite ($ONOO^-$), a key molecule that is implicated in oxidative and nitrosative damage [50]. NO can also take part in redox signaling by modifying proteins and lipids via cysteine S-nitrosation and fatty acid nitration, respectively [51], in this respect affecting the regulation of the vascular system [52].

5.1. Free radical signaling and primary cilia

Information related to primary cilium and free radical signaling emerges mainly from kidney research area. However, how ROS can regulate this mechanosensory organelle is not well described in the literature [17, 30, 53]. It is known that renal primary cilia protrude from the epithelial cell surface into the lumen detecting fluid flow and responding to diverse stimuli [12]. Indeed, several reports show that an alteration of primary cilia length is associated with acute and chronic kidney disease [16].

Information about primary cilia acting as an upstream regulator of ROS comes primarily from *in vitro* experiments, in which immortalized *macula densa* cell line (MMDD1) exposed to an increment in shear stress shows augmented NO production, this effect was blunted by silencing polaris protein in primary cilia using si-RNA methodology [54]. In addition, in isolated perfused juxtaglomerular apparatus preparations incubated with the diuretic furosemide (an inhibitor of Na-K-Cl cotransporter), an increase in tubular flow-induced NO production was observed. This suggests that the NO stimulatory effect is independent of Na^+ concentration in the tubular fluid, as well as volume changes, suggesting a direct FSS-dependent regulation [30]. Also, the results elucidate that FSS can stimulate NO production independently of NaCl delivery to the *macula densa*. Therefore, these results indicate that the primary cilium acts as a mechanosensory organelle for FSS inside the nephron tubule via NO.

The opposite mechanism in which free radical species can regulate primary cilia function is showed mainly in renal ischemia/reperfusion (I/R) experiments. I/R setting is characterized by an increase in free radical species production [55]. Acute tubular necrosis induced by I/R on mice model resulted in changes in primary cilium length. Thus, primary cilium was shortened after 4 h and 1 day of ischemia versus non-ischemic control cells, an effect that was blunted after 16 days [16]. The oxidative stress from I/R derived injury is able to break down cell cytoskeleton and activate various cell death-associated signals, like cell autophagy [45]. As presented by Kim et al. [53], the treatment with the antioxidant molecule Mn(III) tetrakis (1-methyl-4-pyridyl) porphyrin (MnTMPyP) during the reperfusion (i.e., recovery) period of damaged kidneys accelerated the normalization of cilia length in experiments of I/R. Concomitantly, they also showed that MnTMPyP decrease oxidative stress and recover nephron tubule morphology, indicating that the ROS signals are an integral part of cilium length regulation. In addition, cultured kidney cells treated with H_2O_2 released a ciliary fragment into the extracellular medium. MnTMPyP treatment inhibited this deciliation process [17, 53]. Moreover, the extracellular signal-regulated kinase (ERK) inhibitor U0126 blocked the cilium elongation of normal and H_2O_2-treated cells [53]. Taken together, these observations show that primary cilia length can be regulated, at least in part, by H_2O_2 through an ERK-dependent pathway. Similar results were found related in acute kidney injury after hepatic I/R from liver transplantation or resection experiments in the kidney [56]. In particular, transient hepatic ischemia caused functional and histological kidney damage, including brush border loss of tubular epithelial cells and tubule atrophy. This cellular damage also induces a shortening and deciliation of kidney primary cilia via ROS/oxidative stress, suggesting that the presence of ciliary proteins in the urine could be a potential indication of kidney injury [17]. Therefore, remote organ injury model can increase the content of O_2^-, and H_2O_2 subsequently shortening the primary cilium length in several nephron sections [56]. These data confirm that free radical species can modulate the primary cilium length, at least in the kidney, but the mechanism and functional implications of such modulation remain unclear.

6. Vascular endothelial growth factor and shear stress

VEGFs are a complex family of glycoproteins that are structurally related to platelet-derived growth factor (PDGF) [57]. Through alternative RNA splicing, VEGF family is constituted by

several isoforms, including $VEGF_{165}$, which has been named VEGF-A or VEGF, the isoform involved in many of the functions attributed to the VEGF family. All members of the VEGF family activate tyrosine kinase receptors known as VEGF receptors (VEGFRs), which include VEGFR-1 (also known as fms-like tyrosine kinase 1 or Flt-1), VEGFR-2 (or kinase insert-domain containing receptor, KDR) and VEGFR-3 [58, 59]. Activation of VEGFRs has been implicated in several vascular functions, including angiogenesis, vascular tone regulation and endothelial cell survival, among others [59–61].

