Antagonists of bombesin/gastrin-releasing peptide decrease the expression of angiogenic and anti-apoptotic factors in human glioblastoma

Celia A. Kanashiro[a], Andrew V. Schally[a], R.-Z. Cai[a] and Gabor Halmos[a]

We have investigated the antitumor effects and the mechanism of action of antagonists of bombesin/gastrin-releasing peptide (GRP), RC-3940-II and RC-3940-Et, on the growth of U-118MG human malignant glioma xenografted into nude mice. Tumors volume was measured weekly, and after 6 weeks of treatment with GRP antagonists the tumors were analyzed by Western blot assays for the expression of vascular endothelial growth factor (VEGF), protein kinase C (PKC)-α, the anti-apoptotic protein Bcl-2 and the pro-apoptotic protein Bax. A radioreceptor assay was used to characterize the receptors for bombesin/GRP. Specific high-affinity receptors for bombesin were found in U-118MG tumors, and their growth was reduced by 52.5% by RC-3940-II and 72.6% by RC-3940-Et (both $p<0.01$). The tumor doubling time was prolonged by 4.6 and 12 days after treatment with RC-3940-II and RC-3940-Et, respectively, compared to controls ($p<0.05$). Both antagonists caused a significant ($p<0.05$) decrease of about 28% in the levels of VEGF protein and a reduction of approximately 35% in the expression of PKCα. The relative ratio of Bcl-2:Bax was also diminished by around 70% by both analogs, indicating a net apoptotic gain and the efficacy of treatment. Our results suggest that bombesin/GRP antagonists, RC-3940-II and RC-3940-Et, could be of value for the treatment of human glioblastomas. *Anti-Cancer Drugs* 16:159–165 © 2005 Lippincott Williams & Wilkins.

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[a]Endocrine, Polypeptide and Cancer Institute, Veterans Affairs Medical Center and Department of Medicine, Tulane University School of Medicine, New Orleans, LA, USA.

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Correspondence to A. V. Schally, Veterans Affairs Medical Center, 1601 Perdido Street, New Orleans, LA 70112-1262, USA.
Tel: +1 504 589-5230, fax: +1 504 566-1625;
e-mail: aschally@tulane.edu

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Introduction

About 18 400 estimated new cases of primary brain tumors associated with 12 690 deaths are expected to occur in the US in 2004 [1]. Malignant glioblastomas represent the most common type of primary brain tumors in adults [1,2]. The prognosis for individuals with malignant glioma is very poor and, consequently, development of novel therapies is of extreme importance [1,2]. Sharif *et al.* demonstrated that approximately 85% of human glioblastoma cell lines examined expressed functional receptors for bombesin/gastrin-releasing peptide (GRP) [2]. These receptors are members of the G-protein-coupled receptor superfamily, and induce cell proliferation by mechanisms that involve the activation of phospholipase C, the generation of inositol triphosphate, the release of intracellular calcium and the activation of protein kinase C (PKC) [3]. Bombesin-like peptides were first demonstrated to function as autocrine growth factors in small cell lung carcinoma [4] and later were shown to be also implicated in the growth of other cancers [5]. Rosengurt [6] reported that bombesin-mediated mitogenesis in Swiss 3T3 cells could be blocked by different bombesin antagonists through interrupting the signal-transduction process at various post-receptor levels. This mitogenic block is mediated by uncoupling the receptor from its signaling system and is associated with a down-regulation of PKC [6].

We have previously shown that U-87MG and U-373MG human glioblastomas express high-affinity binding sites for bombesin/GRP and mRNA for bombesin receptor subtype (BRS)-1 (GRP receptor) and BRS-2 (neuromedin-B receptor) [7,8]. We have also demonstrated that bombesin/GRP antagonists, RC-3095 and RC-3940-II, inhibited the growth of U-87MG and U-373MG xenografts in nude mice [7,8]. The treatment with RC-3095 reduced the concentration of receptors for bombesin/GRP and significantly prolonged the mean survival time of nude mice inoculated orthotopically with U-87MG cells into the brain [7]. In the present study we evaluated the efficacy of bombesin/GRP antagonists in treatment of brain tumors in another model of human glioblastoma, U-118MG that can be grown in nude mice. We also investigated the mechanism of action of bombesin/GRP antagonists, focusing on angiogenesis and apoptosis.

Angiogenesis is a complex process and vascular endothelial growth factor (VEGF) is the only growth factor known

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to exhibit both potent endothelial mitogenic and vascular permeability activities [9]. Plate *et al.* [10] reported that glioblastomas show a significant up-regulation of VEGF mRNA in certain tumor areas with an important association between VEGF mRNA producer cells and necrosis.

