TEMPORAL OBSERVATION OF DYNAMIC PROCESS OF FOCAL ADHESION-ASSOCIATED PROTEINS IN SHEARED ENDOTHELIAL CELLS

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INTRODUCTION

Vascular endothelial cells adhere to the extracellular matrix at focal adhesions (FAs) and FAs are believed to play an important role for changing cell shape with mechanical forces such as fluid flow. FAs are mainly composed of transmembrane integrin molecules, focal adhesion-associated proteins such as vinculin, and focal adhesion kinase (FAK). Specifically, FAK is known as a prime candidate for a mediator of integrin signaling events. Recent studies have shown that FAK phosphorylation is required for cell migration [1].

We have been interested in the mechanism of mechanical responses of endothelial cells, especially the roles of actin filaments [2,3]. The endothelial cells subjected to fluid shear stress are found to change in actin filament distribution and morphology. The cytoplasmic domain of integrins is indirectly linked to actin filaments in stress fibers via the multiple adhesion proteins and FAK. Taken together, it is expected that FAK and actin filaments may associate in changing endothelial cell shape under fluid shear stress. In order to understand mechanical responses of sheared endothelial cells, it is important to observe dynamic behavior of FAK together with actin filaments.

In the present study, the dynamic behavior of FAK assembly and actin filaments in endothelial cells exposed to shear stress was observed with a confocal laser scanning microscope. Endothelial cells were co-transfected with red fluorescent protein (RFP) combined with focal adhesion targeting (FAT) sequence and enhanced green fluorescent protein (EGFP) combined with actin.

MATERIALS AND METHODS

Cell Culture

Bovine aortic endothelial cells (BAECs) were obtained from thoracic aortas. BAECs were seeded in tissue culture flasks with Dulbecco's modified Eagle medium supplemented with 10% heat-inactivated fetal bovine serum, penicillin and streptomycin, and incubated at 37°C. BAECs were confluent after 4-5 days and then passaged at a 1:4 split ratio in the flasks using trypsin-EDTA. Fully confluent cell populations from the 4th to 10th generation were studied.

Gene Ttransfection

RFP-tagged FAT and EGFP-tagged actin were used in this study. FAT is the amid acid sequence at C terminus of FAK [4]. RFP-FAT and EGFP-actin were co-transfected into the cells planted on the glass-base dish at 50-80% confluence with liposomal method. Co-expressed cells were grown to confluence before a fluid-imposed experiment.

Flow—Imposed Experiment

The confluent endothelial cell monolayer was loaded into a parallel-plate flow chamber, as shown in Figure 1. In the flow chamber, an I/O unit and a gasket were also set in the dish to compose a flow field having a flow section of 0.5 mm in height and 14 mm in width. Laminar shear stress of 2 Pa, which is comparable to the average value in large arteries, was applied to the endothelial cells using a flow circuit. The flow circuit consisted of a flow chamber, two reservoirs, and a roller pump and was filled with the same cultured medium equilibrated with 95% air and 5% CO_2. The system was maintained at 37°C. The observation system consisted of an inverted microscope (IX-70, Olympus, Japan), a confocal laser scanning unit (CSU 10, Yokogawa, Japan), a digital CCD camera (C4742, Hamamatsu, Japan) and a personal computer (PowerMac G4, Apple, USA). Cells expressing RFP-FAT and EGFP-actin were observed under fluid condition. The images were recorded on the computer every 2 min up to 50 min.

RESULTS AND DISCUSSION

An example of time course of changes in FAK assembly and actin filaments is shown in Figure 2. The contrast of the figure is inverted. The panels are typically shown at 10 min intervals up to 50 min. It was confirmed by separate experiments that distribution of FAK was in good agreement with that of the vinculin at FAs, and localization of EGFP-actin coincided with the F-actin filaments stained by rhodamine-phalloidin. Before exposure to shear stress, FAK assembly was observed as dot/streak-like assembly. After exposure to shear stress, some of the dot-like assembly disappeared, and the elongation of the streak-like assembly were observed. In addition, the

appearance/disappearance of FAK assembly was also observed (Arrow 1). Within 20 min, significant changes in the position of FAK assembly did not observed. At 30 min, FAK assembly seemed to slide on the actin filaments with cell slight elongation in the flow direction (Arrow 2, 3). From 40 min to 50 min, the FAK assembly seemed to come together and aggregate. Thus, FAK remodeling involved several prominent features such as appearance/disappearance, elongation, sliding and aggregation. At present, the mechanism of these features remains unclear and should be clarified for better understanding of endothelial cell remodeling.

The separate observation has revealed that the actin filaments developed and formed stress fibers between FAK assemblies, corresponding the filament direction to the direction in the major axis of FAK assembly. This result suggests that the position and orientation of FAK assembly might play an important role in forming actin filament structure under shear flow condition.

