Prolonged Postovulatory Proinflammatory Signaling in the Fallopian Tube Epithelium May Be Mediated through a BRCA1/DAB2 Axis

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Abstract

Purpose: To assess inflammation-related gene expression in nonmalignant fallopian tube epithelium (FTE) from BRCA1/2 mutation carriers and control patients obtained during the luteal and follicular phase, and to determine the impact of BRCA1 and disabled homolog 2 (DAB2) on NF-κB–mediated proinflammatory signaling.

Experimental Design: A list of inflammation-related and NF-κB–responsive genes was compiled through gene set enrichment and PubMed database search, corresponding probes identified, and unpaired t tests conducted to identify differentially expressed genes in previously profiled FTE samples. ES2 and A549 cells were cotransfected with DAB2–or BRCA1-targeting siRNA and an NF-κB–responsive luciferase reporter, treated with TNF-α and luciferase activity determined. To determine whether DAB2 or BRCA1 alters mRNA expression of NF-κB target genes, cells were transfected with siRNA, treated with TNF-α, and harvested for total RNA extraction and quantitative real-time PCR.

Results: A subset of BRCA1–mutated luteal phase samples previously found to group with adnexal high-grade serous carcinomas (HGSCs) differentially expressed 124 inflammation–associated probesets relative to remaining FTE samples. These samples also differentially expressed 264 probes relative to other luteal phase samples exposed to the same postovulatory environment. Both BRCA1- and DAB2–targeting siRNA increased TNF-α–induced NF-κB activity and mRNA expression of NF-κB–dependent target gene SOD2 relative to nontargeting siRNA, suggesting that both proteins repress proinflammatory signaling.

Conclusions: These data provide evidence of elevated proinflammatory signaling in a subset of BRCA1–mutated luteal phase FTE, consistent with an altered response to ovulation-associated cytokines. Furthermore, both BRCA1 and DAB2 affect NF-κB activity, indicating a novel link between BRCA mutation status, ovulation, and predisposition to HGSC. Clin Cancer Res; 1–12. ©2012 AACR.
Recent evidence strongly implicates the fallopian tube as the source of “ovarian” high-grade serous carcinoma (HGSC), the most common and lethal form of epithelial ovarian cancer. In light of this, our work aims to identify molecular alterations in nonmalignant fallopian tube epithelium from BRCA1/2 mutation carriers that may contribute to malignant transformation. Discovery and characterization of these molecular alterations could contribute to a better understanding of the earliest events of serous carcinogenesis, which could lead to improved detection of those patients at greatly enhanced risk, development of prevention strategies, and ultimately significant reductions in ovarian cancer–associated mortality. The data presented in this study further supports the role of ovulation-associated inflammatory signaling in serous carcinogenesis, and could provide targets for prophylaxis to prevent the development of adnexal HGSC in women at known risk.

Translational Relevance

Recent evidence strongly implicates the fallopian tube as the source of “ovarian” high-grade serous carcinoma (HGSC), the most common and lethal form of epithelial ovarian cancer. In light of this, our work aims to identify molecular alterations in nonmalignant fallopian tube epithelium from BRCA1/2 mutation carriers that may contribute to malignant transformation. Discovery and characterization of these molecular alterations could contribute to a better understanding of the earliest events of serous carcinogenesis, which could lead to improved detection of those patients at greatly enhanced risk, development of prevention strategies, and ultimately significant reductions in ovarian cancer–associated mortality. The data presented in this study further supports the role of ovulation-associated inflammatory signaling in serous carcinogenesis, and could provide targets for prophylaxis to prevent the development of adnexal HGSC in women at known risk.

Materials and Methods

Gene expression analysis

Gene expression data derived from laser capture–microdissected FTE and HGSC specimens (GEO #GSE10971) were used to evaluate expression levels of inflammation-associated genes. These samples included histologically normal FTE from 12 confirmed BRCA1/2 mutation carriers and 12 control patients (6 luteal and 6 follicular phase each) and 13 HGSC.

