**BORIS Expression in Ovarian Cancer Precursor Cells Alters the CTCF Cistrome and Enhances Invasiveness through GALNT14**

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**Abstract**

High-grade serous carcinoma (HGSC) is the most aggressive and predominant form of epithelial ovarian cancer and the leading cause of gynecologic cancer-related death. We have previously shown that CTCFL (also known as BORIS, Brother of the Regulator of Imprinted Sites) is expressed in most ovarian cancers, and is associated with global and promoter-specific DNA hypomethylation, advanced tumor stage, and poor prognosis. To explore its role in HGSC, we expressed BORIS in human fallopian tube secretory epithelial cells (FTSEC), the presumptive cells of origin for HGSC. BORIS-expressing cells exhibited increased motility and invasion, and BORIS expression was associated with alterations in several cancer-associated gene expression networks, including fatty acid metabolism, TNF signaling, cell migration, and ECM-receptor interactions. Importantly, GALNT14, a glycosyltransferase gene implicated in cancer cell migration and invasion, was highly induced by BORIS, and GALNT14 knockdown significantly abrogated BORIS-induced cell motility and invasion. In addition, *in silico* analyses provided evidence for BORIS and GALNT14 coexpression in several cancers. Finally, ChIP-seq demonstrated that expression of BORIS was associated with de novo and enhanced binding of CTCF at hundreds of loci, many of which correlated with activation of transcription at target genes, including GALNT14. Taken together, our data indicate that BORIS may promote cell motility and invasion in HGSC via upregulation of GALNT14, and suggests BORIS as a potential therapeutic target in this malignancy.

**Implications:** These studies provide evidence that aberrant expression of BORIS may play a role in the progression to HGSC by enhancing the migratory and invasive properties of FTSEC.

**Introduction**

Although ovarian cancer accounts for less than 2% of all new cancer cases in American women, it is the leading cause of gynecologic cancer-related death, claiming the lives of more than half of all women diagnosed within 5 years. The discordance between prevalence and prognosis is primarily due to its advanced stage at diagnosis, as over 80% of patients present with disseminated cancer that is often resistant or refractory to standard treatment measures. HGSC is the most aggressive and most predominant form of epithelial ovarian cancer (EOC), making up more than 70% of diagnosed cases. Molecular analysis of a large number of HGSC in TCGA uncovered a high frequency of DNA copy number alterations (CNA) and almost ubiquitous occurrence of TP53 mutations, as well as a low but statistically significant frequency of alterations in BRCA1, BRCA2, NF1, RB1, and CDK12 (1). Pathway analysis implicated defective homologous recombination in approximately half of HGSC and the activation of both NOTCH1 and FOXM1 signaling (1, 2).

Although the ovarian surface epithelium (OSE) was originally thought to contain the cell of origin, it is now recognized that most HGSCs originate in secretory cells of the distal fallopian tube (fimbriae) and only later metastasize to the ovary. Providing evidence for this paradigm shift, a large proportion of patients with HGSC have noninvasive lesions called serous tubal intraepithelial carcinoma (STIC) in their fallopian tubes that are histologically similar to and carry identical mutations (including...
TP53) and expression profiles as HGSC (3). STIC has been found in patients who underwent prophylactic salpingo-oophorectomy in the absence of any invasive carcinoma but were known carriers of BRCA1/2 predisposition mutations (4), indicating that this lesion can predate invasive cancer. Moreover, our previous DNA methylome analyses demonstrated that the methylation profile of HGSCs and fallopian tube epithelia (FTE) are more similar than are HGSCs and OSE (5). These findings prompted the development of immortalized fallopian tube secretory epithelial cells (FTSEC) derived from the fimbrial region as a more physiologically relevant experimental model of the disease (6). The validity of this model was supported by the demonstration that ectopic expression of cyclin E1 (CCNE1), which is associated with poor survival in HGSC, imparted malignant properties to untransformed fallopian tube epithelial cells when TP53 function was abrogated, leading to an accumulation of DNA damage and altered transcription of DNA damage response genes related to DNA replication stress (7).

We have previously shown that BORIS (CTCFL) is expressed in a large proportion of EOC and its expression is associated with global genomic DNA hypomethylation and BORIS promoter DNA hypomethylation (8). Importantly, a significant association was found between BORIS expression, increased tumor stage, and poor prognosis (9). BORIS is a cancer germline (CG) or cancer testis (CT) antigen gene, primarily expressed in the germline under normal circumstances and abnormally activated in many malignancies (10). Indeed, BORIS is one of the most commonly activated CG genes in cancer (11) and has been proposed as an activator of other CG genes (12), making it an attractive immunotherapeutic target (13).

