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Inhibition of Constitutively Activated Phosphoinositide 3-Kinase/AKT Pathway Enhances Antitumor Activity of Chemotherapeutic Agents in Breast Cancer Susceptibility Gene 1-Defective Breast Cancer Cells

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Abstract

Loss or decrease of wild type BRCA1 function, by either mutation or reduced expression, has a role in hereditary and sporadic human breast and ovarian cancers. We report here that the PI3K/AKT pathway is constitutively active in BRCA1-defective human breast cancer cells. Levels of phospho-AKT are sustained even after serum starvation in breast cancer cells carrying deleterious BRCA1 mutations. Knockdown of BRCA1 in MCF7 cells increases the amount of phospho-AKT and sensitizes cells to small molecule protein kinase inhibitors (PKIs) targeting the PI3K/AKT pathway. Restoration of wild type BRCA1 inhibits the activated PI3K/AKT pathway and desensitizes cells to PKIs targeting this pathway in BRCA1 mutant breast cancer cells, regardless of PTEN mutations. In addition, clinical PI3K/mTOR inhibitors, PI-103 and BEZ235, showed anti-proliferative effects on BRCA1 mutant breast cancer cell lines and synergism in combination with chemotherapeutic drugs, cisplatin, doxorubicin, topotecan, and gemcitabine. BEZ235 synergizes with the anti-proliferative effects of gemcitabine by enhancing caspase-3/7 activity. Our results suggest that the PI3K/AKT pathway can be an important signaling pathway for the survival of BRCA1-defective breast cancer cells and pharmacological inhibition of this pathway is a plausible treatment for a subset of breast cancers.

Keywords

constitutive activation; PI3K/AKT pathway; BRCA1-defective breast cancer; kinase inhibitor; chemotherapeutic agents; synergism

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Conflicts of interest

The authors declare no conflict of interest.

Introduction

It has now been well established that germline mutations in the breast cancer susceptibility gene 1 (BRCA1) drastically increase the lifetime risk of breast and ovarian cancers in the individuals who carry them [1,2]. In addition, because expression of the BRCA1 protein is often decreased or even absent in sporadic breast and ovarian cancers, deregulation of BRCA1 expression may also have role(s) in nonhereditary tumors [3,4]. Although established functional roles of BRCA1 include the regulation of cell cycle progression, DNA damage signaling and repair, maintenance of genomic integrity, and the regulation of various transcriptional pathways, the specific functions of the BRCA1 gene as a tumor suppressor are still not clear [5]. According to gene expression profiling, tumors from patients carrying BRCA1 mutations segregate within the basal subgroup of breast cancers [6]. To date, evidence suggests that dysfunction of BRCA1 may play a role in the development of basal-like breast cancers [7]. Basal-like breast cancers typically show no expression of the estrogen receptor (ER), the progesterone receptor (PR) and a lack of overexpression of human epidermal growth factor receptor 2 (HER2) [8]. These characteristics overlap with immunohistochemically defined triple-negative breast cancers (TNBCs) [9]. Basal-like breast cancers have been shown to have a poor prognosis and currently respond poorly to targeted therapeutics [7–9]. Despite many efforts to reveal underlying molecular mechanisms, no specific targets to treat BRCA1-related breast cancers are currently available [8].

It was reported that activation of the phosphoinositide 3-kinase (PI3K) pathway is associated with the basal-like phenotype of breast cancer in clinical samples [10]. The PI3K/AKT pathway has an important role in the proliferation of malignant tumor cells, as well as in normal cells [11]. It is suggested that BRCA1 can down-regulate phospho-AKT either by ubiquitin-mediated proteasomal degradation via direct physical interaction or by activating a protein serine/threonine phosphatase, PP2A in MCF7 cells [12,13]. Recently, it was also reported that AKT activation inversely correlates with BRCA1 expression in human breast cancers and that the mammalian target of rapamycin (mTOR) pathway can be used as a therapeutic target for treatment of Brcal-deficient cancers in a mouse model [14]. It is also suggested that PI3K is necessary to activate AKT in MCF7 cells transfected with BRCA1-siRNA, because PI3K inhibitors, LY294002 and Wortmannin, inhibit BRCA1-dependent AKT activation [13]. However, dysregulation of AKT in BRCA1-defective human breast cancer cells is still not well understood. Because AKT is regarded as a central converging node for multiple upstream kinases [15], other upstream kinases may further contribute to the activation of the PI3K/AKT pathway in a BRCA1-dependent manner.

