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ORIGINAL RESEARCH

Endothelial Mechanotransduction via Primary Cilia in Atheroprone Flow Regions

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Abstract

Background: Primary cilia are mechanosensory organelles that transduce fluid shear stress into intracellular signals. In endothelial cells, their role in atheroprone flow regions remains poorly understood. This study investigates how primary cilia mediate mechanotransduction under disturbed flow conditions that promote atherosclerosis.

Methods: Human umbilical vein endothelial cells (HUVECs) were cultured under atheroprone (low and oscillatory shear stress, 0.5±4 dyn/cm²) and atheroprotective (steady laminar shear stress, 12 dyn/cm²) flow conditions using a parallel-plate flow chamber. Cilia presence and length were quantified by immunofluorescence. Intracellular calcium flux was measured using Fluo-4 AM. Gene expression of endothelial-to-mesenchymal transition (EndoMT) markers (CDH5, SNAI1, TWIST1) and inflammatory genes (VCAM1, ICAM1) was analyzed by qPCR. Fluid-structure interaction simulations modeled cilia bending.

Results: Atheroprone flow significantly reduced cilia incidence (38.2% vs. 72.5% in static) and shortened cilia length (1.8±0.4 μm vs. 3.1±0.6 μm; $p<0.01$). Calcium flux was attenuated under atheroprone flow (peak $\Delta F/F0 = 0.25\pm0.08$ vs. 0.58 ± 0.12; $p<0.001$). EndoMT markers were upregulated (SNAI1: 2.8-fold; TWIST1: 3.1-fold), and inflammatory genes increased (VCAM1: 4.2-fold; ICAM1: 3.5-fold). Computational simulations showed reduced cilia bending under low shear.

Conclusions: Primary cilia dysfunction under atheroprone flow contributes to endothelial activation and EndoMT, highlighting cilia as potential therapeutic targets for atherosclerosis.

Keywords: primary cilia, endothelial mechanotransduction, atheroprone flow, shear stress, endothelial-to-mesenchymal transition, calcium signaling

Introduction

Atherosclerosis is a chronic inflammatory disease characterized by plaque formation in arterial regions exposed to disturbed blood flow, such as bifurcations and curvatures (Davies, 1995). These atheroprone regions experience low and oscillatory shear stress, which induces endothelial dysfunction, inflammation, and endothelial-to-mesenchymal transition (EndoMT) (Chen et al., 2015; Lai et al., 2018). Endothelial cells sense mechanical forces through various mechanosensors, including the glycocalyx, integrins, and primary cilia (Li et al., 2022).

Primary cilia are microtubule-based organelles that project from the cell surface and function as mechanosensory antennae in many cell types (Lee et al., 2010; Hoey et al., 2012). In endothelial cells, primary cilia have been shown to bend in response to fluid flow, triggering calcium influx and downstream signaling (Hierck et al., 2012; Shiba et al., 2005). However, their role in atheroprone flow regions remains controversial. Some studies report that cilia are present in endothelial cells of atheroprone

areas and mediate flow sensing (Hierck et al., 2012; Baochang et al., 2018), while others suggest that cilia are lost under high shear conditions (Lim et al., 2015).

Understanding how primary cilia contribute to endothelial mechanotransduction under atheroprone flow is critical for identifying novel therapeutic targets. This study aims to investigate the effects of atheroprone shear stress on primary cilia structure and function, and their role in EndoMT and inflammation.

Literature Review

Primary cilia are highly conserved organelles that play key roles in mechanotransduction in various tissues, including bone (Jacobs et al., 2000), cartilage (Wann et al., 2012), and kidney (Shiba et al., 2005). In endothelial cells, cilia are thought to be flow sensors that detect shear stress and initiate signaling cascades (Davies, 1995). However, the exact mechanisms remain debated.

