BRCA1-IRIS Overexpression Promotes Cisplatin Resistance in Ovarian Cancer Cells
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Abstract
Evasion of apoptosis plays a key role in cancer development, drug resistance, and recurrence. The BRCA1 locus product protein BRCA1-IRIS is overexpressed in several cisplatin-resistant ovarian cancer cell lines, but its relationship to resistance is uncertain. Here, we show that in human ovarian surface epithelial (HOSE) cells, overexpression of BRCA1-IRIS triggers expression of the antiapoptotic protein survivin. Negative modulation of phosphatidylinositol 3-kinase (PI3K) signaling or AKT silencing reduced survivin expression in this setting. Conversely, silencing BRCA1-IRIS in ovarian cancer cell lines derepressed PTEN expression along with the antiapoptotic AKT targets FOXO1 and FOXO3a, suppressing survivin expression. Cisplatin (≤50 μmol/L) exposure was sufficient to activate expression of the BRCA1-IRIS-AKT-survivin cascade in HOSE cells, whereas under similar conditions cisplatin failed to induce apoptosis in ovarian cancer cell lines expressing this regulatory cascade. Mechanistic investigations indicated that BRCA1-IRIS triggers survivin expression through a PI3K/AKT-dependent pathway involving NF-κB, but also through a PI3K/AKT-independent pathway involving PTEN, FOXO1, and FOXO3a. Our findings indicate how BRCA1-IRIS overexpression prevents chemotherapy-induced cell death by upregulating expression of survivin, and they highlight this regulatory cascade as a candidate focus to improve treatment of advanced drug-resistant ovarian cancers.

Introduction
Ovarian cancer is the fourth leading cause of cancer deaths worldwide (1). Cisplatin and its analogues are first-line chemotherapeutic agents for the treatment of human ovarian cancer (2, 3). Cisplatin promotes its cytotoxicity by forming DNA-protein cross-links, DNA mono-adducts, and intrastrand DNA cross-links, which all trigger apoptosis (4, 5). Although the mechanisms involved in cisplatin resistance are not yet fully understood (6), decreased cellular detoxication (7), increased DNA repair (8), p53 mutations (9), as well as defects in intracellular or extracellular survival/apoptotic pathways all have been implicated (10, 11).

Survivin is a bifunctional member of the inhibitor of apoptosis protein family (12) that participates in cell division besides being a suppressor of apoptosis (13). Survivin inhibits apoptosis through binding to caspase-3/7 and inhibits their functions (14). Altered survivin expression is common in many human cancers, such as ovarian, lung, colon, liver, prostate, and breast cancers (15–20), and reducing survivin level sensitizes tumor cells to anticancer drugs (21). Survivin expression is activated, in part, by AKT-dependent mechanism in many cell types, including ovarian cells (22).

AKT is a family of serine/threonine kinases activated in a variety of stimuli, including growth factors (23). AKT can suppress apoptosis by inhibiting proapoptotic proteins, such as BAD, caspase-9, and the transcription factor FKHRL1 (24, 25). AKT deregulation is also involved in the development of chemoresistance (24, 25). AKT2 is amplified in many human ovarian cancer cell lines and primary ovarian carcinomas (26–28), and inhibition of PI3K/AKT2 induces apoptosis in ovarian cancer cells that overexpress AKT2 (29).

BRCA1-IRIS is a recently discovered 1,399-residue BRCA1 locus splice variant (30). Although it and the full-length product of this locus, the tumor suppressor BRCA1/p220 (31, 32), share 1,365 residues, unlike BRCA1/p220, BRCA1-IRIS possesses oncogenic functions. BRCA1-IRIS induces DNA replication by inhibiting Geminin-negative function at DNA replication origins (30) and cell proliferation by upregulating cyclin D1 expression (33, 34). Furthermore, BRCA1-IRIS expression is high in multiple sporadic human breast and ovarian cancer cell lines, as well as in known BRCA1mutant/−cell lines, such as HCC1937 and SNU251 (35, 36).

