



Glucocorticoid-Induced Reversal of Interleukin-1 β -Stimulated Inflammatory Gene Expression in Human Oviductal Cells

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Abstract

Studies indicate that high-grade serous ovarian carcinoma (HGSOC), the most common epithelial ovarian carcinoma histotype, originates from the fallopian tube epithelium (FTE). Risk factors for this cancer include reproductive parameters associated with lifetime ovulatory events. Ovulation is an acute inflammatory process during which the FTE is exposed to follicular fluid containing both pro- and anti-inflammatory molecules, such as interleukin-1 (IL1), tumor necrosis factor (TNF), and cortisol. Repeated exposure to inflammatory cytokines may contribute to transforming events in the FTE, with glucocorticoids exerting a protective effect. The global response of FTE cells to inflammatory cytokines or glucocorticoids has not been investigated. To examine the response of FTE cells and the ability of glucocorticoids to oppose this response, an immortalized human FTE cell line, OE-E6/E7, was treated with IL1 β , dexamethasone (DEX), IL1 β and DEX, or vehicle and genome-wide gene expression profiling was performed. IL1 β altered the expression of 47 genes of which 17 were reversed by DEX. DEX treatment alone altered the expression of 590 genes, whereas combined DEX and IL1 β treatment altered the expression of 784 genes. Network and pathway enrichment analysis indicated that many genes altered by DEX are involved in cytokine, chemokine, and cell cycle signaling, including NF κ B target genes and interacting proteins. Quantitative real time RT-PCR studies validated the gene array data for *IL8*, *IL23A*, *PI3* and *TACC2* in OE-E6/E7 cells. Consistent with the array data, Western blot analysis showed increased levels of PTGS2 protein induced by IL1 β that was blocked by DEX. A parallel experiment using primary cultured human FTE cells indicated similar effects on *PTGS2*, *IL8*, *IL23A*, *PI3* and *TACC2* transcripts. These findings support the hypothesis that pro-inflammatory signaling is induced in FTE cells by inflammatory mediators and raises the possibility that dysregulation of glucocorticoid signaling could contribute to increased risk for HGSOC.

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Introduction

High-grade serous ovarian cancer (HGSOC) is the most common of the epithelial ovarian cancer histotypes and almost invariably presents as late stage disease associated with poor prognosis [1]. While traditionally thought to derive from the ovarian surface epithelium, recent studies indicate that HGSOCs likely originate in the fallopian tube epithelium (FTE) [2,3]. Women with germline mutations in breast cancer susceptibility genes 1 or 2 (*BRCA1* or 2) are at high risk of developing HGSOC and often elect to undergo bilateral salpingo-oophorectomy to reduce this risk [4]. Histomorphological examination of fallopian tubes from these women has revealed putative HGSOC precursor lesions [3,5]. These include regions of FTE exhibiting strong p53 immunostaining, reflective of *TP53* mutations characteristic of HGSOCs, and tubal intraepithelial carcinomas (TICs), which are occult *in situ* adenocarcinomas [6,7,8]. TICs have been found in more than half of patients presenting with HGSOC [7,9,10,11] and share identical *TP53* mutations with the invasive tumor, supporting the concept that they are clonally related [8].

Risk factors for epithelial ovarian cancer are associated with increased lifetime ovulatory years [12,13,14,15], which have led to the concept that ovulation may contribute to malignant transformation of adnexal epithelia. Ovulation is a localized acute inflammatory event during which fimbrial epithelial cells are exposed to follicular fluid containing a complex combination of inflammatory molecules [16]. Prolonged exposure to pro-inflammatory signaling can result in DNA adduct formation, increasing the incidence of gene mutations that can lead to malignant transformation [17,18,19]. Glucocorticoids have been shown to exert anti-inflammatory effects in several tissues [20]. We have previously shown that *BRCA1* enhances glucocorticoid receptor signaling and found evidence of suppressed glucocorticoid activity in luteal phase FTE from *BRCA1* mutation carriers relative to control patients [21]. However, anti-inflammatory activity of glucocorticoids does not occur in all cell types. For example, a stimulatory rather than inhibitory effect of glucocorticoids on expression of prostaglandin-endoperoxide synthase 2 (PTGS2), the rate-limiting enzyme in prostaglandin production, has been shown

Table 1. List of primers used for RT-qPCR.

Gene ID	Primer sequence	NCBI accession #
PTGS2	F: GTTCCACCCATGTCAAAC	NM_000963
	R: ATTCCGGTGTGAGCAGTT	
IL23A	F: CAAGGACTCAGGACAACAG	NM_016584
	R: GCTCCCCTGTGAAAATATCC	
IL8	F: CTCTCTGGCAGCCTTCTC	NM_000584
	R: GGGTGAAAGGTTTGGAGTA	
PI3	F: ATCGTGGTGGTTCCTCAT	NM_002638
	R: ACGGGATCTTGCCATTGAA	
TACC2	F: TCAGGAGAGCCCTGTCAAGT	NM_206861
	R: GTTTTTTCGACAGTGTTC	
TBP	F: TGCACAGGAGCCAAGAGTGAA	NM_003194
	R: CACATCACAGTCCCCACCA	
GAPD	F: CGAGCCACATCGCTCAGA	NM_002046
	R: AGTAAAAGCAGCCCTGGTGA	
B2M	F: CTCCGTGGCCTTAGCTGTG	NM_004048
	R: TTGGAGTACGCTGGATAGCCT	
YWHAZ	F: ACGTCCCTCAAACCTTGCTT	NM_003406
	R: GGCCTTCTGAACCAGCTCAT	

F = forward, R = reverse.

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in amnion fibroblasts [22], placental cytotrophoblasts [23], cardiomyocytes [24], and nasal polyps [25].

Human FTE cells have been shown to respond to IL1 with increased IL-8 expression [26], the global response of fallopian epithelial cells to inflammatory cytokines and/or glucocorticoids has not been investigated. In this study, we used an immortalized human oviductal cell line (OE-E6/E7) to assess changes in gene expression induced by Interleukin-1 β (IL1 β), a pro-inflammatory cytokine, implicated in ovulation [27], and dexamethasone (DEX), a glucocorticoid receptor agonist, to determine whether glucocorticoid signaling alters the response to IL1 β in these cells. OE-E6/E7 cells were derived from ampullary tubal epithelial cells of a patient who underwent surgery for uterine fibromyoma and were immortalized using HPV16 E6/E7 [28]. These cells have been extensively characterized and shown to exhibit properties consistent with oviductal secretory epithelial cells [28,29,30]. Thus, these cells serve as a useful model to explore mechanisms that could relate to early carcinogenesis.

Materials and Methods

Cell Culture

OE-E6/E7 cells [28] were obtained from Dr. William S.B. Yeung, University of Hong Kong (Hong Kong, China). BT20 human breast cancer cells were obtained from ATCC (Manassas, VA). Both cell lines were maintained in Dulbecco's Modified Eagle's medium supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 μ g/ml streptomycin (all from Invitrogen, Burlington, ON). A human fallopian tube sample was obtained from a patient undergoing prophylactic bilateral salpingoophorectomy for a *BRC42* mutation at Mount Sinai Hospital (MSH). The tissue collection and use was made with patient informed consent and was approved by the MSH Research Ethics Board. Epithelial cells were derived from the tissue and

grown in primary culture on collagen-coated transwell membranes as previously described by Fotheringham et al [31]. All cells were grown in a humidified incubator at 37°C and 5% CO₂. DEX (Sigma, St. Louis, MO) was dissolved in ethanol at 1 mM. Recombinant human IL1 β and TNF α (R&D Systems, Minneapolis, MN) were reconstituted in phosphate-buffered saline containing 0.1% bovine serum albumin to 25 μ g/ml and 100 μ g/ml, respectively. Stock solutions were diluted with culture medium just prior to use to achieve final stated concentrations. Cells were transferred to medium containing 0.5% charcoal-stripped FBS 18 h before initiating hormone or cytokine treatments.

Western Blot Analysis

Cells were collected in RIPA lysis buffer (150 mM NaCl, 50 mM HEPES pH 7.25, 1% Triton X-100, 0.1% SDS, 1% sodium deoxycholate) containing Complete protease inhibitor cocktail (Roche Diagnostics, Laval, QC). Clarified lysates were collected by centrifugation at 20,000 *g* for 15 min at 4°C and total protein was quantified using a BCA protein assay (Pierce, Rockford, IL) following the manufacturer's protocol. Aliquots of lysates containing 15 to 20 μ g of total protein were subjected to Western blot analysis as described previously [32] using anti-PTGS2 mouse monoclonal antibody (1:1000; Cayman Chemicals, Ann Arbor, MI) or anti-glucocorticoid receptor rabbit polyclonal (1:500; Abcam, Cambridge, MA) or anti-tubulin mouse monoclonal antibody (1:5000; Sigma). Immunoreactive band intensities were quantified using Molecular Dynamics Image Quant version 5.0 software. Statistical analysis was performed using one-way ANOVA followed by Student-Newman-Keuls post-hoc multiple comparison test (SigmaStat, Systat Software Inc, Chicago, IL). Comparisons were considered statistically significant at $p < 0.05$.

