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Discordant Signal Transduction and Growth Inhibition of Small Cell Lung Carcinomas Induced by Expression of GTPase-deficient $G\alpha_{16}$*

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Small cell lung carcinoma (SCLC) accounts for 20–25% of primary lung cancers and is rapidly growing, widely metastatic, and rarely curable. Autocrine stimulation of multiple G protein-coupled neuropeptide receptor systems contributes to the transformed growth of SCLC. The ability of neuropeptide receptors to stimulate phospholipase C and mobilize intracellular Ca^{2+} indicates that G_q family members of heterotrimeric G proteins are a convergence point mediating autocrine signaling by multiple neuropeptides in SCLC. Expression of a GTPase-deficient, constitutive active form of an α_q family member, α_{16}Q212L, in SCLC markedly inhibited growth of the cells in soft agar and tumor formation in nude mice. SCLC lines expressing α_{16}Q212L exhibited 2–4-fold elevated basal phospholipase C activity, but neuropeptide and hormone-regulated intracellular Ca^{2+} mobilization was nearly abolished. The data suggest that Ca^{2+} mobilization is an obligatory signal in neuropeptide-stimulated growth of SCLC. In addition, the proline-directed c-Jun NH_2-terminal kinases/stress-activated protein kinases, which are members of the mitogen-activated protein kinase family, were stimulated ~2-fold in parental SCLC in response to exogenous neuropeptides and muscarinic agonists and were constitutively activated to the same degree in α_{16}Q212L-expressing SCLC. Thus, α_{16}Q212L expression induced desensitization of neuropeptide-stimulated Ca^{2+} signaling and persistent activation of the c-Jun NH_2-terminal kinase/stress-activated protein kinase pathway. We propose that the induction of discordant signaling by selective perturbation of receptor-regulated effector systems leads to the inhibition of SCLC cell growth.

Small cell lung carcinoma (SCLC)[1] displays neuroendocrine features exemplified by the presence of cytoplasmic neurosecretory granules containing a wide variety of mitogenic neuropeptides including gastrin-releasing peptide, arginine vasopressin, neurotensin, cholecystokinin, and many others (1, 2). Significantly, SCLC also expresses receptors for these neuropeptides, thereby establishing autocrine-stimulated cell growth (3). The number and variability of neuropeptides released from individual small cell carcinomas hampers effective blockade of mitogenic signaling at the level of ligand/receptor binding using specific neuropeptide antagonists. This redundancy at the level of receptor signaling highlights the importance of defining the intracellular components involved in mitogenic signal transduction in SCLC where convergence of multiple neuropeptide receptor systems into common pathways would be anticipated.

Molecular cloning of the receptors for gastrin-releasing peptide, vasopressin, and gastrin/cholecystokinin reveals that they are members of the superfamily of seven membrane-spanning receptors (4–8). As a class, these receptors initiate signaling in response to ligand binding by interacting with heterotrimeric G proteins. Although the repertoire of G proteins potentially involved in neuropeptide signaling in SCLC is quite large, the failure of pertussis toxin to appreciably inhibit *in vitro* growth of SCLC (9) suggests that the pertussis toxin-sensitive G_i and G_o proteins are unlikely to be dominant components of mitogenic signaling in SCLC. In fact, the prominence of phospholipase C activation and Ca^{2+} mobilization (10–12) by neuropeptides in SCLC points to the pertussis toxin-insensitive G_q proteins as likely candidates for mediating autocrine signaling in SCLC because the G_q family of G proteins are known to stimulate several of the phospholipase Cβ isoforms (13, 14).

To examine the role of G_q proteins in SCLC growth pathways, we have used retrovirus-mediated gene transfer to express a GTPase-deficient, constitutively active form of the α_q family member, $G\alpha_{16}$, in SCLC lines. We find that expression of GTPase-deficient $G\alpha_{16}$ in SCLC markedly inhibits their growth and oppositely affected two signal transduction pathways normally engaged by neuropeptides and muscarinic agonists in SCLC; Ca^{2+} mobilization was inhibited, and the c-Jun NH_2-terminal kinase/stress-activated protein kinase pathway was constitutively activated. The data indicate that derangement of coordinated neuropeptide signaling in SCLC leads to strong inhibition of growth.

