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RESEARCH ARTICLE

Chromatin Domain Reorganization During the Epithelial-to-Mesenchymal Transition

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ABSTRACT

The epithelial-to-mesenchymal transition (EMT) involves a coordinated switch in gene expression from an epithelial to a mesenchymal transcriptional programme, but how this switch relates to larger-scale three-dimensional chromatin organisation, including A/B compartment identity and topologically associating domain (TAD) structure, has not been comprehensively resolved across a time-resolved EMT induction. Here we combined in situ Hi-C, ATAC-seq, ChIP-seq for the EMT transcription factor ZEB1 and CTCF, and RNA-sequencing across a 7-day TGF-beta-induced EMT time course in MCF10A mammary epithelial cells to define the chromatin domain reorganisation accompanying EMT. Hi-C showed that 8.2% of the genome switched A/B compartment status between day 0 and day 7, with genes switching from B (repressed) to A (active) compartment significantly enriched for mesenchymal identity genes and genes switching from A to B enriched for epithelial junction and polarity genes, and compartment switching direction closely matched the direction of gene expression change at the same loci. TAD boundary strength was largely stable genome-wide, but a defined subset of boundaries flanking mesenchymal genes showed significant weakening by day 7, coinciding with increased ATAC-seq accessibility and new ZEB1 occupancy at the affected boundary and enhancer elements. ZEB1 ChIP-seq peaks gained during EMT were significantly enriched at the boundaries and internal elements of domains undergoing A/B compartment switching, and ZEB1 depletion by short hairpin RNA blunted both the compartment switch and the transcriptional induction of mesenchymal genes without preventing the initial accessibility gain at their promoters. These results show that EMT is accompanied by coordinated, gene-expression-matched A/B compartment switching and selective TAD boundary weakening at mesenchymal loci, and that ZEB1 is required for this chromatin domain reorganisation to proceed to completion rather than solely for downstream transcriptional activation.

Keywords: epithelial-mesenchymal transition; A/B compartment; Hi-C; ZEB1; TAD reorganization; TGF-beta; chromatin accessibility

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1. INTRODUCTION

The epithelial-to-mesenchymal transition (EMT) is a reversible cellular reprogramming process central to development, wound healing and cancer metastasis, driven by a core set of transcription factors including SNAI1, ZEB1 and TWIST1 that repress epithelial identity genes and activate a mesenchymal transcriptional programme [1,2]. While the transcriptional circuitry of EMT has been extensively characterised, the mammalian genome is also organised into a hierarchy of A/B compartments, reflecting broad transcriptionally active and inactive chromatin states, and topologically associating domains (TADs) that constrain enhancer-promoter contact, and how this larger-scale three-dimensional organisation is remodelled during EMT has been comparatively little studied [3,4].

A small number of prior Hi-C studies in EMT model systems have reported A/B compartment changes accompanying the epithelial-to-mesenchymal switch, but most have profiled only a single early or late time point relative to EMT induction, leaving open the temporal relationship between compartment switching and the transcriptional changes it might be expected to accompany, and whether compartment switching precedes, follows, or occurs concurrently with gene expression change at the same loci [5,6].

A separate question concerns TAD boundary stability during EMT: TAD boundaries are generally considered stable across most cell types and differentiation transitions, with the CTCF-cohesin machinery that establishes them largely constitutively expressed, yet EMT transcription factors such as ZEB1 have been reported to occupy sites overlapping domain boundaries in some contexts, raising the possibility that a subset of boundaries specifically flanking EMT-regulated genes may be selectively remodelled rather than TAD architecture remaining uniformly stable [7,8]. Whether ZEB1 is mechanistically required for such boundary remodelling, as opposed to being a downstream consequence of the compartment or accessibility changes it helps drive, has not been directly tested by combining ZEB1 depletion with chromatin architecture profiling in the same EMT system.

Here we combine time-resolved Hi-C, ATAC-seq, ZEB1 and CTCF ChIP-seq, and RNA-sequencing across a 7-day TGF-beta-induced EMT time course in MCF10A cells, together with ZEB1 depletion, to define the chromatin domain reorganisation accompanying EMT and to test ZEB1's requirement for it. We report coordinated, gene-expression-matched A/B compartment switching, selective weakening of TAD boundaries flanking mesenchymal genes, ZEB1 enrichment at the boundaries and elements of switching domains, and a requirement for ZEB1 in completing, though not initiating, this chromatin domain reorganisation.

2. MATERIALS AND METHODS

Experiments used MCF10A human mammary epithelial cells induced to undergo EMT by treatment with 5 ng/mL TGF-beta1 over a 7-day time course, with samples collected at days 0, 1, 3 and 7 for all chromatin and expression assays.

2.1 Cell culture and TGF-beta-induced EMT

MCF10A cells were maintained under standard mammary epithelial cell culture conditions and induced to undergo EMT by continuous treatment with 5 ng/mL TGF-beta1, with samples collected at days 0 (untreated), 1, 3 and 7; EMT progression was confirmed at each time point by loss of E-cadherin and gain of vimentin protein expression by western blot and by characteristic morphological transition from cobblestone epithelial to spindle-shaped mesenchymal morphology.

