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Activation of the protein-tyrosine kinase associated with the bombesin receptor complex in small cell lung carcinomas

(autocrine growth factor)

G. GAUDINO, D. CIRILLO, L. NALDINI, P. ROSSINO, AND P. M. COMOGLIO

Department of Biomedical Sciences and Oncology, University of Torino Medical School, C.so Massimo D'Azeglio, 52, 10126 Turin, Italy

ABSTRACT **It has been hypothesized that bombesin-like peptides produced by small cell lung carcinomas may sustain deregulated proliferation through an autocrine mechanism. We have shown that the neuropeptide bombesin leads to the activation of a protein-tyrosine kinase that phosphorylates a 115-kDa protein (p115) associated with the bombesin receptor complex in mouse Swiss 3T3 fibroblasts. We now report that phosphotyrosine antibodies recognize a 115-kDa protein, phosphorylated on tyrosine, in four human small cell lung carcinoma cell lines producing bombesin but not in a nonproducer "variant" line. p115 from detergent-treated small cell lung carcinoma cells binds to bombesin-Sepharose and can be phosphorylated on tyrosine in the presence of radiolabeled ATP and Mn^{2+}. As for the p115 immunoprecipitated from mouse fibroblast, the small cell lung carcinoma p115 can be phosphorylated in an immunocomplex kinase assay. However, the latter does not require the presence of exogenous bombesin for activity. Binding data, obtained by using radiolabeled ligand, suggest receptor occupancy in the cell lines producing bombesin. These observations are consistent with the hypothesis that proliferation in some human small cell lung carcinoma lines is under autocrine control, regulated through activation of bombesin receptors.**

The human tumor known as small cell lung carcinoma (SCLC) is characterized by cells closely related to the neuroendocrine components of the respiratory epithelium (1). Therefore, unlike the other major histological types of lung cancer, SCLC cells ectopically synthesize numerous peptide hormones (2–4). Among these, bombesin and bombesin-like peptides—such as the mammalian homologue gastrin-releasing peptide (GRP) (5, 6)—have been proposed as possible autocrine growth factors for SCLC. In fact, specific receptor-binding activity was found in tumor-derived cell lines (7), and a monoclonal antibody raised against the binding moiety of bombesin/GRP prevented growth of tumor cells *in vitro* and of xenografts *in vivo* (8).

Moreover, bombesin was shown to act as a potent mitogen also on human bronchial epithelial cells (9) and on cultured Swiss 3T3 fibroblasts (10), where specific high-affinity receptor sites have been shown (11). By using an affinity-purified phosphotyrosine [Tyr(*P*)] antibody, we showed that bombesin leads to the activation of a protein-tyrosine kinase that phosphorylates a 115-kDa protein (p115) in mice. p115 may, therefore, represent a subunit of the bombesin receptor with intrinsic protein-tyrosine kinase activity or may be a substrate tightly associated with a bombesin-sensitive protein-tyrosine kinase (12). In the work presented here, we used the same antibody and Sepharose-immobilized bombesin to investigate the occurrence of tyrosine phosphorylation in p115 from SCLC cell lines. We compared four cell lines derived from "classic" SCLC lines (13), which produce detectable levels of bombesin (7), with the "variant" SCLC cell line GLC-1, which is devoid of bombesin-like immunoreactivity (14). We found that SCLC cells express the human counterpart of the p115 protein. In agreement with the hypothesis of an autocrine loop (8), in SCLC cells producing bombesin the receptor-associated kinase phosphorylates p115 without addition of exogenous bombesin.