Importantly, VEGF and VEGFRs have also been associated with sensing FSS. High expression of VEGF [62] and the activation of VEGFR2 [63, 64] have been linked to the FSS sensing. Moreover, the activation of VEGFRs generally leads to NO synthesis in many kinds of cells, including endothelial cells [58]. Therefore, it is not surprising that VEGFR2 triggers NO-dependent flow regulation. Jin et al. [63] showed that FSS leads to VEGFR2 activation in a ligand-independent manner and leads to eNOS activation in cultured endothelial cells. Intracellular downstream pathway associated with NO synthesis due to FSS-stimulated VEGFR2 activation included phosphoinositide 3-kinase (PI3K) and PKB/Akt. Interestingly, contrary to PKB/Akt, the PI3K pathway has not been associated to endothelial primary cilium FSS sensory function [29]. Also, *in vivo* experiments confirmed that VEGFR2 is a key mechanotransducer that activates eNOS in response to blood flow [63]. Despite these evidences, as far as we known there is no information related to VEGFRs present in the primary cilium as potential regulator of FSS sensing.

7. Neuropilins and the primary cilium

VEGF can also bind to neuropilins (NRP), a family of transmembrane glycoproteins that play key role in axonal guidance, angiogenesis, tumorigenesis and immunological response [65]. NRPs have been characterized as co-receptors for VEGFRs and plexins, the receptors of the extracellular secreted ligands, belonging to class III semaphorins [60]. In turn, semaphorins are a class of secreted and membrane proteins that were originally identified as axonal growth cone guidance molecules. At least two neuropilin genes, NRP1 and NRP2, have been identified [66]. Genetic studies in mice have confirmed that NRPs are key components of vasculogenesis, angiogenesis and lymphangiogenesis [65, 66]. Nevertheless, NRPs can bind to growth factors such as VEGF, placental growth factor (PlGF), hepatocyte growth factor (HGF) and fibroblast growth factor (FGF), among others. And due to VEGF binding, NRPs can also modulate blood flow [65].

In endothelial cells, NRPs are thought to increase signaling through the VEGFRs acting as a co-receptor of VEGF and by stabilizing the VEGF/VEGFR complexes and therefore enhancing VEGF activity. Thus, the interaction of $VEGF\text{-}A_{165}$ with NRP1 is required for stable binding of $VEGF\text{-}A_{165}$ to VEGFR-2, full activation of VEGFR-2 and downstream signaling and biological responses [65, 67].

Limited information about the localization of NRP in primary cilium is available. Before presenting those evidences, we should give some information about hedgehog (HH) signaling. Briefly, HH signaling is essential for tissue patterning and organ formation during embryonic

and postnatal development, as well as in cancer development and tissue homeostasis renewal and repair in adult animals [68]. The HH pathway acts via activation of transcriptional effectors, such as the glioblastoma (Gli) proteins, a family of transcription factors whose target genes remain enigmatic. The Gli protein family includes Gli1, Gli2 and Gli3 [69].

Referring to the primary cilium, studies conducted by Pinskey et al. [68] found that NRP1 and NRP2 promote the activation of Gli transcription factor. Interestingly, the authors found that a conserved 12 amino acid region of the NRP1 cytoplasmic domain between amino acids 890 and 902 is responsible for the HH-signal promotion. Considering that an intact primary cilium is a main component of the HH signaling, they also looked for the localization of NRP1 in this subcellular compartment and showed the unique evidence until now about the localization of NRP1, but not NRP2, in the primary cilium [68]. Despite the fact that the localization of NRP1 in the primary cilium was not required for HH signaling promotion, it is intriguing why NRP1 is present in primary cilium and what would be its physiological relevance there. This observation is important considering that NRP1, as indicated previously, may interact with growth factors, such as VEGF, PlGF, HGF and FGF, among others, regulating their action. Still more questions than answers emerge and more investigation is required to lighten these intriguing possibilities.

