Diverse activation states of RhoA in human lung cancer cells: Contribution of G protein coupled receptors

HIROKAZU TOUGE[1], HIROKI CHIKUMI[1], TADASHI IGISHI[1], JUN KURAI[1], HARUHIKO MAKINO[1], YOSHISATO TAMURA[1], MIYAKO TAKATA[1], KAZUHIKO YONEDA[1], MASAKI NAKAMOTO[1], HISASHI SUYAMA[1], J. SILVIO GUTKIND[2] and EIJI SHIMIZU[1]

[1]Division of Medical Oncology and Molecular Respirology, Department of Multidisciplinary Internal Medicine, Faculty of Medicine, Tottori University, 36-1 Nishi-machi, Yonago-shi, Tottori-ken 683-0805, Japan; [2]Oral and Pharyngeal Cancer Branch, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD 20892, USA

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Abstract. Rho GTPases play an essential role in the control of various cellular functions. Accumulating evidence suggests that RhoA overexpression contributes to human cancer development. However, the activation states of RhoA are poorly defined in cancer cells. In this study, we examined both the expression levels and the activation states of RhoA in various lung cancer cells by quantitative real-time reverse transcriptase-polymerase chain reaction and *in vivo* Rho guanine nucleotide exchange assay, respectively. Moreover, we dissected the signaling pathway from the cell surface receptors to RhoA using a broad-spectrum G protein coupled receptor (GPCR) antagonist, [D-Arg^{1},D-Trp^{5,7,9},Leu^{11}]Substance P (SP), and a recently reported G$\alpha_{q/11}$-selective inhibitor, YM-254890. We found that RhoA was expressed highly in large cell carcinoma cells but only weakly in adenocarcinoma cells. The activation states of RhoA are considerably different from its expression profiles. We found that four of six small cell lung carcinoma (SCLC) cell lines exhibited a moderate to high activation rate of RhoA. The addition of [D-Arg^{1},D-Trp^{5,7,9},Leu^{11}]SP reduced RhoA activity by almost 60% in H69 SCLC cells. The addition of YM-254890 had no effect on RhoA activity in H69 cells. Our results suggest that RhoA is activated in various lung cancer cells independent of its expression levels, and the high activation state of RhoA in SCLC cells mainly depends on a neuroendocrine peptide autocrine system which signals through Gα_{12} coupled GPCR to RhoA. This study provides new insights into RhoA signaling in lung cancer cells and may help in developing novel therapeutic strategies against lung cancer.

Correspondence to: Dr Hiroki Chikumi, Division of Medical Oncology and Molecular Respirology, Department of Multidisciplinary Internal Medicine, Faculty of Medicine, Tottori University, 36-1 Nishi-machi, Yonago-shi, Tottori-ken 683-0805, Japan
E-mail: chikumi@grape.med.tottori-u.ac.jp

Abbreviations: RT-PCR, reverse transcriptase-polymerase chain reaction; GPCR, G protein coupled receptor; SP, substance P; SCLC, small cell lung carcinoma; RhoGEFs, Rho guanine nucleotide exchange factors; GRP, gastrin releasing peptide; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; RBD, Rho-binding domain; NSCLC, non-small cell lung carcinoma; NMB, neuromedin B; BRS-3, bombesin receptor subtype 3; PLC, phospholipase C; EGF, epidermal growth factor; HGF, hepatocyte growth factor; PDGF, platelet-derived growth factor; LPA, lysophosphatidic acid

Key words: RhoA, lung cancer, YM-254890, G protein coupled receptors, *in vivo* Rho guanine nucleotide exchange assay

Introduction

Lung cancer is the leading cause of cancer-related deaths in the world. Despite recent advances in surgery, chemotherapy, and radiotherapy, the prognosis remains very poor. The poor prognosis of lung cancer indicates an urgent need for the development of innovative treatments. Recent studies of multiple intracellular signaling pathways have revealed mechanisms involved in cell proliferation and the metastasis of cancer cells, and this knowledge is being used to develop therapies directed against novel molecular targets for effective cancer therapy. Therefore, more knowledge regarding the multiple intracellular signaling pathways utilized in lung cancer cells is likely to have clinical relevance.