In the present study, we show that BRCA1-IRIS overexpression triggers survivin expression and ovarian cell survival, in part through triggering the expression and activity of
AKT1, AKT2, and NF-κB and in part by repressing PTEN/FOXO1 and FOXO3a expression. This BRCA1-IRIS-AKT-survivin cascade was upregulated following treatment of human ovarian surface epithelial (HOSE) cells with low cisplatin concentrations and promoted resistance to cisplatin-induced killing. Furthermore, ovarian cancer cell lines endogenously overexpressing BRCA1-IRIS are more resistant to cisplatin. Our data suggest that BRCA1-IRIS overexpression is an important mediator of chemoresistance in ovarian cancer cells. We propose that combinatorial targeted inhibition of the BRCA1-IRIS-AKT-survivin pathway has the potential to enhance the effectiveness of chemotherapy in the treatment of ovarian cancer.

Materials and Methods

Cell culture

OV-90, OVCAR-3, and SK-OV-3 were obtained from the American Type Culture Collection, and IGROV-1, OVCAR-5, OVCAR-8, OVCAR-420, and MCAS were a gift from Drs. S. Mok or R. Drapkin (Harvard Medical School). Immortalized ovarian surface epithelial cell line (HOSE1) was kindly provided by Dr. Nelly Auersperg (University of British Columbia, Vancouver, British Columbia, Canada), and HOSE2 was a gift from Dr. S. Mok. These are normal HOSE cells immortalized with SV40 large T antigen and were grown in medium containing 45% Medium 199, 45% MCDB105, and 10% fetal bovine serum (FBS; HyClone). All other cell lines were grown in RPMI 1640 (Invitrogen) supplemented with 10% FBS. Where indicated, cisplatin/CDDP dissolved in DMSO (Novaplus, Ben Venue Laboratories, Inc.) was added.

HOSE cell line generation

Drs. P. Sellers and S. Korshmyer (Dana-Farber Cancer Institute), A. Abbas (University of California, San Francisco), and D. Alteri (University of Massachusetts) supplied us with the retroviral-expressing vectors HA-tagged Myr-AKT1, Myr-AKT2, and Myr-AKT3; Bcl-2; Bcl-XL; and survivin, respectively. Lentivirus BRCA1-IRIS–expressing vector was constructed by reverse transcription-PCR (RT-PCR) cloning into pLenti/V5-D-TOPO vector (Invitrogen). Recombinant viruses produced in 293T cells were immediately added to cells, and cell lines were selected with appropriate antibiotics.

Small interfering RNA transfection

BRCA1-IRIS small interfering RNA (siRNA) was described previously (30). AKT siRNA that targets AKT1 and AKT2 (not AKT3) and survivin siRNA were purchased from Cell Signaling Technology. Cells were transfected with Oligofectamine (Invitrogen) according to the manufacturer’s instructions.

Quantitative real-time RT-PCR

Real-time RT-PCR for BRCA1-IRIS, AKT1, AKT2, and survivin in some ovarian cancer cell lines. We show using real-time RT-PCR and immunoblotting that all ovarian cancer cell lines, except IGROV-1, express higher levels of BRCA1-IRIS mRNA and protein, respectively, but normal levels of BRCA1/p220 mRNA and protein (Fig. 1A and B). Because AKT and survivin are important inducers of drug resistance in ovarian cancer cells, we examined the levels of AKT (isoforms 1, 2, and 3) and survivin mRNAs and proteins in these ovarian cancer cell lines. AKT1, AKT2 (not AKT3), and survivin mRNAs and proteins were also high in all ovarian cancer cell lines, except IGROV-1 (Fig. 1A and B), implying co-overexpression of BRCA1-IRIS, AKT, and survivin in some ovarian cancer cell lines.

Immunoblotting

Cells treated with different concentrations of cisplatin or not were collected in PBS, sonicated three times, cleared by centrifugation at 10,000 × g for 10 minutes at 4°C, and immunoblotted using antibodies for survivin, AKT isoform specific antibody, PTEN (D4.3) XP, FOXO1 (L27), and FOXO3a (Cell Signaling Technology); Bcl-2 (clone 124; Dako Diagnostics AG); Bcl-XL (Transduction Laboratories); actin (Ab-1, CP01; Oncogene); and phospho-survivin (T34; Novus Biologicals). Mouse antibody against BRCA1-IRIS was generated in our laboratory.

MTT and activated caspase-3 and caspase-7 assays

MTT and activated caspase-3 and caspase-7 assays were done using CellTiter 96 AQone, One Solution Cell Proliferation Assay (Promega) and Caspase-Glo 3/7 Assay (Promega), respectively, as per the manufacturer’s instructions. Measurements were obtained using absorbance at 490 nm. Each experiment was done in eight samples, and the whole experiment was repeated three separate times.

