Bombesin Stimulates Invasion and Migration of Isreco1 Colon Carcinoma Cells in a Rho-dependent Manner

Jean-Christophe Saurin,2,3 Marjorie Fallavier,2 Bernard Sordat, Jean-Claude Gevrey, Jean-Alain Chayvialle, and Jacques Abello

INSERM Unité 45 and IFR 62, Hôpital Edouard-Herriot, Pavillon H, F-69437 Lyon Cedex 3, France [J-C.S., M.F., J-C.G., J-A.C., J.A.] and Department of Experimental Pathology, Swiss Institute for Cancer Research (ISREC), CH-1066 Epalinges, Switzerland [B.S.]

ABSTRACT

The membrane receptor for the neuropeptide bombesin/gastrin-releasing peptide (GRP) is expressed by a large fraction of human colorectal carcinoma cells. We reported previously a stimulation of cell adhesion and lamellipodia formation by the neuropeptide bombesin in the human, bombesin/GRP receptor-expressing, Isreco1 colorectal cancer cell line (J. C. Saurin et al., Cancer Res., 59: 962–967, 1999). Using invasion and motility assays, we demonstrate in this report that bombesin can both enhance the invasive capacity of Isreco1 cells in a dose-dependent manner (maximal effect at 1 μM) and stimulate the closure of wounds formed on confluent Isreco1 cells. These effects were reversed fully by the specific bombesin/GRP receptor antagonist N-Phe6-Bn(6-13)OMe used at 1 μM. MMP-9 and urokinase-type plasminogen activator were expressed by Isreco1 cells, and bombesin did not significantly alter their level of secretion. Interestingly, exoenzyme C3 (10 μg/ml) decreased cell invasiveness induced by bombesin by 70% and completely inhibited the migration of Isreco1 cells. Similarly, the Rho-kinase inhibitor Y-27632 dose-dependently reduced the effect of bombesin on cell invasion. Moreover, pull-down assays for GTP-bound RhoA demonstrated that bombesin was able to activate the small G-protein in Isreco1 cells. These results show that the neuropeptide bombesin is able to modulate invasiveness of Isreco1 colorectal carcinoma cells in vitro through a Rho-dependent pathway, leading to an increase in cell locomotion without a significant effect on tumor-cell associated proteolytic activity. These findings indicate that bombesin/GRP receptor expression may contribute to the cellular events that are critical for invasion/migration of colorectal carcinoma cells.

INTRODUCTION

Several neuroendocrine peptides are expressed throughout the digestive tract. Bombesin and its mammalian counterpart, GRP, are two of these peptides, with predominantly hormone-secreting and gut motor functions in vivo (1). Interestingly, GRP and its receptor, the bombesin/GRP receptor, or BB2, are also frequently expressed in human cancers, with some examples of autocrine growth stimulation in small-cell lung cancer (2). The bombesin/GRP receptor is not detectable by ligand binding or reverse-transcription PCR studies in the normal human colonic mucosa, but is revealed at the mRNA and protein levels in 95% and 20% of human colon cancer samples, respectively (3–5). The reason for this overexpression in colon cancer has been mostly studied in human small cell lung and prostate cancer cell lines. In these models, bombesin can stimulate proliferation and/or invasion by increasing secretion and activation of metalloproteinases (2, 7). Limited knowledge is presently available regarding colon carcinoma cells, and there is no published evidence of a direct invasion-modulating effect of bombesin on this cell type. Moreover, the mechanisms responsible for the potential effects of bombesin on tumor cells have not been determined. We showed previously that bombesin could stimulate adhesion, proliferation, and lamellipodia formation in Isreco1 cells, a human colon carcinoma cell line derived from a primary tumor and expressing a high number of membrane bombesin/GRP receptors (8). Because these biological effects are potentially important modulators of tumor cell invasion, we investigated the following: (a) whether bombesin could stimulate invasion; (b) whether bombesin could modulate tumor-cell associated proteolytic activities; and (c) which intracellular mechanisms are involved in these effects.

