Overexpression of Ganglioside GM1 Results in the Dispersion of Platelet-derived Growth Factor Receptor from Glycolipid-enriched Microdomains and in the Suppression of Cell Growth Signals*

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To investigate the molecular mechanisms of gangliosides for the regulation of cell proliferation, Swiss 3T3 cells were transfected with GM2/GD2 synthase and GM1 synthase cDNAs, resulting in the establishment of GM1-expressing (GM1⁺) lines. Compared with the vector control (GM1⁻) cell lines, GM1⁺ cells showed reduced cell proliferation by stimulation with platelet-derived growth factor (PDGF). In accordance with the reduced cell growth, GM1⁺ cells showed earlier decreases in the phosphorylation levels of PDGFR receptor and less activation of MAP kinases than GM1⁻ cells. To analyze the effects of GM1 expression on the PDGF/PDGF receptor (PDGFR) signals, the glycolipid-enriched microdomain (GEM) was isolated and the following results were obtained. (i) PDGFR predominantly distributed in the non-GEM fraction in GM1⁺ cells, while it was present in both GEM and non-GEM fractions in GM1⁻ cells. (ii) Activation of PDGFR as detected by anti-phosphotyrosine antibody occurred almost in parallel with existing amounts of PDGFR in each fraction. (iii) GM1 binds with PDGFR in GEM fractions. These findings suggested that GM1 regulates signals via PDGF/PDGFR by controlling the distribution of PDGFR in and outside of GEM, and also interacting with PDGFR in the GEM fraction as a functional constituent of the microdomain.

Gangliosides, sialic acid-containing glycosphingolipids are ubiquitously expressed in embryonal and adult tissues of mammals and birds (1). In particular, they are enriched in nervous tissues, and the structures of the carbohydrate moiety are strictly regulated according to the developmental stages and tissue differentiation (1, 2). Biological roles of gangliosides have been investigated in many studies, and various functions have been claimed such as receptors for bacterial toxins (3), receptors for some viruses (4), modulators for Ca²⁺ ions (5), those for adhesion molecules (6–8), and for growth factor receptors (9). Some of them have also been assigned as a messenger of apoptosis signals (10). These functions can be classified into two major groups; recognition molecules for exogenous soluble molecules, and modulators of cis-acting receptor molecules for various growth/differentiation factors (9). However, the molecular mechanisms for ganglioside functions as described above have scarcely been clarified. A clear demonstration of the interaction between gangliosides and other receptor molecules has never been reported except for the binding of nerve growth factor receptor with GM1 (11).

The platelet-derived growth factor (PDGF) receptor is a member of a family of tyrosine kinases that modulate multiple cellular processes in response to ligand binding. This receptor exerts roles through multiple phosphorylation cascades, each of which begins with the phosphorylation of the receptor itself. Recently, many of the participating molecules and substrates of PDGF/PDGFR effects have been identified, and their sites of interaction were mapped (12). These findings suggested the existence of a signaling module associated with PDGFR at the cell surface, consisting of components of the tyrosine kinase mitogen-activated protein kinase (MAPK) pathway. Anderson and co-workers (13) demonstrated that caveolae fractions from unstimulated fibroblasts contained PDGFR, Ras, Raf-1, MAP kinase kinase 1, and MAPK, and PDGF stimulation activated MAP kinase in the caveolae fraction, indicating that these components are functional in vivo.

Caveolae have been thought to be specialized plasmalemmal microdomains originally studied in numerous cell types for their involvement in the transcytosis of macromolecules (14). They are enriched in glycosphingolipids (GSLs), cholesterol, sphingomyelin, and lipid-anchored membrane proteins, and they are characterized by a light buoyant density and resistance to solubilization by Triton X-100 at 4 °C. GSLs are enriched in this detergent-insoluble microdomain, that is almost equal to GEM (15, 16). (GEM was used in this article for this microdomain.) In particular, GM1 has been suggested to be a useful marker of GEM as well as a main structural protein, caveolin. If gangliosides play roles in the modulation of various receptor molecules as described above, it may be possible that gangliosides such as GM1 are involved in the regulation of signaling as a functional component of GEM, being more than an indicator of the microdomain.

