Transcription of carbonyl reductase 1 is regulated by DNA topoisomerase II beta

Mushtaq M. Khazeem1, Ian G. Cowell1, Lauren F. Harkin1, John W. Casement2 and Caroline A. Austin1

1 Faculty of Medical Sciences, Biosciences Institute, Newcastle University, Newcastle upon Tyne, UK
2 Bioinformatics Support Unit, Faculty of Medical Sciences, Newcastle University, Newcastle upon Tyne, UK

Correspondence
Caroline A. Austin, Faculty of Medical Sciences, Biosciences Institute, Newcastle University, Newcastle upon Tyne, UK
Tel: +44 (0)191 208 8864
Email: Caroline.Austin@ncl.ac.uk

(Received 14 May 2020, revised 30 July 2020, accepted 31 July 2020, available online 30 August 2020)
doi:10.1002/1873-3468.13904

Edited by: Claus Azzalin

DNA topoisomerase II beta (TOP2B) has a role in transcriptional regulation. Here, to further investigate transcriptional regulation by TOP2B, we used RNA-sequencing and real-time PCR to analyse the differential gene expression profiles of wild-type and two independent TOP2B-null pre-B Nalm-6 cell lines, one generated by targeted insertion and the other using CRISPR-Cas9 gene editing. We identified carbonyl reductase 1 (CBR1) among the most significantly downregulated genes in these TOP2B-null cells. Reduced CBR1 expression was accompanied by loss of binding of the transcription factors USF2 and MAX to the CBR1 promoter. We describe possible mechanisms by which loss of TOP2B results in CBR1 downregulation. To our knowledge, this is the first report of a link between TOP2B and CBR1.

Keywords: carbonyl reductase 1; cardiotoxicity; CBR1; DNA topoisomerase; TOP2B; topologically associating domains

DNA supercoiling dynamically influences DNA and chromatin structure within cells. During active transcription, positive supercoiling is generated locally in front of a transcribing polymerase and negative supercoiling behind, as postulated in the twin domain model of DNA supercoiling [1]. Topoisomerases modulate DNA topology and relieve the supercoiling stress around the transcriptional machinery. On a larger scale, eukaryotic chromatin is organised into loops [2,3], more recently defined as topologically associating domains (TADs) [4–6]. TADs are conserved between cell types, and TAD boundaries are enriched for CTCF binding sites [6].

The human genome encodes six DNA topoisomerase enzymes TOP2A, TOP2B, TOP3A, TOP3B, TOP1 and TOP1mt. Various lines of evidence point to a role for Top2b/TOP2B in the correct execution of certain transcriptional programmes during differentiation [7] and in nuclear hormone-induced transcription [8–17]. Furthermore, analysis of the DNA sequences within TOP2B ChIP-seq peaks found enrichment for a number of transcription factor motifs including those for CTCF and SP1 [15]; subsequent studies have confirmed a strong overlap between TOP2B and CTCF binding across the genome [13,14]. As CTCF is central to chromatin looping and the organisation of chromatin into TADs [18], the association of TOP2B with CTCF may reflect a requirement for TOP2B to relieve supercoiling within or between TADs [14] that may affect transcription. Clinically, heterozygous mutations in TOP2B patients have been shown to cause B-cell immunodeficiency, with the suspected link being the failure to activate PAX5 transcription, suggesting TOP2B may regulate PAX5 transcription [19].

To further investigate the role of TOP2B in transcriptional regulation, we carried out differential gene expression analysis comparing the pre-B-cell line, Nalm-6 and an established Nalm-6 TOP2B targeted

Abbreviations
CBR1, carbonyl reductase 1; TAD, topologically associating domain; TOP2, DNA topoisomerase II.
null mutation [20]. To confirm high confidence TOP2B regulated genes in this cell line, we generated a new TOP2B null cell line using CRISPR-Cas9 targeting in Nalm-6 cells. Surprisingly, of the most significantly downregulated genes in the former comparison, only one gene, CBR1, was significantly downregulated in the TOP2B CRISPR-Cas9 null cells. We discuss possible mechanisms by which TOP2 could be required for the regulation of CBR1 expression and hypothesise how this may contribute to the cardiotoxicity of anthracycline TOP2 poisons.