Here, we demonstrate that BRCA1 negatively regulates the PI3K/AKT pathway in breast cancer cells. Moreover, the PI3K/AKT pathway is constitutively activated in BRCA1 mutant breast cancer cells and targeting this pathway in combination with chemotherapeutic agents is a potential therapeutic regimen for BRCA1-defective breast cancers.

Materials and Methods

Cell culture and reagents

The SUM149PT and SUM1315MO2 cell lines were maintained as recommended (Asterand, Detroit, MI). All the other cell lines were purchased from American Type Culture Collection (Manassas, VA) and maintained as recommended. Reagents for cell culture were purchased from Invitrogen (Carlsbad, CA), Lonza (Basel, Switzerland), or Cellgro (Manassas, VA). Inhibitors were purchased from the following sources: PI-103, PIK-75, and Perifosine from Selleck Chemicals (Houston, TX); BEZ235 from LC Labs (Woburn, MA); LY294002,

cisplatin, doxorubicin, topotecan, and gemcitabine from Sigma (St. Louis, MO). Stock solutions of compounds were made in appropriate vehicles (Dimethylformamide (DMF) for BEZ235, H₂O for Perifosine and doxorubicin, phosphate-buffered saline (PBS) for gemcitabine, dimethyl sulfoxide (DMSO) for all others except for cisplatin) and stored at -20°C in small aliquots. The stock solution of cisplatin was prepared and stored as previously described [16]. Expression vectors for wild type BRCA1 are described elsewhere [17]. PathScan RTK Signaling Antibody Array was purchased from Cell Signaling Technology (Danvers, MA).

DNA transfection, siRNA transfection and MTT assays

DNA transfection was performed using Lipofectamine Plus reagent (Invitrogen, Carlsbad, CA) as described previously [17]. Twenty-four hours after transfection, cells were plated into either 24- or 48-well plates. The day after plating, cells were treated with normal growth media containing kinase inhibitors in triplicate for 48–72 hr followed by MTT assay.

MCF7 cells pre-treated with 100 nM siRNA (control vs. BRCA1) for 72 hr were re-seeded into either 24- or 48-well plates with DMEM supplemented with 5% fetal bovine serum and grown overnight, then further transfected with 100 nM of fresh siRNA using Lipofectamine 2000 (Invitrogen, Carlsbad, CA). Twenty-four hr after transfection, normal growth media containing small molecule inhibitors were added to the cells in triplicate. The control- and BRCA1-siRNA were obtained from Dharmacon (Lafayette, CO) as previously reported [18].

For MTT assays, cells were subcultured into 96-well plates according to their growth properties. Cell proliferation was assayed at 48–72 hr after treatment of compounds by adding 20 µl of 5 mg/ml 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution per 100 µl of growth medium. After incubating for 3–4 h at 37°C, the media were removed and 150 µl/well of MTT solvent (either absolute DMSO or isopropanol containing 4 mM HCl and 0.1% Nonidet-40) was added to dissolve the formazan. The absorbance of each well was measured by ELx808 (BioTek, Winooski, VT) or Wallac Victor2 (Perkin-Elmer Life Sciences, Boston, MA) Microplate Reader. Viable cells are presented as a percent of the control, vehicle-treated cells. Combination index (CI) was calculated by CompuSyn software V1.0 (ComboSyn, Paramus, NJ).