MATERIALS AND METHODS

Cell Lines and Reagents. Swiss 3T3 mouse fibroblasts were grown in Dulbecco's modified Eagle's medium supplemented with 10% (vol/vol) fetal calf serum (Flow Laboratories). SCLC cell lines were grown in RPMI 1640 medium supplemented with 15% (vol/vol) fetal calf serum. The cell lines NCI-H128, NCI-N592, and GLC-8 were classic SCLC, producing bombesin-like peptides. GLC-1 was a variant nonproducer line. SHP 77/2/trn was a clone selected from an adherent-growing variety of SCLC. The characterization of the cell lines concerning the classic/variant typology, in general, and bombesin-like peptide production, in particular, is described elsewhere (7, 13–15). Since the original lines, obtained from various sources, were found to be rather heterogeneous, sublines were derived by limiting dilutions. Each subline used in the experiments was tested for immunoreactive bombesin production, c-*myc* amplification, and expression. In variant GLC-1 cells, c-*myc* gene was found to be amplified, as described. Classic cells, NCI-H128, NCI-N592, GLC-8, and SHP 77/2/trn, displayed higher levels of c-myc RNA expression, compared with Swiss 3T3 fibroblasts (16), and no further increase occurred following stimulation with mitogenic concentrations of bombesin. GLC-1 had the highest level of c-myc RNA expression, higher than that of the classic cell lines (P.R., unpublished results).

Antibodies and Immobilized Ligand. Tyr(*P*) antibodies were raised in rabbits immunized with azobenzylphosphonate [a phosphatase-resistant synthetic analog of Tyr(*P*)] covalently coupled to keyhole limpet hemocyanin (17). Antibodies were affinity-purified on Tyr(*P*)-bovine serum albumin [bovine serum albumin derivatized with Tyr(*P*) by incubation with *N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide] (18) and coupled to cyanogen bromide-activated Sepharose 4B. Bombesin-Sepharose was prepared with 5 mg of [Lys^3]bombesin and 500 μl of activated CH-Sepharose 4B, by using the carbodiimide coupling procedure as specified by the manufacturer. Controls were performed with the unrelated hydrophobic peptide eledoisin (19) covalently coupled to activated CH-Sepharose 4B.

Immunoblotting. Exponentially growing cells were washed once in serum-free HITES (7) medium, resuspended in HITES medium, and grown for 48 hr. For immunoblotting analysis cells were stimulated with bombesin (Cambridge Research Biochemicals, Cambridge, U.K.) at the mitogenic concentration of 3 nM or with 5 nM GRP (Cambridge Research Biochemicals) for 10 min at 37°C in water-saturated 5%

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Abbreviations: GRP, gastrin-releasing peptide; SCLC, small cell lung carcinoma; Tyr(*P*), phosphotyrosine.

CO_2/95% air or were not stimulated. After stimulation, cells were pelleted and solubilized in a minimal volume of boiling Laemmli buffer (20). Samples were adjusted to an equal protein content of ≈200 μg, electrophoresed in an 8% polyacrylamide slab gel, and transferred to nitrocellulose sheets, as described (21, 22). Blots were probed with purified antibodies at 12 μg/ml followed by ^{125}I-labeled *Staphylococcus aureus* protein A (Amersham). Autoradiograms were exposed with an intensifying screen for 1–2 days. The molecular mass of labeled proteins was estimated relative to the electrophoretic mobility of cotransferred [^{14}C]methylated protein standards (Amersham).

Cell Extraction. Cells were stimulated with bombesin (3 nM) for 30 min, pelleted at 200 × *g* for 5 min at 4°C, and washed twice in an ice-cold buffer (pH 7.2) containing 10 mM Pipes, 100 mM NaCl, 5 mM $MgCl_2$, 300 mM sucrose, and 5 mM EGTA (buffer A). The cells were then extracted with 0.3 ml of the same buffer plus 1% Triton X-100, protease inhibitors (1 mM phenylmethylsulfonyl fluoride, aprotinin at 100 kallikrein inhibiting units/ml, leupeptin at 50 μg/ml, and pepstatin at 4 μg/ml), Tyr(*P*) phosphatase inhibitors (0.1 mM Na_3VO_4 and 1 mM $ZnCl_2$), and 3 nM bombesin (buffer B) for 5 min at 0°C.

