Mutual antagonism between glucocorticoid and canonical Wnt signaling pathways in B-cell acute lymphoblastic leukemia

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Abstract

Glucocorticoids (GCs; i.e., steroids) are important chemotherapeutic agents in the treatment of B-cell precursor acute lymphoblastic leukemia (B-ALL) and *de novo* GC resistance predicts relapse and poor clinical outcome in patients. Glucocorticoids induce B-ALL cell apoptosis through activation of glucocorticoid receptor (GR), a ligand-induced nuclear receptor transcription factor (TF). We previously identified disruptions to glucocorticoid receptor (GR)-bound *cis*-regulatory elements controlling *TLE1* expression in GC-resistant primary B-ALL cells from patients. *TLE1* is a GC-response gene up-regulated by steroids and functions as a canonical Wnt signaling repressor. To better understand the mechanistic relationship between GC signaling and canonical Wnt signaling, we performed diverse functional analyses that identified extensive crosstalk and mutual antagonism between these two signaling pathways in B-ALL. We determined that crosstalk and antagonism was driven by the binding of GR and the canonical Wnt signaling TF LEF1 to overlapping sets of *cis*-regulatory elements associated with genes impacting cell death and cell proliferation, and was further accompanied by overlapping and opposing transcriptional programs. Our data additionally suggest that *cis*-regulatory disruptions at *TLE1* are linked to GC resistance through a dampening of the GC response and GC-mediated apoptosis via enhanced canonical Wnt signaling. As a result of the extensive genomic and gene regulatory connectivity between these two signaling pathways, our data supports the importance of canonical Wnt signaling in mediating GC resistance in B-ALL.

To the Editor

Glucocorticoids (GCs; i.e., steroids) are important chemotherapeutic agents in the treatment of B-cell precursor acute lymphoblastic leukemia (B-ALL)[1] and *de novo* GC resistance predicts relapse and poor clinical outcome[2,3]. Glucocorticoids induce B-ALL cell apoptosis through activation of glucocorticoid receptor (GR), a ligand-induced nuclear receptor transcription factor[4,5]. We previously identified disruptions to glucocorticoid receptor (GR)-bound *cis*-regulatory elements controlling *TLE1* expression, a GC-response gene up-regulated by steroids, as a novel mechanism impacting GC resistance in primary B-ALL cells from patients[6]. Supporting our findings, *TLE1* expression is associated with GC resistance in primary B-ALL cells from patients[7] and *TLE1* was also identified as an indicator of adverse prognosis in B-ALL patients[8]. Notably, because TLE1 functions as a repressor of canonical Wnt signaling[9], and because investigations in other cell systems have suggested interaction between GC and canonical Wnt signaling pathways[10-12], we investigated mechanisms connecting TLE1 to GC resistance and the extent of crosstalk between GC and canonical Wnt signaling pathways in B-ALL.

We first examined the functional effects of a homozygous knockout (KO) of *TLE1* on GC and canonical Wnt signaling using CRISPR-Cas9 genome editing in the Nalm6 human B-ALL cell line (**Figures 1A-B**). Consistent with our previous observations[6], we found *TLE1* KO led to significantly increased resistance to GCs (i.e., prednisolone), with >5-fold increase in prednisolone LC_{50} compared to wild-type (WT) cells (**Figure 1C**). To better understand the broader effects of *TLE1* disruption on GC signaling, we conducted RNA-seq in *TLE1* KO cells treated with prednisolone or vehicle control for 24 hours and compared these data to our previously generated 24-hour GC-response RNA-seq results from WT Nalm6 cells[6]. We identified substantially less GC-response genes in KO cells compared to WT cells (5,734 vs 8,784; FDR<0.05), with 73.5% of *TLE1* KO GC-response genes overlapping WT GC-response genes (**Figure 1D**). Although 90.9% of these overlapping GC-response genes were regulated concordantly between WT and KO cells, fold change comparisons determined that 78.2% exhibited less responsiveness in KO cells (**Figure 1D**), suggesting an overall blunting of the GC transcriptional response from *TLE1* disruption. To identify *TLE1*-dependent changes to canonical Wnt signaling, both WT and *TLE1* KO cells were transfected with the M50 Super 8xTOPFlash reporter plasmid[13] and treated for 24 hours with a canonical Wnt signaling agonist (CHIR-99021) or vehicle control. This assay measures beta-catenin-driven luciferase activity as an indicator of endogenous canonical Wnt signaling. Under both treatment conditions, *TLE1* KO cells showed increased canonical Wnt activity compared to WT cells (**Figure 1E**), consistent with the role of

TLE1 as a repressor of canonical Wnt signaling[9]. Overall, these data support opposing effects on GC and canonical Wnt signaling from *TLE* disruption.

