Downregulation of HER2 by RIG1 involves the PI3K/Akt pathway in ovarian cancer cells

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Interferon-γ (IFN-γ) is known to downregulate HER2 oncoprotein (p185HER2 or briefly p185) in prostate cancer cells. We demonstrate that the IFN-γ-induced retinoid-inducible gene 1 (RIG1) acts as a transrepressor of p185. Furthermore, we exhibit that RIG1 downregulates the activated (phosphorylated) form of p185 and phosphoinositide-3 kinase (PI3K)/serine/threonine-specific protein kinase (Akt) and downstream substrates of HER2. We also elucidate that heregulin (HRG) specifically restores the activation of p185 and Akt after their activities are reduced by RIG1. Additionally, expression of vascular endothelial growth factor (VEGF) increases through the HER2- and Akt/mTOR-signaling pathways, indicating that VEGF is downregulated by RIG1 within the cell. These findings suggest that RIG1 plays a role in IFN-γ-mediated therapy by downregulating p185 and its downstream PI3K/Akt/mTOR/VEGF-signaling pathway. These results may provide a new therapeutic mechanism for the clinical use of IFN-γ and RIG1.

Introduction

Interferon-γ (IFN-γ), one of three IFN cytokines, inhibited the proliferation and regulation of oncogenes and tumor suppressor genes (1,2). Receptor-bound IFN-γ induces Janus kinases 1 and 2 phosphorylation and activates signal transducers and activators of transcription 1 (STAT1), resulting in translocation to the nucleus and the turning on of downstream genes. Although many actions of IFN-γ have been studied, the underlying mechanisms are still not well known.

Retinoid-inducible gene 1 (RIG1), also known as tazarotene-induced gene 3 (TIG3) or retinoic acid receptor responder 3 (RARRES3), belongs to the class II tumor suppressor gene HREV107 family and is one of the IFN-γ downstream genes (3–5). RIG1 is expressed in high levels in well-differentiated skin and colon mucosal tissues but at low levels in cancer tissues derived from the skin, liver, colorectum and biliary tract (6,7). Overexpression of RIG1 suppresses colony formation in T47D breast cancer cells and HacαT keratinocytes. Ectopic RIG1 expression leads to cellular apoptosis and suppression of growth in cancer cells and human keratinocytes (3,8–10). RIG1 also facilitates the terminal stages in keratinocyte differentiation by activating type I transglutaminase (11). Previous studies have demonstrated that RIG1 decreases protein levels and activities of Ras, one of the HER2 downstream proteins, while interacting with Ras protein in the cytoplasmic compartment in HT29 cervical cancer cells (9,12). However, the molecular mechanisms responsible for the effects of RIG1 remain unclear.

The HER2 gene, also known as neu or erbB2, encodes a 185 kDa transmembrane receptor tyrosine kinase belonging to the epidermal growth factor receptor family (13–18). Overexpression of p185 is found in ~30% of human breast cancers and in many other cancer types (19,20). The p185 phosphatases downstream substrates and activates a variety of signaling cascades, including the phosphatidylinositol-3-kinase (PI3K)/serine/threonine-specific protein kinase (Akt) and Ras/mitogen-activated protein kinase (MAPK) pathways. These regulatory signal cascades promote cell survival and tumor growth and metastasis (20–22).

We demonstrated previously that SV40 LT425 (LT425) acts as a transforming and metastasis suppressor of HER2 oncogene (23,24). Microarray and proteome analysis revealed that both IFN-γ and RARRES3 genes are unregulated in LT425 stable transfectants (W.-L.Tsou, T.-C.Chuang, C.-C.Ou, M.-C.Kao et al., unpublished data). Through a literature search and database comparison, we found that IFN-γ downregulates p185 expression in prostate cancer cells and induces RIG1 expression in ovarian cancer cells (2,5). Thus, we hypothesize that IFN-γ-induced RIG1 may contribute to repression of HER2. We also investigate the effects of RIG1 on the HER2-signaling cascade.