Immunoprecipitation and Affinity Chromatography with Bombesin-Sepharose. For both the immunoprecipitation with Tyr(*P*) antibodies and the bombesin-Sepharose chromatography, cell extracts were prepared as above. Cell extracts for immunoprecipitation were first dialyzed against buffer B to remove the nucleotide pool that antagonizes the immunoprecipitation of proteins by Tyr(*P*) antibodies (23, 24). After dialysis, the extracts were centrifuged at 15,000 × *g* for 20 min and incubated with purified Tyr(*P*) antibodies at 16 μg/ml or normal rabbit immunoglobulins at 16 μg/ml for 2 hr at 4°C as described (12, 23). Immunocomplexes were collected on protein A-Sepharose, washed four times in buffer B, and eluted by boiling in Laemmli buffer. Samples were analyzed by $NaDodSO_4$/PAGE and autoradiography with an intensifying screen for 6 hr. Molecular masses were estimated relative to the electrophoretic mobility of [^{14}C]methylated protein standards (Amersham). A control immunoprecipitation was performed with [^{35}S]methionine-labeled unstimulated cells. For affinity chromatography with bombesin-Sepharose, cell extracts were centrifuged at 15,000 × *g* and directly incubated with 100 μl of bombesin-Sepharose beads, for 2 hr at 4°C. Affinity complexes were collected by centrifugation and washed in buffer B.

Phosphorylation Assay. The phosphorylation assays on Tyr(*P*) immunoprecipitates (immunocomplex kinase assay) or on proteins bound to bombesin-Sepharose were done as described (12). Briefly, cells were washed, extracted with buffer B, and immunoprecipitated by Tyr(*P*) antibodies or affinity-purified with bombesin-Sepharose. Immunocomplexes collected on protein A-Sepharose or proteins bound to bombesin-Sepharose were washed four times in buffer B without bombesin and phosphorylated in 40 μl of the same buffer supplemented with 10 mM $MnCl_2$ and 15 μCi of [γ-^{32}P]ATP (5500 Ci/mmol; 1 Ci = 37 GBq; Amersham) at 0°C for 5 min in the presence or in the absence of 3 nM bombesin. The reaction was stopped by dilution in excess ice-cold buffer B/10 mM EDTA, and the immunocomplexes were further washed twice in the same buffer. Samples were eluted from the protein A-Sepharose or the bombesin-Sepharose by boiling in Laemmli buffer, subjected to $NaDodSO_4$/PAGE, and autoradiographed (with an intensifying screen for 2 hr).

Phospho Amino Acid Analysis. For phospho amino acid analysis, ^{32}P-labeled p115 was eluted from the gel in a solution of 50 mM NH_4HCO_3, 0.1% $NaDodSO_4$, 2% (vol/vol) 2-mercaptoethanol, and bovine serum albumin at 10 μg/ml. The eluate was lyophilized, dissolved in water, and precipitated with trichloroacetic acid. The precipitate was washed with ethanol, air-dried, and acid hydrolyzed for 1 hr in 6 M HCl (constant boiling, Pierce) at 115°C under vacuum. The hydrolysate was washed several times with water, dissolved in TLE buffer [pyridine/acetic acid/water, 5:50:945 (vol/vol), pH 3.5] and spotted with phospho amino acid standards onto thin-layer silica-gel plates for electrophoresis at 1100 V. Plates were sprayed with ninhydrin to visualize phospho amino acid standards and exposed 7 days for autoradiography with an intensifying screen.

Binding of ^{125}I-Labeled GRP. For binding at 37°C, SCLC cell lines NCI-N592 and GLC-1 were pelleted, washed once with HITES medium, resuspended in HITES medium containing 0.25% bovine serum albumin and aprotinin at 100 kallikrein inhibiting units/ml (HITES buffer), and transferred to an Eppendorf tube (4 × 10^6 cells per tube). In a total volume of 200 μl, 4 × 10^5 cpm of GRP ^{125}I-labeled at tyrosine-15 (2000 Ci/mmol, Amersham), corresponding to 0.64 nM, was added. After a 45-min incubation at 37°C, cells were pelleted at 200 × *g* for 5 min at 4°C and washed twice with HITES buffer, and total cell-associated radioactivity was determined. Specific binding was calculated by subtracting the nonspecific binding (defined as the cell-associated radioactivity not displaced in the presence of 1 μM GRP) from the total bound ^{125}I-labeled GRP.

