Data in Brief

Genome-wide redistribution of BRD4 binding sites in transformation resistant cells

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ABSTRACT

Hutchinson–Gilford progeria syndrome (HGPS) patients do not develop cancer despite a significant accumulation of DNA damage in their cells. We have recently reported that HGPS cells are refractory to experimental oncogenic transformation and we identified the bromodomain-containing 4 protein (BRD4) as a mediator of the transformation resistance. ChIP-sequencing experiments revealed distinct genome-wide binding patterns for BRD4 in HGPS cells when compared to control wild type cells. Here we provide a detailed description of the ChIP-seq dataset (NCBI GEO accession number GSE61325), the specific and common BRD4 binding sites between HGPS and control cells, and the data analysis procedure associated with the publication by Fernandez et al., 2014 in Cell Reports 9, 248-260 [1].

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Experimental design, materials and methods

The overall aim was to identify specific and common genomic binding sites for BRD4 in experimentally transformed dermal fibroblasts derived from HGPS patients and control individuals through the analysis of BRD4 ChIP-sequencing data.

Cell culture

Transformed cell lines were obtained by retroviral introduction of TERT (T), V12-HRAS (R) and SV40 large and small T antigens (S) of primary dermal fibroblasts from HGPS patients (TRS-HGPS) and age-matched control wild-type individuals (TRS-WT) as previously described [1–3]. Cells were grown in MEM containing 15% fetal bovine serum (FBS), 2 mM L-glutamine, 100 U ml⁻¹ penicillin and 100 µg ml⁻¹ streptomycin, at 37 °C in 5% CO₂. Two independent cell lines from each group were selected for ChIP-sequencing experiments.

ChIP-sequencing

ChIP was performed as previously described [4] with modifications. Briefly, 10 x 10⁶ cells were cross-linked for 10 min with formalin 1% at room temperature, followed by 5 minute quenching with 125 mM glycine. After three washes with chilled PBS, cell pellets were snap-frozen and stored at −80 °C. Cells were resuspended in lysis buffer containing 1 mM EDTA, 0.8% SDS, 20 mM Tris–HCl pH 8 and protease inhibitor

Direct link to deposited data

Deposited data can be found at: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE61325.

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cocktail (Calbiochem) and sonicated in a Diagenode Bioruptor 300 for 12–15 cycles (30 s on, 60 s off). Chromatin was diluted in ChIP dilution buffer (1% Triton X-100, 1 mM EDTA, 20 mM Tris–HCl pH 8, 150 mM NaCl and protease inhibitor cocktail) and immunoprecipitated overnight with pre-coated anti-IgG magnetic beads (Dynabeads, Invitrogen) previously incubated with anti-BRD4 antibody (7 μg, Bethyl labs, lot A301-985) for 6 h at 4 °C. Beads were washed sequentially for 5 min each in low-salt (20 mM Tris–HCl pH 8, 150 mM NaCl, 2 mM EDTA, 1% Triton X-100, 0.1% SDS), high-salt (20 mM Tris–HCl pH 8, 500 mM NaCl, 2 mM EDTA, 1% Triton X-100, 0.1% SDS), LiCl (10 mM Tris pH 8.0, 1 mM EDTA, 250 mM LiCl, 1% NP-40, 1% Na-deoxycholate) and TE (10 mM Tris–HCl pH 8, 1 mM EDTA) buffer at 4 °C followed by a room temperature wash in TE buffer. Beads were eluted in 1% SDS and 100 mM NaHCO₃ buffer for 25 min at 65 °C and cross-linking was reversed for 8 h after addition of NaCl (final concentration 200 mM). After RNase A (1 mg/ml, Qiagen) and proteinase K (2 mg/ml, New England Biolabs) incubations, DNA was column-purified following the manufacturer’s instructions (Qiaquick PCR purification kit, Qiagen).

Library construction (Illumina TruSeq 2.0 protocol) and sequencing were performed at the NCI-Sequencing Facility (SAIC-Frederick, MD) using Illumina HiSeq 2000. Paired-end sequencing with read lengths of 100 bp was performed. 4 input samples and 4 BRD4-ChIP samples (from TRS-WT and TRS-HGPS cells, two independent cell lines in each group) were multiplexed in two lanes. Raw reads files in fastq format were deposited to the SRA database at NCBI (Table 1).

### ChIP-seq data analysis

The data analysis procedure is described in Fig. 1. The raw reads data quality was tested using FastQC [5] (V0.10.1). All samples showed all base quality ≥ 25 and a small number of TruSeq adapter sequences was detected in the WT1 BRD4 reads. Raw reads of each sample in fastq format were then aligned to reference human genome (hg19) using NovoaalignMPI (V3.0.2, Novocraft) and the adapter sequence contamination were then aligned to reference human genome (hg19) using NovoaalignMPI (V3.0.2, Novocraft) and the adapter sequence contamination were then aligned to reference human genome (hg19) using NovoaalignMPI (V3.0.2, Novocraft) and the adapter sequence contamination were then aligned to reference human genome (hg19) using NovoaalignMPI (V3.0.2, Novocraft) and the adapter sequence contamination.

In order to investigate potential differences in BRD4 binding patterns in WT and HGPS samples, a pipeline was developed to detect sample-specific BRD4 bindings. First, the union of the peaks in each biological group was calculated using the “find_union_islands.py” script in SICER, and then the intersection of peaks in each group was achieved using the “intersect” command in bedtools, and finally, the sample-specific peaks were derived by comparing the union peak sets with the intersected peak sets from different samples using “-v” option in the “intersect” command. In this way, 3078 WT-specific and 5574 HGPS-specific BRD4 binding sites were detected and the annotation of the peaks was done using “annotatePeaks.pl” with default options in Homer [12]. Important genomic features e.g., promoter, 5′/3′ UTR, exon, intron, non-coding were used to annotate the peaks and differential enrichment in specific features were compared between wild type and HGPS samples, as interpreted in [1]. Common BRD4 binding sites were detected by “multinter” command in bedtools and annotated using Homer. All annotated specific and common genomic binding sites between WT and HGPS cell lines are provided in Tables 3–5.

### Table 1

| File names   | Sample names | Group             |
|--------------|--------------|-------------------|
| SRR1574701   | HG1 Input    | TRS-HGPS cells    |
| SRR1574702   | HG2 Input    | TRS-HGPS cells    |
| SRR1574697   | HG1 BRD4     | TRS-HGPS cells    |
| SRR1574698   | HG2 BRD4     | TRS-HGPS cells    |
| SRR1574699   | WT1 Input    | TRS-WT cells      |
| SRR1574700   | WT2 Input    | TRS-WT cells      |
| SRR1574695   | WT1 BRD4     | TRS-WT cells      |
| SRR1574696   | WT2 BRD4     | TRS-WT cells      |

### Table 2

| Samples | WT1 | WT2 | HG1 | HG2 |
|---------|-----|-----|-----|-----|
| Peaks   | 29990 | 32239 | 35292 | 42784 |
Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.gdata.2014.11.005.

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