Data in Brief

Topoisomerase I inhibition leads to length-dependent gene expression changes in human primary astrocytes

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ABSTRACT

Topoisomerase I is required for the proper expression of long genes (>100 kb) in mouse and human cortical neurons, including many candidate genes for autism spectrum disorder (ASD) [1]. Given the important role of astrocytes in brain development [2], we investigated whether long genes, including autism susceptibility genes, also require topoisomerase I expression in human primary astrocytes. We carried genome-wide expression profiling of cultured human primary astrocytes following treatment with the topoisomerase I inhibitor Topotecan, using Illumina microarrays. We identified several thousands of differentially expressed genes and confirmed that topoisomerase I inhibition affects gene expression in human primary astrocytes in a length-dependent manner. We also identified over 20 ASD-associated genes that show topoisomerase-dependent gene expression in human primary astrocytes but have not been previously reported as topoisomerase-I-dependent in neurons. The microarray data have been deposited in NCBI GEO (https://www.ncbi.nlm.nih.gov/geo/) under accession number GSE90052.

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1. Introduction

Topoisomerases are important for gene expression as they are required for removing DNA supercoiling generated during transcription. Topoisomerases I and II are expressed throughout the developing and adult brain [3,4]. Interestingly, de novo variants in topoisomerase genes have been identified in patients with autism spectrum disorder (ASD), raising the possibility of a specific role of topoisomerases in brain development [5,6]. It has recently been demonstrated that the inhibition of topoisomerase I and II in human and mouse cortical neurons leads to reduced expression levels of very long genes, over 100 kb, of which many have been implicated in ASD [1]. The effect of topoisomerases on the expression of long genes appears to be occurring at the stage of transcription elongation [1]. In addition, topoisomerase I is required for ligand-dependent enhancer activation [7] and has been shown to generate double-stranded breaks in promoter regions, which in turn are required for the expression of neuronal early-response genes [8]. The role of topoisomerases in regulating gene expression in the brain has been primarily investigated in neurons. However, accumulating evidence supports an important role for glial cells during brain development [2,9–12]. Therefore, we investigated the effect of topoisomerase I inhibition on gene expression in human primary astrocytes, in order to identify genes that may respond to topoisomerase I inhibition in a cell-type-specific manner.
2. Experimental design, materials, and methods

2.1. Cell culture

Human primary astrocytes were isolated from a 14–18-week-old postmortem fetal brain following therapeutic termination with informed consent, following the UNSW ethics protocol HREC 08284. Isolation was carried out as previously described [13]. Cells were cultured in RPMI 1640 medium (Life Technologies, no. 61870-036) supplemented with 10% fetal bovine serum, 1% streptomycin (10,000 μg/ml), 1% penicillin (10,000 units/ml), and 1% Fungizone (2.5 μg/ml).

2.2. Topotecan treatment

Human primary astrocytes were seeded in 6-well cell culture plates at a density of 500,000 cells per well and incubated overnight at 37 °C, with 5% CO₂. After 24 h in culture, cells were treated with either topotecan (Sigma Aldrich, no. T2705) at a final concentration of 300 nM or 0.1% DMSO as a vehicle control. Treatment was carried out in triplicate wells of a 6-well plate for 24 or 48 h.

2.3. RNA extraction

Total RNA was isolated after 24 or 48 h of treatment, using a Qiagen RNeasy kit, with on-column DNA digestion.

2.4. Microarray analysis

100 ng of total RNA from each RNA sample were analyzed on Illumina HumanHT-12 v4 Expression BeadChip. cDNA labeling and array hybridization were carried out at the UNSW Ramaciotti Centre for Genomics using standard Illumina protocols. Raw data were processed in R (http://www.r-project.org). Briefly, raw expression data were log₂ transformed and normalized by quantile normalization using the lumi package [14]. Probes with intensity above the background (i.e. detection p value < 0.05) in at least 3 samples were considered expressed and retained for further analysis. A total of 23,000 probes passed these criteria. Differential expression analysis was carried out using linear model implemented in the Bioconductor package limma, contrasting control cells with cells treated with Topotecan for either 24 or 48 h. p values were adjusted for multiple testing using a Benjamini-Hochberg correction. Probes were considered differentially expressed if the corrected p value was < 0.05 and the absolute fold change was > 1.5. All analyses were carried out at probe level. Gene length for the RefSeq transcript corresponding to each probe was obtained from the UCSC table browser, refGene track for the human genome build hg38, downloaded 11.2016. The list of ASD-associated genes was obtained from the SFARI database’s [15] gene scoring module and included only the “syndromic,” “high confidence,” and “strong candidate” genes (https://gene.sfari.org/autdb/GS_Home.do).

3. Results and discussion

We identified 3303 differentially expressed probes for cells treated with Topotecan for 24 h (1633 down-regulated and 1670 up-regulated), and 3112 differentially expressed probes at 48 h (1565 down-regulated and 1547 up-regulated). We observed a significant inverse correlation between gene length and expression change at both time points (Fig. 1; Spearman rho = −0.57, p value = 4.73 E-252 at 24 h; Spearman rho = −0.38, p value = 2.85 E-97 at 48 h). These results support the notion that topoisomerase I inhibition affects gene expression in a length-dependent manner, leading to down-regulation of long genes in human

Fig. 1. Length-dependent gene expression changes upon topotecan treatment. a) Scatterplot of log₂ fold changes at 24 h versus gene length. Each circle represents one differentially expressed gene. Red dots represent ASD genes. Red vertical line marks 100 kb. b) Boxplot of log₂ fold changes for genes differentially expressed at 24 h, binned by gene length. Green: genes shorter than 100 kb; red: genes longer than 100 kb.
primary astrocytes. Of the 120 genes listed in the SFARI database as “syndromic,” “high confidence,” or “strong candidate,” 26 genes were significantly dysregulated following Topotecan treatment at either time point (Table 1). Seventy-three percent of these genes, i.e. 19 genes, were down-regulated, and only 7 genes were up-regulated. Notably, only one of the ASD genes differentially expressed in astrocytes, CTNND2, has been previously reported to change in expression in response to Topotecan treatment in neurons. Therefore, the present dataset identifies novel effects of topoisomerase I on ASD genes in brain cells.

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