Materials and methods

Cell culture

Nalm-6 and its TOP2B knockout counterpart Nalm-6TOP2B−/− were kindly provided by Noritaka Adachi and were generated as described in Ref. [20]. All cell lines were maintained in RPMI-1640 medium (Gibco by Life Technologies, Invitrogen, Paisley, UK) containing 10% v/v heat-inactivated fetal bovine serum (FBS) (Gibco by Life Technologies, Invitrogen) and 1% v/v antibiotic solution (10 000 units·mL−1 penicillin, 10 000 mg·mL−1 streptomycin, Gibco by Life Technologies, Invitrogen) and incubated at 37 °C and 5% CO2 under standard humidity. Cells were maintained at growing phase and kept at a density of ~1–2×10⁶ cell·mL⁻¹.

RNA-sequencing and analysis

Extraction of total RNA from samples was performed using RNAeasy Mini Kit (Qiagen, Manchester, UK) according to the manufacturer’s instructions. Quantity and quality of the RNA samples were determined using the Agilent 2100 Bioanalyzer System (Agilent Technologies, Stockport, UK). RNA samples were sent to AROS (Denmark) for sequencing. Sequencing libraries were constructed using TruSeq Stranded Total RNA with Ribo-Zero Globin Kit (Illumina, San Diego, CA, USA) according to the manufacturer’s instructions. Input RNA for library preparation was 400 ng per sample. 40 million reads per sample with 75 bp paired-end (PE) sequencing were performed using NextSeq 500 Sequencing System (Illumina). Analysis of raw sequencing files was performed with the assistance of the Bioinformatics Support Unit, Newcastle University. Raw sequencing reads were assessed for quality using fastqc Version 0.11.2 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and trimmed using FASTX-Trimmer from the FASTX-Toolkit version0.0.13 (http://hannonlab. cshl.edu/fastx_toolkit/). Reads were mapped to GRCh37 (Ensembl release 75) using Salmon [21]. Gene and transcript level abundances were analysed for differential expression using the R package EBSeq [22]. Genes whose expression changed by 2-fold or greater with an adjusted P-value of < 0.05 were considered significant. Raw data analysed during this study are available at the NCBI GEO repository with accession number GSE148137.

Reverse transcription quantitative polymerase chain reaction

RNA samples used for RT-qPCR were prepared directly from fresh cell lysates using SingleShot Cell Lysis Kit (Bio-Rad, Watford, UK) according to the manufacturer’s instructions. Primers for RT-qPCR are shown in Table S1. QuantiNova™ SYBR Green RT-PCR (QIAGEN, Cat. 208154) system was used for RT-qPCR according to the manufacturer’s instructions. Briefly, the following were mixed in a PCR: 10 µL (2x SYBR Green RT-PCR), 300 nm primer mix, 0.2 µL (QuantiNova SYBR Green RT Mix which contains HotStaRT-Script reverse transcriptase), 1–2 µL sample volume, and the volume was completed to 20 µL with RNase-free water. CFX96 Touch™ (Bio-Rad) real-time thermal cycler was set to the following parameters: reverse transcription (50 °C for 10 min), DNA polymerase activation and initial DNA denaturation (95 °C for 3 min) for 1 cycle, denaturation, annealing and extension (95 °C for 15 s, 55 °C for 30 s, 72 °C for 30 s) for 40 cycles. Primers were tested and selected for efficiency and specificity. Endpoint PCR results using the primers subsequently employed for RT-PCR are shown in Fig. S1A.