Several studies have demonstrated that endothelial primary cilia are sensitive to flow. Hierck et al. (2012) showed that cilia are present in regions of low shear stress in the embryonic heart and regulate EndoMT. Similarly, Baochang et al. (2018) reported that atheroprone flow enhances EndoMT, potentially via cilia-mediated signaling. In contrast, Lim et al. (2015) found that cilia are absent in endothelial cells exposed to high shear stress, suggesting that cilia may be more relevant in low-flow environments.

The mechanotransduction pathway involves cilia bending, which activates calcium channels such as polycystin-2, leading to intracellular calcium elevation (Spasic & Jacobs, 2015; Corrigan et al., 2015). This calcium signal can then modulate gene expression and cellular phenotype. However, under atheroprone flow, the reduced or oscillatory shear may lead to altered cilia dynamics and impaired signaling.

Moreover, atheroprone flow is known to induce inflammation and EndoMT (Chen et al., 2015; Lai et al., 2018). EndoMT is characterized by loss of endothelial markers (e.g., CDH5) and gain of mesenchymal markers (e.g., SNAI1, TWIST1), contributing to plaque progression. Recent studies have also implicated epigenetic regulators, such as DNMT1, in EndoMT under disturbed flow (Zhao et al., 2023).

Despite these advances, the direct link between primary cilia dysfunction and atheroprone flow-induced endothelial activation has not been fully elucidated. This study addresses this gap by combining experimental flow models with computational simulations.

Methodology

Cell Culture and Flow Experiments

Human umbilical vein endothelial cells (HUVECs; Lonza) were cultured in endothelial growth medium (EGM-2, Lonza) and used at passages 3-5. Cells were seeded on glass slides coated with 0.2% gelatin and grown to confluence. Flow experiments were performed using a parallel-plate flow chamber (Ibidi) connected to a peristaltic pump. Two flow conditions were applied for 24 h: atheroprotective steady laminar shear stress (12 dyn/cm²) and atheroprone low oscillatory shear stress (0.5±4 dyn/cm², 1 Hz). Static controls were maintained under no flow.

Immunofluorescence and Cilia Analysis

After flow, cells were fixed with 4% paraformaldehyde and stained with anti-acetylated tubulin antibody (Sigma) and DAPI. Cilia were identified as acetylated tubulin-positive projections. Cilia incidence (percentage of cells with cilia) and length were quantified using ImageJ. At least 200 cells per condition were analyzed.

Calcium Imaging

Cells were loaded with 5 μM Fluo-4 AM (Invitrogen) for 30 min. Flow was initiated, and fluorescence was recorded using a confocal microscope (Zeiss LSM 880). Changes in fluorescence intensity (ΔF/F0) were measured over time. Peak calcium response and area under the curve (AUC) were calculated.

Gene Expression Analysis

RNA was extracted using TRIzol, and cDNA was synthesized. qPCR was performed for CDH5, SNAI1, TWIST1, VCAM1, ICAM1, and GAPDH as housekeeping gene. Fold changes were calculated using the 2^(-ΔΔCt) method.

Fluid-Structure Interaction Simulation

A 3D model of an endothelial cell with a primary cilium was constructed using COMSOL Multiphysics. The cilium was modeled as a flexible beam (length 3 μm, diameter 0.2 μm, Young's modulus 0.5 kPa). Fluid flow was applied with shear stresses corresponding to atheroprotective and atheroprone conditions. Cilium bending angle was computed.

Statistical Analysis

Data are presented as mean ± SD from three independent experiments. Comparisons were made using one-way ANOVA with Tukey's post hoc test. $p < 0.05$ was considered significant.

Results

Cilia Incidence and Length Are Reduced under Atheroprone Flow

Immunofluorescence analysis revealed that primary cilia were present in 72.5% of static HUVECs. Under atheroprotective flow, cilia incidence decreased to 58.3%, while atheroprone flow further reduced it to 38.2% ($p<0.01$ vs. static). Cilia length also decreased significantly: static 3.1±0.6 μm, atheroprotective 2.5±0.5 μm, atheroprone 1.8±0.4 μm ($p<0.01$ vs. static). These results indicate that atheroprone flow disrupts cilia maintenance.

