Redistribution of Intracellular Calcium Stores with Shear Stress-induced Cytoskeleton Organization in Human Endothelial Cell

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Intracellular gradients of the free calcium concentration are thought to be critical for the localization of functional responses within a cell. The mechanism of mechanotransduction may be associated with the localized accumulation of calcium stores for shear stress-exposed endothelial cells. The distribution of the calcium stores and the formation of the stress fibers were investigated by the indirect double immunofluorescent staining method with the calreticulin antibody and rhodamine phalloidin under flow condition. The shear stress of steady flow reorganized the cytoskeleton structure including the bundling and translocation to focal contacts. The calcium stores translocated from the cytoplasm to the focal contacting area. Consequently, accumulation of the calcium stores may participate in the shear stress-induced cytoskeleton organization of endothelial cells.

Background

In vascular system, calcium influences a variety of responses such as endothelial cell/leukocyte adhesion, leukocyte chemoattraction and migration, release of inflammatory cytokines, and mononuclear cell proliferation in atherogenic process. In cellular system, the intracellular calcium gradient is incorporated in the localization of the functional reaction to adapt the environmental condition [1].

However, endothelial cells are exposed to continuos mechanical stresses, such as pressure, shear and circumferencial strain which regulate the cellular functions. The cell culture studies on the effect of flow showed the elevation in intracellular calcium concentration of the endothelial cell by the sudden onset of a laminar and steady flow [2] and the fast actin cytoskeleton polymerization induced by shear stress [3]. Moreover, in neutrophils, phagocytocing microorganisms requires a focal activation of Ca^{2+}-dependent cellular functions including localized F-actin cytoskeleton polymerization. [4]. These studies provided the rationale of a certain calcium signal pathway of the mechanical signal transductions which is the translocation of the calcium stores.

This study elucidates that the translocation of the calcium stores participates in the shear stress-induced cytoskeleton organization of the human umbilical vein endothelial cells (HUVEC). Figure 1. shows known calcium signal pathways and a proposed one of the movement of calcium stores.

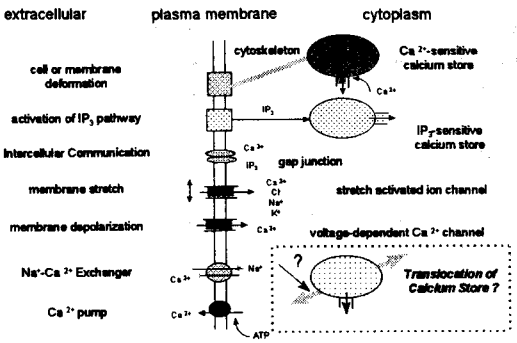

Figure 1. Calcium signaling

Actin and Calcium

Experiment concentrated on the relations of the calcium stores and actin polymerization in mechanical signal cascades hypothesized in Figure 2. Among actin binding protein , gelsolin requires Ca^{2+} to regulate the actin cytoskeleton by capping and severing. We suggest that the accumulation of calcium stores may generate the local increase of calcium concentration to facilitate the accelerated actin cytoskeleton reorganization.

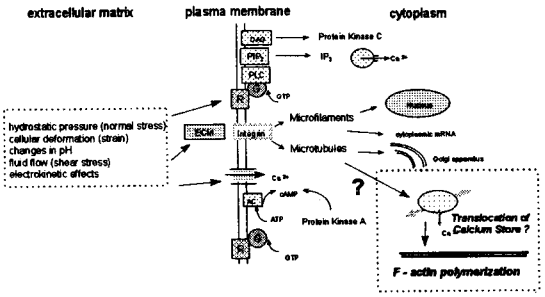

Figure 2. Mechanical signal transduction candidates

Calreticulin

We used the calreticulin as a marker for the Ca^{2+} stores which is the major calcium binding protein in the membrane of smooth muscle sarcoplasmic recticulum (SR) and non-muscle endoplasmic recticulum (ER) . Calreticulin is also known as calregulin, CRP55, CaBP3, calsequestrin-like protein, and Ro/SS-A antigen. It binds calcium with low affinity and high capacity, however it also exhibits a single high affinity binding site. It can act as a modulator of the regulation of gene transcription by nuclear hormone receptors and may also act as a molecular chaperone [5].

