Effect of a neuron-specific actin-binding protein, drebrin A, on cell-substratum adhesion

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Abstract

Drebrin A expression was induced in non-neuronal L cells via transfection with a vector containing the cDNA of rat drebrin A. Following treatment with colcemid (5 μg/ml) and cytochalasin D (0.5 μg/ml), most L cells collapsed into round cells, while drebrin expressing cells were resistant to the treatment, keeping their cell shapes. Simultaneously, actin filaments and microtubules were disrupted in both cell lines. By quantitative analysis, in the presence of cytochalasin D, the extent of cell spreading and cell attachment in drebrin expressing cells was significantly higher than that in control cells. These results suggest that drebrin A modulates cell-substratum adhesion.

Keywords: Drebrin; Cell-substratum adhesion; Actin; Tubulin; Cytochalasin D; Colcemid

Drebrin A is a neuron-specific actin-binding protein which is highly concentrated at the postsynaptic site of dendrites in the adult central nervous system [6,8,11]. We have previously demonstrated that drebrin A bound to actin filaments competes with tropomyosin, and in the case of overexpression of drebrin, tropomyosin binding to actin filaments was replaced by drebrin, resulting in the transformation of actin filaments from stress fibers to thick, curving bundles [4,10]. These results suggest that drebrin A regulates the organization of actin filaments. However, effects of drebrin A on cell-substratum adhesion have not yet been reported. In this study, we demonstrate the cell-substratum adhesion associated with drebrin A expression as changes in the shape of the cells.

The construction of drebrin A expression plasmid MIW-DA and establishment of the clonal cell line MIW-D6, constitutively expressing drebrin A, have been described previously [9]. In brief, the clonal cell line MIW-D6 was obtained after co-transfection of MIW-DA containing rat drebrin A cDNA with β-actin promoter and pSTB-neo into L cells. Neol cell was established by transfection of pSTB-neo alone.

Cell culture and immunocytochemical examination were carried out as described previously [2]. For immunocytochemistry, cells were seeded on 18 mm circular glass coverslips coated with poly-L-lysine in 20 mm diameter wells. Cytochalasin D and colcemid (depolymerization reagents for actin and tubulin, respectively) were added 1 day after inoculation of the cells. Sixty minutes later, the cells were fixed in 4% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.4) for 30 min at room temperature. Monoclonal antibody (MAb) M2F6, and NBD-phallacidin (Molecular Probe) which has affinity for actin filament [1], were used for the double staining of drebrin A and actin filaments. MAb 152H6 was used for the staining of tubulin. The characterization of these MAbs has been described previously [5,7]. Cell shapes were observed with an inverted microscope equipped with Hoffman Module optics in all stages.

Cell adhesion and cell spreading were measured by modification of Grinnell’s method [3]. In brief, to quantify the extent of cell adhesion, $5 \times 10^5$ cells in 4.0 ml of Grinnell’s adhesion medium were incubated in preincubated culture dishes for 60 min at 37°C. At the end of
Fig. 1. Morphological changes in L cells and MIW-D6 cells following treatment with colcemid and cytochalasin D. In the control medium the cell shapes of MIW-D6 (B) were similar to those of L cells (A). After treatment with colcemid and cytochalasin D, L cells changed into round cells (C), but MIW-D6 cells changed into flat cells with short processes (D). Both L cells (E) and MIW-D6 cells (F) were incubated for a further 24 h after removal of colcemid and cytochalasin D. Spread and unspread cells are indicated by arrows and arrowheads. Bar: 20 μm.

Incubation, cell spreading was observed with an inverted microscope and percentages of the cells bearing processes as shown by arrows in Fig. 1D were calculated as spread cells by counting more than 200 cells. Then the dishes were subjected to shaking at 150 rev./min on a shaker for 10 s at room temperature and the cells resuspended by this procedure were transferred to another dish. Percentages of the cells which remained on the shaken dishes were calculated as attached cells by counting more than 200 cells.

In the control medium, the cell shapes of MIW-D6 were similar to those of L cells (Fig. 1A, B). After treatment with 5 μg/ml of colcemid and 0.5 μg/ml of cytochalasin D, L cells collapsed, resembling round cells (Fig. 1C). On the contrary, MIW-D6 cells did not collapse, but changed into flat cells with short processes (Fig. 1D). These morphological changes were reversible both in L cells (Fig. 1E) and in MIW-D6 cells (Fig. 1F).

