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Data in Brief

Microarray-based analysis of plasma cirDNA epigenetic modification profiling in xenografted mice exposed to intermittent hypoxia

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ABSTRACT

Intermittent hypoxia (IH) during sleep is one of the major abnormalities occurring in patients suffering from obstructive sleep apnea (OSA), a highly prevalent disorder affecting 6–15% of the general population, particularly among obese people. IH has been proposed as a major determinant of oncogenetically-related processes such as tumor growth, invasion and metastasis. During the growth and expansion of tumors, fragmented DNA is released into the bloodstream and enters the circulation. Circulating tumor DNA (cirDNA) conserves the genetic and epigenetic profiles from the tumor of origin and can be isolated from the plasma fraction. Here we report a microarray-based epigenetic profiling of cirDNA isolated from blood samples of mice engrafted with TC1 epithelial lung cancer cells and controls, which were exposed to IH during sleep (XenoIH group, $n = 3$) or control conditions, (i.e., room air (RA); XenoRA group, $n = 3$) conditions. To prepare the targets for microarray hybridization, we applied a previously developed method that enriches the modified fraction of the cirDNA without amplification of genomic DNA. Regions of differential cirDNA modification between the two groups were identified by hybridizing the enriched fractions for each sample to Affymetrix GeneChip Human Promoter Arrays 1.0R. Microarray raw and processed data were deposited in NCBI's Gene Expression Omnibus (GEO) database (accession number: GSE61070).

Specifications	
Organism/cell line/tissue	*Mus musculus* (C57BL/6J strain) injected with TC1 murine lung tumor cells/plasma
Sex	Male
Sequencer or array type	GeneChip Mouse Promoter Array 1.0R (Affymetrix, Santa Clara, CA)
Data format	Raw and RMA-processed data
Experimental factors	Xenografted mice exposed to intermittent hypoxia (IH) ($n = 3$, XenoIH group) or room air conditions (RA) ($n = 3$, XenoRA group)
Experimental features	Mice engrafted with TC1 epithelial lung tumor cells were exposed to IH or RA conditions. Large-scale cirDNA epigenetic modification profiles were assessed in plasma cirDNA samples from xenografted mice exposed to IH or to RA conditions, according to previously described methods [1]. After quality control, data were analyzed using the Partek Genomic Suite Software (PGS) (St. Louis, MO).
Consent	NA
Sample source location	Chicago, IL, United States of America

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1. Direct link to deposited data

http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE61070.

2. Experimental design, materials and methods

2.1. Experimental design

Fig. 1 provides a schematic representation of the design for the full study, as previously reported [2]. Mice injected with tumor TC1 cells and control mice were exposed to intermittent hypoxia (IH) during sleep, or room air (RA) conditions (see below for details). Large scale DNA methylation profiles of circulating DNA were produced in plasma cirDNA samples from xenografted mice exposed to IH (XenoIH group, $n = 3$) or to RA (XenoRA group, $n = 3$) conditions using a method that enriches the modified fraction of the cirDNA without amplification of genomic DNA [1] (Fig. 2). Regions of differential cirDNA modification between the XenoIH and XenoRA group were identified by hybridizing the enriched fractions for each sample to Affymetrix GeneChip Human Promoter Array 1.0R.

