Acquired tissue-specific promoter bivalency is a basis for PRC2 necessity in adult somatic cells

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SUPPLEMENTAL MATERIALS

Supplemental Experimental Procedures: Mice and cell isolation; Histology and detection of proteins; Details of ChIP-seq analysis; Published data on gene expression and histone modifications.

Table S1. Summary of ChIP-seq and RNA-seq experiments.

Supplemental References

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Mice and cell isolation. Animals were housed under specific pathogen-free conditions and handled according to protocols approved by the Dana-Farber Cancer Institute's Animal Care and Use Committee. Secretory progenitors (from intestinal crypts of mice ~38 h after treatment with the Notch inhibitor dibenzazepine) and enterocyte progenitors (from crypts of Atoh1-/- small intestines, which lack all secretory cells) were generated and isolated as described previously (Kim et al., 2014).

Histology and detection of proteins. Tissues fixed overnight in 4% paraformaldehyde at 4ºC were washed in PBS, dehydrated in ethanol, embedded in paraffin, and used to prepare 5 µm sections that were deparaffinized, rehydrated, and stained with hematoxylin and eosin or with the alkaline phosphatase substrate nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl-phosphate (Sigma B5655). Following antigen retrieval in 10 mM sodium citrate buffer (pH 6), tissue sections were incubated overnight at 4℃ with EZH2 (Cell Signaling 5246, 1:500), H3K27me3 (Millipore 07-449, 1:1000), BrdU (AbD Serotec OBT0030CX) or KI67 (Vector VP-K452) antibodies (Ab) in PBS, followed by anti-rabbit, anti-rat or anti-mouse IgG conjugated to Cy3 or biotin (Jackson Laboratories, 1:1000), and detection by fluorescence or by staining with Vectastain Elite ABC Kit (Vector) and 3,3' diaminobenzidine tetrahydrochloride (Sigma P8375). Crypts and villi, isolated without digestion or flow sorting, were lysed in Triton Extraction Buffer (TEB) containing 0.5% Triton-X100 and 2 mM phenylmethylsulfonylfluorde (PMSF) in PBS for 10 min. Cell pellets were incubated overnight in 0.2 N HCl at 4℃ and extracted histones were immunoblotted with H3K27me3 (Millipore 07-449, 1:1000) and Histone3 (Cell Signaling 9715S, 1:1000) Ab.

ChIP-seq. To detect H3K27me3 and Pol2, villus epithelial cells and ISC were cross-linked for 25 min with 1% formaldehyde, washed in cold PBS, and resuspended in 50 mM Tris-HCl (pH 8), 10 mM EDTA, 1% SDS, and protease inhibitors (Roche). Chromatin fragments (200-500 bp) obtained by sonication (Covaris) were resuspended in 150 mM NaCl, 20 mM Tris-HCl (pH 8), 2 mM EDTA, and 1% Triton X-100, then incubated overnight at 4℃ with magnetic beads (Dynal) conjugated to H3K27me3 (Millipore 07-449) or Pol2 (Santa Cruz SC-899) Ab. Beads were washed, DNA was eluted, and cross-links were reversed in 1% SDS and 0.1 M $NaHCO_3$ for 6 h at 65℃. DNA was purified using a kit (Qiagen).

Other studies mapped H3K27me3 and H3K4me2 in secretory and enterocyte progenitors, isolated from mouse intestinal crypts, and in wild-type villus epithelial cells prepared as described above. In these experiments, cells were digested with 0.2 U micrococcal nuclease (Sigma N3755) at 37℃ for 6 min in 50 mM Tris-HCl (pH7.6), 1 mM $CaCl_2$, 0.2% Triton X-100, protease inhibitors (Roche), and 0.5 mM PMSF. The nucleosome suspension was dialyzed against RIPA buffer (50 mM HEPES (pH 7.6), 1 mM EDTA, 0.7% Na deoxycholate, 1% NP-40, 0.5 M LiCl) for 3 h at 4℃, isolated by centrifugation, and incubated overnight at 4℃ with magnetic beads (Dynal) conjugated to H3K27me3 (Millipore 07-449), H3K4me2 (Millipore 07-030) or H3K4me3 (Abcam ab8580) Ab. After ChIP, beads were washed 4 times in RIPA buffer and twice in 10 mM Tris-HCL (pH 8) and 1 mM EDTA. The mononucleosome fraction of eluted DNA was isolated using 2% E-gel (Invitrogen).

