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Data in Brief

Genome-wide analysis of HOXC9-induced neuronal differentiation of neuroblastoma cells ☆

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ABSTRACT

Induction of differentiation is a therapeutic strategy in neuroblastoma, a common pediatric cancer of the sympathetic nervous system. The homeobox protein HOXC9 is a key regulator of neuroblastoma differentiation. To gain a molecular understanding of the function of HOXC9 in promoting differentiation of neuroblastoma cells, we conducted a genome-wide analysis of the HOXC9-induced differentiation program by microarray gene expression profiling and chromatin immunoprecipitation in combination with massively parallel sequencing (ChIP-seq). Here we describe in detail the experimental system, methods, and quality control for the generation of the microarray and ChIP-seq data associated with our recent publication [1].

Specifications	
Organism/cell line/tissue	*Homo sapiens*
Strain(s)	Human neuroblastoma cell line BE(2)-C (CRL-2268, ATCC)
Sequencer or array type	Microarray: Affymetrix Human Gene 1.0 ST ChIP-seq: Illumina Genome Analyzer IIX
Data format	Microarray raw data: CEL files ChIP-seq raw data: FASTQ files
Experimental factors	BE(2)-C cells without or with HOXC9 overexpression
Experimental features	Microarray gene expression profiling to identify genes that are regulated by HOXC9 ChIP-seq to map genomic sites that are bound by HOXC9
Consent	N/A
Sample source location	N/A

Direct link to deposited data

Deposited data are available here: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE34422.

Experimental design, materials and methods

Cell line

The human neuroblastoma cell line BE(2)-C was obtained from ATCC (CRL-2268, ATCC). BE(2)-C cells carry *p53* mutation and *MYCN* amplification [2,3]. It has been shown previously that this cell line contains a subpopulation of neuroblastoma cells capable of self-renewal and multi-lineage differentiation [4,5]. BE(2)-C cells can differentiate into neurons following treatment with retinoic acid (RA) [4–7] or into Schwann-like cells when treated with bromodeoxyuridine [4,5,8]. As a result, BE(2)-C cells have been used as a model system for investigating agents and signaling pathways that control neuroblastoma cell differentiation, which represents a promising therapeutic strategy for neuroblastoma [9,10].

We have recently identified HOXC9 as a downstream target gene of RA and an essential mediator of RA action in neuroblastoma differentiation: HOXC9 expression is upregulated by RA and knockdown of HOXC9 expression confers resistance to RA-induced differentiation. Moreover, HOXC9 induction can fully recapitulate the phenotype of RA treatment [7]. To gain a molecular understanding of the mechanism by which HOXC9 induces the neuronal differentiation of neuroblastoma cells, we generated BE(2)-C-derived cells with inducible expression of myc-tagged human HOXC9 in the absence of doxycycline, using the Retro-X Tet-Off Advanced Inducible Gene Expression System (Clontech).

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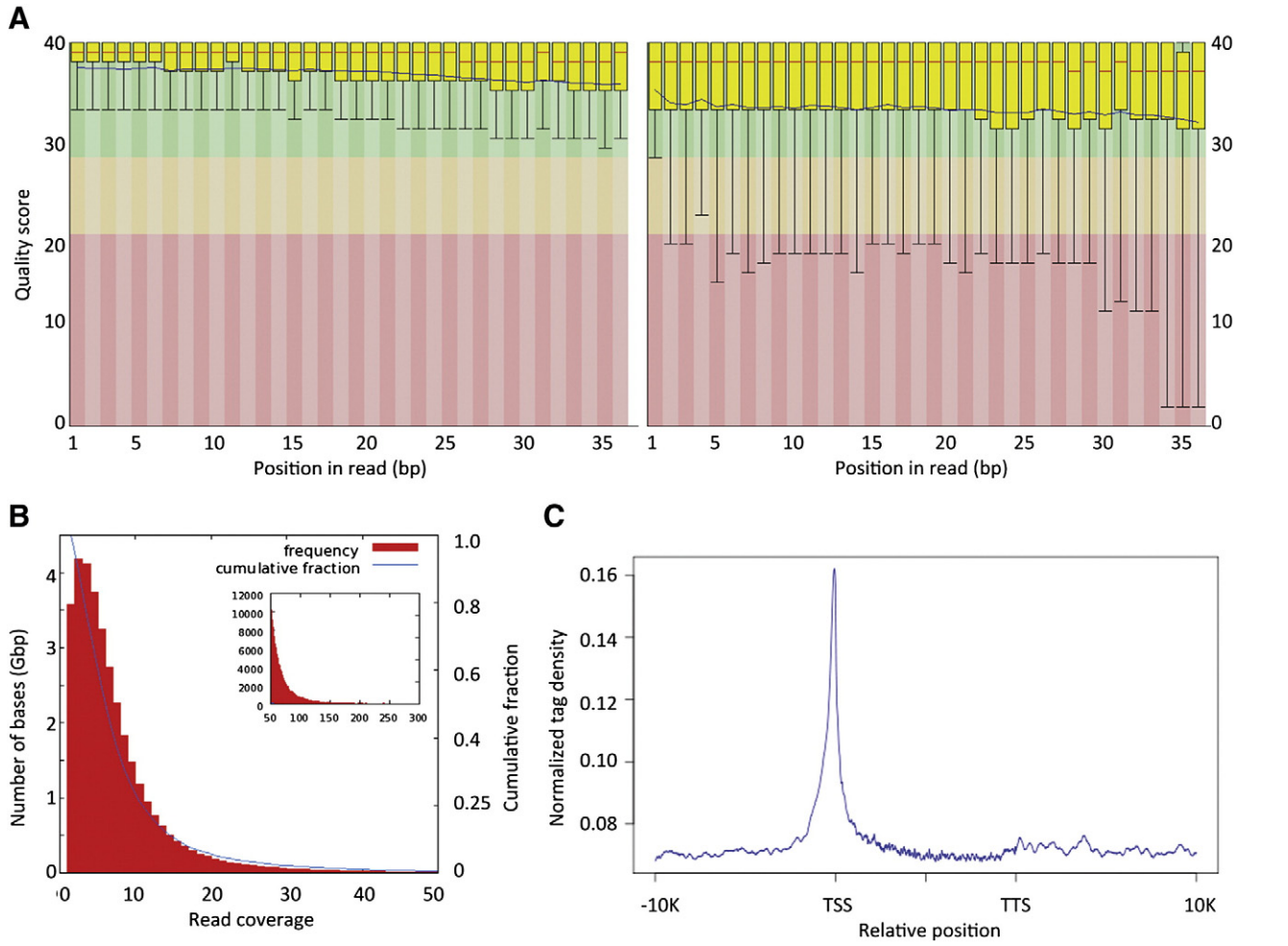