MATERIALS AND METHODS

Materials and Cell Culture Conditions. The human colon carcinoma Isreco1 cell line and the bombesin/GRP receptor antagonist N-Phe6-Bn(6-13)OMe (kind gift from Dr. D. H. Coy, Tulane University Health Sciences Center, New Orleans, LA) have been described previously (9, 10). Bombesin, PMA, and epidermal growth factor were from Sigma (St. Quentin Fallavier, France). Polyclonal rabbit anti-MMP-2 and anti-MMP-9 antibodies were from Chemicon International (Temecula, CA). Mouse monoclonal anti-RhoA antibody was from Santa Cruz Biotechnology (Tebu, Le Perray-en-Yvelines, France). Marimastat (BB-2516) and Y-27632 were obtained from British Biotech (Oxford, United Kingdom) and Welfide Corporation (Osaka, Japan), respectively. The cell-permeant Clostridium botulinum C3 exoenzyme (kind gift from Dr. J. Bertoglio, INSERM U461, Chatenay-Malabry, France) was obtained by cloning the C3 coding sequence, COOH-terminal of the HIV-TAT protein transduction domain, in vector pTAT-HA (11). pGEX-2T vector encoding a GST-fusion protein containing amino acids 7–89 of rhotein, which is the RBD, was kindly provided by Dr. M. Schwartz (Scripps Research Institute, La Jolla, CA) through the courtesy of Dr. P. Jurdic (ENS, Lyon, France). Isreco1 cells, the human keratinocyte HaCaT cell line (12) and HT-1080 fibrosarcoma cells (13) were grown in DMEM (Life Technologies, Inc., Cergy Pontoise, France) supplemented with 10% FCS, 2 mm glutamine and antibiotics (100 IU/ml penicillin + 50 μg/ml streptomycin) in a humidified 5% CO2-95% air incubator at 37°C.

Invasion and Migration Assays. Cells were suspended in DMEM-0.1% BSA at a concentration of 2 × 106 cells/ml and seeded into the upper chamber of a 12-well chemotaxis chamber (Neuroprobe, Gaithersburg, MD). For invasion experiments, lower wells were filled with DMEM-0.1% BSA with or without bombesin at different concentrations and separated from the upper wells by a 12-μm pore Nucleopore polyvinyl pyrrolidone-free polycarbonate membrane (Corning Inc., Acton, MA), precoated for 2 h with 370 μg/ml Matrigel (Becton-Dickinson, Le Pont-de-Claux, France). Cells (2 × 105) were pipetted into each upper well of the chamber and incubated for 18 h at 37°C in 5% CO2 humidified incubator as described (7, 14). Migration assays were
performed in the same manner as for invasion, except that filters were pre-coated for 4 h with 100 μg/ml type I collagen (Becton-Dickinson), and cells were incubated for 3 h at 37°C in 5% CO₂, humidified conditions (7, 14, 15). Cells were then fixed with 4% paraformaldehyde and stained with 1% borax and 1% methylene blue. Cells of the upper surface of the filter were removed with a cotton swab and those underneath were solubilized with 1% SDS and absorbance (630 nm) was measured. To evaluate the effect of the bombesin/GR peptide antagonist, cells were preincubated during 30 min in the presence of 1 μM d-Phe⁶-Bn(6-13)OMe. Marimastat, the MMP inhibitor, was used at a concentration of 20 μM and added into the upper well of the chamber. For experiments using C3 exoenzyme or Y-27632, cells were resuspended in DMEM-0.1% BSA to remove cell debris, then incubated for 36 h (until complete wound closure) in serum-free medium in the presence or absence of the drugs to be tested.

**Western Blot Analysis.** Cells were seeded into 35-mm dishes at 1.5 × 10⁵ cells/cm² and allowed to grow to 90% confluency in DMEM-10% FCS. Medium was then replaced by DMEM-0.1% BSA for 24 h. Cell monolayers were wounded by a plastic tip that touched the plate. For each dish, three to five wounds were performed, and three sites of regular wounds were selected and marked. Wounded monolayers were then washed four times in DMEM-0.1% BSA to remove cell debris, then incubated for 36 h (until complete wound closure) in serum-free medium in the presence or absence of the drugs to be tested.

