GRB-7 facilitates HER-2/Neu-mediated signal transduction and tumor formation

Tao Bai and Shiu-Wen Luoh*

Division of Hematology and Medical Oncology, Oregon Health Sciences University, Portland VA Medical Center, Portland, OR 97239, USA

*To whom correspondence should be addressed.
Fax: +1 503 402 2817.
Email: luohs@ohsu.edu

Growth factor receptor-bound protein-7 (GRB-7), an adaptor molecule, can interact with multiple signal transduction molecules. GRB-7 is amplified concurrently with HER-2/Neu in most, if not all, of breast cancer with chromosome 17q11–21 amplification. GRB-7 gene amplification is associated with RNA overexpression. We show GRB-7 protein is over-expressed by immunoblotting in breast cancer cell lines and primary breast tumors with HER-2/Neu protein overexpression. Over-expression of GRB-7 in MCF-7 breast cancer cells that over-express HER-2/Neu leads to activation of tyrosine phosphorylation of HER-2/Neu. Knockdown of GRB-7 expression in SKBR-3 breast cancer cells with normally occurring HER-2/Neu gene amplification decreases tyrosine phosphorylation of HER-2/Neu. Activation of HER-2/Neu phosphorylation is associated with increase in tyrosine phosphorylation of phosphoinositide-specific lipase C-γ-1 (PLC-γ-1) and recruitment of PLC-γ-1 to HER-2/Neu protein molecule. Activation of downstream protein kinase C (PKC) pathway is evidenced by increase in the phosphorylation of a common PKC substrate—myristoylated alanine-rich protein kinase C substrate (MARCKS). In addition, over-expression of GRB-7 in MCF-7 breast cancer cells that over-express HER-2/Neu leads to activation of AKT phosphorylation. Knockdown of GRB-7 expression in MB-453 and SKBR-3 breast cancer cells results in decrease in AKT phosphorylation. GRB-7 over-expression therefore facilitates activation of phosphorylation of HER-2/Neu and AKT in breast cancer cells with HER-2/Neu over-expression. GRB-7 over-expression in MCF-7 cells over-expressing HER-2/Neu leads to morphologic change of cells and promotes tumor xenograft growth in nude mice. GRB-7 over-expression therefore plays pivotal roles in activating signal transduction and promoting tumor growth in breast cancer cells with chromosome 17q11–21 amplification.

Introduction

Growth factor receptor-bound protein-7 (GRB-7) is an adaptor molecule that mediates signal transduction from multiple cell surface receptors to various downstream signaling pathways (1). GRB-7 has several functional domains including a proline rich domain at its N-terminus, a PH domain and a SH2 domain at its C-terminus. GRB-7 can interact with >20 adaptor molecules, non-receptor tyrosine kinases and receptor tyrosine kinases including HER-1 and HER-2 (2). GRB-7 has been implicated in important cellular and physiological functions such as signal transduction, cell motility and tumor progression. GRB-7 is a member of the GRB-7/10/14 family. GRB-10 and GRB-14 have been implicated in modulation of receptor tyrosine kinase activity (3–5).

Human GRB-7 gene is located at chromosome 17q11–21 in the immediate vicinity of the HER-2/Neu gene. GRB-7 is part of the core of the HER-2/Neu amplicon and is co-amplified with HER-2/Neu in most, if not all, of the breast tumor with 17q11–21 amplification (6–8). Transcript analysis shows GRB-7 RNA is over-expressed concurrently with HER-2/Neu in breast cancer cells with chromosome 17q11–21 amplification (9). The expression pattern and signaling capabilities of GRB-7 strongly suggest its functional involvement in the 25% of breast cancer cells that carry this genetic alteration. Breast cancer with chromosome 17q11–21 amplification is a more virulent disease (10,11). Earlier study on breast cancer with chromosome 17q11–21 amplification focused almost exclusively on the signaling function of HER-2/Neu (12,13). The functional involvement of GRB-7 signaling in breast cancer and, in particular, in the context of HER-2/Neu amplification has not been examined previously.