Fig. 1. Quality assessment of ChIP-seq data. (A) BoxWhisker type plot showing per-base sequence quality scores for read 1 (positive strand, left panel) and read 2 (negative strand, right panel). Higher score indicates better quality. (B) Quality assessment of sequencing sufficiency using histogram and cumulative fraction of base quality scores in peaks. (C) Tag density plot showing the average of the normalized tag densities for all RefSeq genes. HOXC9-binding sites are highly enriched around TSS. TTS, transcription termination sites.

Microarray and quality control

To identify the genes that are regulated by HOXC9, we isolated total RNA from three independent samples of BE(2)-C/Tet-Off/myc-HOXC9 cells cultured in the presence or absence of doxycycline for 6 days using Trizol (Invitrogen). The quantity and quality of the RNA samples were measured and assessed by a NanoDrop spectrophotometer and Agilent 2100 Bioanalyzer (Agilent Technologies). Affymetrix microarray analysis was performed using the Human Gene 1.0 ST microarray chip.

The quality of each CEL file was assessed using Affymetrix Expression Console Software according to the Affymetrix standard protocol (Quality Assessment of Exon and Gene 1.0 ST Arrays, Affymetrix White Paper, 2007). Relative log expression (RLE) was used to identify outlier samples. In order to monitor labeling and hybridization quality, we used polyA-control RNAs (*Lys*, *Phe*, *Thr* and *Dap*) and bacterial spike-in controls (*BioB*, *BioC*, *BioD* and *Cre*), respectively.

CEL files were imported into Partek Genomics Suit using RMA normalization. The probesets were annotated using the HuGene-1_0-st-v1 Probeset Annotations and Transcript Cluster Annotations. The differential expressions were calculated using ANOVA of the Partek package.

ChIP

To identify the genomic sites that are bound by HOXC9, we performed two independent ChIP-seq assays with BE(2)-C/Tet-Off/myc-HOXC9 cells cultured in the absence of doxycycline for 6 days according to the published procedure [11]. Briefly, 4×10^7 cells were fixed with 1% formaldehyde for 10 min and quenched with 0.125 M glycine for 5 min. After cell lysis, cross-linked chromatin DNA was sheared to approximately 250 bp by sonication (Model 150E ultrasonic dismembrator, Fisher Scientific), and immunoprecipitated with 10 μg of mouse anti-myc tag (clone 4A6, Millipore) and 80 μl Dynabeads Protein G (Invitrogen). The immunoprecipitated HOXC9–DNA complexes were washed extensively and eluted with SDS buffer, followed by incubation overnight at 65 °C to reverse cross-linking. The samples were then treated sequentially with RNase A and proteinase K to degrade associated RNA and proteins, and ChIP DNA was purified by phenol–chloroform extraction and ethanol precipitation. Input genomic DNA was purified from an aliquot of chromatin after sonication. DNA concentration was determined using a PicoGreen dsDNA quantitation assay kit (Invitrogen).