MATERIALS AND METHODS

Retrovirus-mediated Gene Transfer and Cell Culture—The PA317 packaging cell line (15) was infected with ecotropic retrovirus secreted from GP+E-86 cell lines (16) that had been stably transfected with LNCX or LNCX-α_{16}Q212L (17). Following selection in G418, individual PA317 clones were screened for expression of α_{16}Q212L polypeptide by immunoblotting. The PA317 packaging cell lines expressing LNCX or LNCX-α_{16}Q212L were allowed to secrete virus into Dulbecco's modified Eagle's medium containing 10% fetal bovine serum for 16–24 h. The medium was collected, supplemented with 8 μg/ml polybrene, filtered through a 0.45-μm filter, and incubated undiluted with the indicated lung cancer cell lines for 16–24 h. The next day, the virus-containing medium was removed from the lung cancer cells and replaced with fresh virus for another 16–24 h. Following the second virus exposure, the lung cancer cell lines were placed in their normal growth medium

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[1] The abbreviations used are: SCLC, small cell lung carcinoma; MAP, mitogen-activated protein; MEK, MAPK/ERK kinase; NSCLC, non-small cell lung carcinoma.

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supplemented with 0.5 mg/ml G418. SCLC cell lines H345 and H1048 were cultured in HITES (RPMI 1640 medium containing 10 nM hydrocortisone, 5 μg/ml insulin, 10 μg/ml transferrin, 10 nM 17β-estradiol, 30 nM sodium selenite, and 0.1% bovine serum albumin), and SCLC cell lines H69 and N417 and NSCLC lines H157 and H2122 were cultured in RPMI 1640 medium containing 10% fetal bovine serum. The studies described herein were performed with pooled populations of G418-resistant cells.

Immunoblot Analysis of α_{16}Q212L Expression—The indicated lung cancer cell lines were collected in phosphate-buffered saline and lysed in 10 mM Tris-Cl (pH 7.4), 1 mM EDTA, 0.5% Triton X-100. Following a 5-min microcentrifugation (10,000 × *g*), soluble proteins (100 μg) were resolved by 10% SDS-polyacrylamide gel electrophoresis, transferred electrophoretically to nitrocellulose, and probed with a rabbit polyclonal anti-α_{16} antiserum (17). The immobilized antibodies were visualized with ^{125}I-protein A and autoradiography.

Determination of Soft Agar Cloning Efficiency—Single cell suspensions of the nonadherent SCLC cell lines were prepared by passage through a 20 guage syringe needle (H69, H345, N417, and H1048) or mild trypsinization of the adherent NSCLC lines (H157 and H2122). The cells were counted with a hemacytometer and 10,000 cells (H345, N417, H157, and H2122) or 25,000 cells (H69 and H1048) were diluted in 1.5 ml of growth medium containing 0.3% agar nobel (Difco) and plated in triplicate on a 2-ml base of growth medium containing 0.5% agar nobel in 35-mm wells of six-well plates. The plates were placed in a humidified CO_2 incubator, and the colonies were counted after 3–4 weeks with a microscope using a 4× objective.

Analysis of Total Inositol Phosphates—The SCLC lines infected with LNCX or LNCX-α_{16}Q212Lwere incubated for 24 h in RPMI 1640 medium containing 0.1% bovine serum albumin and 1 μCi/ml [^{3}H]inositol. The NSCLC lines were incubated in inositol-free Dulbecco's modified Eagle's medium containing 1 μCi/ml [^{3}H]inositol due to low uptake and incorporation of inositol by these lines relative to the SCLC lines. The cells were washed twice with RPMI 1640 medium containing 0.1% bovine serum albumin and once with the same containing 20 mM LiCl. The cells were then incubated in 1 ml of medium containing 20 mM LiCl for 30 min at 37 °C, quenched with 2 ml of MeOH:HCl (100:1), 1 ml of distilled water, and 2 ml of $CHCl_3$, and total inositol phosphates in the aqueous phase were purified by ion exchange chromatography as described previously (18).

Determination of Intracellular Ca^{2+} Levels—Plateau phase H345 and H1048 cells infected with LNCX or LNCX-α_{16}Q212L in HITES medium were washed, and single cell suspensions were prepared. The cells were loaded with the calcium indicator Indo-1 AM (Molecular Probes, Eugene, OR) as described (12, 19). The changes in intracellular Ca^{2+} levels were determined with an EPICS752 cell sorter (Coulter Electronics, Hialeah, FL) by quantitating the ratio of fluorescence at 410–490 nm using an excitation wavelength of 360 nm.