The role of apoptosis in tumorigenesis is a subject of intense investigations, and among the regulatory proteins in the Bcl-2 family are Bcl-2, Bcl-xl, Bag-1, Mcl-1 and A-1 (acting as inhibitors), and Bcl-x_S, Bax, Bak, Bad and Bid (acting as promoters) [11]. An increase in the relative ratio of anti-apoptotic and pro-apoptotic Bcl-2 family proteins through overexpression of Bcl-2 and/or down-regulation of Bax has been correlated with a poor prognosis in patients with brain tumors [12,13]. It was also demonstrated that the overexpression of exogenous PKCα results in an increased Bcl-2 phosphorylation and enhanced chemoresistance, suggesting a mechanism whereby PKCα mediates Bcl-2 phosphorylation and accounts for the increased cell survival observed following chemotherapy [14].

To identify some of the molecular mechanisms involved in the anti-cancer effect of bombesin/GRP antagonists, we evaluated their effects on the expression of VEGF, PKCα, as well as on the apoptotic markers, Bcl-2 and Bax.

Materials and methods

Peptides

The bombesin/GRP antagonist [Hca^6, Leu$^{13}\Psi$ (CH_2N)-Tac^{14}]BN(6–14) (RC-3940-II) (D-24197), originally synthesized in our laboratory [15,16], was made and provided by Zentaris (Frankfurt/Main, Germany). The bombesin/GRP antagonist RC-3940-Et is the analog of RC-3940-II with a C-terminal ethylamide modification. RC-3940-Et was synthesized in our laboratory by methods similar to those described for RC-3940-II [15,16]. For injections, peptides were dissolved in 0.1% dimethylsulfoxide in sterile aqueous 10% propylene glycol.

Cell line and animals

Human glioblastoma cell line U-118MG obtained from the ATCC (Manassas, VA) was cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, penicillin and streptomycin.

Male athymic nude mice (Ncr *nu*/*nu*) were obtained from the National Cancer Institute (Frederick Cancer Research and Development Center, Frederick, MD) and maintained under pathogen-limited conditions. To obtain donor animals with tumors, two nude mice received s.c. injections of cell suspension containing 2×10^7 U-118MG cells. The developed tumors were dissected and minced, and 3-mm^3 pieces of tumor tissue were transplanted s.c. to both flank areas of the experimental animals. When tumors became measurable, control and experimental groups were formed with animals bearing tumors of about the same size. Tumor volumes and body weights were measured weekly. The volume of tumors was measured in a blinded manner and calculated as length × width × height × 0.5236. Tumor volume doubling time (TDT) was calculated between the start and the end of the treatment using the formula: days of treatment/[log (final volume)–log (initial volume)]/log2. Antitumor activity of the compounds was evaluated by calculation of the tumor growth reduction (TGR) using the following formula: $TGR\% = 100 - 100 \times (T-t)/(C-c)$, where t is the volume of treated tumors at the beginning of therapy, T is the volume of the same tumors at the end of the experiment, c is the volume of controls at the start of treatment and C is the volume of controls at the end of the experiment. After 6 weeks of treatment, mice were sacrificed, and various organs were removed and weighed. Tumors were excised, weighed, snap-frozen and stored at –70°C until further analyses. All animal experiments were reviewed by the institutional animal care and use committee, and were performed in accordance with institutional guidelines for animal care.

Experimental protocol

The experiment was started when tumors had grown to approximately 90 mm^3. Mice were divided into groups containing eight to 10 animals each, and received s.c. injections of vehicle or 10 μg/day/animal of the analogs as follows: group 1, control, vehicle solution; group 2, bombesin/GRP antagonist RC-3940-II; group 3, bombesin/GRP antagonist RC-3940-Et.

Receptor binding assays

The binding characteristics of receptors for bombesin/GRP were determined on tumor membrane fractions of control animals. For *in vitro* binding studies, radioiodination of [Tyr^4]bombesin and separation of the monoiodinated radioligands by HPLC were performed [17]. To characterize membrane receptors for bombesin/GRP, ligand competition assays were performed, based on binding of the radiolabeled ligands to tumor membrane homogenates [17]. The ligand PC computerized curve-fitting program was used to determine the type of receptor-binding, dissociation constant (K_d) and maximal binding capacity (B_{max}) of the receptors [17].