Western blots and antibodies

Western blot analyses were performed using cleared cell lysates resolved on sodium dodecyl sulfate (SDS)-polyacrylamide gels, transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA), and probed with specific antibodies using standard procedures [18]. Antibodies used in this study were purchased from following sources: BRCA1 (C-20) (sc-642) from Santa Cruz Biotechnology (Santa Cruz, CA); phospho-GSK3β (Ser9) (#9323), GSK3β (#9332), phospho-S6 ribosomal protein (Ser235/236) (#4856), S6 ribosomal protein (#2217), phospho-Akt (Thr308) (#4056), phospho-Akt (Ser473) (#9271), Akt (#9272), phospho-mTOR (Ser2448) (#2971), mTOR (#4517), phospho-Bad (Ser112) (#9291) from Cell Signaling Technology (Danvers, MA); β-actin; horseradish peroxidase-conjugated secondary antibodies from Sigma (St. Louis, MO). Chemiluminescence reagent was purchased from Santa Cruz Biotechnology (Santa Cruz, CA) or Thermo Scientific (Rockford, IL). Densitometric analysis was performed by ImageJ software [19].

Caspase-3/7 Assay

Activity of Caspase-3/7 was measured by Casapase-Glo 3/7 Assay Kit (Promega, Madison, WI) according to manufacturer's instructions. The day after subculture, cells were treated

with either gemcitabine, BEZ235 alone, or combination of both drugs for indicated times and caspase-3/7 activity was measured from cell lysates. Relative luminescence units were normalized by protein concentration and adjusted to the value from vehicle-treated cells as 100.

Statistical Methods

The two-tailed Student's t-test was applied for statistical analysis when only 2 groups of interest were compared. For comparisons with multiple groups, one-way or two-way ANOVA were implemented. After the overall analysis was done for each data set, Turkey tests controlling the type one error have been performed to make the pairwise comparisons between the treatment groups. (*) indicates $P < 0.05$; (**) indicates $P < 0.01$; and (***) indicates $P < 0.001$.

Results

BRCA1 negatively regulates phospho-AKT in breast cancer cell lines

To determine if defective BRCA1 affects signaling pathways of breast cancer cells, we chose the MCF7 cell line as a model system. First, we performed antibody microarray analysis of lysates from MCF7 cells transiently transfected with BRCA1-siRNA using an antibody array chip which can detect several phospho-proteins. We identified elevated levels of several phospho-proteins including phospho-AKT (T308 and S473) and phospho-S6 ribosomal protein (S235/236) in BRCA1-knockdown (BRCA1-KD) MCF7 cells as compared to control-siRNA-transfected cells (Figure 1A). To further confirm the antibody microarray results, we performed western blot analysis for the AKT pathway in BRCA1-KD MCF7 cells. Significant up-regulation of phospho-AKT (S473) was detected in BRCA1-KD MCF7 cells compared to controls (Figure 1B). To exclude cell-type specificity, we performed knockdown of BRCA1 in the UWB1.289+BRCA1 ovarian cancer cell line. This cell line was established by stable expression of wild type BRCA1 in the BRCA1-null ovarian cancer cell line, UWB1.289 [20]. Knockdown of BRCA1 in UWB1.289+BRCA1 cells also increased levels of phospho-AKT (Figure 1B).

Recently, several breast cancer cell lines, such as MDA-MB-436, SUM149PT and HCC1937, were reported as carrying deleterious mutations in the BRCA1 gene (Table 1). Because AKT is a well-known convergent kinase for the activation of multiple upstream effector molecules [15], we first determined the status of phospho-AKT (S473) and phospho-GSK3 β (S9) in several BRCA1-defective breast cancer cell lines. Western blot analysis of these cell lines showed marked increase of phospho-AKT in BRCA1 mutant breast cancer cells (SUM149PT, MDA-MB-436, and HCC1937) as compared to wild type BRCA1 breast cancer cells (MCF7 and MDA-MB-231) (Figure 2, left and Supplementary figure 1). The phosphorylation of GSK3 β was also elevated in BRCA1-defective breast cancer cell lines, as compared to wild type BRCA1 breast cancer cell lines. In addition, the phosphorylation of AKT (S473) in BRCA1-defective cells was not abolished after deprivation of growth factors by serum starvation (Figure 2, right and Supplementary figure 1). By contrast, phospho-AKT levels were barely detectable in serum-starved MCF7 and MDA-MB-231, irrespective of PIK3CA mutation status (Table 1).