Assay of MAP Kinase and c-Jun NH_2-terminal Kinase Activities—Plateau phase H345 cells expressing α_{16}Q212L or the empty LNCX vector were collected by centrifugation (5 min, 1000 × *g*) and lysed in 1 ml of lysis buffer (0.5% Triton X-100, 50 mM β-glycerophosphate (pH 7.2), 0.1 mM sodium vanadate, 2 mM $MgCl_2$, 1 mM EGTA, 1 mM dithiothreitol, 2 μg/ml leupeptin, and 4 μg/ml aprotinin). In addition, H345 cells expressing the empty vector were incubated (37 °C) for 10 min with 100 nM 12-*O*-tetradecanoylphorbol-13-acetate, collected by centrifugation and lysed as described above. Following a 5-min centrifugation (10,000 × *g*) to remove nuclei and cell debris, portions of the soluble extracts (0.5 ml, 1 mg protein) were applied to a Pharmacia HR5/5 MonoQ column equilibrated in 50 mM β-glycerophosphate (pH 7.2), 0.1 mM sodium vanadate, 1 mM EGTA, and 1 mM dithiothreitol and eluted with a 30-ml gradient of 0–350 mM NaCl in the same buffer. Fractions (1 ml) were collected and assayed for MAP kinase activity with $EGFR_{662-681}$ peptide (20). Aliquots (20 μl) of the fractionated extracts were mixed with 20 μl of 50 mM β-glycerophosphate (pH 7.2), 0.1 mM sodium vanadate, 20 mM $MgCl_2$, 200 μM [γ-^{32}P]ATP (5000 cpm/pmol), 50 μg/ml IP-20 (TTYADFIASGRTGRRNAIHD), 1 mM EGTA, and 400 μM $EGFR_{662-681}$ peptide (RRELVEPLTPSGEAPNQALLR). The kinase reactions were incubated for 15 min at 30 °C and terminated with 10 μl of 25% trichloroacetic acid, and $EGFR_{662-681}$ peptide phosphorylation was assessed by binding to phosphocellulose filters as described (20).

The activity of c-Jun NH_2-terminal kinases was determined essentially as described (21). SCLC cells were collected by centrifugation (5 min, 1000 × *g*) and lysed (4 °C, 30 min) in 0.5 ml of 25 mM HEPES (pH 7.7), 20 mM β-glycerophosphate, 0.1 mM sodium vanadate, 0.1% Triton X-100, 0.3 M NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM dithiothreitol, 2 μg/ml leupeptin, and 4 μg/ml aprotinin. Following a 5-min microcentrifugation (10,000 × *g*), aliquots of the soluble extracts containing 400 μg of protein were incubated for 2 h at 4 °C with GST-c-Jun(1–79) adsorbed to glutathione-agarose as described (21). The GST-c-Jun(1–79) beads were washed four times by repetitive centrifugation in 20 mM HEPES (pH 7.7), 50 mM NaCl, 2.5 mM $MgCl_2$, 0.1 mM EDTA, and 0.05% Triton X-100 and then incubated for 20 min at 30 °C in 40 μl of 50 mM β-glycerophosphate (pH 7.6), 0.1 mM sodium vanadate, 10 mM $MgCl_2$, and 20 μM [γ-^{32}P]ATP (25,000 cpm/pmol). The reactions were terminated with 10 μl of SDS-polyacrylamide gel electrophoresis sample buffer, boiled, and submitted to 10% SDS-polyacrylamide gel electrophoresis. The GST-c-Jun(1–79) polypeptides were identified in Coomassie-stained gels, excised, and counted in a scintillation counter.

RESULTS AND DISCUSSION

To test the role of G_q family members in SCLC growth, we analyzed the effects of expression of GTPase-deficient forms of α_q family members on neuropeptide-stimulated signaling and growth in SCLC lines. We have previously used retrovirus-mediated gene transfer as a standard method to express exogenous gene products in many different cell lines (17, 20). However, repeated attempts to establish a stable PA317 retroviral packaging line expressing the GTPase-deficient form of α_q (α_qQ209L) failed, suggesting that the α_qQ209L polypeptide was cytotoxic in these lines and did not permit retroviral packaging. Subsequent attempts with the GTPase-deficient form of the α_q family member, α_{16}Q212L, were successful such that a stable PA317 retroviral packaging line that secreted virus encoding the α_{16}Q212L polypeptide was established.