Materials and methods

Cell culture and plasmids

SKOV-3, OVCAr-3 and TOV-21G human ovarian cancer cell lines were grown in McCoy’s 5A, RPMI 1640 (Gibco BRL, Gaithersburg, MD) and MCDB 105/Medium 199 (Sigma–Aldrich, St. Louis, MO) media, respectively, supplemented with 10–20% fetal bovine serum (HyClone, Logan, UT). BT-474, MCF-7/HER (MCF-7 of a HER2-transfected stable line) and MCF-7 human breast cancer cell lines were grown in Dulbecco’s modified Eagle’s medium/F12 medium supplemented with 10% fetal bovine serum. Cells were grown at 37°C in a humidified incubator with 5% CO2. The plasmids pRIG1-EGFP and pRIG1-myc were kindly provided by Dr S.Y.Jiang (Department of Education and Research, Buddhist Tzu Chi General Hospital, Taipei, Taiwan, Republic of China).

Cell transfection

A liposome-mediated transfection method was used to transfect cells. In brief, small interference RNAs (siRNAs) (Dharmacon, Lafayette, CO and Ambion, Austin, TX) or plasmids were diluted in Opti-MEM I (Gibco BRL) medium and then mixed with Lipofectamine 2000 (Gibco BRL) at room temperature for 20 min. The lipoplex complex was added to the cells for 6 h, and the cells refreshed with complete medium.

Western blot analysis

Cell lysates were prepared and sedimented as described previously (23). The supernatant was used for sodium dodecyl sulfate–polyacrylamide gel electrophoresis analysis. Sixty micrograms of each cell sample was loaded onto the NuPAGE 4–12% Bis–Tris Gel (NP0322, Invitrogen, Grand Island, NY). After electrophoresis, the gels were blotted onto nitrocellulose membranes. The nitrocellulose membranes were subjected to blocking with 5% skim milk in Tris-buffered saline Tween-20 buffer (10 mM Tris, pH 8.0, 150 mM NaCl and...
0.1% Tween 20) for 2 h at room temperature. The membranes were then incubated with the primary anti-neu antibody (Ab-3; 1:1000 dilution; Oncogene Science, Cambridge, MA), anti-phospho-neu (Tyr1248) antibody (Ab-18; 1:500 dilution; NeoMarkers, CA), anti-akt antibody (1:1000 dilution; Cell Signaling), anti-phospho-Akt (Ser473) antibody (1:500 dilution; Cell Signaling, Beverly, MA), anti-GFP antibody (Santa Cruz Biotechnology, CA), anti-PI3K antibody (Cell Signaling, Beverly, MA), anti-phospho-(Tyr) p85 PI3K antibody (Cell Signaling), anti-mammalian target of rapamycin (mTOR) antibody (Cell Signaling), anti-phospho-mTOR (Ser2448) antibody (Cell Signaling), anti-β-actin (Chemicon, San Diego, CA 1:5000 dilution) antibody or other antibodies (Cell Signaling) in fresh 5% skim milk–Tris-buffered saline Tween-20 buffer for 1 h at room temperature or 4°C overnight. The membranes were washed and incubated with the secondary antibody of horseradish peroxidase-conjugated goat anti-mouse or goat anti-rabbit IgG (1:1000 dilution; Santa Cruz Biotechnology). Visualization was performed in an Amersham ECL chemiluminescence system. Bands were quantified by Adobe Photoshop software. IFN-γ, heregulin (HRG) and all chemicals were purchased from Sigma–Aldrich.