RESULTS

p115 Is the Major Protein Phosphorylated on Tyrosine in SCLC Lines. In immunoblots of all classic SCLC cell lines probed with Tyr(*P*) antibodies, a major 115-kDa band (p115) was labeled. This protein comigrated with the p115 phosphorylated on tyrosine in Swiss 3T3 fibroblasts stimulated by bombesin (Fig. 1 and ref. 12). A few other additional bands were also labeled in classic SCLC cells, having molecular masses distinct from those detected in Swiss 3T3 cells stimulated by bombesin. The extent of tyrosine phosphorylation in these bands differed among the various cell lines. In the variant cell line GLC-1 no polypeptides were labeled by Tyr(*P*) antibodies at levels comparable to those of bombesin-stimulated fibroblasts or "classic" SCLC cell lines (Figs. 1 and 2).

While the tyrosine phosphorylation of the fibroblast p115 was dependent on stimulation with exogenous bombesin (Fig. 1 and ref. 12), the SCLC p115 appeared to be consti-

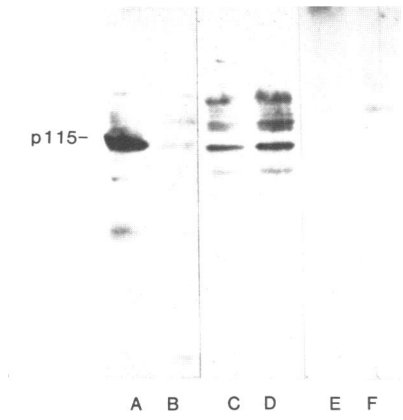

FIG. 1. Tyrosine phosphorylation of proteins solubilized from Swiss 3T3 fibroblasts (lanes A and B) and SCLC lines (lanes C–F). The latter are the NCI-H128 (lanes C and D), a classic bombesin producer, and the GLC-1 (lanes E and F), a variant nonproducer. Proteins after $NaDodSO_4$/PAGE were transferred to nitrocellulose sheets and probed with Tyr(*P*) antibodies and radiolabeled protein A. Lanes: A, C, and E, cells were stimulated with 3 nM bombesin before solubilization; B, D, and F, unstimulated cells.

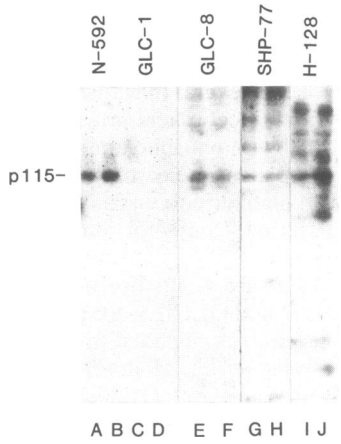

FIG. 2. Tyrosine phosphorylation of proteins in various human SCLC cell lines. All the lines shown are classic SCLC cells producing bombesin, with the exception of the GLC-1 nonproducer variant. Cell cultures were either stimulated with 3 nM bombesin before solubilization (lanes B, D, F, H, and J) or unstimulated (lanes A, C, E, G, and I). Samples were analyzed by immunoblotting with Tyr(*P*) antibodies, as shown in Fig. 1.

tutively phosphorylated and not substantially affected by the addition of exogenous peptide. A moderate enhancement of phosphorylation was detected only in one of the cell lines (NCI-N592) upon addition of exogenous bombesin to cells prior to extraction (Table 1). This finding is consistent with the hypothesis that there is complete or partial occupancy of receptor binding sites by endogenous bombesin produced by the classic SCLC lines. The availability of binding sites was thus assessed by determining the amount of ^{125}I-labeled GRP specifically bound to various SCLC cells upon incubation with ^{125}I-labeled GRP. Among the classic cell lines tested only the NCI-N592 displayed a binding activity of 20 fmol/mg of protein. This value is comparable with that obtained in the same line by another group (7) and does correlate with the fact that the phosphorylation of p115 is modulated by exogenous bombesin only in these cells. The variant GLC-1 did not exhibit detectable binding activity of ^{125}I-labeled GRP (Table 1). However, since a ligand-independent measure for receptor quantitation is not available, it is possible that some cells express fewer receptors rather than express both receptor and ligand.