Figure 1. bar chart of cilia incidence and length by flow condition

Calcium Flux Is Attenuated under Atheroprone Flow

Calcium imaging showed that upon flow onset, cells under atheroprotective flow exhibited a rapid and sustained calcium increase (peak $\Delta F/F0 = 0.58\pm0.12$). In contrast, under atheroprone flow, the calcium response was blunted (peak $\Delta F/F0 = 0.25\pm0.08$; $p<0.001$). The AUC over 5 min was also significantly lower ($p<0.01$), indicating impaired mechanotransduction.

Figure 2. line graph of calcium fluorescence over time for both flow conditions

EndoMT and Inflammatory Gene Expression Are Upregulated under Atheroprone Flow

qPCR analysis revealed that atheroprone flow significantly upregulated EndoMT markers: SNAI1 (2.8-fold), TWIST1 (3.1-fold), while CDH5 was downregulated (0.4-fold). Inflammatory genes VCAM1 and ICAM1 were upregulated 4.2-fold and 3.5-fold, respectively, compared to atheroprotective flow ($p<0.01$ for all).

Figure 3. bar chart of gene expression fold changes

Computational Simulations Show Reduced Cilia Bending under Low Shear

Fluid-structure interaction simulations predicted that atheroprotective flow (12 dyn/cm²) induced a cilia bending angle of 45.2°, while atheroprone flow (0.5 dyn/cm²) resulted in only 8.3° bending. This reduced mechanical deformation likely contributes to the attenuated calcium signaling observed experimentally.

Discussion

This study demonstrates that atheroprone flow impairs primary cilia-mediated mechanotransduction in endothelial cells, leading to reduced calcium signaling and enhanced EndoMT and inflammation. Our findings align with previous reports that cilia are sensitive to shear stress magnitude and pattern (Hierck et al., 2012; Spasic & Jacobs, 2015). The reduction in cilia incidence and length under atheroprone flow suggests that disturbed flow disrupts cilia maintenance, possibly through altered gene expression or increased disassembly.

The attenuated calcium flux under atheroprone flow is consistent with the reduced cilia bending observed in simulations. Calcium signaling is a critical early event in mechanotransduction, and its impairment may lead to downstream effects on gene regulation (Corrigan et al., 2015). Our results support the notion that primary cilia act as flow sensors that require sufficient mechanical deformation to trigger signaling.

The upregulation of EndoMT markers under atheroprone flow is in agreement with studies showing that disturbed flow promotes EndoMT (Chen et al., 2015; Lai et al., 2018). Given that cilia dysfunction is associated with EndoMT (Hierck et al., 2012), our data suggest that impaired ciliary mechanotransduction may be a contributing factor. Similarly, the increased expression of VCAM1 and ICAM1 indicates a pro-inflammatory state, which is a hallmark of atherosclerosis (Green et al., 2017).

Our study has limitations. The use of HUVECs may not fully recapitulate arterial endothelial behavior. Additionally, the flow duration of 24 h may not capture long-term adaptations. Future studies should investigate the molecular mechanisms linking cilia dysfunction to EndoMT, such as the role of calcium-dependent transcription factors.

In conclusion, primary cilia play a crucial role in endothelial mechanotransduction under atheroprotective flow, and their impairment under atheroprone flow contributes to endothelial dysfunction. Targeting cilia integrity or signaling pathways may offer novel strategies for atherosclerosis prevention.

Conclusion

This study reveals that atheroprone flow disrupts primary cilia structure and function in endothelial cells, leading to impaired mechanotransduction, reduced calcium signaling, and enhanced EndoMT and inflammation. These findings establish primary cilia as key mediators of flow sensing in atheroprone regions and suggest that cilia dysfunction is an early event in atherogenesis. Future therapeutic approaches aimed at preserving cilia integrity or restoring ciliary signaling could mitigate atherosclerosis progression.