RNA isolation and reverse transcription–polymerase chain reaction
Total RNAs were extracted using TRIzol solution (Invitrogen). cDNA was prepared by incubation at 37°C for 1 h in 20 μl of a mixture containing 3 μg total RNA, 1 μl Moloney murine leukemia virus reverse transcriptase (Promega, Madison, WI), 1 μl deoxyribonucleotide triphosphate, 1 μl oligo-dT and 1 μl RNaseout recombination RNAse inhibitor. Polymerase chain reaction (PCR) was performed in 25 μl of a reaction containing 100 ng cDNA and 200 nM each of HER2 (sense: 5′-AGCCGCGACCACTAGTT-3′ and antisense: 5′-TTGGTGTCGCCGGTAGTTGTT-3′), RIG1 (sense: 5′-ACCAAGCCTCCCTCTTGCGC-3′ and antisense: 5′-GAAGGGCAGATGGCGTTG-3′) or β-actin (sense: 5′-CATAGAGAAGAGATCC-3′ and antisense: 5′-CTTGGGTGGTGAAGCCGTAG-3′) primers. The PCR products were quantified by ethidium bromide staining on a 0.8% agarose gel.

Quantitative PCR
Quantitative PCR was performed on a LightCycler (Roche Diagnostics, Mannheim, Germany) using SYBR Green I as a double-strand DNA-specific binding dye and continuous fluorescence monitoring. Amplification was carried out in a total volume of 20 μl containing 0.5 μM of each primer, 4 μM MgCl₂, 2 μl LightCycler DNA Master SYBR Green I [containing 1.25 U Taq polymerase, 10× Taq buffer (500 μM KCl and 100 μM Tris–HCl, pH 8.3), 2 μM of each deoxyribonucleotide triphosphate and 10× SYBR Green; Roche Diagnostics] and 1 μl of cDNA prepared as described above. The PCR primers were identical to those used for semiquantitative reverse transcription–PCR. Melting curve analysis of amplification products was performed at the end of each PCR by cooling the samples to 40°C and then increasing the temperature to 95°C at 0.2°C/s.

Fluorescence-activated cell sorter analysis
The fluorescence-activated cell sorter (FACS) analysis of p185 was performed as described (25). In brief, phycoerythrin-conjugated anti-HER2 monoclonal antibody (Becton Dickinson, San Diego, CA) was diluted with washing buffer (0.1% NaN₃ and 1.0% fetal bovine serum in phosphate-buffered saline) and the cell pellets were resuspended to a concentration of 2 × 10⁷ cells/ml. The cell–antibody complex was incubated at 4°C for 30 min in the dark and then washed twice with washing buffer. The supernatant was removed after each centrifugation. Five hundred microliters of washing buffer was added to wash the cell pellets to Falcon round-bottom tubes (Becton Dickinson), and the cells were analyzed using either a FACS Caliber or FACS Vantage SE (Becton Dickinson).

Enzyme-linked immunosorbent assay
Sorted cells (1 × 10⁶) were plated in a 60 mm tissue culture plate and allowed to attach overnight. The cells were then washed and incubated in a serum-free medium for 24 h. The serum-free conditioned medium was collected and centrifuged at 15000 g for 15 min to remove debris. Enzyme-linked immunosorbent assay was performed using the vascular endothelial growth factor (VEGF) Quantikine enzyme-linked immunosorbent assay kit (R&D Systems, Minneapolis, MN) (26).

HER2 promoter activity assay
Cells were cotransfected with pNu1it (kindly provided by Dr M.C. Hung, Department of Molecular and Cellular Oncology, University of Texas MD Anderson Cancer Center, Houston, TX) and pCMV-β-gal plasmids and various amounts of pKRG1-myC. Cell lysates were prepared before the luciferase activity assay by following the manufacturer’s (Promega) instructions. The measured luciferase activity levels were normalized against the β-galactosidase (β-gal) standard. The β-gal activity was measured by mixing equal amounts of cell extract and the 2× assay buffer (120 mM Na₂HPO₄, 80 mM NaH₂PO₄, 2 mM MgCl₂, 100 mM β-mercaptoethanol and 1.33 mg/ml orthonitrophenyl galactoside). After incubation at 37°C, the enzyme activity of β-gal was determined at A₄₂₀ nm (27).
Statistical analysis
Data were analyzed by using the paired t-test for comparison of independent means.