To further determine the consequence of AKT activation in BRCA1-KD MCF7 cells, we utilized several small molecule PI3K/AKT pathway inhibitors (Table 2). In BRCA1-KD MCF7 cells, treatment of PI-103, a PI3K/mTOR inhibitor, abolished phosphorylation of AKT and its substrate GSK3 β , in a dose-dependent manner (Figure 3A and Supplementary figure 2A). Because PI-103 specifically inhibits PI3K, mTOR, and DNA-PK without significantly affecting AKT activity [25], these results suggest that loss of BRCA1 activates

AKT via more upstream kinases. As previously reported [27], inhibition of AKT reduced the level of BRCA1 in control MCF7 cells.

The effects of PI3K/AKT pathway inhibition were further determined in BRCA1-defective breast cancer cells. Treatment of PI-103 reduced the phosphorylation of AKT in all BRCA1 mutant breast cancer cells tested (Figure 3B, left and Supplementary figure 2).

Phosphorylations of downstream targets of AKT, such as phospho-GSK3 β (S9) and phospho-BAD (S112) were also reduced by PI-103 treatment. Phosphorylation of mTOR at S2448, which is also known to be phosphorylated by AKT [28], was also reduced by PI-103 resulting in reduced phosphorylation of S6 ribosomal protein at S235/236 (Figure 3B and Supplementary figure 2). The effect of PI-103 was much more potent than LY294002 in MDA-MB-436 cells (Figure 3B, right and Supplementary figure 2).

Anti-proliferative effects of the PI3K/AKT pathway inhibition were also determined. Cells were incubated with different concentrations of inhibitors for 72 hr and viable cells were measured by MTT assay. As expected, PI-103 inhibited the proliferation of SUM149PT, HCC1937 and MDA-MB-436 cells in a dose-dependent manner (Supplementary figure 3). An AKT translocation inhibitor, Perifosine [29], showed less anti-proliferative effects on HCC1937 and MDA-MB-436 cells than the other tested inhibitors did (Supplementary figure 3). By contrast, BEZ235 showed the most potent anti-proliferative effects in BRCA1-defective breast cancer cells.

Loss of BRCA1 enhances anti-proliferative effects of PI3K/AKT pathway inhibitors

MCF7 cells transiently transfected with either control-siRNA or BRCA1-siRNA were treated with different doses of inhibitors for up to 48 hr and viable cells were determined by MTT assay. Under these conditions, knockdown of BRCA1 can sensitize the MCF7 cells to Perifosine in a dose-dependent manner (Figure 4A). BRCA1-KD also sensitizes the MCF7 cells to dual PI3K/mTOR inhibitors, such as PI-103 or BEZ235 (Figure 4B, D). Another inhibitor, PIK-75 which specifically inhibits PI3K α and PI3K γ , but not mTOR [25], also showed similar effects on proliferation of BRCA1-KD MCF7 cells (Figure 4C). These results further support the idea that BRCA1 negatively regulates the activation of upstream kinase(s) of AKT.

To further verify BRCA1-dependency of PI3K/AKT pathway regulation, expression of wild type BRCA1 was restored by transient transfection. Wild type BRCA1 expressing plasmids were transiently transfected into MCF7, SUM149PT, or HCC1937 cells. Expression of wild type BRCA1 was confirmed by western blot (Figure 5A). In MCF7 cells, overexpression of wild type BRCA1 further decreased the basal level of phospho-AKT at both Ser473 and Thr308 (Figure 5A). Overexpression of wild type BRCA1 was also sufficient to significantly decrease levels of phospho-AKT in SUM149PT cells (Figure 5A).

In addition, overexpression of wild type BRCA1 conferred resistance to PI-103. After transfection of the wild type BRCA1 expressing plasmid, the cells were treated with increasing amounts of PI-103 and viable cells were measured by MTT assay. In MCF7 cells, overexpression of wild type BRCA1 de-sensitizes the cells to PI-103 (Figure 5B). Restoration of wild type BRCA1 in BRCA1-defective cells (SUM149PT and HCC1937) also made cells resistant to PI-103 compared to control-transfected cells carrying BRCA1 mutations (Figure 5C, D). All these results suggest that BRCA1 negatively regulates the PI3K/AKT pathway despite the phosphatase and tensin homolog (PTEN) mutation (Table 1).