Cell cycle analysis

Cell cycle analysis was carried out by flow cytometry after propidium iodide staining using standard protocol.

Results

BRCA1-IRIS, AKT1, AKT2, and survivin are overexpressed in ovarian cancer cell lines

Using total RNAs and proteins from two immortalized human surface epithelial cell lines (HOSE1 and HOSE2) and several ovarian cancers cell lines, we show using real-time RT-PCR and immunoblotting that all ovarian cancer cell lines, except IGROV-1, express higher levels of BRCA1-IRIS mRNA and protein, respectively, but normal levels of BRCA1/p220 mRNA and protein (Fig. 1A and B). Because AKT and survivin are important inducers of drug resistance in ovarian cancer cells, we examined the levels of AKT (isoforms 1, 2, and 3) and survivin mRNAs and proteins in these ovarian cancer cell lines. AKT1, AKT2 (not AKT3), and survivin mRNAs and proteins were also high in all ovarian cancer cell lines, except IGROV-1 (Fig. 1A and B), implying co-overexpression of BRCA1-IRIS, AKT, and survivin in some ovarian cancer cell lines.

Ectopic BRCA1-IRIS expression triggers AKT and survivin expression and activation

To test the hypothesis that BRCA1-IRIS triggers AKT/survivin expression in ovarian cells, HOSE1 and HOSE2 cell lines stably expressing BRCA1-IRIS (HOSE/IRIS), myristoylated HA-tagged AKT1, AKT2, and AKT3 (HOSE/AKT1, HOSE/AKT2, and HOSE/AKT3), or survivin (HOSE/survivin) were generated. The data in Fig. 2A to C represent results obtained using cell lines generated in HOSE1 cells; however, identical results were obtained using cell lines generated in HOSE2.
Compared with control, HOSE/survivin showed normal levels of BRCA1-IRIS and total AKT (detected using pan-antibody; Fig. 2A). HOSE/AKT1, HOSE/AKT2, and HOSE/AKT3 showed normal level of BRCA1-IRIS (Fig. 2B) but high levels of phospho-AKT (detected using a mixture of anti–phospho-T308 and anti–phospho-S473 antibodies), survivin, and phospho-survivin (detected using anti–phospho-T34 antibody; Fig. 2B). HOSE/IRIS, on the other hand, showed high levels of AKT1 and AKT2 (not AKT3), phospho-AKT, survivin, and phospho-survivin (Fig. 2C). Increase in AKT1, AKT2 (not AKT3), and survivin mRNA levels was also detected following BRCA1-IRIS overexpression in HOSE1 and HOSE2 cell lines (Supplementary Fig. S1A). Taken together, these data suggest that BRCA1-IRIS overexpression triggers AKT1, AKT2, and survivin expression and activation.

**BRCA1-IRIS–induced survivin expression is partially AKT dependent**

AKT (siRNA silences AKT1 and AKT2), BRCA1-IRIS, survivin, or AKT and BRCA1-IRIS were silenced in SK-OV-3 cells (endogenously overexpressing all proteins; see Fig. 1B) for 72 hours. Compared with control, AKT silencing had no effect on BRCA1-IRIS expression, but partially reduced survivin expression (perhaps due to BRCA1-IRIS–related effect; Fig. 2D). BRCA1-IRIS silencing reduced the expression of AKT1 and AKT2, and also only partially reduced survivin expression (perhaps due to an AKT related effect; Fig. 2E). Simultaneous silencing of BRCA1-IRIS and AKT, like survivin silencing, completely abolished survivin expression (Fig. 2F and G). Survivin silencing had no effect on BRCA1-IRIS or AKT expression (Fig. 2G). Identical results were obtained using MCAS cell line. Moreover, the levels of AKT1, AKT2 (not AKT3), and survivin mRNAs also decreased in SK-OV-3 and MCAS cells depleted from BRCA1-IRIS (Supplementary Fig. S1B).

Furthermore, AKT silencing in HOSE/IRIS cells (siRNA targets AKT1 and AKT2) for 72 hours, while completely abolishing AKT expression, only partially decreased survivin expression (Fig. 2H). These data suggest that BRCA1-IRIS–induced survivin expression is partially AKT dependent.

