



A multi-omics approach to characterise epithelial to mesenchymal transition in lung cancer metastasis

Cells undergoing phenotypic transition from epithelial to mesenchymal states can be evaluated using full length transcriptomics, targeted methylation, and chromatin conformational changes with Pore-C

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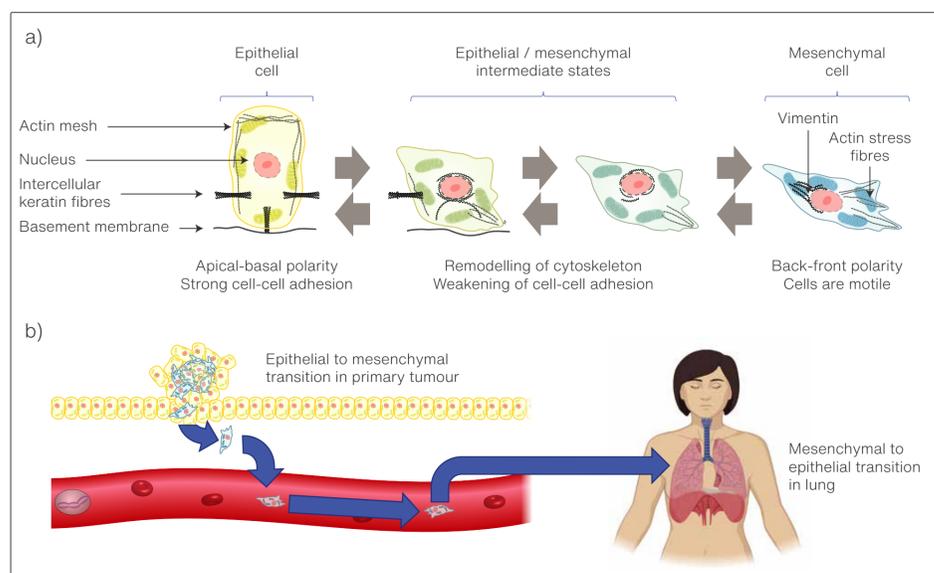


Fig. 1 Investigating complex cellular morphology changes during EMT in metastatic cancer

EMT in metastatic cancer involves extensive changes to cell morphology

Epithelial-mesenchymal transition (EMT) is a complex cellular process by which cancer cells lose the apical-basal polarity and cell-cell adhesive properties that are characteristic of epithelial cells, and gain the properties of mesenchymal cells (Fig. 1a). The newly motile mesenchymal cells are able to migrate from the site of the primary tumour and to spread via the bloodstream to a secondary site (Fig. 1b). This process is known as metastasis. Once cells reach the secondary site the process reverses, and the mesenchymal cells revert to epithelial cells. The metastasised cells multiply and eventually form another tumour. Many epigenetic, transcriptomic, and genomic changes are known to occur during EMT.

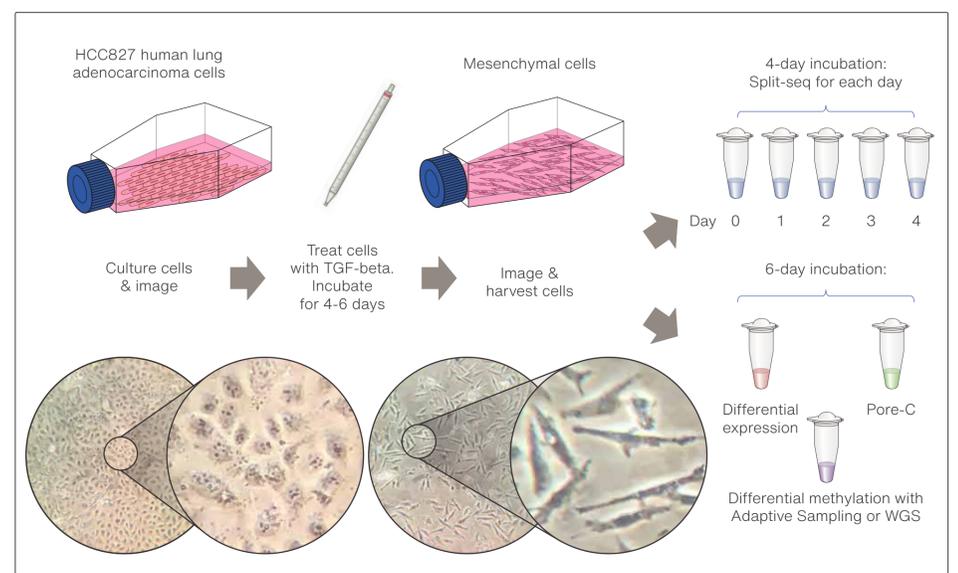


Fig. 2 Treating human lung cancer cells with TGF- β induces EMT

A comprehensive DNA- and RNA-based investigation of cells after EMT induction

We cultured human HCC827 lung adenocarcinoma cells in T75 culture flasks and treated them with transforming growth factor beta (TGF- β) to induce EMT. The mesenchymal phenotype fully developed over the course of several days and is characterised as a physical change to the cell morphology when viewed under a microscope. Following transition, we harvested cells and extracted DNA or RNA (Fig. 2). We then prepared several different library types following multiple experimental approaches, including whole-genome, transcriptomic and Pore-C. We generated multiple datasets and performed multi-omic analyses to obtain as complete a picture of the EMT process as possible.