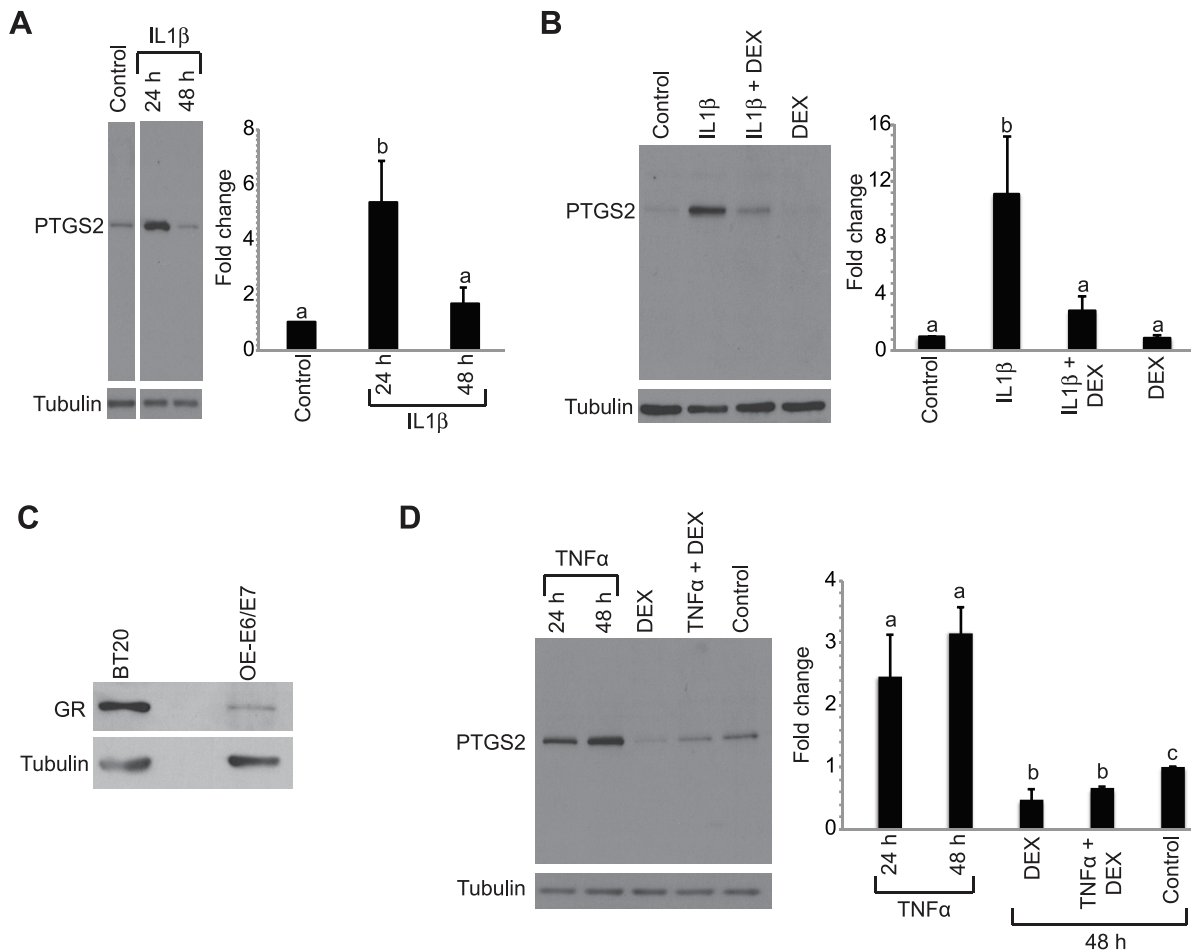


Figure 1. Increased PTGS2 levels in OE-E6/E7 cells by IL1 β and TNF α treatment is blocked by DEX. A, Cells were treated with 50 ng/ml IL1 β for 24 h or 48 h and Western blot analysis was performed for PTGS2 and tubulin. B, Cells were treated with 10 nM DEX or vehicle 30 h prior to treatment with IL1 β or vehicle and harvested 24 h later. Western blot analysis was performed for PTGS2 and tubulin. C, Western blot analysis was performed on OE-E6/E7 cells for glucocorticoid receptor and tubulin levels with BT20 breast cancer cells used as positive control. D, Cells were treated with DEX or vehicle 30 h prior to treatment with TNF α or vehicle. Cells treated with TNF α alone were harvested at 24 and 48 h after treatment and cells treated with DEX+TNF α were harvested at the 48 h time point. Western blot analysis was performed for PTGS2 and tubulin. Histograms summarize quantification of PTGS2 levels normalized to tubulin in 3 to 6 immunoblots. Bars represent the mean \pm SEM relative to control. Bars with different letters are statistically different from one another as determined by ANOVA followed by a Student-Newman-Keuls post-hoc multiple comparison test ($p < 0.05$).

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Gene Expression Analysis

Total RNA extracted using Trizol reagent (Invitrogen) and verified for integrity and purity, was labeled using Illumina TotalPrep RNA amplification kit (Ambion, Austin, TX) as per manufacturer's instructions. The cRNA generated was hybridized to Illumina HT-12 v4.0 BeadChips at 58°C for 18 h. The BeadChips were then washed, stained, and scanned using iScan (Illumina). Data files were quantified in GenomeStudio v2011.1 (Illumina), quantile normalized using the lumi package of Bioconductor [33], and filtered using varFilter to retain the 10% most variable probes. Data for multiple probes targeting the same gene were collapsed to their median value and annotated with gene names using GenePattern [34]. The collapsed data were analyzed using the linear models for microarray analysis (LIMMA) package [35] to identify differentially expressed genes. To understand the relationship among differentially expressed genes further, we performed a network-based analysis using the Reactome Functional Interaction (FI) Network plugin for Cytoscape [36]. The gene expression profiling data can be

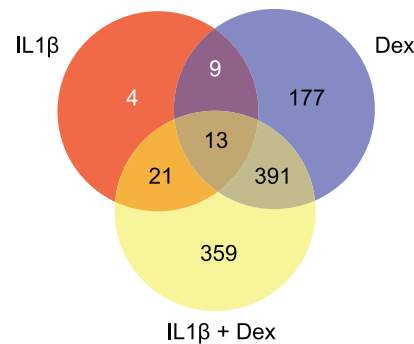


Figure 2. Number and overlap of genes differentially expressed due to IL1 β , DEX, and IL1 β +DEX treatment. Cells were treated with 10 nM DEX or vehicle 30 h prior to treatment with 50 ng/ml IL1 β or vehicle and harvested 18 h later. Total RNA was extracted and gene expression profiling was performed on Illumina HT-12 v4.0 BeadChips. The venn diagram shows the overlap of genes differentially expressed by the different treatments relative to control treated cells. doi:10.1371/journal.pone.0097997.g002

Table 2. Genes with altered expression due to IL1 β treatment.