Western blotting assays

Protein-matched samples (40 μg/lane) were separated by 10–12% SDS–PAGE. Proteins were immunodetected on nitrocellulose membranes using a chemiluminescence detection system (Amersham, Arlington Heights, IL). The membranes were incubated overnight at 4°C in 5% non-fat dry milk in TBS-Tween. The blots were probed with polyclonal antibodies (1:1000) to VEGF (1-147), Bax (N-20) or PKCα, and monoclonal antibody (1:1000) to

Bcl-2, before incubation with horseradish peroxidase-conjugated secondary antibodies (all from Santa Cruz Biotechnology, Santa Cruz, CA; except for PKCα, which was from Sigma, St Louis, MO) and exposure to the chemiluminescence substrate. The protein bands were quantified by normalizing the signals of different proteins to β-actin signal (1:2000, Santa Cruz) using the Kodak EDAS 290 imaging system with Kodak 1D Image Analysis Software (Kodak, Rochester NY).

Statistical analyses

SigmaStat software was used for the statistical analysis of data. Results were evaluated by one-way ANOVA, and the Bonferroni and Student–Neuman–Keul's tests. Results were considered statistically significant when $p < 0.05$.

Results

Receptor-binding studies

Radiolabeled [Tyr^4]bombesin was bound to a single class of specific receptors for bombesin/GRP in U-118MG human glioblastoma tumors with high affinity (dissociation constant, $K_d = 1.32 \pm 0.07$ nM) and with a mean maximal binding capacity (B_{max}) of 493.5 ± 83.1 fmol/mg protein).

Effects of therapy on growth of U-118MG glioblastoma *in vivo*

The treatment of nude mice bearing U-118MG tumors with bombesin/GRP antagonists, RC-3940-II and RC-3940-Et had a strong inhibitory effect on tumor volumes. We observed a significant ($p < 0.05$ for both) tumor growth reduction at the end of the second week of treatment (Fig. 1), but the greatest suppression ($p < 0.01$ for both) in the tumor volume was observed from weeks 4 to 6 of treatment when the experiment was ended (Fig. 1 and Table 1). After treatment with antagonists RC-3940-II and RC-3940-Et, the tumor growth reduction rate ($p < 0.01$ versus controls) was 52.5 and 72.6%, respectively, and the TDT was also significantly ($p < 0.05$) prolonged by 4.6 and 12 days, respectively (Table 1). We also detected a decrease in the tumor weight in the treated groups, but this inhibitory tendency was not statistically significant (Table 1).

Effects of bombesin/GRP antagonists on the expression of VEGF in U-118MG human glioblastoma

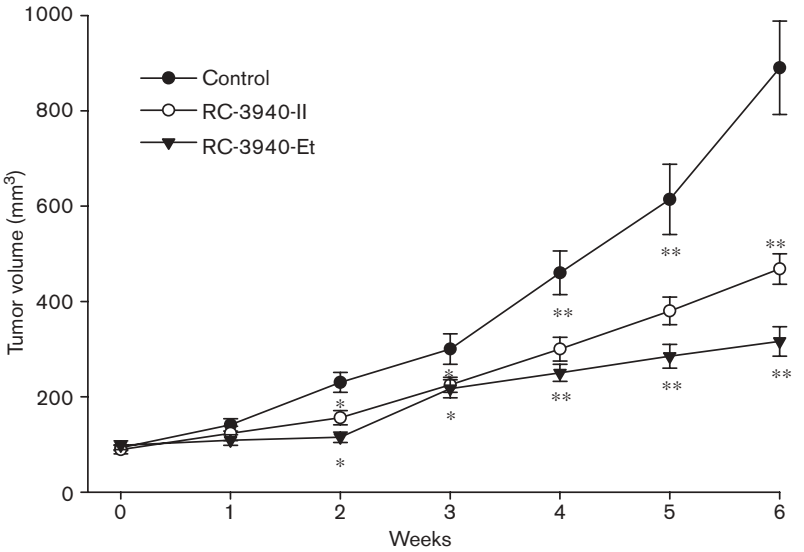
We investigated the expression of VEGF protein by Western blot assays with specific antibody in U-118MG tumors from animals treated with bombesin/GRP antagonists. Figure 2(A) shows three representative tumors from each group. Densitometric analyses of the bands representing VEGF, after normalization to β-actin levels, demonstrated that RC-3940-II and RC-3940-Et significantly ($p < 0.05$) reduced VEGF expression by 27 and 29%, respectively (Fig. 2B).

