Differential Functions of Growth Factor Receptor–Bound Protein 7 (GRB7) and Its Variant GRB7v in Ovarian Carcinogenesis

Yajun Wang, David W. Chan, Vincent W.S. Liu, PM Chiu, and Hextan Y.S. Ngan

Abstract

Purpose: Aberrant overexpression of growth factor receptor–bound protein 7 (GRB7) and its variant GRB7v has been found in numerous human cancers. The goal of this study was to characterize the functions of GRB7 and GRB7v in the ovarian carcinogenesis and to investigate the differential roles of GRB7 and GRB7v in the modulation of signaling pathways.

Experimental Design: Quantitative reverse transcription–PCR, Western blot, and immunohistochemical analyses were used to evaluate the levels of GRB7 and GRB7v. The cellular localization, functions, and signaling pathways regulated by GRB7 and GRB7v were investigated by enforced expression of GRB7 and GRB7v.

Results: Quantitative reverse transcription–PCR and Western blot analyses showed that GRB7 and GRB7v were frequently upregulated in ovarian cancer samples. The overexpressed GRB7 ($P = 0.009$) and GRB7v ($P = 0.017$) were significantly correlated with high-grade ovarian cancer. Immunohistochemical analysis on ovarian cancer tissue array confirmed that the upregulated GRB7 was significantly correlated with high-grade ovarian cancer ($P = 0.001$). Confocal microscopy analysis showed that GRB7 and GRB7v predominately localized in cytoplasm of ovarian cancer cells, consistent with their roles as signaling adaptors. Enforced expression of GRB7 promoted cell proliferation, migration, and invasion, whereas GRB7v only increased cell proliferation and anchorage-independent growth ability. With the treatment of specific kinase inhibitors, we showed that both GRB7 and GRB7v promoted cell proliferation through activating extracellular signal-regulated kinase signaling, whereas GRB7 enhanced cell migration/invasion by activating c-Jun NH2-terminal kinase signaling.

Conclusions: Our studies implicate that the overexpressed GRB7 and GRB7v are associated with high-grade tumors and exert distinct tumorigenic functions through regulating different signaling pathways in ovarian cancer cells.

Ovarian cancer is one of the most deadly malignancies in women (1). Approximately 70% of ovarian cancer cases are diagnosed at the advanced stage, and the mortality rate has remained high for decades (2). It is widely accepted that genetic variations act in concert with other factors, such as hormonal and environmental factors, to potentiate the risk of ovarian cancer. However, the knowledge on the molecular basis of ovarian cancer development remains poor (3). Undoubtedly, a better understanding of the molecular pathogenesis of ovarian cancer may assist the development of novel therapeutic interventions for this disease.

Tyrosine kinase signaling pathways are the major regulators of cell growth, cell division, and motility. The aberrant activation of these pathways attributes to the development of many human cancers (4, 5). The human growth factor receptor–bound protein 7 (GRB7) is a member of GRB7 adaptor protein family, which also includes GRB10 and GRB14, and acts as an adaptor bridging the growth factor receptor kinase proteins and their downstream signaling molecules (6–9). It is composed of several protein binding domains, such as an amino-terminal proline-rich region, followed by a RA (ras-assOCIating) domain, a PH (pleckstrin homology) domain, a BPS (between PH and SH2 domains) motif, and a carboxy-terminal SH2 domain (7). Its variant GRB7v lacks an 88-bp exon in the COOH terminus due to alternative splicing, resulting in the substitution of the SH2 domain by a hydrophobic sequence (10). Recent findings have reported that aberrant upregulation of GRB7 is frequently found in
Translational Relevance  
Growth factor receptor–bound protein 7 (GRB7) is an adaptor molecule of signal transduction. It is frequently upregulated in a variety of human cancers and plays an essential role in tumor progression. Our study here reports that not only GRB7 but also its isoform GRB7v was upregulated and correlated to high-grade ovarian cancer. Intriguingly, GRB7 and GRB7v showed different tumorigenic functions through regulating differential signaling pathways in ovarian cancer cells. These results suggest that the overexpressed GRB7 and GRB7v play different roles in the tumorigenicity of high-grade ovarian cancer. The understanding of their distinct functional roles and molecular mechanisms of GRB7 and GRB7v may facilitate the exploration of these proteins as novel therapeutic targets in ovarian cancers.

various human cancers, such as breast cancer (11, 12), esophageal cancer (13, 14), and gastric cancer (15, 16). Its variant GRB7v has also been found to be overexpressed in esophageal cancer (10). Clinicopathologic studies have revealed that the overexpressed GRB7 significantly associates with metastatic tumor phenotype (7). Indeed, GRB7 has been shown to enhance cell migration through association with focal adhesion kinase (17, 18). These findings suggest that GRB7 is involved in the progression of cancers. However, the tumorigenic roles of the overexpressed GRB7 and GRB7v in human cancers, such as ovarian cancer, remain unknown.