In this study, we show that GRB-7 over-expression facilitates the activation of phosphorylation of both AKT and HER-2/Neu in a cellular context with HER-2/Neu protein over-expression. In addition, GRB-7 over-expression promotes formation of tumor xenograft by HER-2/Neu-expressing cells in nude mice. Our study indicates that GRB-7 promotes HER-2/Neu-mediated signaling and tumor formation. GRB-7 protein over-expression therefore contributes significantly to the biological behaviors conferred by 17q11–21 amplification in breast cancer and may serve as a rational target for therapy.

Materials and methods

Cell culture and reagents

Human breast cancer cell lines BT-474, MCF-7, ZR75-1, ZR-75–15, BT-20, SKBR-3, MB-453, MB-361, MB-231, MB-175, T-47D, HCC-1954 were obtained from American Type Culture Collection (Manassas, VA). MCF-7::HER-2 was MCF-7 cell that over-expressed HER-2/Neu as described previously (12). Human breast cancer cell lines were maintained in RPMI (Invitrogen, Grand Island, NY) supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 µg/ml streptomycin at 37°C in a humidified atmosphere containing 5% CO₂. MCF-7::HER-2 cell line was maintained in growth medium containing 400 µg/ml geneticin (active form) (Invitrogen).

Primary breast tumor tissue

Snap-frozen breast tumor tissues were purchased from National Disease Research Interchange or obtained from Tissue Procurement at Oregon Health Sciences University. These were all primary breast tumor tissues whose pathology was verified. Tumor specimen were anonymized at the sites of origin and obtained according to protocol approved by local institute review board.

Immunoblotting and immunoprecipitation

Cell lines were washed with ice cold phosphate-buffered saline once and resuspended on ice for 5-10 minutes with 20 mM Tris–HCl (pH 7.5), 150 mM NaCl, 1 mM Na₂EGTA, 1% Triton, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride, 1× Protease Inhibitor Cocktail Set I (EMD, La Jolla, CA) and 1× Phosphatase Inhibitor Cocktail Set III (EMD). Cell lysates were cleared with centrifugation. Aliquots of each cell lysates containing 40 µg of protein were mixed with Laemmli lysis buffer, boiled for 5 min, chilled on ice, separated by 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane by electro-blotting (Trans-Blot) (Bio-Rad, Hercules, CA). After blocking, immunoblotting was performed with different antibody reagents at appropriate dilution according to the manufacturer’s recommendation. This was followed by incubation with appropriate horseradish peroxidase conjugated-secondary antibody. Bands were analyzed using an enhanced chemiluminescence protocol (LumiGLO) (Cell Signaling Technology, Beverly, MA) and visualized on radiographic films (Eastman Kodak, Rochester, NY). For immunoprecipitation, cleared cell lysates containing 0.5–1 mg of protein were incubated with primary antibody with gentle rocking overnight. Protein-A/G plus conjugated agarose beads (EMD) (20 µl of 50% bead slurry) were added followed by gentle rocking for 1 h at 4°C. Pellets were collected with centrifugation and washed for at least three times with 1× lysis buffer at 4°C. Pellets were resuspended in Laemmli lysis buffer, boiled and loaded onto sodium dodecyl sulfate–polyacrylamide gel electrophoresis gels followed by western analysis. Each western blotting and immunoprecipitation experiment was repeated at least three times with identical results. One representative result was presented here.

