Loss of BRCA1 in the Cells of Origin of Ovarian Cancer Induces Glycolysis: A Window of Opportunity for Ovarian Cancer Chemoprevention

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Abstract

Mutations in the breast cancer susceptibility gene 1 (BRCA1) are associated with an increased risk of developing epithelial ovarian cancer. However, beyond the role of BRCA1 in DNA repair, little is known about other mechanisms by which BRCA1 impairment promotes carcinogenesis. Given that altered metabolism is now recognized as important in the initiation and progression of cancer, we asked whether the loss of BRCA1 changes metabolism in the cells of origin of ovarian cancer. The findings show that silencing BRCA1 in ovarian surface epithelial and fallopian tube cells increased glycolysis. Furthermore, when these cells were transfected with plasmids carrying deleterious BRCA1 mutations (5382insC or the P1749R), there was an increase in hexokinase-2 (HK2), a key glycolytic enzyme. This effect was mediated by MYC and the STAT3. To target the metabolic phenotype induced by loss of BRCA1, a drug-repurposing approach was used and aspirin was identified as an agent that countered the increase in HK2 and the increase in glycolysis induced by BRCA1 impairment. Evidence from this study indicates that the tumor suppressor functions of BRCA1 extend beyond DNA repair to include metabolic endpoints and identifies aspirin as an ovarian cancer chemopreventive agent capable of reversing the metabolic derangements caused by loss of BRCA1. Cancer Prev Res; 10(4); 255–66. © 2017 AACR.

Introduction

Breast cancer susceptibility gene 1 (BRCA1) was cloned in 1994. Since then, the relationship between germline mutations in this gene and a predisposition to ovarian cancer have become clear. Carriers of a BRCA1 mutation have up to a 46% risk of developing ovarian cancer by age 70 (1); in contrast, the general population lifetime risk of ovarian cancer is only 1.4% (2). Unfortunately, screening for ovarian cancer is ineffective (3); therefore, to prevent ovarian cancer, clinical guidelines recommend that BRCA1 mutation carriers have their fallopian tubes and ovaries removed between the ages of 35 and 40 years (4). To date, nonsurgical strategies for ovarian cancer prevention have been limited to the use of oral contraceptive pills, which reduce ovarian cancer risk by 50% with five years of use (5).

Given that neither of these options is optimal for all patients, increased understanding of the functions of BRCA1 might elucidate new approaches for ovarian cancer prevention. In cells lacking BRCA1, double-stranded DNA breaks are repaired by error-prone nonhomologous end joining, leading to chromosomal instability (6). Impairment of homologous recombination is one means by which BRCA1 loss promotes carcinogenesis; however, there are other functions of BRCA1 that are less understood (7). Just as our understanding of cancer has moved beyond a focus on the fidelity of the genome, so may our understanding of BRCA1.

Metabolism is one of the newest frontiers in cancer biology. The observation that cancer cells have altered metabolism dates back to the 1920s, when the German Nobel laureate, Otto Warburg, reported that cancer cells preferentially utilize the inefficient system of glycolysis to produce ATP, in contrast to normal cells, which use highly efficient mitochondrial respiration (8). The reliance of cancer cells on aerobic glycolysis, even in the presence of oxygen, is now termed the "Warburg effect" and the reprogramming of metabolism in cancer cells has been recognized as a hallmark of cancer (9, 10). In pancreatic cancer, the Warburg effect has been shown to be an early event in carcinogenesis, induced by activation of oncogenic Kras (11). Others have shown that suppression of the Warburg effect with a liver-X-receptor (LXR) inverse agonist inhibits growth of colon cancer (12). Moreover, metabolic alterations in the tumor microenvironment, as well as the intrinsic metabolism of the cancer cell, can contribute to carcinogenesis (13). In ovarian cancer, we have reported that...
metabolic alterations in cancer cells allow them to scavenge energy substrates from adjacent adipocytes to fuel tumor growth (14).

The role of BRCA1 in metabolism is unknown and, with the exception of a limited number of studies, has not been investigated. In vitro, metabolite profiling of breast cancer cell lines indicated that mutations in BRCA1 reprogram metabolism (15). In MCF7 breast cancer cells, BRCA1 suppresses lipid synthesis through binding to phosphorylated acetyl coenzyme A carboxylase alpha (ACCoA; ref. 16). In a study of BRCA1 mutation carriers, Friebel and colleagues showed that weight loss was associated with a reduced risk of breast cancer (17). While the data are limited, in combination, given these studies (15–17) and with the important role of glycolysis in tumorigenesis, we reasoned that BRCA1 impairment could induce metabolic changes that promote the initiation of cancer. We, therefore, investigated whether the loss of BRCA1 alters the metabolic phenotype in the cells that are thought to be the origin of ovarian cancer, and whether these metabolic changes could be exploited as a means of ovarian cancer prevention.