Gene ID	Gene Name	Log2 Fold-Change*		
		IL1 β	DEX	IL1 β +DEX
13 genes altered in all 3 treatment groups				
<i>NGLY1</i>	N-glycanase 1	0.35	0.30	0.43
<i>TRIML2</i>	Tripartite motif family-like 2	0.63	-1.03	-0.67
<i>FOXQ1</i>	Forkhead box Q1	0.97	0.69	1.58
<i>CCL20</i>	Chemokine (C-C motif) ligand 20	0.43	0.49	0.84
<i>C7ORF10</i>	Chromosome 7 open reading frame 10	0.40	1.11	0.98
<i>EBI3</i>	Epstein-Barr virus induced 3	0.46	0.79	1.53
<i>GJB2</i>	Gap junction protein, beta 2	0.75	-0.39	0.36
<i>SYTL2</i>	Synaptotagmin-like 2	-0.85	-1.22	-1.58
<i>PLD5</i>	Phospholipase D family, member 5	-0.73	2.59	2.25
<i>PRSS23</i>	Protease, serine, 23	-0.64	-0.56	-1.53
<i>MAMDC2</i>	MAM domain containing 2	-0.55	-0.59	-1.38
<i>LTB</i>	Lymphotoxin beta	-0.81	-0.88	-1.92
<i>CAMK2N1</i>	Ca ⁺⁺ /calmodulin-dependent protein kinase II inhibitor 1	-0.59	0.91	0.38
21 genes altered by IL1β and by IL1β+Dex				
<i>BATF3</i>	Basic leucine zipper transcription factor, ATF-like 3	0.64	NS	0.91
<i>SAT1</i>	Spermidine/spermine N1-acetyltransferase 1	0.45	NS	0.29
<i>CES1</i>	Carboxylesterase 1	0.81	NS	0.88
<i>E2F2</i>	E2F transcription factor 2	0.71	NS	0.45
<i>ST6GAL1</i>	ST6 beta-galactosamide alpha-2,6-sialyltransferase 1	0.47	NS	0.45
<i>CXCL5</i>	Chemokine (C-X-C motif) ligand 5	0.70	NS	0.71
<i>APPL2</i>	Adaptor protein, phosphotyrosine interaction, PH domain and leucine zipper containing 2	0.37	NS	0.24
<i>CXCL6</i>	Chemokine (C-X-C motif) ligand 6	0.65	NS	0.60
<i>IL17C</i>	Interleukin 17C	1.22	NS	1.45
<i>ARG2</i>	Arginase, type II	0.79	NS	0.68
<i>PI3</i>	Peptidase inhibitor 3	1.18	NS	1.66
<i>RAB38</i>	RAB 38, member RAS oncogene family	0.68	NS	0.68
<i>FLJ10986</i>	FGGY, Carbohydrate kinase domain containing	0.64	NS	0.40
<i>FAM19A3</i>	Chemokine (C-C motif)-like protein TAFA-3	-0.68	NS	-0.84
<i>TMEM166</i>	Transmembrane protein 166	-0.61	NS	-0.40
<i>OXTR</i>	Oxytocin receptor	-0.71	NS	-0.67
<i>SPOCK2</i>	Testican	-0.41	NS	-0.58
<i>CXCL1</i>	Chemokine (C-X3-C motif) ligand 1	-0.99	NS	-0.69
<i>ITGA3</i>	Integrin, alpha 3	-0.50	NS	-0.64
<i>C15ORF52</i>	Chromosome 15 open reading frame 52	-0.34	NS	-0.26
<i>GBP4</i>	Guanylate binding protein 4	-0.62	NS	-0.45
9 genes altered by IL1β and by DEX				
<i>PTGS2</i>	Prostaglandin-endoperoxide synthase 2	1.10	-1.57	NS
<i>NFKBIZ</i>	I-Kappa-B-zeta	0.46	-0.32	NS
<i>FGFRL1</i>	Fibroblast growth factor receptor-like 1	0.43	-0.31	NS
<i>S100A9</i>	S100 calcium binding protein A9	1.19	-0.96	NS
<i>LOC728454</i>	Similar to Beta-defensin 2 precursor	0.69	-0.83	NS
<i>PTPN20</i>	Protein tyrosine phosphatase, non-receptor type 20	0.51	-0.44	NS
<i>DEFB4</i>	Defensin, beta 4	0.69	-0.76	NS
<i>LI10RB</i>	Interleukin 10 receptor, beta	-0.50	0.42	NS
<i>NFKBIE</i>	I-Kappa-B-epsilon	-0.47	0.45	NS
4 genes altered by IL1β only				
<i>LXN</i>	Latexin	0.63	NS	NS

Table 2. Cont.

Gene ID	Gene Name	Log ₂ Fold-Change*		
		IL1 β	DEX	IL1 β +DEX
<i>MAGED1</i>	Melanoma antigen family D, 1	0.40	NS	NS
<i>CCDC24</i>	Coiled-coil domain containing 24	-0.43	NS	NS
<i>IL1R2</i>	Interleukin 1 receptor, type II	-0.52	NS	NS

*Values represent log₂ fold change relative to control-treated cells with adjusted p-value <0.05.

NS = non significant (adjusted p-value >0.05).

doi:10.1371/journal.pone.0097997.t002

accessed from the NCBI Gene Expression Omnibus (GEO; <http://www.ncbi.nlm.nih.gov/geo>. Accessed 2014 May 1), accession number GSE54608.

Quantitative real-time RT-PCR (RT-qPCR)

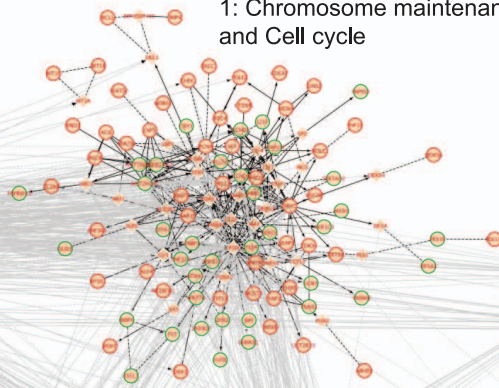
Total RNA from OE-E6/E7 and primary FTE cells was isolated using Trizol reagent (Invitrogen) following the manufac-

turer's protocol. Traces of genomic DNA from RNA isolated from OE-E6/E7 cells were removed using a TURBO DNA-free Kit (Applied Biosystems, Foster City, CA) following the manufacturer's protocol. RNA from primary FTE cells was purified using the RNeasy MinElute Clean up kit (Qiagen, Toronto, ON). RNA purity and integrity was determined using a bioanalyzer (Agilent Technologies, Inc, Mississauga, ON) and only samples with a

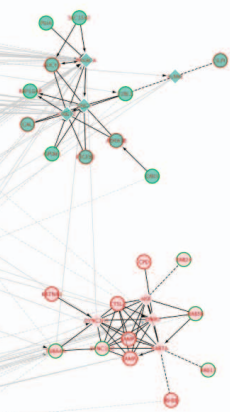
0: Inflammatory response, Interleukin signaling and NF κ B signaling



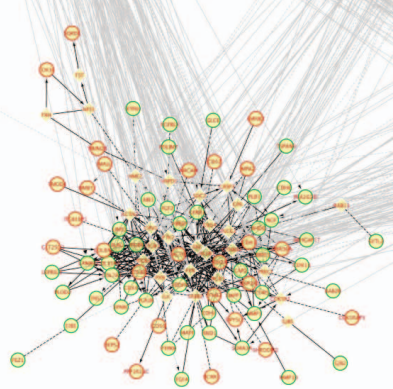
1: Chromosome maintenance and Cell cycle



4: GPCR signaling and downstream targets



2: Integrin signaling, and Extracellular matrix organization



3: Ubiquitin mediated proteolysis, and Antigen processing and presentation



Figure 3. Network analysis of differentially expressed genes in human FTE OE-E6/E7 cells treated with DEX. Module annotations were performed with false discovery rate (FDR) <0.05. Genes up-regulated in human FTE OE-E6/E7 cells treated with DEX are depicted by red outer circles, whereas genes down-regulated in human FTE OE-E6/E7 cells treated with DEX are depicted by green outer circles. Linker genes are depicted as diamond shaped nodes. Direct activating or inhibitory interactions are indicated with the symbols \rightarrow and $-\mid$, respectively. Indirect interactions involving additional proteins are depicted with dashed lines.
doi:10.1371/journal.pone.0097997.g003