8. Purinergic receptors and the primary cilium

Since early 1970s [70], adenosine triphosphate (ATP) has been recognized as an extracellular signaling molecule activating a pathway defined as "purinergic signaling" where ATP, ADP and adenosine are involved. The signaling pathway starts with the activation of a family of membrane receptors. At this moment, separate families for adenosine purinergic (P1) and ATP and ADP purinergic (P2) receptors have been characterized. Briefly, adenosine receptor or P1 family includes at least four members of G-protein-coupled receptor subtypes identified as A_1, A_{2A}, A_{2B} and A_3. In contrast, the P2 family encompasses seven members of purinergic receptor type X (P2X), a family of ion channels receptor subtypes (P2X1–7) and at least eight members of P2Y G-protein-coupled receptor subtypes ($P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$ and $P2Y_{14}$) [71]. $P2Y_1$, $P2Y_2$, $P2Y_4$ and $P2Y_6$ are associated with the intracellular calcium (iCa^{2+}) signaling pathway, whereas $P2Y_2$, $P2Y_{13}$ and $P2Y_{14}$ are associated with cyclic adenosine monophosphate (cAMP) signaling. In contrast, $P2Y_{11}$ has been shown to be associated with both iCa^{2+} and cAMP signaling [71].

ATP is released by almost all cell types after gentle mechanical stimulation and acts in an autocrine or paracrine manner [72]. Living cells under stressful conditions (i.e., hypoxia) or dying cells release ATP [72]. Interestingly, purinergic signaling parallel to flow sensor activity of the primary cilium [73]. Purinergic signaling associated with flow sensing was detected in several structures such as kidney tubules [20], intrahepatic bile ducts [74, 75], endothelial cells [31], among other lining cells. Therefore, deflection of the primary cilium has been related with ATP release leading to autocrine or paracrine activation of purinergic receptors.

The relationship between ATP, purinergic signaling and primary cilium has been studied in the kidney tubular system [73]. The main physiological area that established a relationship

between these three elements is related to urinary flow sensor, where a flow-stimulated increase of iCa^{2+} has been characterized. Initial investigation suggested that deflecting the cilium releases a paracrine factor, such as ATP that can activate a G-protein-coupled receptor and generate inositol triphosphate (IP_3) leading to iCa^{2+} increase throughout the cytosol due to the release of Ca^{2+} from the intracellular stores. Also, increasing the tubular flow triggered an increase in iCa^{2+}. The same experiments were also performed in renal tubules from mice lacking $P2Y_2$ receptors or cells lacking the primary cilium. In those experiments, the response to tubular flow was markedly reduced only in those cells lacking the $P2Y_2$ [76]. These results strongly suggested that tubular flow triggers ATP release, followed by auto- and paracrine activation of epithelial P2 receptors. However, a direct link to the primary cilium could not be established in these experiments. Despite that, information regarding how purinergic signaling can be associated or not to function of primary cilia is missing.

9. Ciliopathies: an insight into some clinical consequences of impaired ciliary function

9.1. Polycystic kidney disease

As indicated above, the relevance of the primary cilium function has been well established in the kidney, as evidenced in polycystic kidney disease [37, 77, 78]. Also, previous reports suggest that the outcome of I/R in kidneys is associated with the change of primary cilia length [17]. Physiologically, urinary flow through the nephron is a highly variable process. In the short term, flow changes can be caused by variations in glomerular filtration rate [79], tubuloglomerular feedback [80] and fluid absorption along the nephron [79]. In the long term, urinary flow fluctuations can be caused by a high salt [81] or high protein diet [82], as well as due to hypertension [83] or early stages of diabetes [84]. Variations in luminal urinary flow alter the mechanical forces (shear stress, stretch and pressure) that affect epithelial cells in the nephron.