**BRCA1-IRIS overexpression–induced survivin expression is partially PI3K/AKT independent**

To study this further, MCAS or SK-OV-3 cells were exposed to the PI3K inhibitor LY294002 for 24 hours or were transfected with BRCA1-IRIS siRNA for 48 hours before they were exposed to LY294002 and left for another 24 hours. Compared with untreated cells, although LY294002 alone did not change the level of total AKT, it decreased the level of phospho-AKT (Fig. 3A) and survivin in both cell lines (Fig. 3A). In contrast, treatment of BRCA1-IRIS–silenced cells with LY294002 completely abolished survivin expression (Fig. 3A). These data suggest that BRCA1-IRIS–induced survivin expression is partially PI3K/AKT independent. Indeed, LY294002 decreased survivin expression in HOSE/vector cells but had little effect in HOSE/IRIS cells (Fig. 3B). Furthermore, our unpublished data show that silencing of BRCA1-IRIS in HOSE/AKT1 or HOSE/AKT2 cells (expression driven from heterologous promoter and proteins is myristoylated/activated) reduces the expression of the exogenous proteins as measured by HA immunoblotting. The mechanism is still under investigation.

Finally, in chromatin immunoprecipitation (ChIP) experiments using HOSE1 or SK-OV-3, we found that although BRCA1-IRIS was bound to the cyclin D1 promoter (known BRCA1-IRIS target; ref. 35), it was not bound to AKT1, AKT2, or survivin promoter (data not shown). Moreover, in cotransfection experiments, BRCA1-IRIS overexpression did not activate transcription from AKT1, AKT2, and survivin promoter-driven reporter plasmids (data not shown).
Taken together, we propose that BRCA1-IRIS–induced AKT1 and AKT2 are not transcriptional but posttranscriptional driven (e.g., promotes their mRNA and/or protein stabilities; see ref. 38), and that survivin expression is partially induced by BRCA1-IRIS–dependent/PI3K/AKT–independent mechanism.

**BRCA1-IRIS overexpression induces survivin expression through suppressing PTEN/FOXO1 and FOXO3a expression**

Besides inducing the expression of the known surviving transcription inducer, NF-κB (Fig. 3C; see also refs. 37, 38), BRCA1-IRIS overexpression in HOSE cells also reduced the expression of PTEN and its downstream survivin transcription suppressors, FOXO1 and FOXO3a (Fig. 3C; ref. 39). Moreover, BRCA1-IRIS silencing in SK-OV-3 (Fig. 3C) or MCAS (data not shown) cells derepressed the expression of these three proteins (Fig. 3C). It is possible that BRCA1-IRIS induces survivin expression by inducing AKT expression and activation or by suppressing PTEN/FOXO1 and/or FOXO3a expression. Indeed, when BRCA1-IRIS was silenced in equal number of HOSE/vector, HOSE/AKT1, or HOSE/AKT2 cells, the increase in cell number observed following AKT1 and AKT2 overexpression was dramatically reduced (Fig. 3D, gray columns). Cosilencing of PTEN restored the increase in cell number in AKT1- and AKT2-overexpressing cells (Fig. 3D, black columns), suggesting that BRCA1-IRIS silencing kills cells even when AKT1 or AKT2 is overexpressed, most likely due to increase in PTEN/FOXO1 and FOXO3a expression, leading to survivin expression decrease.

**Low cisplatin concentrations trigger BRCA1-IRIS-AKT-survivin expression in HOSE cells**

Ovarian cancer cells expressing high level of survivin usually are cisplatin resistant. To investigate whether BRCA1-IRIS overexpression induces acquired cisplatin resistance, HOSE cells were treated with increasing concentrations of cisplatin, and BRCA1-IRIS, AKT, and survivin expression was investigated. Compared with untreated cells, low concentrations of cisplatin (≤50 μmol/L) triggered BRCA1-IRIS, AKT, and survivin expression (Fig. 4A), whereas high concentrations (>50 μmol/L) abolished their expression (Fig. 4A). In FACScan analysis, no G2-M arrest was detected in HOSE cells treated with any of the cisplatin concentrations used (survivin expression increases in G 2-M cells; Supplementary Fig. S2). Thus, low cisplatin concentration–induced survivin expression must be due to the induction in AKT and/or BRCA1-IRIS expression by the same treatment.