BORIS is the sole paralog of CTCF (CCCTC-binding factor) gene which encodes a multifunctional DNA-binding protein (14). BORIS and CTCF share high homology in their central zinc finger core and can potentially bind identical or similar DNA sequences; however, ChIP-seq experiments in cancer and germ cells have shown that BORIS binds only a subset of CTCF-binding sites (15) which consist of two closely located CTCF binding motifs (termed 2xCTSes) either as a CTCF-BORIS heterodimer or a BORIS homodimer (15). The 2xCTSes are generally enriched in active chromatin regions including H3Kme2, H3Kme3, H3K27ac, H3K79me2, and H3K9ac, in addition to the histone variant H2A.Z (15, 16). Unlike single-motif CTCF target sites (1xCTSes), which are suggested to have insulator function, 2xCTSes are evidenced by embryonic lethality of whole body CTCF knockout mice, suggesting that the normal function of BORIS is to regulate male germ cell development. This function may be due to its regulating expression of the important testis-specific genes Gal3st and Pers50 (15, 20, 21) possibly by cooperating with CTCF (at 2xCTSes) and other testis-specific transcription factors (22). In cancer, BORIS has been proposed to be an oncogene with various studies demonstrating its activity in repressing tumor suppressor genes including CTCF and inducing other oncogenes (23). BORIS has been shown to induce protumorigenic phenotypes in cell line models (24–26), including increased cellular proliferation (27–29) and invasion (26, 29). Although the mechanism(s) of action of BORIS is still being elucidated, it may function in a dominant-negative fashion by displacement of CTCF and the reorganization of the chromatin landscape (15, 30) thus impacting gene expression and contributing to the cancer phenotype. Consistent with this idea, our previous studies showed that an elevated BORIS/CTCF expression ratio correlated with disease progression in ovarian cancer (9).

In this study, we assessed the phenotypic and molecular changes that BORIS expression imposes on a physiologically relevant model of HGSC precursor cells. BORIS was expressed in FT282 cells, an immortalized nontransformed cell line derived from FTSEC, the proposed initiating cells of many HGSCs (7). Ectopic expression of BORIS in FT282 cells induced changes indicative of cellular transformation including increased migration and invasion through Matrigel. Phenotypic changes were accompanied by significant alterations in gene expression and alteration of key cancer-associated pathways. In particular, BORIS induced expression of the cancer-associated glycosyltransferase GALNT14 gene, which contributed to the downstream invasive phenotype. Moreover, BORIS was directly implicated in many of the observed gene expression changes, as its binding was enriched at differentially expressed genes (DEG). Finally, ectopic expression of BORIS in FT282 cells was associated with both quantitative and qualitative increases in genomic CTCF binding. Taken together, our results support a role for BORIS in increasing the invasive potential of HGSC precursor cells. These data, coupled with widespread increased expression of BORIS in HGSC, suggests that BORIS is a potential therapeutic target in this malignancy.

Materials and Methods

FTSE cell culture and ectopic BORIS-expressing cell line

A clonal derivative of FT282 was generated as described previously (31), and cultured in DMEM/Ham F-12 50/50 Mix (DMEM:F12) without L-glutamine (Corning) with 10% FBS (Pepsi Serum) and 1% penicillin–streptomycin (Gibco). To generate a BORIS-expressing FT282 cell line, a CgG-free BORIS cDNA (15) was cloned into SparQ IRES lentivector containing GFP (System Biosciences). FT282 cells were transduced with BORIS containing lentivirus or empty vector (EV) in the presence of 8 μg/mL polybrene. Infected cells were propagated for 14 days and sorted to obtain GFP-positive cells containing lentivirus.

Protein extraction and Western blotting

Cells at approximately 80% confluency in 6-well plates were washed 2 × with 1 mL ice-cold PBS containing protease inhibitors (Pierce Protease Inhibitor Mini Tablets, EDTA Free) and lysed in
Propidium iodide staining/cell-cycle analysis

except inserts coated with Matrigel were used and assays were performed in triplicate transwells and quantified using the RPCCC Genomics Shared Resource using an Illumina HiSeq2500. FASTQ files were mapped to the UCSC Human reference (build hg19) using TopHat2 with the default parameter setting of 20 alignments per read and up to two mismatches per alignment. The aligned reads (BAM files) were then analyzed with Cufflinks 2.0.0 to estimate transcript relative abundance using the UCSC reference annotated transcripts (build hg19). The expression of each transcript was quantified as the number of reads mapping to a transcript divided by the transcript length in kilobases and the total number of mapped reads in millions (FPKM).