Rho GTPases, a subfamily of low molecular weight GTP-binding proteins, control many aspects of basic cellular functions, such as cell morphology, motility, cell proliferation, and cell cycle progression (1). To date, Rho GTPases are composed of 18 family members of proteins and can be subdivided further into several groups according to sequence similarity; those that are most similar to RhoA, those that are most similar to Rac1 and CDC42, and those that lack GTPase activity. RhoA, Rac1, and CDC42 are the most thoroughly studied members of the Rho GTPases. Recently, accumulating data suggest that aberrant regulation of RhoA contributes to human cancer development (2-5). For example,

constitutively active RhoA can transform murine fibroblasts (6), and these Rho-transformants develop distant lung metastases in animal models (7). Studies on clinical specimens have revealed that RhoA is overexpressed in a wide variety of human cancers, such as colon (2), breast (2), head and neck (8), and lung cancers (2). Moreover, its expression levels were reported to correlate positively with progression of the disease in breast (9) and testicular germ-cell cancer (4). These data suggest that RhoA plays an important role in human cancer development by its aberrant expression levels. Although aberrant expression levels of RhoA in cancer have been well described as cited above, its activation states and regulation in cancer are still poorly defined.

The activation states of Rho GTPases depend on their ability to cycle between an active GTP-bound form and an inactive GDP-bound form. Rho GTPases are active when bound to GTP and are inactivated upon hydrolysis of GTP to GDP. This cycling is controlled by a large family of Rho guanine nucleotide exchange factors (RhoGEFs) that stimulate the exchange of GDP for GTP (10-12). RhoGEFs are regulated by a diverse array of cell surface receptors, including receptor tyrosine kinases, G protein-coupled receptors (GPCR), and integrins, thus mediating the activation of Rho GTPases in response to external stimulation (12). Therefore, in theory, there are three possible mechanisms by which RhoA leads to cancer development; a high expression level, constitutively activated mutants or aberrant activation by abnormal external stimuli. The expression levels of Rho GTPases have been well documented but the presence of an activating mutation similar to that of oncogenic Ras has not been reported in human cancers. On the other hand, aberrant activation states of RhoA in human cancers have not been well studied. *In vivo* Rho guanine nucleotide exchange assay was recently developed that permits the measurement of the amount of an active GTP-bound form of Rho GTPases in targeted cells (13). However, few studies have applied this technique to estimate the pathologic impact of RhoA in cancer cells.

To investigate the potential role of RhoA in cancer and its regulation, we examined not only the expression levels of RhoA but also its activation states in various lung cancer cell lines. Furthermore, we dissected the signaling pathway from the cell surface receptors to RhoA in a variety of lung cancer cell lines using a broad-spectrum GPCR antagonist, [D-Arg^{1},D-Trp^{5,7,9},Leu^{11}]Substance P (SP) (14,15), and a recently reported $G\alpha_{q/11}$-selective inhibitor, YM-254890 (16).

Materials and methods

Reagents. [D-Arg^{1},D-Trp^{5,7,9},Leu^{11}]SP, an inhibitor of G protein-coupled receptors (GPCR), was purchased from Bachem AG (Bubendorf, Switzerland). YM-254890, a selective $G\alpha_{q/11}$ inhibitor and targets the exchange step of GDP for GTP in a $G\alpha_{q/11}$ activation state, was obtained from Astellas Pharma (Tokyo, Japan) (16). Gastrin releasing peptide (GRP) was purchased from Peptide Institute (Osaka, Japan).

Cell lines. Ms1, Ms13, and Ma29 lung cancer cell lines were provided by Dr T. Hirashima (Osaka Prefectural Habikino Hospital, Osaka, Japan). RERF-LC-KJ lung cancer cell line was obtained from RIKEN cell bank (Tsukuba, Japan). LU65, LSCS, EBC-1, LK-2, OBA-LK1, and Lu99 lung cancer cell lines were provided by Cell Resource Center for Biomedical Research, Institute of Development, Aging and Cancer, Tohoku University (Sendai, Japan). 87-5, 11-18, LK87, 1-87, LK79, Sq19, Sq-1, and 86-2 lung cancer cell lines were provided by Dr S. Kobayashi (Miyagi Prefectural Semine Hospital, Miyagi, Japan) through the Cell Resource Center for Biomedical Research. N417 and H69 small cell lung carcinoma (SCLC) cell lines were provided by Drs A.F. Gazdar and F.J. Kaye (NCI-Navy Medical Oncology Branch, NIH, Bethesda, MD). These 22 lung cancer cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM; Nissui, Tokyo, Japan) with 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 50 U/ml penicillin, and 50 mg/ml streptomycin. The cells were cultured in a humidified 5% CO_2 atmosphere at 37˚C.