In fact, Bremer et al. (17, 18) reported that GM3 and/or GM1 added to the culture medium of cells suppressed the cell growth

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1 The abbreviations used are: PDGF, platelet-derived growth factor; PDGFR, platelet-derived growth factor receptor; MAPK, mitogen-activated protein kinase (meaning ERK1/2); GSL(s), glycosphingolipid(s); GEM, glycolipid-enriched microdomain; mAb, monoclonal antibody; β1,4-GalNAc-T, β1,4-N-acetylgalactosaminyltransferase or GM2/GD2 synthase; β1,3-Gal-T, β1,3-galactosyltransferase or GM1 synthase; FITC, fluorescein isothiocyanate; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; MES, 4-morpholineethanesulfonic acid. The nomenclature of gangliosides is based on that of Svennerholm (54).
and/or phosphorylation of epidermal growth factor receptor and PDGFR. Exogenous GM1, in turn, enhanced the phosphorylation of nerve growth factor receptor TrkA, resulting in neurite extension of rat pheochromocytoma PC12 cells (11). In this study, we have established transfectant cells of mouse fibroblast line Swiss 3T3 highly expressing GM1 using cloned cDNAs of GM2/GD2 synthase (19) and GM1 synthase (20). Two kinds of cDNAs were transfected into cells with LipofectAMINE +/H262 transfection were plated in a 60-mm plastic tissue culture plate (Falcon, University). The cell surface expression of gangliosides was analyzed by flow cytometry (Beckton Dickinson, Mountain View, CA), and cultured up to 90% confluency. Then the cells were serum-starved for 6 h, and treated with PDGF (50 ng/ml) for 10 min. After treatment, cells were washed with phosphate-buffered saline containing 1 mM NaCl collected, suspended in 1 ml of MNE buffer (25 mM MES, pH 6.5, 150 mM NaCl, 5 mM EDTA) containing 1% Triton X-100, then DNase/treated were recognized 20 times, and incubated with an equal volume of 80% sucrose (w/v) in MNE buffer. Then, samples were placed on the bottom of Ultra-Clear Centrifuge Tubes (Beckman Instruments). Two ml of 30% sucrose in MNE buffer was overlaid, and 1 ml of 5% sucrose in MNE buffer was layered on the top. The samples were centrifuged for 16 h at 20,000 × g. The entire procedure was performed at 4 °C. Five hundred ml each was fractionated from the top. An opaque band located immediately above the 5% interface (fraction 3) was collected and designated the GEM fraction. A sample from the bottom fraction (fraction 10) was collected and designated the non-GEM fraction.

**Immunoprecipitation of PDGFR in the GEM and Non-GEM Fractions**—Sample fractions isolated as described above were vortexed. Fifty μl of each sample fraction was precipitated with trichloroacetic acid, washed with acetone 3 times, and subjected to SDS-PAGE and immunoblotted. The remaining samples were dialyzed twice for 4 h against the dialysis buffer (50 mM HEPES, pH 7.4, 150 mM NaCl, 50 mM NaF, 10 mM Na pyrophosphate, 0.2% Nonidet P-40, 1 mM NaVO3, 1 mM EDTA) before immunoprecipitation with anti-PDGFR antibody. After immunoprecipitation, beads were washed 4 times with washing buffer (50 mM Tris-HCl, pH 7.5, 0.3 mM NaCl, 0.5% (w/v) sodium deoxycholate, 0.5% Triton X-100, 1 mM Pefabloc SF, 1 μg/ml PMSF, 1 mM NaVO3, 1% SDS), and subjected to SDS-PAGE and immunoblotting.

**Cytoskeleton of GEM and PDGFR**—Cells were plated at a density of 1 × 10^5 in five 15-cm plates, and serum starved for 6 h before PDGF treatment. Then, cells were washed three times with phosphate-buffered saline containing 1 mM NaCl, and crosslinked at room temperature using 8.7 mM bis(sulfosuccinimidyl)suberate (BS3; Pierce, Rockford, IL) in 50 mM HEPES (pH 8.5) containing 137 mM NaCl, 6 mM KCl, 1 mM MgCl2, 10 mM EDTA. The reaction was terminated after 30 min. Cells were then subjected to GEM isolation.

