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Abstract

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Controlled oxygen tension regulates vascular endothelial cell migration through p21-activated kinase signaling

[1]Yugo Tabata,[1]Naoyuki Takahashi,[2]Daisuke Yoshino,[3]Kenishi Funamoto

[1]Graduate School of Biomedical Engineering, Tohoku University, Japan
[2]Frontier Research Institute for Interdisciplinary Sciences, Tohoku University, Japan
[3]Institute of Fluid Science, Tohoku University, Japan

Abstract Body (Objectives, Methods, Results and Conclusions):

Hypoxic environment *in vivo* contributes to maintenance of homeostasis, but an abnormal oxygen tension in blood vessel is a trigger that leads to onset and progression of various diseases. Vascular endothelial cell monolayer has been shown to increase permeability and collective cell migration speed, resulting from the cell-cell junction lessened by hypoxic exposure. However, the underlying mechanisms are not fully understood yet. Here, we investigated signal transduction which relates to the changes in collective cell migration of vascular endothelial cells under hypoxic condition. Especially, a contribution of p21-activated kinase (PAK), which is a protein involved in motility of the cells and vascular permeability, was examined under controlled oxygen tension.

We first evaluated several proteins involved in a change of collective cell migration by hypoxic exposure. Human umbilical vein endothelial cells (HUVECs) were cultured with endothelial cell basal medium supplemented with growth factors (EGM-2) in cell cultured dish. HUVECs were collected with SDS buffer after 5-hour incubation under hypoxic (3% O_2) or normoxic (21% O_2) condition, and the collected proteins were examined by SDS-PAGE and Western blotting. Time-lapse observation of migration of HUVECs was then performed using a microfluidic device with oxygen tension controllability. The device had a central gel channel flanked with media channels, and gas channels were placed next to the media channels being separated by polydimethylsiloxane (PDMS) wall. It was fabricated with PDMS embedding a polycarbonate film. The type â… collagen gel (2.5 mg/mL) was placed in the gel channel, and the surface of the media channels was coated with Matrigel (2.0 mg/mL). HUVECs were seeded at a density of 2.5×10^6 /mL in the media channels and cultured by EGM-2 for three days to form a vascular endothelial cell monolayer covering the channel. In order to investigate a contribution of intracellular PAK, the cell culture medium was changed to EGM-2 supplemented with an inhibitor of PAK at 100 nM one day before the experiment. The device was placed in a stage incubator mounted on microscope. Phase-contrast images were captured every 10 minutes for five hours while controlling oxygen tension by supplying gas mixtures to the gas channels. The migration speed of cells was measured by particle image velocimetry (PIV) using the time-series phase-contrast images.

Phosphorylated level of PAK in HUVECs exposed to hypoxia was lower than that under normoxia. PIV analysis of time-lapse microscope images revealed that the hypoxic exposure increased the migration speed of HUVECs. However, the migration speed of HUVECs under supply of the PAK inhibitor decreased even though they were exposed to hypoxic condition. In addition, the HUVECs represented similar values, which were lower than that under normoxia without the PAK inhibitor, regardless the oxygen condition. These

results indicate PAK contribute to the changes of cell migration under hypoxic exposure.

We presented the increased migration speed and decreased phosphorylation of PAK in HUVECs exposed to hypoxia for 5 h. However, inhibition of PAK decreased the migration speed, implying PAK contributes to changes of the cell migration.