Nevertheless, these results clearly show that Tyr(*P*) antibodies capable of recognizing the phosphorylated p115 associated with the bombesin receptor in Swiss 3T3 celis detect a constitutively phosphorylated protein of the same molecular mass in classic SCLC cell lines.

p115 Solubilized from SCLC Cells Is Phosphorylated on Tyrosine upon Immunoprecipitation. The p115 associated

Table 1. Surface binding of ^{125}I-labeled GRP and p115 phosphorylation in various cell lines

Cell line	Specific binding, fmol/mg of protein	p115 Tyr phosphorylation	
		− BM	+ BM
SWISS 3T3	185 ± 36	0.000	0.730
NCI-N592	20 ± 6	0.140	0.310
GLC-1	<3	0.000	0.000
GLC-8	<3	0.080	0.065
SHP-77/2/trn	<3	0.060	0.060
NCI-H128	<3	0.130	0.150

Intensity of the p115 band visualized by autoradiography after immunoblot analysis with Tyr(*P*) antibodies and ^{125}I-labeled protein A was quantitated with a densitometer. Values shown are optical density (arbitrary units) obtained in different experiments and are comparable within each cell line. BM, bombesin.

with the bombesin receptor in mouse 3T3 fibroblasts could be phosphorylated on tyrosine *in vitro*, during solubilization in nonionic detergent and immunoprecipitation with Tyr(*P*) antibodies. The reaction required 10 mM Mn^{2+}, was optimal at 30°C, and was enhanced by bombesin (12). NCI-H128 cells, a classic SCLC, were extracted with Triton X-100, immunoprecipitated with Tyr(*P*) antibodies, and assayed for kinase activity (see above). Two major proteins of 115 kDa and 70 kDa were phosphorylated on tyrosine (Fig. 3). The presence of Tyr(*P*) in the proteins phosphorylated *in vitro* was assessed by resistance to alkali treatment and by phospho amino acid analysis (Fig. 3). The latter showed that the phosphorylation of SCLC p115 in immunoprecipitates took place exclusively on tyrosine. As for the immunoblot analysis, exogenous bombesin did not affect the extent of phosphorylation when added to cells before the extraction or to the immunoprecipitate in the reaction buffer. The phosphorylation reaction took place also at 0°C, in contrast to the 30°C required for the phosphorylation of p115 solubilized from Swiss 3T3 fibroblasts. Thus, unlike normal fibroblasts, in SCLC cells the immunoprecipitation of p115 by Tyr(*P*) antibodies and p115 phosphorylation in immunocomplexes were independent of exogenous bombesin stimulation.

p115 from Detergent-Solubilized Classic SCLC Cells Binds to Immobilized Bombesin and Can Be Phosphorylated on Tyrosine. Since exogenous bombesin could not be used to assess the participation of 115-kDa protein in the SCLC bombesin receptor complex, a solid-phase ligand affinity test coupled with the phosphorylation assay was developed. Nonionic detergent extracts of NCI-H128 cells were incubated with bombesin-Sepharose and the bound protein was incubated with [^{32}P]ATP as described above. A protein comigrating with the p115 precipitated from SCLC by Tyr(*P*) antibodies was phosphorylated. The presence of Tyr(*P*) in the SCLC