Analysis of mRNA. For quantitative real-time reverse transcriptase-polymerase chain reaction (RT-PCR), DNA-free RNA was obtained using the RNeasy Mini kit (Qiagen, Tokyo, Japan). One microgram of total RNA was reverse-transcribed with random hexamers and SuperScript II RNase H^{-} reverse transcriptase (Invitrogen, Tokyo, Japan). Quantitative real-time RT-PCR was carried out with the LightCycler (Roche, Tokyo, Japan) and LightCycler FastStart DNA Master SYBR-Green I (Roche) using appropriate primers. Ten-fold serial dilutions of cDNAs were used to generate curves of log input amount versus threshold cycle, and comparable slopes were obtained for the group of cDNAs being tested. The amount of each mRNA was normalized relative to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA. The oligonucleotide primers used were: RhoA, 5'-AAGGACCAGTTCCCAGAGGT-3' and 5'-TTCTGGGGTCCACTTTTCTG-3' (Sigma, Osaka, Japan), and GAPDH, 5'-GTCATCCATGACAACTTTGG-3' and 5'-GCAGGTCAGGTCCACCACTG-3' (Sigma).

In vivo Rho guanine nucleotide exchange assay. The *in vivo* Rho activation states were assessed by a modified assay as described (13). Briefly, after serum starvation for 24 h, treated cells were lysed at 4˚C in buffer containing 20 mM HEPES, pH 7.4, 0.1 M NaCl, 1% Triton X-100, 10 mM EGTA, 40 mM ß-glycerophosphate, 20 mM $MgCl_2$, 1 mM Na_3VO_4, 1 mM dithiothreitol, 10 μg/ml aprotinin, 10 μg/ml leupeptin, and 1 mM phenylmethylsulfonyl fluoride. The lysates were incubated for 45 min with GST-rhotekin-RBD (Rho-binding domain) previously bound to glutathione-Sepharose beads and washed twice with lysis buffer. The associated GTP-bound forms of Rho were released with protein loading buffer and detected by Western blot analysis using a monoclonal antibody against RhoA (26C4, Santa Cruz Biotechnology, Santa Cruz, CA).

Western blot analysis. The proteins obtained from the *in vivo* Rho guanine nucleotide exchange assay were quantified by Western blot analysis (17) after SDS-polyacrylamide gel electrophoresis and visualized by enhanced chemiluminescence detection (Amersham Biosciences, Piscataway, NJ) using goat anti-mouse (Amersham Biosciences) IgGs coupled to horseradish peroxidase as a secondary antibody. Monoclonal

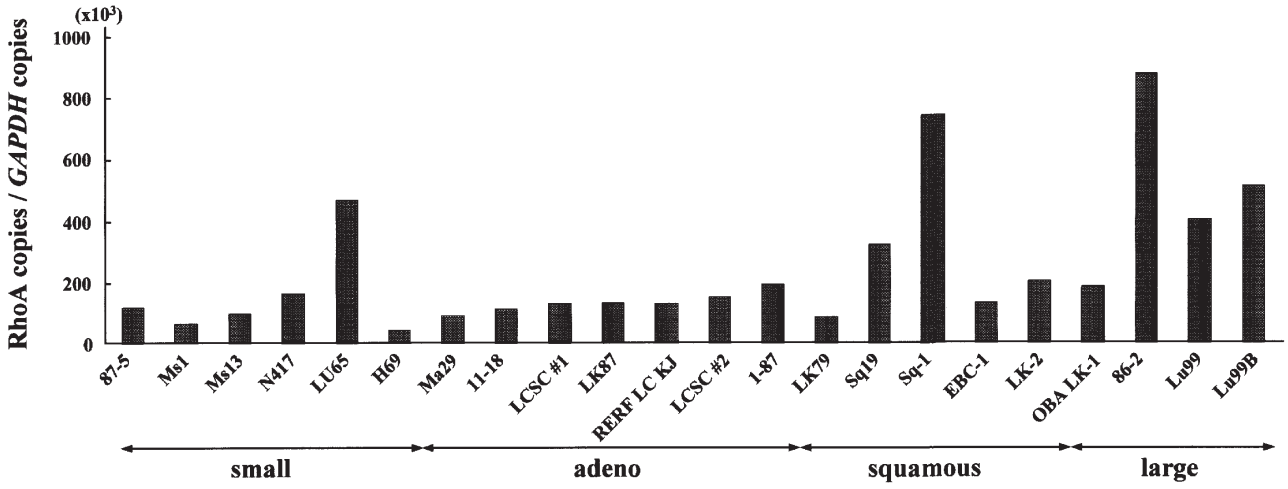

Figure 1. Quantitative real-time RT-PCR analysis of RhoA mRNA expression in lung cancer cells. The copy numbers of RhoA mRNA were normalized relative to GAPDH mRNA. The mRNA samples representing six SCLC cell lines, seven adenocarcinoma cell lines, five squamous cell carcinoma cell lines, and four large cell carcinoma cell lines were analyzed. The lung cancer cells were grown in DMEM supplemented with 10% fetal bovine serum.