In all cases, libraries were prepared from the ChIP material using the ThruPLEX DNA-seq kit (Rubicon) and sequenced on Illumina HiSeq 2000 (50-bp reads) or NextSeq 500 (75-bp reads) instruments. Unique ChIP-seq reads were aligned to the mouse genome (Mm9, NCBI build 37) using Bowtie2. Raw files were filtered to remove PCR duplicates and reads that aligned to multiple locations. Library information is provided in Table S1. Mapped regions were visualized using the Integrated Genomics Viewer (IGV 2.3 (Robinson et al., 2011)). To compensate for differences in sequencing depth of multiple H3K27me3 ChIP-seq libraries, we down-sampled the larger libraries to the depth of the smallest library using Downsample BAM tool (version 1.136.0) in Galaxy Tools (https://usegalaxy.org) and generated wiggle files for visualization using bamCoverage tool in the DeepTools package (Ramirez et al., 2014). For quantitative comparison of H3K27me3 signals within cell types, libraries were normalized through the DiffReps pipeline (Shen et al., 2013), which uses a window-based approach and calculates library normalization factors as the median of region–wise normalization ratios after removing from all libraries the regions with low counts.

Published data on gene expression and histone modifications. We used ChIP- and RNA-seq datasets from the skin (matrix cells), blood (LSK cells), and undifferentiated ESCs. Raw data (FASTQ or BAM files) from the following datasets in the Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) were used in the analysis:
Skin (matrix cells) - ChIPseq (H3K4me3, H3K27me3): GSE31239 (Lien et al., 2011), RNA-seq: GSE61505 (Genander et al., 2014), Gene expression in Ezh1/2 KO mice: GSE26616 (Ezhkova et al., 2011).
Blood (LSK Cells) - ChIPseq (H3K4me3, H3K27me3) GSE43007 (Hasemann et al., 2014),

RNA-seq: GSE60101 (Lara-Astiaso et al., 2014), Gene expression in Eed KO LT-HSCs: GSE51084 (Xie et al., 2014).

ES cells - ChIPseq (H3K4me3, H3K27me3): GSE40065 (Subramanian et al., 2013), GSE43229 (Das et al., 2014), and GSE58391 (Shpargel et al., 2014); SUZ12 ChIP-seq: GSE58019 (Riising et al., 2014).

Table S1

Summary of ChIP-seq and RNA-seq experiments

(Related to Figures 1, 2, 3, 5, 6, 7)

Cell Type	Experiment	Uniquely mapped reads	GEO accession
WT ISC Replicate-1	H3K27me3 ChIP (Sonicated)	17,357,789	GSM1843532
WT ISC Replicate-2	H3K27me3 ChIP (Sonicated)	49,35,8827	GSM2065674
WT Villus Replicate-1	H3K27me3 ChIP (Sonicated)	16,558,898	GSM1843530
WT Villus Replicate-2	H3K27me3 ChIP (Sonicated)	42,799,090	GSM2065675
WT Villus Replicate-3	H3K27me3 ChIP (Sonicated)	65,114,772	GSM2065676
WT Villus	H3K4me3 ChIP (MNase)	17,587,823	GSM1843531
WT Enterocyte Progenitors	H3K27me3 ChIP (MNase)	22,439,356	GSM2065679
WT Enterocyte Progenitors	H2K4me2 ChIP (MNase)	26,163,006	GSM2065681
WT Secretory Progenitors	H3K27me3 ChIP (MNase)	26,739,653	GSM2065680
WT Secretory Progenitors	H2K4me2 ChIP (MNase)	34,059,738	GSM2065682
WT Villus Replicate-1	RNA Pol-II ChIP (Sonicated)	35,85,3219	GSM2065677
WT Villus Replicate-2	RNA Pol-II ChIP (Sonicated)	34,99,8477	GSM2065678
WT ISC Replicate-1	RNA-seq	27,294,167	GSM1843524
WT ISC Replicate-2	RNA-seq	20,834,649	GSM1843525
WT ISC Replicate-3	RNA-seq	48,980,494	GSM1843526
WT Villus Replicate-1	RNA-seq	27,811,300	GSM1843521
WT Villus Replicate-2	RNA-seq	27,687,974	GSM1843522
WT Villus Replicate-3	RNA-seq	23,148,182	GSM1843523
Eed -/- Villus Replicate-1	RNA-seq	23,308,614	GSM1843527
Eed -/- Villus Replicate-2	RNA-seq	23,540,166	GSM1843528
Eed -/- Villus Replicate-3	RNA-seq	31,315,825	GSM1843529

SUPPLEMENTAL REFERENCES

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