Antibody reagents

Antibody sources were as follows: phospho-tyrosine (PY-100), HER-2/Neu, FAK, SRC, total AKT, PLC-γ-1, ERK-1/2, Phospho-AKT (Ser 473), Phospho-AKT (Ser 308), Phospho-PLC-γ-1 (Tyr783), Phospho-MARCKS (Ser152/156),

Abbreviations: GRB-7, growth factor receptor-bound protein-7; MARCKS, myristoylated alanine-rich protein kinase C substrate; siRNA, small interfering RNA.
Phospho-HER-2 (Tyr1248), Phospho-HER-2 (Tyr877) were from Cell Signaling Technology. Tubulin and phospho-tyrosine (PY-20) antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA). MARCKS (myristoylated alanine-rich protein kinase C substrate) antibody was from Proteintech (Chicago, IL). Anti-HER-2/Neu antibody used for immunoprecipitation was directed against the extracellular domain of HER-2/Neu (AB-5) (EMD). In the case of immunoprecipitation with general anti-phospho-tyrosine antibody, PY-20 antibody conjugated with agarose was used directly (Santa Cruz Biotechnology). Anti-rabbit and anti-mouse IgG, horseradish peroxidase-conjugated secondary antibodies were obtained from Cell Signaling Technology.

Generation of anti-GRB-7 antibody

The sequence of the synthetic peptide used as immunogen was the following: N’ - CTTRGRK LRE EER RAT S’ - C’. The segment was from a portion of GRB-7 that was divergent from GRB-10 and GRB-14. Anti-GRB-7 antisera was therefore not expected to show cross-reactivity against GRB-10 or GRB-14. The titer of polyclonal rabbit antisera was monitored serially by enzyme-linked immunosorbent assay after each boosting. Anti-GRB-7 antisera was affinity purified with affinity chromatography conjugated with peptide immunogen. The specificity of this antisera was verified as it detected wild-type GRB-7 protein and a fusion protein of GRB-7 and Green Fluorescence Protein (GFP) in transfectants but not in non-transfectants (not shown).

Plasmid construction and transfection

Full-length human GRB-7 cDNA from Image clone # 1440 (GenBank accession number no. BC006535) was released as an EcoRI and Sall fragment and inserted in the EcoRI and XhoI sites of pCMV-IRE2EGFP bi-cistronic expression vector (Clontech, Mountain View, CA). EGF-C3 vector (Clontech) was inserted in the EcoRI and XhoI sites of pCMV-IRES2EGFP bi-cistronic expression vector (Clontech, Mountain View, CA). IRES-GFP was verified by western analysis (Figure 2A).

GRB-7 protein over-expression in primary breast tumors and breast cancer cell lines

We examined the expression of GRB-7 protein in primary breast tumor samples and breast cancer cell lines. We first generated a GRB-7 specific, affinity purified, anti-peptide antibody. The peptide sequence chosen was from a region of GRB-7 that showed significant sequence divergence from that of GRB-10 and GRB-14, avoiding cross-reactivity. This antibody specifically recognizes a protein of expected molecular weight in MCF-7::HER-2/Neu cells that expressed GRB-7 or GRB-7 and GFP fusion protein, respectively, but none in parental cell line (see below and not shown).

Western blotting analysis of breast cancer cell lines showed that GRB-7 over-expression was present in breast cancer cell lines that had HER-2/Neu protein over-expression and were known to carry HER-2/Neu gene amplification. No GRB-7 over-expression was noted in breast cancer cell lines without HER-2/Neu protein over-expression or HER-2/Neu gene amplification (Figure 1A). Analysis of anonymized, primary breast tumors with western blotting showed that GRB-7 expression was present in tumor samples that had high levels of HER-2/Neu expression. GRB-7 expression was absent in primary breast tumors with low or no HER-2/Neu protein expression (Figure 1B).

Earlier studies showed concurrent amplification and over-expression of HER-2/Neu and GRB-7 in breast cancer cell lines based on DNA and RNA analysis (15,9,14). Our study showed concordant GRB-7 protein over-expression in breast cancer cells with HER-2/Neu gene amplification, consistent with results from an earlier report (2).