ChIP library and quality control

ChIP libraries were generated according to the Illumina ChIP-seq library construction procedure. Briefly, 10 ng of ChIP DNA was end repaired with T4 DNA polymerase, Klenow fragment and T4 polynucleotide kinase (all from Enzymatics), and an adenosine base was then added to the 3′ end of the blunt phosphorylated DNA fragments by Klenow fragment (3′ → 5′ exo-) (Enzymatics). This was followed by ligation of Illumina genomic adapters. Ligated DNA around the size of 250–300 bp was isolated by electrophoresis through a 3% NuSieve 3:1

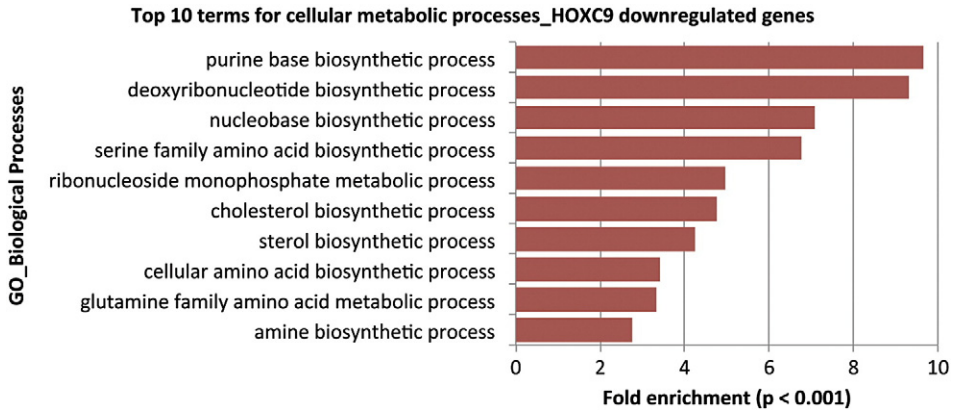

Fig. 2. HOXC9-induced neuroblastoma cell differentiation is characterized by reduced production of biosynthetic precursors. Gene ontology analysis of downregulated HOXC9-responsive genes reveals the enrichment of genes involved in the biosynthesis of nucleotides, sterol, and amino acids (enrichment fold >2.0, $p < 0.001$).

agarose gel and amplified by PCR using Phusion DNA Polymerase (Thermo Scientific). The PCR products were then purified using an Agencourt Ampure kit (Beckman Coulter) to remove primer dimmers.

Agilent 2100 Bioanalyzer was employed to examine the normal size distribution and possible primer or linker contamination of each library. The library concentration was measured using both Qubit dsDNA BR Assay Kit (Invitrogen) and real-time qPCR method. Real-time qPCR was performed on StepOne Plus Real-Time PCR systems (Applied Biosystems) using KAPA SYBR FAST universal qPCR kit (KAPA Biosystems). qPCR primers, 1.1 and 2.1, were designed based on Illumina Sequencing Library qPCR Quantification Guide. PhiX Control v3 (Illumina) was used in the reaction to plot the standard curve.

ChIP-seq data quality control

ChIP DNA was sequenced using Illumina Genome Analyzer IIX with a read length of 36 (paired-end). We performed several analyses to assess the quality of the sequencing data. Box whisker type plot analysis shows per base sequence quality scores for read 1 (Fig. 1A, left panel) and read 2 (Fig. 1A, right panel). Red and blue lines represent the median and mean value, respectively; the yellow box indicates the inter-quartile range (25–75%); and the upper and lower whiskers indicate the 10% and 90% points (FASTQC) [12]. Fig. 1B shows the quality assessment of sequencing sufficiency using histogram and cumulative fraction of base quality scores in peaks. We also analyzed tag density to visualize the quality of sequencing and mapping, and to examine the global profile of HOXC9 ChIP-seq based on genes, which shows that HOXC9-binding peaks are highly enriched around transcription start sites (TSS) (Fig. 1C).

Discussion

We described here a dataset composed of microarray gene expression profiling of HOXC9-responsive genes and ChIP-seq data for genome-wide mapping of HOXC9-binding sites in the human neuroblastoma BE(2)-C cells. With this dataset, we were able to demonstrate that HOXC9 directly controls and coordinates the expression of a large number of genes involved in neuron genesis and differentiation, cell cycle progression, and DNA damage repair [1]. We believe that this dataset would be particularly valuable for investigating the cellular processes associated with differentiation and underlying molecular mechanisms. For example, we found that HOXC9-induced differentiation is associated with a marked reduction in cellular metabolism, as a result of global downregulation of genes involved in the biosynthesis of nucleotides, sterol, and amino acids (Fig. 2). This finding sheds new light on the interplay between metabolic reprogramming and cancer cell differentiation, and suggests that reprogramming cellular metabolism may represent a promising strategy for promoting cancer cell differentiation.

Conflict of interest

The authors have no conflicts of interest.

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