Table 1 Effects of therapy with bombesin/GRP antagonists, RC-3940-II and RC-3940-Et, on the growth of U-118MG human glioblastomas xenografted in nude mice

Groups	Tumor weight (g)	Final tumor volume (mm^3)	TGR (%)	TDT (days)
Control	1.43 ± 0.17	890 ± 98	0.0 ± 2.1	12.9 ± 1.1
RC-3940-II	1.25 ± 0.15	468 ± 32[b]	52.5 ± 4.4[b]	17.5 ± 1.5[a]
RC-3940-Et	1.01 ± 0.10	316 ± 31[b]	72.6 ± 6.9[b]	24.9 ± 2.4[a]

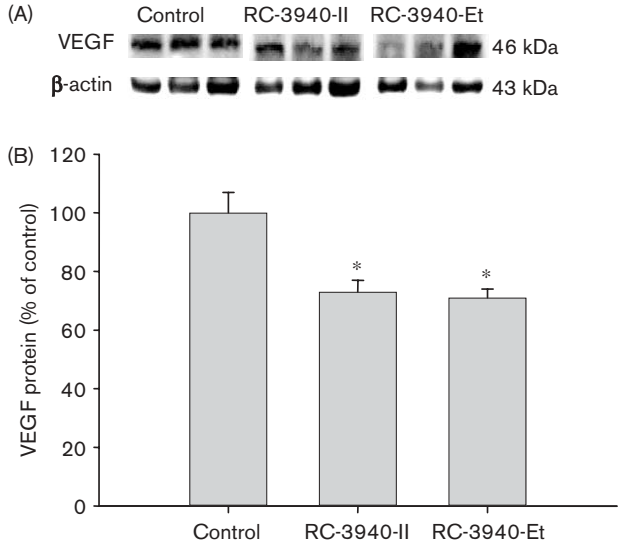
[a]$p < 0.05$ and [b]$p < 0.01$ versus control.

Fig. 1



Changes in tumor volumes in nude mice bearing subcutaneous xenografts of U-118MG glioblastomas, during therapy with bombesin/GRP antagonists, RC-3940-II and RC-3940-Et. The data represent means ± SE. *$p < 0.05$ and **$p < 0.01$ versus control.

Fig. 2



(A) Representative Western blotting for VEGF in U-118MG glioblastomas. (B) Levels of VEGF protein expressed as a percentage of control after standardization to the expression of β-actin levels. The experiments were performed in triplicate. The data are expressed as means ± SE. *$p < 0.05$ versus control.

Expression of Bcl-2 and Bax proteins in tumors treated with RC-3940-II and RC-3940-Et

The levels of Bcl-2 and Bax proteins were determined in U-118MG tumors by Western blot analysis. As shown in Figure 3, the levels of Bcl-2 were significantly ($p < 0.05$) decreased by treatment with bombesin/GRP antagonists, RC-3940-II and RC-3940-Et, while the level of Bax protein were significantly ($p < 0.05$) increased by both compounds. Since Bcl-2 and Bax are thought to have opposite effects in determination of the net apoptotic gain, a relative ratio of Bcl-2:Bax was calculated. Given that this ratio has anti-apoptotic protein in the numerator and the pro-apoptotic protein in the denominator, lower ratio values are observed with lower levels of the anti-apoptotic proteins and/or higher levels of the pro-apoptotic proteins. In the treated groups, the ratio of the Bcl-2 to Bax proteins, which is a parameter of a net apoptotic gain, was about 70% lower than the control values, indicating the effectiveness of treatment (Fig. 3).

Expression of PKCα in U-118MG tumors treated with bombesin/GRP antagonists

Assuming that an increase in the expression of PKCα might result in more phosphorylated and consequently more active Bcl-2, which may offer enhanced protection against apoptosis, we measured the levels of PKCα. Immunoblots of whole tissue homogenate of U-118MG tumors revealed significant immunoreactive bands at 79.5 kDa corresponding to PKCα (Fig. 4). The bands could be eliminated by inclusion of the antigenic peptide (data not shown). Both bombesin/GRP antagonists caused a significant ($p < 0.05$) inhibition in the expression of PKCα. RC-3940-II induced a decrease of 32% and after treatment with RC-3940-Et a 38% reduction was also observed, compared to controls (Fig. 4).