Materials and Methods

Patient samples and primary fallopian tube isolation

Tissue specimens were collected under protocols approved by the Institutional Review Board at the University of Chicago (Chicago, IL) with written consent from the patients. Primary fallopian tube secretory epithelial cells (FTEC) were isolated and cultured as described previously (18). To isolate fallopian tube stromal cells (FTSC), fallopian tube tissue was minced with dissectors and digested on an orbital shaker with hyaluronidase (Worthington Biochemical, 100 U) and collagenase type 3 (Worthington Biochemical, 1,000 U) in 20-mL PBS for 6 hours (Worthington Biochemical, 100 U) and collagenase type 3 (Worthington Biochemical, 1,000 U) in 20-mL PBS for 6 hours at 37°C. FTSCs were plated in DMEM supplemented with 10% FBS, 100 U/mL penicillin, 100 μg/mL streptomycin, nonessential amino acids, and vitamins.

Reagents, plasmids, and cell lines

The IOSE397 cell line was provided by Dr.nelly Auersperg (University of British Columbia) and the fallopian tube cells (FT33-TAg and FT33-MYC; ref. 18) were provided by Dr. Ronny Drapkin (University of Pennsylvania). The TYK was from Dr. Gottfried Konecny (UCLA Medical Center). The HeyA8 ovarian cancer cell line was provided by Dr. Gordon Mills (MD Anderson Cancer Center). The CAOV3, Kuramochi, and SNU119 ovarian cancer cell line was provided by Dr. Gordon Mills (MD Anderson Cancer Center). The CAOV3, Kuramochi, and SNU119 ovarian cancer cell lines were purchased from ATCC, respectively. The MCF7 and HCC1937 breast cancer cell lines were purchased from ATCC, the Japanese Collection of Research Bioresources Cell Bank, and the Korean Cell Line Bank, respectively. The MCF7 and HCC1937 breast cancer cell lines were purchased from ATCC. All cell lines were genotyped to confirm their authenticity (IDEXX Bioresearch short tandem repeat marker profiling every 3 months; all cell lines last validated January-July 2016). AllStars negative control siRNA and siBRCA1 oligos were obtained from Qiagen. Hs_BRCA1_2 FlexiTube siRNA is designated siBRCA1 no. 1 and Hs_BRCA1_25 FlexiTube siRNA is designated siBRCA1 no. 2. siBRCA1 no. 2 is used unless otherwise noted. pcDNA BRCA1 siRNA and pcDNA BRCA1 siRNA were purchased from Qiagen. Hs_BRCA1_2 FlexiTube siRNA is designated siBRCA1 no. 1 and Hs_BRCA1_25 FlexiTube siRNA is designated siBRCA1 no. 2. siBRCA1 no. 2 is used unless otherwise noted. pcDNA BRCA1 P1749R were described previously (17,19). pcDNA BRCA1 5382insC, and pcDNA BRCA1 P1749R were transfected with siRNA and 24 hours after transfection, cell viability was determined by MTT assay with 0.5 mg/mL thiazolyl blue tetrazolium bromide (Sigma-Aldrich), as described previously (19).

Cell viability assays

Cells were plated in 96-well plates in quintuplicate overnight and subsequently treated as indicated. Cell viability was determined via a MTT assay with 0.5 mg/mL thiazolyl blue tetrazolium bromide (Sigma-Aldrich), as described previously (19).

Immunoblotting

For Western blots, lysates were prepared and immunoblotting was performed as described previously (20). Coimmunoprecipitation was performed with rabbit polyclonal MYC antibody [c-MYC antibody (9E10), Santa Cruz Biotechnology] or mouse monoclonal HA antibody (16B12, Covance) using protein G agarose (Roche). Intensity of bands were quantified using the ImageJ software (NIH, Bethesda, MD).

