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The role of endothelial cilia in shear stress sensing in (patho)physiology.

Bachelor Thesis

Pathophysiology Research

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5th of July 2016

Abstract

Endothelial cells are constantly exposed to shear stress. When the shear stress becomes higher, endothelial cells sense the change and release vasodilation agents. To sense shear stress, endothelial cells contain mechanosensors, such as primary cilia. When the cilium senses shear stress by bending, a calcium influx is created. This calcium influx activates a cascade of reactions, which eventually leads to an activation of nitric oxide. Primary cilia are relatively new as a mechanosensor and the mechanism in which the cilium senses shear stress is not fully understood. In some diseases, such as Atherosclerosis, the expression of primary cilia is changed. This review will inquire the role of cilia in mechanosensing in (patho)physiology.

Index

Introduction

Endothelial cells are constantly exposed to the blood flow. The flowing of the blood exerts a drag force on the vessel wall, called shear stress. Endothelial cells are activated by shear stress and respond to a change therein.

Shear stress is influenced by the velocity of the blood flow, the viscosity of the blood and the circumference of the vessel. Therefore, shear stress is not the same throughout the vasculature. In arteries the shear stress is higher than in veins, because the velocity of the blood flow in arteries is higher. Normal shear stress ranges from 10-20 dynes/cm^2 (1-2 Pa) in the aorta, from 10-40 dynes/cm^2 (1-4 Pa) in the large arteries (Resnick et al., 2003) and from 1-6 dynes/cm^2 (0.1-0.6 Pa) in veins (Kamiya et al., 1984).

Through the changes in shear stress in the blood vessel, endothelial cells need to respond to it. To sense these changes endothelial cells have mechanosensors. Mechanosensors are molecules on or in the plasma membrane that react to changes in shear stress. The sensors are able to activate other molecules. This activation cascade eventually leads to an intracellular calcium influx followed by NO production. NO stimulates smooth muscle cells to relax, which leads to vasodilation and thus lowering of the shear stress.

There are many known mechanosensors responding to shear stress (figure 1). There are cellular structures like cilia, caveolae (Parton et al., 2007) and the glycocalyx layer (Tarbell et al., 2014). Other mechanosensors are receptors, such as receptor tyrosine kinases (TRK) (Quillon et al., 2015). Ion-channels and adhesion molecules like Vascular Endothelial-cadherin (VE-cadherin) and Platelet Endothelial Cell Adhesion Molecule-1 (PECAM-1) are also able to function as a mechanosensor (Quillon et al., 2015). Some of these molecules work together in a mechanosensor complex like caveolae, PECAM-1 and TRK (Chatterjee et al., 2014; Goedicke-Fritz et al., 2015).

Cilia are present on many cell types and are relatively new as mechanosensors in blood vessels. In renal epithelial cells, cilia have been extensively studied in relation to Polycystic Kidney disease (PKD). In PKD epithelial and possibly endothelial cilia are not functioning properly, due to a mutation. Cilia are also related to atherosclerosis, since cilia are present in places of the vasculature that are atheroprone. Therefore, this review will focus on the role of endothelial cilia in shear stress sensing in physiology and pathology.