Over-expression of GRB-7 in MCF-7::HER-2 cells

To study the functional significance of GRB-7 over-expression in breast cancer cells, we stably over-expressed GRB-7 in MCF-7::HER-2 cells. MCF-7::HER-2 cells are MCF-7 cells that were stably transfected to over-express wild-type human HER-2/Neu gene (12). We first made a bi-cistronic expression vector with GRB-7 positioned upstream of GFP in a pCMV-IRE2-EGFP vector (Clontech). MCF-7::HER-2 cells were transfected with pCMV-GRB-7-ires-GFP or a control pCMV-EGFP vector (Clontech). Polyclonal stable transfectants were obtained through FACS based on GFP expression. GRB-7 expression in MCF-7::HER-2 cells transfected with pCMV-GRB-7-ires-GFP was verified by western analysis (Figure 2A).
GRB-7 facilitates HER-2 Neu-mediated signal transduction and tumor formation

protein from MCF-7::HER-2 cells that over-expressed GRB-7 plus GFP and those that expressed GFP alone, respectively. The immunoprecipitates were subject to western analysis. As shown in Figure 3A, the tyrosine phosphorylation of HER-2/Neu and more specifically the tyrosine phosphorylation of HER-2/Neu at tyrosine 877 (Y877) and tyrosine 1248 (Y1248) positions were activated with GRB-7 over-expression.

Next, we immunoprecipitated tyrosine phosphorylated protein from MCF-7::HER-2 cells that over-expressed GRB-7 plus GFP and those that expressed GFP alone with PY-20 antibody. The immunoprecipitates were subject to western analysis. Tyrosine phosphorylation of HER-2/Neu and PLC-γ-1 was increased with GRB-7 over-expression. On the contrary, tyrosine phosphorylation of Src, Shc and FAK was not significantly altered with GRB-7 over-expression (Figure 3B).

Knockdown of GRB-7 expression in SKBR-3 cells attenuates HER-2 Neu phosphorylation

SKBR-3 is a breast cancer cell line that carries chromosome 17q11–21 amplification. Both HER-2/Neu and GRB-7 proteins are over-expressed in SKBR-3 (Figure 1A). Knockdown of GRB-7 expression was achieved with siRNA transfection. Transfection with a non-targeting control siRNA was used as a negative control. Western blotting analysis of cell lysates from SKBR-3 cells transfected with a GRB-7-targeting siRNA or a non-targeting control siRNA was performed. Tyrosine phosphorylation of HER-2/Neu at tyrosine 1248 position (Y1248), but not the total amount of HER-2/Neu, was decreased with GRB-7 knockdown (Figure 3C).

In confirmation of this study, we immunoprecipitated HER-2/Neu protein from SKBR-3 cells that were transfected with siRNA targeting GRB-7 or non-targeting siRNA control. The immunoprecipitates were subject to western analysis. We found tyrosine phosphorylation of HER-2/Neu at tyrosine 1248 position (Y1248) was decreased with GRB-7 knockdown (Figure 3D). Decrease in GRB-7 protein expression as a result of siRNA transfection was verified with western analysis of input cell lysates (Figure 3D). Tyrosine phosphorylation of HER-2/Neu, either total or at Y1248, was present at a lower level in MB-453 cells. No reduction in tyrosine phosphorylation at Y1248 could be convincingly demonstrated with GRB-7 knockdown in MB-453 cells (not shown).

Taken together, the above clearly showed that GRB-7 protein over-expression was both sufficient and necessary for activation of tyrosine phosphorylation of HER-2/Neu.