Metabolic assays

Glycolysis, glycolytic capacity (extracellular acidification rate, ECAR), and oxidative phosphorylation (OXPHOS) were measured with the Seahorse Extracellular Flux (XF-96) analyzer (Seahorse Bioscience) as described previously (20). The glucose uptake assay was performed as described previously (19). Briefly, cells were transfected with siRNA and 48 hours after transfection, glucose uptake was determined using a fluorescein labeled glucose analogue, 2-deoxy-2-[7-nitro-2,1,3-benzoxadiazol-4-yl]amino]-D-glucose (2-NBDG; Cayman Chemical Company), according to the manufacturer's instructions. Relative fluorescence was normalized to protein concentration.

Fatty acid oxidation was assessed using a previously described method (14), with minor modifications. Briefly, cells were cultured in media containing [9,10(n)-3H] palmitic acid. 3H2O secreted into the medium was quantified by scintillation counting and normalized to the cellular protein content in each well. To measure NADPH/NADP+ 48 hours after siRNA transfection, NADPH and NADP+ were measured using NADP/NADPH-Glo Assay (Promega) according to the manufacturer’s protocol.

IHC

Samples were formalin-fixed, paraffin-embedded, sectioned, and mounted on slides. For IHC, slides were deparaffinized and incubated with anti-HK2 (Cell Signaling Technology) at 1:200 dilution. Antigen retrieval was performed using 1 mol/L sodium citrate in a rice cooker for 30 minutes. Slides were stained using the VECTASTAIN Elite ABC Kit (Vector Laboratories) and counterstained with hematoxylin. Intensity of staining was determined by gynecologic pathologists (S.M. McGregor and R.R. Lastra).

Promoter luciferase assay

A dual luciferase assay was performed according to the manufacturer’s protocol (Promega). 293T cells were transfected with BRCA1 siRNA and 24 hours later cotransfected with Firefly luciferase and pRL-SV40 using Lipofectamine 2000 (Invitrogen). The dual luciferase assay was done 48 hours after BRCA1 siRNA transfection and 24 hours after luciferase construct transfection. All experiments were performed in triplicate and normalized to Renilla luciferase activity.

Chromatin immunoprecipitation

Fragmented chromatin was immunoprecipitated using a chromatin immunoprecipitation (ChIP) Assay Kit (EMD Millipore). 293T cells transfected with pBabe or pBabe HA BRCA1 were cross-linked by formaldehyde. Fragmented chromatin was immunoprecipitated with rabbit polyclonal HA (Y-11; Santa Cruz Biotechnology) or rabbit IgG (Santa Cruz Biotechnology). The qPCR...
**Figure 1.**

**BRCA1** mutations increase glycolysis in IOSE and fallopian tube (FT) cells. 

**A.** Glycolysis. IOSE cells were transfected with plasmids carrying deleterious **BRCA1** mutations (P1749R or 5382insC) and the glycolytic response to pharmacologic inhibitors of metabolism (1, 2, and 3) was determined using a Seahorse SF96 Extracellular Flux Analyzer. ECAR for 3 groups are shown. Bar graph represents glycolysis and glycolytic capacity. 

**B.** Glycolysis. ECAR measured in IOSE cells with **BRCA1** silenced using siRNA. 

**C.** Glycolysis. ECAR measured in immortalized fallopian tube cells transfected with a **BRCA1** siRNA. 

1, glucose 10 mmol/L; 2, oligomycin 1 mmol/L; 3, 2-deoxyglucose 100 mmol/L treatments; n = 5/group. Cont, control. 

**D.** Glucose uptake assay. The uptake of fluorescently labeled deoxyglucose analogue (2-NBDG) was measured by fluorescent plate reader in IOSE and fallopian tube cells transfected with **BRCA1** siRNA. **y**-axis, fluorescence units normalized by protein concentration; n = 3. P values were calculated using an unpaired t test. Data in **A–D** represent mean value ± SD. *, P < 0.05; ***, P < 0.01; ****, P < 0.001; or *****, P < 0.0001 between the indicated values calculated using an unpaired t test; N.S., not significant.
was performed using SYBR Select Master Mix (Applied Biosystems). The primer sequences for the ChIP assay were described previously (21).

Statistical analysis

Specific analyses performed for each assessment are described in the figure legends. In all analyses, data were evaluated using a two-tailed t test or Fisher exact test; \( P < 0.05 \) was considered statistically significant (GraphPad).

