Available online at www.sciencedirect.com
ScienceDirect
European Journal of Cell Biology 88 (2009) 701–709
European Journal of Cell Biology
www.elsevier.de/ejcb

Vascular smooth muscle cells promote endothelial cell adhesion via microtubule dynamics and activation of paxillin and the extracellular signal-regulated kinase (ERK) pathway in a co-culture system

Yan-Hua Wang, Zhi-Qiang Yan, Bao-Rong Shen, Lu Zhang, Ping Zhang, Zong-Lai Jiang*

Institute of Mechanobiology & Medical Engineering, School of Life Sciences and Biotechnology, Box 888, Shanghai Jiao Tong University, 800 Dong Chuan Road, Minhang, Shanghai 200240, China

Received 6 January 2009; received in revised form 11 June 2009; accepted 12 June 2009

Abstract

Interaction between endothelial cells (ECs) and vascular smooth muscle cells (VSMCs) plays an important role in vascular biology. Cell adhesion to the extracellular matrix provides critical environmental information necessary for cell migration, proliferation, differentiation and survival. In this study, the role of VSMCs in EC adhesion was demonstrated by using a co-culture system. It was shown that the co-cultured VSMCs significantly increased the number of adherent ECs, and induced an increase of total focal adhesion area in ECs. These changes were associated with a low microtubule-to-tubulin ratio, and activation of extracellular signal-regulated kinase (ERK) and paxillin. Both the EC adhesion state and activation of the ERK/paxillin pathway by the co-cultured VSMCs could be inhibited by trichostatin A (TSA). As an inhibitor of histone deacetylase, TSA acts by modulating microtubule polymerization state. Taken together, these data suggest that the co-cultured VSMCs promote EC adhesion by modulating the microtubule cytoskeleton polymerization state, which in turn activates the ERK pathway and up-regulates phosphorylated paxillin expression to accelerate focal adhesion formation.

Keywords: Co-culture; Endothelial cell; Vascular smooth muscle cell; Trichostatin A; Paxillin; ERK

Introduction

Cell adhesion is an essential process for migration, survival and proliferation of endothelial cells (ECs) in the events of vascular physiology and pathophysiology. ECs and vascular smooth muscle cells (VSMCs) are the major cellular components of the vessel wall. The interaction between ECs and VSMCs plays a significant role to keep the homeostasis of blood vessels during vascular remodeling. Adhesion of ECs has been described by many authors, but in the majority of these works, ECs were cultured alone in vitro (Cheng et al., 2007; Reinhart-King et al., 2005; Ivanov et al., 2004). Consequently, the effects and mechanisms of the VSMC/EC interaction on EC adhesion have not yet been well elucidated in a physiological mimicking model system.

Cell adhesion is modulated by a series of intracellular events including membrane receptor-ligand binding,

*Corresponding author. Tel.: +86 21 3420 4863; fax: +86 21 3420 4118.
E-mail address: zljiang@sjtu.edu.cn (Z.-L. Jiang).

0171-9335/$ - see front matter © 2009 Elsevier GmbH. All rights reserved.
doi:10.1016/j.ejcb.2009.06.001

integrin activation, establishment of focal adhesions (FAs), cytoskeleton reorganization, and signaling cascade activation (Kirchner et al., 2003; Wang et al., 2007; Small et al., 2002). In cells, the depolymerization of microtubules can lead to the depolarization of cell shape (Vasiliev and Gelfand, 1976), an increase in the contractility of the cytoskeleton (Danowski, 1989) and an amplification in the size of FAs (Kirchner et al., 2003). Intimate interaction between microtubules and FAs has been shown to play an important role in modulating adhesion site dynamics (Kaverina et al., 2000).