Interestingly, whereas silencing of BRCA1-IRIS in HOSE cells completely abolished survivin induction by low cisplatin concentrations (Fig. 4B), AKT silencing only partially blocked survivin induction by low cisplatin concentrations in HOSE cells (Fig. 4B), reinforcing the view that BRCA1-IRIS–induced survivin expression is partially PI3K/AKT independent (see above). Finally, AKT and survivin were also activated by low cisplatin concentration treatment, because we observed phosphorylation of AKT on T308/S437, of its downstream target, GSK3β, on S9 (Fig. 4C; see ref. 40), and of survivin on T34 (an event that occurs by cdc2 in viable and proliferating cells; Fig. 4C; see ref. 41).
Ectopic BRCA1-IRIS expression protects HOSE cells from cisplatin-induced cell death

HOSE/vector, HOSE/IRIS, HOSE/AKT1, HOSE/AKT2, or HOSE/AKT3 cells were untreated or treated with low (35 μmol/L) or high (70 μmol/L) concentration of cisplatin for 24 hours, and the expression of survivin was measured by immunoblotting. In untreated HOSE/IRIS, HOSE/AKT1, and HOSE/AKT2, survivin levels were higher compared with HOSE/vector cell (Fig. 5A). As expected, treatment of HOSE/vector and HOSE/AKT3 cells with 35 μmol/L cisplatin increased survivin expression slightly, whereas 70 μmol/L cisplatin did not (Fig. 5A). In HOSE/IRIS cells, 35 μmol/L, and not 70 μmol/L, cisplatin increased BRCA1-IRIS and survivin expression (Fig. 5A). In HOSE/AKT1 and HOSE/AKT2 cells, 35 μmol/L cisplatin did not increase survivin expression and 70 μmol/L actually decreased it slightly (Fig. 5A). It is possible that BRCA1-IRIS and, to a lesser extent, AKT1 and AKT2 overexpression protects survivin from cisplatin-induced downregulation.

Furthermore, HOSE/vector, HOSE/IRIS, HOSE/AKT1, HOSE/AKT2, HOSE/AKT3, and HOSE/survivin were plated at equal numbers and incubated with 0, 5, 10, 20, 30, 50, or 100 μmol/L cisplatin for 48 hours followed by measurement of cell viability using MTT assay (Fig. 5C, solid lines) or cell death using activated caspase-3/-7 assay (Fig. 5C, dashed lines). HOSE/vector or HOSE/AKT3 cells were sensitive to high cisplatin concentrations, with IC50 of 15 to 20 μmol/L (Fig. 5C), which coincided with an increase in activated caspase-3/-7 (Fig. 5C). HOSE/IRIS, HOSE/AKT1, HOSE/AKT2, and HOSE/survivin were resistant to high cisplatin concentration-induced cell death, and the IC50 was ~75 μmol/L in these cell lines (Fig. 5C), with low yet measurable levels of active caspase-3/-7 detected (Fig. 5C). Taken together, BRCA1-IRIS overexpression protects HOSE cells from high cisplatin concentration-induced cell death by protecting survivin from cisplatin-induced downregulation.

Endogenous BRCA1-IRIS overexpression protects ovarian cancer cells from cisplatin-induced cell death

IGROV-1, MCAS, OVCAR-5, and SK-OV-3 cell lines were treated with 0, 35, or 70 μmol/L cisplatin for 24 hours. IGROV-1 cells showed no detectable expression of BRCA1-IRIS, AKT, or survivin proteins even after treatment with 35 or 70 μmol/L cisplatin (Fig. 5B). In contrast, untreated MCAS, OVCAR-5, and SK-OV-3 showed high levels of BRCA1-IRIS, AKT, and survivin proteins (Fig. 5B). Treatment with 35 μmol/L cisplatin increased BRCA1-IRIS in all cell lines (Fig. 5B), did not affect AKT expression in all cell lines.

Figure 3. BRCA1-IRIS silencing in ovarian cancer cell lines reduces AKT and survivin expression and phosphorylation. A, expression of the indicated proteins in MCAS and SK-OV-3 untreated, treated with 10 μmol/L LY294002 for 24 h, or transfected with BRCA1-IRIS siRNA first for 48 h and then treated with LY294002. B, expression of the indicated proteins in HOSE/vector or HOSE/IRIS treated or not with LY294002 (10 μmol/L) for 24 h. C, expression of indicated proteins in HOSE/IRIS (left) or SK-OV-3 cells after transfection with siLuc or siIRIS (72 h, right). D, viability of HOSE/vector, HOSE/AKT1, and HOSE/AKT2 cells following transfection with siIRIS or siIRIS + siPTEN. Columns, mean of 24 samples done in three different times; bars, SD. *, P < 0.05.
and AKT3-expressing cells (Fig. 6A, left), but had no such
35 μmol/L cisplatin (Cis) for 24 h. B, expression of the indicated proteins in HOSE cells or HOSE cells transfected
with siIRIS or siAKT for 48 h exposed to 0, 10, 30, and 50 μmol/L cisplatin for 24 h. C, expression of the indicated proteins in HOSE cells exposed to
0, 5, 10, 30, 50, or 100 μmol/L cisplatin for 24 h.