Results

Mutations in *BRCA1* increase aerobic glycolysis in ovarian surface epithelial and fallopian tube cells

To determine whether the mutations in *BRCA1* alter metabolism, glycolysis was measured in noncancerous immortalized ovarian surface epithelial (IOSE) cells transfected with plasmids carrying deleterious *BRCA1* mutations, 5382insC or P1749R, mutations associated with increased risk of breast and ovarian cancer (22). The results showed increased glycolytic activity (glycolysis and glycolytic capacity) in IOSE cells transfected with either of the *BRCA1* mutations when compared with mock-transfected cells (Fig. 1A). Glycolytic reserve was not significantly increased (average glycolytic reserve: mock, 25.0 mpH/min; *BRCA1* P1749R, 30.6 mpH/min; and *BRCA1* 5382insC, 31.9 mpH/min; \( P > 0.05 \)). To confirm that loss of *BRCA1* increases glycolytic activity, *BRCA1* was silenced in IOSE cells using siRNA and efficiency of the knockdown was confirmed (Supplementary Fig. S1A and S1B). The results showed that IOSE cells with *BRCA1* silenced had increased aerobic glycolysis as compared with control-transfected cells (Fig. 1B; Supplementary Fig. S2). Recent studies suggest that ovarian cancer may actually originate from the fallopian tube (23, 24), rather than from ovarian surface epithelial cells; therefore, the next group of experiments tested whether loss of *BRCA1* in immortalized fallopian tube cells increased aerobic glycolysis. As with IOSE cells, immortalized noncancerous fallopian tube cells with *BRCA1* knockdown had elevated glycolytic activity (Fig. 1C). Moreover, glucose uptake was increased in both IOSE and fallopian tube cells with *BRCA1* knockdown (Fig. 1D).

*BRCA1* loss increases a key regulator of glycolysis, hexokinase-2

To understand how loss of *BRCA1* increase glycolysis, a screen of several kinases in the glycolytic pathway was performed including known major glycolytic enzymes in cancer cells. Hexokinase-2 (HK2) and pyruvate kinase muscle isoenzyme type 2 (PKM2). The findings show that knockdown of *BRCA1* increased HK2 protein and mRNA levels in IOSE and immortalized fallopian tube cells, whereas other regulators of glycolysis, including HIF1α and HK1, were not significantly changed (Fig. 2A and B; Supplementary Fig. S3). HK2 expression was also higher in cells transfected with plasmids carrying either the 5382insC or the P1749R deleterious *BRCA1* mutations when compared with mock-transfected cells (Fig. 2C). Next, we tested whether reexpressing *BRCA1* would reverse the increase in HK2 expression induced by *BRCA1* loss. In both IOSE (left) and fallopian tube cells (right), the increased HK2 expression induced by loss of *BRCA1* was reversed when *BRCA1* was reconstituted (Fig. 2D; lane 3 vs. 4).

The p53 mutation in the immortalized IOSE and fallopian tube cells could alter metabolism (25); therefore, nonimmortalized primary human FTECs and FTSCs were also tested. Consistent with the findings in immortalized cells, both primary human fallopian tube cell types transfected with *BRCA1* siRNA demonstrated higher HK2 expression compared with control-transfected cells (Fig. 2E). The next experiments were aimed at testing whether, like in noncancerous IOSE and fallopian tube cells, loss of *BRCA1* induces metabolic changes in ovarian cancer cells. Three cell lines that represent high-grade serous ovarian cancer (TYK-nu, SNU119, and Kuramochi) were selected: all have wild-type *BRCA1* (26). As the case with the IOSE and fallopian tube cells, knockdown of *BRCA1* in the TYK-nu and SNU119 ovarian cancer cell lines resulted in increased HK2 expression (Fig. 2F). In the Kuramochi ovarian cancer cell line, baseline HK2 expression was very high and not increased further by *BRCA1* knockdown (Supplementary Fig. S4A) and HK2 mRNA levels were increased with one of the siRNA *BRCA1* transfections (Supplementary Fig. S4B). Overall, these findings indicate that in both noncancerous IOSE and fallopian tube cells and in ovarian cancer cell lines *BRCA1* loss increases HK2 expression.