**Extraction of Glycolipids**—Lipid fractions were extracted from about 200 μl of packed cells using chloroform/methanol (2:1, 1.1, 1.2) sequentially. After desalting, gangliosides were isolated by anion exchange column chromatography with DEAE-Sephadex A-50 (Amersham Biosciences Inc.). Thin layer chromatography (TLC) was performed with high performance TLC plates (Merck, Darmstadt, Germany) using a solvent system of chloroform, methanol, 0.2% CaCl2 (55:45:10).

**In Vitro Kinase Assay of PDGFR**—GEM fraction was isolated from V1 cells and served for immunoprecipitation with anti-PDGFR antibody. The precipitates were mixed with 20 μl of tyrosine kinase assay buffer (50 mM HEPES, pH 7.4, 20 mM MnCl2, 5 mM MgCl2, 1 mM diithiothreitol, 100 μM NaClO4). Then, 5 μCi of [γ-32P]ATP and GM1 were added before incubation at 30 °C for 10 min. Then, samples were subjected to 8% SDS-PAGE, and relative kinase activity was measured with autoradiography.

**Cytoskeleton of GEM and PDGFR**—Intracellular localization of GM1 and PDGFR was analyzed by immunohistochemistry. Cells were cultured on a coverslip and fixed with cold acetone containing 10% phosphate-buffered saline at −20 °C for 10 min. PDGFR staining was performed in the presence of anti-PDGFR antibody (958) and FITC anti-rabbit antibody (BIOSOURCE Int., Camarillo, CA). GM1 was stained with rhodamine-conjugated cholera toxin B (LIST Biological Laboratories Inc.). Staining pattern was analyzed with confocal microscopy (μRadiance™, Bio-Rad Microscience Lab, Tokyo).
RESULTS

Expression of Gangliosides by Transfection of Genes—After the transfection of Swiss 3T3 cells with β1,4-GalNAc-T expression vector (pMIKneo/β1,4-GalNAc-T) and β1,3-Gal-T expression vector (pMIKneo/β1,3-Gal-T), or pMIKneo alone, two transfectants (M1 and M3) and two vector control lines (V1 and V2) were established. Expression profiles of gangliosides were examined by flow cytometry (Fig. 1). Among the gangliosides examined, expression levels of GM1 markedly increased in the transfectants (M3 and M6). Expression levels of other gangliosides showed no change compared with those in the vector control lines (V1 and V2).

Effects of GM1 Expression on Cell Proliferation—The proliferation of the GM1 transfectants (M3 and M6) and the vector control lines (V1 and V2) were compared by MTT assay. As shown in Fig. 2, the GM1 transfectants showed a reduced growth rate in the presence of PDGF. The absorbance (590 nm) of transfectants (M3 and M6) in MTT assay was about 30–40% of that of vector control lines (V1 and V2). In the absence of PDGF, the growth of the transfectants was similar to that of vector control cells (data not shown). Consequently, we found endogenously expressed GM1 suppressed PDGF-dependent growth of Swiss 3T3 cells.

Phosphorylation of MAPK—To analyze the alteration of PDGF receptor (PDGFR) and its downstream signaling molecules, the activation of MAPK was examined by Western immunoblotting (Fig. 3A). After 6 h of serum deprivation, 50 ng/ml PDGF was added and incubated for 5, 10, 30, 60, or 120 min. Then cells were lysed, resolved by SDS-PAGE, and immunoblotted with anti-MAPK antibody. After that, the membrane was rebotted with anti-phosphorylated MAPK antibody. The intensity of MAPK bands was almost equivalent between transfectants (M3 and M6) and control lines (V1 and V2) and no apparent change in the intensity was observed during the incubation. For 5–10 min after PDGF addition, the phosphorylation level of MAPK was equivalent between the two groups. However, after 30–120 min, the transfectants showed earlier reduction in the activation levels of MAPK than those of vector control lines (Fig. 3B).

Phosphorylation of PDGFR—After analysis of the phosphorylation level of MAPK, the activation of PDGFR was examined by immunoprecipitation and immunoblotting (Fig. 4). After 6 h of serum deprivation, 50 ng/ml PDGF was added and incubated for 5 or 60 min. Then, lysates were immunoprecipitated with anti-PDGFR antibody. The immunoprecipitates were subjected to SDS-PAGE and immunoblotted with anti-phosphotyrosine antibody (PY20), (Fig. 4A, upper). Subsequently, the membrane was rebotted with anti-PDGFR antibody (Fig. 4A, lower). Phosphorylation levels of PDGFR in the transfectants were generally lower than those in vector control lines (Fig. 4B).