**Zymographic Analyses.** For measurement of MMP activation, confluent cells in 60-mm Petri dishes were incubated for 24 h in DMEM-0.1% BSA, then washed three times and incubated for 24 h in serum-free medium in the presence or absence of 1 nM bombesin. At 24 h, the supernatants were collected, concentrated 20-fold with centrifugal filters (molecular cutoff at 30 kDa; Millipore), and equal volumes (20 μl) of conditioned medium were mixed with 4× nonreducing buffer [200 mM Tris-HCl (pH 7.4), 10% SDS, 4% sucrose, and 0.4% Coomassie blue] for 30 min at 25°C. Each sample was then subjected to electrophoresis on a 10% polyacrylamide gel containing 0.1% gelatin (Sigma). After electrophoresis, gels were incubated in buffer containing 2.5% Triton X-100 (2 × 30 min at room temperature), rinsed in distilled water, and incubated overnight at 37°C in 100 mM Tris buffer (pH 7.4) containing 15 mM CaCl₂. Gels were fixed with 30% methanol and 7% acetic acid, stained with 0.1% Coomassie blue, destained, and dried. Plasminogen activator activities were qualitatively analyzed by fibrin zymography. Serum-free conditioned media were collected by centrifugation (4400g for 10 min at 4°C) and stored at −70°C until thawing for SDS-PAGE zymographic analysis using copolymerized plasminogen-rich fibrinogen as described (16).

**ADP-ribosylation of Rho Proteins with Recombinant C3 Exoenzyme.** Isreco1 cells (0.4 × 10⁶) were incubated in serum-free DMEM for 24 h, then pretreated at time intervals with or without 10 μg/ml C3 exoenzyme. Cells were washed twice in PBS, collected with trypsin/EDTA, centrifuged and homogenized in 20 mM HEPES (pH 7.5), 10 mM thymidine, 1 mM EDTA, 5 mM MgCl₂, 1 mM ATP, 100 μM GTP, 100 IU/ml aprotinin, 20 μM leupeptin, and 0.2 mg/ml PMSF. The ADP-ribosylation reaction was carried out for 30 min at 37°C with 100 μg of cell homogenate, 0.5 μg (0.15 μM) of [³²P]NAD (30 Ci/mmol, 2 mCi/ml; NEN Life Science Products, Paris, France) and 10 μg/ml of C3 exoenzyme in a final volume of 120 μl. Proteins were then precipitated with 10% (v/v) trichloroacetic acid, centrifuged (13,000g for 15 min at 4°C), and dissolved in Laemmli buffer for SDS-polyacrylamide gel separation. Labeled proteins were revealed by autoradiography, and spot intensities were analyzed by scanning densitometry.

**Rho-activation Assay.** GST-RBD was produced in Escherichia coli (BL21 strain) transformed with pGEX-2T-thioketin RBD (17). Bacterial cultures were grown to A₅₀₀ = 0.6 and induced with 0.5 mM isopropyl-β-D-thiogalactopyranoside for 2 h at 37°C. Bacteria were harvested in lysis buffer [50 mM Tris (pH 7.5), 1% Triton X-100, 150 mM NaCl, 5 mM MgCl₂, 1 mM DTT, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 1 mM PMSF] and sonicated (five times for 15 s on ice). After centrifugation (30,000g for 20 min at 4°C), GST-RBD was collected from the supernatant by rocking at 4°C for 1 h with glutathione-Sepharose 4B beads (Amersham Pharmacia, Orsay, France). The Sepharose was washed three times with assay buffer, resuspended in fresh buffer containing 10% glycerol, aliquoted, and stored at −70°C. Isreco1 cells (10⁶) were plated onto 100-mm dishes in DMEM-0.1% BSA for 48 h before agonist stimulation at indicated times. Cells were lysed in 50 mM Tris-HCl buffer (pH 7.5) containing 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 500 mM NaCl, 10 mM MgCl₂, 1 μg/ml aprotinin, 1 μg/ml leupeptin, 2 μg/ml pepstatin, and 0.1 mM PMSF. Cell lysates were centrifuged (14,000g for 5 min at 4°C) and incubated with 40 μg of Sepharose-bound GST-RBD for 1 h at 4°C. Beads were washed four times with cold 50 mM Tris buffer (pH 7.5) containing 0.5% Triton X-100, 150 mM NaCl, 5 mM MgCl₂, 1 mM DTT, 1 μg/ml leupeptin, and 1 μg/ml aprotinin. Activated Rho bound to beads and total Rho in cell extracts were detected by Western blotting using a monoclonal RhoA-specific antibody.