We next assessed GC and canonical Wnt signaling pathway crosstalk in B-ALL cells independent of *TLE1* KO. Two human B-ALL cell lines (Nalm6 and 697) were treated with different prednisolone concentrations for 72 hours in the presence or absence of Wnt agonist CHIR-99021. At all GC concentrations and in both cell lines, co-treatment with the Wnt agonist significantly increased cellular viability relative to prednisolone-only treatment (**supplemental Figure 1).** We additionally confirmed this effect through *ex vivo* GC drug viability studies in B-ALL patient-derived xenograft cell samples (**supplemental Figure 2)**. These findings suggest that Wnt activation opposes GC-induced apoptosis, which is consistent with the role for canonical Wnt signaling in cell proliferation and cancer predisposition[14], and prior findings from B-ALL cell viability experiments using Wnt antagonist[15]. To further determine the extent that GCs impact canonical Wnt signaling, we measured beta-catenin-driven luciferase reporter activity in B-ALL cells treated with prednisolone and/or CHIR-99021. We found that GC treatment significantly dampened canonical Wnt signaling in the presence or absence of Wnt agonist, with consistent results also observed in TLE1 *KO* cells (**supplemental Figure 3**). Collectively, these data indicate mutual antagonism between these two signaling pathways.

We further investigated genomic mechanisms that promote mutual antagonism between GC and canonical Wnt signaling pathways. Using our published GC-response RNA-seq data from Nalm6 cells[6], we find that in addition to up-regulation of the *TLE1* canonical Wnt repressor gene, GCs repressed canonical Wnt activating transcription factor (TF) genes *LEF1* and *TCF7L2* (**supplemental Figure 4**). Consistent effects on *TLE1* and *LEF1* expression were also reported in a panel of 19 human B-ALL cell lines after dexamethasone treatment[16]. We therefore next asked if a 24-hour GC treatment resulted in decreased genome occupancy of *LEF1*. Using the CUT&RUN assay, we determined that LEF1 binding was decreased at 53.4% (32105) and increased at only 1.2% (730) of sites after 24 hours of prednisolone treatment (**Figures 2A-B**). Strikingly, we also uncovered that 58.3% of GR binding sites [previously mapped in [6]] overlap with LEF1 binding sites (examples in **Figure 2C**), and genes associated with these overlapping sites were enriched for cell proliferation (FDR=1.53×10^{-25}) and apoptotic signaling (FDR=6.13×10^{-20}) pathways. To determine if overlapping TF occupancy impacts crosstalk between GC and canonical Wnt signaling transcriptional responses, RNA-seq was performed on WT Nalm6 cells treated for 24 hours with CHIR-99021 or vehicle control. We identified 1,549 Wnt-response genes (FDR<0.05) and found that 75.5% of these genes were also GC-response genes in Nalm6 cells

(**supplemental Table 1**; **Figure 2D**). Supporting a mutual antagonism between these two signaling pathways, 80% of these common response genes exhibited discordant changes in gene expression (**Figure 2E**). These discordantly regulated response genes were significantly enriched in programmed cell death (FDR<$3x10^{-9}$) and cell cycle (FDR=$1.1x10^{-9}$) pathways, and 51.4% of them were associated with overlapping GR and LEF1 binding events. H3K27ac HiChIP in Nalm6 cells after 24 hours of prednisolone treatment[6] additionally identified looping between promoter-distal overlapping GR and LEF1 binding sites and the promoters of 246 discordantly regulated response genes. Forty-nine discordantly regulated response genes were also previously linked to GC resistance in primary B-ALL cells from over 200 patients [[7]; **supplemental Table 1**], supporting a broader impact on GC resistance, and most of these genes (34 of 49) were associated with overlapping GR and LEF1 binding events. To determine if this transcriptional antagonism translates to the proteome level, we performed mass spectrometry after a 24-hour treatment with prednisolone or CHIR-99021 and uncovered that 33% of discordantly regulated response genes exhibited consistent patterns as proteins (**Figure 2F** and **supplemental Table 1**). Concordant with our previous analyses of overlapping binding sites and response genes, these proteins were also enriched for cell death (FDR=$2.97x10^{-8}$) and cell cycle (FDR=$8.16x10^{-6}$) pathways.