Retrovirus encoding the α_{16}Q212L polypeptide as well as the vector, LNCX, lacking a cDNA insert were used to infect four SCLC lines (H69, H345, N417, and H1048) as well as two NSCLC cell lines (H157 and H2122). Stable populations of tumor cells were selected for resistance to G418. Immunoblotting with an α_{16}-specific antibody verified that an α_{16} polypeptide was indeed expressed in the α_{16}Q212L-transfected lines (Fig. 1*A*) relative to the LNCX-transfected controls. The lung cancer cell lines lack detectable endogenous α_{16} because it is normally restricted in expression to cells of hematopoetic origin (22). As predicted for an α_q family member, expression of the α_{16}Q212L polypeptide in the SCLC and NSCLC lines persistently stimulated phospholipase C activity as shown by the 2–4-fold increase in total inositol phosphate content observed in the α_{16}Q212L-expressing cells relative to the LNCX-transfected lines (Fig. 1*B*). Expression of the α_{16}Q212L polypeptide in the H1048 line, a small cell carcinoma of extrapulmonary origin (23), stimulated basal phospholipase C activity at least 10-fold. Repeated attempts to stably express α_{16}Q212L in another extrapulmonary small cell line (H510) failed, suggesting that small cell carcinomas of extrapulmonary origin may be particularly sensitive to α_{16}Q212L. Thus, the data show that α_{16}Q212L was functionally expressed in SCLC and NSCLC lines and constitutively activated phospholipase C activity.

Colony formation in semi-solid medium was used to monitor the transformed phenotype of SCLC and NSCLC expressing α_{16}Q212L. The ability of the α_{16}Q212L-expressing SCLC to form colonies in soft agar was inhibited approximately 70% respective to lines infected with the vector containing no cDNA insert (Fig. 2). In contrast, α_{16}Q212L expression did not influence the growth of two NSCLC lines (H157 and H2122). Note that the absolute cloning efficiency of the different lung cancer cell lines varied considerably, although the percentage of inhibition of soft agar cloning efficiency by α_{16}Q212L was similar. The soft agar cloning efficiencies of H345 cells expressing GTPase-deficient forms of α_{i2} and α_o were 96 and 139%, respectively, of the cloning efficiency of H345 cells infected with LNCX (data not shown), indicating that inhibition of SCLC growth is specific for the α_q family members. This finding is consistent with the inability of pertussis toxin to significantly stimulate or inhibit the *in vitro* growth of SCLC lines (9). As a

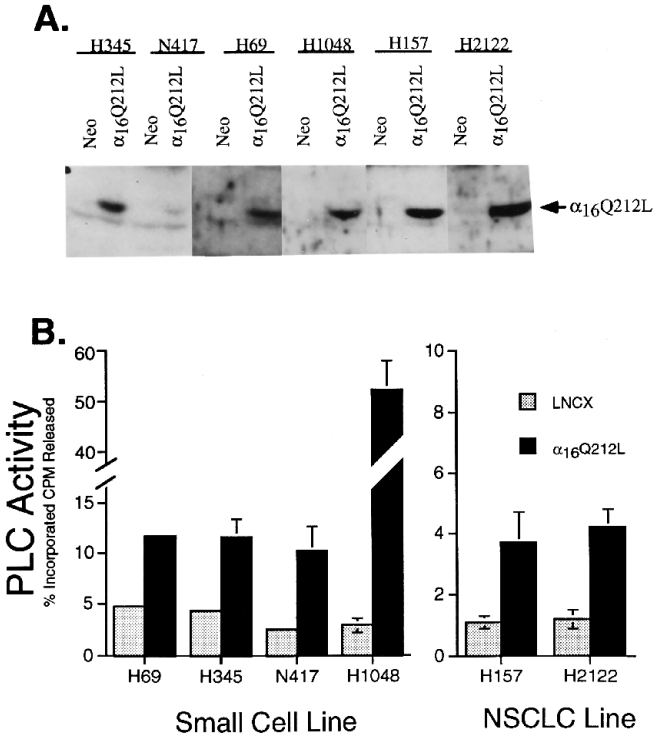

FIG. 1. **Functional expression of Gα_{16}Q212L in lung cancer cell lines.** *A*, immunoblot analysis of α_{16}Q212L expression in SCLC and NSCLC lines. Extracts from the indicated lung cancer cell lines were resolved by 10% SDS-polyacrylamide gel electrophoresis, transferred electrophoretically to nitrocellulose, and immunoblotted with a rabbit polyclonal anti-α_{16} antiserum. *B*, basal phospholipase C (*PLC*) activation in lung cancer cell lines expressing α_{16}Q212L. The indicated cell lines were labeled for 16–24 h with [^{3}H]inositol, and the accumulation of total inositol phosphates was determined as described under "Materials and Methods." The data are presented as the means ± S.E. of three independent experiments, except for H69, which depicts the average of two experiments.

more stringent indicator of the transformed growth properties of SCLC lines expressing α_{16}Q212L, the H1048 lines infected with LNCX or α_{16}Q212L were implanted in the flanks of nude mice. Of the three mice injected with H1048-LNCX cells (10^7 cells per mouse), all formed large tumors after 6–8 weeks. In contrast, none of the three mice injected with H1048-α_{16}Q212L cells developed tumors within 8 weeks. Thus, expression of α_{16}Q212L in SCLC but not NSCLC significantly inhibited their *in vitro* and *in vivo* growth properties.