**Statistical Analysis.** Results were analyzed by one-way ANOVA followed by post hoc Fisher comparison. Differences between two means with P < 0.05 were regarded as significant. All values were expressed as means ± SE of at least three separate experiments.

**RESULTS**

**Effect of Bombesin on Matrigel Invasion by Isreco1 Cells.** We previously reported that bombesin could stimulate several invasion-related processes in Isreco1 cells, including adhesion, proliferation, and lamellipodium extension (8). Therefore, we investigated the ability of bombesin to stimulate invasion of Isreco1 cells using an invasion chamber and Matrigel. In bombesin-free conditions, invading cells represent ~10% of initial loading after 18 h of incubation (not shown). As shown in Fig. 1, bombesin dose-dependently stimulated the invasion of Isreco1 cells through the Matrigel-coated filter, with no significant effect at a concentration of 10 pM (113.7 ± 6.0% of control), a maximal effect at 1 nM (190.7 ± 7.7% of control; P < 0.05), and a submaximal effect at 100 nM (138.4 ± 5.5% of control; P < 0.05). To determine whether bombesin stimulation of cell invasion resulted from a specific interaction of the peptide with the bombesin/GRP receptors expressed by Isreco1 cells, we tested the ability of the bombesin/GRP receptor antagonist d-Phe⁶-Bn(6-13)-OMe to block bombesin stimulation of invasion.

**Fig. 1.** Effect of bombesin on invasion of Isreco1 cells. Isreco1 cells (2 × 10⁶/ml) were seeded on a Matrigel precoated filter into an invasion chamber, with or without increasing concentrations of bombesin (lbs) in the absence or presence of 1 μM bombesin/GRP receptor antagonist d-Phe⁶-Bn(6-13)-OMe (antagonist) (4 μM [A]) added 30 min before agonist stimulation. Invading cells were counted and stained. Cells from the upper side of the filter were removed, and cells from the lower surface of the filter were dissolved in 1% SDS and assessed by measuring absorbance at 630 nm. Data are the means ± SE of five independent experiments, each performed in triplicate. *, P < 0.05 versus control; **, P < 0.05 versus stimulation with 1 nM bombesin.
**Effect of Bombesin on Isreco1 Cell Migration.** Using a wound-closure assay, we investigated whether bombesin-stimulated invasion of Isreco1 cells could be related to enhanced cell motility. The repair process of wounds performed on Isreco1 cell monolayers in serum-free medium is illustrated in Fig. 2A. Compared with control wounds, in the presence of bombesin (1 nM), wound repair was accelerated, leading to an almost complete closure at 36 h. The specific bombesin/GRP receptor antagonist D-Phe6-Bn(6-13)OMe, at a concentration of 1 μM, completely inhibited this effect (Fig. 2A). To determine whether cell proliferation accounted for some part of wound repair in the course of the 36-h assay, we looked for nuclear Ki-67 expression cell proliferation accounted for some part of wound repair in the absence or presence of 1 nM bombesin (bbs) without or with 1 μM D-Phe6-Bn(6-13)OMe (bbs + A). Scale bar, 300 μm. The pictures are representative of at least three separate experiments. B, cells (2 × 10^5/ml) were seeded on a type 1 collagen-precultured filter into an invasion chamber in the absence or presence of 1 nM bombesin (bbs) without or with 1 μM D-Phe6-Bn(6-13)OMe (bbs + A). After a 3-h incubation, migrating cells were measured as described in Fig. 1. Data are the means ± SE of three independent experiments; each performed in triplicate. *, P < 0.05 versus control; +, P < 0.05 versus stimulation with 1 nM bombesin.