GRB-7 activates PLC-γ-1

PLC-γ-1 is an important signaling molecule downstream of HER-2 Neu. Earlier study showed tyrosine phosphorylation of HER-2 Neu at tyrosine 1248 (Y1248) was coupled with PLC-γ-1 activation (16–19). We studied the effect of GRB-7 over-expression on the phosphorylation status of PLC-γ-1. In addition, we examined the phosphorylation status of myristoylated alanine-rich protein kinase C substrate (MARCKS), a widely distributed protein kinase C substrate (20). Western blotting analysis demonstrated that phosphorylation of PLC-γ-1 and MARCKS was both increased in MCF-7::HER-2 transfectant that over-expressed GRB-7 (Figure 4A). When we brought down HER-2/Neu protein molecule with immunoprecipitation, we found increase in association of PLC-γ-1 to HER-2/Neu in GRB-7 transfectants (Figure 4B). The above experiments demonstrated increase in both phosphorylation of PLC-γ-1 and recruitment of PLC-γ-1 to HER-2/Neu with GRB-7 over-expression. In addition, activation of PLC-γ-1 was associated with activation of downstream PKC pathway as evidenced by the increase in MARCKS phosphorylation.

GRB-7 activates AKT pathway

Western blotting analysis was performed to examine the AKT activation status as the result of GRB-7 over-expression. We showed in Figure 5A that AKT phosphorylation at serine 473 position (S473) and threonine 308 position (T308) was increased in MCF-7::HER-2

In our subsequent studies, we compared the signaling and biological behavior of MCF-7::HER-2 cells that over-expressed GRB-7 and GFP with those that expressed GFP alone to study the contribution of GRB-7.

There was morphological difference between MCF-7::HER-2 cells that over-expressed GRB-7 plus GFP and those that expressed GFP alone. MCF-7::HER-2 cells with GRB-7 over-expression appeared to be more transformed. Instead of being flat, these cells had shiny margins, were more spindle shaped and piled on top of one another (Figure 2B).

**GRB-7 activates tyrosine phosphorylation of HER-2 Neu**

We examined the status of tyrosine phosphorylation of HER-2 Neu upon GRB-7 over-expression. Western blotting analysis of cell lysates isolated from MCF-7::HER-2 cells that over-expressed GRB-7 plus GFP and those that expressed GFP alone were performed with pan anti-phospho-tyrosine specific antibodies (PY-20 and PY-100) and anti-p-HER-2 Neu (Y1248) antibody. A 185 kD signal, expected of HER-2/Neu phosphorylation, was present in much increased intensity in GRB-7 over-expressing cells relative to that in GFP-expressing control (not shown).

To verify that tyrosine phosphorylation of HER-2 Neu was activated by GRB-7, we performed the following immunoprecipitation and western blotting analysis: We first immunoprecipitated HER-2 Neu
cells that over-expressed GRB-7. In support of this observation, we knocked down GRB-7 protein expression with transfection of siRNA in breast cancer cell lines with naturally occurring HER-2/Neu gene amplification. In both SKBR-3 and MB-453 cells, knockdown of GRB-7 protein expression was associated with decrease of AKT phosphorylation at serine 473 (S473) and threonine 308 (T308) positions (Figure 5B). The results demonstrated that GRB-7 over-expression played important roles in activation of AKT pathway in the context of HER-2/Neu protein over-expression.

**Fig. 3.** Activation of tyrosine phosphorylation of HER-2/Neu by GRB-7 over-expression. MCF-7::HER-2 cells over-expressing GFP only or both GFP and GRB-7 were subject to immunoprecipitation with anti-HER-2/Neu antibody (A) or anti-P-tyrosine antibody (PY-20) (B). The immunoprecipitates were then probed with various antibodies in western analyses. (C) SKBR-3 cells were transiently transfected with siRNA that targeted GRB-7 or control siRNA. Knockdown of GRB-7 protein expression was verified with western analysis. Tyrosine phosphorylation status of HER-2/Neu was analyzed with western blotting using anti-HER-2/Neu and anti-p-HER-2/Neu (Y1248) antibodies. (D) SKBR-3 cells were transiently transfected with siRNA that targeted GRB-7 or control siRNA. Knockdown of GRB-7 protein expression in cell lysates were verified with immunoblotting. These cell lysates were subject to immunoprecipitation with anti-HER-2/Neu antibody followed by western blotting with anti-HER-2/Neu and anti-p-HER-2/Neu (Y1248) antibodies.