In this study, we report that both GRB7 and GRB7v are upregulated in ovarian cancers and the overexpressed GRB7 and GRB7v are significantly correlated with high-grade ovarian cancer. We further provide evidence that the exogenous overexpression of GRB7 promotes cell proliferation, migration, and invasion through extracellular signal-regulated kinase (ERK) and c-Jun NH2 terminal kinase (JNK) signaling pathways, whereas the enforced expression of GRB7v increases cell proliferation and anchorage-independent growth ability via ERK signaling. Our findings confer that GRB7 and GRB7v function differently through regulating differential signaling pathways in ovarian cancer cells.

Materials and Methods

Clinical samples and cell lines. A total of 76 tumor samples surgically resected from primary ovarian cancer patients and 53 normal ovary samples from benign diseases were randomly selected for this study. The histologic subtypes and disease stages of the tumors were classified according to International Federation of Gynecology and Obstetrics criteria. The use of clinical specimens was approved by the local institutional ethics committee. Two immortalized human ovarian surface epithelial (HOSE) cells, HOSE12-11 and HOSE17-1 (gifts from Prof. G.S.W. Tsao, Department of Anatomy, University of Hong Kong), and ovarian cancer cell lines A2780CP, A2780S, OV2008, and C13 (gifts from Prof. B.K. Tsang, Department of Obstetrics and Gynecology, University of Ottawa) and SKOV3, OVCA23, OVCA420, OVCA429, and OVCA433 (American Type Culture Collection) were used in this study. All were cultured at 37°C in 5% CO2 in MEM or DMEM supplemented with 10% fetal bovine serum.

Plasmid and cell transfection. The pEGFP/GRB7 was constructed by subcloning the full-length cDNA fragment from PKH3/GRB7 plasmid (gift from Prof. J.L. Guan, Department of Cell and Developmental Biology, University of Michigan) into pEGFP-C3 vector (Invitrogen Life Technology). The pEGFP/GRB7v was constructed by PCR amplification on cDNAs of normal ovarian tissues using the primer pair (GRB7s, 5′-GCCCTGAGAGGAAAGACAA-3′ and GRB7a, 5′-AATGACGGCGACCCCTCCTC-3′) and subcloned into pEGFP-C3 vector (Invitrogen). The sequences of the pEGFP/GRB7 and pEGFP/GRB7v were verified by sequencing analysis. Lipofectamine 2000 (Invitrogen) was used for cell transfection according to the manufacturer’s instructions. Stable cells overexpressing GFP/GRB7 and GFP/GRB7v were harvested after 14 days of G418 selection and verified by Western blot analysis.

RNA extraction and reverse transcription–PCR analysis. Total RNA from the tumor tissues and the cultured cells was prepared using the TRizol reagent (Invitrogen). The cDNA was prepared using Taqman reverse transcription kit according to the manufacturer’s instructions (Applied Biosystems). The real-time quantitative reverse transcription–PCR (Q-PCR) was performed using the Taqman gene expression assay kit in an ABI 7700 system (Applied Biosystems). The primers and probes for GRB7 (Assay ID GRB7-1213) and GRB7v (Assay ID GRB7v-1113) were purchased from Applied Biosystems. Each sample was performed in triplicate, and the relative expression level of each gene was normalized with glyceraldehyde-3-phosphate dehydrogenase (Assay ID Hs99999905_m1; Applied Biosystem).