Discussion

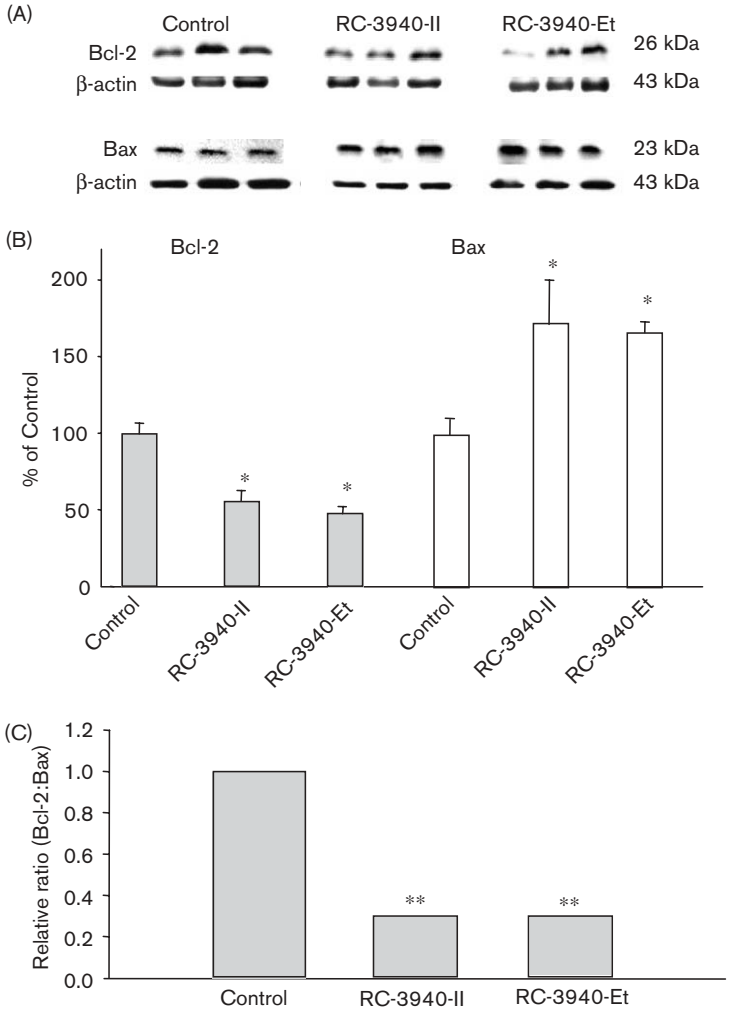
The present study demonstrates a significant inhibitory effect of bombesin/GRP antagonists RC-3940-II and RC-3940-Et on the growth of xenografts of U-118MG human glioblastoma in nude mice. Thus, antagonists RC-3940-II and RC-3940-Et inhibited the growth of U-118MG tumors by 52.5 and 72.6%, respectively, and also extended significantly the tumor doubling time by 4.6 and 12 days, respectively.

Our findings showing the presence of high affinity-binding sites for bombesin/GRP on membranes of U-118MG tumors are in agreement with previous studies demonstrating the expression of these receptors in two other glioblastoma cell lines, U-373MG and U-87MG [7]. We also have reported that U-87MG tumors express mRNA for BRS-1 (GRP receptor) and BRS-2 (neuromedin-B receptor), but not mRNA for BRS-3 [8]. In those studies, the inhibition of growth of both glioblastomas, U-87MG and U-373MG, was accompanied by a decrease in the concentration of bombesin/GRP receptors on membranes of these tumors [7]. In addition to the down-regulation of the bombesin/GRP receptors, the inhibition of growth of various cancers including brain, lung, pancreatic, prostatic, gastric and mammary by bombesin/GRP antagonists was associated with a major decrease in receptor levels for the epidermal growth factor (EGF) on the tumor membranes [7,15]. Thus, bombesin/GRP antagonists may act locally by various mechanisms that result in a reduction in the binding sites for EGF, which have been shown to be present in many brain tumors [18–22]. However, the exact molecular mechanism and the heterologous regulation of EGF by bombesin/GRP antagonists receptors remain unclear.

Bombesin initiates a series of intracellular signals that causes an increase in inositol-1,4,5-trisphosphate, a mobilization of intracellular Ca^{2+} and diacylglycerol production, leading to activation of PKC [23]. Activation of PKC causes the phosphorylation of EGF receptors on threonine residues. Bombesin and GRP were shown to enhance the phosphorylation of EGF receptors and this effect was inhibited by treatment with bombesin/GRP antagonist [24]. These results suggest that bombesin/GRP may function by up-regulating EGF receptors and that bombesin/GRP antagonists prevent this upregulation [24].