GEM Isolation—It is well known that GM1 localizes in GEM and has been used as a marker of GEM. PDGFR was found in caveolae by Anderson and co-workers (21). Therefore, we isolated GEM to compare the phosphorylation levels of PDGFR in GEM and non-GEM. To confirm the technique for the isolation

![Fig. 1. Expression levels of gangliosides in the transfectant cells.](image) The cell surface expression levels of gangliosides in each cell line were analyzed by flow cytometry as described under “Experimental Procedures.” Cells were stained with the anti-ganglioside mAbs. Controls were prepared with second antibody alone, and presented by thinner lines with dark color. The main synthetic pathway of gangliosides was shown at right side. Thick arrows represent the action of transfected gene products. V1 and V2 are control lines transfected with vector alone, and M3 and M6 are transfectant lines expressing high levels of GM1.

![Fig. 2. Effects of GM1 expression on the cell growth.](image) Two × 10⁴ cells were seeded in 48-well plates. After serum deprivation for 24 h, they were cultured in the presence of PDGF (50 ng/ml) and 1% fetal calf serum. At day 0, 1, and 2 of culture, MTT assay was performed as described under “Experimental Procedures.” Same experiments were repeated 3 times showing similar results. Note that the growth of two transfectant cells highly expressing GM1 was suppressed.
of GEM, we isolated GEM of transfectant cells (M3) and immunoblotting was performed with anti-caveolin-1 antibody and cholera toxin B subunit to identify GM1. As shown in Fig. 5, both caveolin-1 and GM1 were detected mainly in fraction 3. Both were present only in the low density fractions, and not in high density fractions such as fractions 9 and 10 in which soluble proteins were present. Therefore, we used fraction 3 as the GEM, and fraction 10 as the non-GEM fraction in the following experiments.

Alteration of PDGFR Localization in the Transfectants—We isolated the GEM fraction and the non-GEM fraction from each cell line. In all cell lines, the isolated GEM fraction contained 0.05 mg of total protein, and the non-GEM fraction contained 0.5 mg of total protein. They were subjected to immunoprecipitation with anti-PDGFR antibody. Immunoprecipitates were then immunoblotted with anti-phosphotyrosine antibody (PY20) to determine the phosphorylation of PDGFR (Fig. 6, A). Surprisingly, PDGFR in GEM of the transfectants (M3 and M6) was clearly and significantly reduced compared with that in non-GEM, although the vector control lines (V1 and V2) showed an almost equal distribution of PDGFR between GEM and non-GEM (Fig. 6, A and B, and Table I). Then, the membrane was reblotted with anti-phosphotyrosine antibody (PY20) to determine the phosphorylation of PDGFR (Fig. 6, A). The phosphorylation of PDGFR was detected only in PDGF-treated samples in both the transfectants and vector controls, and the intensity of the phosphorylated bands was almost proportional to that of PDGF receptor bands stained with anti-PDGFR antibody (Fig. 6, A). Phosphorylation levels of PDGFR decreased more rapidly in cells highly expressing GM1. Two bands in the immunoblots represent ERK1 and ERK2.

Alteration of Dimer Formation in the Transfectants—Cross-linking of PDGFR revealed that dimer bands could be detected in GEM fractions of control cells after PDGF treatment, but not in GEM fractions of the transfectant cells. Only a faint dimer band appeared in non-GEM of M6, but not in that of M3 (Fig. 7).