The following process is expected that the FAK remodeling at focal adhesion site of endothelial cells in response to shear stress might mainly consist of 1. appearance/disappearance, 2. elongation, 3. sliding, 4. aggregation. In addition, the distributions of actin filaments might be associated with the location and orientation of FAK assembly.

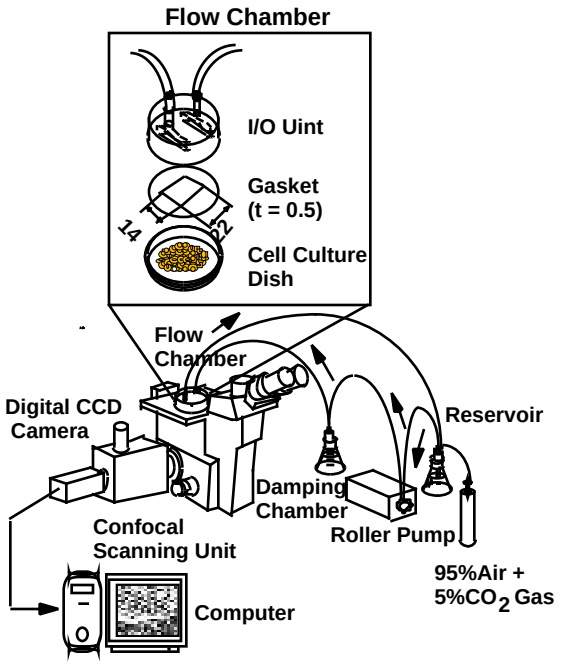

Figure 1. Schematic diagram of experimental setup.

CONCLUSIONS

Dynamic behavior of FAK and actin filaments in cultured endothelial cells co-transfected with RFP-FAT and EGFP-actin was observed during exposure to fluid shear stress. It was found that FAK remodeling involved several prominent features, and the location and orientation of FAK assembly were important to actin filament development.

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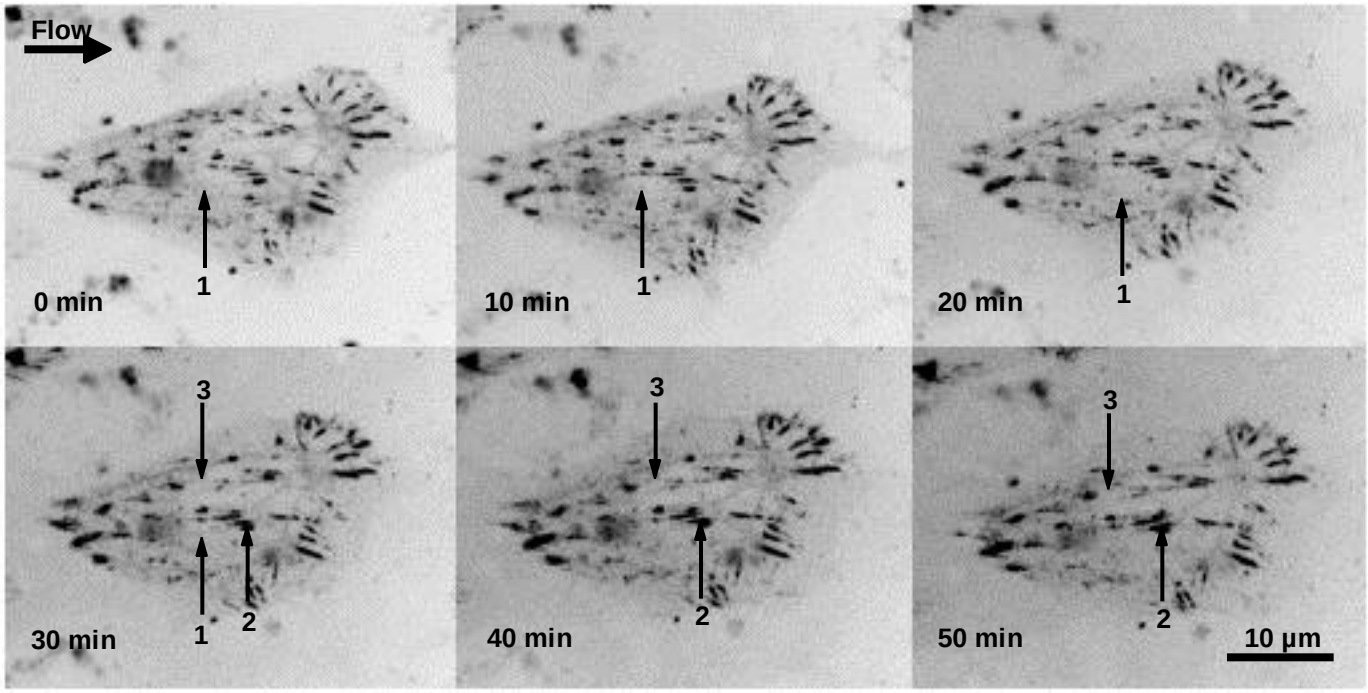

Figure 2. Time course of changes in FAT assembly and actin filamens in a cell exposed to shear stress.