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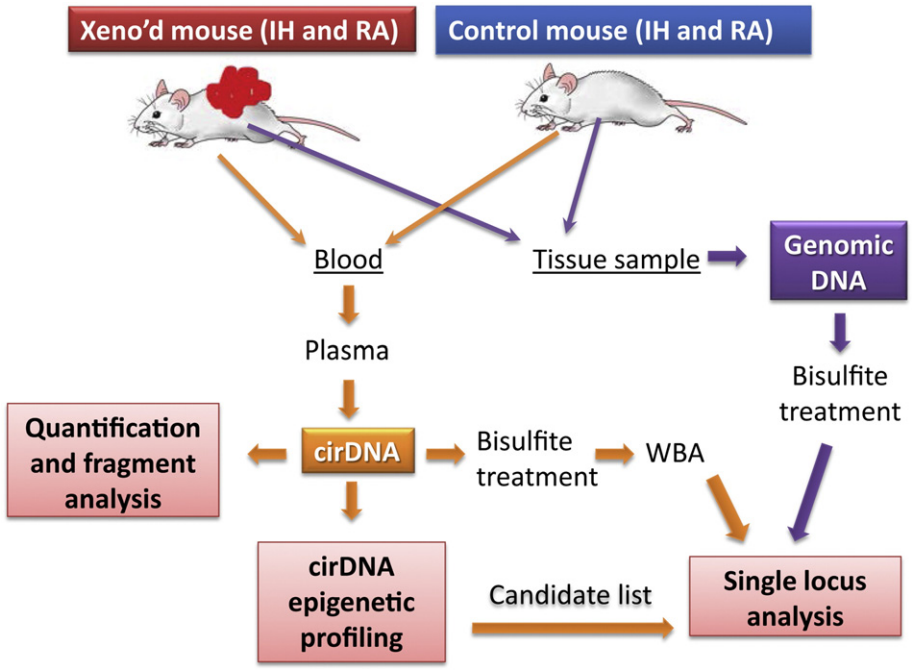
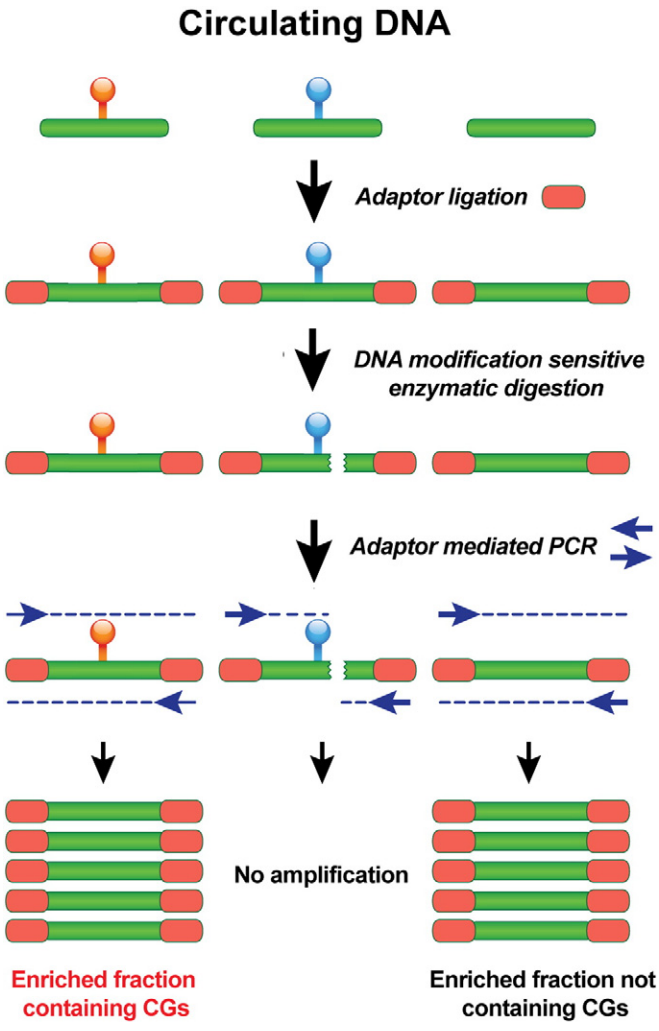


Fig. 1. Experimental setup for the full study. Xenografted and control mice were exposed to intermittent hypoxia (IH) during sleep — XenoIH and CtrlIH groups, and room air (RA) conditions — XenoRA and CtrlRA groups. cirDNA was isolated from blood plasma and characterized by quantification, fragment analysis and DNA methylation profiling. In addition, genomic DNA was isolated from peripheral blood lymphocytes, tumor and skeletal muscle. DNA methylation status of candidate loci was studied in plasma cirDNA and tissue genomic DNA by single locus analysis.