Collectively, our data uncovered extensive crosstalk and mutual antagonism between GC and canonical Wnt signaling pathways in B-ALL cells through binding of GC and Wnt TFs to common *cis*-regulatory elements associated with cell death and cell proliferation genes. This overlap in TF occupancy was further accompanied by overlapping and opposing transcriptional programs that impacted protein expression. Mutual antagonism is additionally promoted by GC-mediated repression of the canonical Wnt activating TF genes *LEF1* and *TCF7L2*, and the activation of the canonical Wnt repressor gene *TLE1*. Overall, these data suggest *cis*-regulatory disruptions at *TLE1* are linked to GC resistance in primary B-ALL cells from patients through a dampening of the GC response and GC-mediated apoptosis via enhanced canonical Wnt signaling. As a result of the deep genomic and gene regulatory connectivity between these two signaling pathways, our data supports the importance of canonical Wnt signaling in mediating GC resistance in B-ALL.

Disclosures

No conflicts of interest to disclose.

Contributions

Conceptualization, D.S.; Methodology, B.P.B, K.R.B, R.J.M., J.P., S.M.P., D.S.; Investigation, B.P.B, K.R.B, R.J.M., B.S.H., A.B., K.K.; Formal Analysis, B.P.B, K.R.B, R.J.M., A.B., K.K., D.S.; Writing – Original Draft, B.P.B, D.S.; Writing – Review & Editing, B.P.B, K.R.B., R.J.M., B.S.H., A.B., K.K., J.P., S.M.P., D.S.; Funding Acquisition, D.S.

Data-sharing statement

All functional genomic data from cell lines have been deposited into the Gene Expression Omnibus (GSE215188).

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Figure Legends

Figure 1. (A) Schematic of CRISPR/Cas9 genome editing to exon 7 of the TLE1 gene with deep sequencing confirmation of edit to both strands in Nalm6 cells **(B)** Western blot showing protein expression of TLE1 (~83kD; Abcam ab183742) in both *TLE1* knockout Nalm6 (KO) and Nalm6 cells (WT). Ponceau staining for total protein below. **(C)** Prednisolone drug response curves in *TLE1* knockout Nalm6 cells (red) and wild-type Nalm6 cells (black) after 72-hours of prednisolone treatment is shown at the left, n = 3 per group. Concentrations of prednisolone used were: 5pM, 50pM, 0.5nM, 5nM, 50nM, 0.5uM, 5uM, 50uM, and 500uM. LC50 fold change in *TLE1* gene knockout (TLE1 KO) Nalm6 cells compared to wild-type (WT) Nalm6 cells is shown at the right. **(D)** Venn diagram shows the number of overlapping GC-responsive genes between wild-type Nalm6 cells (light red) and *TLE1* KO Nalm6 cells (dark red) at the left. Histogram provides a log_2-transformed fold change comparison between wild-type Nalm6 cells and *TLE1* KO Nalm6 cells at the right. A percentage denote the total number of overlapping genes regulated in a concordant manner that exhibit higher GC-responsiveness in wild-type cells. **(E)** Beta-catenin luciferase reporter assay for wild-type Nalm6 (WT) and *TLE1* KO (KO) cells treated with or without CHIR-99021 (0.5uM) for 24-hours, n = 6 per group.

Figure 2. (A) CUT&RUN sequencing read enrichment at LEF1 binding sites +/- 1kb (Cell Signaling antibody # 2230). Enrichment is shown for a 24-hour treatment with vehicle control (left) or prednisolone (5uM; GC, right). **(B)** Log_2-transformed normalized CUT&RUN sequencing read counts at LEF1 binding sites treated for 24 hours with vehicle control (x-axis) or prednisolone (y-axis). Binding sites exhibiting significant differences in occupancy (FDR<0.05) are shown in pink. **(C)** IGV genome browser images of signal tracks providing examples of GR and LEF1 co-occupancy at *BCL6* (left) and *PHLDA1* (right) gene loci (blue highlights). **(D)** Venn diagram shows overlap between GC-response (red, left) and Wnt-response (green, right) genes. **(E)** Log_2-transformed fold changes of genes commonly regulated by GC and Wnt signaling pathways. GC-response (x-axis) and Wnt-response (y-axis) log2 fold changes are provided. The percentage of genes in each quadrant is provided, and genes showing opposing effects are highlighted in quadrants 2 and 4. Red denotes genes involved in cell death and/or cell cycle pathways and notable genes with GR and LEF1 co-occupancy are labeled. **(F)** Protein expression volcano plot of proteins that maintain expression patterns that are consistent with transcriptomics following GC or Wnt agonist treatment for 24 hours. Red denotes proteins involved in cell death and/or cell cycle pathways and notable proteins with GR and LEF1 co-occupancy are labeled.