Co-precipitation of GM1 with PDGFR—To analyze the complex formation of PDGFR with GM1, the GEM fraction and signal suppression of PDGFR with GM1 overexpression...
FIG. 6. Localization of PDGFR in the GEM/non-GEM fractions during PDGF stimulation. A. after 6 h of serum deprivation, cells were stimulated by PDGF (50 ng/ml) for 10 min. Then cells were lysed. GEM (G) and non-GEM (N) fractions were isolated as described in the legend to Fig. 5, and PDGFR was immunoprecipitated. The immunoprecipitates were immunoblotted using an anti-phosphotyrosine antibody (PY20) or anti-PDGFR receptor as described under "Experimental Procedures." Note that levels of PDGFR in GEM were decreased in cells highly expressing GM1. Localization of caveolin-1 and GM1 was also examined using a caveolin-1 antibody or cholera toxin B, respectively. Same experiment was repeated at least 3 times with similar results. B, ratio of PDGFR bands in GEM to those in non-GEM in the cells before and after PDGF treatment. Average values ± S.D. from three experiments are presented. Note that the GEM/non-GEM ratios were extremely decreased in the transfectant cells. C, ratio of phosphorylated PDGFR bands in GEM to total phosphorylated PDGFR (non-GEM + GEM). Relative intensities to V2 bands (GEM + non-GEM:100) are presented. Three experiments showed similar results, and mean value ± S.D. is presented. Statistical significance was summarized in Table 1.

TABLE I

PDGFR was dispersed from GEM/DIM in GM1+ transfectant cells

A. Significant differences in the ratio of PDGFR as GEM/non-GEM shown in Fig. 6B

|        | M3 | M6 | M3 | M6 |
|--------|----|----|----|----|
| −PDGFR |     |    |    |    |
| V1     | 0.006* | 0.007 | 0.013 | 0.009 |
| V2     | 0.023 | 0.013 | 0.009 | 0.030 |
| +PDGFR |     |    |    |    |

B. Significant differences in the relative intensity of phospho-PDGFR bands shown in Fig. 6C

|        | GEM | GEM + non-GEM |
|--------|-----|---------------|
| M3 | M6 | M3 | M6 |
| V1 | 0.008* | 0.022 | 0.012 | 0.010 |
| V2 | 0.006 | 0.008 | 0.015 | 0.011 |

*p values obtained with paired t test.

non-GEM fraction were subjected to immunoprecipitation with anti-PDGFR antibody. Then, immunoprecipitates were extracted with chloroform/methanol (1:1), and the extracted samples were subjected to TLC immunostaining. The polyvinylidene difluoride membrane was blotted with cholera toxin B subunit, resulting in the detection of definite GM1 bands in the GEM fraction and faint bands in the non-GEM fraction (Fig. 8A). There was no significant difference in the intensity of GM1 bands between transfectants and vector controls (Fig. 8B), suggesting that the majority of the increased GM1 in the transfectants existed in GEM, but it was difficult for them to bind to PDGFR. The ratio of immunoprecipitated GM1 to PDGFR in GEM was markedly and significantly high in GM2+ cells (Fig. 8C). The immunoprecipitates were also subjected to immunoblotting with anti-caveolin-1 antibody. Caveolin-1 could not be detected (data not shown).

Effects of GM1 on Kinase Activity of PDGFR—To analyze the effects of GM1 on the PDGFR kinase activity, immunoprecipitated PDGFR served for kinase assay in the presence of various amounts of GM1. As shown in Fig. 9, a low concentration of GM1 rather enhanced the kinase activity, while relatively high concentration of GM1 (higher than 100 μM) suppressed the kinase in a dose-dependent manner.

Alteration of Intracellular Localization of PDGFR in the Transfectants as Analyzed by Immunocytochemistry—To confirm the dispersion of PDGFR from GEM in the transfectants, cytoplasm staining of GM1 and PDGFR was performed. GM1 was stained mainly on the cell membrane in both vector controls and transfectants, whereas the intensity was much stronger in the transfectants as expected (Fig. 10, left column). As for PDGFR, it was stained in membrane and cytoplasm as a granular pattern (Fig. 10, middle column). Merge of these two staining patterns revealed contrasting results between GM1+ cells and GM1− cells. The majority of GM1 was overlapped with PDGFR showing yellow color mainly around the cell membrane in the control cells (Fig. 10, right column). The faint green color seemed to represent PDGFR in the non-GEM fraction. In contrast, the yellow color indicating co-localizing GM1 and PDGFR was scarcely detected in the transfectants, and either the single red color (GM1/GEM) or single green color (PDGFR/non-GEM) was prominently detectable. These results as shown with higher magnification at the bottom were in good accordance with the results of the biochemical fractionation experiments as summarized in Fig. 11.