Generation of TOP2B knockout clones using CRISPR

Exon 1 of TOP2B gene was targeted with a single guide RNA (gRNA) (CCGCCTGCAGC-CACCCGACT). The gRNA was cloned into pSpCas9(BB)-2A-GFP (PX458) (Addgene plasmid # 48138). The plasmid was transfected into Nalm-6 cells by Nucleofection with Amaxa Nucleofector II system and the Cell Line Nucleofector® Kit T (Lonza, Cat. VCA-1002, Basel, Switzerland) according to the manufacturer’s instructions. Cells were selected and sorted based on GFP expression using a BD FACSAria™ Fusion cell sorter (BD Bioscience, Wokingham, UK) through the 488 nm wavelength channel. GFP-positive cells were sorted into a single cell per well of 96-well plates and incubated at 37 °C for 2–3 weeks until colonies formed. Screening for TOP2B knockout clones was performed using genotyping and immunofluorescence (see Fig. S1).

Immunofluorescence

Cells were allowed to adhere onto poly-L-lysine-coated microscope slides followed by fixation in 4% paraformaldehyde. Cells were permeabilised in KCMT buffer (120 mM
KCl, 20 mM NaCl, 10 mM Tris/HCl pH8.0, 1 mM EDTA, 0.1% Triton X-100) and blocked overnight in (KCM-T + 2% BSA and 10% dried milk powder). Immunofluorescence staining was performed using anti-TOP2B MA-BMAB6348 (Bio-Techne, Abingdon, UK) and Alexa 488-coupled secondary antibody (Thermo Fisher, Paisley, UK) prior to mounting with Vectashield with DAPI (Vector Laboratories, Burlingame, CA, USA).

**Chromatin immunoprecipitation**

Cell suspensions containing (2*10^7 cells per immunoprecipitate) were harvested by centrifugation at 300 × g for 5 min at 4 °C, and the pellet was resuspended in ice-cold PBS. Cells were then fixed with 1% formaldehyde for 10 min on ice with gentle mixing every 2 min. Formaldehyde was quenched with glycine to a final concentration of 0.125 M for 5 min at room temperature. Cell lysis was performed using Farnham lysis buffer (5 mM PIPES pH 8.0, 85 mM KCl, 0.5% NP-40) with protease inhibitor cocktail (Expedeon, Cambridge, UK) and mixed by pipetting. Lysate was then pipetted 20 times through a 20-gauge needle. This treatment breaks the cells while keeping the nuclei mostly intact. The lysate was centrifuged at 2000 rpm at 4 °C for 5 min. Pellets were then resuspended with RIPA buffer (1X PBS, 1% NP-40, 0.5% Na deoxycholate, 0.1% SDS) with protease inhibitor cocktail and mixed by pipetting. Chromatin was then fragmented to achieve (200–600 bp) size range using probe sonication (Bandelin Sonopuls HD2070 sonicator) by applying 10 rounds of 7 pulsed cycle for 15 s, and 20% power and 10% of the sonicated chromatin were used as input for analysis.

ChIP-Grade Protein A/G Magnetic Beads (Thermo Scientific, Cat. 26162) were washed 3 times and resuspended in BSA (fraction V) in PBS solution. The mixture was then incubated on a rotator platform for 4 h at 4 °C to bind the following antibodies: 5 μg of anti-acetylated histone 3 (Ac-H3) (Millipore, Watford, UK, 06-599B), 2 μg per 500 μg of total protein of GFP (Santa Cruz, Heidelberg, Germany, sc-8334), 5 μg of TOP2B (3535, In house[15]), 5 μg per 1000 μg of total protein of USF2 (Abcam, Cambridge, UK, ab125184) or 5 μg per 1000 μg of total protein of MAX (Abcam, ab53570).

Beads were then washed with BSA solution and then incubated at 4 °C overnight with chromatin (amount equivalent to 100 μg DNA per IP). Immunoprecipitated chromatin–bead complexes were then washed 4 times with LiCl IP Wash Buffer (100 mM Tris pH 7.5, 500 mM LiCl, 1% NP-40, 1% sodium deoxycholate), followed by one wash TE Wash Buffer (10 mM Tris/HCl pH 7.5, 0.1 mM Na2EDTA) and resuspended in IP Elution Buffer (1% SDS, 0.1 M NaHCO3) with vortexing. Chromatin cross-linking was reversed, and then, DNA was purified using QIAquick PCR Purification Kit (Qiagen, Cat. 28104) according to the manufacturer’s instructions.