Figure 4. Cisplatin triggers BRCA1-IRIS, AKT, and survivin expression and activation in HOSE cells. A, expression of the indicated proteins in HOSE cells exposed to 0, 5, 10, 30, 50, or 100 μmol/L cisplatin (Cis) for 24 h. B, expression of the indicated proteins in HOSE cells or HOSE cells transfected
with siIRIS or siAKT for 48 h exposed to 0, 10, 30, and 50 μmol/L cisplatin for 24 h.

Identical numbers of these four cell lines were incubated
with 0, 5, 10, 20, 30, 50, or 100 μmol/L cisplatin for 48 hours
followed by MTT and active caspase-3/7 assays. As expected,
low viability and high level of active caspase-3/7 were detected
in IGROV-1 (Fig. 5C), showing an IC_{50} of ~5 μmol/L. On the other hand, MCAS, OVCAR-5, and SK-OV-3 cells expressing high level of BRCA1-IRIS-AKT-survivin were more resistant
to cisplatin-activated caspase-3/7 and cell death (Fig. 5C) and showed IC_{50} of ~25 μmol/L for OVCAR-5 and ~40 μmol/L for MCAS and SK-OV-3 cell lines. Taken together, these data suggest that higher level of BRCA1-IRIS-AKT1/AKT2 induces persistence survivin expression in the face of high concentrations of cisplatin and that it protects
ovarian cancer cells against cisplatin-induced cell death.

BRCA1-IRIS silencing sensitizes HOSE cells to cisplatin-induced survivin downregulation and cell death

If BRCA1-IRIS-AKT-survivin cascade were important to protect against cisplatin-induced cell death, one would expect that BRCA1-IRIS depletion would sensitize cells to cisplatin-induced loss of survivin expression and cell death. First, HOSE cell lines stably expressing two known survival factors (Bcl-2 and Bcl-XL; Fig. 6C, left; ref. 42) were generated. HOSE/IRIS, HOSE/AKT1, HOSE/AKT2, HOSE/AKT3, HOSE/Bcl-2, or HOSE/Bcl-XL cells were transfected with control (luciferase or scrambled IRIS siRNA) or BRCA1-IRIS-specific siRNA for 48 hours before treatment with DMSO or cisplatin (35 or 70 μmol/L) for an additional 24 hours.

BRCA1-IRIS silencing had no effect on the expression of BRCA1/p220 in HOSE/vector or actin expression in HOSE/AKT1, HOSE/AKT2, or HOSE/AKT3 cell lines (Fig. 6A, right and middle). Treatment of HOSE/vector cells with 35 μmol/L cisplatin induced BRCA1-IRIS expression, which was effectively blocked by BRCA1-IRIS siRNA but had no effect on BRCA1/p220 expression (Fig. 6A, right). BRCA1-IRIS silencing reduced survivin expression regardless of AKT1, AKT2, or AKT3 overexpression (Fig. 6A, left). Treatment with 35 μmol/L cisplatin induced survivin expression in vector and AKT3-expressing cells (Fig. 6A, left), but had no such effect in AKT1- and AKT2-expressing cells (Fig. 6A, left).

Presilencing of BRCA1-IRIS further reduced the level of survivin in all cell lines (Fig. 6A, left). Moreover, these effects were even more pronounced when cells were treated with 70 μmol/L cisplatin (Fig. 6A, left).

