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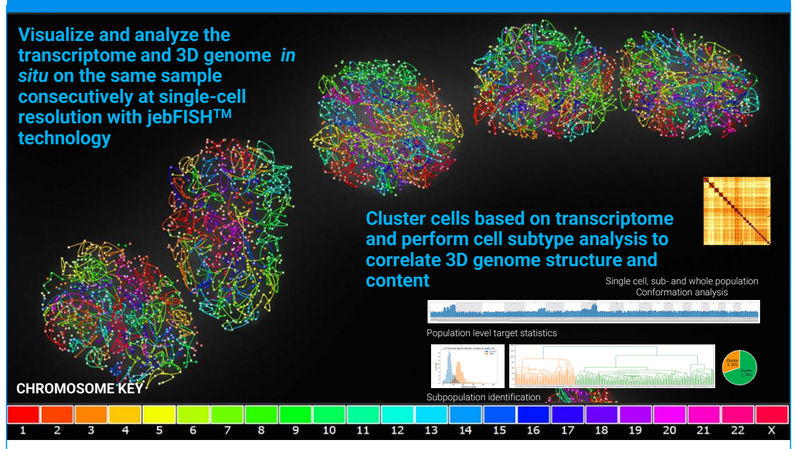
Integrated 3D genome, transcriptome and proteomic assays – simultaneously observing genome architecture and transcriptional activity at single-cell resolution

Jude Dunne[1], David King[1], Nagasangeethavani Anandakrishnan[1], Johnson Huynh[1], Serdar Tulu[1], Wyatt Eng[1], Marc Glazer[1], Brian Smart[1], David Castillo[1], Kenny Chung[1], Huy Nguyen[1], Shyamtanu Chattoraj[1], and Doug Werner[1]

[1]Bruker Spatial Genomics Inc., Research and Development, San Jose, California, USA

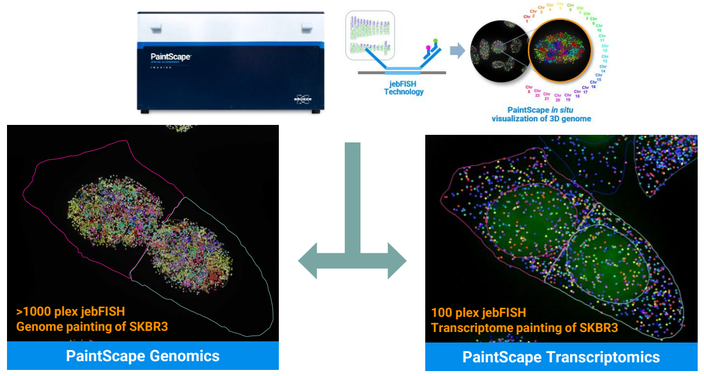
Introduction



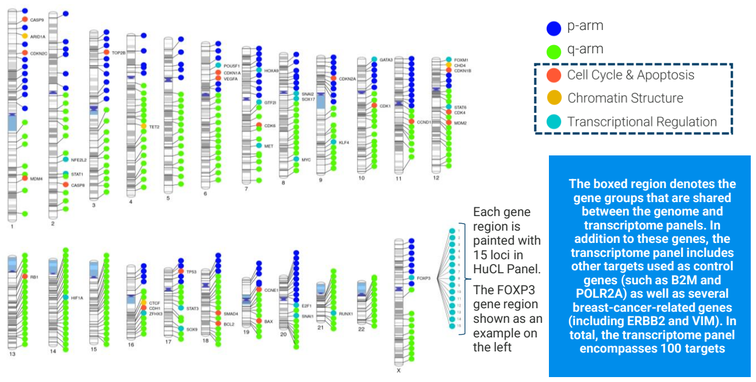
- Here we present a novel jebFISH™ protocol on the PaintScape platform used to characterize differences in genome structure, gene expression, and phenotype at subcellular resolution between HER2⁺ breast cancer cell lines HCC1954 and SKBR3.
- Variation in chromatin structure, including whole or partial chromosome copy gain or loss, genome openness or compartment switching, and TAD boundary disruptions can directly alter transcriptional activity and protein expression.
- Cancer cells heavily rely on dysregulated cell cycle and signaling pathways for proliferation and invasion to further fuel abnormal cell growth and epithelial-mesenchymal transition. These signaling pathways consist of a complex network of interactions involving several key oncogenes.
- HCC1954 and SKBR3, both HER2⁺ breast cancer cell lines used in this study, contain hotspots of clustered breakpoints on specific chromosomal DNA or extra chromosomal DNA (ecDNA) regions and often show complex focal amplifications[1-4]. Selective copy gain, rearrangement, and altered 3D architecture of underlying oncogenes and their regulatory elements cause dysregulation of oncogenic signaling pathways and increases cancer cell proliferation providing positive selection for cancer progression.
- We present a multiomics assay on the PaintScape System using our OncoPaint™ Oncogenic Pathways Panels with associated transcriptome targets that seeks to characterize these structural genomic changes and the impact they have on gene expression.
- Automated imaging decodes both DNA and RNA targets *in situ*. Optical barcodes label more than 1,000 genomic loci across the genome, including dense coverage of 45 gene regions spanning multiple cancer-relevant signaling pathways. The panel also includes probes for transcripts from key cancer-associated pathways, enabling simultaneous assessment of pathway-specific transcriptional outputs. Together, this integrated approach allows analysis of chromatin structure and gene expression within the same cells, enabling direct comparison between local chromatin organization and transcriptional activity.