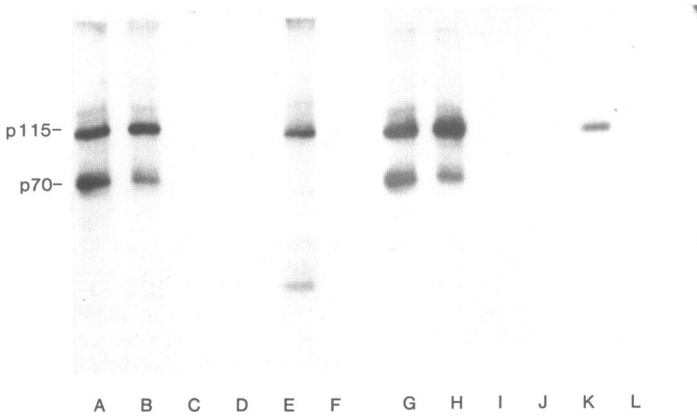

FIG. 3. Protein-tyrosine kinase activity in detergent extracts of SCLC. NCI-H128 cells were solubilized with nonionic detergent, and proteins were immunoprecipitated with Tyr(*P*) antibodies (lanes A and B) or chromatographed on bombesin-Sepharose (lane E). Controls with nonimmune immunoglobulin (lanes C and D) and with Sepharose coupled with an unrelated peptide (eledoisin) (lane F) are shown. [γ-^{32}P]-ATP was added to immunoprecipitates and to proteins bound to bombesin-Sepharose under conditions allowing protein-tyrosine kinase activity. Samples were analyzed by NaDodSO$_4$/PAGE and autoradiography. The gel whose autoradiograph is shown in lanes A–F has been subjected to alkali treatment and autoradiographed again (lanes G–L). The presence of authentic Tyr(*P*) in p115 was confirmed by phospho amino acid analysis (lane M). P, phosphate; PSer, phosphoserine; PThr, phosphothreonine; PTyr, Tyr(*P*); O, origin.

p115 bound to bombesin-Sepharose was confirmed by resistance to alkali treatment (Fig. 3). It should be noted that several additional protein bands were eluted from the bombesin- as well as the eledoisin-Sepharose columns, when control experiments were performed with extracts solubilized from [^{35}S]methionine-labeled cells (data not shown). However, the p115 phosphorylated on tyrosine was specifically bound only by the bombesin-Sepharose column.

The other four SCLC lines were extracted in nonionic detergent, and the presence of p115 was investigated after affinity chromatography on bombesin-Sepharose and phosphorylation with [^{32}P]ATP, as described above. All classic SCLC lines examined, namely NCI-N592, GLC-8, and, at a lower extent, SPH-77, had a phosphorylated protein comigrating with the p115 from NCI-H128 and with the p115 phosphorylated on tyrosine in bombesin-stimulated Swiss 3T3 fibroblasts that was not detectable in the variant GLC-1 line (Fig. 4).

DISCUSSION

Several independent reports have strengthened the hypothesis that the malignant behavior of some tumors may be sustained by an autocrine loop, whereby cancer cells produce growth factors that stimulate their own growth (25–27). Several polypeptide growth factors, including epidermal growth factor, platelet-derived growth factor, transforming growth factor, and nerve growth factor, have been shown to influence propagation of normal and malignant cells *in vitro* (17, 18, 28–30). However, so far, the strongest evidence for the involvement of a growth factor in autocrine stimulation *in vivo* has been provided for bombesin-like peptides produced by SCLCs (8, 13). Several human SCLCs or carcinoid lung tumors do produce bombesin-related peptides (2, 4, 7, 31–33). Among these, the GRP shares with bombesin the 7 amino acids long C-terminal sequence responsible for receptor binding and biological activity (34–36). Bombesin and GRP induce a variety of responses in cells possessing specific receptors, such as induction of proliferation in Swiss 3T3 fibroblasts (10) and in human bronchial epithelial cells (9). Bombesin-like peptides can stimulate the clonal growth of SCLC cells in serum-free medium (37). Both Swiss 3T3 fibroblasts and SCLC cells express a single class of high-affinity receptors for bombesin at the cell surface (7, 11). Moreover, in fibroblasts, the binding of bombesin to cell surface receptors is rapidly followed by the activation of transcription of c-*fos* and c-*myc* (16), two oncogenes involved in the regulation of cell proliferation (38). Finally, high levels of c-*myc* expression have also been reported in SCLC cell lines, including those that are characterized for bombesin/GRP production (39).