Construction of endothelial cell-extracellular matrix system

Human umbilical vein cells (HUVECs) were isolated from human umbilical vein with modified collagenase digestion method and then seeded on the human fibronectin (Boehringer Mannheim, Germany, 5 mg/cm^2) coated on micro slide glass. Seeding density is about 5 x 10^4cell/cm^2

Immunofluorescent staining protocols

Double-labeling indirect immunofluorescence protocol was used with Rhodamine Phalloidin (Molecular Probe Inc.) for actin filaments , Polyclonal(Rabbit) Anti-Calreticulin Antibody (Affinity Bioreagents Inc.) for calcium store and FITC-conjugated mouse anti-rabbit IgG (Sigma Inc) as a secondary antibody of calreticulin antibody.

Stress exposure protocols

HUVECs were exposed to a laminar and steady flow of culture media with different durations in figure 3. Shear stress is adjusted at 15 dyne/cm^2. For control condition, HUVECs were cultured in static condition. Exposure times of shear stress are 5 min, 20 min and 60 min respectively.

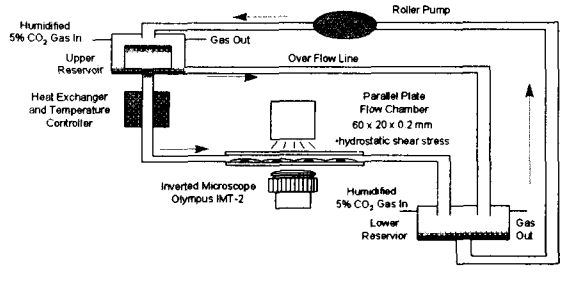

Figure 3. Flow chamber

Image processing of Immonofluorescent light microscopy for 2D distribution

The photograph was taken at the same exposure time (10 sec for actin and 15 sec for Calreticulin) with the final magnification of 400. PV-WAVE (Visual Numerics Inc., Boulder, CO, USA) running for the data analysis on Sun workstation was applied to convert the image intensity to the contour line to visualize the 2-dimensional distribution of calcium stores in HUVECs. See figure 4.

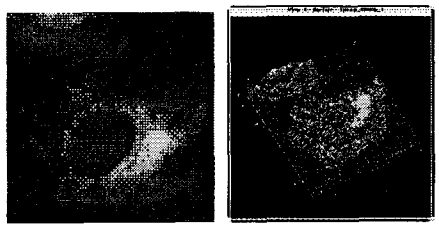

Figure 4. Calreticulin images

Laser confocal microscopy study for 3D distribution

Whole specimens were examined by laser scanning confocal microscope (Carl Zeiss, LSM410, Germany) with an argon laser for illumination. The wavelength of excitation was 488 nm. Sliced Image were acquired with 1μm interval from the basal membrane of the HUVECs.

Results

Morphologic changes of HUVECs by shear stress are one sided translocation of nucleus (to the down stream direction) , reducing the cell height (from 9μm to 6μm), and the formation of lamellipodium and focal contacting area.

Actin cytoskeleton changes its shape and location ; from the thin filaments to the thick filaments including the bundling process, from the distribution of the upper and central membrane surface to the lower and peripheral focal contacts, and the disappearance of dense peripheral bands and formation of stress fibers aligned with the direction of flow.

Calreticulin as a calcium store was redistributed to one sided region of the EC body (to the up stream direction),and from the upper and central position to lower and peripheral focal contacts. See figure 5. for 2-D changes and figure 6. for 3D.