**Bombesin Does Not Increase MMP-9 and uPA Secretions.** Bombesin has been shown previously to modulate transcription and/or secretion of MMP-9 and secretion of uPA in human prostate cancer cells (7, 18). Western blotting was used to study the expression of these proteases, and zymography was used to determine whether bombesin could regulate their secretion in Isreco1 cells. Western blotting of total Isreco1 cell lysate showed expression of the type IV collagenase MMP-9, whereas MMP-2 could not be detected (Fig. 3A). As shown in Fig. 3B (top), zymography of Isreco1 cell-conditioned medium revealed a low basal secretion of MMP-9, that did not increase after treatment with 1 nM bombesin for 24 h. No secreted MMP-2 was found. Increasing bombesin concentration to 100 nM, or bombesin exposure time to 48 h, led to similar results (not shown). In contrast, PMA, at a concentration of 100 nM, slightly increased secretion of MMP-9 in Isreco1 cells (Fig. 3B, top). As a positive control, the high basal MMP-9 secretion in human HaCaT keratinocytes increased significantly after stimulation with either 20 ng/ml epidermal growth factor or 100 nM PMA for 24 h (Fig. 3B, bottom). The absence of detectable MMP-2 in Isreco1 cells was not related to the experimental procedure nor to the nature of the MMP-2 antibody used, because MMP-2 could be detected by Western blotting in HT-1080 fibrosarcoma cell lysates (Fig. 3A) and by zymography in conditioned media from HaCaT keratinocytes (Fig. 3B, bottom). With regard to the plasminogen activator system (Fig. 3C), zymographic analyses of Isreco1 cell-conditioned medium showed a high basal secretion of uPA, and incubation in the presence of 1 nM bombesin for 24 or 48 h did not lead to a detectable increase of expression. Additional experiments were performed to test the effect of Marimastat, a large-spectrum inhibitor of metalloproteinases (19). Marimastat alone at a concentration of 20 μM slightly inhibited the basal invasion of Isreco1 cells through Matrigel (77.2 ± 6.4% of control, P < 0.05;
The GTPase Rho and the Rho-associated Kinase ROCK Are Involved in the Bombesin-stimulated Invasive Process of Isreco1 Cells. The small GTPase Rho and the ROCK have been shown to be crucial for the transmigration of tumor cells (20). Moreover, Rho drives the morphological and cytoskeletal changes induced by bombesin in nontransformed murine Swiss 3T3 fibroblasts (21). We therefore investigated whether the Rho-ROCK pathway accounted for some part in the stimulation of Isreco1 cell invasion by bombesin. We first tested the ability of a recombinant C3 exoenzyme of C. botulinum, which is known to specifically inactivate the small GTPase Rho, to ADP-ribosylate Rho in our model. In total Isreco1 cell lysate, the HIV-TAT-coupled C3 exoenzyme catalyzed the [$^{32}$P]ADP-ribosylation of a protein exhibiting a molecular mass of 21 kDa corresponding to p21$^\text{Rho}$ proteins (Fig. 5A, inset). Pretreatment of cultured Isreco1 cells for 18 h with 10 $\mu$g/ml C3 exoenzyme before total cell lysis and subsequent in vitro ADP-ribosylation led to a reduction of $\sim$80% in the amount of the 21-kDa band (Fig. 5A), demonstrating the ability of C3 exoenzyme to penetrate into intact Isreco1 cells and inactivate p21$^\text{Rho}$ proteins by ADP-ribosylation. We then studied whether the C3 exoenzyme was able to inhibit bombesin-stimulated invasion of Isreco1 cells through Matrigel. The exoenzyme alone at a concentration of 10 $\mu$g/ml had no effect on the basal level of Isreco1 cell invasion (Fig. 5B). In contrast, treatment of cells with C3 exoenzyme resulted in $\sim$80% inhibition of the invasion stimulated by 1 nm bombesin (117.1 $\pm$ 5.2% versus 191.3 $\pm$ 17.2% for bombesin alone; $P < 0.05$). Using the wound closure assay, we found that incubating cells with the exoenzyme in serum-free medium resulted in an almost complete inhibition of the bombesin-stimulated wound repair (Fig. 5C). We then tested whether ROCK activity was required for the stimulation of Isreco1 cell invasion by bombesin using the Rho kinase inhibitor Y-27632 (22). Y-27632 was identified as a selective inhibitor of ROCK-I and ROCK-II, with inhibitory effects on protein kinase C and protein kinase A only at 200-fold higher concentrations (22). The compound inhibited ROCK-I and ROCK-II 20–30-fold more potently than the Rho-dependent kinases citron kinase and protein kinase N/PRK1 (23). Y-27632 was reported more recently to preferentially inhibit ROCK-II but also PRK2 (a kinase that is closely related to PRK1) with a potency similar to that for ROCK-II (24). Pretreatment of Isreco1 cells with Y-27632 at a concentration of 10 $\mu$M (Fig. 6) significantly reduced bombesin-mediated cell invasion (162.7 $\pm$ 9.9% versus 209.4 $\pm$ 17.1% without inhibitor; $P < 0.05$) and abolished it at 100 $\mu$M, whereas the inhibitor alone did not modify the basal level of cell invasion. Thus, our results show that enhanced Isreco1 cell invasion by bombesin is dependent on the small GTPase Rho and on the Rho-associated kinase ROCK.