DISCUSSION

Cellular events regulated with signals via PDGF/PDGFR include cytoskeletal rearrangement and migration (22, 23), mitogenesis (24), differentiation (25), calcium mobilization (26), and apoptosis (27). These phenotypic changes induced via PDGF/PDGFR need sequential activation of various signaling molecules and substrates, and the mapping of the critical sites in the cytoplasmic domain of PDGF for the activation of individual pathways has been achieved (12, 28–30). The mechanisms for the PDGF/PDGFR to differentially regulate its multiple effects have been investigated by considering the existence of modular intermediates (31–33) and a functional unit at...
largely affected the intracellular localization of PDGFR, and consequently altered the quantity of the PDGF/PDGFR signals for cell proliferation, by reducing MAP kinase activation levels in response to PDGF stimulation. The discrepancy between the marked reduction in cell growth and MAPK activation and the mild decrease in PDGFR phosphorylation levels might imply the importance of GEM as a site of efficient signal transduction, i.e. PDGFR phosphorylation outside of GEM might be less efficient. This is the first study to demonstrate the role of GM1 to regulate the localization of a growth factor receptor inside/outside of the GEM fraction. GM1 also significantly binds to PDGFR, although the meaning of the binding is not clear at this moment.

A few examples of the association of glycosphingolipids with growth factor receptors/signal molecules have been known; GM1 and nerve growth factor receptor TrkA (11), GM3 and Srf/Rho (41), GD3 and Lyn (42), globotriaosylceramide (Gb3) and Yes (43). However, they just showed the association of protein molecules with membrane residing or exogenously added GSLs, and effects of the modification of the carbohydrate structures on the signaling functions have not been analyzed. Thus, findings obtained in the present study proposed a novel insight into the roles of glycolipids as a regulator of the organization of GEM and of the magnitude of the signals.

Bremer et al. (17, 18) reported suppression of cell growth and PDGFR phosphorylation in response to PDGF by exogenous GM1 as well as GM3. Yates et al. (44) analyzed the mechanisms for the inhibition of PDGFR-stimulated mitogenesis with gangliosides, showing reduced dimerization of PDGFR as a main ganglioside function (45). Inhibition of signaling events mediated by PDGFR with GM1 in glioma cells (46, 47) or with a few gangliosides in neuroblastoma cells (48) was also reported. The findings obtained in the present study appear almost similar, but the suppression levels are milder than those observed in the experiments with exogenous GM1. The conditions of the present experiments with GM1 synthase gene transfectants should be more physiological, and reflect the natural functions of GM1 in situ.

Since PDGF-dependent di- or multimerization of PDGFR is considered to be essential for the signal transduction, cross-linking experiments were performed. As shown in Fig. 7, dimer
PDGFR from GEM seemed to have induced the poor phosphorylation and reduced proliferation signals.

In general, anchoring of receptors/signal molecules to caveolae/GEM are determined by lipid modification, such as acylation and glycosylphosphatidylinositol linkage. The former targets the cytoplasmic signaling molecules to the inside, and the latter targets glycosylphosphatidylinositol-anchored surface proteins to the outside of the caveolae/GEM microdomain. However, PDGFR should be targeted to the microdomains mainly by the hydrophobic nature of its transmembrane domain. Therefore, the alteration in the localization of PDGFR observed in this study might be due to either the modified lipid core of GEM with the carbohydrate change, or the modified nature of the transmembrane domain of PDGFR caused by overexpressed GM1. Whatever the mechanisms, this is the first study to find that modification of glycosphingolipids resulted in the dispersion of receptor/signaling proteins from the microdomain. The molecular mechanism is now under investigation in our laboratory.

There have been a few studies in which signaling proteins moved toward or from GEM after phosphorylation with outside stimulation (43, 49–52). In the case of c-Ret, it is concentrated into rafts after stimulation of glial cell line-derived neurotrophic factor (53). In the present study, activation of PDGFR did not appear to cause significant changes in the localization of PDGFR, although the concentration of She, Syp, and MAPK in caveolae increased (21). The phosphorylation levels of PDGFR appeared almost parallel to its amounts in the GEM/non-GEM fractions. Consequently, the most critical factor to determine the quantity of PDGF/PDGFR signals in Swiss 3T3 cells appears to be the ratio of PDGFR localization between GEM and non-GEM.