Gene Ontology (GO) analysis was performed in R 3.5.1 with clusterProfiler 3.8.1. Biological process annotation was performed with the enrichGO() command and redundant annotations were collapsed with simplify (up_ego, cutoff = 0.7, by = "p.adjust", select_fun = min) prior to plotting. Full GO results are provided in Supplementary Table S2. Significantly dysregulated genes (P < 0.006, logFC > 0.25), were then tested for pathway enrichment utilizing DAVID (https://david.ncifcrf.gov) and the KEGG database (http://www.genome.jp/kegg/) and the Reactome (https://reactome.org/) database. Complete RNA-seq data is available in GEO under accession number GSE131931.

RNA-sequencing, DEG identification, and pathway analysis

Sequencing libraries were prepared from 1 μg total RNA using the TruSeq Stranded Total RNA Kit (Illumina). Library preparation, sequence enrichment, and sequencing were carried out by the RPCCC Genomics Shared Resource using an Illumina HiSeq2500. Sequencing libraries were prepared from 1 μg total RNA using the TruSeq Stranded Total RNA Kit (Illumina). Library preparation, sequence enrichment, and sequencing were carried out by the RPCCC Genomics Shared Resource using an Illumina HiSeq2500. FASTQ files were mapped to the UCSC Human reference (build hg19) using TopHat2 with the default parameter setting of 20 alignments per read and up to two mismatches per alignment. The aligned reads (BAM files) were then analyzed with Cufflinks 2.0.0 to estimate transcript relative abundance using the UCSC reference annotated transcripts (build hg19). The expression of each transcript was quantified as the number of reads mapping to a transcript divided by the transcript length in kilobases and the total number of mapped reads in millions (FPKM).

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qRT-PCR

One microgram of DNase-treated RNA was converted to cDNA using the iScript Advanced cDNA Synthesis Kit (Bio-Rad). cDNA was diluted 1:10 with water for use in subsequent qRT-PCR reactions. qRT-PCR was performed using the CFX96 Real-Time PCR Detection System (Bio-Rad) with SsoAdvanced Universal SYBR Green Supermix (Bio-Rad). Primers used for qPCR were designed using Beacon Designer (Premier Biosoft) and sequences included in Supplementary Table S7. HPRT1 mRNA levels were assayed as an endogenous control.

siRNA knockdown of GALNT14

Knockdown of GALNT14 was by transient transfection of Silencer Select siRNA targeted against GALNT14 (Thermo Fisher Scientific, assay s35936); the Silencer Select Negative Control No. 1 was used as a baseline control. Cells were grown to approximately 80% confluence in 6-well dishes. For each transfection, 3 μL of Lipofectamine RNAiMAX reagent was added to 150 μL of Opti-MEM medium followed by 30 pmol of siRNA. The diluted siRNA (150 μL) was added to the diluted Lipofectamine RNAiMAX reagent and incubated for 5 minutes in a heat block at 23°C. A total of 250 μL of the siRNA Lipofectamine RNAiMAX complex was then added dropwise to the cells. Cells were harvested for protein 48 hours after transfection and GALNT14 knockdown was confirmed by Western blot analysis.

ChIP-sequencing

Chromatin immunoprecipitation sequencing (ChIP-seq) and bioinformatics analysis was performed essentially as described using approximately 10⁶ cells (15). Formaldehyde-fixed chromatin was sheared to 200–500 bp (Bioruptor), incubated overnight with DiaMag magnetic beads (Diagenode, Inc.) and CTCF or BORIS monoclonal or polyclonal antibodies (15), and DNA isolated from precipitated chromatin. ChIP DNA was amplified using a TruSeq ChIP Sample Preparation Kit (Illumina, Inc.) and
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used for single-end sequencing on an Illumina Genome Analyzer. Sequences were aligned with Bowtie and peaks called using MACS; the Peak Splitter was used to call sub-peaks and summits of peaks and improve peak resolution. ChIP-seq data were visualized using IGV. Peak overlaps between CTCF and BORIS ChIP-seq datasets were determined with BedTools Suite. Peaks were defined as overlapping if at least 1 bp of reciprocal peaks intersected (CTCF & BORIS); the remaining peaks were defined as nonoverlapping (CTCF-only and BORIS-only). Normalized tag density profiles were generated using the BedTools coverage option from the BedTools Suite, normalized to the number of mapped reads, and plotted in Microsoft Excel. The heatmaps were generated using the seqMINER 1.3.3. We used either k-means ranked or linear method for clustering normalization. The summits of either CTCF or BORIS peaks were extended ±5 kb. seqMINER was also used to generate the average profiles of read density for different clusters. Position weight matrices for CTCF and BORIS-bound regions were searched using Multiple EM for Motif Elicitation (MEME). The sequences under the summit of either CTCF or BORIS peaks extended 100 bp upstream and downstream were used for motif discovery. We ran MEME with parameters (– mod oops-revcomp -w 20) to identify 20-bp-long motifs considering both DNA strands. To analyze the occurrence of CTCF motifs in the sequences occupied by CTCF or BORIS, or both proteins, FIMO software (MEME suite) was employed with default parameters. The position weight matrices found for CTCF-binding regions by MEME were used for FIMO software. Each CTCF motif occurrence had a P < 0.0001 in the sequences of 200 bp around the summits of either CTCF (CTCF-only, CTCF & BORIS-bound regions) or BORIS (BORIS-only bound regions) peaks. Genomic distribution of CTCF and BORIS ChIP-seq peaks relative to reference genes was performed using the Cis-regulatory Element Annotation System (CEAS). Complete ChIP-seq data are available in GEO under accession number GSE131931.

