MOVEMENTS OF PDGF b RECEPTORS IN VASCULAR ENDOTHELIAL CELLS

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ABSTRACT

The motility of cell membrane receptors has been implicated in the regulation of cell migration. We demonstrate here the movement of platelet-derived growth factor (PDGF) β receptor aggregates in endothelial cells (ECs) and possible mechanisms for regulating such movement in a rat aortic EC-denudation model. The movement of PDGF β-receptors was found in ECs in the denuded area, but not in control ECs without denudation. PDGF β-receptor movements were higher in the leading and middle regions of an EC than that in the trailing region. While PDGF β-receptor aggregates moved randomly in the cell leading region, those in the middle and trailing regions moved toward the leading region. Motile actin filaments interacted with PDGF β-receptor aggregates, enhancing the aggregate movement. Purified PDGF β-receptor was able to bind actin filaments in vitro. While PDGF-BB enhances PDGF β-receptor movements, cytochalasin D, AG1296 (a PDGF β-receptor tyrosine kinase inhibitor), PD98059 (an inhibitor for the ERK 1/2 activator), or ML-7 (a myosin light chain kinase inhibitor) reduced the movement. The movement of PDGF β-receptor was correlated with EC migration. These observations suggest that actin filaments possibly facilitate PDGF β-receptor movements in ECs following EC denudation, and PDGF-BB-related signaling kinases possibly participate in the regulation of such movements.

INTRODUCTION

Cell membrane receptors, including integrins and growth factor receptors, undergo random and directed movements in cultured cells [1-4]. Such movements have been implicated in regulating cell division and migration [1, 2, 4]. Vascular ECs express PDGF β receptors after mitogenic stimulations. The movement of PDGF β-receptors may be involved in regulating EC activities. We demonstrate here the movement of PDGF β-receptor aggregates in ECs and possible mechanisms for regulating such movement in a rat aortic EC-denudation model.

METHODS

Endothelial denudation A polyethylene catheter (1.8 mm) was inserted into the rat abdominal aorta (~1.6 mm) and moved back and forth for 5 times within a region of 3 mm in length. Aortic specimens were collected from the EC denuded area and a control area without denudation at day 3, 5, 7, and 10 (n=5 each time) for observation. These procedures were approved by the Animal Care and Use Committee of Northwestern University.

Immunohistochemistry At a specified time, collected aortic specimens were cut into axial strips, each including the entire EC-denuded area and a control area. Selected specimens were used to stain ECs with silver nitrate. EC migration was measured from the edge of denudation to the leading front of EC migration. Specimens were incubated in host serum with supplements of penicillin 50 units/ml and streptomycin 0.05 mg/ml, anti PDGF β-receptor antibody 0.1 μg/ml, rhodamine-phalloidin 2 nM, and Hoechst 33258 3 nM at 37° C for 1 hr, incubated with a rhodamine-conjugated secondary antibody 0.1 μg/ml, and observed using a fluorescence microscope in a mini-culture system on the microscopic stage. The density, speed, and direction of motile PDGF β-receptor aggregates were measured in ECs in denuded and control areas.

Administration of inhibitors PDGF-BB was applied to specimens at 0.5 μg/ml. Cytochalasin D (0.5 μM) was used to assess the role of actin filaments in regulating the movement of PDGF β-receptors AG1296, PD98059, and ML-7 were used to examine the influence of PDGF β-receptor tyrosine kinase, ERK 1/2, and myosin light chain kinase, respectively, on the movement of PDGF β-receptors These protein kinases are known to regulate actin-myosin interactions.

In vitro binding assay Purified actin filaments (10 μg) were incubated with purified, fluorescein-conjugated PDGF β-receptor (2 μg) in reaction buffer containing 2 nM rhodamine-phalloidin for 10 min, and observed using a fluorescence microscope. Fluorescein-conjugated bovine serum albumin, mouse IgG, and rat host serum proteins were used as controls.

RESULTS AND DISCUSSION

Movements of PDGF b-receptor aggregates

PDGF β-receptor aggregates (including motile and stationary) were mainly found in ECs in denuded areas but not in control areas. At day 5, the density of PDGF β-receptor aggregates was 4456+/-1404 aggregates/mm^2 in control ECs and 51544+/-13684 aggregates/mm^2 in ECs in the denuded area. The density decreased significantly at day 10 when the EC-denuded area was fully covered with ECs.

The movement of PDGFR-β aggregates was found mainly in ECs in the denuded area, but not in control ECs (Fig. 1). The aggregate movement was dependent on not only the location within the EC-denuded area, but also the location within a cell. In the periphery of the denuded area (partial EC coverage), the movement of PDGF β-receptor aggregates was higher in the leading and middle regions of an EC (0.238+/-0.020 and 0.222+/-0.030 μm/sec, respectively, at day 5) than that in the trailing region (0.125+/-0.020 μm/sec at day 5). While PDGF β-receptor aggregates moved randomly in the leading region of an EC, those in the middle and trailing regions moved toward the leading edge (Fig. 2).