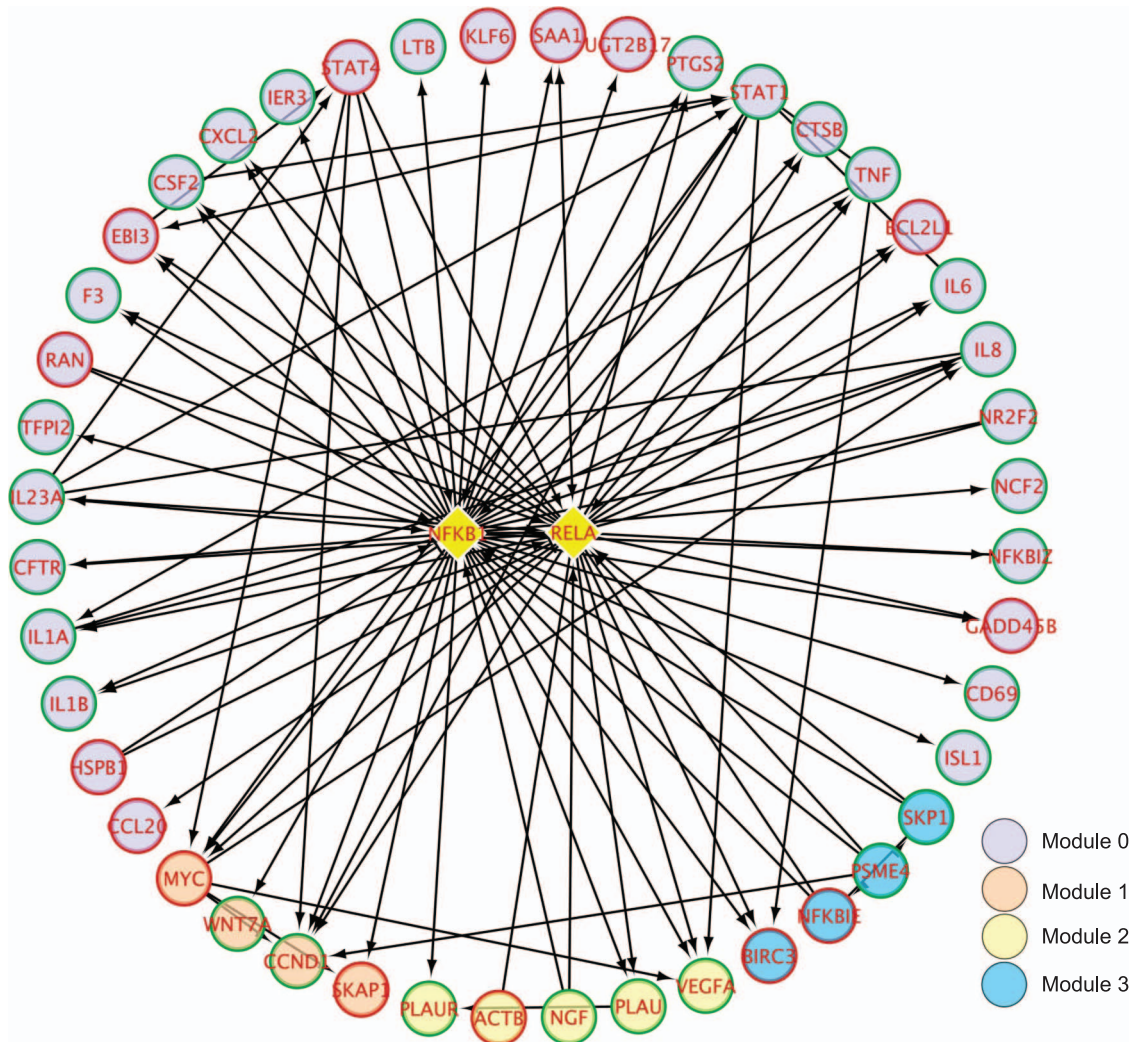


Figure 4. DEX affected NF κ B target genes and interacting proteins across four network modules identified by network-based analysis. Genes up-regulated by DEX are depicted by red outer circles, whereas genes down-regulated by DEX are depicted by green outer circles. NF κ B subunits are depicted by yellow diamonds. Direct activating or inhibitory interactions are indicated by the symbols \rightarrow and $-|$, respectively. Indirect interactions involving additional proteins are depicted with dashed lines. doi:10.1371/journal.pone.0097997.g004

RNA integrity number greater than 9 were used. The RNA was then reverse transcribed using Superscript III Reverse Transcriptase and random hexamers (Invitrogen) following the manufacturer's protocol. Primers were designed to span an intron/exon boundary using Primer3 (v.0.4.0.) and NCBI Primer-BLAST programs (Table 1) and their specificity was verified by BLAST analysis. Real-time qPCR was performed using a Perkin Elmer-JAMUS automated liquid handling system (Perkin Elmer, Waltham, MA) and a C1000 Thermal cycler (Bio-Rad, Hercules, CA) with LuminoCt SYBER Green (Sigma) detection using a total reaction volume of 5 μ l containing 2.5 μ l SYBER Green, 300 nM of each primer pair and 75 ng cDNA in 384 well plate. The cycling conditions were 95°C for 30 sec, 95°C for 5 sec, and 60°C for 20 sec. At the end of the PCR cycle, a dissociation curve was performed to confirm amplification of a single product. Each template was run with reference genes (Glyceraldehyde-3-phosphate dehydrogenase, *GAPD*; Beta-2-microglobulin, *B2M*; and Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, Zeta polypeptide, *YWHAZ*; and/or TATA-binding

protein, *TBP*). RT-qPCR data were analyzed using Bio-Rad CFX manager 2.0 software (Bio-Rad) and the targeted Cq values were normalized to *TBP* (OE-E6/E7 cells) or the geometric mean of *TBP*, *GAPD*, *B2M* and *YWHAZ* values using standard curves for each gene to account for any possible differences in primer efficiency. Statistical analysis was performed using two-way ANOVA followed by Student-Newman-Keuls post-hoc multiple comparison test. In cases of inhomogeneity of variance, data were log-transformed or analyzed using Kruskal-Wallis ANOVA on ranks followed by Student-Newman-Keuls post-hoc multiple comparison test. Comparisons were considered statistically significant at $p < 0.05$.

Results

We first established that OE-E6/E7 cells respond to IL1 β treatment with increased PTGS2 expression. Western blot analysis showed a more than 5-fold increase in levels of PTGS2 at 24 h, but not 48 h after treatment with 50 ng/ml IL1 β , relative to

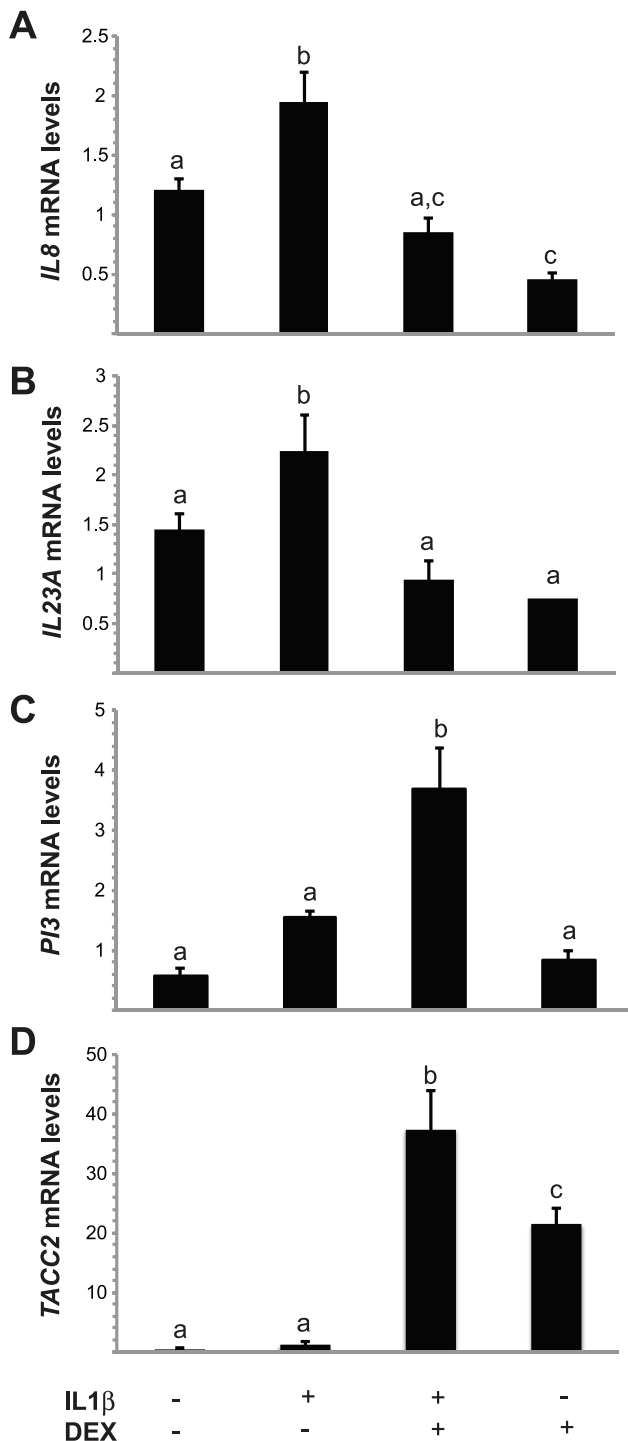


Figure 5. Transcript levels of *IL8*, *IL23A*, *PI3* and *TACC2* following IL1 β and/or DEX treatment. OE-E6/E7 cells were treated with 10 nM DEX or vehicle 30 h prior to treatment with 50 ng/ml IL1 β or vehicle and harvested 18 h later. Total RNA was extracted and RT-qPCR was performed for *IL8* (A), *IL23A* (B), *PI3* (C), *TACC2* (D) and were normalized to *TBP*. Bars represent the mean \pm SEM (n=3 biological replicates performed in triplicate). Bars with different letters are statistically different from one another as determined by ANOVA followed by a Student-Newman-Keuls post-hoc multiple comparison test ($p < 0.05$). doi:10.1371/journal.pone.0097997.g005

control-treated cells (Figure 1A). Pre-treatment of cells with 10 nM DEX reversed the IL1 β -induced increase at 24 h but did not affect uninduced PTGS2 levels (Figure 1B). Western blot analysis indicated OE-E6/E7 cells express glucocorticoid receptor but at levels below that of BT20 used as a positive control [37] (Figure 1C). A similar inhibitory effect on PTGS2 was observed with TNF α (Figure 1D). Treatment of cells with 100 ng/ml TNF α increased PTGS2 levels with the highest levels observed at 48 h after treatment. TNF α induction of PTGS2 at 48 h was blocked by pre-treatment with DEX (Figure 1D).

The global impact of IL1 β and glucocorticoid treatment on gene expression in OE-E6/E7 cells was determined by whole genome microarray analysis. Cells were treated with DEX or vehicle 30 h prior to treatment with IL1 β or vehicle. Cells were harvested 18 h after IL1 β treatment and RNA was extracted, labeled, and hybridized to Illumina HT-12 v4.0 BeadChip arrays. Genes differentially expressed between treatment groups and the control group were identified by t-test with correction for multiple testing. Altogether, a statistically significant alteration due to treatment was detected for expression of 974 of the 26,737 genes. Relative to control, IL1 β treatment altered the expression of 47 genes, whereas DEX altered the expression of 590 genes (Table S1). Combined IL1 β and DEX treatment altered the expression of 784 genes as compared to control-treated cells (Table S1). Of the 47 genes with altered expression due to IL1 β treatment, 4 were unique to IL1 β and 9 were also altered by DEX alone (Figure 2). IL1 β increased the expression of 7 and decreased the expression of 2 of these 9 genes with DEX treatment alone having an opposite effect on their expression relative to control-treated cells (Table 2). None of the 13 genes were altered by combined IL1 β and DEX treatment, as compared to control group (Figure 2). Thus, DEX treatment opposed the effect of IL1 β on the expression of these 13 genes. Thirteen genes were commonly regulated by all three treatment groups relative to the control group (Figure 2). Pre-treatment with DEX reversed the impact of IL1 β on 4 while enhancing the effect on 8 of these 13 genes (Table 2).