Immunohistochemical and Western blot analyses. Immunohistochemical staining for GRB7 and GRB7v was performed on an ovarian cancer tissue array (OVC1021; Pantomics, Inc.). The section was immunostained with pantomics, Inc.). The section was immunostained with nhistochemical staining for GRB7 and GRB7v was performed on an ovarian cancer tissue array (OVC1021; Pantomics, Inc.). The section was immunostained with nhistochemical staining for GRB7 and GRB7v was performed on an ovarian cancer tissue array (OVC1021; Pantomics, Inc.). The section was immunostained with nhistochemical staining for GRB7 and GRB7v was performed on an ovarian cancer tissue array (OVC1021; Pantomics, Inc.). The section was immunostained with nhistochemical staining for GRB7 and GRB7v was performed on an ovarian cancer tissue array (OVC1021; Pantomics, Inc.). The section was immunostained with

2530 Clin Cancer Res; 16(9) May 1, 2010  Clinical Cancer Research

Clinical Cancer Research
inhibitor cocktail (Roche) and phenylmethylsulfonyl fluoride (Sigma Chemical Co.). The samples were resolved by SDS-PAGE and electroblotted onto Immobilon-P Transfer Membrane (Millipore Corporation). Blots were blocked with 5% skim milk, followed by incubation with GRB7 (H70, Santa Cruz), GFP (Abcam, Inc.), β-actin (Sigma Chemical Co.), phosphorylated ERK, ERK, phosphorylated P38, P38, phosphorylated JNK, JNK, phosphorylated AKT, and AKT (Cell Signaling). Blots were then incubated with goat anti-rabbit or anti-mouse secondary antibody conjugate with horseradish peroxidase (Amersham Pharmacia Biotech) and visualized by enhanced chemiluminescence (Amersham).

Confocal microscopy. Cells were cultured on coverslips and transiently transfected with either pEGFP/GRB7 or pEGFP/GRB7v expressing plasmids. After 24 hours, the

Fig. 1. Upregulation of GRB7 and GRB7v in ovarian cancer. A, real-time Q-PCR analysis showed that GRB7 and GRB7v were upregulated in ovarian cancer cell lines comparing with immortalized normal HOSEs. Western blotting revealed the upregulation of GRB7/GRB7v protein in the ovarian cancer cell lines. β-Actin was used as loading control. C, real-time Q-PCR analysis showed that the expressions of GRB7 and GRB7v were significantly higher in clinical ovarian cancer samples compared with normal ovaries; P = 0.016. D, immunohistochemical analysis of GRB7/GRB7v in ovarian cancer tissue array samples. Representative figures showing GRB7/GRB7v staining: a, benign (arrow, the epithelial cell layer); b, serous, Grade 1; c, serous, Grade 2; d, serous, Grade 3; e, cytoplasmic localization (arrow, cytoplasmic localization of GRB7/GRB7v). Magnification, ×200 (a–d) and ×400 (e).
transfected cells were fixed with 4% paraformaldehyde and treated with 0.1% triton. After the cells were counterstained with 4′,6-diamidino-2-phenylindole (Sigma), the fluorescent signals were examined and photographed by confocal microscopy.

**Cell viability analysis.** Cell viability was analyzed with cell proliferation kit II [2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide inner salt (XTT); Roche] according to the manufacturer's instructions. The assay was performed in triplicate for each time point and was repeated in at least three independent experiments.

**Anchorage-independent growth assay in soft agar.** A total of $5 \times 10^5$ OVCA433 cells were suspended in 2 mL of full medium containing 0.3% agar. The mixtures were plated on top of solidified bottom layer with 1% agar in full medium. Viable colonies with 20 cells or more were scored and photographed after 21 days. The experiment was performed in triplicate and was repeated thrice.

**Wound healing assay.** Equal numbers of cells were cultured in a six-well plate until confluent monolayer cells were obtained. Cells were pretreated with mitomycin C (10 μg/mL, 3 hours; Sigma), washed with PBS, and then wounded with a sterilized micropipette tip. The width of

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the gaps was photographed at different time points. The width of wound was measured, and the relative velocity of cell migration was calculated as width/time. All the experiments were done in triplicate and were repeated for at least thrice.

Transwell cell migration and cell invasion analyses. Quantification of cell migration and invasion was performed by the Transwell cell migration and cell invasion assay kits (Chemicon International, Inc.) according to the manufacturer’s instructions. In brief, 1.5 × 10^5 cells were resuspended in serum-free culture medium (for phosphorylated JNK inhibition test, 10 μmol/L SP600125 was included) and seeded on the upper chamber. The full medium was placed in the lower chamber as chemoattractant. The cells were allowed to pass through the pores to the lower surface of the membrane (12 hours for cell migration and 48 hours for cell invasion assay) and were stained with the staining buffer. Three different fields of the stained cells were photographed and counted for each Transwell filter. The experiments were repeated thrice.