Technology and Methods

A Multiomic jebFISH workflow on PaintScape platform



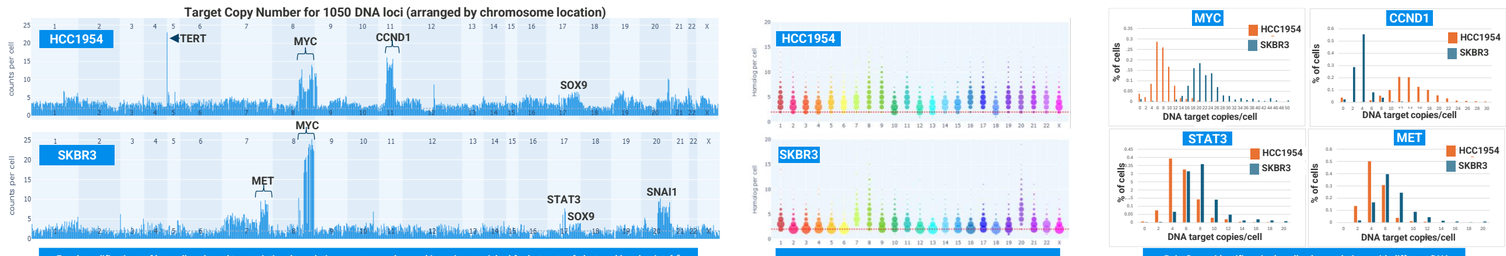
B OncoPaint™ HuCL panel (>1000 plex) with high resolution mapping around genes in cancer pathways. Transcriptome Panel (100 plex) targets these same genes.



The boxed region denotes the gene groups that are shared between the genome and transcriptome panels. In addition to these genes, the transcriptome panel includes other targets used as control genes (such as B2M and POLR2A) as well as several breast-cancer-related genes (including ERBB2 and VIM). In total, the transcriptome panel encompasses 100 targets

PaintScape system directly visualizes 3D genome structure copy number and interactions between genes *in situ* in single breast cancer cells

A PaintScape system identifies DNA amplification differences of oncogenic genes in HER2⁺ breast cancer cells