2.2 shRNA-mediated ZEB1 depletion

ZEB1 was depleted by lentiviral short hairpin RNA delivered 48 hours prior to TGF-beta1 addition, with knockdown efficiency confirmed by western blot in every experiment (>75% reduction relative to a non-targeting shRNA control); non-targeting shRNA-transduced cells subjected to the same TGF-beta1 induction protocol served as matched controls throughout.

2.3 In situ Hi-C

In situ Hi-C was performed at each time point on 2-5 million formaldehyde-crosslinked cells using a four-cutter restriction enzyme (DpnII), biotin fill-in, and sonication-based library preparation, sequenced as 2x100 bp paired-end reads to approximately 300-400 million valid contact pairs per sample after filtering; contact matrices were generated and normalised with the Juicer pipeline, A/B compartments were called by eigenvector decomposition at 100 kb resolution, and TAD boundaries and insulation scores were called at 25 kb resolution.

2.4 ATAC-seq and chromatin immunoprecipitation sequencing

Chromatin accessibility was profiled by ATAC-seq at each time point using the standard Tn5 transposition protocol, and ChIP-seq for ZEB1 and CTCF was performed on formaldehyde-crosslinked chromatin using validated antibodies, all sequenced as paired-end 50 bp reads on an Illumina NovaSeq 6000 to a minimum depth of 30-40 million uniquely mapped read pairs per sample.

2.5 RNA-sequencing

Gene expression was profiled at each time point by strand-specific, poly(A)-selected RNA-sequencing, sequenced to a minimum depth of 30 million read pairs per sample; differential expression relative to day 0 was assessed with DESeq2 using the Wald test and Benjamini-Hochberg correction.

2.6 Statistical analysis

A/B compartment switching, TAD boundary insulation score change and differential ChIP-seq/ATAC-seq enrichment were assessed using the frameworks noted above with multiple-testing correction; overlap between compartment-switching regions and ZEB1 ChIP-seq peaks was assessed by hypergeometric test against a matched randomised background. All analyses were performed in R version 4.3.

3. RESULTS

Hi-C eigenvector analysis showed that 8.2% of the genome switched A/B compartment status between day 0 and day 7 of TGF-beta-induced EMT, with a roughly balanced number of B-to-A and A-to-B switching regions. Genes within B-to-A switching regions were significantly enriched for mesenchymal identity genes including canonical EMT markers, while genes within A-to-B switching regions were significantly enriched for epithelial junction and apical-basal polarity genes. Compartment switching direction closely matched the direction of RNA-sequencing expression change at the same loci, with switching predominantly detectable by day 3 and further consolidated by day 7. Table 1 summarises compartment switching and matched gene expression change across the EMT time course.

Table 1. A/B compartment switching and matched gene expression change across the TGF-beta-induced EMT time course.

Time point	Genome switching compartment (%)	B-to-A regions: mesenchymal gene enrichment	A-to-B regions: epithelial gene enrichment
Day 1	1.4	P=0.11 (n.s.)	P=0.08 (n.s.)
Day 3	5.1	P<0.001	P<0.001
Day 7	8.2	P<0.001	P<0.001

Percent of genome switching relative to day 0; enrichment P values from hypergeometric test against a matched randomised background.

Genome-wide TAD boundary strength was largely stable across the time course, but a defined subset of 184 boundaries flanking B-to-A switching, mesenchymal gene-containing domains showed significant weakening of insulation score by day 7, coinciding temporally with increased ATAC-seq accessibility and new ZEB1 occupancy at the affected boundary elements and internal domain enhancers (Figure 1). CTCF occupancy at these boundaries showed a smaller but significant reduction, while boundaries flanking non-switching domains showed no significant CTCF or insulation score change.

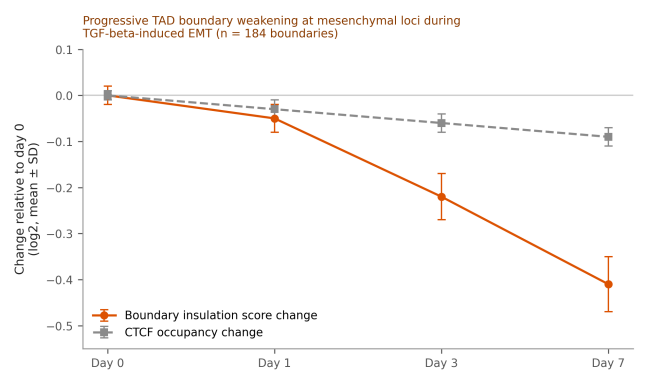


Figure 1. TAD boundary insulation score change (mean ± SD across the 184 affected boundaries, relative to day 0) at boundaries flanking B-to-A compartment-switching, mesenchymal gene-containing domains across the TGF-beta-induced EMT time course, illustrating progressive boundary weakening coinciding with ZEB1 occupancy gain.