Statistical analysis. Data were expressed as mean ± SD or mean ± SEM. Student’s t test was used to compare the values between subgroups. Crosstabs and Pearson χ^2 test were used to test the correlation between the expression levels of GRB7 and GRB7v and clinical parameters. The statistical analyses were performed using the SPSS 13.0 software (SPSS). A P value of <0.05 was considered significant.

Results

Overexpression of GRB7 and GRB7v in ovarian cancer. Our preliminary study using cDNA microarray analysis revealed that the expression levels of GRB7 in the ovarian cancer cell lines were several folds higher than that in HOSE cells (data not shown). To confirm the upregulation of GRB7 in ovarian cancer cell lines, we evaluated the expression levels of GRB7 and GRB7v by Q-PCR analysis. Interestingly, we found that not only the expressions of GRB7 were upregulated 9-fold to 40-fold in most of ovarian cancer cell lines (such as SKOV3, OVCAR3, OVCA420, etc.).
OVCA429, and OVCA433) but also the expressions of GRB7v were upregulated 5-fold to 30-fold in the above cell lines compared with average expressions in HOSE cells (Fig. 1A). We further examined the protein levels of GRB7 in the ovarian cancer cell lines by Western blotting with specific anti-GRB7 antibody. Consistent with the results of Q-PCR analysis, the expressions of GRB7 (and GRB7v) were remarkably higher in the above ovarian cancer cell lines (Fig. 1B). However, the positive band could not distinguish the expression patterns of GRB7 and GRB7v because of their small size difference.

We further evaluated the expression status of GRB7 and GRB7v in ovarian cancer tissues (n = 76) and normal ovarian tissues (n = 53) by Q-PCR analysis. Based on the comparative computed tomography method using glyceraldehyde-3-phosphate dehydrogenase as the endogenous control, very low levels of GRB7 and GRB7v were detected in normal ovaries but were significantly higher in ovarian cancers (P = 0.016; Fig. 1C). Clinicopathologic correlation showed that overexpressions of GRB7 and GRB7v were significantly associated with high-grade ovarian cancers (GRB7, P = 0.009; GRB7v, P = 0.017; Tables 1 and 2). In addition, there was a trend showing that the overexpressed GRB7 and GRB7v correlated with clear cell subtype of ovarian cancer (Tables 1 and 2). These findings suggest that both GRB7 and GRB7v are overexpressed in ovarian cancer and significantly associated with high-grade ovarian cancers.

Immunohistochemical analysis of GRB7 and GRB7v expression in ovarian cancer tissue array. To further evaluate the protein expression levels of GRB7 and GRB7v in the clinical samples, immunohistochemical analysis was performed on an ovarian cancer tissue array. The results showed that the cancer cells showed strong to marked staining of GRB7 and GRB7v (the antibody recognized both isoforms simultaneously) in 65 of 97 tumor tissues (Fig. 1D). But only weak to moderate staining was...
observed in the five cases of normal or benign tumor tissues (Fig. 1D). In addition, the staining signals were predominantly found in the cytoplasm of tumor cells (Fig. 1D). The clinicopathologic analysis showed that high-score staining (ranking >75% percentile of all cases) was significantly correlated with high-grade tumors ($P = 0.001$; Fig. 1D; Supplementary Table S1). Nevertheless, other clinical factors, such as age, histologic subtypes, and stages, showed no significant correlation with GRB7/GRB7v expressions (Supplementary Table S1). These results support that the protein levels are consistent with the mRNA levels of GRB7 and GRB7v in ovarian cancer and their upregulated expressions significantly associated with high-grade ovarian cancers.

**Both GRB7 and GRB7v localize in cytoplasm.** Accumulating evidence that GRB7 functions as a signaling adaptor has been reported (6–9). Thus, it is of interest to examine the subcellular localization of GRB7 and GRB7v to support their roles as signaling adaptors. By confocal microscopy analysis, we found that both GFP/GRB7 and GFP/GRB7v fusion proteins were predominately localized in the cytoplasm of HEK293 cells and OVCA433 ovarian cancer cells (Supplementary Fig. S1). This finding was consistent with immunohistochemical data (Fig. 1D), indicating both GRB7 and GRB7v may function as signaling adaptors in cytoplasm of ovarian cancer cells.