To define the mechanism by which α_{16}Q212L inhibits SCLC growth, the influence of α_{16}Q212L expression on neuropeptide signaling was examined. A prominent indicator of neuropeptide signaling in SCLC is intracellular Ca^{2+} mobilization (10–12), which can be readily detected by changes in fluorescence of the Ca^{2+}-sensitive dye, Indo-1. The H1048 and H345 SCLC lines infected with the LNCX vector alone show marked Ca^{2+} responses following exposure to exogenous cholecystokinin (Fig. 3*A*) and the muscarinic agonist, carbachol (Fig. 3*C*), respectively. The H1048-LNCX cells also exhibit a measurable Ca^{2+} mobilization response to bradykinin (Fig. 3*B*). The H1048 and H345 SCLC lines expressing α_{16}Q212L exhibited markedly blunted Ca^{2+} mobilization responses following application of cholecystokinin, bradykinin, and carbachol. The data are consistent with a mechanism whereby α_{16}Q212L expression desensitizes hormone and neuropeptide-regulated Ca^{2+} mobilization in a heterologous fashion. Previously, workers have shown that chronic activation of phospholipase Cβ systems induces desensitization or down-regulation of the the inositol trisphosphate-gated Ca^{2+} channel in the endoplasmic reticulum (24) as well as induction of polyphosphatidyinositol phosphatases.[2]

Besides a blunted growth rate, SCLC H345 cells expressing α_{16}Q212L are significantly increased in cell size relative to LNCX-infected controls. In addition, both H345 and H1048 exhibited increased adherence to tissue culture plastic upon expression of α_{16}Q212L. Thus, expression of α_{16}Q212L alters the morphologic properties of some of the SCLC lines. Interestingly, a rat neuroendocrine tumor cell line, PC12 pheochromocytoma cells, undergoes marked growth arrest and neuronal differentiation upon introduction of α_{16}Q212L by retrovirus-mediated gene transfer.[3] The inhibition of SCLC growth observed with α_{16}Q212L expression is likely to be related, at least in part, to loss of Ca^{2+} signaling, indicating that the Ca^{2+} mobilization is a major mitogenic signal in SCLC. However, it seemed possible that additional signal pathways involved in regulation of growth and differentiation were being regulated in response to expression of α_{16}Q212L, especially in relation to altered cellular morphology. The activity of the p42/44 MAP kinases was assessed because they have been previously shown to be persistently stimulated in morphologically differentiated PC12 cells (20). Extracts from LNCX and α_{16}Q212L-expressing H345 cells were fractionated on MonoQ fast protein liquid chromatography and analyzed for MAP kinase activity with an $EGFR_{662-681}$ peptide phosphorylation assay. The data in Fig. 4 reveal that increased p42/44 MAP kinase activity relative to control cell extracts was not observed in fractionated extracts from α_{16}Q212L-expressing H345 cells. In addition, incubation of the various SCLC lines with neuropeptides including bombesin and vasopressin failed to significantly stimulate p42/44 MAP kinase activity (data not shown), although treatment of H345 cells with the phorbol ester 12-*O*-tetradecanoylphorbol-13-acetate markedly increased MAP kinase activity (Fig. 4).

The recently defined c-Jun NH_2-terminal kinases or stress-activated protein kinases are members of the MAP kinase family that exhibit homology to the p42/44 MAP kinases (26, 27) and are strongly activated by exposure to heat shock and ultraviolet light (21). Analysis of c-Jun NH_2-terminal kinase activity using an immobilized GST-c-Jun(1–79) assay revealed a statistically significant 1.8-fold increase in c-Jun NH_2-terminal kinase/stress-activated protein kinase activity in extracts from H345 cells expressing α_{16}Q212L relative to control cells (Fig. 5*A*). The magnitude of c-Jun NH_2-terminal kinase activation by α_{16}Q212L is similar to the level of activation observed in response to acute treatment of H345 cells with a neuropeptide, bombesin, or a muscarinic agonist, carbachol (Fig. 5*B*). Furthermore, c-Jun NH_2-terminal kinase activity is strongly stimulated (~10-fold) by exposure of H345 cells to ultraviolet irradiation (UV-C, 96 J/M^2), but not by strong activation of protein kinase C with 12-*O*-tetradecanoylphorbol-13-acetate (Fig. 5*C*) that leads to marked p42/44 MAP kinase activation (Fig. 4). Thus, the findings demonstrate a significant activation of protein kinases characteristic of the c-Jun NH_2-terminal kinases/stress-activated protein kinases but not the p42/44 MAP kinases following expression of α_{16}Q212L in a SCLC line.