A list of potential inflammation–related and NF-kB-responsive genes was compiled using 2 broad categorical definitions. The first was a gene set enrichment ("VSNFKAPPAB65_01") identifying 190 genes with promoters matching the RelA consensus-binding site (16). The second was an in-house MySQL database containing all publications before April 2010 found in PubMed indexed to the complete set of human and mouse Entrez gene symbols (and synonyms) found in their abstracts. Through a series of SQL joins, keyword searching retrieved a list of all potential gene names mentioned, along with the number of article abstracts in which they were found. Results were restricted to those genes with more than 50 articles for the keyword “inflammatory” and more than 2 articles for the keywords “p65/RelA.” The resulting set of genes was combined with the aforementioned gene set enrichment category and a nonredundant list of corresponding probes was identified (n = 2,510) on the Affymetrix array.

The expression of these probes was assessed in our previously profiled samples. CEL files were imported into GeneSpringGX (v10.0, Agilent), and normalization was conducted using the Robust Multi-Array Average algorithm, followed by the median measurement for each probeset across all samples. An unpaired t-test with a Benjamini and Hochberg false discovery rate of P < 0.05 was used to
identify differentially expressed probes in FTEb(S) samples compared with the remaining FTE samples, irrespective of ovarian cycle stage. Regrouping and analysis of the dataset were then conducted to identify differentially expressed probes in FTEb(S) samples relative to the remaining luteal phase samples (uncorrected unpaired t-test P < 0.05). The expression of significantly altered probesets among samples was visualized by hierarchical clustering (Pearson-centered correlation distance metric with average linkage rules).

Cell culture
ES2 ovarian cancer cells, MCF7 breast cancer cells, A549 lung cancer cells, and MIA PaCa-2 pancreatic cells were verified by short tandem repeat profiling by The Centre for Applied Genomics Genetic Analysis Facility (Toronto, ON, Canada). All cells were grown in Dulbecco's modified Eagle's medium/f-12 supplemented with 10% FBS, 100 U/mL penicillin, 100 µg streptomycin and fungizone (Gibco Cell Culture Products/Invitrogen). Medium containing charcoal-stripped FBS was used during experiments requiring hormone/cytokine treatments. Dexamethasone was dissolved in ethanol and recombiant human TNF-α (R&D Systems) was reconstituted in PBS containing 0.1% bovine serum albumin, and were further diluted with culture medium. Vehicle treatment consisted of an equivalent diluted amount of ethanol.

Cell lines were selected on the basis of expression of proteins of interest [glucocorticoid receptor, DAB2, RelA, and BRCA1], ease of transfection, and responsiveness to dexamethasone and/or TNF-α treatment. A549 cells are a commonly used cell line for studies on NF-kB signaling.

Western blot analysis
Western blot analysis was conducted as previously described (17) using monoclonal anti-DAB2 (1:500; BD Transduction Laboratories), monoclonal anti-BRCA1 (1:50, EMD Biosciences), rabbit polyclonal anti-NF-κB p65(A) (1:50, EMD Biosciences), monoclonal anti-glucocorticoid receptor (DNA-free kit, Ambion), and reverse transcription conducted using Superscript III Reverse Transcriptase and Oligo(dT)20 primers (Invitrogen). Samples were diluted to 1.6 ng/µl and quantitative PCR was conducted as previously described (2). Primer sequences (and relative primer concentrations) used included: ACTB forward 5’-GCATTGTTACAGGAATTGCCCTTG-3’ and reverse 5’-CTATGACCTCCCCCTGTTGAGA-3’ (900 nmol/L forward: 300 nmol/L reverse); DAB2 forward 5’-CTAGCTATTGCAATGAGGGGAG-3’ and reverse 5’-GG-TAATACACTTGG-AACCGAGGACCA-3’ (300 nmol/L forward:300 nmol/L); BRC1 forward 5’-CTCTGGCAAGAACGGTACGTG-3’ and reverse 5’-TCTCCGAATTTCTCTCCAC-3’ (300 nmol/L forward:300 nmol/L); BIRC3 forward 5’-CCATTGTTACAGGAATTGCCCTTG-3’ and reverse 5’-CTATGACCTCCCCCTGTTGAGA-3’ (900 nmol/L forward: 300 nmol/L reverse); DAB2 forward 5’-CTAGCTATTGCAATGAGGGGAG-3’ and reverse 5’-GG-TAATACACTTGG-AACCGAGGACCA-3’ (300 nmol/L forward:300 nmol/L). 