In denuded areas fully covered with ECs, the movement of the aggregates (0.119+/-0.024, 0.117+/-0.019, and 0.121+/-0.017 μm/sec for the leading, middle, and trailing regions, respectively, at day 5) was lower than that in the peripheral ECs, and there was no significant difference between the 3 intracellular regions.

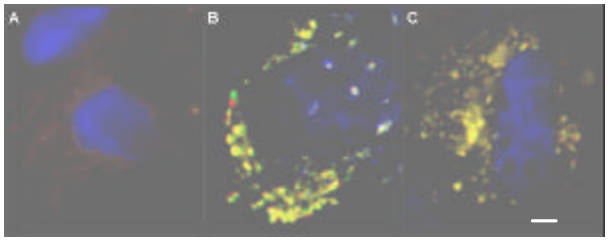

Fig. 1. Micrographs showing the movement of PDGF β-receptor aggregates in ECs. A. Control ECs. B and C. ECs from the denuded area. Each panel is an overlay of two successive images with a time gap of 1 sec. The green and red colors indicate the location of PDGF β-receptor aggregates at time 0 and 1 sec, respectively. The yellow color indicates that the location of the aggregates did not change. The blue color represents cell nuclei. Bar: 1 μm.

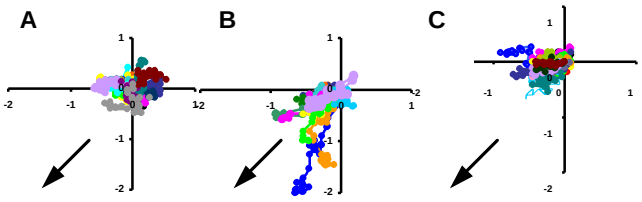

Fig. 2. Trajectories of PDGF β-receptor aggregates, recorded within 30 seconds, in the leading (A), middle (B), and trailing region (C) of an EC from the denuded area at day 5.

Interactions of Actin Filaments with PDGF b-receptor Aggregates

Motile actin filaments, observed mainly in ECs in the denuded area, interacted with PDGF β-receptor aggregates, enhancing the movement of the aggregates. A fraction of actin filaments initiated transient interactions with PDGF β-receptor aggregates, while others were associated permanently with PDGF β-receptor aggregates. A treatment with cytochalasin D reduces the motility of the PDGFR β-receptor aggregates. Furthermore, purified PDGFR-β was able to bind actin filaments in vitro, whereas albumin, IgG, and serum proteins were not (Fig. 3). These observations suggest that motile actin filaments possibly interact with and facilitate the movement of PDGF β-receptor aggregates in ECs in the denuded area.

Regulation of PDGF b-receptor Movements

It is known that PDGF-BB enhances the migration of cultured ECs via the mediation of PDGF β-receptor tyrosine kinase (TK) and ERK 1/2, which phosphorylates myosin light chain kinase (MLCK) [5]. Activated MLCK induces actin-myosin interactions, enhancing the motility of actin filaments. To elucidate whether these kinases mediate PDGF β-receptor movements, we applied PDGF-BB and selective kinase inhibitors, including AG1296 (for PDGF β-receptor TK), PD98059 (for MEK, an ERK 1/2 activator), and ML-7 (for MLCK), to aortic specimens. While PDGF-BB enhanced PDGF β-receptor movement, AG1296, PD98059, and ML-7 significantly reduced the movement. These observations suggest that PDGF-BB-related signaling mechanisms possibly mediate PDGF β-receptor movements via regulating the function of actin filaments.

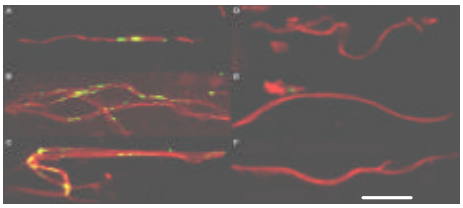

Fig. 3. Binding of purified PDGF β-receptor to purified actin filaments in vitro. A., B, and C. Different forms of actin filaments (red) with bound PDGF β-receptor (green). D, E, and F. Actin filaments incubated with host serum, albumin, and IgG, respectively. Bar: 1 μm.

Relation of PDGF b-receptor Movement to EC Migration

The movement of PDGF β-receptors varied with time (0.144+/0.044, 0.195+/-0.052, and 0.153+/-0.049 μm/sec at day 3, 5, and 7, respectively), so did the migration of ECs (5.44+/-0.86, 8.82+/-1.27, and 7.48+/-0.66 μm/hour at day 3, 5, and 7, respectively). The movement of PDGF β-receptors was correlated with EC migration ($p<0.05$).

In addition, motile PDGF β-receptor aggregates and phosphorylated Src (on tyrosine 418) were co-localized to the leading region of ECs in the denuded area. It is known that activated PDGF β-receptor tyrosine kinase induces the phosphorylation of Src, a non-receptor tyrosine kinase playing a critical role in regulating focal contact assembly and cell migration. Our observations suggest that PDGF β-receptor movements may facilitate interactions of PDGF β-receptor and Src. This mechanism may play a role in regulating EC migration following EC denudation.

ACKNOWLEDGMENT

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