Elevated cAMP and activation of cAMP-dependent protein kinase are growth inhibitory in many cell systems. It has been previously established that expression of α_{16}Q212L in Swiss 3T3 fibroblasts significantly stimulates cAMP-dependent protein kinase through a protein kinase C-dependent mechanism (17), a finding that may be partially responsible for the growth inhibition observed in Swiss 3T3 cells. However, the basal activity of cAMP-dependent protein kinase in α_{16}Q212L-expressing H345 and H69 cells was 85 and 117% of LNCX-

[2] Lobaugh, L. A., Eisfelder, B., Johnson, G. L., and Putney, J. W., Jr., submitted for publication.

[3] L. E. Heasley, B. Storey, and J. Zamarripa, unpublished results.

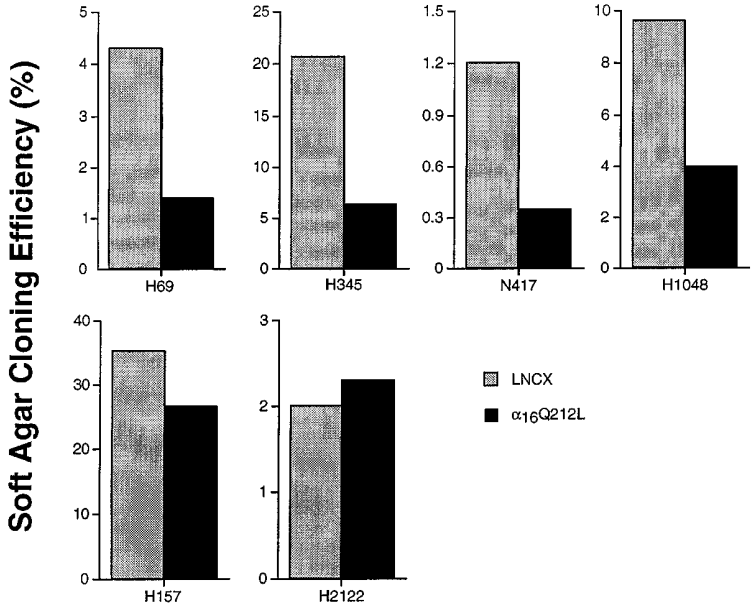

FIG. 2. **Soft agar cloning efficiencies of control and α_{16}Q212L-expressing lung cancer cell lines.** Single cell suspensions of the indicated cell lines were prepared and cultured in growth medium containing 0.3% agar nobel as described under "Materials and Methods." The data are presented as cloning efficiencies (percent of seeded cells that formed a colony) and are the means of two independent experiments where each experiment was assessed in triplicate.

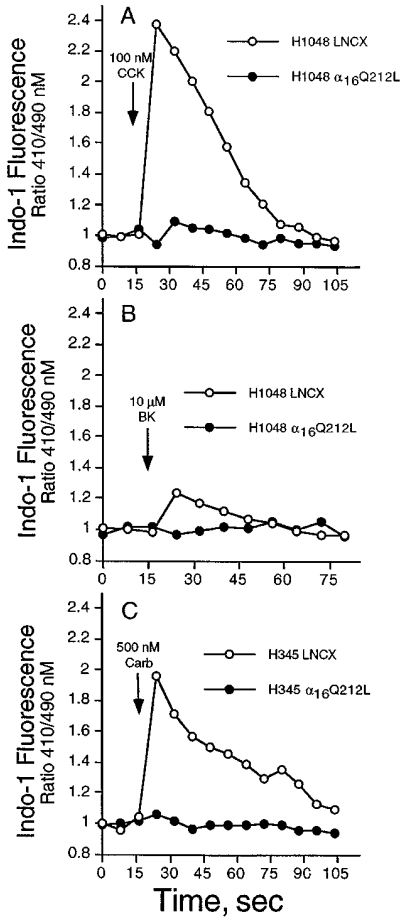

FIG. 3. **Influence of $G\alpha_{16}$Q212L on the regulation of intracellular Ca^{2+} levels by neuropeptides and hormones in SCLC lines.** LNCX and α_{16}Q212L-expressing H345 and H1048 cells in HITES medium were loaded with the calcium indicator Indo-1 AM, and the changes in intracellular Ca^{2+} levels were determined as described under "Materials and Methods." The data are representative of three independent experiments with the indicated cell lines.