Results
BORIS expression in EOC correlates with increased stage and decreased survival

We previously showed that BORIS expression in EOC correlated with advanced tumor stage and decreased survival; interestingly, this correlation was even more significant when the ratio of BORIS/CTCF mRNA expression was used in the analysis. Furthermore, a significant correlation was observed between BORIS promoter hypomethylation, increased tumor stage, and poor prognosis (9). To corroborate these findings in an independent and larger dataset, we queried CSIOVDB (Ovarian Cancer Database of Cancer Science Institute Singapore), a microarray-based gene expression database of EOC (33). Analyses showed that BORIS expression is significantly higher in fallopian tube carcinoma (“FTE tumors”) and ovarian carcinomas (“Tumors”) compared with normal fallopian tube epithelium (FTE) and ovarian surface epithelium (OSE; Supplementary Fig. S1). Moreover, BORIS expression is highest in serous tumors compared with other EOC subtypes (Supplementary Fig. S2A). Consistent with our previous findings, BORIS expression was significantly higher in advanced stage (Fig. 1A) and higher-grade tumors (Supplementary Fig. S2B). A similar analysis of 47 ovarian cancer cell lines in the Cancer Cell Line Encyclopedia (CCLE) showed no significant differences in levels of BORIS expression across histologic subtypes from which the cell lines were derived (Supplementary Fig. S2C); stage and grade information was not available. Also consistent with our earlier study, BORIS expression correlated with reduced disease-free and overall survival (Fig. 1B). When the ratio of BORIS/CTCF expression was used in these analyses, the same associations were observed (Supplementary Fig. S3). We also analyzed RNA-seq data from The Cancer Genome Atlas (TCGA) and found that ovarian cancers (i.e., HGSC) exhibited the highest expression of BORIS compared with other cancers (data not shown). However, no correlation was observed between BORIS mRNA expression, or BORIS:CTCF mRNA expression ratio, and overall survival in HGSC (Supplementary Fig. S4). This was also true when TCGA microarray expression data was used (not shown). The discrepancy between CSIOVDB and TCGA is likely explained by the fact that CSIOVDB data contains all EOC subtypes while TCGA data is composed solely of HGSC. Indeed, when similar analysis was carried out using only the serous carcinoma samples in CSIOVDB, no correlation with survival was observed (Supplemental Fig. S5) consistent with increased expression of BORIS in HGSC (Supplementary Fig. S2A).

Ectopic expression of BORIS in FTE cells induces cellular changes typical of cellular transformation

To determine the impact of ectopic BORIS expression in a physiologically relevant in vitro model of HGSC, we employed a clonal derivative of the immortalized, nontransformed human fallopian tube epithelial cell line, FT282, originally described by Karst and colleagues (7). The FT282 cell line was established from a clonal derivative of the immortalized, nontransformed human fallopian tube epithelial cell line, FT282, originally described by Karst and colleagues (7). The FT282 cell line was established from a clonal derivative of the immortalized, nontransformed human fallopian tube epithelial cell line, FT282, originally described by Karst and colleagues (7). The FT282 cell line was established from a clonal derivative of the immortalized, nontransformed human fallopian tube epithelial cell line, FT282, originally described by Karst and colleagues (7).

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Ectopic BORIS expression modulates expression of cancer-associated genes and pathways

During spermatogenesis, BORIS acts as a transcriptional regulator of several essential testis-specific genes, Gal3st and Prss50 (15, 20). To access the impact of ectopic BORIS expression in FTSE cells, we performed RNA-sequencing (RNA-seq) analysis in FT282-BORIS cells compared with the FT282-EV control, which revealed substantial differences in gene expression between the cell populations (Fig. 3A). The total number of differentially expressed genes (DEG) in FT282-BORIS compared with FT282-EV was 1,656, including 773 genes significantly upregulated and 883 genes significantly downregulated ($P < 0.006$; Supplementary Table S1). To gain insight into the functional significance of these changes, we performed gene ontology (GO) analysis. Consistent with the increase in migration observed in FT282-BORIS cells, upregulated genes were significantly enriched for biological processes including “regulation of chemotaxis” and “positive regulation of cell migration” while downregulated genes were enriched for processes such as “extracellular matrix organization” and “cell–substrate interaction” (Fig. 3B; Supplementary Table S1). These observations suggest that BORIS enhances FT282 cell motility by upregulating positive regulators of cell motility and downregulating genes involved in cell adhesion.