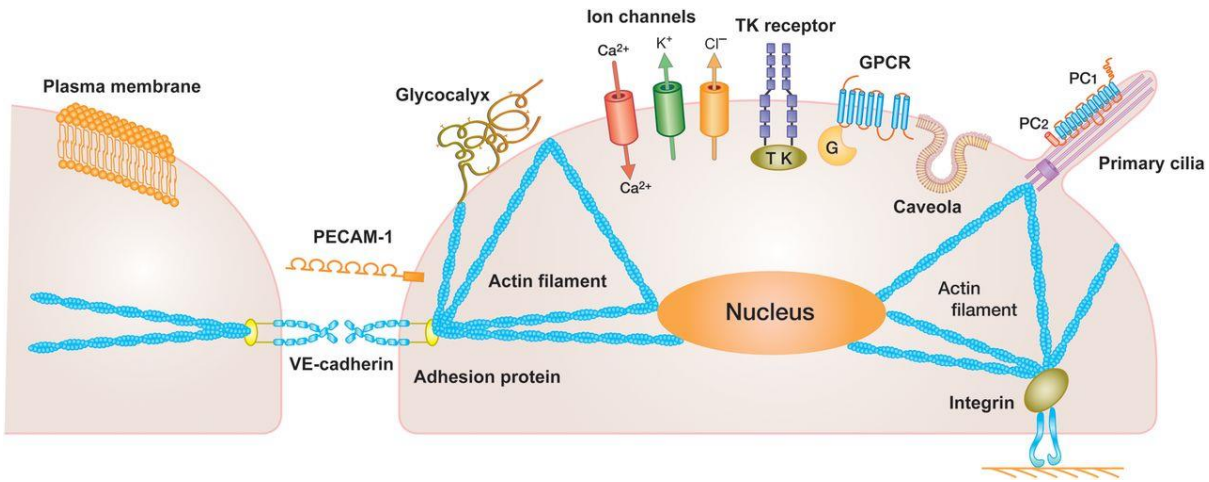

Figure 1: Overview of possible mechanosensors. Glycocalyx, Ion-channels, tyrosine kinase receptor, G-protein coupled receptor (GPCR), caveolae, primary cilia, adhesion proteins (integrin, VE-cadherin, and PECAM-1), cytoskeleton, and plasma membrane may act as a mechanosensor. (Ando et al., 2013)

Structure of endothelial cilia

Cilia are present on many cell types, including endothelial cells. Cilia are hair like structures on the surface of the cell. The length of the cilium is about 2-3 µm in vivo and is up to 30 µm in vitro (Wheatley et al., 2000). In figure 2 the inner structure of the cilium is shown. The cilium consists of an axoneme. The axoneme is made out of a ring of 9 doublet microtubules. A microtubule is a hollow cylinder that is formed out of α-tubulin and β-tubulin proteins that bind together. One doublet consists of one whole microtubule and one half of a microtubule that are bound together. Radial spokes link the 9 doublets to the two centre microtubules. The 9 doublet microtubules are linked by bridges formed by Dynein and other accessory proteins. The cilium is able to bent on its own due to the Dynein molecules. When Dynein, on one side of the axoneme, slides down and the other stays in the same position, the cilium is bent. In this fashion a swinging motion is established as seen in sperm flagella. The axoneme is anchored to the cells cytoskeleton by a basal body (Hierck et al., 2008; Jensen et al., 2004). The basal body consists of a ring of 9 triplet microtubules.

There are two types of cilia, motile cilia and non-motile cilia. Non-motile cilia lack the two centre microtubules. Therefore, they are referred to as a 9+0 cilia or primary cilia. Primary cilia are unable to move on its own, because they lack the radial spokes and dynein proteins. Primary cilia are found on endothelial cells, epithelial cells, secretory cells and many more cells. They exhibit multiple sensing functions such as, chemo-sensing and mechanosensing (Praetorius et al., 2005).

On the other hand, motile cilia do possess the two centre microtubules, radial spokes and dynein proteins. Therefore, they are able to move and are referred to as 9+2 cilia or secondary cilia. Secondary cilia are found among others in the trachea, where they swing the mucus upwards to the oesophagus.