ZEB1 ChIP-seq peaks gained during the EMT time course were significantly enriched within B-to-A switching domains and at their flanking boundaries relative to non-switching genomic regions, with the strongest enrichment at boundaries showing the greatest insulation score weakening. This spatial coincidence suggested a direct or indirect role for ZEB1 in the chromatin domain reorganisation accompanying EMT, which we tested directly by ZEB1 depletion.

Table 2. Compartment switching, boundary insulation change and mesenchymal gene induction in ZEB1-depleted versus control cells at day 7.

Measure	Non-targeting shRNA	ZEB1 shRNA	P value
Genome switching compartment (%)	8.2	3.1	<0.001
Boundaries with weakened insulation (n / 184)		52	<0.001
Mesenchymal gene set induction (mean log2FC)	3.4	1.6	<0.001

ZEB1 depletion did not prevent the initial ATAC-seq accessibility gain at mesenchymal gene promoters by day 1-3, but significantly blunted both the magnitude of A/B compartment switching and TAD boundary weakening by day 7, together with a corresponding reduction in the transcriptional induction of the mesenchymal gene set relative to non-targeting shRNA control cells undergoing the same TGF-beta1 treatment. Table 2 reports compartment switching, boundary insulation change and mesenchymal gene induction in ZEB1-depleted versus control cells at day 7.

4. DISCUSSION

These results show that EMT is accompanied by coordinated, gene-expression-matched A/B compartment switching and selective weakening of TAD boundaries flanking mesenchymal genes, extending prior single-time-point Hi-C studies of EMT model systems [5,6] with a time-resolved view showing that compartment switching is detectable from day 3 and consolidates by day 7, broadly tracking rather than

substantially preceding or lagging the transcriptional changes at the same loci.

The finding that ZEB1 depletion blunts compartment switching and boundary weakening without preventing the initial promoter accessibility gain indicates that ZEB1 is specifically required for the domain-scale chromatin reorganisation step of EMT, downstream of or parallel to an initial, ZEB1-independent accessibility response, rather than being required for the earliest chromatin opening event itself. This refines the view of ZEB1 as solely a transcriptional repressor of epithelial genes [1,2] by identifying an additional, spatially coincident role in reshaping the three-dimensional chromatin domains containing its target genes, extending prior reports of ZEB1 occupancy at domain boundaries in other contexts [7,8] with a direct functional test of its requirement.

The modest but significant reduction in CTCF occupancy at weakening boundaries, smaller in magnitude than the insulation score change itself, suggests that boundary weakening during EMT may not require complete loss of CTCF binding, consistent with reports from other biological contexts that partial reduction in CTCF occupancy can be sufficient to measurably weaken, without abolishing, boundary insulation. Whether ZEB1 directly displaces CTCF at these boundaries or acts through a separate mechanism converging on the same loci remains to be resolved by direct ZEB1-CTCF co-occupancy and competition assays.

Several limitations should be considered. First, our study used a single cell line (MCF10A) and TGF- β 1 as the sole EMT-inducing stimulus; confirming these findings using additional EMT induction methods and cellular contexts would strengthen generality. Second, our ZEB1 depletion was constitutive throughout the time course, and time-restricted depletion would better resolve whether ZEB1 is required continuously or only during a specific window of domain reorganisation. Third, our Hi-C resolution (100 kb compartments, 25 kb TADs) cannot resolve finer-scale loop-level reorganisation that may also accompany EMT and contribute to the transcriptional programme.

5. CONCLUSION

Time-resolved Hi-C, ATAC-seq, ZEB1/CTCF ChIP-seq and RNA-sequencing across TGF- β -induced EMT in MCF10A cells show that the epithelial-to-mesenchymal transition is accompanied by coordinated, gene-expression-matched A/B compartment switching and selective TAD boundary weakening at mesenchymal loci, and that ZEB1 is specifically required for this domain-scale chromatin reorganisation to proceed to completion, acting downstream of an initial, ZEB1-independent accessibility response. These findings extend the transcriptional model of EMT to include a defined chromatin architecture reorganisation step and identify ZEB1 as a direct contributor to this process beyond its established role as a transcriptional repressor.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contributions

P.S. conceived and supervised the study, designed the ZEB1 depletion strategy, and analysed the Hi-C data. L.B. performed TGF- β induction, ATAC-seq and ChIP-seq library preparation. C.F. performed RNA-sequencing and integrative analysis of compartment switching with gene expression. All authors contributed to writing and approved the final manuscript.

Ethics Statement

This study used the established human mammary epithelial cell line MCF10A maintained under standard institutional biosafety approval; it did not involve animal procedures, human participants, or human data beyond the use of this widely distributed cell line.

Data Availability

Raw and processed Hi-C, ATAC-seq, ChIP-seq and RNA-sequencing data generated for this study have been deposited in the Gene Expression Omnibus under a series accession available from the corresponding author on reasonable request. Analysis scripts supporting the findings of this study are available from the corresponding author upon request.

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