Inhibition of the PI3K/AKT pathway enhances anti-proliferative effects of chemotherapeutic agents in BRCA1-defective cancer cells *in vitro*

The findings above motivated us to search combinatorial effects of drugs showing BRCA1-dependency. To determine whether there are any combinatorial benefits with PI3K/AKT pathway inhibitors, we selected conventional chemotherapeutic agents, cisplatin, doxorubicin, topotecan, and gemcitabine. The viability of SUM149PT cells treated with one PKI and one conventional anti-tumor agent was measured after 72 hr treatment by MTT assay. Combination of PI-103 with cisplatin, doxorubicin, or topotecan reduced the viable cells, as compared to each anti-tumor agent alone (Figure 6). The combination of BEZ235 and gemcitabine was also synergistic in SUM149PT cells with CI50 value of 0.72 ± 0.075 (Figure 7A). To further assess the synergism of BEZ235 with gemcitabine, we measured apoptotic cell death in SUM149PT cells by measuring caspase-3/7 activity. BEZ235 (0.25 μ M) alone did not significantly activate caspase-3/7 activity at 24 hr after treatment. In contrast, gemcitabine induced caspase-3/7 activity by ~3-fold after 24 hr treatment (Figure 7B). Gemcitabine-induced caspase-3/7 activity was further increased by prolonged treatment. In addition, co-treatment of BEZ235 enhanced gemcitabine-induced caspase-3/7 activity after 24 hr treatment (Figure 7B). These results suggest that the combination of BEZ235 with gemcitabine enhances caspase-3/7-mediated apoptosis.

Discussion

BRCA1-related cancers show basal-like phenotype [7–9], but the origin of these cancers is not fully understood yet. A recent study shows that the BRCA1 breast cancers originate not from basal stem cells but from luminal epithelial progenitors [30]. Because the MCF7 cell line expressing wild type BRCA1 shows the luminal phenotype [21], we chose MCF7 cells as one of the cell models to investigate BRCA1-dependent signal activation.

Although AKT is activated in BRCA1-KD MCF7 cells in our study and others [12,13], the contribution of the AKT pathway in BRCA1-defective breast cancer cells has not been well elucidated. BRCA1 may directly down-regulate phospho-AKT either by ubiquitin-mediated proteasomal degradation [12] or indirectly by activating PP2A [13]. It is also suggested that PI3K plays a role in AKT activation because treatment of PI3K inhibitors (LY294002 or Wortmannin) reduce phospho-AKT in BRCA1-KD MCF7 cells [13]. Full activation of AKT requires phosphorylation at two specific amino acid residues, T308 and S473, and these phosphorylations are achieved specifically by PDK1 on T308 and mTORC2 on S473, respectively [15]. Regarding these, our data further support the involvement of upstream effectors in activation of the PI3K/AKT pathway in a BRCA1-dependent manner: 1) Increased phosphorylation of AKT at T308 observed in antibody microarray analysis in BRCA1-KD MCF7 cells (Figure 1A) implies that the activation of upstream kinases can directly or indirectly phosphorylate AKT; 2) Perifosine inhibits proliferation of breast cancer cell lines in a BRCA1-dependent manner. Unlike other kinase inhibitors targeting ATP-binding pockets, Perifosine inhibits translocation of AKT from the cytoplasm to the plasma membrane by targeting the pleckstrin homology domain, thereby preventing phosphorylation of AKT by upstream kinases [29]; 3) Several PI3K inhibitors preferentially reduce proliferation of BRCA1-defective breast cancer cells.