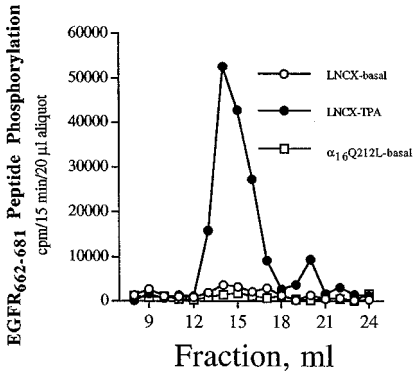

FIG. 4. **MonoQ fast protein liquid chromatography analysis of p42/44 MAP kinase activity in H345 cells expressing $G\alpha_{16}$Q212L.** Extracts of H345 cells expressing α_{16}Q212L or the empty LNCX vector were fractionated on MonoQ fast protein liquid chromatography. Fractions were collected and assayed for MAP kinase activity with $EGFR_{662-681}$ peptide. The results are from one experiment that is representative of two other independent experiments.

expressing H345 and H69 cells, respectively, indicating that the cAMP-dependent protein kinase pathway is not involved in the α_{16}Q212L-induced growth inhibition observed in SCLC lines.

The results show that cellular expression of α_{16}Q212L inhibits the growth of SCLC but not NSCLC. The selective inhibition of SCLC growth relative to NSCLC growth is likely to be explained by the different cellular pathways that exert dominance in growth regulation in the two lung cancer cell types. Oncogenic activation of Ras is frequently observed in NSCLC. In fact, H157, a squamous cell carcinoma, and H2122, an adenocarcinoma, have been previously shown to express mutated forms of Ki-Ras (28). In addition, NSCLC frequently overexpress specific receptor tyrosine kinase systems such as the epidermal growth factor receptor (29). In contrast, SCLC is characterized by the notable absence of GTPase-deficient forms of Ras, and overexpression of epidermal growth factor receptors is rare (28, 29). Instead, neuropeptide autocrine loops utilizing seven membrane-spanning receptors and heterotrimeric G proteins are proposed to be the primary mitogenic inputs in SCLC. Previous findings that α_qQ209L expression in NIH 3T3 fibroblasts leads to cellular transformation (30, 31) is consistent with a role for G_q proteins, phospholipase Cβ, and Ca^{2+} mobilization in mitogenic signaling. However, other studies revealed that α_{16}Q212L expression in Swiss 3T3 fibroblasts profoundly inhibited bombesin (gastrin-releasing peptide) signal transduction including calcium mobilization, MAP kinase acti-

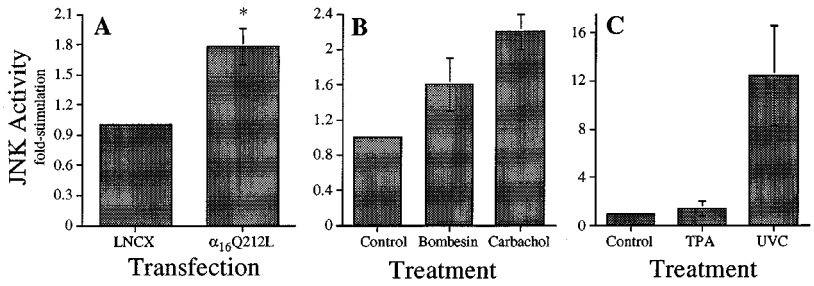

FIG. 5. **Regulation of c-Jun amino-terminal kinase activity in H345 cells by Gα_{16}Q212L, hormones, and ultraviolet light.** *A*, H345 cells expressing the LNCX vector or α_{16}Q212L were incubated for 10 min at 37 °C and soluble extracts were prepared and assayed for c-Jun NH_2-terminal kinase activity as described under "Material and Methods." *B*, H345 cells were incubated at 37 °C for 15 min in the presence or the absence of 100 nM bombesin or 10 μM carbachol. The cells were collected by centrifugation, lysed, and assayed for c-Jun NH_2-terminal kinase activity as in *A*. *C*, H345-LNCX cells were UV irradiated (192 J/M^2), incubated for 30 min at 37 °C or incubated for 10 min with 100 nM 12-*O*-tetradecanoylphorbol-13-acetate, and assayed for c-Jun NH_2-terminal kinase activity as described above. The data are the mean of five, three, and three independent experiments for *A*, *B*, and *C*, respectively. * indicates $p < 0.05$.