![Figure 1.](image_url)

**Ectopic BORIS expression modulates expression of cancer-associated genes and pathways**

It might be anticipated that the more differentially expressed genes in a biological process may be driving identification of the GO category. Using this criterion, a number of genes from several different GO categories (Supplementary Table S2) were chosen to validate the RNA-seq data using qRT-PCR. These included 6 genes upregulated in BORIS-expressing cells, GALNT14 and CHSY3 (glycoprotein biosynthesis), SERPINB2 and PCOLCE2 (regulation of peptidase activity), ITGA4 (positive regulation of cell migration), and CXCL2 (regulation of cell migration and chemotaxis), as well as 4 genes downregulated in FT282-BORIS cells, KLK5 (ECM organization), SFRP1 and IGFBP5 (gland development), and BCAM (cell substrate adhesion). All 6 upregulated genes were validated by qRT-PCR (Fig. 3C), while 3 of 4 of the downregulated genes also exhibited decreased expression by qRT-PCR (Fig. 3D); IGFBP5 did not validate possibly because of cross reaction with other IGFBP gene family members.

KEGG pathway analysis revealed several cancer-associated pathways significantly impacted by ectopic BORIS expression (Supplementary Table S3). In this analysis, the pathway for biosynthesis of unsaturated fatty acids was the most enriched for DEGs upregulated in FT282-BORIS cells; fatty acids are essential for cancer cell proliferation due to a high demand for lipids (35).
Consistent with this, several downregulated genes are involved in fatty acid degradation. In addition, the TNF signaling pathway was among the most highly upregulated. The NFκB signaling pathway, which is activated by proinflammatory cytokines, including those in the TNF pathway was also significantly upregulated as well as the cytokine–cytokine receptor interaction and the rheumatoid arthritis pathway. Among pathways enriched for genes downregulated in FT282-BORIS cells, genes involved in signaling of the p53 tumor suppressor gene. Notably, a separate pathway analysis using the Reactome database also identified several inflammatory pathways including IL10 signaling, chemokine receptors, and the inflammasome pathway as enriched in upregulated genes in FT282-BORIS cells, as well as several pathways related to p53 signaling enriched for downregulated genes (Supplementary Table S4). Consistent with the observed increases in migration and invasion, several related pathways were also enriched for DEGs; for example KEGG pathways for adherens junction and focal adhesion were enriched for a large number of genes downregulated in FT282-BORIS cells, some of which (e.g., WASF3, ACTN2, TCN, VCL, ZYX) are known to enhance cell motility or metastasis when lost in certain cell types (e.g., ref. 36). Among upregulated genes, the "Mucin type O-Glycan biosynthesis" pathway was significantly enriched in the KEGG analysis (Supplementary Table S3). Similarly, analysis of upregulated DEGs in the Reactome database revealed enrichment in pathways for "O-linked glycosylation of mucins"
Abnormal glycosylation, which is often attributed to deregulated expression of glycosyltransferases, is a common feature of human cancers and affects a number of cellular properties including proliferation, apoptosis, differentiation, migration, invasion, transformation, and immune responses (37). Thus, BORIS expression in normal FTSE cells resulted in a deregulation of multiple cell pathways related to cancer development and progression.

BORIS expression of BORIS in FT282 cells induces gene expression changes in biological processes associated with cancer. A, RNA-seq was carried out on FT282-EV and FT282-BORIS cells using three independent RNA isolates each. Differentially expressed genes between FT282-EV and FT282-BORIS cells (q-value < 0.05 and the log2 fold change > ± 0.25) were used to generate the heatmap. B, Bar plots of GO analysis. The length of the bar indicates the number of DEGs in each enriched category and the color of each bar indicates the adjusted P-value for each ontology. C and D, qRT-PCR results for 6 genes corresponding to upregulated GO categories (C) and 3 genes for downregulated categories (D). For each gene, there were three “biological” replicates (i.e., 3 cell cultures, 3 RNA preps, 3 cDNA syntheses) and three technical replicates biological replicate. The plots show the mean expression level in FT282-BORIS cells relative to FT282-EV cells and SE of the three biological replicates; the solid dots are the individual replicates. Statistical analysis was by unpaired t test.

(Supplementary Table S4). Abnormal glycosylation, which is often attributed to deregulated expression of glycosyltransferases, is a common feature of human cancers and affects a number of cellular properties including proliferation, apoptosis, differentiation, migration, invasion, transformation, and immune responses (37). Thus, BORIS expression in normal FTSE cells resulted in a deregulation of multiple cell pathways related to cancer development and progression.