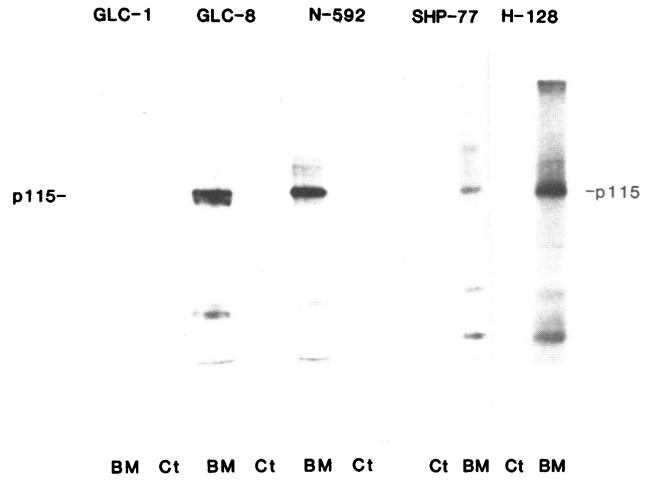

FIG. 4. Protein-tyrosine kinase activity of proteins bound to bombesin-Sepharose. Samples were solubilized with nonionic detergent, affinity-chromatographed on bombesin-Sepharose (lanes BM) or on control uncoupled Sepharose (lanes Ct), and incubated with [γ-^{32}P]ATP, as in Fig. 3. GLC-8, NCI-N592 (N-592), SHP 77/2/trn (SHP 77/2), and NCI-H128 (H-128) are classic SCLC lines; GLC-1 is a variant SCLC line.

These data suggest, but do not prove, that bombesin or bombesin-like peptides could function as autocrine growth factors for SCLC tumors. Evidence for constitutive activation (i.e., without stimulation by exogenous ligand) of the receptors for bombesin in SCLC cells would substantiate the autocrine hypothesis.

We have identified (12) a membrane protein, p115, in Swiss 3T3 fibroblasts that is phosphorylated in response to nanomolar concentrations of bombesin or GRP (12). p115 was immunoprecipitated from bombesin-stimulated cells by Tyr(*P*) antibodies by using a method that proved to be suitable to identify the autophosphorylated form of class 1 oncogene products (40), of platelet-derived growth factor receptors, and of epidermal growth factor receptors (41). Immunocomplexes between p115 and Tyr(*P*) antibodies did bind bombesin or GRP in a specific and saturable manner and showed ligand-dependent kinase activity. Phosphorylation took place at tyrosine residues. p115 could be either a protein-tyrosine kinase associated with the mouse bombesin receptor complex or a substrate of a kinase sensitive to bombesin (12). Cross-linking experiments indicate that a protein of 60–80 kDa possessing a binding site for bombesin/GRP is part of the receptor complex (42, 43); a cross-linked protein of higher molecular mass (100–110 kDa) has also been reported (44, 45). It is known that a number of biological responses triggered by bombesin are mediated by the activation of protein kinase C that is in turn modulated by the products of inositol phospholipid turnover (46). Coupling of the bombesin receptor to a pertussis-toxin-sensitive guanine-nucleotide-binding protein, related to the p21 *ras* oncogene product, has also been proposed (47, 48). The present finding of a tyrosine kinase activity associated with the bombesin receptor complex is reminiscent of the properties of the platelet-derived growth factor receptor, which is both endowed with a strong protein-tyrosine kinase activity and also triggers intracellular signals by way of the inositol phospholipid pathway (46).

The data presented in this paper show that a 115-kDa protein, phosphorylated on tyrosine, is detectable in the four classic SCLC lines studied but not in a variant line that do not produce bombesin. This molecule (SCLC p115) comigrates with the mouse p115 phosphorylated on tyrosine in response to bombesin, binds—after detergent treatment—to bombesin-Sepharose beads, and can be phosphorylated on tyrosine after either immunoprecipitation with Tyr(*P*) antibodies or binding to bombesin-Sepharose.

Phosphorylation of p115 detected in normal fibroblasts is dependent upon added bombesin, and the phosphorylation is transient (12). The p115 found in SCLC cells is phosphorylated in absence of exogenous ligand. This may be explained by autocrine activation by way of permanent occupation of receptor binding sites, as suggested by the observations that no available binding sites for the radiolabeled ligand were detected and that the kinase activity was not affected by addition of exogenous bombesin. Notably, modulation of p115 tyrosine phosphorylation by exogenous bombesin was observed only in the NCI-N592 line, the line with residual free receptor available for ^{125}I-labeled GRP binding. The observed binding of p115 to the bombesin affinity column does not contradict the idea of receptor occupancy in SCLC cells: it may have several explanations. These include partial dissociation of the ligand in the presence of detergent or multivalency of the receptor complex. It needs to be established whether the binding of bombesin-related peptides to

their receptors in classic SCLC cells takes place at the cell surface, at the end of a complete autocrine circuit, or occurs inside the cell in the exocytotic pathway. Moreover, a partial lesion of the bombesin-sensitive protein-tyrosine kinase leading to receptor uncoupling and constitutive activation cannot be excluded.

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