Investigating bombesin/GRP antagonists, Kiaris *et al.* [8] demonstrated that GRP induces the expression of c-*fos*

Fig. 3



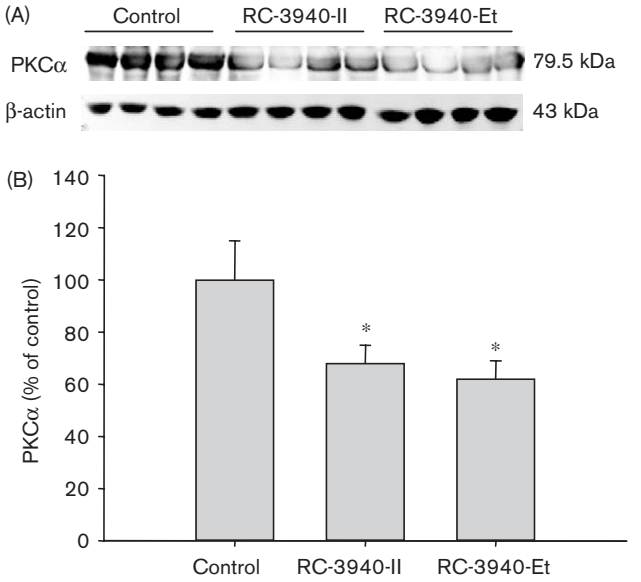
(A) Representative Western blotting for Bcl-2 and Bax in U-118MG tumors. (B) Bcl-2 and Bax were normalized to β-actin levels and expressed as percentage of control. (C) Relative ratio (Bcl-2:Bax). The experiments were performed in triplicate. The data are means ± SE. *$p < 0.05$ and **$p < 0.01$ versus control.

oncogene in U-87MG and U-373MG glioblastomas, suggesting that c-*fos* might be involved in the mechanism of action of bombesin-like peptides including GRP. Since the products of c-*fos* and c-*jun* oncogenes are proteins involved in the mechanism of regulation of mRNA for GRP, this finding suggests that the paracrine production of GRP could be implicated in the pathogenesis of brain tumors [8].

To better understand the molecular mechanism of the bombesin/GRP antagonists, their effects on the expression of the growth signal proteins (VEGF and PKCα) and apoptosis-related proteins (Bcl-2 and Bax) were analyzed in the present study. Studies of angiogenesis in glioblastomas have shown an up-regulation of VEGF in these cell lines [25]. To further examine this phenomenon, we investigated the effect of bombesin/GRP antagonists on the expression of VEGF in U-118MG glioblastomas. Our results indicate that treatment of U-118MG tumors with bombesin/GRP antagonists, RC-3940-II and RC-3940-Et, caused a significant inhibition in the expression of VEGF. This finding is in agreement with our previous work showing that bombesin/GRP antagonists significantly decreased the expression of mRNA for VEGF-A in human experimental breast cancers [26]. Considering that glioblastomas are highly vascularized, the therapies that cause reduction in the neovascularization process would be of major clinical importance.

Many investigations have suggested that the deregulation of the apoptotic proteins could affect tumor progression and the resistance to treatment as well as the initiation of

Fig. 4



Expression of PKCα in U-118MG tumors untreated or treated with bombesin/GRP antagonists. (A) Representative Western blot. (B) Percentages of protein measurements are presented as means ± SE of eight to 10 samples from each group after standardization to the expression of β-actin levels. *$p < 0.05$ versus control.

oncogenesis [27]. The expression of Bcl-2-related proteins was increased in untreated patients with gliomas, suggesting that the changes in the expression of Bcl-2 could reflect the natural course of the disease [28]. Bcl-2 and Bax are functionally antagonistic proteins that control apoptosis, and the measurements of the expression of these proteins could be of prognostic value. The relative ratio of the anti-apoptotic Bcl-2 and the pro-apoptotic Bax protein is positively correlated with a poor prognosis for patients with brain tumors [29,30]. Thus, we compared the expression of two apoptotic markers, Bcl-2 and Bax, using immunoblot assays. We found that U-118MG tumors treated with bombesin/GRP antagonists, presented a low level of Bcl-2 and a high level of Bax, compared to controls. Our results show that antagonists, RC-3940-II and RC-3940-Et, caused a down-regulation of Bcl-2 protein by 44 and 52%, respectively. On the contrary, the levels of Bax protein were significantly increased by 73 and 67% in the groups treated with RC-3940-II and RC-3940-Et, respectively. The relative ratio of the Bcl-2 to Bax was reduced in both treated groups by about 70% as compared to controls. A low Bcl-2:Bax ratio is an indicator of a net apoptotic gain and a good prognosis for the patients [29,30]. However, the discrepancies between Bcl-2:Bax ratios and the sensitivity of cells to chemotherapy suggest that other factors are also involved. Thus, chemosensitivity cannot be explained by simple quantitative differences in the expression of Bcl-2 or Bax [31]. In addition to phosphorylation of EGF receptors, a classical isoform of PKC family, PKCα, was shown to increase the phosphorylation of Bcl-2 [14]. Therefore, the net apoptotic signal resulting from the Bcl-2 family may depend not only on the relative ratios of pro- and anti-apoptotic members, but also on the degree of phosphorylation of Bcl-2 members [14]. Based on this view, we also measured the expression of PKCα in U-118MG tumors after treatment with bombesin/GRP antagonists. Interestingly, we observed that in U-118MG tumors, treatment with RC-3940-II and RC-3940-Et, inhibited PKCα by 32 and 38%, respectively, as compared to untreated tumors. This inhibitory effect of bombesin/GRP antagonists on the expression of PKCα, exhibited a positive correlation with the inhibitory effect induced by the same compounds in the expression of Bcl-2 protein.