Results
Downregulation of p185 by IFN-γ-induced RIG1
IFN-γ has been shown to have inhibitory effects on p185 in prostate cancer cells (2). Thus, it is reasonable to ask whether IFN-γ downregulates p185 in ovarian and breast cancer cell lines. As expected, IFN-γ downregulated p185 expression in SKOV-3, OVCAR-3, and TOV-21G ovarian cancer cell lines, as well as in BT-474, MCF-7/Htr and MCF-7 breast cancer cell lines (Figure 1A). IFN-γ also downregulated p185 expression in a dose- and time-dependent manner in HER2-overexpressing SKOV-3 cells, as shown in the western blot analysis (Figure 1B and C). We then examined whether IFN-γ could upregulate RIG1 gene expression in SKOV-3 cells. Since there is no effective anti-RIG1 antibody available, we used a quantitative reverse transcription–PCR approach to measure the RIG1 messenger RNA (mRNA) levels. As expected, increasing RIG1 mRNA levels after treatment with increasing doses of IFN-γ were observed. The level of RIG1 mRNA increased >2-fold after treatment with 1000 U/ml of IFN-γ (Figure 2A). The suppressive effects of IFN-γ on p185 expression by induction of RIG1 were examined by treating several ovarian cancer cell lines with different doses of IFN-γ (100 and 1000 U/ml). After IFN-γ treatment for 24 h, cells were transfected with RIG1 siRNA or negative control siRNA. In cells treated with 1000 U/ml of IFN-γ, RIG1 siRNA restored the IFN-γ-mediated repression of p185 level to 2.9-folds in TOV-21G and 51% in OVCAR-3 cell lines. In addition, the p185 level in SKOV-3 cells could be restored by 40% by RIG1 siRNA with 100 U/ml of IFN-γ treatment (Figure 2B). These results were also revealed by different set of RIG1 siRNA (supplementary Figure, available at Carcinogenesis Online). Taken together, these data suggest that IFN-γ downregulates p185 by induction of RIG1 (Figures 1 and 2).

To further explore whether RIG1 is an important suppressor of p185 in SKOV-3 cells, the protein levels of p185 detected with the phycoerythrin-conjugated anti-HER2 monoclonal antibody were analyzed by FACS. We transfected the pEGFP vector only or pRIG1-EGFP into SKOV-3 cells, and then measured the red fluorescence (p185) and green fluorescence (pEGFP or pRIG1-EGFP) in the cells. RIG1 could significantly downregulate p185 expression by ~52% after 24 h of transfection of RIG1 (Figure 3A). At the same time, we isolated the successfully transfected RIG1-overexpressing cells 18 and 24 h after plasmid transfection (Figure 4A). Western blot analysis demonstrated that these FACS-sorted RIG1-overexpressing cells were able to effectively reduce the p185 level by 24 h (Figure 4A). In addition, phospho-p185 (the activated form of p185) was remarkably downregulated by RIG1 at both 18 and 24 h (Figure 4A). These results suggest that RIG1 downregulates p185 protein levels and suppresses p185 activation.

RIG1 represses the HER2 gene activity
To unravel the mechanisms of downregulation of p185 by RIG1, we assayed the mRNA levels of different sorted transcripts. As shown in Figure 3B, consistent with the p185 protein levels, the HER2 mRNA levels were downregulated by RIG1 at 24 h but not at 18 h. RIG1 obviously reduced HER2 RNA by >50% at 24 h (Figure 3B). The transcriptional activity of the HER2 promoter was also analyzed. In transient expression assays, using NIH3T3 (Figure 3C) and SKOV-3 (Figure 3D) as recipient cells, cotransfection of pNull and pCMV-β-gal and different amounts of pRIG1-myc for 24 h led to a significant decrease in luciferase activity (Figure 3C and D, left), which was normalized by β-gal. The HER2 promoter inhibitory rates increased in relation to an increasing amount of RIG1, and an inhibition rate of more than two times was observed at a ratio of pNull:pRIG1-myc as low as 1:1 (Figure 3C and D, right). Taken together, these data suggest that downregulation of p185 protein by RIG1 occurs because of the repression of HER2 promoter activity.