Paxillin is associated with the cytoplasmic domains of integrins that are localized at FAs (Tang et al., 2008). It functions as an adaptor protein that interacts with both cytoskeletal and signaling proteins to regulate the assembly, disassembly, and signaling of FAs (Brown and Turner, 2002). Paxillin is also a cellular target for tyrosine or serine/threonine kinases that are activated as a result of integrin signaling after either cell adhesion or stimulation of quiescent cells with soluble growth factors and cytokines (Huang et al., 2008; Lee et al., 2006). Liu et al. (2002) found that extracellular signal-regulated kinase (ERK) phosphorylates paxillin in hepatocyte growth factor-stimulated epithelial cells, and that paxillin phosphorylation in turn enhances paxillin-FAK association. These observations suggest ERK might initially promote FA complex assembly by phosphorylation of paxillin. But whether this process also occurs in ECs co-cultured with VSMCs remains undetermined.

In the present study, the effect of VSMCs on EC adhesion and the possible underlying mechanism were investigated in a VSMC/EC co-culture system. It was demonstrated that the co-cultured VSMCs significantly promoted the adhesion of ECs. This process was mediated by the up-regulation of the paxillin phosphorylation and the activation of the ERK pathway in the ECs co-cultured with VSMCs. These data provide important insights into the mechanisms of VSMC-regulated EC function that may be essential in vascular remodeling.

Materials and methods

Cell culture

ECs were isolated by means of the collagenase perfusion technique, and VSMCs cultures were prepared by the explant technique from fresh human umbilical veins as described previously (Wang et al., 2007). Each experiment used secondary passage of ECs from a different donor. In all experiments, VSMCs between passages 2–7 were used. Cell populations with more than 95% purity were used in all experiments. Human periodontal ligament fibroblasts (HPLFs) were obtained from normal human periodontal tissue attached to permanent lower premolar teeth, and these fibroblasts were extracted during orthodontic therapy and subsequently cultured in DMEM (Gibco) supplemented with 10% fetal bovine serum. For all experiments, only fibroblasts between the fourth and eighth passages were used.

All procedures used conform to the principles outlined in the Declaration of Helsinki for use of human tissue.

VSMC/EC co-culture model

A co-culture system was established as previously described (Wang et al., 2007). Briefly, ECs were first seeded onto the outer side of a 10 μm thick porous polyethylene terephthalate (PET) membrane of a cell culture insert (Becton Dickinson Labware, NJ) at a density of 3×10^5 cells/cm^2. Prior to seeding VSMCs on the opposite side of the membrane at a density of 1×10^5 cells/cm^2, the VSMCs were collected, and incubated in 2% serum in DMEM for 2 h to eliminate the effect of trypsinization (VSMC/EC). ECs cultured alone in the same conditions were used as a control (Ø/EC). In other experiments, HPLFs were used instead of VSMCs (FB/EC).

For inhibitor studies, DMSO (Sigma) and ethanol were used as a solvent for PD98059 (Sigma) and trichostatin (TSA; Sigma), respectively. ECs were pretreated with either PD98059 (10 μM), or TSA (300 nM) or vehicle (M199 + DMSO or M199 + ethanol) for 20 min before co-cultured with VSMCs.

Cell adhesion assay

Tissue culture dishes (24-well plates; BD Biosciences) were incubated with fibronectin for 2 h at room temperature, and then washed three times with phosphate-buffered saline (PBS). Non-specific adhesion was blocked by incubation with 1% bovine serum albumin (BSA) in PBS. ECs (0.3 ml at a concentration of 2×10^5 cells/ml) cultured with or without VSMCs were added to 24-well plates. After incubating different samples for 30, 60 and 90 min, respectively, at 37 °C, cell adhesion was stopped in all samples by removal of the medium. Non-adherent cells were washed off, and attached cells were fixed with 0.5 ml acetone-methanol (1:1) (Kappert et al., 2000). Bright-field images of marked microscope fields were collected at 40× magnification at defined times. The number of attached cells in eight randomly chosen fields was counted and the data were analyzed. Each experiment was performed in triplicate.