antibodies against RhoA (26C4) were purchased from Santa Cruz Biotechnology.

Quantification of RhoA activation rate. Band intensity was quantified using the NIH Image software for Macintosh (version 1.63). The intensity of GTP-RhoA divided by the intensity of total RhoA multiplied by 100 was defined as the activation rate of RhoA.

Results

Quantitative real-time RT-PCR analysis of RhoA mRNA expression in lung cancer cells. First, we analyzed RhoA mRNA expression in lung cancer cells by quantitative real-time RT-PCR. RhoA mRNA was expressed in all cell lines tested in this study (Fig. 1). RhoA mRNA was highly expressed in one of six SCLC cell lines (LU65), two of five squamous cell carcinoma cell lines (Sq19, Sq-1), and three of four large cell carcinoma cell lines (86-2, Lu99, Lu99B). In contrast, all seven adenocarcinoma cell lines showed relatively low RhoA mRNA expression. These data indicate that RhoA is expressed to varying degrees in all types of lung cancer cell lines, with a tendency to be expressed highly in large cell carcinoma cell lines but only weakly in adenocarcinoma cell lines.

Activation rate of RhoA in lung cancer cell lines. The biologic significance of RhoA is determined not only by its expression levels but also by its activation states (1,18). To reveal the activation states of RhoA in each lung cancer cell line, we measured the intracellular levels of an active GTP-bound form of RhoA using a recently available *in vivo* Rho guanine nucleotide exchange assay (13). In this assay, a precipitated active GTP-bound form of RhoA and the total cellular amount of RhoA was visualized using Western blot analysis, and then the activation rate of RhoA was calculated as described in Materials and methods. We found that two of six SCLC cell lines (Ms13 and Lu65) exhibited a high activation rate (≥80%) and another two cell lines (N417 and H69) exhibited a moderate activation rate (20-40%) (Fig. 2). On the other hand, three of seven adenocarcinoma cell lines (Ma29, LCSC#1, and 1-87), one of five squamous cell carcinoma cell lines (Sq-1), and one of four large cell carcinoma cell lines (Lu99) exhibited a moderate activation rate, and the rest of the cell lines exhibited little (≤20%) or no activation. These data indicate that the activation states of RhoA differ considerably from its expression profiles, with a tendency to be higher in SCLC cell lines than in other types of cell lines.

[D-Arg^{1},D-Trp^{5,7,9},Leu^{11}]SP inhibits the RhoA activation states in H69 cells. We next focused on the mechanism of the highly activated state of RhoA in SCLC cell lines. To examine the involvement of the cell surface protein GPCR in RhoA activation in these cell lines, we utilized a broad-spectrum GPCR antagonist, [D-Arg^{1},D-Trp^{5,7,9},Leu^{11}]SP, which inhibits a broad range of neuropeptide receptors such as the bombesin receptors (14,15). In this experiment, we used H69, 1-87, Sq-1, and Lu99 cells as representative cells of SCLC cell lines, adenocarcinoma cell lines, squamous cell carcinoma cell lines, and large cell carcinoma cell lines, respectively. As shown in Fig. 3A, the addition of [D-Arg^{1},D-Trp^{5,7,9},Leu^{11}]SP at 10 μM reduced RhoA activation by almost 60% in H69 cells. However, in 1-87 and Sq-1 cells, the addition of [D-Arg^{1}, D-Trp^{5,7,9},Leu^{11}]SP at 10 μM did not affect the RhoA activation states. In Lu99 cells, the addition of [D-Arg^{1},D-Trp^{5,7,9},Leu^{11}]SP at 10 μM increased the RhoA activation states by almost 20%. These data indicated the highly activated state of RhoA in the SCLC cells; H69 was mediated most by the G protein coupled neuropeptide receptors.