Loss of *BRCA1* has diverse metabolic consequences

To determine whether the loss of *BRCA1* induces changes beyond glycolysis, several other metabolic pathways were evaluated. Fatty acid oxidation activity was noted to be lower in IOSE and fallopian tube cells with a *BRCA1* knockdown compared with control transfected cells (Fig. 3A). Consistent with this finding, the mRNA levels of carnitine palmitoyl transferase 1 (CPT1), the rate-limiting enzyme of fatty acid oxidation, were also decreased in IOSE and fallopian tube cells with a *BRCA1* knockdown (Fig. 3B). Evaluation of fatty acid synthase (FASN) after *BRCA1* silencing showed a slight increase in FASN mRNA levels in IOSE cells and no change in FASN mRNA in fallopian tube cells (Fig. 3C). Consistent with our finding that *BRCA1* appears to have a minimal impact on fatty acid synthesis, silencing *BRCA1* did not change phosphorylated ACC or AMPK, an ACC substrate (Supplementary Fig. S3). Cellular NADPH production was also evaluated in IOSE and fallopian tube cells with silenced *BRCA1*, and NADPH was found to be elevated in cells with *BRCA1* knockdown (Fig. 3D). Two measures of mitochondrial OXPHOS, basal and maximal respiration, were elevated in the fallopian tube cells with *BRCA1* knockdown, but not in IOSE cells (Fig. 3E). Together, these findings indicate that silencing *BRCA1* increases glycolysis, reduces fatty acid oxidation, increases NADPH production, and upregulates OXPHOS, providing multiple metabolic pathways by which loss of *BRCA1* could increase ATP and anabolic substrate production in IOSE and fallopian tube cells.

*BRCA1* impairment activates HK2 via MYC and STAT3

Having identified glycolysis as the primary metabolic pathway altered by *BRCA1* loss, the next experiments tested the molecular mechanism mediating this effect. Because *BRCA1* is known to regulate transcription through chromatin remodeling by histone deacetylase (HDAC1 and HDAC2; ref. 27), the possibility of direct transcriptional regulation of HK2 by *BRCA1* was investigated. ChIP assays performed in HEK293T cells transfected with non-mutated *BRCA1* indicated that the *BRCA1* protein did not bind to the HK2 promoter (Fig. 4A) and co-immunoprecipitation assays demonstrated no direct binding of the *BRCA1* and HK2 proteins (Supplementary Fig. S5). Next, HK2 promoter activity was evaluated using luciferase assays. The results show increased HK2 promoter activity in cells with *BRCA1* silenced compared with
Figure 2.
BRCA1 impairment upregulates the glycolytic enzyme HK2. **A**, Immunoblot for glycolytic enzymes. IOSE and fallopian tube cells were transfected with BRCA1 siRNA and the expression of kinases involved in glycolysis were measured. **B**, qRT-PCR. IOSE and fallopian tube cells were transfected with BRCA1 siRNA and the mRNA levels of BRCA1, HK2, and HIF1A were measured. Results expressed as relative quantity over GAPDH control gene. n = 3. **C**, Immunoblot for HK2 and BRCA1. 293T cells were transfected with pcDNA mock, BRCA1 P1749R, or BRCA1 5382insC plasmids and Western blotting analysis for designated protein was completed 48 hours after transfection. **D**, Immunoblot for HK2 and BRCA1. BRCA1 was silenced in IOSE or fallopian tube cells by transfection of 3’-untranslated region (UTR) targeted BRCA1 siRNA then BRCA1 was reconstituted by transfecting cells with BRCA1 (pBabe-BRCA1). Control is mock (pBabe). Immunoblotting for BRCA1 and HK2 was performed after BRCA1 silencing with siRNA and again after restoration of BRCA1 with pBabe-BRCA1 transfection. The results show that silencing BRCA1 increased HK2 expression (lane 1 vs. 3) and that restoration of BRCA1 rescues HK2 upregulation induced by BRCA1 knockdown (lane 3 vs. 4). Note, exogenous BRCA1 is not entirely abolished by the siBRCA1 used here because the siRNA targets 3’-UTR. **E**, Immunoblot for HK2 and BRCA1 in primary human fallopian tube cells. Primary human FTECs, passage = 0 and FTSCs, passage = 1 were extracted from a patient and BRCA1 was silenced using siRNA. Western blotting for designated protein was completed 48 hours after transfection. **F**, Immunoblot in ovarian cancer cell lines. Two different epithelial ovarian cancer cell lines (TYK-nu and SNU119) were transfected with two different BRCA1 siRNAs and the expression of HK2 and BRCA1 was determined by immunoblotting. Data in “**B**” represent mean value ± SD; ��, P < 0.001; ���, P < 0.0001; or ���, P < 0.0001 between the indicated values calculated using an unpaired t test. N. S., not significant. HIF1α, hypoxia-inducible factor 1-alpha, PDHK1, pyruvate dehydrogenase kinase type 1.
**Figure 3.**

BRCA1 knockdown induces diverse metabolic changes in IOSE and fallopian tube cells. 