Recently, Liu et al. (35) demonstrated that caveolae fractions from unstimulated fibroblasts contain PDGFR, Ras, Raf-1, MAPKK, and ERK-2, and they functionally associate upon PDGF exposure (35). Activated MAP kinase was not detected in non-caveolae fractions even after PDGF stimulation, suggesting that caveolae are a cell surface domain where MAP kinase is functionally linked to the PDGFR. Provided that PDGF/PDGFR signals are mainly transduced via GEM as described for the glial cell line-derived neurotrophic factor/GFRα1/c-Ret signals, it seems reasonable to consider that dislocation of PDGFR in the transfectant cells causes the reduction of activation levels of PDGFR and of the downstream signaling molecules.

REFERENCES

1. Wiegandt, H. (ed) (1985) in Glycolipids, pp. 199–260, Elsevier Science Publishing Co., Inc., New York
2. Vanier, M. T., Holm, M., Ohman, R., and Svennerholm, L. (1971) J. Neurochem. 18, 581–592
3. King, C. A., and van Heyningen, W. E. (1975) J. Infect. Dis. 131, 643–648
4. Markwell, M. A., Svennerholm, L., and Paulson, J. C. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 5406–5410
5. Wu, G. S., Yasawat, K. K., Lu, Z. H., and Ledeen, R. W. (1990) J. Neurochem. 55, 484–491
6. Kleiman, H. K., Martin, G. R., and Fishman, P. H. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 3367–3371
7. Cheres, D. A., Pierschbacher, M. D., Herrig, M. A., and Mujo, K. (1986) J. Cell Biol. 102, 690–696
8. Blackburn, C. C., Swank-Hill, P., and Schnaar, R. L. (1986) J. Biol. Chem. 261, 2873–2881
9. Hakomori, S. (1990) J. Biol. Chem. 265, 18713–18716
10. DeMali, K. A., Whiteford, C. C., Ulug, E. T., and Kazlauskas, A. (1997) J. Biol. Chem. 272, 9011–9018
11. Mutoh, T., Takada, A., Miyadai, T., Hamaguchi, M., and Fuji, N. (1995)/Proc. Natl. Acad. Sci. U. S. A. 92, 5087–5091
12. DeMali, K. A., Whiteford, C. C., Ulug, E. T., and Kazlauskas, A. (1997) J. Biol. Chem. 272, 9011–9018
13. Shaul, P. W., and Anderson, R. G. (1998) Am. J. Physiol. 275, L834–L851
14. Anderson, R. G., Renneman, B. A., Rothberg, K. G., and Lacey, S. W. (1992) Science 255, 410–411
15. Harder, T., and Simons, K. (1997) Curr. Opin. Cell Biol. 9, 534–542
16. Brown, D. A., and London, E. (1998) Annu. Rev. Cell Dev. Biol. 14, 111–136
17. Bremer, E. G., Hakomori, S., Bowen-Pope, D. F., Raines, E., and Ross, R.
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(1984) J. Biol. Chem. 259, 6818–6825
18. Bremer, E. G., Schlessinger, J., and Hakomori, S. (1986) J. Biol. Chem. 261, 2434–2440
19. Nagata, Y., Yamashiro, S., Yodoi, J., Lloyd, K. O., Shiku, H., and Furukawa, K. (1992) J. Biol. Chem. 267, 12082–12089
20. Miyazaki, H., Fukumoto, S., Okada, M., Hasegawa, T., and Furukawa, K. (1997) J. Biol. Chem. 272, 24784–24789
21. Liu, P., Ying, Y., Ko, Y. G., and Anderson, R. G. (1996) J. Cell Biol. 129, 584–588
22. Seppa, H., Grotendorst, G., Seppa, S., Schifflmann, E., and Martin, G. R. (1982) J. Cell Biol. 92, 286–290
23. Moolenaar, W. H., Tertoolen, L. G., and de Laat, S. W. (1984) Nature 312, 321–324
24. Cochran, B. H., Reffel, A. C., and Stiles, C. D. (1983) J. Cell Biol. 97, 1569–1573