G$\alpha_{q/11}$ inhibitor (YM-254890) does not inhibit the RhoA activation states in H69 cells. We next investigated which class of G protein was involved in the neuropeptide receptors of the RhoA pathway in the SCLC cell line H69. Generally, neuropeptide receptors, such as bombesin receptors, are reported to couple with G$\alpha_{12/13}$ and Gα_{q} (19), and both are believed to activate RhoA (20). To dissect this signaling pathway, we used a newly developed G$\alpha_{q/11}$-selective inhibitor, YM-254890 (16), because there are no G$\alpha_{12/13}$ specific

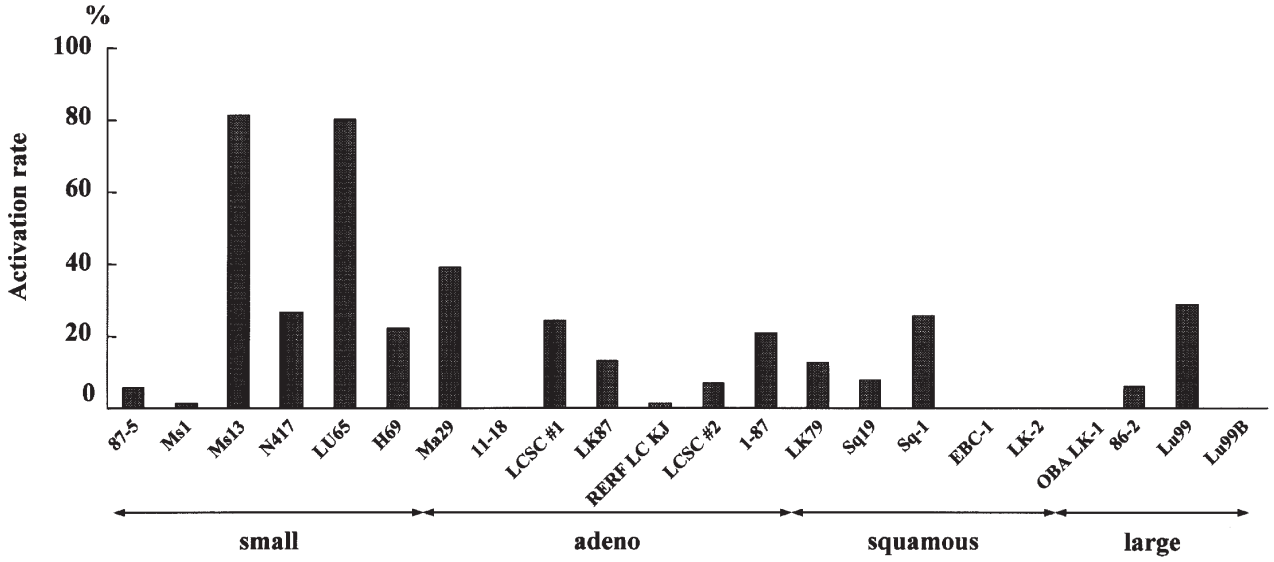

Figure 2. Activation rate of RhoA in lung cancer cells. Lung cancer cells were grown in 10-cm plates to 80% confluence and serum-starved overnight. The next morning, the cells were lysed in HEPES buffer. The cell lysates were incubated with GST-RBD beads. The beads were washed, and the amount of active RhoA bound to the beads (GTP-RhoA) and the total RhoA (total RhoA) in each cell lysate was analyzed by Western blot analysis with a monoclonal antibody against RhoA. The activation rate of RhoA was calculated as described in Materials and methods.

inhibitors. First, to test the contribution of $G\alpha_q$ to RhoA activation in GRP stimulated cells, we used Swiss 3T3 cells. Swiss 3T3 cells express GRP receptors and have been used most extensively for signal transduction studies of GRP receptors (21-23). As shown in Fig. 4A, RhoA was activated by GRP stimulation, and treatment with YM-254890 reduced this RhoA activation caused by GRP to 60%. This indicates that RhoA is activated by GRP receptors partially through $G\alpha_q$ in Swiss 3T3 cells. We then tested this $G\alpha_q$ contribution to RhoA activation in the lung cancer cells. As shown in Fig. 4B, the addition of YM-254890 at 10 μM had no effect on the RhoA activation states in the SCLC cell line H69. This result suggests that $G\alpha_q$ is not involved in the GPCR-mediated RhoA activation pathway and indicates the possible involvement of $G\alpha_{12/13}$ to this signaling pathway in the SCLC cell line H69. In addition, in several non-small cell lung carcinoma (NSCLC) cell lines, such as 1-87, Sq-1, and Lu99, the addition of 10 μM YM-254890 also had no effect on RhoA activation.