Downregulation of phospho-P3K/Akt and phospho-mTOR by IFN-γ and RIG1
Among HER2 stimulated intracellular signaling pathways, two main pathways, the Ras/MAPK and the PI3K/Akt cascades, were shown to be activated by HER2 (22). RIG1 was reported to inhibit the Ras/MAPK pathway by suppressing the activity of Ras and Ras protein in HTA cervical cancer cells (12). Here, we examined whether RIG1 could interfere with the Ras/MAPK pathway in ovarian cancer cells.

![Fig. 2. (A) Upregulation of RIG1 expression by IFN-γ treatment in SKOV-3 ovarian cancer cells. Cells were harvested after 2 h incubation with the indicated doses of IFN-γ. The PCR products were stained with ethidium bromide on a 0.8% agarose gel (upper panel). The quantitative PCR quantification was performed by adding the threshold cycle values of RIG1 cDNA and normalizing them to glyceraldehyde-3-phosphate dehydrogenase cDNA (Roche Diagnostics) (lower panel). (B) RIG1 siRNA restored the reduction of p185 caused by IFN-γ. Cells were treated with 1000 or 100 U/ml of IFN-γ for 24 h and then transfected with negative control siRNA (NCi) or RIG1 siRNA (RIG1i). Cell lysates were immunoblotted with antibodies specific for neu or β-actin (upper panel). Reverse transcription–PCR for RIG1 shows the inhibitory effect of RIG1 siRNA (lower panel).]
As demonstrated in Figure 4A, both Ras and extracellular signal-regulated kinase proteins were not altered in RIG1-overexpressing cells. The activation of extracellular signal-regulated kinase was not significantly reduced after ectopic expression of RIG1 (Figure 4A). We then investigated if RIG1 affected the PI3K/Akt-signaling cascade. After 18 and 24 h of transfection with RIG1, phospho-PI3K, phospho-Akt and phospho-mTOR were all dramatically downregulated ($P < 0.01$). More than 40% of inhibition rate was observed at 24 h (Figure 4A). In addition, a new finding that RIG1 has an inhibitory effect on mTOR protein (Figure 4A) suggests that mTOR is another target for RIG1 inhibition. These data clearly demonstrate that RIG1 exerts inhibitory effects on phospho-PI3K/phospho-Akt/phospho-mTOR-signaling pathway. This is the first report showing that RIG1 could affect PI3K/Akt pathway.

To further evaluate the effects of IFN-γ on the PI3K/Akt pathway, SKOV-3 cells were treated with 1000 U/ml of IFN-γ. After 24 h of IFN-γ administration, the expression of p185, phospho-p185, phospho-PI3K, phospho-Akt and VEGF all remarkably decreased, but the protein levels of PI3K and Akt did not change (Figure 4B). These results were identical to those obtained for RIG1 ectopic expression, as shown in Figure 4A. In summary, these data demonstrate that both IFN-γ and RIG1 can abrogate the HER2 and PI3K/Akt pathways.

**Downregulation of VEGF by RIG1**

HER2-overexpressing tumors tend to be more angiogenic than other tumors (28). VEGF is one of the most potent inducers of angiogenesis (28). The HER2- and PI3K/Akt-signaling pathway has been implicated in the regulation of VEGF through the activation of mTOR/p70S6K (26). Thus, we proposed that RIG1 downregulated VEGF by inactivating mTOR. As expected, western blot analysis of intracellular VEGF demonstrated that RIG1 downregulated VEGF at 18 and 24 h after transfection (Figure 5A). To further explore the participation of VEGF in the medium, we used enzyme-linked immunosorbent assay to quantify and compare the VEGF secreted into serum-free conditioned medium after 24 h of incubation with different transfectants. As shown in Figure 5B, RIG1 significantly reduced the secreted VEGF level by −50%, indicating that RIG1 could decrease VEGF expression by downregulating the HER2- and PI3K/Akt/mTOR-signaling pathway.