Furthermore, HOSE/Bcl-2 or HOSE/Bcl-XL cell lines were treated in the same manner except that we tested the effect of 35 μmol/L cisplatin only. Overexpression of Bcl-2 or Bcl-XL did not protect survivin against BRCA1-IRIS loss-induced, cisplatin-induced, or both loss- and cisplatin-induced downregulation (Fig. 6C, right). Moreover, an equal number of HOSE/vector, HOSE/Bcl-2, or HOSE/Bcl-XL cells were transfected with AKT, survivin, or BRCA1-IRIS siRNAs separately or in combination and then were treated or not with 35 μmol/L cisplatin followed by measurement of cell viability using MTT assay. Silencing of single molecule had little effect on HOSE/vector, HOSE/Bcl-2, or HOSE/Bcl-XL cell viability (Fig. 6D), whereas treatment of single-molecule silenced cells with cisplatin significantly reduced HOSE cell viability by ~25% (Fig. 6D). The combination of each two siRNAs reduced the viability by ~50% (Fig. 6D), suggesting that each molecule controls overlapping as well as distinct cell survival and/or proliferation pathways. Adding low concentration of cisplatin (35 μmol/L) to any of the siRNA combinations decreased cell viability even further to ~75% (Fig. 6D), suggesting that reducing BRCA1-IRIS, AKT, or survivin expression (or activity) kills more cells in combination with low cisplatin concentration.

BRCA1-IRIS downregulation sensitizes ovarian cancer cell lines to cisplatin-induced survivin downregulation and cell death

In addition to the intrinsically high levels of BRCA1-IRIS in ovarian cancer cells, their stress-induced upregulation of AKT and/or survivin in part through upregulation of BRCA1-IRIS likely enhances the resistance to cisplatin or other anticancer agents. To test this hypothesis, ovarian cancer cell lines MCAS, OVCAR-5, and SK-OV-3 were transfected with control or BRCA1-IRIS siRNAs for 72 hours and were exposed to DMSO or 35 μmol/L cisplatin during the last 24 hours. As expected BRCA1-IRIS silencing downregulated the expression of AKT1, AKT2, and survivin to various degrees in all cell lines (Fig. 6B). Cisplatin (35 μmol/L) alone...
modestly decreased the expression of these proteins (Fig. 6B), whereas the combination abolished the expression of all proteins in all cell lines (Fig. 6B).

Equal numbers of MCAS and SK-OV-3 cells were transfected with control or BRCA1-IRIS siRNAs for 72 hours and exposed to DMSO or 35 μmol/L cisplatin during the last 24 hours followed by MTT assay to measure cell viability. BRCA1-IRIS silencing or treatment with 35 μmol/L cisplatin decreased cell viability by 25% to 35% in both cell lines (Fig. 6D), and the combination decreased it by >65% (Fig. 6D), suggesting that reducing BRCA1-IRIS levels sensitizes ovarian cancer cells to low doses of cisplatin.

Discussion

Here, we showed that BRCA1-IRIS mRNA and protein are overexpressed in several ovarian cancer cell lines together with the mRNAs and proteins of AKT1, AKT2, and survivin, and that ectopic expression of BRCA1-IRIS in HOSE cells induced AKT and survivin protein levels, whereas silencing of BRCA1-IRIS in ovarian cancer cell lines, overexpressing BRCA1-IRIS, AKT, and survivin (25–27), reduced the expression of AKT (1 and 2) and survivin. PI3K inactivation failed to completely abolish survivin expression in BRCA1-IRIS–overexpressing HOSE cells, whereas silencing of BRCA1-IRIS cooperated with the PI3K inhibitor LY294002 in completely abolishing survivin expression in ovarian cancer cell lines overexpressing the protein, suggesting that BRCA1-IRIS activates survivin expression by a PI3K/AKT-dependent and PI3K/AKT-independent mechanisms or activates AKT by other pathways other than PI3K. Indeed, our data show that BRCA1-IRIS overexpression can induce survivin expression by upregulating the expression of NF-κB (37, 39) or by suppressing the expression of PTEN and its downstream transcription suppressors FOXO1 and FOXO3a (39). Other pathways, such as CKII (43), mTORC1 and mTORC2 (44, 45), or STAT-3 (46), can also be considered.