Quantitative PCR was performed using iTaq Universal SYBR Green Supermix (Bio-Rad) and primers described in Table S1 by mixing the following: 10 μL iTaq Universal SYBR Green Supermix (2X), 1 μL (F + R primer mixture 10 μM stock), 2 μL ChIP DNA and complete volume to 20 μL with nuclease-free water.

Parameters for qPCR were as follows: initial DNA denaturation (95°C for 3 min) for 1 cycle, denaturation, annealing and extension (95 °C for 15 s, 55 °C for 20 s, 72 °C for 30 s) for 40 cycles, melting curve analysis 65–95 °C at 0.5 °C increment for 5 s. Signal of enrichment of protein of interest was quantified as % of input using the formula:

\[
\text{Input ct- IP ct} / \text{Input dilution factor} \times 100
\]

**Epigenetic analysis**

Figure 3 depicting epigenetic information in the 100kb surrounding the CBR1 gene was prepared using Broad Integrative Genome Viewer (IGV) [23,24]. The following tracks were imported into IGV for display: Nalm-6 end-seq data (highlighting genomic sites of etoposide-induced DNA cleavage) [13] were imported from GEO (GSE2635574); GM12878 immortalised B-cell tracks were from ENCODE [25]. Mouse activated B-cell end-seq data and mouse activated B-cell Ctcf, Rad21, Top2b and Top2a ChIP data [13] were imported from GEO: GSE99194 (GSM2635560 & GSM2635561, GSM2635592, GSM2635597, GSM2635606 & GSM2635602, respectively). Mouse thymus DNA-seq data were imported from GEO (GSE83185, GSM2195840), and mouse spleen H3K4Me3 data were from ENCODE. Mouse liver Top2b Chip data were from Ref. [14].

For Fig. 4, publicly available Hi-C data sets were accessed via the Hi-C data browser (promoter.bx.ed) [26]. The selected Hi-C data were plotted to highlight potential TADs in the vicinity of the CBR1 gene.

**Results**

**Differential expression analysis by RNA-seq**

In addition to the general role for topoisomerases in the relief of over- and underwinding of DNA ahead and behind an elongating polymerase [1], TOP2B has been implicated in the activation or repression of specific genes during differentiation or after addition of a stimulus [17]. Furthermore, through its association with CTCF, TOP2B has been hypothesised to have a role in controlling supercoiling within or between TADs [13,14]. To further investigate the role of TOP2B in transcriptional regulation, we carried out a comparative exome analysis in the pre-B-cell line Nalm-6 and previously described Nalm-6 cells null for TOP2B (Nalm-6 TOP2B−/−) that were generated by...
Transcription of CBR1 is regulated by TOP2B

M. M. Khazeem et al.

RNA-seq analysis was carried out for four replicates for each cell line, resulting in the identification of a number of up- or down-regulated genes. The pattern of differential gene expression is shown in a volcano plot in Fig. 1A (red dashed lines indicate 2-fold change in gene expression), and genes significantly down- or upregulated in the TOP2B null cells (Nalm-6 toplb+/−) compared to wild-type are listed in Tables S2 and S3. Genes are determined to be significantly differentially expressed where the log2 fold change is greater than 1 or less than −1, and the Benjamini–Hochberg adjusted P-value is less than 0.05. Strong downregulation was observed for ZNF43, ZSCAN18, YOD1, MTMR1, PRKACB, ZN5312, ZNF626, ZNF573 and CBR1. The reduced expression of these genes in Nalm-6 toplb+/− cells was also assessed by quantitative RT-PCR. CBR1, YOD1, PRKABC, ZSCAN18 and ZNF573 were significantly under expressed in the TOP2B null cells by qRT-PCR (Fig. 1B, Table S2), and none of the genes that appeared upregulated in the RNA-seq analysis were significantly changed when assayed by RT-PCR (data not shown).