BORIS induces expression of GALNT14 in FTSE cells that contributes to the migratory and invasive phenotype. GALNT14, a member of the Mucin type O-Glycan biosynthesis pathway, was the most highly induced gene \( \log_2 \text{fold change} = 5.85 \) (Supplementary Table S1), a finding confirmed by qRT-PCR (Fig. 3C). Western blot analysis confirmed that GALNT14 protein was also significantly upregulated in FT282-BORIS cells compared with FT282-EV (Fig. 4A). In addition, we found that BORIS
and GALNT14 were coexpressed in several cancer datasets, providing further evidence for a link between these two genes (Supplementary Table S5).

On the basis of the transcriptional induction of GALNT14, and its proposed involvement in cancer cell migration and invasion in ovarian cancer (38), we sought to determine whether the BORIS-associated increase in cellular migration and invasion was related to the observed increase in expression of GALNT14. FT282-BORIS cells were transfected with siRNAs targeted against GALNT14 mRNA, or with a nontargeting negative control. GALNT14 protein was markedly reduced by siRNA knockdown (KD; Fig. 4B) and this correlated with significantly decreased migration (Fig. 4C), supporting the notion that the GALNT14 glycosyltransferase is involved in the BORIS associated increase in cell motility. Moreover, KD of GALNT14 significantly diminished the ability of FT282-BORIS cells to invade through Matrigel compared with the negative control (Fig. 4D). These results implicate GALNT14 as a contributor to BORIS-induced cellular and molecular changes in FT-282 cells. Taken together, these results corroborate previous studies implicating BORIS in upregulation of inflammatory genes and pathways (15) as well as provide a novel link between BORIS expression and aberrant O-linked glycosylation of mucins, which may function in cell migration and invasion.

BORIS binding is enriched at the promoters of deregulated genes

To investigate the genomic targets of ectopically expressed BORIS, and to compare BORIS binding with CTCF occupancy, we performed genome-wide ChIP sequencing (ChIP-seq) in FT282-BORIS and FT282-EV cells. ChIP-seq identified a total of more than 47,000 CTCF-binding sites (ChIP-seq peaks) in the two cell populations with 95% present in both FT282-BORIS and FT282-EV cells. In addition, almost 24,000 BORIS peaks were identified in FT282-BORIS cells (Fig. 5A, left). Overlapping of CTCF and BORIS ChIP-seq peaks in FT282-BORIS cells revealed
Figure 5.