**A**, Fatty acid oxidation assay. IOSE and fallopian tube cells were transfected with two different BRCA1 siRNAs and [9,10-(n-3H)] palmitate oxidation converted to 3H2O was quantified by scintillation counting. (negative control: etomoxir), n = 3.

**B**, qRT-PCR. IOSE and fallopian tube cells were transfected with two different BRCA1 siRNAs. mRNA expression of the rate-limiting fatty acid oxidation enzyme, CPT1 was measured. Results expressed as relative quantity over GAPDH control gene. n = 3. **C**, qRT-PCR. IOSE and fallopian tube cells were transfected with two different BRCA1 siRNAs. mRNA expression of the FASN which catalyzes fatty acid synthesis was measured. n = 3. **D**, NADPH and NADH+ measurements. NADPH and NADP+ were measured using NADP/NADPH-Glo Assay (Promega) in IOSE and fallopian tube cells after BRCA1 knockdown. n = 4. **E**, Mitochondrial function. OXPHOS was evaluated by determining the oxygen consumption rate (OCR) in IOSE and fallopian tube cells with BRCA1 knockdown using a Seahorse SF96 Extracellular Flux Analyzer. Bar graph represents basal respiration and maximal respiration. 1, oligomycin 1 μmol/L; 2, FCCP 0.5 μmol/L; 3, antimycin A 1 μmol/L plus rotenone 1 μmol/L treatment; n = 5. Data in A–E represent mean value ± SD. *, P < 0.05; **, P < 0.01; ***, P < 0.001; or ****, P < 0.0001 between the indicated values calculated using an unpaired t test; N.S., not significant.
Figure 4.
BRCA1 impairment upregulates HK2 via MYC and STAT3. A, ChIP. To test for binding of BRCA1 to the HK2 promoter, HEK293T cells were transfected with BRCA1 and ChIP assays with qRT-PCR were performed using an anti-HA antibody (or rabbit IgG as a specificity control). Relative quantitation of input DNA (not immunoprecipitated) is shown. Primers were selected from across the HK2 promoter (schematic). B, HK2 promoter luciferase assay. A luciferase reporter gene assay was used to evaluate activity following silencing BRCA1 in HEK293T. BRCA1 was silenced in HEK293T cells followed by cotransfection with a HK2 gene promoter luciferase vector and a Renilla luciferase vector to normalize transfection efficiency. Relative luciferase activity (RLU) was calculated; n = 3. C, qRT-PCR. IOSE and fallopian tube cells were transfected with two different BRCA1 siRNAs and mRNA levels of MYC were measured; n = 3. D, Immunoblot. IOSE and fallopian tube cells were transfected with BRCA1 siRNA and the expression of BRCA1, MYC, and pSTAT3 measured. E, Immunoblot. HEK293T cells were transfected with pcDNA mock, BRCA1 P1749R, or BRCA1 5382insC plasmids and Western blotting analysis for the indicated proteins completed. F, Coimmunoprecipitation of STAT3 with BRCA1. HEK293T cells were transiently transfected with HA-BRCA1–expressing construct or empty vector. Proteins were precipitated by anti-HA antibody and examined by immunoblot using Abs against STAT3 and BRCA1. G, HK2 promoter assay. BRCA1 was silenced in HEK293T cells and luciferase reporter gene assay was used to measure HK2 promoter activity using a HK2 promoter construct after treatment with designated doses of a STAT3 inhibitor (Stattic), a MYC inhibitor (10058-F4), or a HIF1α inhibitor (PX-478) for 24 hours. n = 3. Data in A–C, G represent mean value ± SD. *, P < 0.05; **, P < 0.01; ***, P < 0.0001 between the indicated values using an unpaired t test; N.S., not significant.
control cells (Fig. 4B). Together, these findings indicate that silencing BRCA1 upregulates HK2 activity via a transcription factor, rather than through the direct binding of BRCA1 to the HK2 promoter or through direct interactions between the BRCA1 and HK2 proteins.