The original Nalm-6 TOP2B gene knockout line used in the RNA-seq analysis above (referred to from now as Nalm-6 toplb+/−) was generated by Adachi et al in 2008 [20]. To determine which gene expression changes were due directly to TOP2B downregulation rather than later changes or adaptions in the cell line, a new TOP2B null Nalm-6 cell line (Nalm-6 toplbKO4) was generated using CRISPR-Cas9 gene inactivation (Fig. 1C, Fig. S1). The relative expression of genes that were altered in the Adachi Nalm-6 toplb+/− RNA-seq data (Fig. 1A,B) was assessed by RT-PCR in the new knockout cell line, designated Nalm-6 toplbKO4. Of the genes tested, only CBR1 was expressed at a significantly lower level in Nalm-6 toplbKO4 compared to WT cells (Fig. 1D). The much lower expression of CBR1 in both TOP2B null lines (<10% by RT-PCR and RNA-seq) compared to WT cells strongly suggests that TOP2B is involved in maintaining the normal expression level of CBR1.

CBR1 promoter analysis by ChIP-seq

Bioinformatic examination revealed prominent USF2 and MAX binding in the promoter region of CBR1 in publicly available GM12878 immortalised B-cell data [25] (Fig. 2A). In order to explore the mechanism by which TOP2B is required for normal expression of CBR1, we examined the binding of TOP2B and of USF2 and MAX in the CBR1 promoter and at sites flanking the gene using ChIP on WT Nalm-6 and Nalm-6 toplbKO4. We could detect TOP2B in the promoter region (primers CBR1-c1). However, no difference in TOP2B ChIP signal was observed when comparing the promoter and the flanking sites Fig. 2B. As anticipated, the signal obtained for TOP2B in the TOP2B null cell line (Nalm-6 toplbKO4) was much lower than in the WT cells, attesting to the specificity of the TOP2B ChIP signal. As expected for the promoter of a actively transcribed gene, the CBR1 promoter displays prominent histone acetylation (H3K9Ac, Fig. 2A) and this was confirmed by ChIP in Nalm-6 cells (Fig. 2C).

However, perhaps surprisingly, the acetyl histone (Ac-H3) signal detected at the promoter region (CBP-c1) was not significantly diminished in the Nalm-6 toplbKO4 cell line even though CBR1 is expressed at a much lower level in this cell line. Next, we examined the effect of TOP2B depletion on USF2 occupancy at the CBR1 promoter. Consistent with the publicly available ChIP-seq data for GM12878 pre-B cells (Fig. 2A), we could readily detect USF2 at the promoter (CBR1-c1) of CBR1 in Nalm-6 cells, but not at a site (CBR1-p4) downstream of CBR1 (Fig. 2D). Significantly, USF2 binding was greatly reduced (~10X) at the CBR1 promoter in Nalm-6 toplbKO4 cells that lack TOP2B, but although reduced, the signal was still higher at the promoter site than at the downstream site, suggesting that USF2 binding at the promoter is not completely lost in Nalm-6 toplbKO4 cells. To determine whether the dramatically reduced presence of USF2 at the CBR1 promoter was due to altered USF2 expression in Nalm-6 toplbKO4 cells, we compared steady-state USF2 RNA levels in these cells and their TOP2B WT counterparts; we found no significant difference in the expression of USF2 in the WT versus TOP2B null cells (Fig. 2E). Following the analysis of USF2, we examined recruitment of MAX to the CBR1 promoter in the same way. As was observed for USF2, recruitment of MAX to the CBR1 promoter was dramatically reduced in Nalm-6 toplbKO4 cells (Fig. 2F), but the steady-state RNA level for MAX was not significantly different between the WT and TOP2 depleted cells (Fig. 2G). Thus, depletion of TOP2B appears to lead to reduced recruitment of USF2 and MAX to the promoter of the CBR1 gene in the absence of altered USF2 or MAX expression.