As microtubules and actin filaments in L and MIW-D6 cells were disrupted by the treatment (Figs. 2 and 3), the flat shape seen in the MTW-D6 cells was possibly associated with the enhanced cell-substratum adhesion. In control medium, adhesion plaques were visualized by vinculin immunostaining both in MIW-D6 and L cells. After treatment, vinculin-positive adhesion plaques still remained in MIW-D6 cells, while the plaques disappeared in L cells (data not shown). NeoL cells were similar to L cells with regard to the changes in the cell shapes and cytoskeletons caused by the treatment (data not shown), therefore, the difference between L and MIW-D6 cells is thought to be not due to transfection of the plasmids, but to drebrin A expression. Taking the results together, the characteristic cell shape of the drebrin expressing cells treated with colcemid and cytochalasin D may be caused by enhanced cell-substratum adhesion based on the adhesion plaques. Both L and MIW-D6 cells showed no remarkable morphological changes after treatment with either colcemid or cytochalasin D alone, while either microtubules or actin filaments were disrupted by each depolymerization reagent (data not shown).

Effects of drebrin A on cell-substratum adhesion are also suggested by the following result. By quantitative analysis, all of the MIW-D6, NeoL, and L cells showed similar ability in both cell spread and cell attachment in the control medium. However, after depolymerization of the actin filaments by treatment with cytochalasin D, the extent of cell spreading and cell attachment in drebrin expressing cells was significantly higher than that in L and NeoL cells (Fig. 4). It is said that spread cells are actively spreading, depending on their adhesion plaques, while cell-substratum adhesion in attached cells is based on not only adhesion plaques but also passive adhesion of their membrane to the substrate [3]. Because the differ-
Fig. 3. Effects of colcemid and cytochalasin D on actin filaments and microtubules in MIW-D6 cells. Cells in control medium (A–C). Cells in the medium supplemented with colcemid and cytochalasin D (D–F). Cells were double-labeled with MAb 152H6 for actin filaments (B,E), and with MAb M2F6 for drebrin (C,F), respectively. Curving bundles of actin filaments co-localized with drebrin were observed in the control medium, which were similar to those in the fibroblasts transiently expressing drebrin at a high level as reported previously [9]. After treatment, mesh-like structures (A) in the cells in control medium disappeared (D), and actin bundles (B) in the cells in control medium were transformed into patchy dot-like aggregates (E) co-localized with drebrin (F) in the cytoplasm. Bar: 20μm.

ence in the extent of cell spreading between L and MIW-D6 cells is nearly the same as that of cell attachment, the difference in cell-substratum adhesion between L and MIW-D6 cells might be mainly due to the difference of active cell-substratum adhesion based on adhesion plaques. Treatment with colcemid did not affect the ability of cell-substratum adhesion in any of the cell lines and created no significant difference between the L, NeoL, and MIW-D6 cells (data not shown). Effects of treatment with cytochalasin D and colcemid on cells were difficult to assay, because this treatment remarkably prolonged the time for the cells to spread on the dishes.

Adhesion plaques defined as the ends of stress fibers are responsible for the cell-substratum adhesion in cultured cells [12], but stress fibers are not observed within neurons in vivo. Therefore, there is the possibility that following the removal of stress fibers by cytochalasin D, the adhesion mechanism in cultured cells may be replaced by another adhesion mechanism which is similar to that in vivo. In the present study, the adhesion mechanisms in the MIW-D6 and L cells treated with cytochalasin D may be similar to those in neurons with and without drebrin expression, respectively. In the case of L cells treated with cytochalasin D, their adhesion mechanism is thought to be based not on adhesion plaques but on attachment of their membrane to the substrate. On the other hand, the adhesion mechanism in the MIW-D6 cells treated with cytochalasin D may be based on still another kind of adhesion plaques that is not defined as the ends of actin fibers. There are two possible ways of forming these adhesion plaques. One possibility is that the expression of drebrin A induces the formation of new adhesion plaques after disruption of actin fibers. The other possibility is that the adhesion plaques localized at the ends of actin fibers remain with the aid of expression of drebrin A.

Drebrin A may correlate with the regulation mechanisms of cell-substratum adhesion in vivo. Quantitative

Fig. 4. Effects of cytochalasin D on cell-substratum adhesion in L, NeoL, and MIW-D6 cells. Cells were first treated with cytochalasin D for 60 min, and then inoculated. After another 60 min inoculation, percentages of spread cells and attached cells were calculated. In the presence of cytochalasin D, the extent of cell spreading and cell attachment in drebrin expressing cells was significantly higher than that in L and NeoL cells. The difference of the extent of cell spreading between L and MIW-D6 cells (19.5%) was nearly the same as that of cell attachment (18.2%).
analysis of adhesion plaque-associated molecules such as vinculin is necessary to investigate the above mechanisms.

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