Polycystic kidney disease is a genetic disease characterized by bilateral enlarged cystic kidneys. It is caused by mutations of genes encoding for PKD1 and PKD2 linked to polycystic kidney disease type 1 (*pkd1*) and polycystic kidney disease type 2, respectively [37, 77, 78]. The frequencies of cardiovascular complications are very high in polycystic kidney disease patients. Hypertension occurs in 50–70% of patients even before any substantial kidney disorder [85]. Polycystic kidney disease has been associated with abnormalities in FSS sensing due to primary cilia dysfunction [36]. Mechanistically, polycystic kidney disease patients exhibit impairment of endothelium-dependent relaxation and a decrease of eNOS activity, impaired release of NO and, therefore, endothelial dysfunction [86]. Furthermore, polycystic kidney disease has been associated to the inability of renal epithelia [87] or vascular endothelia [9] to induce Ca^{2+} influx in response to FSS. Endothelial cells isolated from mice and humans with polycystic kidney disease lack PKD1 and/or PKD2 in the primary cilium and fail to produce NO in response to FSS [2, 9, 29].

Abnormal PKD2 function or expression has been associated with hypertension [88]. Mutations of *pkd2* gene abolish Ca^{2+} and NO increases in endothelial cells showing that PKD2 mediates FSS sensing in endothelial cells [29]. In addition, PKD2 sensory function as a Ca^{2+} channel

depends on its localization at the primary cilium and on PKD1. Thus, impaired function and expression of PKD2 are associated with endothelial dysfunction. Interestingly, prolonged exposure of endothelial cells to high FSS induces the disassembly of cilia [28] and inactivation of PKD1 by proteolytic cleavage [9], reducing the ability of endothelial cells to properly sense alterations in blood pressure.

9.2. Atherosclerosis

Initial evidence showed that primary cilia were present in the vascular beds where flow is disturbed, and related to atherosclerosis [89]. Particularly, the primary cilia were found in the endothelial cells of human aortic fatty dots and streaks, but not in those of the normal intima [89]. Moreover, recent evidences also found the primary cilium in cells exposed to laminar blood flow [27]. Regarding atherosclerotic plaque, primary cilia have been shown to be located at the atherosclerotic predilection sites, where flow is disturbed and around atherosclerotic lesions in the aortic arch in wild-type mice and apolipoprotein E-deficient mice, respectively [23]. In addition, experimentally induced pathologic turbulent flow in mice leads to induction of primary cilia, and subsequently to atherogenesis, suggesting a role of primary cilia in endothelial activation and dysfunction [23].

Contrary, another evidence found an inverse correlation between the presence of endothelial primary cilia and vascular calcified areas, although the signaling mechanisms involved remain unknown [22]. In order to analyze this phenomenon, Sanchez-Duffhues et al. [22] used the Tg737 cilium-defective mouse model and they found that non-ciliated aortic endothelial cells acquire the ability to trans-differentiate into mineralizing osteogenic cells. The mechanism for this trans-differentiation requires the presence of bone morphogenetic proteins (BMP). Therefore, these data emphasize the role of the endothelial cells in vascular calcification and generation of atherosclerosis. Whether these findings are associated or not to iCa^{2+}, eNOS activation and NO synthesis remains unclear.

Apparently, differences in blood flow patterns along the endothelium trigger abnormal vascular responses that have been associated with pathophysiological consequences, such as atherosclerosis. While endothelial cells exposed to laminar blood flow are protected from atherosclerosis formation, turbulent blood flow, which occurs at bends and bifurcations of blood vessels, facilitates the process of atherosclerosis. Primary cilia presence and function have barely been studied in both endothelial activation and dysfunction. Hence, more studies are required to better understand these issues.

10. Concluding remarks

Phenotypic cell alterations resulting from flow-induced mechanical strains and their implication in diseases are a growing field of research in many cell types such as vascular endothelial, smooth muscle, kidney epithelial cells and chondrocytes.

In the chapter, we presented the role of the primary cilium as one of the multiple physiological mechanosensors for FSS in endothelial and renal cells, where it regulates vascular homeostasis

and epithelial function. To respond to FSS, a functional primary cilium requires the constitutive proteins, PKD1, PKD2 and polaris. The primary cilium is functional under normal FSS and activates the Ca^{2+} and NO signaling cascade; nevertheless, it becomes dysfunctional after prolonged exposure to high FSS analogous to a hypertensive situation present in any kind of biological fluid. Respectively, growing evidence implicates the primary cilium and the disruption of its function in many diseases such as hypertension, atherosclerotic lesions and acute and chronic kidney disease.