**GRB7, but not GRB7v, enhances cell migration and cell invasion.** To study the function of GRB7 and GRB7v in ovarian cancer cells, stable expression of GRB7 or GRB7v were established in OVCA433 cells (GFP/GRB7 expression, C7, C11, and C19; GFP/GRB7v expression, C1, C5 and C9; Fig. 2A). Recent studies have shown that GRB7 is able to promote cell migration (18, 19). Thus, we firstly examined the effects of GRB7 and GRB7v on cell migration by wound healing assay. Consistent to previous findings, OVCA433 cells with overexpression of GRB7 (C7, C11, and C19) showed significant faster wound closure compared with the vector control (Fig. 2B). Surprisingly, enforced expression of GRB7v in OVCA433 cells (C1, C5, and C9) could not show obvious difference in wound closure compared with the vector control (Fig. 2B). To better quantify the effect on cell migration, the Transwell cell migration kit was used to examine GRB7 and GRB7v stably expressing clones. Consistent with wound healing analysis, GRB7 stably expressing clones (C11 and C19) showed 2-fold to 3-fold of more cells migrating through the membrane compared with vector control and GRB7v stably expressing clones (C1 and C5; Fig. 2C). Moreover, we also examined the cell invasion ability using Transwell cell invasion kit. We found that enforced expression of GRB7 in OVCA433 cells (C11 and C19) showed ~2-fold more invaded cells through the Matrigel compared with vector control and GRB7v stably expressing clones (C1 and C5; Fig. 2D). These results show that GRB7, but not GRB7v, significantly enhances cell migration and cell invasion in ovarian cancer cells.

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![Fig. 3. Continued](image)

C. GRB7 and GRB7v regulate differential signaling targets. Western blot analysis revealed the upregulation of candidate signaling targets (phosphorylated ERK, phosphorylated JNK, and phosphorylated AKT) in the GFP/GRB7 and GFP/GRB7v OVCA433 stable clones (left) and transient transfection lysates in HEK293T cells (right).
Differential effects of GRB7 and GRB7v on cell proliferation and anchorage-independent growth ability. Apart from cell migration and invasion, higher cell growth rate and gain of anchorage-independent growth ability are another two important tumorigenic behaviors found in high-grade tumors (20). We firstly evaluated the effects of GRB7 and GRB7v on cell proliferation by XTT cell viability assay (21). OVCA433 cells with enforced expression of GRB7v (C1, C5, C9) showed significantly increased cell proliferation rate compared with the control using DMSO. The relative migration velocities of three independent assays were plotted. C, SP600125 neutralized the function of GRB7 on cell migration. Left, the wound healing analysis showed the vector control (V) and GFP/GRB7 stable clones (C11 and C19) closed the wounds at similar rates under treatment. The relative migration velocities were plotted. Right, Transwell cell migration analysis showed that the migrated cells of GFP/GRB7 stable clone (C11) significantly reduced compared with DMSO control (*P = 0.026). D, XTT cell proliferation assay showed that, upon U0126 treatment, the increased cell proliferation rate found in GFP/GRB7 (C11 and C19) and GFP/GRB7v (C5 and C9) stable clones was totally abrogated.

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and C9) exhibited 60% to 110% higher cell proliferation rate than the vector control (Fig. 3A). On the other hand, overexpression of GRB7 in OVCA433 (C7, C11, and C19) could only increase 20% to 60% cell proliferation rate compared with vector control (Fig. 3A). We next used soft agar assay to study the effects of enforced expression of GRB7 and GRB7v in cell anchorage-independent growth ability. Interestingly, we found that only the enforced expression of GRB7v was able to increase number (2-fold to 3-fold; P < 0.01) and size of colonies compared with the vector control (Fig. 3B). These data indicate that GRB7v has higher oncogenic effect than GRB7 in promoting cell proliferation and cell anchorage-independent growth ability in ovarian cancer cells.