25. Noble, M., Murray, K., Stroobant, P., Waterfield, M. D., and Riddle, P. (1988) Nature 334, 280–284
26. Moolenaar, W. H., Tertoolen, L. G., and de Laat, S. W. (1984) J. Biol. Chem. 259, 8066–8069
27. Kim, H. R., Upadhyay, S., Li, G., Palmer, K. C., and Deuel, T. F. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 9500–9504
28. Franke, T. F., Yang, S. I., Chan, T. O., Datta, K., Kazlauskas, A., Morrison, D. K., Kaplan, D. R., and Tsichlis, P. N. (1995) Cell 81, 727–736
29. Mahadevan, D., Yu, J. C., Salsnaha, J. W., Thakur, N., McPhie, P., Uren, A., LaRochelle, W. J., and Heidaran, M. A. (1995) J. Biol. Chem. 270, 27595–27600
30. Klinghoffer, R. A., Duckworth, B., Valius, M., Cantley, L., and Kazlauskas, A. (1996) Mol. Cell. Biol. 16, 5995–5914
31. Lawson, T. (1995) Nature 373, 573–578
32. Cohen, G. B., Ben, R., and Baltimore, D. (1995) Cell 80, 237–248
33. Marshall, C. J. (1995) Cell 80, 179–185
34. Hakomori, S., Yamamura, S., and Handa, A. K. (1998) Ann. N. Y. Acad. Sci. 845, 1–10
35. Liu, P., Ying, Y., and Anderson, R. G. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 13666–13670
36. Schnitzer, J. E., McIntosh, D. P., Dvorak, A. M., Liu, J., and Oh, P. (1995) Science 269, 1435–1439
37. Fra, A. M., Williamson, E., Simons, K., and Parton, R. G. (1994) J. Biol. Chem. 269, 30745–30748
38. Parton, R. G. (1994) J. Histochem. Cytochem. 42, 155–166
39. Iwabuchi, K., Yamamura, S., Prinetti, A., Handa, K., and Hakomori, S. (1998) J. Biol. Chem. 273, 9130–9138
40. Iwabuchi, K., Handa, K., and Hakomori, S. (1998) J. Biol. Chem. 273, 33766–33773
41. Yamamura, S., Handa, K., and Hakomori, S. (1997) Biochem. Biophys. Res. Commun. 236, 218–222
42. Kasahara, K., Watanabe, Y., Yamamoto, T., and Sanai, Y. (1997) J. Biol. Chem. 272, 29547–29553
43. Katagiri, Y. U., Mori, T., Nakajima, H., Katagiri, C., Taguchi, T., Takeda, T., Kyukawa, N., and Fujimoto, J. (1999) J. Biol. Chem. 274, 35278–35282
44. Yates, A. J., Van Brocklyn, J., Sagar, H. E., Guan, Z., Stokes, B. T., and O’Donoros, M. S. (1993) Exp. Cell Res. 204, 38–45
45. Yates, A. J., Sagar, H. E., and Van Brocklyn, J. (1995) J. Neurooncol. 24, 65–73
46. Van Brocklyn, J., Bremer, E. G., and Yates, A. J. (1995) J. Neurochem. 61, 371–374
47. Farquhert, T., Kelley, T., Cogeshall, K. M., Rampersaud, A. A., and Yates, A. J. (1999) Anticancer Res. 19, 5007–5013
48. Hynds, D. L., Summers, M., Van Brocklyn, J., O’Donoros, M. S., and Yates, A. J. (1995) J. Neurochem. 65, 2251–2258
49. Montixi, C., Langley, C., Bernard, A. M., Thimonier, J., Dubois, C., Wurbel, M. A., Chauvin, J. P., Piers, M., and He, H. T. (1998) EMBO J. 17, 5334–5348
50. Zhang, W., Trible, R. P., and Samelson, L. E. (1998) Immunity 9, 239–246
51. Xavier, R., Brennan, T., Li, Q., McCormack, C., and Seed, B. (1998) Immunity 8, 723–732
52. Yashiro, T., Zhou, X. Y., Toyo-Oka, K., Tai, X. G., Park, C. S., Hamaoka, T., Abe, R., Miyake, K., and Fujimoto, H. (2000) J. Immunol. 164, 1251–1259
53. Tansey, M. G., Baloh, R. H., Milbrandt, J., and Johnson, E. M. (2000) Neuron 25, 611–623
54. Svennerholm, L. (1963) J. Neurochem. 10, 613–623