In this regards, we have summarized evidences implicating that polycystic kidney disease, a pathology characterized by lack of PKD1 and/or PKD2 expression, leads to impaired vascular endothelial FSS sensing. Even when the primary organ affected by the disease is the kidney, the endothelial dysfunction is a common extra renal symptom observed in polycystic kidney disease. Those patients exhibit an impairment of endothelium-dependent relaxation and a decrease of primary cilia-dependent NO production leading to hypertension.

Contrary to its physiological role in sensing FSS, it has also been described that primary cilium is related to plaque formation, since this organelle was present in the endothelial cells of human aortic fatty dots and streaks. Indeed, primary cilium has been shown to be located at the atherosclerotic predilection sites, where flow is disturbed and around atherosclerotic lesions in the aortic arch in wild-type mice and apolipoprotein E-deficient mice, respectively [31]. In addition, primary cilia have been involved in endothelial activation and dysfunction present in atherosclerosis. Despite relevance of these evidences, it is highlighted in this review that more studies are required to better understand the role of endothelial primary cilium in normal and pathological conditions, such as atherosclerosis.

We also presented examples of regulatory signals that control NO bioavailability or might participate as modulators of primary cilium. For instance, ROS can modulate cilia length and deciliation process in tubular kidney cells. Whether these effects could be extrapolated to endothelial cells is worth of more investigation.

Finally, we presented the interconnected coreceptors VEGF and VEGFRs, neuropilins, ATP, adenosine and purinergic receptors. All have been suggested to be involved in FSS sensing and/or colocalization in the primary cilium. To this respect, we can provide more questions than answers. NRP1, a VEGFR2 receptor, localizes to the primary cilium but its physiological relevance is still unknown. On the other hand, ATP and adenosine are involved in sensing FSS, in a primary cilium-independent manner. Moreover, information regarding whether or not purinergic signaling can be associated to the primary cilia function is missing.

In conclusion, these data emphasize the role of the primary cilium present in endothelial cells as a microsensory organelle transducing FSS. Impairment in the ciliogenesis, cilia length and intracellular pathways can be involved in cardiovascular diseases. The participation of ROS, VEGF and purinergic signaling pathways is being described, but more research is required to elucidate their participation in the primary cilium-mediated sensing of FSS in normal and pathological conditions, such as hypertension, atherosclerosis or polycystic kidney disease.

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Conflict of interest

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Abbreviations

eNOS	Endothelial nitric oxide synthase
ERK	Extracellular signal-regulated Kinase
FGF	Fibroblast growth factor
FSS	Fluid shear stress
Gli	Glioblastoma transcription factors
HH	Hedgehog signaling pathway
HGF	Hepatocyte growth factor
HUVECs	Human umbilical vein endothelial cells
MMDD1	Immortalized macula densa cell line

InsP3R	Inositol 1,4,5-triphosphate receptor
IP_3	Inositol triphosphate
iCa^{2+}	Intracellular calcium
ift88 or Tg737	Intraflagellar transport particle 88
IFT	Intraflagellar transport particles
I/R	Ischemia/reperfusion
MT	Microtubules
MnTMPyP	Mn(III) tetrakis (1-methyl-4-pyridyl) porphyrin
NRP	Neuropilins
L-NAME	N^G-nitro-L-arginine methyl ester
Nox	Nicotidamine adenine dinucleotide phosphate oxidase
NO	Nitric Oxide
$ONOO^-$	peroxynitrite
PI3K	Phosphoinositide 3-kinase
PlGF	Placental growth factor
PDGF	Platelet-derived growth factor
PKD	Polycystic kidney disease
PKD1	Polycystin-1
PKD2	Polycystin-2
PKB	Protein kinase B
PKC	Protein kinase C
PT	Proximal tubule
ROS	Reactive oxygen species
RyR	Ryanodine receptors
siRNA	Small interference RNA
VEGF	Vascular endothelial growth factor
VSMC	Vascular smooth muscle cells
VEGFRs	VEGF receptors
VEGFR-1	Also known as fms-like tyrosine kinase 1 or Flt-1
VEGFR-2	Or kinase insert-domain containing receptor, KDR

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