vation, and arachidonic acid release. The net effect was a nearly complete inhibition of bombesin (gastrin-releasing peptide)-stimulated DNA synthesis (17). Similar to the latter studies, our findings show that expression of α_{16}Q212L in SCLC lines resulted in strong inhibition of neuropeptide-regulated Ca^{2+} mobilization (Fig. 3) and dramatically reduced the ability of the cells to growth in soft agar (Fig. 2). These conflicting results concerning cellular actions of GTPase-deficient Gα_q proteins may reflect different levels of expression of the α-subunits in the various cell lines where low expression results in transformation and high expression leads to negative modulation of intracellular signaling pathways and growth arrest. Alternatively, the ability of Gα_q, phospholipase Cβ, and Ca^{2+} mobilization to stimulate DNA synthesis may be quite variable among different cell types.

Expression of GTPase-deficient α_{16}Q212L oppositely affected two signal transduction pathways normally engaged by neuropeptides and muscarinic agonists in SCLC; Ca^{2+} mobilization was inhibited, and the c-Jun NH_2-terminal kinase/stress-activated protein kinase pathway was constitutively activated. Thus, α_{16}Q212L expression induced discordant signaling in SCLC. We have found that α_{16}Q212L expression also constitutively activates the c-Jun NH_2-terminal kinase/stress-activated protein kinase pathway in PC12 cells where growth arrest and neuronal differentiation is observed.[3] The failure of phorbol esters to significantly activate the c-Jun NH_2-terminal kinase/stress-activated protein kinase pathway (Fig. 5*C*) in SCLC indicates that the phorbol ester-regulated forms of protein kinase C are not likely to mediate the activation of the c-Jun NH_2-terminal kinase/stress-activated protein kinase pathway, although the potential involvement of nonphorbol ester-sensitive protein kinase C isoforms cannot be eliminated. Constitutive activation of the c-Jun NH_2-terminal kinase/stress-activated protein kinase pathway in the absence of Ca^{2+} mobilization and p42/44 MAP kinase activation is correlated with a strong growth arrest in two cell types, SCLC and PC12 cells. It will be of interest to observe the spectrum of tumor cells where this response is growth inhibitory.

Ultraviolet irradiation of H345 cells strongly activates the c-Jun NH_2-terminal kinase/stress-activated protein kinase pathway (Fig. 5*C*) and leads to a rapid apoptotic-like cell death.[4] The 2-fold activation of the c-Jun NH_2-terminal kinase/stress-activated protein kinase pathway with α_{16}Q212L expression relative to the greater than 10-fold activation by UV irradiation may, in addition to inhibited Ca^{2+} mobilization, explain the strong growth arrest observed in the SCLC lines. We hypothesize that high level constitutive activation of the c-Jun NH_2-terminal kinase/stress-activated protein kinase pathway in the absence of neuropeptide-stimulated Ca^{2+} mobilization may have lethal consequences in SCLC. Consistent with this possibility was our inability to isolate α_{16}Q212L-expressing clones in some SCLC lines such as H510, despite repeated attempts. We have found that a proximal regulator of c-Jun NH_2-terminal kinases is a MEK kinase distinct from Raf (25, 32). MEK kinase activation is Ras-regulated and independent of Raf (32). Expression of activated MEK kinase-1 leads to cell death in several fibroblast systems and differentiation of PC12 cells[5] similar to the phenotype observed with activated α_q members such as α_{16}Q212L. Thus, the MEK kinase/c-Jun NH_2-terminal kinase regulatory pathways may contribute to growth inhibition and possibly cell death, a sharply contrasting phenotype from that observed with activated Raf expression.

These findings indicate that it is possible to inhibit cell growth of selected tumor cell types by selectively disrupting coordinated signal transduction pathways. Increasing awareness that signals controlling growth and apoptosis are overlapping is insightful in regards to these results. We suggest that the balance and magnitude of specific signal transduction pathways regulated by hormones and growth factors can determine commitment to tumor cell growth or arrest. Identifying tumors that have a strong growth inhibitory response resulting from activated α_q expression will allow a new pharmacologic and genetic approach to cancer therapeutics. The ability of G_q family members of G proteins to regulate the c-Jun NH_2-terminal kinase/stress-activated protein kinase pathway and to cause growth inhibition in SCLC and some fibroblasts focuses our attention on the α_q effectors capable of inhibiting tumor cell growth.

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