The transcriptional regulation of HK2 is primarily driven by HIF1α (28), MYC (21), and STAT3 (29). HIF1α was not upregulated by BRCA1 knockdown (Fig. 2A and B), but IOSE and fallopian tube cells with BRCA1 silenced did have increased MYC mRNA levels (Fig. 4C) and increased protein levels of MYC and phosphorylated STAT3 (pSTAT3; Fig. 4D). MYC and pSTAT3 were also increased in HEK293T cells transfected with plasmids carrying deleterious BRCA1 mutations (5382insC or P1749R) compared with mock-transfected cells (Fig. 4E). Similar findings were noted in breast cancer cell lines (Supplementary Fig. S6A and S6B). Coimmunoprecipitation experiments showed binding of BRCA1 to STAT3 (Fig. 4F) and others have also reported binding of MYC to BRCA1 (30). Consistent with the finding that MYC and STAT3 activate HK2 in BRCA1 knockdown cells, HK2 promoter activity induced by knockdown of BRCA1 was partially reversed by either a STAT3 (Stat3c) or a MYC (10058-F4) inhibitor, whereas a HIF1α inhibitor (PX-478) had no effect (Fig. 4G).

Aspirin counteracts the increase in glycolysis induced by BRCA1 mutations

The metabolic phenotype induced by BRCA1 loss may represent a window of opportunity for chemoprevention; therefore, we sought to identify agents that could reverse this phenotype by decreasing HK2 expression and glycolysis. Using a drug repurposing approach, several drugs that are used for noncancer indications and are reported to be protective against ovarian cancer were tested (Fig. 5A; refs. 20, 31–33). IOSE cells were treated with the candidate chemopreventive agents and glycolysis, as well as the expression of HK2, MYC, pSTAT3, and HIF1α, measured. Two agents, aspirin and luteolin, induced a dose-dependent decrease in the protein levels of HK2 and reduced MYC expression (Fig. 5B). Glycolytic activity was decreased in IOSE and immortalized fallopian tube cells treated with aspirin compared with control-treated cells (Fig. 5C and D). Two other drugs also suppressed glycolysis, but these agents either did not change HK2 expression (lovastatin) or increased it (resveratrol; Supplementary Fig. S7A and S7B) and were not evaluated further.

HK2 has been reported to be necessary for oncogenic transformation (34); therefore, suppressing HK2 may protect against ovarian cancer. Consistent with this, treatment with an HK2 inhibitor [bromopyruvic acid (3-BP); ref. 35] induced dose-dependent toxicity in MYC-transformed fallopian tube secretory cells (FT33-MYC) and in the ovarian cancer cell lines, TKYnu and Kuramochi (Fig. 6A). As 3-BP is not approved for use by noncancer indications and are reported to be protective against ovarian cancer were tested (Fig. 5A; refs. 20, 31–33). IOSE cells were treated with the candidate chemopreventive agents and glycolysis, as well as the expression of HK2, MYC, pSTAT3, and HIF1α, measured. Two agents, aspirin and luteolin, induced a dose-dependent decrease in the protein levels of HK2 and reduced MYC expression (Fig. 5B). Glycolytic activity was decreased in IOSE and immortalized fallopian tube cells treated with aspirin compared with control-treated cells (Fig. 5C and D). Two other drugs also suppressed glycolysis, but these agents either did not change HK2 expression (lovastatin) or increased it (resveratrol; Supplementary Fig. S7A and S7B) and were not evaluated further.

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Discussion

There is increasing evidence that metabolic derangements facilitate cancer growth (10) and it is well-known that mutations in BRCA1 significantly increase the risk of ovarian cancer. In this study, to identify novel means by which BRCA1 loss promotes carcinogenesis, we asked whether the loss of BRCA1 induces protumorigenic metabolic changes in the two cell types thought to be the origin of ovarian cancer (fallopian and ovarian surface epithelial cells). The main finding is that loss of BRCA1 in ovarian surface epithelial and fallopian tube cells increases glycolysis through HK2 activation. With the goal of improving care for patients who carry BRCA1 mutations, we next asked whether the metabolic changes induced by loss of BRCA1 could be reversed by chemopreventive agents. Using a drug-repurposing approach, we found that aspirin reverses the metabolic derangements caused by loss of BRCA1.