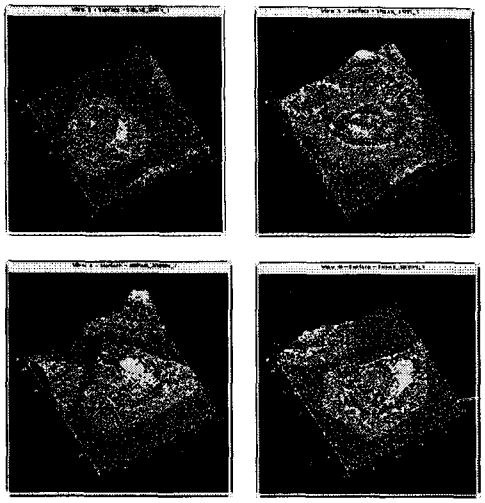

Figure 5. 2D distribution of calcium stores with different shear stress exposure time
(0min, 5min, 20min, 60min; clockwise direction from upper left side)

Conclusion

Calcium stores and actin cytoskeleton were redistributed from the central and upper cytoplasmic area to peripheral and lower contacting area. Calcium stores translocated to the focal contacting area in which integrins in the plasma membrane connect intracellular actin filaments to the extracellular matrix in figure 7. A certain calcium signaling such as movement of Ca^{2+} stores participates in the mechano-transduction of the actin cytoskeleton polymerization to shear stress in HUVECs

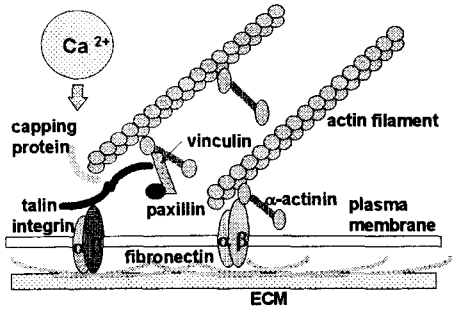

Figure 7. Schematics of integrin, actin, and extracellular matrix

Acknowledgment

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Reference

1. Clapham, Calcium Signaling, *Cell,* 80:259-268,1995
2. Shen et.al., Fluid shear stress modulates cytosolic free calcium in vascular endothelial cells, *Am. J. Physiol.* 262 (Cell Physiol. 31):C384-390,1992
3. J.K. Chang, Mechanotransduction in endothelial cells adhered on the extracellular matrix, *Ph.D. thesis*, 1994
4. O. Stendahl, et al, Redistribution of intracellular Ca2+ stores during phagocytosis in human neutrophils, *Science*, 265(5177): 1439-1441, 1994
5. M. Michalak, et al, Calreticulin, *Biochem*, 285: 681-692, 1992

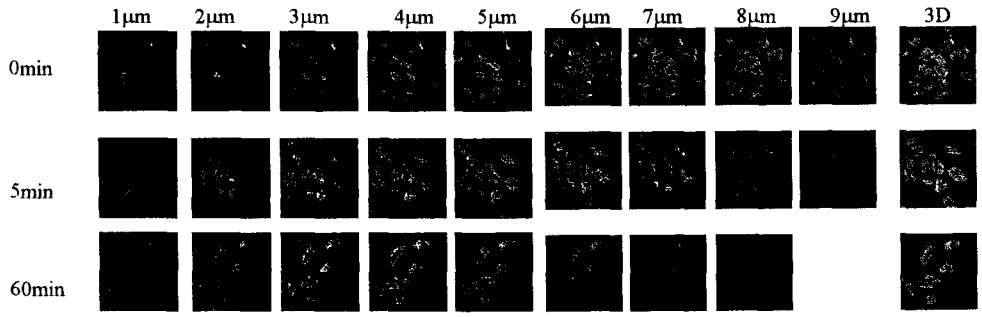

Figure 6. 3D distribution of calcium stores and actin filaments with different shear stress exposure time