**HRG restores the RIG1-induced decrease in phospho-p185 and phospho-Akt**

HRG is a member of the neuregulin family of ligands that interact with and activate epidermal growth factor receptor family members. We found that RIG1 suppresses the activation of p185 and wondered...
whether HRG could reactivate p185. As shown in Figure 6A, phospho-p185 was downregulated by RIG1 at 24 h and HRG restored phospho-p185 at this time point. Since RIG1 downregulated both p185 levels and phospho-p185 expression, HRG might not effectively restore the phosphorylation levels to the vector control level. In addition, HRG restored the downregulated phospho-Akt to the control level, implying that RIG1 acts as a suppressor of the HER2 and PI3K/Akt pathway. HRG was added to the cells before they were applied to FACS, so VEGF expression would not have been increased by HRG (Figure 5A). We also found that p185 protein expression slightly decreased 24 h after addition of HRG (Figure 6A, lower panel). This finding is consistent with a previous report that ligand can induce endocytosis and degradation of HER2 (29).

**Discussion**

Type II IFNs possess antiproliferative effects in various tumor cell types. The binding of IFN-γ to its cognate receptor activates the Janus kinase/STAT signal transduction pathway, in which STAT1 molecules are phosphorylated and translocated to the nucleus where they bind to IFN-γ-activated sites and activate transcription of target genes. Here, we demonstrated that IFN-γ suppresses p185 expression in both ovarian and breast cancer cell lines. The phosphoprotein levels of p185, PI3K and Akt and the protein levels of p185 and VEGF were all downregulated by IFN-γ treatment (Figure 4B). The repressive effect of IFN-γ-induced RIG1 on p185 expression was verified by the RIG1 siRNA. Treatment with 1000 U/ml of IFN-γ together with RIG1
siRNA restored the IFN-γ-mediated repression of the p185 level to 2.9-folds in TOV-21G cells and to 51% in OVCAR-3 cells (Figure 2B), whereas p185 was only partially restored by RIG1 siRNA in SKOV-3 cells after treatment with 1000 U/ml of IFN-γ (data not shown). This phenomenon was also observed in the HER2-overexpressing breast cancer BT-474 cell line when treated with 1000 U/ml of IFN-γ (data not shown). However, the p185 level in both SKOV-3 and BT-474 cells could be restored by RIG1 siRNA under a low dose of IFN-γ (100 U/ml). Therefore, inhibition of RIG1 appeared insufficient to restore the repression of p185 expression after a high dose of IFN-γ treatment. It has been reported that downregulation of p185 by a high dose (5000 U/ml) of IFN-γ occurs through the interaction of phosphorylated STAT1 with p300 (2). Thus, RIG1 may play an important role after IFN-γ treatment, but other mechanisms may be involved in the regulation of p185, especially with a high dose of IFN-γ.

Using a transient transfection method and cell sorter, we isolated RIG1-overexpressing SKOV-3 cells. Inside these cells, both p185 protein and its phosphorylated form were downregulated. We also showed that repression of p185 protein was caused by the inhibition of HER2 promoter activities (Figure 3C and D). Therefore, we propose that RIG1 may possess phosphatase activities, and the interaction and colocalization of RIG1 with p185 in the cytoplasmic compartment may contribute to the decreased p185 activation (data not shown). In addition to downregulation of phospho-p185, the amount of activated PI3K, Akt and mTOR decreased 18 h after transfection of RIG1 (Figure 4A). Thus, IFN-γ-induced RIG1 may play an important role in the HER2 and PI3K/Akt pathways (Figure 6B).