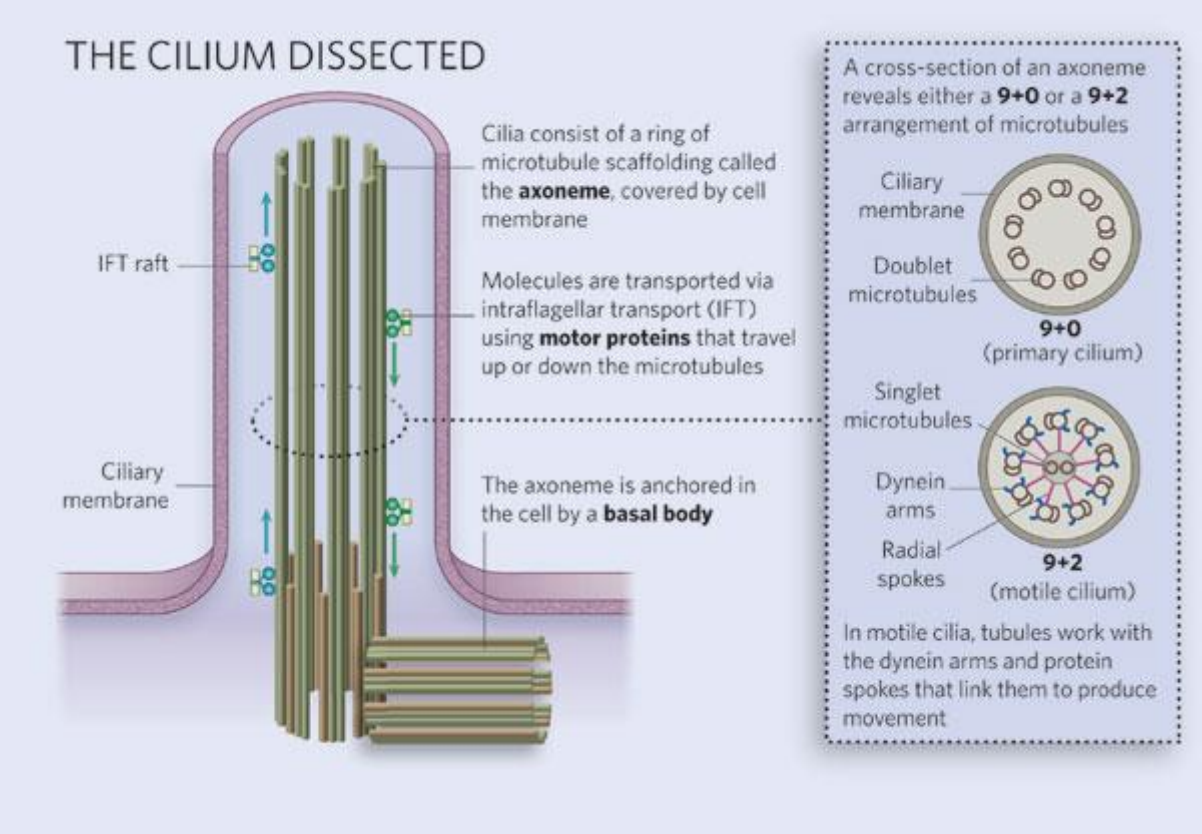

Figure 2: The inner structure of the cilium. The structure of the cilium with a cross-section of an axoneme of a primary (non-motile) and secondary cilium (motile). (Ainsworth, 2007)

Mechanosensing in endothelial cilia

Nearly every endothelial cell has one cilium at the apical side of the cell (Nauli et al., 2008). There the endothelial cell senses the shear stress, because of the bending of the cilium through the force. The bending causes an influx of extracellular calcium. Eventually, nitric oxide (NO) is produced. NO is a vasodilator produced by endothelial cells. When NO is excreted by endothelial cells, it causes a relaxation of the smooth muscle cells surrounding the endothelial cells in the blood vessel wall. As a result of this relaxation, shear stress is due to the circumference of the blood vessel becoming wider.

Through the force of the shear stress, the cilium is bent. After the bending of the cilium an extracellular calcium influx occurs. The influx of calcium is needed to initiate a cascade of reactions, in order for NO to be produced by the endothelial cell (Praetorius et al., 2005).

Upon bending of the cilium, Polycystin-1 is activated (Nauli et al., 2003). Polycystin-1 is an integral protein, located in the cilium, that is activated by shear stress. This activation takes place by cleavage of the tail of the protein (Chauvet et al., 2004). Activated Polycystin-1 binds to Polycystin-2, by which Polycystin-2 is activated. Polycystin-2 is a Transient Receptor Potential (TRP) non-selective cation channel. When Polycystin-2 becomes activated, calcium enters the cell through the channel (Nauli et al., 2003; Zimmerman et al., 2015). Polycystin-1 and Polycystin-2 are both localised in the primary cilium (AbouAlaiwi et al., 2009; Nauli et al., 2003; Yoder et al., 2002). When either Polycystin-1 or Polycystin-2 are blocked, calcium influx fails. With that the induction of an intracellular calcium influx and NO production will also fail (Nauli et al., 2003; Wang et al., 2007).

Contradictory, shear stress can also be too high or last too long for cilia. When cilia were exposed to laminar shear stress for 1 hour they started to disassemble (Iomini et al., 2004; Van der Heiden et al., 2008). This disassembly was part of a cytoskeletal rearrangement (Iomini et al., 2004). Calcium can increase the rate of depolymerisation of microtubules (O'Brien et al., 1997), which can result in loss of cilia. Thus, because shear stress induces a calcium influx, cilia disassemble and disappear (Egorova et al., 2011; Iomini et al., 2004). Resulting in a loss of the ability of mechanosensing by cilia. This is what may happen in hypertension.