The genomic position of CBR1 is close to a TAD boundary

From the ChIP analysis above, TOP2B appears to be present in the vicinity of the CBR1 gene (Fig. 2B) and depletion of TOP2B leads to reduced promoter recruitment of USF2 and MAX. TOP2 activity may be
necessary to resolve DNA topological problems at diverse regulatory elements and a substantial proportion of TOP2B binding locations in the genome coincide with CTCF/cohesin bound regions [13,14,19], an association that has been proposed to reflect the necessity to relieve supercoiling at chromosome loop and/or topologically associating domain (TAD) boundaries [13,14]. Furthermore, TOP2B activity has been linked to transcriptional activation in a number of systems [8–11,27,28]. Bioinformatic analysis of the 100kB region of human chromosome 21 centred on CBR1 shows that it and the adjacent SETD4 gene are present in an 80k genomic region flanked by CTCF/cohesion (RAD21) binding sites (Fig. 3A) in ChIP-seq tracks from GM12878 immortalised B cells (ENCODE). A similar configuration of CTCF/RAD21 binding was also found in primary mouse B cells [13] (Fig. 3B). Interestingly, murine primary B-cell and liver Top2b ChIP-seq (Fig. 3B,C) [13,14] show Top2b signal somewhat concentrated at the 5’-end of Cbr1 but spanning the cbr1 locus and beyond including the regions equivalent to those sampled in the Nalm-6 ChIP analysis in
Transcription of CBR1 is regulated by TOP2B

M. M. Khazeem et al.
between the three human cell lines/tissue, and even between these and the syntenic mouse locus. In each case, the \textit{CBR1}/\textit{cbr1} gene is located close to the TAD boundary and the cluster of CTCF/end-seq peaks described above in relation to Fig. 3.

**Discussion**

We show that \textit{CBR1} expression is downregulated in two independently generated Nalm-6 cell lines null for \textit{TOP2B} (Fig. 1). To our knowledge, this is the first report of a link between \textit{TOP2B} and \textit{CBR1}. \textit{CBR1} is the predominant doxorubicin reductase in human liver [30]. \textit{CBR1} catalyses the reduction of doxorubicin to doxorubicinol. Doxorubicinol is reported to be more cardiotoxic than doxorubicin \textit{in vivo} which is important for studies on the cardiotoxicity of doxorubicin-based therapies [31]. In previous studies, the loss of one allele of \textit{cbr1} protected murine cardiac tissue from the damage caused by doxorubicin [32] and in parallel, \textit{TOP2B} null cardiomyocytes have also been reported to be protected from doxorubicin-induced damage [33]. With the caveat that this work employed B-cell lines rather than tissues or organs \textit{in vivo}, we propose that \textit{TOP2B} depletion may impact the expression of \textit{CBR1} in other cell types. It would be of interest to study the cardioprotective effect of \textit{TOP2B} depletion due to concomitant reduced expression of \textit{CBR1}.