Expression constructs and siRNA
Human DAB2 cDNA (MEC-1764; American Tissue Type Collection) was cloned into pcDNA3.1/Hygro (Invitrogen). Human BRCA1 cDNA in pcDNA3.1 was obtained from Dr. Irene Andrulis (Mount Sinai Hospital, Toronto, Ontario, Canada). All expression constructs were sequenced before use. siGENOME SMARTpool DAB2–targeting siRNA, BRCA1-targeting siRNA, and nontargeting siRNA were purchased from Dharmacon. Western blotting confirmed specific knockdown of DAB2 (70%) and BRCA1 (80%) relative to nontargeting siRNA control (shown in Fig. 2).

Luciferase assays
To determine the impact of DAB2 and BRCA1 on endogenous NF-kB transcriptional activity, cells were plated at 100,000 cells/well in 24-well plates and transiently transfected with 0.2 µg/well pNF-kB–luciferase or pTAL-luciferase (negative control; Clontech), 0.1 µg/well β-galactosidase, and 50 pmol each of DAB2-, BRCA1- or nontargeting siRNA 24 hours after plating using Lipofectamine LTX (Invitrogen) according to the manufacturer’s protocol. Cells were treated with 10 ng/mL TNF-α or vehicle 48 hours following transfection, and harvested in Reporter Lysis Buffer (Promega) 8 hours after treatment. The impact of DAB2 and BRCA1 on glucocorticoid receptor activity was determined by cotransfection with 0.2 µg/well mouse mammary tumor virus luciferase, 0.1 µg/well β-galactosidase, and either 50 pmol of siRNA (DAB2-, BRCA1-, or nontargeting) or varying concentrations of expression constructs (DAB2, BRCA1, or pcDNA3 empty vector). Cells were treated with 10 nmol/L dexamethasone or vehicle and harvested 24 hours later. Luciferase activity was measured following addition of Luciferase Assay Substrate (Promega) and was normalized to β-galactosidase activity. Triplicate wells were included for each condition within each experiment. Normalized data are expressed as average fold change (± SEM) relative to nontargeting siRNA/empty vector–transfected cells receiving active treatment (TNF-α or dexamethasone). Statistical analysis was conducted using one-way ANOVA, followed by Newman–Keuls multiple comparison post hoc test (P < 0.05).

Quantitative real-time PCR
To determine the impact of DAB2 and BRCA1 on mRNA expression of selected NF-kB–regulated genes, ES2 cells (100,000 cells/well in 24-well plates) were transfected with 50 pmol DAB2-, BRCA1-, or nontargeting siRNA. Cells were treated with 10 ng/mL TNF-α or vehicle 48 hours following transfection, and harvested 8 hours later in 250 µL TRIzol reagent (Invitrogen). Two wells per condition were pooled to increase yield. RNA was isolated, genomic DNA was removed (DNA-free kit, Ambion), and reverse transcription conducted using Superscript III Reverse Transcripate and Oligo(dt)20 primers (Invitrogen). Samples were diluted to 1.6 ng/µL and quantitative PCR was conducted as previously described (2).
(300 nmol/L:900 nmol/L). All experiments included triplicate wells of each sample for both target and reference gene. The comparative CT method for relative quantitation was conducted and normalized to \( \text{ACTB} \) expression. Results are expressed as average fold change \((\pm \text{SEM})\) relative to TNF-\(\alpha\)-treated cells transfected with nontargeting siRNA \((n = 4\) experiments). A one-way ANOVA followed by Newman–Keuls multiple comparison test \((P < 0.05)\) was conducted for statistical comparisons.