Discussion

The present study was designed to evaluate not only RhoA expression levels but also its activation states in human lung cancer cells. We showed that RhoA was expressed to varying degrees in all types of lung cancer cell lines, with a tendency to be expressed highly in large cell carcinoma cell lines but only weakly in adenocarcinoma cell lines. On the other hand, RhoA activation states were considerably different from its expression profiles. There was a trend toward higher RhoA activation states in SCLC cell lines than in any other type of lung cancer. In addition, we found that this high activation state of RhoA in SCLC cell lines is mediated by neuropeptide receptors possibly through the $G\alpha_{12/13}$ class of G proteins. Our results indicate that RhoA is activated to varying degrees irrespective of its expression levels in lung cancer cell lines, and this activation is caused mostly by neuropeptide receptor stimulation in SCLC cell lines, but by another stimulus in NSCLC cell lines. We believe this is the first demonstration of the difference in the activating pathway of RhoA between SCLC and NSCLC cell lines.

Many studies recently have suggested that Rho GTPases might contribute to cancer development. Rho GTPases have been shown to regulate cell morphology and actin cytoskeleton, gene expression, cell proliferation, and survival (1). As these cellular functions are important in tumorigenesis, subsequent studies have shed light on how Rho GTPases contribute to cancer development. It has been confirmed that there is overexpression of RhoA in human cancers (2), and it is associated with advanced stage (4), invasion (3), and metastasis (5). These previous studies on RhoA and cancer focused on RhoA expression levels in cancer cells or cancer tissue. However, as the biologic effect of RhoA is exerted by its activation states, it is essential to study the activation states of RhoA in cancer. To our knowledge, only one study has addressed this issue. Using four SCLC cells and four NSCLC cells, Varker *et al* reported that both the expression and GTPγS-dependent activation states of RhoA were generally greater in the SCLC cell lines than in NSCLC cell lines (24). Our result is partially in line with their study. With respect to the expression of RhoA, contrary to their study, we found that RhoA is highly expressed in not only SCLC cell lines but also in some NSCLC cell lines such as large cell carcinoma. This discrepancy may be due to differences in the cell lines tested. Our study assayed 16 NSCLC cell lines including four large cell carcinoma cell lines which highly expressed RhoA, whereas their study tested only two adenocarcinoma cell lines and two squamous cell carcinoma cell lines. Indeed, we similarly found that RhoA is only weakly expressed in all seven adenocarcinoma cell lines tested. With respect to the activation state of RhoA, our finding that the

