Primary cilia on endothelial cells : component of the shear stress sensor localized to athero-prone flow areas

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Chapter 7

Deciphering the Endothelial Shear Stress Sensor

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The single non-motile primary cilium, protruding several microns from the apical surface, contains cytoskeletal elements in a specific fashion. It consists of 9 circularly arranged microtubule doublets, as revealed by transmission electron microscopy[1], but lacks the central ones characteristic for motile cilia and flagellae. The primary cilium is anchored to the basal body and is thereby connected to the cytoskeletal apparatus. Sorokin[1] concluded that primary cilia were probably vestigial remnants of motile cilia. We now know that ciliary functions are abundant. Examples include processes involving Hedgehog and Wnt signaling[2] and determining left-right asymmetry[3]. Cilia functioning as sensory antennas in insect ears and the human retina are well established[4]. Because of the widespread presence of primary cilia, it does not come as a surprise that many diseases, often syndromic, are caused by ciliary dysfunction. Several diseases are related to disruption of intraflagellar transport in the case of mutation in, for example, Polaris, *Kif3a* or various Bbs proteins that can lead to such conditions as obesity and Bardet Biedl Syndrome[5]. Other cilium-related proteins involve cell membrane-bound Ca^{2+} channels such as the complex formed by polycystin-1 and -2, encoded from *Pkd1* and *Pkd2*, respectively. Mutations cause polycystic kidney diseases[6,7].

Primary cilia were first described in the cardiovascular system in human embryos and adults more than 20 years ago[8]. The geometry of the heart and vascular tree strongly influences hemodynamics with repercussions on the pattern of ciliation. Endothelial ciliation is restricted to areas of low and oscillatory blood flow and is absent in areas of high flow and ensuing wall shear stress and shear-responsive gene expression[9]. Endothelial cells in the adult mouse are ciliated in areas of low and disturbed blood flow[10] in the inner curve of the aortic arch and the branching points of the major arteries. These are areas that are prone to developing atherosclerosis. The ciliated phenotype is typically induced by disturbed or oscillating shear stress. It is noteworthy that patients with polycystic kidney disease present with an increased risk for atherosclerosis and hypertension[11].

Nauli *et al.*[12] in the current issue of *Circulation*, proceeding from their thorough and extensive program related to polycystic kidney disease[6,13], describe the ability of immortalized and cultured murine endothelial cells to sense low shear ranges using primary cilia. *Polycystin-1*- and *polaris*-deficient cells were used that are unable to transmit flow-related shear stress into Ca^{2+} signaling and nitric oxide (NO) synthesis. They mention that polycystin-1 in the basal body of the cilium is not in itself sufficient for a fluid shear stress response, whereas optimal shear stress does not change cilium structure but rather modifies the responsiveness to high stress by proteolytic modification of polycystin-1. In their article, Nauli *et al.*[12] demonstrate that nearly all immortalized endothelial cells carry a single primary cilium and that mechanical probing of a single cell in a monolayer causes a Ca^{2+} flux throughout the epithelium in a time frame of many seconds. These experiments carried out *in vitro* demonstrate the response potentials of endothelial cells.

If one examines the response time of the endothelial cells to fluid shear stress in terms of a Ca^{2+} flux and NO release, which is effectively 10 to 15 seconds from the trigger, one finds that the activation mechanism *in vivo* needs further exploration. Because the heart rate is 1 to 2 Hz in humans and even 7 to 8 Hz in mice, the pulse frequency is much faster than the endothelial response time. This difference would imply a refractory period for endothelial activation. Furthermore, we have demonstrated that *in vivo*, only a minority of endothelial cells (< 25%) are ciliated, which leaves large areas non-ciliated, nevertheless leading to relatively homogeneous expression patterns of shear responsive genes, such as *Krüppel-like factor-2 (KLF2)*, *Endothelin-1 (ET1)*, or *endothelial NO synthase*[14,15]. Figure 1 shows the presence of ciliated ventricular and atrial endocardial cells in wild-type and

Pkd1$^{-/-}$ mouse embryos (kindly provided by Dr. D.J.M. Peters, LUMC, The Netherlands), demonstrating the presence of ciliated endothelial cells but also the lack of cilia on most neighboring cells. The intercellular Ca^{2+} exchange, nicely demonstrated by Nauli and colleagues[12], may serve in cilia-poor areas to synchronize the reaction of endothelial cells that are non-ciliated. This effect is most probably facilitated through intercellular ion channels but does not result in activation of NO synthesis and release. The latter aspect appears to be restricted to cells with functional cilia.

Iomini and colleagues[16] demonstrated that endothelial cells lose their primary cilia after 1 to 2 hrs exposure to steady fluid flow. It is obvious that both prolonged steady and pulsed flows, which cause de-ciliation, affect the expression profiles and phenotypes of endothelial cells. These flow profiles typically result in the alignment of cells in the direction of the shear forces and in induction of, for example, *Klf2* and repression of NF-κB-mediated endothelial activation[17]. In contrast, disturbed or oscillatory flow induces a ciliated phenotype, induction of *Et1* expression, and endothelial activation. We must realize that sensitivity in ciliary mechanosensation is an important factor, as well, and is a matter of ongoing debate[18].