Bombesin Induces RhoA Activation in Isreco1 Cells. Because C3 exoenzyme and the Rho-associated kinase inhibitor Y-27632 inhibited bombesin-enhanced invasion, we hypothesized that bombesin could activate RhoA in Isreco1 cells. Therefore, we measured the intracellular levels of the active, GTP-bound form of RhoA using a pull-down assay system in the presence or absence of bombesin. As shown in Fig. 7, no GTP-bound RhoA was detected in Isreco1 cell extracts when cells were grown in serum-free medium without bombesin, although Western blotting revealed the presence of nonactivated RhoA. At a concentration of 10 nm, in contrast, bombesin increased the level of GTP-bound RhoA in a time-dependent manner, reaching a maximum at 15–30 min and decreasing at 60 min (Fig. 7, top). Because the total amount of RhoA in each lysate was almost constant (Fig. 7, bottom), these results demonstrate that bombesin can stimulate RhoA activation in Isreco1 cells.
DISCUSSION

The receptor for bombesin/GRP is expressed by carcinoma cells in a very large panel of human cancers, (4, 25–27) suggesting that this receptor may play a significant role in human carcinogenesis. However, studies comparing expression of bombesin/GRP receptor and tumor progression in vitro failed to clearly demonstrate a relationship between this expression and the prognosis of human colorectal cancer (3, 4, 6), although one series showed a correlation between high levels of bombesin/GRP receptor mRNA and the invasion of peritumoral lymphatics, a well-recognized independent prognostic factor in these tumors (3). Experimentally, bombesin was shown to dramatically increase lymphatic and peritoneal invasiveness of chemically induced gastric and colonic tumors (28, 29), but no difference in colorectal tumor stage and dissemination was observed between bombesin/GRP receptor knock-out mice and wild-type littermates under azoxymethane treatment (30). In contrast, in vitro studies on cell lines strongly suggested that activation of the bombesin/GRP receptor might promote tumor progression and metastasis. Bombesin-stimulated motility has been reported in nontransformed cells, such as murine Swiss 3T3 fibroblasts and human monocytes, with both chemotactic and chemokinetic effects (31, 32). Interestingly, bombesin induced the phosphorylation of several focal-adhesion, motility-related proteins including p125Fak and p130Cas in Swiss 3T3 fibroblasts (33). In carcinoma cells, bombesin is primarily known to act as a growth factor, stimulating the proliferation of a large variety of tumor cell lines expressing the bombesin/GRP receptor (8, 34) and has been reported to act as an invasion-promoting agent in prostate cancer cell lines (35). We reported previously lamellipodia formation in Isreco1 colon carcinoma cells maintained in the presence of bombesin (8), a phenotype that has been associated with cell motility (36), and demonstrate in this report that bombesin enhances invasive and migratory properties of these cells.

The process of cell invasion relies on several cell properties including actin dynamics, adhesion, and proteolysis. In human prostate carcinoma cells, activation of the bombesin/GRP receptor was shown to stimulate in vitro invasion of cancer cells together with increased MMP-9 and uPA secretion (18, 37), indicating that pericellular proteolysis could be the major mechanism of bombesin-stimulated invasion in prostate tumor cells. In contrast, our results suggest that bombesin stimulates cell motility rather than pericellular proteolysis in Isreco1 cells. Nevertheless, we cannot exclude that proteolytic enzymes other than plasminogen activators or MMP-2 and -9 could contribute to the effect of bombesin on cell invasion. In this context, bombesin was shown to stimulate the formation of lamellipodia in Isreco1 cells, structures that are known to be a major site of proteolytic activity in invasive cells (38). One possible mechanism would be
that bombesin stimulates expression of the uPAR and activates the uPA-uPAR pathway (39). Further work is needed to test whether anti-uPA or anti-uPAR antibodies are able to inhibit bombesin-stimulated invasion and whether bombesin increases PA activity in a membrane fraction of Isreco1 cells.