Results

**Differential expression of NF-\(\kappa\)B–regulated and inflammation-related genes**

In our initial study (2), FTE from 12 BRCA carriers and 12 control women were stratified by whether they were in the follicular phase or luteal phase \((n = 6\) for each genotype and cycle phase). When analyzed as a group, the 6 luteal phase BRCA mutation carrier specimens partitioned more closely with HGSC and distant from the follicular phase and the control luteal phase samples. Upon closer examination considering the individual profiles, 4 of the \(\text{BRCA}\) luteal phase samples emerged in both analyses \(\text{(shown at bottom of each panel)}\). The cluster groups are shown at the top, with each line representing one sample. The type of sample is indicated at the bottom of each heatmap \(\text{(blue, FTEn; red, FTEx)}\), with the stage of the ovarian cycle at the time of surgery indicated below \(\text{(purple, follicular; yellow, luteal)}\). The distinct \(\text{FTEb(S)}\) sample subgroup is outlined in yellow in each panel.
Four hundred and sixty-six probes were significant without a multiple testing correction (P < 0.05, data not shown). Hierarchical clustering was used to visualize how well expression of these probes categorized patient samples. As expected, FTEb(S) samples grouped together and were more closely related to other luteal samples. In contrast, no clear separation of samples based solely on BRCA1/2 mutation status was observed (Fig. 1A). Regrouping and analysis of the dataset using only luteal phase samples found 264 differentially expressed inflammatory probesets (uncorrected t test P < 0.05) in the FTEb(S) relative to the remaining luteal phase samples exposed to a similar postovulatory environment (Supplementary Table S3). Because of the smaller numbers of samples in this comparison, an FDR-corrected t test revealed no significant changes (P < 0.05). Clustering of patient samples using this list separated samples into two distinct groups not directly related to mutation status; however, as expected, the unique pattern of gene expression in BRCA1-mutated FTEb(S) samples was evident (Fig. 1B). Notable genes with greatly increased expression in FTEb(S) samples in both comparisons included chemokines such as interleukin (IL)-8, detoxifying enzymes such as glutathione peroxidase 3 (GPX3), and superoxide dismutase 2 (SOD2), and survival genes such as baculoviral IAP repeat-containing 3 (BIRC3). Altogether, these data provide compelling evidence of altered inflammatory signaling in a subset of BRCA1-mutated luteal phase samples.

**DAB2 and BRCA1 directly impact TNF-α–induced NF-κB transcriptional activity**

These findings, combined with the inverse pattern of DAB2 and RelA protein expression within secretory FTE cells during the luteal phase, led us to investigate whether DAB2 or BRCA1 impact NF-κB transcriptional activity. Cytokine-responsive ES2 ovarian cancer cells were cotransfected with DAB2-targeting, BRCA1-targeting, or nontargeting siRNA construct. Cells were subsequently treated with 10 ng/mL TNF-α or vehicle, and harvested 8 hours later for determination of luciferase activity. As expected, TNF-α treatment induced NF-κB–dependent luciferase activity in ES2 cells transfected with nontargeting siRNA (Fig. 2A and C). Importantly, both DAB2- (Fig. 2A, p < 0.001) and BRCA1-targeting (Fig. 2C, P < 0.001) siRNA led to enhanced TNF-α–dependent induction of NF-κB activity relative to control siRNA. Similar results were also observed when these experiments were repeated in A549 lung cancer cells (Fig. 2B and D), indicating that the impact of DAB2 and BRCA1 on NF-κB is not cell line specific.

![Figure 2](image-url)
We next determined whether the observed differences in NF-κB–dependent activity resulted in altered expression of known endogenous target genes, specifically those showing increased expression in FTEb(S) samples. ES2 cells were transfected with either DAB2-, BRCA1-, or non-targeting siRNA, treated with TNF-α or vehicle, and harvested 8 hours later for total RNA extraction and qRT-PCR for previously identified NF-κB–induced genes with increased expression in FTEb(S) samples (n = 4 wells/condition). The impact of DAB2/BRCA1 status on SOD2 (A and C) and IL-8 (B and D) mRNA expression is shown. Circles in each panel represent expression levels in individual samples [normalized to β-actin and expressed relative to cells transfected with nontargeting siRNA (“Ntsi”) and treated with TNF-α], whereas horizontal lines represent average relative mRNA expression for each experimental condition. Groups with different letters are statistically different from one another, as determined by one-way ANOVA followed by the Newman–Keuls multiple comparison test (P < 0.05).

Because DAB2 levels were found to be further decreased in luteal samples from BRCA mutation carriers in our previous study, we tested the impact of BRCA1-targeting siRNA on the expression of DAB2. TNF-α treatment decreased DAB2 mRNA levels in cells transfected with nontargeting siRNA (p < 0.05), whereas BRCA1-targeting siRNA had no effect (Fig. 4). This suggests that the...
previously observed loss of DAB2 in luteal phase FTE from both mutation carriers and controls (2) may in part be due to the local elevation of TNF-α following ovulation, and that BRCA1 does not directly affect DAB2 expression.