Ectopically expressed BORIS binds to a subset of CTCF sites enriched in >1 CTCF-binding motif. A, Left, Venn diagram depicting overlap of CTCF and BORIS binding sites in FT282-BORIS cells determined by ChIP-seq. Right, heatmap depicts CTCF (red) and BORIS (blue) occupancy at 55061 loci in FT282-BORIS cells. The tag density of CTCF and BORIS ChIP-seq data was collected within a 4-kb window around the summit of CTCF (CTCF & BORIS and CTCF-only) and BORIS peaks (BORIS-only). The collected data were subjected to k-means clustering using linear normalization based on similar tag density profiles using seqMiner. B, Left, heatmap showing ChIP-seq tag density at the top 1,000 CTCF-only, BORIS-only, and CTCF/BORIS peaks chosen for CTCF motif analysis. Right, proportion of peaks in each class found by FIMO (MEME suite) to contain >1 CTCF motif. Statistical analysis by \( \chi^2 \) test. C, Genomic distribution of the three classes of CTCF- and BORIS-binding sites in FT282 cells ectopically expressing BORIS. D, Prevalence of CTCF- and BORIS-binding sites at CpG islands in FT282-BORIS cells. E, Proportions of differentially expressed genes bound by BORIS FT282-BORIS cells. Comparisons tested for significance by \( \chi^2 \) analysis. F, The GALNT14 locus shows enhancement of CTCF binding at its promoter and the appearance of a ”new” CTCF-binding site associated with enhanced gene expression following binding of BORIS.
three classes of differential occupancy: 29,112 sites bound by CTCF alone (CTCF-only), 15,513 sites bound by both CTCF and BORIS (CTCF/BORIS shared), and 8,140 sites bound by BORIS alone (BORIS-only; Fig. 5A, right). In a previous study using three cancer cell lines that express BORIS endogenously, we showed that BORIS preferentially binds and forms CTCF-BORIS heterodimers at 2xCTSes (15). Thus, to determine whether ectopically expressed BORIS in FT282-BORIS cells was also associated with 2xCTSes, we carried out CTCF motif analysis using FIMO (MEME suite) within the sequence 100-bp upstream and downstream of the summit of the top 1,000 CTCF, CTCF/BORIS shared, or BORIS (BORIS-only) peaks (Fig. 5B, left). More than one CTCF motif was identified under 53% of BORIS-only peaks and 42% of CTCF/BORIS shared peaks compared with only 12% for CTCF-only peaks (Fig. 5B, right), thus confirming previous findings that BORIS preferentially occupies 2xCTSes either together with CTCF or alone. Also, in agreement with previous work (15), BORIS binding in the FT282-BORIS cell line was enriched at promoter regions [defined as plus or minus 2 kb from the transcription start site (TSS)] while CTCF-only sites were relatively depleted at promoters and comparatively enriched at intergenic regions (Fig. 5C). Consistent with this, and in contrast to CTCF alone, BORIS preferentially bound to CpG islands (CGI; Fig. 5D). Compared with genes not affected by BORIS expression, binding of BORIS was significantly enriched at both upregulated and downregulated DEGs, 83% and 61%, respectively (Fig. 5E), suggesting direct involvement of BORIS in gene regulation at these loci. Importantly, upregulation of GALNT14 was accompanied by BORIS binding at sites within the promoter and gene body (Fig. 5F). Similarly, upregulation of BIRC2, a component of the TNF signaling pathway, was accompanied with increased binding of BORIS at its promoter (Supplementary Fig. S8). Taken together, these results further demonstrate that, when aberrantly expressed, BORIS binds to a subset of CTCF-bound sites (CTCF/BORIS), as well as a number of sites not bound by CTCF (BORIS-only). Moreover, comparison of BORIS binding and RNA-seq data suggests that, in many cases, BORIS is influencing the transcription of DEGs as a consequence of direct promoter occupancy. CTCF ChIP-seq in the FT282-BORIS and FT282-EV lines also suggested a second mechanism beyond competition at CTCF-binding sites by BORIS at genomic loci already bound by CTCF. Compared with FT282-EV cells, more than 500 loci in BORIS-expressing cells exhibited enhanced binding of CTCF, defined as a tag density more than three times higher in BORIS-expressing cells compared with FT282-EV (Supplementary Fig. S7). In some cases, CTCF binding was clearly evident in FT282-BORIS cells but undetectable in BORIS-EV cells and thus appeared as "new" CTCF-binding sites (Fig. 5F; Supplementary Fig. S8). For example, the GALNT14 gene showed increased CTCF binding at its promoter and a novel CTCF-binding site in the gene body (Fig. 5F), indicating that increased CTCF occupancy may be important for its increased expression in FT282-BORIS cells. Interestingly, only about 50% of these new or enhanced CTCF-binding sites were also bound by BORIS in FT282-BORIS cells. These new or enhanced CTSes appeared to be nonrandom with respect to genomic location. More than two-thirds (382/561) of them overlap with known genes, 74 of which are DEGs. Fifty-three (53) of these 74 (~72%) DEGs were upregulated at the mRNA level in FT282-BORIS cells, while 21 (~28%) were downregulated (Supplementary Table S6). This phenomenon indicates that BORIS binding may lead to a more open chromatin conformation at upregulated DEGs by making these regions more accessible to CTCF binding.

**Discussion**

Identification of oncogenic drivers of HGSC is expected to lead to new therapeutic targets in this malignancy. Our earlier findings that expression of BORIS was positively correlated with advanced stage disease and poor prognosis of patients with ovarian cancer (9) suggested that this CT gene might be an oncogene in this malignancy. Here, we corroborated these findings by showing in an independent dataset (CSIOVDB), that BORIS is highly expressed in HGSC and correlates with advanced stage and reduced disease-free and overall survival. The correlation is even more significant when the ratio of BORIS/CTCF mRNA is used in the analysis, possibly reflecting the competitive nature of BORIS and CTCF binding.