Deregulated survivin expression is associated with a negative prognosis in various cancer types (47, 48). Several mechanisms have been implicated, including amplification of survivin locus on 17q25, demethylation of the survivin exon 1, overexpression of H-Ras, and loss of p53 function (49, 50). We here add to this list overexpression of
Figure 6. BRCA1-IRIS silencing sensitizes ovarian cells to low cisplatin concentration–induced survivin downregulation and cell death. Expression of indicated proteins in HOSE/vector, HOSE/AKT1, HOSE/AKT2, and HOSE/AKT3 cells (A) or MCAS, OVCAR-5, and SK-OV-3 cells (B) transfected or not with BRCA1-IRIS siRNA and treated or not with 0, 35, or 70 μmol/L CDDP. C, generation of HOSE/Bcl-2 and HOSE/Bcl-XL cell lines (left) and expression of indicated proteins in HOSE/Bcl-2 and /Bcl-XL transfected or not with BRCA1-IRIS siRNA and treated with 0 or 35 μmol/L cisplatin (right). D, viability of HOSE/vector, HOSE/Bcl-2, or HOSE/Bcl-XL transfected with control, AKT, BRCA1-IRIS, or survivin siRNAs alone or in combination ± 35 μmol/L cisplatin. Columns, percentage of control of the mean of 24 samples in three different experiments; bars, SD. *, P < 0.05; **, P < 0.01; ***, P < 0.001. E, cell viability measured using MTT assay of MCAS and SK-OV-3 cells transfected or not with BRCA1-IRIS siRNA and treated with 0 or 35 μmol/L cisplatin. Columns, percentage of control of the mean of 24 samples in three different experiments; bars, SD. *, P < 0.05; **, P < 0.01.
BRCA1-IRIS and propose that this contributes, at least in part, to the development of aggressive and/or drug-resistant ovarian cancers. Many studies on clinical specimens have shown that survivin expression is associated with resistance to chemotherapy or radiation therapy, and linked to poor prognosis, suggesting that cancer cells survive with survivin. It is tempting to speculate that because BRCA1-IRIS overexpression upregulates survivin expression, it plays a fundamental role in the development of (acquired) drug resistance and/or recurrent ovarian cancer.

In fact, in the current study, the anticancer drug cisplatin reduced survivin expression in ovarian cancer cells only when administered at concentrations above 50 μmol/L. Concentrations below 50 μmol/L triggered survivin expression, which we showed following the upregulation in BRCA1-IRIS by the same treatment. These findings have significant clinical implication because they provide direct evidence that anticancer agents can itself trigger prosurvival events in ovarian cancer cells that attenuate their therapeutic efficacy. Further studies will be necessary to determine whether cisplatin upregulates BRCA1-IRIS-AKT-survivin expression cascade in vivo and whether this is implicated in the development of the deadly ovarian cancer recurrence.

Tumor cells differ from normal cells by aberrant cell cycle progression, resistant to growth-inhibitory signals and insensitive DNA damage checkpoints. These abnormalities can be the result of overexpression of a proto-oncogene(s) even in the face of reduced or absence of growth factors. Constitutive oncogene activation might play a key role in carcinogenesis and tumor progression; this phenomenon is known as oncogene addiction. BRCA1-IRIS-AKT-survivin pathway might represent such an oncogenic pathway in human ovarian cells. In fact, we showed that ovarian cancer cells overexpressing BRCA1-IRIS were more sensitive to cisplatin-induced cell death than HOSE cells, raising the interesting possibility that BRCA1-IRIS, at least in this setting, might act as a fate-determining factor because those ovarian cancer cells are already addicted to it. The question that remains to be answered is how BRCA1-IRIS expression is induced in cancer cell. Although speculative at the moment, our recent data\(^1\) show a cross talk between BRCA1/p220 mutation/low expression and BRCA1-IRIS overexpression by a posttranscriptional mechanism.

Our working hypothesis is that cells in an ovarian tumor mass are exposed to different concentration of any given drug, depending on factors such as the distance the drug travels in the circulation before reaching the mass, the diffusion rate of the drug to the inside of the mass, etc. It is likely that the cells in the inner core of this tumor mass are exposed to the least concentration of the drug. If this triggers BRCA1-IRIS overexpression followed by AKT and/or survivin overexpression, then this could lead to the production of drug-insensitive tumor cells that may initiate the recurrence.

Whether BRCA1-IRIS is a critical target for ovarian cancer chemointervention remains an open question. The fact that BRCA1-IRIS overexpression upregulated survivin expression in normal ovarian epithelial cells and survivin antipapoptic activity in ovarian cell lines is maintained by BRCA1-IRIS and/or AKT pathways, especially following treatment with a chemotherapeutic drug, adds support to that hypothesis. In this context, BRCA1-IRIS silencing should increase cell death in ovarian cancer cells and sensitized cells to apoptosis induced by low concentrations of cisplatin.

**Disclosure of Potential Conflicts of Interest**

W.M. ElShamy submitted a patent application. The other authors disclosed no potential conflicts of interest.

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