Mechanotransduction in endothelial cilia

Thus, when flow is sensed by Polycystin-1, a calcium influx is initiated by the activation of Polycystin-2. This initial extracellular calcium influx activates ryanodine receptors on the endoplasmic reticulum (ER) membrane, which stimulates an intracellular release of calcium (AbouAlaiwi et al., 2009; Nauli et al., 2003). The ryanodine receptor is a calcium channel that is activated when cytosolic calcium binds to the channel. The receptor will release calcium from the ER lumen to increase the cytosolic calcium levels further.

The increased cytosolic calcium will activate Protein Kinase C (PKC) and bind to calmodulin. PKC is a protein kinase that acts as a second messenger. Calmodulin is a calcium sensor and is activated when calcium binds to it. Akt is phosphorylated by the calcium-calmodulin complex. Akt is a cytosolic protein kinase.

Together, PKC, calcium-calmodulin and Akt activate eNOS. eNOS will then synthesise NO (AbouAlaiwi et al., 2009). When either polycystin-1 or 2 was knocked out, calcium influx and NO production was less or failed (AbouAlaiwi et al., 2009; Nauli et al., 2003).

eNOS expression is regulated among others by Krüppel-like Factor-2 (KLF-2). KLF-2 is a shear sensitive transcription factor, which regulates gene expression of eNOS (Dekker et al., 2005). When shear stress is applied to endothelial cells KLF-2 becomes upregulated (Dekker et al., 2005). KLF-2 binds on the eNOS promoter and eNOS transcription is activated (SenBanerjee et al., 2004). KLF-2 is mainly expressed in areas of high laminar shear stress and depends on a stable cytoskeleton (Dekker et al., 2004; Hierck et al., 2008). However, primary cilia are less expressed in areas of laminar shear stress (Van der Heiden et al., 2008), ciliated endothelial cells express more KLF-2 than non-ciliated cells under laminar shear stress (Hierck et al., 2008). Thus, for a functional eNOS expression promoted by KLF-2, cilia are needed.

Without shear stress, KLF-2 expression is suppressed by binding of Histone Deacetylase 5 (HDAC5). HDA5 is a transcription factor called, myocyte enhancer factor 2 (MEF2) (Kwon et al., 2014; Wang et al., 2010). HDAC5 is phosphorylated by calcium-calmodulin kinases, through which it separates from MEF2 and is transported out of the nucleus (Kwon et al., 2014). HDAC5 can also bind to KLF-2, to inhibit eNOS transcription (Kwon et al., 2014). Thus, shear stress induces phosphorylation of HDAC5. KLF-2 and eNOS is not inhibited anymore. NO is produced by the endothelial cell and relaxation of smooth muscle cells is induced, which lowers the blood pressure.

Endothelial cilia in disease

Shear stress is not the same throughout the vasculature. At bifurcations, such as the aortic arch, shear stress is non-laminar, whereas in straight parts of the vasculature shear stress is laminar. At bifurcations endothelial cells are not aligned (Hajra et al., 2000) and atherosclerotic lesions occur most of the time (Chiu et al., 2011). Primary cilia are mostly found in these regions. In the cilia rich areas only 1-4 cells possess a cilium (Van der Heiden et al., 2008).

To examine the correlation between patterns of shear stress, the frequency of primary cilia and sites of atherogenesis, Van der Heiden et al. (2008) used an atherosclerotic mouse model (*Apoe*$^{-/-}$*).* In *Apoe*$^{-/-}$ mice atherosclerotic lesions developed in the inner curvature of the aortic arch and at the upstream side of the branch points in the aorta. Primary cilia are present on the unaffected endothelium and on the endothelium overlying the lesions. Lesion-free *Apoe*$^{-/-}$ arteries had the same distribution of primary cilia as the wildtype. When the researchers placed a cast to represent a lesion and alter shear stress, they found that primary cilia where present in the areas with low and oscillating shear stress. However, in the areas where shear stress was high, due to the cast, primary cilia were absent (Van der Heiden et al., 2008).