Expression of BORIS in human FT282 cells, a physiologically relevant cell culture model of HGSC precursor cells in the fallopian tube (6, 7), resulted in enhanced cellular migration and invasion. Changes in cell-cycle progression were also observed, as BORIS expression led to cell-cycle arrest at G2–M. Because CTCF has previously been shown to regulate cell-cycle progression (39, 40), it might be anticipated that this function could be impaired by BORIS-induced disruption of CTCF regulation at key cell-cycle genes. Moreover, this phenotype is consistent with previous studies linking BORIS expression to deregulation of the cell cycle (26). Alternatively, cell-cycle arrest in BORIS-expressing cells may reflect DNA damage due to disruption of CTCF function in limiting oxidative stress (41). Related to this, RNA-seq analysis indicated a significant upregulation in ataxia telangiectasia mutated (ATM) mRNA in BORIS-expressing cells (42). There was also significant upregulation of genes involved in inflammatory pathways and the O-linked glycosylation of mucins, including a more than 50-fold (RNA-seq) upregulation of GALNT14, a glycosyltransferase with an established link to ovarian and breast cancer cell migration (38, 43). siRNA knockdown of GALNT14 partially abrogated both cellular migration and invasion in FT282-BORIS cells, supporting its role as a downstream effector of these BORIS-induced phenotypes. Importantly, based on ChIP-seq analysis, GALNT14 is a direct target of BORIS. Although BORIS and GALNT14 expression appear to be correlated in several other cancer types, this association was not observed in primary HGSC (Supplementary Table S5). One possibility for this inconsistency is that BORIS regulates GALNT14 expression only early on in HGSC progression; alternatively, BORIS and GALNT14 may be coexpressed in only a subset of tumor cells, similar to the intratumor heterogeneity observed previously for other CT genes (44). GALNT14, and most of the other genes examined by qRT-PCR (Fig. 3C and D), exhibited coexpression (i.e., maintained high log ORs in cooccurrence and mutual exclusivity analysis, cBioPortal) in TCGA ovarian cancer expression data. This is consistent with our finding that all but one of these genes (CXCL2) bound BORIS and CTCF at their promoter regions and/or intragenic regions in BORIS-expressing FT282 cells. Despite this, only expression of CHSY3 and SERPINB2 (both upregulated) mirrored BORIS expression with respect to increased expression in later stage and higher grade tumors in the CSIOVDB database; similarly, only increased expression of CHSY3 and SERPINB2 correlated with either overall or disease-free survival (Supplementary Fig. S9) similar to BORIS expression (Fig. 1). It may be that other BORIS
transcriptional targets, or the overall chromatin state induced by BORIS, are driving the survival association for BORIS. In some contexts, BORIS has been implicated in the activation of other CT antigen (CTA) genes (12, 45); however, in our study only 26 of 1,019 identified CTA genes (11) were significantly upregulated in FT282-BORIS cells (not shown), a finding consistent with other studies that have failed to verify BORIS-mediated induction of CT genes (46, 47). BORIS expression had no detectable effect on FT282 cell proliferation. This might be explained by the unique pattern of progression of ovarian cancer; initial cellular transformation may begin in the FTSE, but the ovary is often the site of the overt cancerous mass, suggesting that transformed cells of the FTSE may in some instances acquire a migratory and invasive phenotype before they metastasize to the ovary and begin to proliferate at an increased rate.

ChIP-sequencing revealed that BORIS preferentially bound at gene promoters and CGL with this tendency strongest for BORIS-only sites. In addition, BORIS binding was more likely to be found at BORIS-induced DEGs, implicating BORIS in a direct regulatory role of these loci. Although ChIP-seq analyses of FT282 cells, or its derivatives, for histone modifications and other transcription factors have yet to be carried out, it is likely that BORIS-binding sites coloclate with active chromatin marks such as H3Kme2, H3Kme3, H3K27ac, H3K79me2, and H3K9ac, in addition to the histone variant H2AZ, as observed in other cell systems (15, 16).

Interestingly, we identified more than 500 CTCF-binding sites in FT282-BORIS cells that were either undetectable in FT282-EV cells or exhibited enhanced binding in BORIS-expressing cells. Roughly half of these sites were also bound by BORIS suggesting that, at least for these sites, binding of BORIS may provoke a more open chromatin conformation that facilitates CTCF binding. For the remaining new or enhanced CTCF sites that do not exhibit binding of BORIS, it is possible that a similar mechanism is in play but occurred very early after the induction of BORIS expression where BORIS functioned in a “hit and run” fashion. The mechanism by which BORIS facilitates new or increased binding of CTCF is unknown. Consistent with evidence that BORIS binding is less sensitive to methylation (48), it is possible that BORIS promotes DNA demethylation at these regions, thereby allowing CTCF binding, as previously shown for other loci (12, 27) perhaps by recruitment of TET proteins and conversion of 5mc to 5hmC (49). An alternative mechanism may be that BORIS acts as a pioneer factor at these sites, either allowing CTCF to bind via protein–protein interactions, or recruiting chromatin remodelers that reposition nucleosomes to allow CTCF binding (50).

In summary, our results provide evidence supporting a protumorigenic role for BORIS in HGSC. In FT282 cells, ectopic BORIS expression significantly increased cellular migration and invasion, deregulated the cell cycle, and significantly altered gene expression. We provide further support for BORIS as an important mediator of inflammation as has been suggested previously and confirm that BORIS binding is not uniform across CTCF sites. Relevant future investigations of BORIS-related oncogenic mechanisms should include the process by which CTCF binding is altered and the function of aberrant O-linked glycosylation of mucins.

**Disclosure of Potential Conflicts of Interest**

A. Stablewski has received speakers bureau honoraria from Transnetyx and is a consultant/advisory board member for the International Society for Transgenic Technologies. No potential conflicts of interest were disclosed by the other authors.

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