2.2. Materials and methods

2.2.1. Animals, hypoxic exposures, and epithelial lung tumor model

C57BL/6J male mice (7-week old) were acquired from Jackson Laboratories (Bar Harbor, ME). Mice were pre-exposed during 2 weeks to either RA or IH during the daylight phase corresponding to the preferred sleep period, and were then injected with 1×10^5 TC1 murine lung tumor cells in the left flank. The protocol for IH exposure was previously described by our group [3] and consisted of alternating cycles of 90 s (6% fraction of inspired oxygen (FiO_2) followed by 21% FiO_2) for 12 h/day (7 AM to 7 PM). With this paradigm, the oxyhemoglobin saturation at the end of the hypoxic period reaches to 65%–72% mimicking that experienced by moderate to severe OSA patients [4]. For the rest of the day (7 PM to 7 AM) the mice were in normoxic conditions (21% FiO_2). Gas mixture was electronically controlled by an internal analyzer which can receive in real-time the O_2 values inside of the chamber and can automatically modify by a computerized system of solenoid valves the gas mixture to follow the programmed gas profile. After 4 weeks from tumor injection, mice were sacrificed and tumors excised and weighed. All experimental procedures were approved by the Institutional Animal Care and Use Committee of the University of Chicago.

2.2.2. Plasma cirDNA and genomic DNA isolation

Blood samples were collected after being sacrificed and immediately processed. The plasma fraction was separated by centrifugation and

Fig. 2. Method for cirDNA modification epigenetic profiling. Universal DNA adaptors (red blocks) were ligated to the ends of cirDNA fragments (green bars), followed by digestion with DNA modification-sensitive enzymes (*HpaII*, *HinP1* and *HpyCH4IV*). cirDNA fragments that survive enzymatic hydrolysis were amplified by adaptor-mediated PCR and labeled with biotinylated nucleotides. During the PCR reaction, DNA polymerase extends primers (dashed blue lines) according to its processivity and the optimized reaction conditions. PCR products will be obtained only from undigested short templates that have ligated adaptors at both sides. In longer template (as expected from genomic DNA), the DNA polymerase cannot extend primers in the distance between 5′ and 3′ adaptors and therefore they will not be amplified. This enriched differentially modified DNA fraction was hybridized to Affymetrix GeneChip Human Promoter Array 1.0R, which contains more than 25,000 promoter regions.

cirDNA was isolated using the QIAamp Nucleic Acid isolation kit (Qiagen, Valencia, CA) according to the manufacturer's instruction.

2.2.3. cirDNA modification profiling

Large-scale cirDNA epigenetic modification profiles were assessed according to previously described methods [1]. Briefly, universal DNA adaptors were ligated to the ends of cirDNA fragments, followed by digestion with DNA modification-sensitive enzymes and amplification by adaptor-mediated PCR (Fig. 2).

2.2.4. Microarray hybridization and processing

The enriched differentially cirDNA modified fraction was fragmented, biotin-labeled, and hybridized on Affymetrix GeneChip Mouse Promoter Array 1.0R (Affymetrix, Santa Clara, CA) and scanned, according to the manufacturer's protocol. The array consisted of over 4.6 million probes tiled to interrogate over 28,000 mouse promoter regions. Promoter regions were selected from annotated genes in public databases (33,559 Ensembl genes (version 30_33f), 18,167 RefSeq mRNAs (NCBI GenBank) and 27,707 complete-CDS mRNAs (NCBI GenBank)). Probes were 25-mer long, leaving 10-mer separation between adjacent probes, providing a 35 base pair resolution. Each promoter region was cover by a 10 kb segment.

2.2.5. Microarray data analysis

Microarray raw and processed data were deposited in NCBI's Gene Expression Omnibus (GEO) database (accession number: GSE61070). Raw files (.cel) were produced using GCOS 1.3 software (Affymetrix).

Data quality control. Data quality control was performed using the STARR package [5] in the R statistical environment (version 3.0.2) [6]. Probe annotation was provided by the manufacturer (Mm_PromPR_v02-1_NCBIv36.bpmap file; Affymetrix). The absence of hybridization artifacts was verified by building pseudoimage plots for each array. Paired scatter plots were produced to determine the signal distribution correlation between each array (Fig. 3). Signal distributions before and after normalization were assessed by density plots, as well as the correction of bias due to GC-content differences (Supplementary Fig. S2 in [[2]]). Microarray signals in each microarray were loess-normalized and M–A plots produced to detect technical variation that may mask true biological differences [7] (Fig. 4). No outliers were detected and all arrays were included in the assessment of differential cirDNA modification.