In conclusion, this study in U-118MG human glioblastomas xenografted into nude mice reinforces previous findings on effectiveness of bombesin/GRP antagonists for therapy of brain tumors. The present investigation also shows that bombesin/GRP antagonists act by decreasing the levels of the angiogenic factor VEGF protein, as well as by reducing the relative ratio of the apoptotic proteins (Bcl-2:Bax) and also by diminishing the expression of PKCα. These data, together with the results of our previous investigations in other glioblastoma cell lines, suggest that bombesin/GRP antagonists might be useful for the treatment of patients with malignant brain tumors expressing receptors for bombesin/GRP. However, further experimental work, followed by clinical trials is necessary.

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References

1 Jemal A, Tiwari RC, Murray T, Ghafoor A, Samuels A, Ward E, *et al.* Cancer statistics, 2004, CA. *Cancer J Clin* 2004; **54**:8–29.
2 Sharif TR, Luo SW, Sharif M. Functional expression of bombesin receptor in most adult and pediatric human glioblastoma cell lines; role in mitogenesis and in stimulating the mitogen-activated protein kinase pathway. *Mol Cell Endocrinol* 1997; **130**:119–130.
3 Fathi Z, Way JW, Corjay MH, Viallet J, Sausville EA, Battey JF. Bombesin receptor structure and expression in human lung carcinoma cell lines. *J Cell Biochem Suppl* 1996; **24**:237–246.
4 Moody TW, Pert CB, Gazdar AF, Carney DN, Minna JD. High levels of intracellular bombesin characterize human small-cell lung carcinoma. *Science* 1981; **214**:1246–1248.
5 Schally AV, Comaru-Schally AM. Hypothalamic and other peptide hormones. In: Bast RC, Kufe DW, Pollock RE, Weichselbaum RR, Holland JF, Frei III E (editors): *Cancer Medicine*, 6th edn. Lewiston, NY: Decker; 2003, pp. 911–926.
6 Rozengurt E. Bombesin stimulation of mitogenesis, Specific receptors, signal transduction, and early events. *Am Rev Respir Dis* 1990; **142**: S11–S15.
7 Pinski J, Schally AV, Halmos G, Szepeshazi K, Groot K. Somatostatin analogue and bombesin/gastrin-releasing peptide antagonist RC-3095 inhibit the growth of human glioblastomas *in vitro* and *in vivo*. *Cancer Res* 1994; **54**:5895–5901.

8 Kiaris H, Schally AV, Sun B, Armatis P, Groot K. Inhibition of human malignant glioblastoma in nude mice by antagonists of bombesin/gastrin-releasing peptide. *Oncogene* 1999; **18**:7168–7173.

9 Carrol RS, Zhang J, Bello L, Melnick MB, Maruyama T, Black PM. KDR activation in astrocytic neoplasms. *Cancer* 1999; **86**:1335–1341.

10 Plate KH, Breier G, Weich HA, Mennel HD, Risau W. Vascular endothelial growth factor and glioma angiogenesis: coordinate induction of VEGF receptors, distribution of VEGF protein and possible *in vivo* regulatory mechanisms. *Int J Cancer* 1994; **59**:520–529.

11 Kroemer G. The proto-oncogene *bcl-2* and its role in regulating apoptosis. *Nat Med* 1992; **3**:614–620.

12 Kaliberov SA, Buchsbaum DJ, Gillespie GY, Curiel DT, Arafat WO, Carpenter M, *et al.* Adenovirus-mediated transfer of BAX driven by the vascular endothelial growth factor promoter induces apoptosis in lung cancer cells. *Mol Therapy* 2001; **6**:190–198.