**GRB-7 facilitates HER-2/Neu-mediated tumor xenograft growth in nude mice**

We examined the biological outcome of GRB-7 over-expression in MCF-7::HER-2 cells. We did not see difference in proliferation or anchorage-independent growth in tissue culture in either serum replete or serum starved and growth factor-depleted conditions (not shown). We then examined the ability of MCF-7::HER-2 transfectants to form tumor xenograft in estrogen primed and ovariectomized female nude mice. Four million transfectants were injected subcutaneously to the left (or right) flank per animal. MCF-7::HER-2 cells that expressed GFP alone formed small tumors, if at all. In contrast, MCF-7::HER-2 cells that expressed both GRB-7 and GFP formed larger tumors readily and the difference was statistically significant (P = 0.016) (Figure 6). Our study therefore showed that GRB-7 could facilitate HER-2/Neu-mediated signaling and xenograft tumor growth in nude mice. Our work demonstrated that GRB-7 played a pivotal role in the signaling and tumor growth in breast cancer cells with chromosome 17q11–21 amplification.
Discussion

Gene amplification is a common occurrence in solid tumor. It is associated with disease progression, adverse prognosis or development of drug resistance (21). Selective retention and over-expression of amplified sequences in cancer cells suggest that evolutionary advantage is conferred by the amplified and over-expressed genes. In the case of amplification of chromosome 17q11–21 in breast cancer, a 280 kb segment was identified as the core of the amplicon (6–8). Multiple genes including HER-2/Neu reside in this region. The transforming function of HER-2/Neu has been described previously (22). We hypothesize that GRB-7 may play important roles in breast cancer with 17q11–21 amplification for the following reasons: (i) GRB-7 protein is over-expressed concurrently with HER-2/Neu; (ii) GRB-7 is an adaptor molecule capable of interacting with multiple signal transduction molecules and (iii) GRB-7 has been previously implicated in functions relevant to cancer biology. We show in this study that GRB-7 over-expression is both sufficient and necessary for activation of HER-2/Neu and AKT phosphorylation. In addition, GRB-7 promotes the growth of tumor xenograft formed by HER-2/Neu-expressing cells in nude mice. Our work shows that GRB-7 has important functional involvement in the development and/or progression of breast cancer.

GRB-7 is a member of the GRB-7/-10/-14 family (1). Members of this family have been shown to interact with receptor tyrosine kinases, non-receptor tyrosine kinases and a variety of signaling molecules (3–5). GRB-10 and -14 can interact with insulin receptor and insulin growth factor-1 receptor, respectively (23–25). GRB-7 can interact with Ephrin receptor (EphB1) when the latter is autophosphorylated (26). GRB-7 can associate with HER-2/Neu but the functional significance of this interaction has not been elucidated (2). Activation of HER-2/Neu phosphorylation as a result of GRB-7 over-expression, for example, has not been described previously. An earlier study found that GRB-7, probably through its SH2 domain, could interact preferentially with phosphorylated form of HER-2/Neu (2). The mechanism of activation of HER-2/Neu phosphorylation by GRB-7 remains to be elucidated. One earlier study showed GRB-10 could bind to and activate AKT by tethering the latter to the cell membrane (27). The ability of GRB-7 to activate AKT phosphorylation has not been reported. Our observation that GRB-7 activates phosphorylation of AKT and HER-2/Neu is novel. As the phosphorylation status of HER-2/Neu and AKT reflects the activity of the signaling cascades directed by these two molecules, our study indicates that GRB-7 has important signaling functions in breast cancer with 17q11–21 amplification. Indeed, the increase in HER-2/Neu phosphorylation is associated with activation of downstream PLC-γ-1 and PKC pathway.
GRB-7 has been implicated in cell motility through its association with focal adhesion kinase, phosphoinositides, ephrin receptor (EphB1) and calmodulin (26,28–30). GRB-7 therefore likely plays a role in tumor progression. GRB-7 has been shown to be an adverse prognostic factor in esophageal cancer (31). In chronic lymphocytic leukemia, the level of GRB-7 RNA expression is significantly higher in stage IV than in stage I disease (32). Amplification of chromosome 17q11–21 has been observed in germ cell tumor. Expression analysis shows GRB-7 rather than HER-2/Neu is over-expressed in germ cell tumor with chromosome 17q11–21 amplification (33). Selective retention and preferential expression of GRB-7 indicate its functional involvement in germ cell tumorigenesis that is independent of HER-2/Neu. Recently, a ‘knock-in’ mouse model has been created with a HER-2/Neu gene carrying an activating mutation inserted into the wild-type HER-2/Neu locus in the mouse genome (34). These mice develop mammary tumors at high frequency. Analysis of the resulting tumors reveals amplification of the genomic segment around HER-2/Neu gene in much the same way as human HER-2/Neu gene amplification event. GRB-7 is part of the amplified sequence (34) which strongly supports that GRB-7 is important to the biology of these tumors.