Assessment of differential cirDNA modification. Data were analyzed using the Partek Genomic Suite Software (PGS) (St. Louis, MO). Signals were adjusted according to the probe sequence and background corrected using the Robust Microarray method (RMA)[8]. One-way ANOVA was used to detect probes showing differential cirDNA modification between the groups. The significance level was set at $p < 0.05$ and fold changes higher than 2. Model-based analysis of tiling-arrays (MAT) [9] was used to identify regions of differential cirDNA modification by combining adjacent probes showing significant differences between the groups. A sliding window of 500 bp was set, according to the average size of the fragments produced in the amplicon preparation step [1].

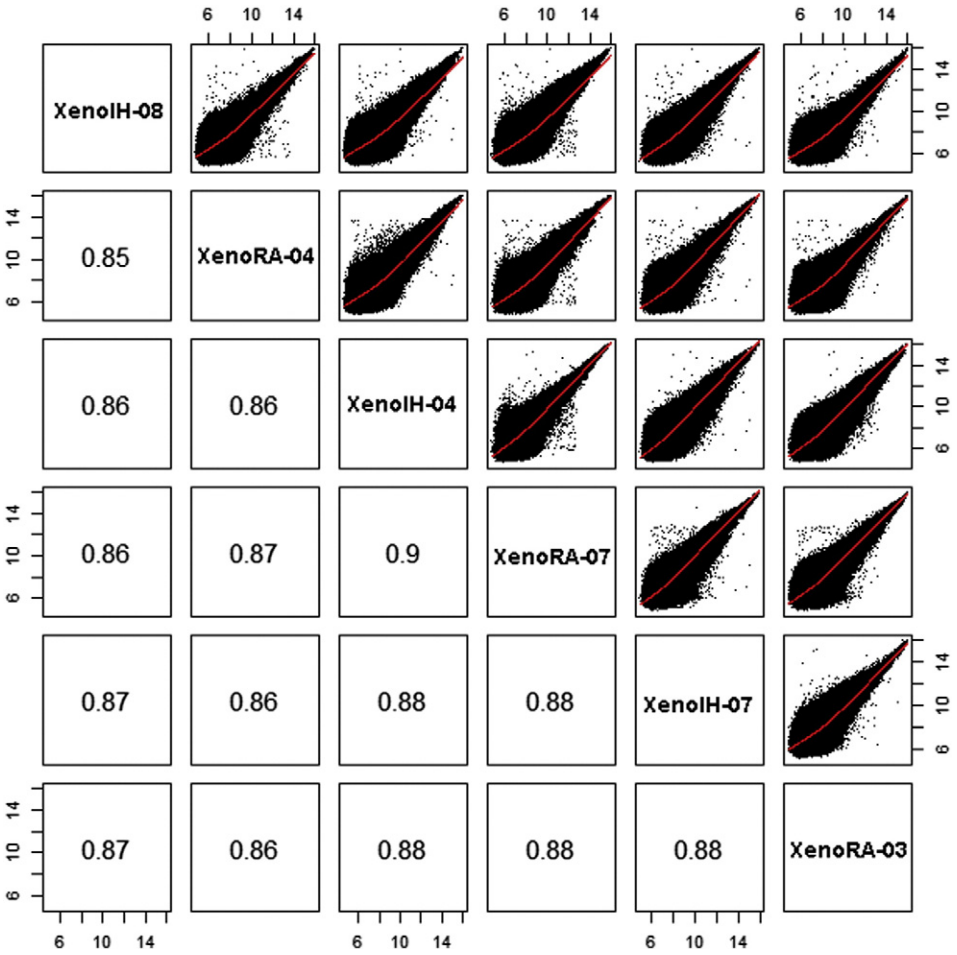


Fig. 3. Signal intensity correlation among arrays before normalization. Paired scatter plots of signal intensity for each array in the set. Signal intensity values (before normalization) for each array are plotted in the X- and Y axes, respectively. Red line depicts the correlation trend lines. Correlation coefficient for each pair is indicated.