Twenty-one genes were common between the IL1 β +DEX and IL1 β alone versus control comparisons, but not DEX alone versus control (Figure 2 and Table 2). The impact of treatment on the expression of these genes reflects IL1 β since DEX neither further enhanced nor inhibited the IL1 β effect. This was confirmed by direct comparison of the IL1 β alone and IL1 β +DEX treatment groups (data not shown).

DEX treatment altered the expression of a greater number of genes than IL1 β . We used the Reactome Functional Interaction (FI) Network to explore the relationships among differentially expressed genes in OE-E6/E7 cells treated with DEX [36]. In brief, the FI network covers ~50% of the human proteome representing nearly 210,000 FIs. Of the 590 genes that were differentially expressed in response to DEX, 356 (60%) were projected onto the FI network. The average-shortest path calculation demonstrated that the interactions between the 365 genes were more highly connected with each other than by chance alone ($p < 0.001$). A minimum spanning tree algorithm was used to add 128 "linker" genes from the FI network to create a single fully connected subnetwork [38]. A spectral partitioning clustering algorithm was then used to identify clusters of genes that were highly interconnected with each other [39]. The clustering algorithm identified 5 functionally related network modules (0 to 4), which consisted of 11 or more genes (Figure 3 and File S1). All network diagrams were visualized using Cytoscape [40]. Using functional enrichment analysis, the modules were annotated for BioCarta, KEGG, NCI-PID, Panther, and Reactome pathways with a false discovery rate (FDR) < 0.05 . Of interest were a number

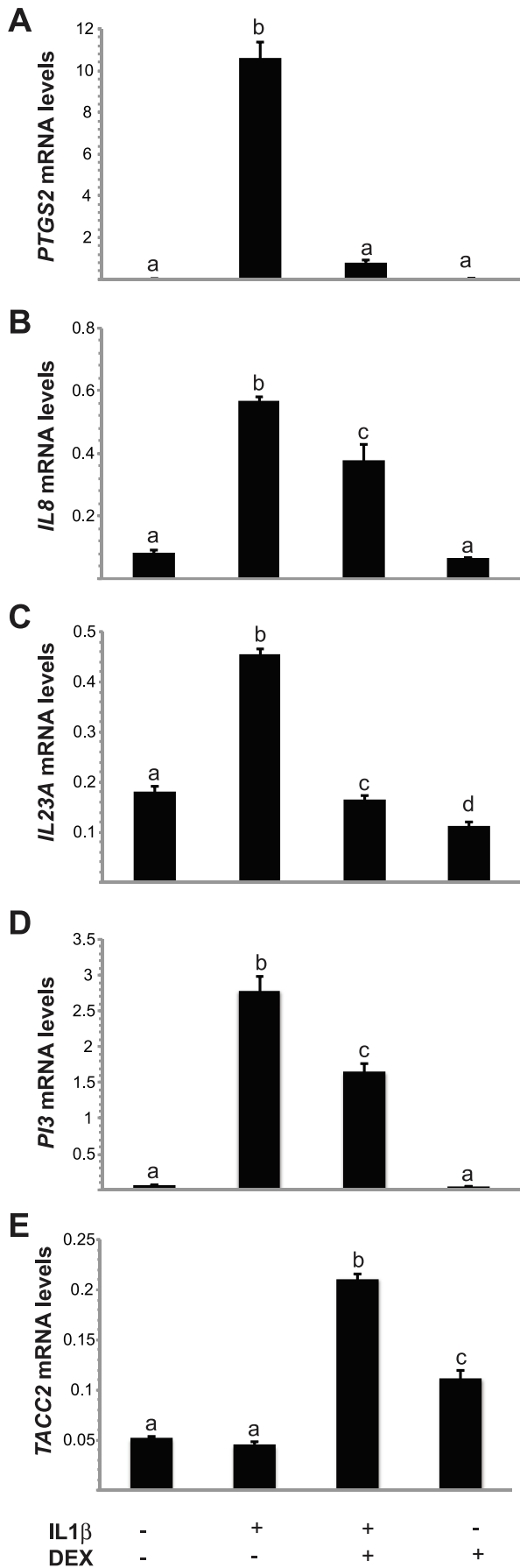


Figure 6. Transcript levels of *PTGS2*, *IL8*, *IL23A*, *PI3* and *TACC2* following IL1 β and/or DEX treatment. Primary FTE cells were treated with 10 nM DEX or vehicle 30 h prior to treatment with 50 ng/ml IL1 β or vehicle and harvested 18 h later. Total RNA was extracted and RT-qPCR was performed for *PTGS2* (A), *IL8* (B), *IL23A* (C), *PI3* (D), *TACC2* (E) and were normalized to the geometric mean of 4 reference genes. Bars represent the mean \pm SEM (n=3). Bars with different letters are statistically different from one another as determined by ANOVA followed by a Student-Newman-Keuls post-hoc multiple comparison test ($p < 0.05$).
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of pathways contained within the modules, including inflammatory response, interleukin and NF κ B signaling (module 0; Figure S1 in File S1), chromosome maintenance and cell cycle (module 1; Figure S2 in File S1), integrin signaling and extracellular matrix organization (module 2; Figure S3 in File S1), ubiquitin mediated proteolysis and antigen processing/presentation (module 3; Figure S4 in File S1), and G protein coupled receptor signaling and downstream targets (module 4; Figure S5 in File S1). Using *NF κ B1* and *RELA1* 'linker' genes of module 0, NF κ B target genes and interacting proteins across four network modules were visualized in Cytoscape (Figure 4).

Four genes were selected for validation as differentially expressed genes due to IL1 β or DEX treatment based on their potential involvement in carcinogenesis. OE-E6/E7 cells were treated as described for the gene expression profiling study and levels of Interleukin 8 (*IL8*), Interleukin-23 alpha subunit p19 (*IL23A*), Peptidase Inhibitor 3 (*PI3*) and Transforming Acidic Coiled-Coil Containing protein 2 (*TACC2*) transcripts were measured by RT-qPCR. Microarray analysis indicated increased expression of NF κ B target gene *IL23A* by IL1 β that did not attain statistical significance with a FDR-corrected t-test ($P = 0.051$, Table S1); however, a statistically significant increase was detected by RT-qPCR (Figure 5B). A similar increase was also observed for the NF κ B target gene *IL8* (Figure 5A). Consistent with an anti-inflammatory role, DEX pre-treatment inhibited the IL1 β -induced expression of both genes. In agreement with our gene expression profiling data, DEX alone reduced expression of *IL8*. Also consistent with our microarray data, DEX treatment increased *TACC2* expression, which was further increased by IL1 β (Figure 5D). *PI3* expression was increased by IL1 β in our microarray analysis (Table 1) and the increase detected by RT-qPCR approached statistical significance ($P = 0.096$; Figure 5C). Consistent with our microarray data, increased *PI3* expression was detected with combined IL1 β and DEX treatment.

To determine if similar effects are observed in non-immortalized FTE cells, a primary culture of human FTE cells derived from excess surgical material was treated with IL1 β with or without DEX as described for OE-E6/E7 cells and RT-qPCR was performed for *PTGS2*, *IL8*, *IL23A*, *PI3* and *TACC2* transcripts. IL1 β treatment increased the expression of *PTGS2*, *IL8*, *IL23A*, *PI3* transcripts and DEX treatment inhibited the IL1 β -induced increase (Figure 6). DEX alone had no effect on *PTGS2*, *IL8*, or *PI3* transcript levels but decreased expression of *IL23A*. Similar to OE-E6/E7 cells, DEX treatment increased *TACC2* transcript levels, which were further elevated by IL1 β co-treatment (Figure 6).

Discussion

Inflammation plays an important role in the initiation and progression of several cancers [18,41]. In healthy cycling women, the fallopian tube may be exposed to an inflammatory milieu at the time of ovulation and in the event of retrograde menstruation

[42]. Ovulation is a highly-localized, acute inflammatory event that exposes the distal FTE to follicular fluid containing pro-inflammatory cytokines and mediators, including IL1 and TNF, as well as high concentrations of steroid hormones [43,44]. Likewise, retrograde menstruation can expose the entire length of the fallopian tube to pro-inflammatory agents within the menses. Rapid resolution of inflammatory signaling is thus important to limit cellular damage to the epithelium. Approximately 80% of TICs within the FTE are found in the fimbria or distal portion of the tube [3], consistent with exposure to ovulation-associated inflammation. Molecular profiling of microdissected FTE from *BRCA* mutation carriers and control patients indicated luteal FTE from mutation carriers differed from that of control patients, whereas follicular FTE were highly similar between the two groups [45]. This difference in luteal phase FTE from *BRCA* mutation carriers predominantly reflected a pro-inflammatory gene signature consistent with increased NF κ B signaling and diminished glucocorticoid receptor signaling [21,46]. The FTE samples were not obtained immediately following ovulation and the response of tubule epithelial cells to inflammatory cytokine or glucocorticoid exposure has not been characterized.

