Protein Aggregation of Endothelial Cells with Fluid Shear Stress

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Introduction

Many environmental stresses, such as heat shock, Ca^{2+} overload and fluid shear stress, cause rapid aggregation of protein in the cytoplasm of mammalian cells. The aggregation of cytoskeletal protein and heat shock protein (HSP) are increased by many chemical stresses as well as heat shock. These stress-response-proteins are thought to help solublize and refold denatured proteins. Thus stress-induced protein aggregation may be the one of key points in the mechanism of cell responses to various stresses [1].

The objectives of our study are to compare Triton(X-100)-insoluble (TIS) cell protein aggregation of human umbilical vein endothelial cell (HUVEC) from two different kinds of stress, shear stress and heat shock. Stress-induced protein aggregation was assessed by measuring the increase in TIS cell protein to analyze whether shear stress and heat shock can cause the aggregation of stress-response-proteins in HUVECs, such as cytoskeletal proteins and HSPs.

Materials and Methods

Cell Culture and Stress Treatment

HUVECs were isolated from human umbilical veins and cultured in Medium-199 containing 20 % fetal bovine serum [2]. HUVECs were seeded on fibronectin (FN)-coated glass and grown for 4 days before the experiment. One group of HUVECs was exposed to the fluid shear stress of 20 dyne/cm^2 with laminar flow chamber apparatus [3], and another group was exposed to 43 °C heat shock for 1 hour. For the control group, HUVECs were incubated in Medium-199 at 37 °C without any exposure of stresses.

Electrophoresis and Immunoblotting.

For the preparation of TIS-fraction of HUVECs, 1 ml cell suspension of cells was extracted in 1 ml of 40 mM Tris-HCl (pH 7.5), containing 2 % Triton X-100, 10 mM ethylene diamine tetraacetic acid (EDTA), aprotinin and leupeptin (30 μg/ml, each) by incubation on ice. Then, the extract was centrifugated at 6000 g for 10 min, and the pellet was washed twice in the extracting solution and dissolved in the sample buffer with 4% sodium dodecyl sulfate (SDS) and 5 % 2-ß-mercaptoethanol. Protein contents were measured with bicinchoninic acid protein assay kit (Pierce, Co.). Then, they were separated by 12.5 % SDS-PAGE

under reducing conditions. Gels were stained with brilliant blue R-250 and silver staining (Bio-Rad, Co.). Contents of HSP were assayed by immunoblotting using monoclonal antibody to human-HSP70 (Affinity BioReagent, Co.) and anti-mouse IgG peroxidase conjugates.

Results and Discussion

As shown in Figure 1, shear stress and heat shock induced the changed expression of triton-insoluble proteins of HUVECs. The expression of 50~50 kDa protein and approximately 70 kDa proteins were different under two types of stress. To access the expression of HSP70 in each stress, we will compare the relative amounts of HSP70 by the immunoblotting with anti-HSP70 antibody.

In conclusion, the different expression of insoluble protein aggregation by fluid shear stress and/or heat shock shows the different pathophysiologic mechanisms were underlying for these environmental stresses.

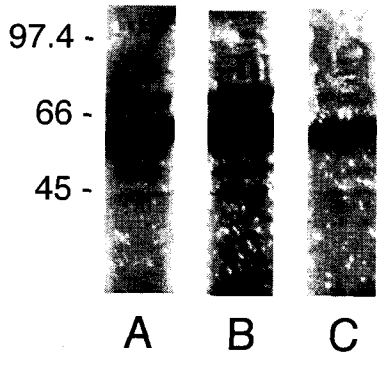

Figure 1. Gel pattern of the control (lane A) , and stressed cells (lane B: heat shock, laneC: shear stress). They were extracted with Triton (X-100)-containing buffer. The gels were stained with the silver staining.

References

1. A.E.kabakov and V.L Gabai, "Protein aggregation as primary and characteristic cell reaction to various stresses " *Experientia* 49 1993, 706-710.
2. Hyun Ah Chang, et al., "Morphological changes of endothelial cell induced by disruption of cytoskeleton.", *Proc. of KOSOMBE*, 1994, 16, 181-184.
3. Jun Keun Chang, "Mechanotransduction in endothelial cells adhered on the extracellular matrix.", Ph.D. Thesis, Seoul National University, 1995.
4. Vladimir L. Gabai, Alexander E. TiKabakov, AlexeiF.Mosin. "Association of blebbing with assembly of cytoskeletal proteins in ATP-depleted EL-4 ascites tumour cells." *Tissue and Cell.* 1992, 24 (2) , 171-177.
5. Milton J. Schlesinger, "Heat shock protein", *J. Bio. Chem.* 1990, 122111-12114.