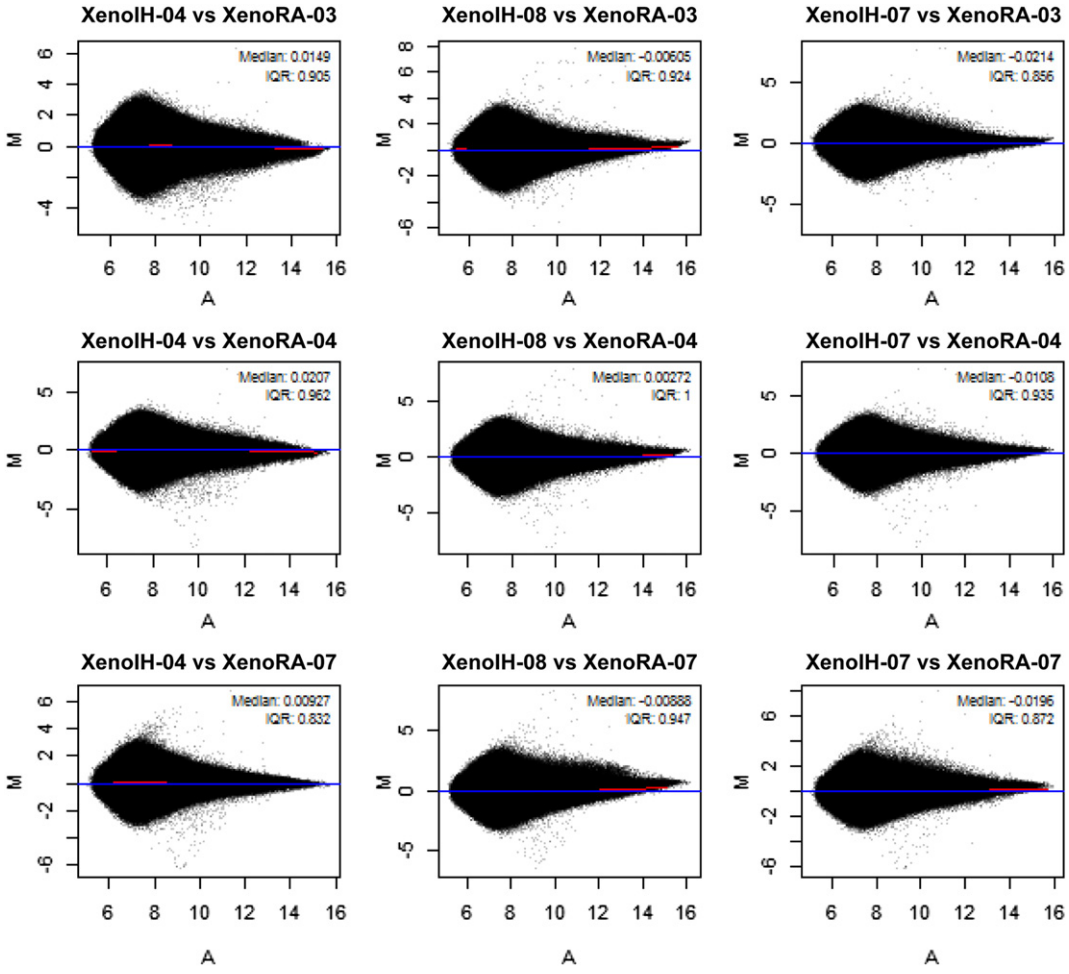


Fig. 4. Probe-wise signal intensity differences among arrays after Loess-normalization. M–A plots of normalized signal intensity for each possible pair of microarrays in the set. X-axis represents the mean average of the normalized signal intensity ($A = [\log2(\text{signal array1}) + \log2(\text{signal array2})] * 1/2$). Y-axis represents the log ratios of the normalized signal intensity ($M = \log2(\text{signal array 1}) - \log2(\text{signal array 2})$). The loess lines and the horizontal axis ($M = 0$) are shown in red and blue, respectively. The inter-quartile range (IQR) and median are reported for each comparison.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

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