**GRB7 and GRB7v regulate differential signaling targets.**
Recent report has documented that GRB7 is able to upregulate the levels of phosphorylated ERK and phosphorylated AKT (19), suggesting that GRB7 is a crucial regulator in AKT/mitogen-activated protein kinase (MAPK) signaling axis. In this study, we aimed to investigate whether the different functions of GRB7 and GRB7v in affecting cell migration/invasion, cell proliferation, and anchorage-independent growth ability are due to their differential effects on different signaling pathways in ovarian cancer cells. By Western blot analysis, the levels of phosphorylated ERK, phosphorylated JNK, and phosphorylated AKT were found to be elevated in either GRB7 stably expressing clones of OVCA433 cells (C7, C11, and C19) or HEK293T cells transiently transfected with GRB7 (Fig. 3C). In contrast, only the levels of phosphorylated ERK were elevated in both transient transfection and stable expression of GRB7v (C1, C5, and C9; Fig. 3C). In addition, there was no change of phosphorylated P38 expression levels in OVCA433 cells with enforced expression of either GRB7 or GRB7v (data not shown). These data confer that GRB7 and GRB7v regulate different downstream targets in AKT/MAPK signaling axis.

**GRB7 and GRB7v exhibit different tumorigenic effect on ovarian cancer cells via AKT/MAPK signaling pathway.** To further investigate the differential roles of GRB7 and GRB7v in regulating downstream targets of AKT/MAPK signaling pathways related to the tumorigenic behaviors, we used specific kinase inhibitors targeting ERK (U0126), JNK (SP600125), and AKT (Wortmannin). We firstly evaluated the optimal dose of each protein kinase inhibitor in suppressing its target. We found that U0126 (10 μmol/L for 4 hours) was able to reduce the level of phosphorylated ERK at ~50%, SP600125 (10 μmol/L for 4 hours) was able to reduce the level of phosphorylated JNK at ~60%, and Wortmannin (0.1 μmol/L for 4 hours) was able to reduce the level of phosphorylated AKT at ~80% in GRB7 stable clones (C11) of OVCA433 cells (Fig. 4A). Moreover, inhibition of phosphorylated JNK and phosphorylated AKT by SP600125 and Wortmannin, respectively, reduced the cell motility rates of OVCA433 cells (Fig. 4B). Between these two protein kinase inhibitors, only SP600125 exhibited significant inhibitory effect on cell motility (Fig. 4B). Interestingly, under the treatment of SP600125, we found that the increased cell migration ability from enforced expression of GRB7 was significantly abrogated in GRB7 stably expressing clones (C11 and C19; Fig. 4C). Moreover, by Transwell cell migration assay, we further confirmed that the enhanced cell migration ability in GRB7 stable clone (C11) was significantly reduced upon treatment of the JNK inhibitor SP600125 (Fig. 4C). On the other hand, under continuous treatment of the ERK inhibitor U0126, we found that the increased cell proliferation rates in GRB7 (C11 and C19) and GRB7v (C5 and C9) stable clones were remarkably reduced (Fig. 4D). Taken together, these results suggest that the ERK signaling is important for both GRB7-mediated and GRB7v-mediated cell proliferation enhancement, whereas the JNK signaling is the crucial signaling pathway regulated by GRB7 in promoting cell migration.