NF κ B, a principal intracellular mediator of pro-inflammatory cytokines, has been proposed as a mechanistic link between inflammation and cancer [47,48] and its pro-inflammatory signaling targets are activated by both IL1 and TNF. Consistent with this, we found well-established direct transcriptional targets of NF κ B signaling to be up-regulated by IL1 β treatment in OE-E6/E7 cells.

Glucocorticoids exert pleiotropic effects critical to maintaining tissue homeostasis. As such, they play a key role in moderating and resolving inflammatory environments by inhibiting downstream signaling by inflammatory cytokines and by inducing cell-type specific apoptosis of monocytes, macrophages, and T-lymphocytes [49]. In addition to reversing the impact of IL1 β on many inflammation-associated genes, our data indicate that glucocorticoid receptor signaling impacts the expression of multiple genes unaffected by IL1 β . Activated glucocorticoid receptors heterodimerize with the NF κ B subunit RelA, resulting in inhibition of RelA signaling [50,51]. Additionally, the glucocorticoid receptor can affect the expression of anti-inflammatory genes through transactivation. Our pathway analysis indicated genes involved with inflammatory response, cell cycle, extracellular matrix organization, antigen processing, and G-protein coupled receptor signaling are affected by DEX treatment. Many of the genes in these pathways are NF κ B target genes or encode NF κ B interacting proteins. Several of these were not found to be affected by IL1 β treatment, which could reflect differences in NF κ B family members involved, the timing of the treatments, or regulation through independent pathways [52,53]. Nonetheless, this finding is consistent with a more generalized anti-inflammatory effect of glucocorticoid receptor activation that is not restricted to inhibition of IL1 β -activated NF κ B signaling.

The genes validated in this study – *PTGS2*, *IL8*, *IL23A*, *TACC2* and *PI3* – were selected based upon their differential response to treatment and their implication for cancer initiation. IL1 is a potent stimulator of *PTGS2* expression and prostaglandin production in ovarian tissue. Mice with targeted deletion of *PTGS2* fail to ovulate [54], indicating the importance of prostaglandin production in ovulation. Within the fallopian tube, prostaglandins may play a non-inflammatory role in muscular contraction and the resulting transport of the oocyte/embryo toward the uterus [55]. We found that treatment of OE-E6/E7 cells with peri-ovulatory follicular fluid collected from patients undergoing *in vitro* fertilization increased *PTGS2* protein levels (data not shown). *PTGS2* is a

major inflammatory mediator up-regulated in several cancers, most notably colorectal carcinoma [56,57]. Prostaglandins evoke further cytokine expression to promote the inflammatory response, which could contribute to carcinogenesis. Indeed, a protective effect of *PTGS2* inhibition has been shown for certain cancers, such as colorectal cancer [56,57]. Moreover, recent studies suggest aspirin, an inhibitor of both *PTGS1* and 2, may exert a protective effect for invasive ovarian cancer [58,59]. In the present study, we found *PTGS2* expression was increased by IL1 β , an effect that was blocked by glucocorticoid signaling. A stimulatory, rather than inhibitory effect of DEX on *PTGS2* expression has been reported in a variety of cell types [22,23,24,25]. While the mechanism underlying this paradoxical response are not fully understood, Wang et al [23] recently showed that DEX treatment of placental cytotrophoblast cells up-regulated expression of *MAP3K14/NIK*, a serine/threonine kinase that mediates activation of non-canonical NF κ B signaling by processing NF κ B2/p100 to active p52 [52]. In the present study, we did not see evidence of DEX altering the expression of *MAP3K14* or of NF κ B subunits in OE-E6/E7 cells.

IL8 accentuates local pro-inflammatory signaling by attracting monocytes, neutrophils and T-cells and its expression is increased in the distal fallopian tube during the peri-ovulatory period [45,60]. IL8 is elevated in the serum of ovarian cancer patients and enhances tumorigenicity of ovarian cancer cell mouse xenographs [61,62]. Since *IL8* is a known downstream target of IL1 [26] and our profiling data indicated an inhibitory effect of DEX, we examined its expression by RT-qPCR. Our results are consistent with IL1 β acting upon these cells and primary cultured FTE cells to induce *IL8*, and verified the inhibitory impact of DEX on this expression.

IL23 is a heterodimeric cytokine, consisting of a p19 (IL23A) and p40 (IL12 β) subunit, produced by multiple cell types [63]. Although IL23 shares a subunit with IL12, it activates a distinct receptor and exerts effects that differ from those of IL12 [64]. In contrast to IL12, IL23 promotes pro-inflammatory responses and diminishes tumor infiltration of cytotoxic T cells as determined in a DMBA/TPA-induced skin papilloma mouse model [65]. More recently, Teng et al [66] showed IL23 promotes methylcholanthrene-induced fibrosarcoma in mice by antagonizing anti-tumor innate immune responses. This combination of stimulating inflammatory signaling while diminishing immune surveillance would facilitate early events in carcinogenesis. We found that IL1 β stimulated expression of *IL23A* in both OE-E6/E7 and primary FTE cells and that this increase was blocked by DEX.

TACC2 is a member of a family of highly conserved proteins that associate with the centrosome-spindle apparatus and are thought to play a role in cell division [67]. In mammals, both TACC2 and 3 function as nuclear receptor coactivators [60,67]. TACC2 associates with retinoic acid receptor RXR β and with histone acetyltransferases and the Switch/sucrose nonfermentable (swi/snf) complex involved with chromatin remodeling and transcription [67,68,69]. While both tumor promoting and suppressive activities of TACC2 have been reported in various cancers [70,71,72], Lauffart et al [73] have shown that TACC2 and 3 interact with the BARD1/BRCA1 complex and may play a role in DNA double-strand break repair. Our data indicate *TACC2* expression is increased by DEX in both OE-E6/E7 cells and primary FTE cells, which is consistent with a previous report showing increased expression of TACC2 by DEX in ovarian cancer cells [74]. The functional impact of this increase remains to be determined.

PI3 encodes a serine protease inhibitor that functions as an anti-inflammatory mediator. Ghosh et al [75] reported that epithelial cells within the female reproductive tract, including the fallopian

tube, express PI3 with higher levels observed during the luteal phase. Although PI3 has been shown to reduce neutrophil and macrophage accumulation and increase G-CSF levels, consistent with a role in innate immunity, a role in adaptive immunity has also been suggested [76]. Consistent with our data showing increased PI3 expression by IL1 β , other studies have shown increased expression of PI3 in IL1 β -treated endocervical cells and in OE-E6/E7 cells exposed to *Chlamydia trachomatis* [77,78], likely through NF κ B signaling [79]. Since NF κ B signaling is in turn inhibited by PI3 [80] this increase in PI3 may be directed at containing the pro-inflammatory response. Our finding that DEX treatment further increased PI3 expression in the presence of IL1 β in OE-E6/E7 cells was surprising, given the inhibitory impact of glucocorticoid activation on NF κ B signaling. However, an inhibitory effect of DEX on IL1 β -induced *PI3* transcript levels was observed in primary FTE cells.

In summary, we demonstrate that IL1 β , a cytokine associated with ovulation, induces a pro-inflammatory gene expression signature in an immortalized human fallopian tube epithelial cell line. Activation of glucocorticoid receptor by a synthetic agonist reversed the expression of several IL1 β -induced inflammatory genes and altered the expression of additional inflammation-associated genes. These findings were confirmed for key representative genes in a primary culture of FTE cells. Thus, this study provides support for the hypothesis that pro-inflammatory signaling is induced in FTE cells by inflammatory mediators, such as those present in follicular fluid at the time of ovulation, and that this signaling is opposed by glucocorticoids. Dysregulation of glucocorticoid receptor signaling could therefore contribute to increased risk for HGSOE.