We have clearly demonstrated that \textit{TOP2B} depletion dramatically reduces the expression level of \textit{CBR1} and that this is accompanied by reduced binding of USF2 and MAX in the \textit{CBR1} promoter. However, the mechanism by which \textit{TOP2B} affects binding of these transcription factors and \textit{CBR1} expression is not clear. Potentially \textit{TOP2B} could be directly or indirectly required for efficient recruitment of USF2, MAX or other transcription factors to the promoter region, perhaps through a requirement for local supercoiling modulation. Notably, other work has demonstrated a role for SP1 in \textit{CBR1} expression via GC boxes in the...
Fig. 3. Epigenetic configuration surrounding the human CBR1 and mouse Cbr1 loci. (A) A 100kb region of chromosome 21 (hg19) bounded by peaks of CTCF/RAD21 binding and containing the human CBR1 and adjacent genes is plotted with etoposide-induced end-seq signal, ChIP-seq data for CTCF, RAD21, USF2, Max, H3K4Me3 and H3K27Ac and DNase-seq signal intensity. Etoposide-treated and control end-seq data (Nalm-6) are from GEO: GSE99194 (GSM2635574 & GSM2635574) [13]. The ChIP-seq-derived tracks and DNase-seq data were derived from GM12878 immortalised B cells (ENCODE) [25]. The bottom red bars represent predicted TAD boundaries derived from GM12878 Hi-C data [38]. TAD coordinates were downloaded from promoter.bx.psu.edu/hi-c/ [4,26]. (B) The equivalent region of mouse chromosome 16 (mm10) containing the Cbr1 and surrounding genes including CTCF, RAD21, USF2, Max, H3K4Me3 and H3K27Ac and DNase-seq signal intensity. Etoposide-treated and control end-seq data (primary mouse B cells (aB)) are from GEO: GSE99194 (GSM2635560 & GSM2635561, GSM2635592, GSM2635597, GSM2635606 & GSM2635602 respectively) [13]. DNase-seq data were from mouse thymus (GEO: GSE83185, GSM2195840) [25]. Mouse liver Top2b ChIP data were from Ref.[14], and mouse H3K4Me3 ChIP-seq data were from spleen (ENCODE) [25]. TAD boundaries were identified as in (A), and Hi-C data were from mouse ES cells [39]. (C) A 10kb region of mouse chromosome 16 centred on Cbr1.
promoter region [34], and these sequence motifs were found to frequently occur in TOP2B ChIP peaks in a genome wide analysis [15]. Furthermore, SP1 and USF proteins have previously been shown to physically and functionally interact [35]. However, another possibility is presented by the juxtaposition of CBR1 with a TAD boundary, associated with a cluster of CTCF/RAD21/TOP2B binding sites (the latter deduced from end-seq data in Nalm-6 cells but directly observed in mouse B cells). In this scenario, depletion of TOP2B would lead to altered boundary function or inability to resolve supercoiling problems at the TAD boundary that could affect the expression of nearby genes. Thirdly, CBR1 expression can be induced by the classical arylhydrocarbon activator β-naphthoflavone via the arylhydrocarbon receptor (ARH) and/or the NRF2 transcription factor [36,37]. Although our experiments have not addressed the inducibility of CBR1, several cases have been reported where TOP2B plays an important role in the regulation of ligand-inducible genes in a way that involves chromatin binding and catalytic activity of TOP2B [8–11,16,27,28]. Thus, it is possible that the reduced expression of CBR1 that we have observed in the uninduced state in TOP2B null Nalm-6 cells reflects this aspect of the transcriptional regulation of CBR1.

Acknowledgements

We thank Robert Stones and Simon Cockell for bioinformatics input and George Schlossmacher and Niall Kenneth for instruction and advice on the use of CRISPR-Cas9. We acknowledge the Newcastle University Flow Cytometry Core Facility (FCCF) for assistance with the sorting of GFP-positive CRISPR/Cas9 clones. We acknowledge studentship funding for Mushtaq M. Khazeem from the Higher Committee for Education Development in Iraq.

Author Contributions

MMK performed the majority of the experimental work, IGC carried out the TAD analysis, LFH prepared RNA for RNA-seq, JWC provided bioinformatics assistance, CAA conceived of experiments and supervised the study. All authors contributed to writing the manuscript.
Transcription of CBR1 is regulated by TOP2B

M. M. Khazeem et al.

References


Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig. S1. TOP2B CRISPR knockouGenotyping and specificity of qRT primers and ChIP qPCR primers.

Fig. S2. The genomic position of CBR1 is close to a TAD boundary.

Table S1. Primers used for reverse transcription quantitative PCR and quantitative PCR for ChIP analysis.

Table S2. Genes whose expression is downregulated in TOP2B null versus WT Nalm-6 cells (Log2 fold change >1).

Table S3. Genes whose expression is upregulated in TOP2B null versus WT Nalm-6 cells.