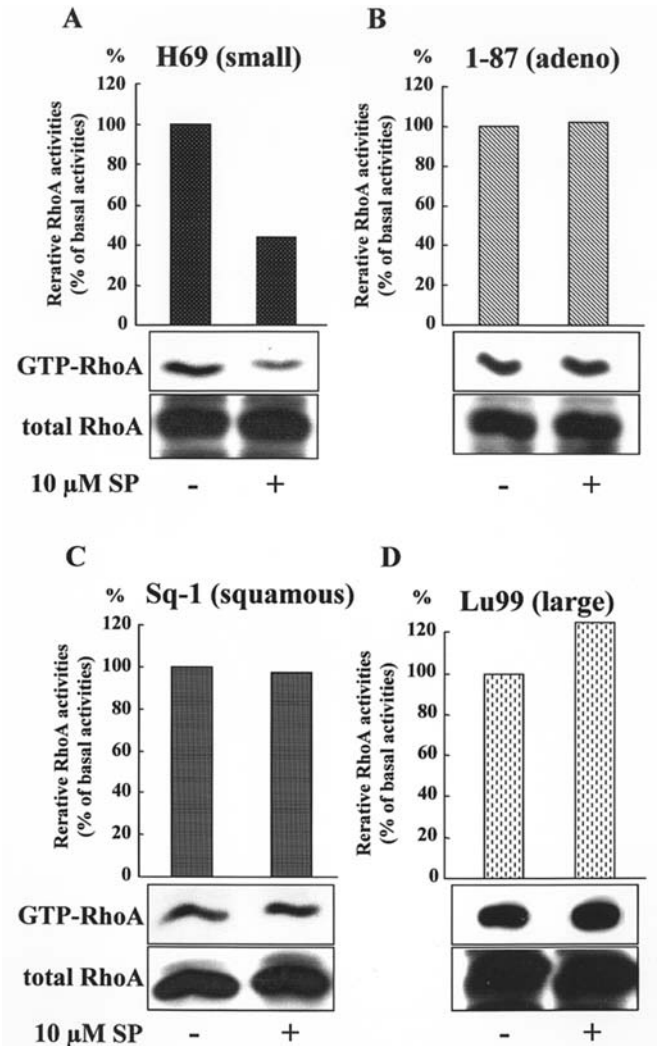

Figure 3. Influence of [D-Arg^{1},D-Trp^{5,7,9},Leu^{11}]SP treatment on RhoA activation in lung cancer cells. After serum starvation, the lung cancer cells were incubated with or without 10 μM [D-Arg^{1},D-Trp^{5,7,9},Leu^{11}]SP for 30 min at 37˚C. The cells were lysed and the lysates were incubated with GST-RBD beads. The beads were washed, and the amount of active RhoA bound to the beads (GTP-RhoA) and the total amount of RhoA (total RhoA) in each cell lysate were analyzed by Western blot analysis with a monoclonal antibody against RhoA. The band intensity of GTP-RhoA was quantified using NIH Image software. The relative RhoA activities are expressed as the percent of the basal RhoA activities.

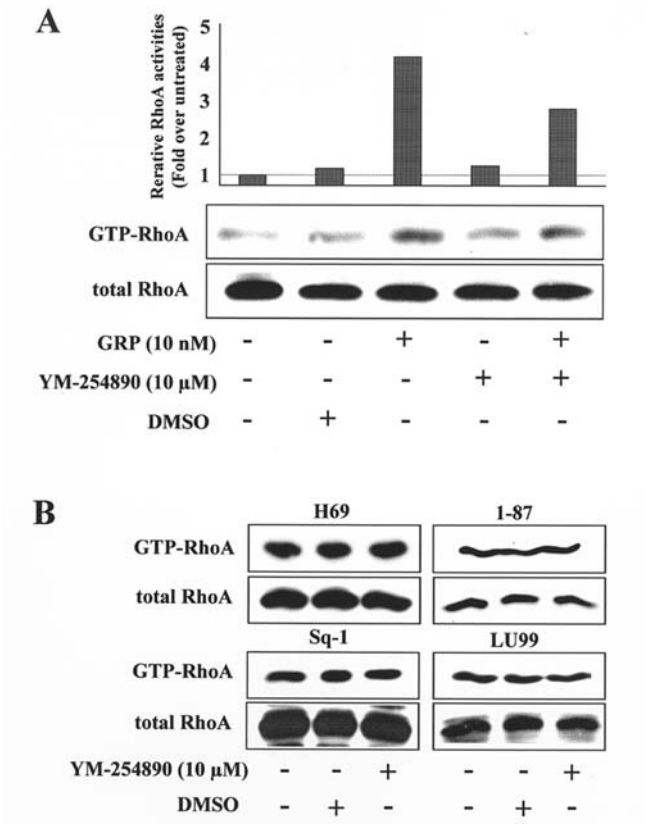

Figure 4. G$\alpha_{q/11}$ inhibitor YM-254890 inhibited RhoA activation states in Swiss 3T3 cells but not in lung cancer cells. A, Swiss 3T3 cells were grown on 10-cm plates to 80% confluence and serum-starved for 24 h. Next, the cells were treated with either vehicle control (DMSO) or 10 μM YM-254890 for 17 h. The cells then were left without further treatment or stimulated with GRP (10 nM) for 15 min. The cells were lysed and the lysates were incubated with GST-RBD beads. The amount of RhoA bound to the beads (GTP-RhoA) and the total amount of RhoA (total RhoA) in each cell lysate was analyzed by Western blot analysis with a monoclonal antibody against RhoA. The band intensity of GTP-RhoA was quantified using NIH Image software. The relative RhoA activities are expressed relative to the untreated set. B, lung cancer cells were serum-starved for 24 h, and treated with either vehicle control (DMSO) or 10 μM YM-254890 for 17 h. The cells were lysed and GTP-RhoA and total RhoA were visualized using an *in vivo* Rho guanine nucleotide exchange assay as described above.

activation states are higher relative to the expression levels in SCLC cell lines is consistent with their study. This provides a more complete understanding of a biologic feature of SCLC cell lines. Certain clinical aspects of SCLC, such as its high metastatic potential and high mortality rate, might be a consequence of its high RhoA activation state.