References

- Soslow RA (2008) Histologic subtypes of ovarian carcinoma: an overview. *Int J Gynecol Pathol* 27: 161–174.
- Crum CP, Drapkin R, Miron A, Ince TA, Muto M, et al. (2007) The distal fallopian tube: a new model for pelvic serous carcinogenesis. *Curr Opin Obstet Gynecol* 19: 3–9.
- Lee Y, Miron A, Drapkin R, Nucci MR, Medeiros F, et al. (2007) A candidate precursor to serous carcinoma that originates in the distal fallopian tube. *J Pathol* 211: 26–35.
- Sogaard M, Kjaer SK, Gayther S (2006) Ovarian cancer and genetic susceptibility in relation to the BRCA1 and BRCA2 genes. Occurrence, clinical importance and intervention. *Acta Obstet Gynecol Scand* 85: 93–105.
- Piek JM, van Diest PJ, Zweemer RP, Jansen JW, Poort-Keesom RJ, et al. (2001) Dysplastic changes in prophylactically removed Fallopian tubes of women predisposed to developing ovarian cancer. *J Pathol* 195: 451–456.
- Finch A, Shaw P, Rosen B, Murphy J, Narod SA, et al. (2006) Clinical and pathologic findings of prophylactic salpingo-oophorectomies in 159 BRCA1 and BRCA2 carriers. *Gynecol Oncol* 100: 58–64.
- Kindelberger DW, Lee Y, Miron A, Hirsch MS, Feltmate C, et al. (2007) Intraepithelial carcinoma of the fimbria and pelvic serous carcinoma: Evidence for a causal relationship. *Am J Surg Pathol* 31: 161–169.
- Kuhn E, Kurman RJ, Vang R, Schdev AS, Han G, et al. (2012) TP53 mutations in serous tubal intraepithelial carcinoma and concurrent pelvic high-grade serous carcinoma—evidence supporting the clonal relationship of the two lesions. *J Pathol* 226: 421–426.
- Carlson JW, Miron A, Jarboe EA, Parast MM, Hirsch MS, et al. (2008) Serous tubal intraepithelial carcinoma: its potential role in primary peritoneal serous carcinoma and serous cancer prevention. *J Clin Oncol* 26: 4160–4165.
- Seidman JD, Zhao P, Yemelyanova A (2011) “Primary peritoneal” high-grade serous carcinoma is very likely metastatic from serous tubal intraepithelial carcinoma: assessing the new paradigm of ovarian and pelvic serous carcinogenesis and its implications for screening for ovarian cancer. *Gynecol Oncol* 120: 470–473.
- Tang S, Onuma K, Deb P, Wang E, Lytwyn A, et al. (2012) Frequency of serous tubal intraepithelial carcinoma in various gynecologic malignancies: a study of 300 consecutive cases. *Int J Gynecol Pathol* 31: 103–110.
- Hunn J, Rodriguez GC (2012) Ovarian cancer: etiology, risk factors, and epidemiology. *Clin Obstet Gynecol* 55: 3–23.

Supporting Information

Table S1 Annotation of Genes in Venn diagram shown in Figure 2.

(XLSX)

File S1 Supporting Figures. Figure S1. Network Module 0, representing ‘Inflammatory response, Interleukin signaling and NF κ B signaling’ shown in Figure 3. **Figure S2.** Network Module 1, representing ‘Chromosomal maintenance and Cell cycle’ shown in Figure 3. **Figure S3.** Network Module 2, representing ‘Integrin signaling and Extracellular matrix organization’ shown in Figure 3. **Figure S4.** Network Module 3, representing ‘Ubiquitin mediated proteolysis and Antigen processing and presentation’ shown in Figure 3. **Figure S5.** Network Module 4, representing ‘G protein coupled receptor signaling and downstream targets’ shown in Figure 3.

(DOC)

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Author Contributions

Conceived and designed the experiments: SB AK TJB. Performed the experiments: SB AK. Analyzed the data: RH LS SB AK TJB. Contributed reagents/materials/analysis tools: RH LS. Wrote the paper: SB AK RH TJB.

- McGuire V, Felberg A, Mills M, Ostrow KL, DiCioccio R, et al. (2004) Relation of contraceptive and reproductive history to ovarian cancer risk in carriers and noncarriers of BRCA1 gene mutations. *Am J Epidemiol* 160: 613–618.
- McLaughlin JR, Risch HA, Lubinski J, Moller P, Ghadirian P, et al. (2007) Reproductive risk factors for ovarian cancer in carriers of BRCA1 or BRCA2 mutations: a case-control study. *Lancet Oncol* 8: 26–34.
- Tung KH, Wilkens LR, Wu AH, McDuffie K, Nomura AM, et al. (2005) Effect of anovulation factors on pre- and postmenopausal ovarian cancer risk: revisiting the incessant ovulation hypothesis. *Am J Epidemiol* 161: 321–329.
- Buscher U, Chen FC, Kentenich H, Schmiady H (1999) Cytokines in the follicular fluid of stimulated and non-stimulated human ovaries; is ovulation a suppressed inflammatory reaction? *Hum Reprod* 14: 162–166.
- Karihtala P, Soini Y, Vaskivuo L, Bloigu R, Puistola U (2009) DNA adduct 8-hydroxydeoxyguanosine, a novel putative marker of prognostic significance in ovarian carcinoma. *Int J Gynecol Cancer* 19: 1047–1051.
- Lu H, Ouyang W, Huang C (2006) Inflammation, a key event in cancer development. *Mol Cancer Res* 4: 221–233.
- Valavanidis A, Vlachogianni T, Fiotakis C (2009) 8-hydroxy-2'-deoxyguanosine (8-OHdG): A critical biomarker of oxidative stress and carcinogenesis. *J Environ Sci Health C Environ Carcinog Ecotoxicol Rev* 27: 120–139.
- Singh N, Rieder MJ, Tucker MJ (2004) Mechanisms of glucocorticoid-mediated anti-inflammatory and immunosuppressive action. *Paediatric and Perinatal Drug Therapy* 6: 107–115.
- Tone AA, Virtanen C, Shaw P, Brown TJ (2012) Prolonged postovulatory proinflammatory signaling in the fallopian tube epithelium may be mediated through a BRCA1/DAB2 axis. *Clin Cancer Res* 18: 4334–4344.
- Zhu XO, Yang Z, Guo CM, Ni XT, Li JN, et al. (2009) Paradoxical stimulation of cyclooxygenase-2 expression by glucocorticoids via a cyclic AMP response element in human amnion fibroblasts. *Mol Endocrinol* 23: 1839–1849.
- Wang B, Palomares K, Parobchak N, Cece J, Rosen M, et al. (2012) Glucocorticoid Receptor Signaling Contributes to Constitutive Activation of the Noncanonical NF- κ B Pathway in Term Human Placenta. *Mol Endocrinol* 27: 203–211.
- Sun H, Sheveleva E, Xu B, Inoue H, Bowden TG, et al. (2008) Corticosteroids induce COX-2 expression in cardiomyocytes: role of glucocorticoid receptor and C/EBP-beta. *Am J Physiol Cell Physiol* 295: C915–922.