The molecular mechanisms leading to bombesin-stimulated effects in tumor cells remain poorly understood. In nontransformed murine Swiss 3T3 fibroblasts, morphological and cytoskeletal changes induced by bombesin have been shown to be dependent on activation of the Rho GTPase and on tyrosine phosphorylation of multiple proteins such as p125 FAK, p130Cas, and paxillin (33). The Rho pathway has also been shown to modulate the invasive potential of tumor cells in different models, either in vitro or in vivo. For example in colon carcinoma cells, expression of a dominant negative RhoA resulted in the attenuation of membrane ruffling, lamellipodia formation, and cell invasion stimulated by the integrin α6β4 (40). In the same way, cells transformed by the activated RhoA gene (41) or cells expressing a constitutively active form of RhoA (42) have greatly promoted invasive ability, contributing to the acquisition of a metastatic phenotype in vivo. However, the involvement of the Rho pathway in bombesin-stimulated carcinoma cell invasion and the demonstration of a direct activation of Rho by bombesin, as reported in the present study, have not been described previously. We do not know at this time which effector protein(s) transmits the bombesin signal downstream of the Rho-ROCK pathway in Isreco1 cells. To date, several Rho/ROCK effectors have been described, including Dia proteins, myosin light chain kinase, LIM kinase, and proteins of the ezrin-radixin-moesin family or phospholipid metabolizing kinases (43, 44). Additional experiments are needed to appreciate the role of these proteins in the bombesin-stimulated invasive process in Isreco1 cells. Similarly, very few data are available regarding how bombesin/GRP receptor can activate Rho in either nontransformed or tumor cells. It has been shown that G-protein subunits Gi2 and Gq3 were able to activate directly or indirectly the Rho-dependent pathway (45, 46). However these G proteins were different from that activated by the bombesin/GRP receptor, i.e., members of the Gq family (47). Whether Goq or other effectors are involved in bombesin-stimulated Rho activation in Isreco1 cells remains to be determined. Finally, Rho is only one of the three major small GTPases involved in the control of cell morphology, motility, and invasion. Several interactions and control loops have been shown to interplay among Rac, Rho, and Cdc42 (44). In this context, it would be important to determine the role of each of these G-proteins in the control of the invasive potential of colon carcinoma cells.

The prognosis of colorectal cancer depends mostly on the ability of tumor cells to escape from the primary tumor site, leading to the development of regional or distant metastases, and 30–40% of patients treated by surgery in a curative intent will develop such metastases (48). The present study brings new insights into the mechanisms of tumor invasion using a model of colorectal carcinoma cells: deciphering the mechanisms of bombesin-mediated enhanced invasive migration in Isreco1 cells may help in identifying novel therapeutic targets to prevent malignant invasion.

ACKNOWLEDGMENTS

We would like to thank Drs. J. Bertoglio and C. Crouin (INSERM U461, Chatenay-Malabry, France) for Tat-C3, Dr. D. H. Coy (Tulane University Health Sciences Center, New Orleans, LA) for supplying the bombesin-GRP receptor antagonist o-Phe6-Bn(6-13)OMe, Dr. T. Kondo (Wellfide Corporation, Osaka, Japan) for the gift of the p16ROCK inhibitor Y-27632, and Dr. M. Schwartz (Scirpps Research Institute, La Jolla, CA) for providing pGEX-2T–rhotekin RBD. We are also grateful to Drs. M-F. Bader (INSERM U338, Strasbourg, France), P. Jurdic (ENS, Lyon, France), and M. Serres (INSERM U346, Lyon, France) for their help during this study.

REFERENCES


4834

Downloaded from cancerres.aacrjournals.org on May 2, 2017. © 2002 American Association for Cancer Research.
Bombesin Stimulates Invasion and Migration of Isreco1 Colon Carcinoma Cells in a Rho-dependent Manner

Jean-Christophe Saurin, Marjorie Fallavier, Bernard Sordat, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/62/16/4829

Cited articles
This article cites 45 articles, 15 of which you can access for free at:
http://cancerres.aacrjournals.org/content/62/16/4829.full.html#ref-list-1

Citing articles
This article has been cited by 5 HighWire-hosted articles. Access the articles at:
/content/62/16/4829.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.