Although, Van der Heiden et al. found a correlation, it is still unclear whether primary cilia dysfunction or the curvature is the cause of the atherosclerotic lesions. Therefore, Dinsmore et al. (2016) developed a mouse model that lacked endothelial primary cilia by deleting the intraflagellar transport 88 gene (*Ift88*$^{-/-}$*)*. This gene is needed for ciliogenesis and cilia maintenance. *Ift88*$^{-/-}$ and *Apoe*$^{-/-}$ mice had more atherosclerotic lesional surface area than control mice. The distribution did not change, only the surface became larger. Also, eNOS activity was lower in *Ift88*$^{-/-}$ and *Apoe*$^{-/-}$ mice. This indicates that altered eNOS activity is a cause of increased atherosclerosis observed in *Apoe*$^{-/-}$ mice lacking endothelial primary cilia and is not a consequence of atherosclerotic lesions.

Another disease where primary cilia are involved is Autosomal Dominant Polycystic kidney disease (ADPKD). ADPKD is characterised by the formation of renal cysts and cardiovascular complications. ADPKD is caused by a dysfunction in mechanosensing of the primary cilia (Nauli et al., 2003), due to a mutation in the Polycystin-1 and/or Polycystin-2 gene (D'Angelo et al., 2009; Xu et al., 2007). Research on ADPKD is mainly focussed on the renal epithelial cells, since their dysfunction leads to the forming of renal cysts and eventually renal failure. However, in epithelial cilia the same proteins are expressed as in endothelial cilia namely, Polycystin-1 and Polycystin-2. Therefore, it is possible that the cardiovascular complications are a consequence of an impaired relaxation of the vasculature due to the mutation in one of the proteins.

For example, AbouAlaiwi et al. (2009) inhibited *pkd2* in endothelial cells using small interfering RNA to mimic the mutation in the polycystin-2 gene in ADPKD. They found that these cells had a lower calcium influx and NO production compared to normal endothelial cells. Part of the failure to respond to shear stress is contributed to the loss of polycystin-2, but a part of the failure is also because of the loss of polycystin-1 (AbouAlaiwi et al., 2009).

Thus, the mutation in the Polycystin-1 or Polycystin-2 protein causes a dysfunction in the extracellular calcium influx when the cilium is bent. Due to the loss of the initial calcium influx, eNOS is not activated. As a consequence, NO is not being produced and smooth muscle cell relaxation is weaker. The shear stress stays high, which can cause hypertension (AbouAlaiwi et al., 2009)

Eventually, ADPKD patients' become hypertensive much earlier than in healthy people (Kellerher et al., 2004). Hypertension increases the risk of developing Atherosclerosis. Also, patients with ADPKD develop atherosclerosis, because of an impaired renal-angiotensin-aldosterone-system (Kocaman et al., 2004).

Discussion

In conclusion, when the primary cilium is bent through shear stress, polycystin-1 becomes activated. Polycystin-1 activates the calcium channel polycystin-2. This activation results in an influx of extracellular calcium. The extracellular calcium activates ryanodine receptors, which causes an intracellular influx of calcium. Calcium activates PKC and binds to calmodulin. The calmodulin-calcium complex enhances the Akt/PKB activity. eNOS is activated by PKC, the calmodulin-calcium complex and Akt/PKB. NO is produced by eNOS (AbouAlaiwi et al., 2009).

However, when one of the cascade molecules is dysfunctional, the endothelial cell fails to produce NO. Sometimes a mutation in a gene of the cilium occurs, like in ADPKD. People with ADPKD develop hypertension earlier in live and are more prone for atherosclerosis. Mostly, primary cilia are found in areas of low shear stress. This is a result of disassembly of the primary cilia under high shear.

However, in areas of high shear stress other mechanosensors may be present, such as PECAM-1 (Bagi et al., 2005). The other mechanosensors are still able to respond to the change in shear stress in these areas when primary cilia fail or disappear. Thus, when primary cilia are not present in these areas of the vasculature, the absence of them will not automatically lead to cardiovascular diseases.

Because, primary cilia disassemble at high shear stress, they are presumably mechanosensors of low shear stress. Their antenna like structure could make it easier for them to sense minor changes in shear stress. Other mechanosensors are present in the plasma membrane, whereas primary cilia reach into the lumen of the blood vessel.

Thus, endothelial cilia are very delicate structures that are easily disturbed by high shear stress. They play a significant role in the development of atherosclerosis. Perhaps endothelial cilia can be an early target for therapy in hypertension. However, the precise mechanism in which endothelial cilia sense shear stress and their relation with the other mechanosensors remain unclear.

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