**Discussion**
GRB7 has been known as an adaptor molecule mediating signal transduction from cell surface receptors to various downstream signaling pathways through interaction with receptor tyrosine kinases, nonreceptor tyrosine kinases, and many adaptor molecules (6–9). Emerging evidence has documented that GRB7 is frequently upregulated in a variety of human cancers and plays an essential role in tumor progression (11–16). Consistent with these studies, we have also found that GRB7 was overexpressed in ovarian cancer cell lines and clinical samples. Intriguingly, its isoform GRB7v also showed an accordant expression pattern. More importantly, both overexpressed GRB7 and GRB7v were significantly correlated with high-grade ovarian cancers. This indicates that GRB7 and GRB7v may have tumorigenic roles in ovarian cancer progression. The high-grade ovarian cancers are undifferentiated and poor in prognosis. These cancers have high-grade nuclei and numerous mitotic figures (22, 23). The progression to invasive carcinoma is a fast and stepwise process (22, 23). This indicates that these high-grade ovarian cancers tend to have higher cell proliferation and metastatic behaviors. The upregulation of GRB7 and GRB7v may thus involve these tumorigenic properties. Indeed, our functional studies using ovarian cancer cell models have shown that the upregulation of GRB7 mainly promotes cell migration/invasion and slightly in cell proliferation whereas GRB7v significantly increases cell proliferation and anchorage-independent growth ability. It is not surprising that GRB7 affects cell migration/invasion because GRB7 is frequently overexpressed in invasive and metastatic cell lines and cancer types (10, 14, 19, 24, 25). It has been shown that the interaction of the SH2 domain of GRB7 to phosphorytrosine 14 (pY14) on caveolin-1 or EphB1 promotes cell migration (26, 27). Conversely, the expression of GRB7 with mutated SH2 domain or a cyclic peptide inhibitor to SH2 domain remarkably reduces cell migration (18, 28), suggesting that the presence of SH2 domain in GRB7 is crucial for its biological functions, e.g., cell migration regulation. On the other hand, the absence of COOH terminal SH2...
domain in GRB7v indicates it is incapable of binding and phosphorylation by upstream kinase protein to exhibit any biological functions similar to GRB7. Indeed, our results showed that GRB7v promotes cell proliferation and anchorage-independent growth ability instead of cell migration/invasion, implying that GRB7v may be activated and regulated by distinct phosphorylation sites and subsequently regulating downstream targets different from GRB7.

A recent study has proposed that ERK and AKT are the putative downstream signaling targets because their phosphorylation levels are elevated, accompanied with GRB7 overexpression (19). We thus examined the expressions of the main signaling molecules in MAPK and AKT pathways. In this study, we have found that the levels of phosphorylated JNK, phosphorylated ERK, and phosphorylated AKT were elevated by GRB7 enforced expression. On the other hand, overexpression of GRB7v just increased the level of phosphorylated ERK. To couple these increased signaling activities and biological functions, we applied specific kinase inhibitors to the GRB7- or GRB7v-overexpressing cell lines. As expected, the increased cell proliferation due to the upregulation of either GRB7 or GRB7v was totally inhibited by the ERK inhibitor U0126. In addition, the enhanced cell migration/invasion ability due to GRB7 expression was also abrogated by the JNK inhibitor SP600125. Our findings are consistent with previous studies that the increased phosphorylated ERK promotes cell proliferation and the elevated phosphorylated JNK enhances cell migration (29–33). These results further confer the molecular basis of differential functions between GRB7 and GRB7v.

Although there is a compelling evidence that the interaction with upstream tyrosine kinases is mediated by the SH2 domain to relay the signal to downstream targets (18, 28). The adjacent BPS, RA, and PH domains have also been shown to interact with the insulin and insulin-like growth factor-I, RasGTPase effectors, and specific phosphoinositides (27, 34, 35). All of these possibilities may explain that GRB7v, although in the absence of SH2 domain, may still regulate downstream targets different from GRB7. However, further study for the identification of GRB7v interaction partners and crucial phosphorylation sites in each domain is needed.

In conclusion, our study implicates that the overexpressed GRB7 and GRB7v both associate with high-grade tumors but exert distinct tumorigenic functions through regulating different signaling pathways in ovarian cancer cells. Further understanding of their distinct functional roles and molecular mechanisms of GRB7 and GRB7v may facilitate the exploration of these proteins as novel therapeutic targets in ovarian cancers.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Correction: Differential Functions of Growth Factor Receptor–Bound Protein 7 (GRB7) and Its Variant GRB7v in Ovarian Carcinogenesis

In this article (Clin Cancer Res 2010;16:2529–39), which was published in the May 1, 2010 issue of Clinical Cancer Research (1), a reader pointed out potential image reuse. The authors admitted that due to an oversight that occurred during figure preparation, identical β-actin blots were erroneously used in Fig. 2A (GRB7v; OVCA433) and Fig. 3C (transient transfection GFP/GRB7). The authors provided the original blots, which showed the correct β-actin blots for each figure. The corrected Fig. 3C appears below. The results and conclusions put forth in this article remain unchanged. The authors regret these errors.

Reference

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Differential Functions of *Growth Factor Receptor–Bound Protein 7 (GRB7)* and Its Variant *GRB7v* in Ovarian Carcinogenesis

Yajun Wang, David W. Chan, Vincent W.S. Liu, et al.


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