**DAB2 and BRCA1 enhance dexamethasone-induced glucocorticoid receptor activity**

The proinflammatory cascade leading to ovulation also activates a compensatory anti-inflammatory cascade to limit tissue damage and promote luteinization. This is achieved in part by cytokine-induced upregulation of 11β-hydroxysteroid dehydrogenase type 1, which catalyzes the conversion of cortisol from its inactive precursor cortisone, in granulosa and OSE cells at the site of follicular rupture. This results in elevation of cortisol in follicular fluid, which acts upon the intracellular glucocorticoid receptor to mediate anti-inflammatory signaling and restore tissue homeostasis (4, 8, 18). To determine whether DAB2 and BRCA1 impact anti-inflammatory signaling and restore tissue homeostasis upon the intracellular glucocorticoid receptor to mediate glucocorticoid receptor activity. Consistently, transfection of ES2 cells with increasing amounts of DAB2 resulted in a dose-dependent increase in dexamethasone-induced luciferase activity (Fig. 5B, P < 0.001). Similar results were observed upon transfection with increasing amounts of BRCA1 in MIA PaCa-2 cells (Fig. 5D, P < 0.001). Finally, enhanced dexamethasone-induced glucocorticoid receptor activity was observed following upregulated DAB2 expression in MIA PaCa-2 cells and either DAB2 or BRCA1 in MCF7 breast cancer cells (Supplementary Fig. S3), indicating that both DAB2 and BRCA1 promote glucocorticoid receptor transactivation activity in multiple cancer cell lines.

**Discussion**

The cell of origin for pelvic HGSC is now thought to lie within the FTE, regardless of whether the cancer is diagnosed as ovarian, peritoneal, or tubal (19). Women with a germline mutation in BRCA1 or BRCA2 have up to a 60%

![Figure 5](https://www.aacrjournals.org/clin-cancer-research/article-pdf/18/18/6955/4854593/clin-cancer-research-2012-18-18-6955.pdf)

Figure 5. DAB2 and BRCA1 enhance dexamethasone-induced glucocorticoid receptor transactivation activity. To determine the impact of DAB2 and BRCA1 on endogenous glucocorticoid receptor transactivation activity, ES2 cells were cotransfected with either DAB2-targeting ("Dsi"; A, n = 3 wells/condition) or BRCA1-targeting ("Bsi"; C, n = 6 wells/condition) siRNA and a glucocorticoid-responsive luciferase reporter (mouse mammary tumor virus, MMTV). Cells were treated with 10 nmol/L of the synthetic glucocorticoid dexamethasone or vehicle and harvested 24 hours later for luciferase activity determination. The dose-dependent effect of DAB2 and BRCA1 were determined by transfecting ES2 or MIA PaCa-2 cells with increasing amounts of DAB2 ("D"; B) or BRCA1 ("B"; D), respectively (n = 9 wells/condition). Circles in panels A and C indicate the MMTV luciferase activity (normalized to β-galactosidase) in individual wells, expressed relative to cells transfected with nontargeting siRNA ("NTsi") and treated with dexamethasone. Normalized individual values in the remaining panels are expressed relative to dexamethasone-treated cells transfected with pcDNA3 empty vector. Statistically significant differences in average MMTV luciferase activity were determined by one-way ANOVA followed by the Newman–Keuls multiple comparison test. Groups with different letters are statistically different from one another (P < 0.05).
and 30% chance of developing HGSC, respectively, compared with a 1.6% chance in the general female population (20, 21); however, the underlying mechanisms contributing to an increased propensity for malignant transformation in mutation carriers are incompletely understood.

To identify alterations that may predispose to HGSC development, we previously molecularly characterized nonmalignant FTE from BRCA1/2 mutation carriers and control patients, as well as tubal and ovarian HGSC (2). Importantly, BRCA1-mutated samples obtained during the postovulatory luteal phase showed global gene expression profiles closely resembling HGSC, suggesting that the luteal phase milieu may contribute to serous carcinogenesis. In contrast, BRCA1/2-mutated samples obtained during the follicular phase, and both follicular and luteal phase samples from normal controls, clustered separately from HGSC. The separation of luteal and follicular phase FTE was similarly observed in an independent set of cases (22), further highlighting the potential role of the luteal phase milieu.