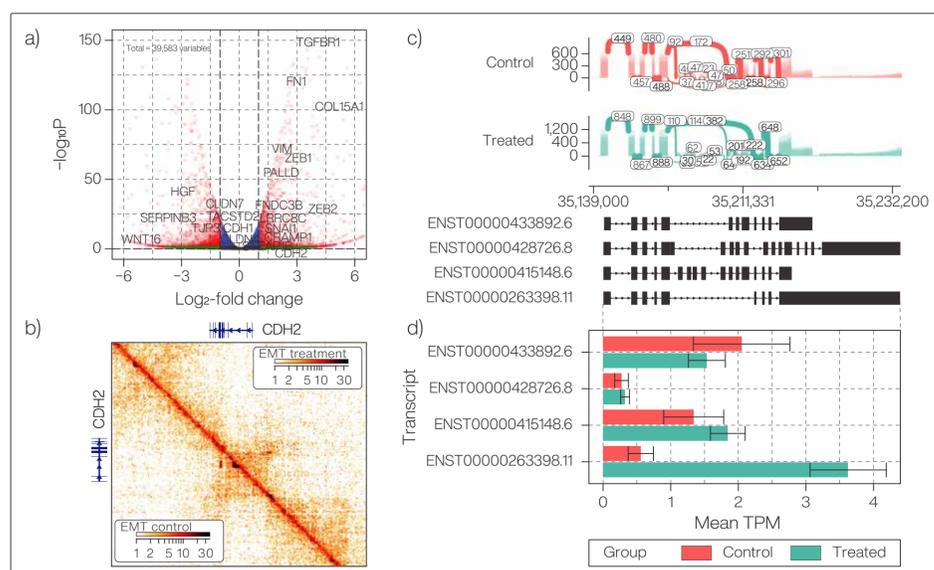


Fig. 3 Differential expression, isoform, and chromatin analysis show changes at marker genes

Changes at EMT marker genes revealed by expression, isoform and chromatin analyses

We performed differential expression analysis on genes between the control and treatment flasks using DESeq2 to identify mesenchymal markers. These included vimentin (VIM), E-cadherin (CDH1), N-cadherin (CDH2) and zinc finger E-box-binding homeobox 2 (ZEB2) (Fig. 3a). We observed differential chromatin loop architecture for CDH2 using Pore-C (Fig. 3b), which suggests a reorganisation of chromatin during cadherin switching. Our differential transcript-usage analysis revealed upregulation of a specific isoform of CD44 in the treated sample, although CD44 was not differentially expressed when looking across all isoforms (Figs. 3c and 3d). CD44 is a cell-surface adhesion molecule with known alternatively spliced transcripts which have been implicated in tumour metastasis.

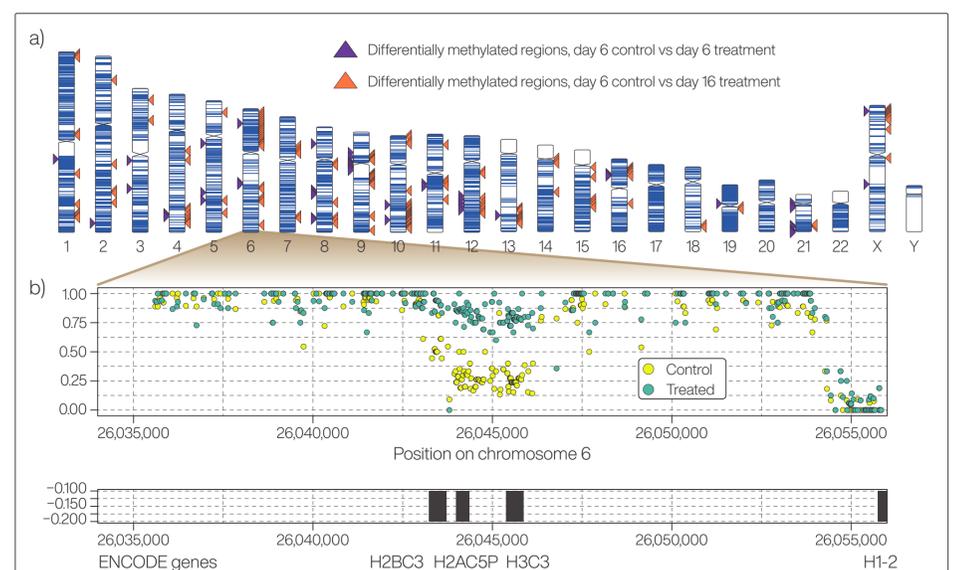


Fig. 4 Adaptive sampling shows targeted methylation changes at EMT loci

Using adaptive sampling for finding changes in methylation status at EMT loci

We used adaptive sampling with a customised methylation target panel to investigate methylation differences at the genomic loci corresponding to differentially expressed genes. We adding a 10 kb buffer region upstream and downstream of each gene to include promoters and nearby regulatory regions. Target regions are represented as blue bands across the ideogram (Fig. 4a). We called differentially methylated regions between the control and treatment after 6 days (purple triangles on the left of chromosomes) or 16 days (orange triangles on the right) using DSS and bsseq. 53 differentially methylated loci were seen after 6 days, and 127 differentially methylated regions were seen after 16 days. Methylation at a histone cluster on chromosome 6 is up-regulated during treatment (Fig. 4b).