13 Wang ZH, Ding MX, Yuan JP, Jin ML, Hao CF, Chew-Cheng SB, *et al.* Expression of Bcl-2 and Bax in EGFR-antisense transfected and untransfected glioblastoma cells. *Anticancer Res* 1999; **19**: 4167–4170.

14 Ruvolo PP, Deng X, Carr BK, May WS. A functional role for mitochondrial protein kinase C α in Bcl-2 phosphorylation and suppression of apoptosis. *J Biol Chem* 1998; **273**:25436–25442.

15 Schally AV, Comaru-Schally AM, Nagy A, Kovacs M, Szepeshazi K, Plonowski A, *et al.* Hypothalamic hormones and cancer. *Front Neuroendocrinol* 2001; **22**:248–291.

16 Reile H, Cai R-Z, Armatis P, Schally AV. New antagonists of bombesin/gastrin-releasing peptide with C-terminal Leuø(CH_2N) Tac-NH_2. *Int J Oncol* 1995; **7**:749–754.

17 Halmos G, Pinski J, Szoke B, Schally AV. Characterization of bombesin/gastrin-releasing peptide receptors in membranes of MKN45 human gastric cancer. *Cancer Lett* 1994; **85**:111–118.

18 Pinski J, Halmos G, Shirahige Y, Wittliff JL, Schally AV. Inhibition of growth of the human malignant glioma cell line (U87MG) by the steroid hormone antagonist RU486. *J Clin Endocrinol Metab* 1993; **77**: 1388–1392.

19 Libermann A, Razon N, Bartal AD, Yarden Y, Schlessinger J, Soreq H. Expression of epidermal growth factor receptors in human brain tumors. *Cancer Res* 1984; **44**:753–760.

20 Baugnet-Mahieu L, Lemaire M, Brotchi J, Levivier M, Born J, Gilles J, *et al.* Epidermal growth factor receptors in human tumors of the central nervous system. *Anticancer Res* 1990; **10**:1275–1280.

21 Reubi JC, Horisberger U, Lang W, Koper JW, Braakman R, Lamberts SWJ. Coincidence of EGF receptors and somatostatin receptors in meningiomas but inverse, differentiation-dependent relationship in glial tumors. *Am J Pathol* 1989; **134**:334–337.

22 Shiurba RA, Eng LF, Vogel H, Lee Y-L, Horoupian SS, Urich H. Epidermal growth factor receptor in meningiomas expressed predominantly on endothelial cells. *Cancer* 1988; **62**:2139–2144.

23 Zachary I, Sinnett-Smith JW, Rozengurt E. Early events elicited by bombesin and structurally related peptides in quiescent Swiss 3T3 cells. I. Activation of protein kinase C and inhibition of epidermal growth factor binding. *J Cell Biol* 1986; **102**:2211–2222.

24 Liebow C, Crean DH, Lee MT, Kamer AR, Mang TS, Schally AV. Synergistic effects of bombesin and epidermal growth factor on cancers. *Proc Natl Acad Sci USA* 1994; **91**:3804–3808.

25 Ke LD, Fueyo J, Chen X, Steck PA, Shi YX, Im SA, *et al.* A novel approach to glioma gene therapy: down-regulation of the vascular endothelial growth factor in glioma cells using ribozymes. *Int J Oncol* 1998; **12**:1391–1396.

26 Bajo AM, Schally AV, Groot K, Szepeshazi K. Bombesin antagonists inhibit proangiogenic factors in human experimental breast cancers. *Br J Cancer* 2004; **90**:245–252.

27 Reed JC. Dysregulation of apoptosis in cancer. *J Clin Oncol* 1999; **17**:2941–2953.

28 Strik H, Deininger M, Streffer J, Wickboldt J, Dichgans J, Weller M, *et al.* BCL-2 family protein expression in initial and recurrent glioblastomas: modulation by radiochemotherapy. *J Neurol Neurosurg Psychiatry* 1999; **67**:763–768.

29 Yacoub A, Mitchell C, Lister A, Yacoub A, Mitchell C, Lister A, Lebedeva IV, *et al.* Melanoma differentiation-associated 7 (interleukin 24) inhibits growth and enhances radiosensitivity of glioma cells *in vitro* and *in vivo*. *Clin Cancer Res* 2003; **9**:3272–3281.

30 Mizoguchi M, Inamura T, Shono T, Ikezaki K, Inoha S, Ohgami S, *et al.* A comparative study of apoptosis and proliferation in germinoma and glioblastoma. *Neurooncology* 2000; **2**:96–102.

31 Yang E, Korsmeyer SJ. Molecular thanatopsis: a discourse on the BCL2 family and cell death. *Blood* 1996; **88**:386–401.