Our results also suggest a major contribution of neuropeptide receptors to the activation of RhoA in SCLC cell lines. Most SCLC cell lines are reported to express an autocrine growth system utilizing bombesin-like peptides and one or more of the known bombesin-like peptides receptor subtypes; the GRP receptor, the neuromedin B (NMB) receptor, and the bombesin receptor subtype 3 (BRS-3) (25,26). These receptors have seven transmembrane-spanning domains that are coupled to the heterotrimeric G proteins Gα_{q} and Gα_{12} (19). On the binding of GRP, these receptors activate multiple signal transduction pathways including proliferation (27-30) through a p42mapk/p44mapk pathway or actin cytoskeletal reorganization through a RhoA pathway. Therefore, the finding of a relatively high activation state of RhoA compared to its low expression level in the SCLC cell lines indicate the possible contribution of on autocrine system via neuropeptide receptors to RhoA activation. To test this hypothesis, we inhibited this autocrine loop using a broad spectrum neuropeptide antagonist, [D-Arg^{1},D-Trp^{5,7,9},Leu^{11}]SP. [D-Arg^{1},D-Trp^{5,7,9},Leu^{11}]SP, a substance P analogue, and reported to act as an antagonist of neuropeptide GPCRs (31). The finding that [D-Arg^{1},D-Trp^{5,7,9},Leu^{11}]SP reduced RhoA activation states by 60% in only the SCLC cell lines but not in the NSCLC cell lines is in line with this hypothesis. Together, our data indicate that, in SCLC cell lines, RhoA is highly activated mostly by a neuropeptide-mediated autocrine growth system.

To assess which class of G protein interacts with the neuropeptide receptors in the RhoA activation pathway in SCLC cells, we used a newly reported G$\alpha_{q/11}$-selective inhibitor,

YM-254890 (16). Neuropeptide receptors, such as the bombesin-like peptide receptors, are coupled to the $G\alpha_q$ and $G\alpha_{12}$ class of G protein. It was believed previously that the main effector molecules of $G\alpha_q$ and $G\alpha_{12}$ were phospholipase C (PLC) and Rho GTPases, respectively. Recently, however, we and other investivators have reported that $G\alpha_q$ also activates RhoA in certain cell types (20,32). Thus, in SCLC cell lines, it is possible that RhoA is activated by neuropeptide receptors through both $G\alpha_q$ and $G\alpha_{12}$. To test this hypothesis, we used the $G\alpha_q$ selective inhibitor YM-254890 because there is no available $G\alpha_{12}$ inhibitor. The novel compound YM-254890 is a cyclic depsipeptide discovered from the culture broth of Chromobacterum sp. It is the first known $G\alpha_{q/11}$ selective inhibitor, blocking the exchange step of GDP for GTP *in vitro* and *in vivo* (16). Using this compound, we observed that YM-254890 did not have any affect on the RhoA activation states in SCLC cell lines. This indicates that the RhoA activation states may not be mediated by $G\alpha_q$ but rather may be mediated by the $G\alpha_{12}$ family of G protein in SCLC cell lines.

In NSCLC cell lines, there are several possible mechanisms other than a neuropeptide system which can activate RhoA. Many growth factors such as epidermal growth factor (EGF), hepatocyte growth factor (HGF), or platelet-derived growth factor (PDGF) are reported to activate RhoA (33-35). Transforming growth factor-ß also is reported to activate RhoA and lead to cell motility (36). Lysophosphatidic acid (LPA) is known to activate RhoA, and this pathway is reported to use GPCR and the $G\alpha_{12/13}$ family of G proteins (18). Besides these humoral factors, cell adhesion also is reported to activate RhoA through integrins or cadherins (13). Since all the NSCLC cell lines tested in this study are adherent and all the SCLC cell lines are nonadherent, it is possible that the integrin or cadherin signal pathway contributes to a certain degree to the RhoA activation states in NSCLC cell lines. Further study is needed to determine what stimulation is important to RhoA activation states in NSCLC cells.

In conclusion, RhoA is activated in various lung cancer cells independent of its expression levels. Importantly, the high activation state of RhoA in SCLC cell lines is mainly dependent upon the neuroendocrine peptide autocrine system which signals through a $G\alpha_{12}$ coupled GPCR to RhoA. We conclude that to assess the pathogenicity of RhoA signaling, we need to consider its activation states in addition to its expression levels. This study provides new insights into RhoA signaling in lung cancer cells and may help in developing novel therapeutic strategies against lung cancer.

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