To our knowledge, although PI-103 specifically inhibits PI3K, it also inhibits mTOR, DNA-PK, and PI3KC2 β , but does not inhibit either PDK1 or AKT at 10 μ M concentration *in vitro* [25]. Because the AKT/mTOR pathway is regulated by a complex feedback loop [31] and inhibition of mTOR by rapalogs can increase AKT phosphorylation both at S473 and T308 by activating the upstream kinase signaling in certain types of cancer cells [32], we further utilized a more specific PI3K α and PI3K γ inhibitor such as PIK-75 (Table 2). PI-103, Perifosine, and BEZ235 showed similar anti-proliferative effects on BRCA1-KD MCF7

cells as PIK-75 (Figure 4C vs. A, B, D). Both PI-103 and PIK-75 also potentially inhibit DNA-dependent protein kinase catalytic subunit (DNA-PKcs) *in vitro* [25]. Previously, DNA-PKcs has been identified as a putative AKT (S473) kinase in response to ionizing radiation (IR) [33]. However, subsequent reports revealed that AKT (S473) phosphorylation is not dependent on DNA-PKcs but the MRE11-ATM pathway in response to DNA double strand breaks [34]. Moreover, during the course of this study, it has been reported that BEZ235 inhibits not only PI3K and mTOR, but also ATM, ATR, and DNA-PKcs with similar *in vitro* potency [35]. Based on these data, we cannot rule out the possible involvement of DNA-PK or ATM pathways in up-regulation of the PI3K/AKT pathway in BRCA1-defective breast cancer cells. However, specific inhibitors of DNA-PK (NU 7441) [36] or ATM (KU-55933) [37] did not significantly affect proliferation of BRCA1-defective breast cancer cells (SUM149PT, MDA-MB-436, and HCC1937) as compared to PI-103 and PIK-75 (unpublished observations).

All BRCA1 mutated breast cancer cell lines used in this study contain gross PTEN mutations and are negative in expression of PTEN [24]. Because PTEN is a negative regulator of PI3K/AKT, it is possible that activation of AKT in these cells is solely dependent on loss of PTEN function. However, overexpression of wild type BRCA1 could further reduce basal phospho-AKT (S473/T308) levels in PTEN wild type MCF7 cells. Transient expression of wild type BRCA1 also abolished phospho-AKT (S473/T308) in PTEN-negative SUM149PT cells. In addition, overexpression of wild type BRCA1 in MCF7, SUM149PT, or HCC1937 cells conferred resistance to PI-103. These results suggest that BRCA1 may regulate the PI3K/AKT pathway by acting on upstream kinases of AKT regardless of PTEN status.

Up till now, the success of many targeted cancer therapies including protein kinase inhibitors has been based on their efficacy when used in combination with established chemotherapies [38]. Therefore, one of the major issues in recent anti-cancer drug development is identifying effective combinatorial regimens of drugs [39]. We demonstrated that combination of PI3K pathway inhibitors with chemotherapeutic drugs such as cisplatin, doxorubicin, topotecan, or gemcitabine results in enhancing cell killing effects in BRCA1-defective breast cancer cells *in vitro*. Our findings suggest that the PI3K/AKT pathway is constitutively activated in BRCA1-defective breast cancer cells and targeting this pathway in combination with chemotherapeutic agents is a plausible strategy for treatment of these cells. It is worth noting that PI3K pathway activation is commonly found in the basal-like breast cancer in clinical samples [10] and AKT phosphorylation has an inverse correlation with BRCA1 expression in human breast cancers [14].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

BRCA1	breast cancer susceptibility gene 1
PKIs	protein kinase inhibitors

PI3K	phosphoinositide 3-kinase
mTOR	mammalian target of rapamycin
PTEN	phosphatase and tensin homolog

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Figure 1.

Knockdown of BRCA1 activates the PI3K/AKT pathway. (A) Lysates were prepared from MCF7 cells that had been transiently transfected with BRCA1-siRNA and analyzed by antibody microarray. Relative intensities were calculated from two replicative spots by ImageJ software [19]. (B) Wild type BRCA1-expressing cells (MCF7 and UWB1.289+BRCA1) pre-treated with 100 nM siRNA for 72 hr were re-seeded with normal growth media and grown overnight, then further transfected by 100 nM of fresh siRNA. Cell lysates were subjected to western blot analysis with the indicated antibodies.

Figure 2.

The AKT pathway is constitutively activated in BRCA1-defective breast cancer cell lines. Cells were cultured in normal growth conditions (left) or deprived of growth factors by serum starvation for 24 hr (right) and cell lysates were analyzed by western blot with the indicated antibodies. Numbers indicate the relative levels of p-AKT normalized to β-actin. Further normalized values are indicated in Supplementary figure 1.