In addition to BRCA1/2, epidemiologic studies have identified additional modifiers of ovarian cancer risk, mostly associated with reproductive history. Several studies have reported a substantial protective effect of oral contraceptives (OCP) in both the general population and BRCA1/2 mutation carriers, with greater risk reduction observed with increasing duration of use (23, 24). Parity and breastfeeding have also consistently been associated with risk reduction in both populations, with greater protection provided by a increased number of full-term pregnancies and longer duration of total lactation time (23, 24). Consistent with suppressive effects of OCP use, pregnancy, and breastfeeding on ovulation, a population-based case-control study found a positive association between estimated lifetime ovulatory (log)years and ovarian cancer risk, specifically among premenopausal women (25). Particular relevance of ovulatory process in HGSC development.

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In addition to providing evidence that altered ovulation-associated inflammatory signaling in a subset of FTE samples may contribute to serous carcinoma development; the present study suggests a novel underlying mechanism for this phenomenon by demonstrating an impact of both BRCA1 and DAB2 on the transcriptional activity of NF-kB and glucocorticoid receptor, the main mediators of pro- and anti-inflammatory signaling, respectively. The importance of BRCA1 in HGSC has long been appreciated. Several functions of BRCA1 likely contribute to its tumor suppressive role, including but not limited to maintenance of genomic integrity through facilitation of DNA repair, regulation of cell-cycle progression, and modulation of steroid hormone (including progesterone, estrogen, and androgen) receptor activity (10–13). DAB2 has similarly been implicated as an important tumor suppressor by us and others. It was originally identified in a screen for transcripts downregulated in ovarian cancer cell lines compared with normal OSE (34), and subsequent studies have observed decreased DAB2 in breast, prostate, pancreatic, esophageal, and squamous cell carcinoma (35–37). DAB2 has been implicated in various cellular processes, including proliferation, differentiation, polarity, endocytosis, adhesion, and migration. It has also been shown to regulate several important signaling pathways, including androgen

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receptor signaling (14), TGF-β signaling (38), and the canonical Wnt pathway (36). Most notably, DAB2 has been shown to inhibit AP-1 signaling (15); this transcriptional complex activates a subset of inflammatory genes and is a target of transrepression by glucocorticoid receptor (39). DAB2 primarily functions as an adaptor protein in these pathways, resulting in alteration of the phosphorylation status of key molecules, disruption of protein complexes, and altered subunit expression. Our results suggest an additional function for BRCA1 and DAB2 in balancing anti-versus pro-inflammatory signaling, providing further explanation for their tumor suppressive roles in HGSC.

Interestingly, although BRCA1 and DAB2 similarly affect overall NF-κB transcriptional activity, the impact on expression of specific target genes appears to be more complex. Consistent with our luciferase data, both DAB2 and BRCA1 inhibit TNF-α–dependent expression of NF-κB target gene SOD2. This suggests that both proteins may act in concert to regulate the response to elevated (sublethal) ROS levels adjacent to the ovulatory site in vivo. Superoxide anions, released in large amounts by invading inflammatory leukocytes, are dismutated by enzymes (such as SOD2) into H₂O₂. H₂O₂ is further decomposed into molecular oxygen and water by "H₂O₂-detoxifying enzymes" (such as catalase and thioredoxin) to prevent the formation of highly reactive hydroxyl radicals by excess H₂O₂. Both of these steps are crucial, as hydroxyl radicals can readily generate DNA adducts, principally 8-oxo-7,8-dihydro-2'-deoxycytidine (8-oxo-dG), if not prevented by antioxidant enzymes (40, 41). In contrast to SOD2, FTEb(S) samples show decreased expression of thioredoxin and catalase relative to the remaining luteal phase samples (not shown). Increased expression of SOD2 in the absence of catalase and thioredoxin could lead to accumulation of potentially mutagenic 8-oxo-dG adducts, in addition to preventing induction of p53-dependent apoptosis in damaged cells (41). The connection between ovulation and 8-oxo-dG adducts has been shown in (ovine and human) OSE cells (9), but similar studies have not been conducted in FTE cells. 8-Oxo-dG adducts could result in DNA double-strand breaks and increased risk for mutations/malignant transformation (40) if repair pathways are not activated in the ensuing luteal phase. Interestingly, BRCA1 has previously been reported to increase the expression of several H₂O₂-detoxifying enzymes, and to regulate antioxidant response element-driven transcription (42). In addition, a recent study reported an increased level of 8-oxo-dG in leukocyte DNA obtained from BRCA1 mutation carriers (with or without symptoms of breast or ovarian cancer) compared with controls (43).