FIGURE 1

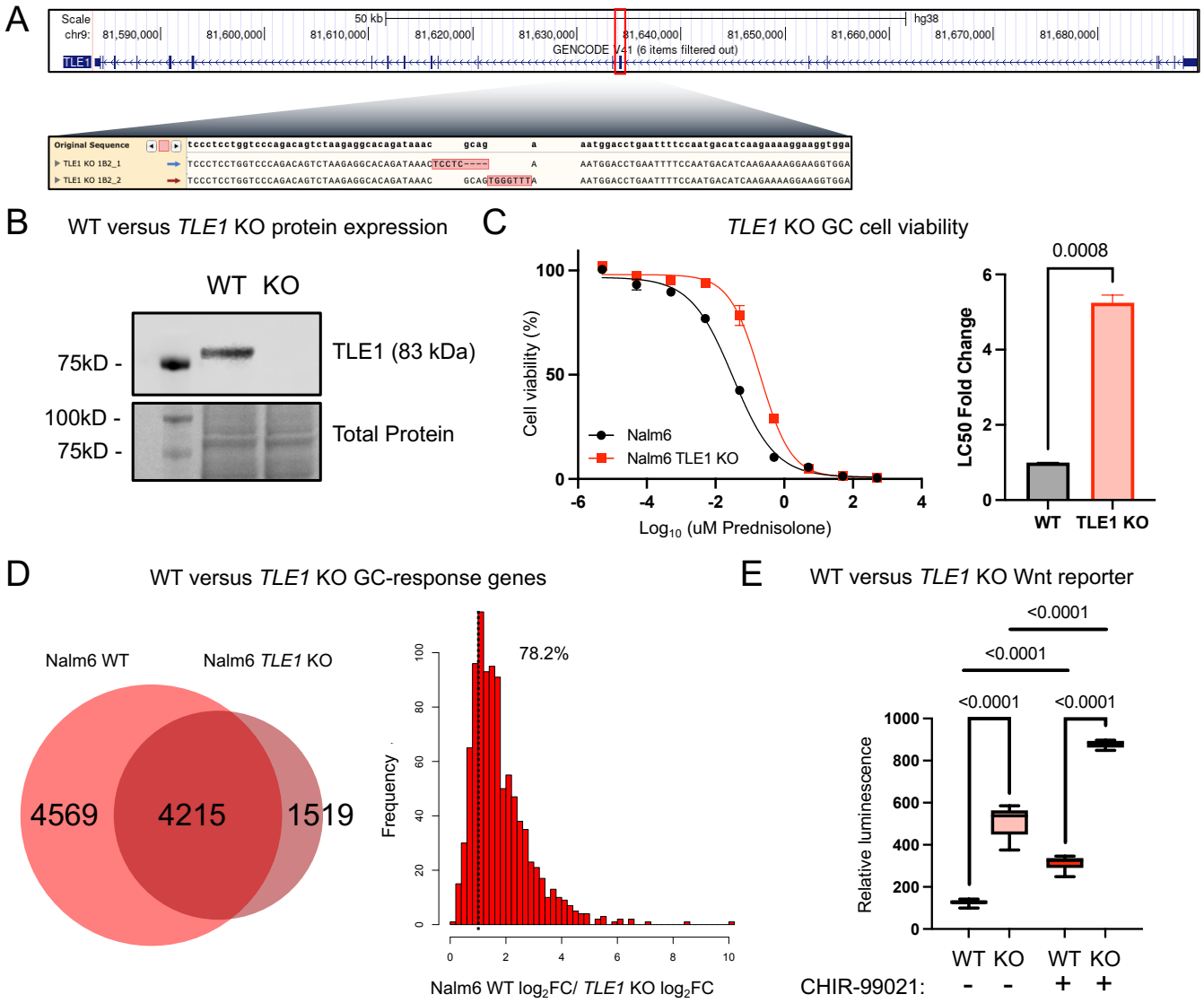
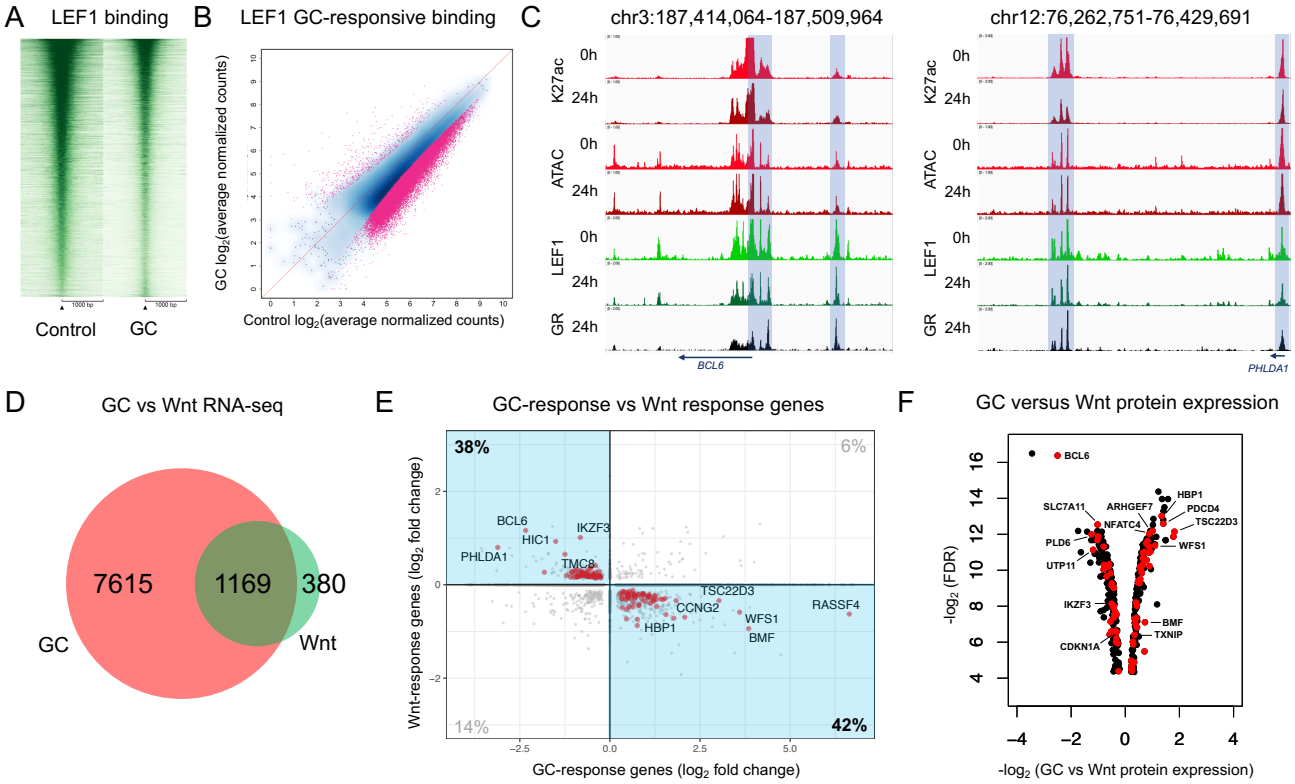