25. Pujols L, Benitez P, Alobid I, Martinez-Anton A, Roca-Ferrer J, et al. (2009) Glucocorticoid therapy increases COX-2 gene expression in nasal polyps in vivo. *Eur Respir J* 33: 502–508.
26. Mulayim N, Palter SF, Selam B, Arici A (2003) Expression and regulation of interleukin-8 in human fallopian tubal cells. *American journal of obstetrics and gynecology* 188: 651–656.
27. Gerard N, Caillaud M, Martoriati A, Goudet G, Lalmanach AC (2004) The interleukin-1 system and female reproduction. *J Endocrinol* 180: 203–212.
28. Lee YL, Lee KF, Xu JS, Wang YL, Tsao SW, et al. (2001) Establishment and characterization of an immortalized human oviductal cell line. *Mol Reprod Dev* 59: 400–409.
29. Monkkonen KS, Aflatoonian R, Lee KF, Yeung WS, Tsao SW, et al. (2007) Hormonal regulation of Galphai2 and mPRalpha in immortalized human oviductal cell line OE-E6/E7. *Mol Hum Reprod* 13: 845–851.
30. Ling L, Lee YL, Lee KF, Tsao SW, Yeung WS, et al. (2005) Expression of human oviductin in an immortalized human oviductal cell line. *Fertil Steril* 84 Suppl 2: 1095–1103.
31. Fotheringham S, Levanon K, Drapkin R (2011) Ex vivo culture of primary human fallopian tube epithelial cells. *J Vis Exp* 51. Available: <http://www.jove.com/details.php?id=2728>, doi:10.3791/2728.
32. Kollara A, Brown TJ (2006) Functional interaction of nuclear receptor coactivator 4 with aryl hydrocarbon receptor. *Biochem Biophys Res Commun* 346: 526–534.
33. Gentleman RC, Carey VJ, Bates DM, Bolstad B, Detting M, et al. (2004) Bioconductor: open software development for computational biology and bioinformatics. *Genome Biol* 5: R80.
34. Reich M, Liefeld T, Gould J, Lerner J, Tamayo P, et al. (2006) GenePattern 2.0. *Nat Genet* 38: 500–501.
35. Wettenhall JM, Smyth GK (2004) limmaGUI: a graphical user interface for linear modeling of microarray data. *Bioinformatics* 20: 3705–3706.
36. Wu G, Feng X, Stein L (2010) A human functional protein interaction network and its application to cancer data analysis. *Genome Biol* 11: R53.
37. Mikosz CA, Brickley DR, Sharkey MS, Moran TW, Conzen SD (2001) Glucocorticoid receptor-mediated protection from apoptosis is associated with induction of the serine/threonine survival kinase gene, *sgk-1*. *J Biol Chem* 276: 16649–16654.
38. Gross JL, Yellen J (1998) *Graph Theory and Its Applications*. Boca Raton, FL: CRC Press.
39. Girvan M, Newman ME (2002) Community structure in social and biological networks. *Proc Natl Acad Sci USA* 99: 7821–7826.
40. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, et al. (2003) Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res* 13: 2498–2504.
41. Trinchieri G (2012) Cancer and inflammation: an old intuition with rapidly evolving new concepts. *Annu Rev Immunol* 30: 677–706.
42. Maccio A, Madeddu C (2012) Inflammation and ovarian cancer. *Cytokine* 58: 133–147.
43. Johnson ML, Murdoch J, Van Kirk EA, Kaltenbach JE, Murdoch WJ (1999) Tumor necrosis factor alpha regulates collagenolytic activity in preovulatory ovine follicles: relationship to cytokine secretion by the oocyte-cumulus cell complex. *Biol Reprod* 61: 1581–1585.
44. Revelli A, Delle Piane L, Casano S, Molinari E, Massobrio M, et al. (2009) Follicular fluid content and oocyte quality: from single biochemical markers to metabolomics. *Reprod Biol Endocrinol* 7: 40.
45. Tone AA, Begley H, Sharma M, Murphy J, Rosen B, et al. (2008) Gene expression profiles of luteal phase fallopian tube epithelium from BRCA mutation carriers resemble high-grade serous carcinoma. *Clin Cancer Res* 14: 4067–4078.
46. Tone AA, Virtanen C, Shaw PA, Brown TJ (2011) Decreased progesterone receptor isoform expression in luteal phase fallopian tube epithelium and high-grade serous carcinoma. *Endocr Relat Cancer* 18: 221–234.
47. Karin M (2009) NF-kappaB as a critical link between inflammation and cancer. *Cold Spring Harb Perspect Biol* 1: a000141.
48. Aggarwal BB, Sung B (2011) NF-kappaB in cancer: a matter of life and death. *Cancer Discov* 1: 469–471.
49. Almawi WY, Beyhum HN, Rahme AA, Rieder MJ (1996) Regulation of cytokine and cytokine receptor expression by glucocorticoids. *J Leukoc Biol* 60: 563–572.
50. Caldenhoven E, Liden J, Wissink S, Van de Stolpe A, Raaijmakers J, et al. (1995) Negative cross-talk between RelA and the glucocorticoid receptor: a possible mechanism for the antiinflammatory action of glucocorticoids. *Mol Endocrinol* 9: 401–412.
51. Necela BM, Cidlowski JA (2004) Mechanisms of glucocorticoid receptor action in noninflammatory and inflammatory cells. *Proc Am Thorac Soc* 1: 239–246.
52. Sen R, Smale ST (2010) Selectivity of the NF-kB response. *Cold Spring Harb Perspect Biol* 2: a000257.
53. Chaturvedi MM, Sung B, Yadav VR, Kannappan R, Aggarwal BB (2011) NF-kappaB addiction and its role in cancer: 'one size does not fit all'. *Oncogene* 30: 1615–1630.
54. Lim H, Paria BC, Das SK, Dinchuk JE, Langenbach R, et al. (1997) Multiple female reproductive failures in cyclooxygenase 2-deficient mice. *Cell* 91: 197–208.
55. Wanggren K, Stavreus-Evers A, Olsson C, Andersson E, Gemzell-Danielsson K (2008) Regulation of muscular contractions in the human Fallopian tube through prostaglandins and progestagens. *Hum Reprod* 23: 2359–2368.
56. Menter DG, Schilsky RL, DuBois RN (2010) Cyclooxygenase-2 and cancer treatment: understanding the risk should be worth the reward. *Clin Cancer Res* 16: 1384–1390.
57. Wang D, Dubois RN (2010) The role of COX-2 in intestinal inflammation and colorectal cancer. *Oncogene* 29: 781–788.
58. Ammundsen HB, Faber MT, Jensen A, Hogdall E, Blaakaer J, et al. (2012) Use of analgesic drugs and risk of ovarian cancer: results from a Danish case-control study. *Acta Obstet Gynecol Scand* 91: 1094–1102.
59. Baandrup L, Faber MT, Christensen J, Jensen A, Andersen KK, et al. (2013) Nonsteroidal anti-inflammatory drugs and risk of ovarian cancer: systematic review and meta-analysis of observational studies. *Acta Obstet Gynecol Scand* 92: 245–255.
60. Palter SF, Mulayim N, Senturk L, Arici A (2001) Interleukin-8 in the human fallopian tube. *J Clin Endocrinol Metab* 86: 2660–2667.
61. Xu L, Fidler IJ (2000) Interleukin 8: an autocrine growth factor for human ovarian cancer. *Oncol Res* 12: 97–106.
62. Lokshin AE, Winans M, Landsittel D, Marrangoni AM, Velikokhatnaya L, et al. (2006) Circulating IL-8 and anti-IL-8 autoantibody in patients with ovarian cancer. *Gynecol Oncol* 102: 244–251.
63. Oppmann B, Lesley R, Blom B, Timans JC, Xu Y, et al. (2000) Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12. *Immunity* 13: 715–725.
64. McKenzie BS, Kastlein RA, Cua DJ (2006) Understanding the IL-23-IL-17 immune pathway. *Trends Immunol* 27: 17–23.
65. Langowski JL, Zhang X, Wu L, Mattson JD, Chen T, et al. (2006) IL-23 promotes tumour incidence and growth. *Nature* 442: 461–465.
66. Teng MW, Andrews DM, McLaughlin N, von Scheidt B, Ngwi SF, et al. (2010) IL-23 suppresses innate immune response independently of IL-17A during carcinogenesis and metastasis. *Proc Natl Acad Sci USA* 107: 8328–8333.
67. Still IH, Vettaikorumakankau AK, DiMatteo A, Liang P (2004) Structure-function evolution of the transforming acidic coiled coil genes revealed by analysis of phylogenetically diverse organisms. *BMC Evol Biol* 4: 16.
68. Gangisetty O, Lauffart B, Sondarva GV, Chelsea DM, Still IH (2004) The transforming acidic coiled coil proteins interact with nuclear histone acetyltransferases. *Oncogene* 23: 2559–2563.
69. Lauffart B, Gangisetty O, Still IH (2003) Molecular cloning, genomic structure and interactions of the putative breast tumor suppressor TACC2. *Genomics* 81: 192–201.
70. Takayama K, Horie-Inoue K, Suzuki T, Urano T, Ikeda K, et al. (2012) TACC2 is an androgen-responsive cell cycle regulator promoting androgen-mediated and castration-resistant growth of prostate cancer. *Mol Endocrinol* 26: 748–761.
71. Cheng S, Douglas-Jones A, Yang X, Mansel RE, Jiang WG (2010) Transforming acidic coiled-coil-containing protein 2 (TACC2) in human breast cancer, expression pattern and clinical/prognostic relevance. *Cancer Genomics Proteomics* 7: 67–73.
72. Lauffart B, Vaughan MM, Eddy R, Chervinsky D, DiCioccio RA, et al. (2005) Aberrations of TACC1 and TACC3 are associated with ovarian cancer. *BMC Womens Health* 5: 8.
73. Lauffart B, Gangisetty O, Still IH (2007) Evolutionary conserved interaction of TACC2/TACC3 with BARD1 and BRCA1: potential implications for DNA damage response in breast and ovarian cancer. *Cancer Therapy* 5: 409–416.
74. Runnebaum IB, Bruning A (2005) Glucocorticoids inhibit cell death in ovarian cancer and up-regulate caspase inhibitor cIAP2. *Clin Cancer Res* 11: 6325–6332.
75. Ghosh M, Shen Z, Fahey JV, Cu-Uvin S, Mayer K, et al. (2010) Trappin-2/Elafin: a novel innate anti-human immunodeficiency virus-1 molecule of the human female reproductive tract. *Immunology* 129: 207–219.
76. Roghanian A, Williams SE, Shelldrake TA, Brown TE, Oberheim K, et al. (2006) The antimicrobial/elastase inhibitor elafin regulates lung dendritic cells and adaptive immunity. *Am J Respir Cell Mol Biol* 34: 634–642.
77. King AE, Wheelhouse N, Cameron S, McDonald SE, Lee KF, et al. (2009) Expression of secretory leukocyte protease inhibitor and elafin in human fallopian tube and in an in-vitro model of Chlamydia trachomatis infection. *Hum Reprod* 24: 679–686.
78. Stock SJ, Duthie L, Tremaine T, Calder AA, Kelly RW, et al. (2009) Elafin (SKALP/Trappin-2/proteinase inhibitor-3) is produced by the cervix in pregnancy and cervicovaginal levels are diminished in bacterial vaginosis. *Reprod Sci* 16: 1125–1134.
79. Bingle L, Tetley TD, Bingle CD (2001) Cytokine-mediated induction of the human elafin gene in pulmonary epithelial cells is regulated by nuclear factor-kappaB. *Am J Respir Cell Mol Biol* 25: 84–91.
80. Henriksen PA, Hitt M, Xing Z, Wang J, Haslet C, et al. (2004) Adenoviral gene delivery of elafin and secretory leukocyte protease inhibitor attenuates NF-kappa B-dependent inflammatory responses of human endothelial cells and macrophages to atherogenic stimuli. *J Immunol* 172: 4535–4544.