In contrast to SOD2, and opposite to that expected on the basis of the pattern of expression observed in FTEb(S) samples, BRCA1 appears to increase TNF-α–dependent expression of NF-κB target genes IL8 and BIRC3. First, elevated expression of IL8 (a chemokine responsible for recruitment of neutrophils to inflammatory sites) in luteal phase FTE is consistent with previous observations of increased IL-8 immunopositivity in distal FTE during the periovulatory period (44), and infiltration of the fallopian tube (including the epithelium) by neutrophils and macrophages (45, 46). Second, increased expression of BIRC3 in luteal samples is consistent with previous reports of its induction by genotoxic stress, whereupon it contributes to antiapoptotic NF-κB activation (47). The discrepancy between the impact of BRCA1 on NF-κB luciferase activity and IL8/BIRC3 expression has several possible explanations. For instance, there are likely important differences between loss of wild-type BRCA1 expression versus the presence of inherited mutations; furthermore, specific mutations could lead to specific changes of function and/or downstream gene expression. Second, we have treated cells with TNF-α alone, not the complex milieu of the luteal phase/follicular fluid. Third, we have altered the expression of one gene at a time and assayed for target gene expression, which would not recapitulate complex patterns of gene expression in vivo, including the expression of potential genes with redundant and/or opposing functions. For example, AP-1 is also known to regulate IL8 expression (48), whereas BIRC3 is a target of glucocorticoid receptor transactivation (49). An additional possibility is that BRCA1 has promoter-specific effects, consistent with a previous study reporting a coactivator role in TNF-α and IL-1β–dependent transcription of NF-κB target genes Fas and interferon-β (50). This was achieved in part through interaction of endogenous BRCA1 and RelA proteins; however, the effect of BRCA1 on global NF-κB–dependent transcription was not determined.

Despite complex effects on specific targets, the data presented herein support the generation of a refined model of serous carcinogenesis, in which malignant transformation of secretory cells of the distal FTE is promoted by an altered balance of pro-versus anti-inflammatory signaling during the postovulatory luteal phase, and furthermore, that this balance is determined by the underlying status of key genes such as BRCA1 and DAB2 (Fig. 6). We propose that homeostasis would be restored shortly following ovulation in FTE cells with sufficient levels of wild-type BRCA1 and/or DAB2, through induction of anti-inflammatory glucocorticoid receptor and inhibition of proinflammatory NF-κB by an as yet unidentified mechanism. In contrast, FTE cells with insufficient levels of BRCA1 and/or DAB2 would be unable to mount an adequate anti-inflammatory response following each ovulatory event. Incessant activation of the inflammatory response by NF-κB during successive luteal phases could therefore eventually lead to genomic instability and increased tumor risk, through continued production of mutagenic ROS and induction of cell cycle and antiapoptotic genes. Importantly, unlike previous theories suggesting a role for ovulation-associated inflammation in HGSC, the current proposed model emphasizes the differential response to this environment as the predisposing factor, rather than the nature of the environment itself. Proinflammatory signaling is recognized to play a role in carcinogenesis for multiple cancers. It is likely that BRCA1 and DAB2 would affect resolution of a proinflammatory environment in multiple tissues other than the fallopian tube. In our study, we observed results with breast and pancreatic cell...
Figure 6. Proposed model for high-grade serous carcinogenesis which incorporates a central role for restoring homeostasis following ovulation. In this model, the risk of malignant transformation of distal (secretory) FTE is increased by an altered balance of pro- versus anti-inflammatory signaling during the postovulatory luteal phase, as determined by DAB2 and BRCA1 expression or mutation status. In FTE cells with sufficient DAB2 and/or BRCA1 (top), these proteins enhance glucocorticoid-induced glucocorticoid receptor transactivation activity and inhibit cytokine-induced NF-kB activity shortly following ovulation. This shifts the balance in favor of anti-inflammatory signaling, leading to a timely resolution of the local inflammatory milieu that could promote mutagenesis. In contrast, FTE cells with deficient DAB2 and/or BRCA1 (bottom) would be unable to adequately mount a glucocorticoid receptor-mediated anti-inflammatory response or inhibit NF-kB-dependent signaling following ovulation. This would result in a sustained proinflammatory environment within the distal fallopian tube following each ovulatory event, contributing to an increased propensity for malignant transformation over a woman’s reproductive lifespan.


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