A
Scale
chr9:
50 kb
hg38
81,590,000
81,600,000
81,610,000
81,620,000
81,630,000
81,640,000
81,650,000
81,660,000
81,670,000
81,680,000
GENCODE V41 (6 items filtered out)
TLE1
Original Sequence
TLE1 KO 1B2_1
TLE1 KO 1B2_2
B
WT versus TLE1 KO protein expression
WT KO
75kD -
100kD -
75kD -
TLE1 (83 kDa)
Total Protein
C
TLE1 KO GC cell viability
Cell viability (%)
Nalm6
Nalm6 TLE1 KO
Log10 (uM Prednisolone)
0.0008
LC50 Fold Change
WT
TLE1 KO
D
WT versus TLE1 KO GC-response genes
Nalm6 WT
Nalm6 TLE1 KO
4569
4215
1519
78.2%
Frequency
Nalm6 WT log2FC/ TLE1 KO log2FC
E
WT versus TLE1 KO Wnt reporter
<0.0001
<0.0001
<0.0001
<0.0001
Relative luminescence
WT KO WT KO
CHIR-99021: - - + +

FIGURE 2


A
LEF1 binding
Control
GC
B
LEF1 GC-responsive binding
GC log2(average normalized counts)
Control log2(average normalized counts)
C
chr3:187,414,064-187,509,964
chr12:76,262,751-76,429,691
K27ac
ATAC
LEF1
GR
0h
24h
BCL6
PHLDA1
D
GC vs Wnt RNA-seq
7615
1169
380
GC
Wnt
E
GC-response vs Wnt response genes
38%
6%
14%
42%
BCL6
HIC1
IKZF3
PHLDA1
TMC8
TSC22D3
CCNG2
WFS1
BMF
HBP1
RASSF4
Wnt-response genes (log2 fold change)
GC-response genes (log2 fold change)
F
GC versus Wnt protein expression
BCL6
HBP1
SLC7A11
ARHGEF7
PDCD4
NFATC4
TSC22D3
PLD6
WFS1
UTP11
IKZF3
BMF
TXNIP
CDKN1A
-log2 (FDR)
-log2 (GC vs Wnt protein expression)