The experiments presented here show that glycolytic activity is significantly increased in ovarian surface epithelial cells that are transfected with either the 5382insC or the P1749R deleterious BRCA1 mutation (Fig. 1) and in fallopian tube cells with BRCA1 silenced. To the best of our knowledge, this is the first report describing metabolic changes induced by BRCA1 mutations in noncancerous cells of origin of ovarian cancer. In fact, little is known about BRCA1’s role in metabolism in normal tissue. In cardiomyocytes, loss of BRCA1 has been reported to reduce glucose levels and fatty acid oxidation (36) and in skeletal muscle, BRCA1 loss reduces fatty acid synthesis (37). Menendez and colleagues use targeted metabolomics to show that breast epithelial cells transfected with a BRCA1 mutation (185delAG) undergo metabolic reprogramming to increase anabolic processes, including the tricarboxylic acid cycle and lipogenesis (38). Our findings add to these studies by demonstrating in ovarian cancer that loss of BRCA1 decreases fatty acid oxidation and increases NADPH and MYC expression, indicating that BRCA1 impairment may also upregulate the pentose phosphate pathway and glutaminolysis. The most prominent finding in the IOSE and fallopian tube cells is that when BRCA1 is silenced glycolytic activity is significantly increased.

To understand how loss of BRCA1 increases glycolytic activity, we evaluated several kinases in the glycolytic pathway and found that enhanced HK2 activity was central to the process. Elevated HK2 expression was noted in ovarian surface, fallopian, ovarian cancer, and breast cancer cells when BRCA1 was silenced. We found that silencing BRCA1 induces the MYC and STAT3 transcription factors, resulting in increased HK2 promoter activity. This finding adds to those of others who have shown that high levels of HK2 expression are needed for tumor initiation (34), and that HK2-mediated glycolysis is required for tumorigenesis in Pten-/P53-; mouse embryonic fibroblasts (39). Analysis of patient samples demonstrates uniformly high HK2 expression in fallopian tubes, specifically in the secretory cells. Previously, HK2 expression has been reported to be limited to embryonic tissue.
skeletal muscle, and adipose (40). Our results suggest that high basal HK2 levels combined with loss of BRCA1 in fallopian tube cells can further push these cells of origin of ovarian cancer into a state of metabolic derangement.

To target the metabolic phenotype induced by loss of BRCA1, we tested drugs that are already FDA approved for noncancer indications and identified aspirin and luteolin as agents that reduced both HK2 and glycolysis. Luteolin is a natural flavonoid
in several fruits and vegetables with reported activity against cancer (32, 41). In our study, luteolin reduced glycolysis by suppressing MYC-dependent HK2 expression but only at supra-physiologic concentrations. In contrast, aspirin, at physiologically relevant concentrations (2 mmol/L; ref. 42), efficiently suppressed both HK2 expression and glycolysis. The in vitro findings support that assertion that suppression of glycolysis by aspirin may protect against ovarian cancer; however, these findings are limited by the lack of confirmation in an animal model. Because of the limitations of animal models ovarian cancer (43), testing aspirin as a
chemopreventive agent in a murine model was beyond the scope of the study. A protective effect in murine ovarian cancer has been suggested in epidemiologic studies (44, 45). A recent analysis of the Iowa’s Women’s Health Study found that use of aspirin was associated with a decrease in incidence of ovarian cancer and other aspirin-sensitive cancers [HR, 0.87; 95% confidence interval, 0.72–1.06; ref. 46]. Similar findings were reported in an analysis of the Danish Cancer Registry (47). For colorectal cancer prevention, the US Preventive Service Task Force is recommending 81 mg of aspirin daily (48), based on two large randomized clinical trials (49, 50). For ovarian cancer, precedent already exists for the development of therapeutics directed against the pathologic effects of BRCA1 impairment, the most prominent example being PARP inhibitors (51). Aspirin’s ability to suppress HK2 and glycolysis induced by loss of BRCA1 provides a rationale for repurposing aspirin as a chemoprevention in BRCA1 mutation carriers.

Historically, the evaluation of BRCA1 has largely focused on its role in DNA repair. Less is known about the other functions of BRCA1, including any effects on metabolism. Given that aerobic glycolysis and other metabolic alterations are vital for initiation and growth of cancer (52), the increase in glycolysis in fallopian tube and OSE cells induced by loss of BRCA1 reported here is a new mechanism by which BRCA1 impairment promotes ovarian cancer growth. The loss of BRCA1 may metabolically prime the cells of origin of ovarian cancer for oncogenic transformation and provide a window of opportunity for primary cancer prevention with agents such as aspirin.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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