Figure 3.

PI-103, a PI3K/mTOR inhibitor, reduces activated phospho-AKT and its downstream targets. (A) MCF7 cells pre-treated with 100 nM siRNA for 72 hr were reseeded with normal growth media and grown overnight, then further transfected by 100 nM of fresh siRNA. Twenty-four hr after transfection, the cells were further treated with PI-103 for 24 hr and the cell lysates were immunoblotted with the indicated antibodies. (B) Breast cancer cells carrying BRCA1 mutations were treated with 1 μ M of PI-103 for 24 hr (left) or increasing amounts of PI-103 for 24 hr (right). Cell lysates were analyzed by western blotting with the indicated antibodies. Numbers indicate the relative levels of p-AKT normalized to β -actin. Further normalized values are indicated in Supplementary figure 2.

Figure 4.

Knockdown of BRCA1 sensitizes cells to PI3K/AKT pathway inhibitors. MCF7 cells transfected with either BRCA1-siRNA or control-siRNA were treated with increasing amounts of inhibitors targeting the PI3K/AKT pathway for 48 hr in triplicate. Viable cells were measured by MTT assay. Data are means $\pm$ SEMs from at least two independent experiments performed in triplicate. (**) indicates $P < 0.01$; and (***) indicates $P < 0.001$.

Figure 5.

Wild type BRCA1 de-sensitizes breast cancer cells to PI-103. (A) MCF7 or SUM149PT cells were transfected with wild type BRCA1 expressing vectors and cell lysates were analyzed by antibodies for BRCA1 and phospho-AKT (S473 and T308). (B–D) Cells were transfected with either pcDNA3-BRCA1 or pcDNA3 for 48 hr and treated with increasing amounts of PI-103 for 72 hr. The viable cells were measured by MTT assay. Data are means $\pm$ SEMs from at least two independent experiments performed in triplicate. (*) indicates $P < 0.05$; (**) indicates $P < 0.01$.

Figure 6. Combination of PI-103 with chemotherapeutic agents. SUM149PT cells were treated with PI-103 in combination with cisplatin (A), doxorubicin (B), and topotecan (C) for 72 hr and viable cells were measured by MTT assay. Data are means $\pm$ SEMs from two independent experiments performed in triplicate. (*) indicates $P < 0.05$.

Figure 7. BEZ235 sensitizes BRCA1-defective cells to gemcitabine via caspase-3/7-dependent apoptosis. (A) SUM149PT cells were treated with either BEZ235 or gemcitabine alone or in combination for 72 hr and MTT assays were performed to measure viable cells. Data are means $\pm$ SEMs from three independent experiments performed in triplicate. (*) indicates $P < 0.05$. (B) BEZ235 enhances the gemcitabine-induced caspase-3/7 activity in SUM149PT cells. SUM149PT cells were treated with BEZ235 and gemcitabine as indicated amounts for upto 48 hr. Caspase-3/7 activity was measured by colorimetric assays. Data are means $\pm$ SEMs from two independent experiments. Significant differences ($P < 0.05$) were found between all pairs except for control vs. 0.25 μM BEZ235.

Table 1

Breast cancer cell lines used in this study

Cell Line	Gene Cluster	BRCA1	PI3KCA	PTEN
MCF7	Luminal	Wild type	E545K	Wild type
MDA-MB-231	Basal	Wild type	Wild type	Wild type
SUM149PT	Basal	2288delT	Wild type	Gross mutation
HCC1937	Basal	5382insC	Wild type	Gross mutation
MDA-MB-436	Basal	5396+1G>A	Wild type	Gross mutation
Reference	21	22	23	24

Table 2

PI3K/AKT pathway inhibitors used in this study

Inhibitor	Major known targets and reported Biochemical IC50 (nM)							
	PI3K α	PI3K β	PI3K δ	PI3K γ	mTORC1	mTORC2	Reference	
PI-103	8	88	48	150	20	83	25	
PIK-75	58	1300	510	76	~1,000	~10,000	25	
BEZ235	4	75	7	5	20.7		26	