RIG1 belongs to the HREV107 family of class II tumor suppressor genes. A notable feature of class II tumor suppressor is loss of the growth-constraining function in tumor cells because of blocked expression, but not because of DNA mutation or deletion, such as p53 (30,31). Previous studies have shown the induction of RIG1 protein by retinoids (3,4), but the mechanisms of induction of RIG1 by IFN-γ are not well understood. Evidence suggests that the RIG1 promoter contains a retinoic acid response element, which is also referred to as a putative p53 response element (32). The presence of putative p53 response element suggests that the class II tumor suppressor, RIG1, might be a direct transcriptional target of the class I tumor suppressor p53. However, SKOV-3 is a p53-null cell line, meaning that the regulation of RIG1 by IFN-γ is p53 independent. Treatment of A27/80 and OVCAR-3 human ovarian carcinoma cells with IFN-γ can stimulate HREV107-1 expression, which is directly related to the activation of interferon regulatory factor-1 (IRF-1), indicating that HREV107-1 is a target of IRF-1 and is involved in IFN-γ-induced angiogenesis.
cell death (5). In this study, we demonstrated that RIG1 mRNA levels increased by >2-fold after administration of IFN-γ for 2 h (Figure 2A). This result confirmed an earlier report that IRF-1 is detectable within 3 h after IFN-γ administration (5). Thus, the mechanisms responsible for the IFN-γ induction of RIG1 probably involve IRF-1 and should be investigated further.

It has been hypothesized that RIG1 is involved in a negative feedback loop that normally controls the level of MAPK activation (33). Overexpression of RIG1 downregulates MAPK activities in HTA cervical and T47D breast cancer cells and enhances MAPK-mediated suppression of RIG1 (33). In our study, the activity of extracellular signal-regulated kinase is not significantly reduced by RIG1. Therefore, we focused on PI3K/Akt pathway. As shown in Figure 4A, PI3K/Akt activities were dramatically reduced (P < 0.01) after ectopic expression of RIG1. In SKOV-3 cells, Akt signaling is dominantly activated as a result of increased HER2 activation and in the absence of p53 and phosphatase and tensin homolog (34–37). p53 and phosphatase and tensin homolog are known to repress PI3K/Akt activities (34–37). Although MAPK is also activated in ovarian carcinoma cells, it is not the major pathway for RIG1-mediated suppression of HER2 signaling in SKOV-3 cells in our study.

HER2 overexpression increases the metastatic potential of human cancer cells (20,21). Patients with HER2-overexpressing tumors have increased incidences of metastasis and a poorer survival rate compared with HER2 normal patients (19). Angiogenesis is a key component of cancer metastasis (38) and is tightly controlled under normal physiological conditions. However, in pathological diseases such as cancer, the fine balance between proangiogenic and antiangiogenic factors is disrupted. VEGF is one of the most potent inducers of angiogenesis and induces endothelial cell proliferation and migration (28). VEGF expression in human breast cancers correlates with increased microvessel density and reduced survival (39). HER2 has been implicated in the regulation of VEGF, which is activated by the mTOR/p70S6K pathway. mTOR is a 289 kDa phosphoinositide kinase-related serine/threonine kinase (40). Through the formation of multimolecular complexes, rictor or raptor, the evolutionarily conserved TOR pathway controls an array of fundamental cell functions, such as translation initiation, protein stability, transcription of ribosome and stress response genes, ribosomal biogenesis and transfer RNA synthesis, translation initiation, protein stability, transcription of ribosome and stress response genes, p53 and phosphatase and tensin homolog (34–37). p53 and phosphatase and tensin homolog are known to repress PI3K/Akt activities (34–37). Although MAPK is also activated in ovarian carcinoma cells, it is not the major pathway for RIG1-mediated suppression of HER2 signaling in SKOV-3 cells in our study.

Supplementary Material

Supplementary Figure can be found at http://carcin.oxfordjournals.org/

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References


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