Breast cancer with chromosome 17q11–21 amplification is a more virulent disease (10). Current HER-2/Neu-targeted monoclonal antibody therapy induces response in 50% of these patients when they present with advanced disease (35). For those patients with early-stage breast cancer, ~40% reduction in the risk of recurrence has been noted (36, 37). Additional effective therapies are urgently needed to battle this more aggressive form of breast cancer. Our work reveals the important contribution of GRB-7 in the signaling of breast cancer with 17q11–21 amplification and establishes GRB-7 as a rational target for therapy (38).

Over-expression of GRB-7 in MCF-7::HER-2 cells lead to increase in the growth of tumor xenograft in nude mice. Morphological changes were noted in these cells that over-expressed both HER-2/Neu and GRB-7. We did not observe difference in proliferation or anchorage-independent growth either in serum replete or growth factor deplete conditions (not shown). An earlier study, however, showed knockdown of GRB-7 expression in breast cancer cells with naturally occurring HER-2/Neu amplification lead to a decrease in cell proliferation (39).

Mapping studies pinpointed a small minimal common region (280 kb) of amplification at the HER-2/Neu locus in breast cancer with 17q11–21 amplification (6–8). A number of genes from this region, including GRB-7, were demonstrated to have consistently elevated expression when amplified (9). It was postulated that coordinated effects of more than one over-expressed genes provided a growth advantage for cancer cells. Our study provides the first detailed analysis to show that GRB-7 indeed plays an important role in facilitating the signaling and tumor growth mediated by HER-2/Neu.

In conclusion, our study shows GRB-7 protein over-expression is present in breast cancer cells with HER-2/Neu over-expression. GRB-7 over-expression facilitates activation of HER-2/Neu and AKT phosphorylation. Activation of HER-2/Neu is associated with activation of downstream PLC-γ–PKC pathways. GRB-7 over-expression promotes the growth of xenograft tumor formed by HER-2/Neu-expressing cells in nude mice. GRB-7 contributes to HER-2/Neu-mediated signal transduction and tumor growth in breast cancer cells and may serve as a novel target for therapy.

Funding

Biostatistics Shared Resource of the Oregon Health Sciences University Cancer Institute (P30CA069533); VA Merit Review Award.

Acknowledgements

We thank Drs Shannon McWeeny, X.-J.Wang, SuEllen Pommier, Craig Okada and Michael Deininger for careful review of the manuscript.

Conflict of Interest Statement: None declared.

References

ETP-7 facilitates HER-2/Neu-mediated signal transduction and tumor formation

16. Peles,E. et al. (1991) Oncogenic forms of the neu/HER2 tyrosine kinase are permanently coupled to phospholipase C gamma. EMBO J., 10, 2077–86.

Received July 13, 2007; revised September 17, 2007; accepted September 21, 2007