Changing the shape of the cell and consequently the three-dimensional relations within the cytoskeleton have been described as a basis for mechanosensing and transduction of mechanical signals[19] even in cells without cilia. Cytoskeletal deformation is instrumental in the response to prolonged shear forces by activating membrane proteins through conformational changes[20] or through activation of small G-proteins[21]. We have preliminary data that the cilium assists cytoskeletal deformation and acts as a signal amplifier in endothelial cells[22].

Combining the now available data from *in vivo* and *in vitro* studies we are still left with some conflicting features: (1) The relation between pulse frequency and endothelial response time, (2) the endothelial heterogeneity in ciliated phenotype *in vivo* and the fact that non-ciliated cells are responsive to shear stress, and (3) the role of the cytoskeleton in mechanosensation. It is tempting to solve the dilemma by considering the shear stress sensing mechanism as a 2-step process: (1) an immediate response that involves ciliary bending, activation of polycystin-1 and -2, a rise in intracellular Ca^{2+}, and a concomitant synthesis and release of stored (vasoactive) substances like NO and endothelin, and (2) a prolonged response that is coordinated through cytoskeletal conformational changes and that involves transcriptional activation of shear responsive genes, such as *KLF2*, which drive phenotypic adaptation of endothelial cells to this environmental epigenetic cue. Combining our efforts, we will be able in the near future to arrive at a more detailed plan for mechanosensation in the hemodynamic complexity of the cardiovascular system, both in development and in disease.

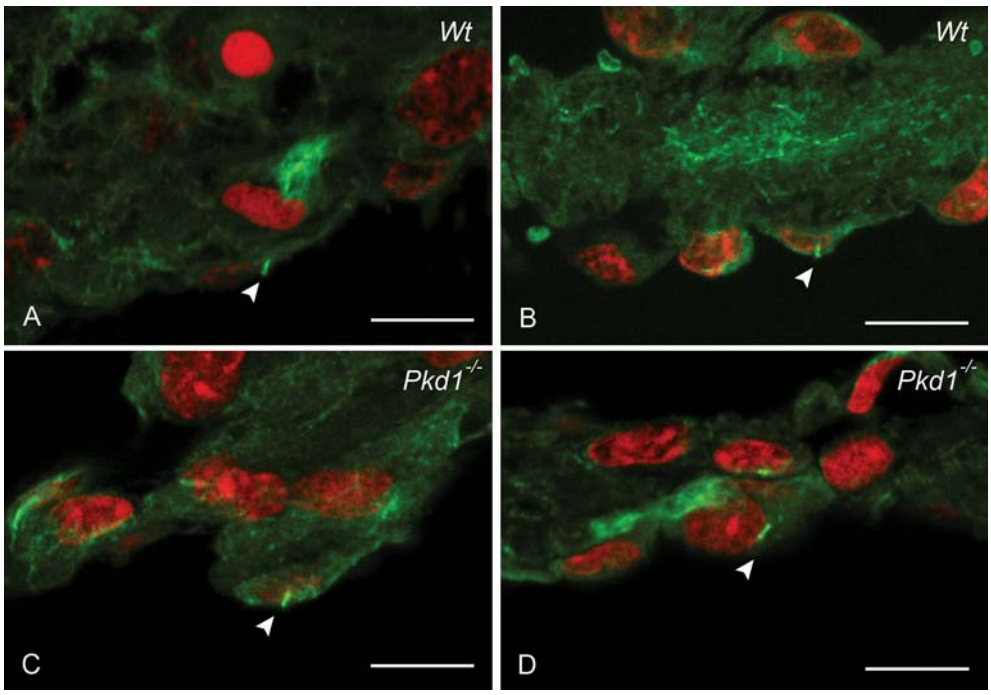

Figure 1. Confocal images of the endocardial cell layer of the ventricle (A and C) and the atrium (B and D) of a wild-type (*Wt*) C57BL/6 (A and B) and a *Pkd1*$^{-/-}$ (C and D) mouse embryo of embryonic day 13.5. Only a subset of endocardial cells is ciliated (arrowheads), leaving many neighboring cells unciliated. *Pkd1* deficiency does not affect ciliation. In both *Wt* and *Pkd1*$^{-/-}$ mouse embryos primary cilia are approximately 2 µm in length and protrude from the endocardial cell into the lumen. Ciliated endocardial cells are only present in areas of disturbed or oscillatory blood flow such as the atrial and ventricular chambers, and are absent from high-shear areas such as the atrioventricular canal and outflow tract, Acetylated α-tubulin (green), and nuclei (red; propidium iodide). Scale bar = 10 µm.

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