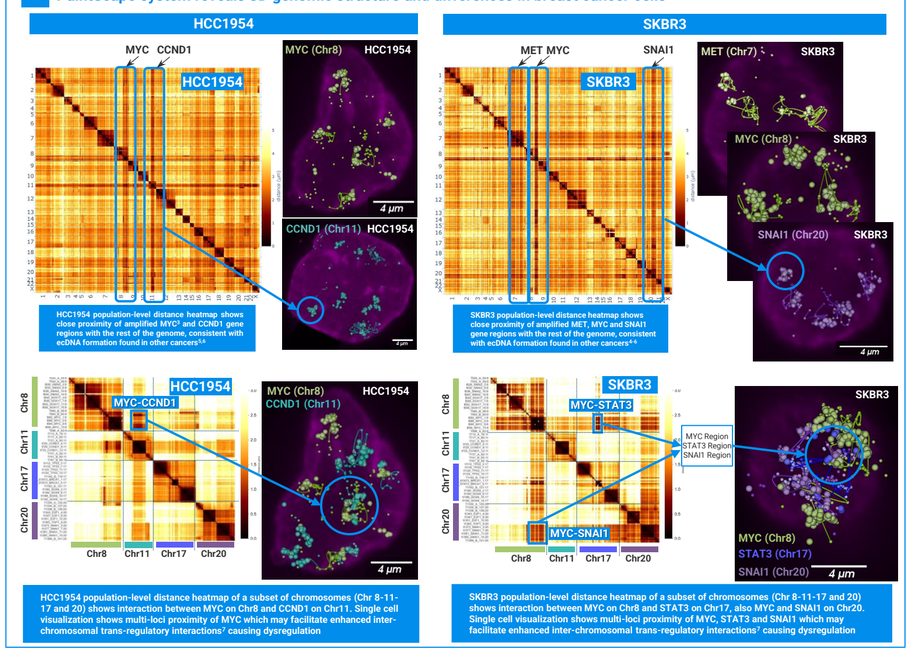


Focal amplifications of key cell cycle and transcriptional regulation oncogenes observed in regions enriched for hotspots of clustered breakpoints[1,2]
HCC 1954: TERT(5p) , MYC (8q) and CCND1 (11q) region SKBR3: MET (7p), MYC(8q) and SNAI1 region

Distributions of chromosome counts per cell show copy gain in regions enriched for hotspots of clustered breakpoints[1,2]

PaintScape identifies single cell sub-populations with different DNA copy numbers of key oncogenes, suggesting intra-tumoral heterogeneity of cancer cells

B PaintScape system reveals 3D genomic structure and differences in breast cancer cells



HCC1954 population-level distance heatmap shows close proximity of amplified MYC[2] and CCND1 gene regions with the rest of the genome, consistent with ecDNA formation found in other cancers[5,6]

SKBR3 population-level distance heatmap shows close proximity of amplified MET, MYC and SNAI1 gene regions with the rest of the genome, consistent with ecDNA formation found in other cancers[4-6]

HCC1954 population-level distance heatmap of a subset of chromosomes (Chr 8-11-17 and 20) shows interaction between MYC on Chr8 and CCND1 on Chr11. Single cell visualization shows multi-loci proximity of MYC which may facilitate enhanced inter-chromosomal trans-regulatory interactions[7] causing dysregulation

SKBR3 population-level distance heatmap of a subset of chromosomes (Chr 8-11-17 and 20) shows interaction between MYC on Chr8 and STAT3 on Chr17, also MYC and SNAI1 on Chr20. Single cell visualization shows multi-loci proximity of MYC, STAT3 and SNAI1 which may facilitate enhanced inter-chromosomal trans-regulatory interactions[7] causing dysregulation

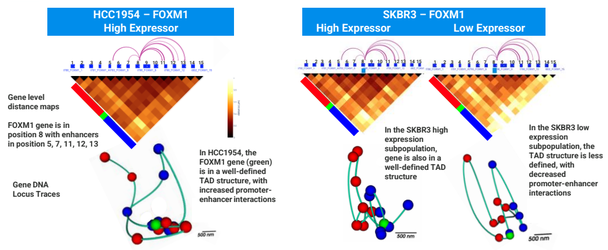
PaintScape system enables assessment of genome structure in cell subpopulation based on transcriptomic expression analysis of same cells

A PaintScape system visualizes 3D genome structure and RNA expression in the same cells

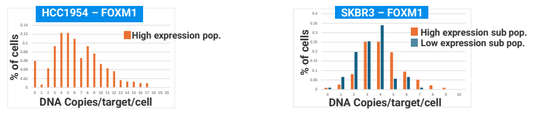
In situ visualization of genome structure and gene expression in the same single cells

FOXM1 gene, a downstream target of HER2,[10] is highly expressed in a majority of cells for HCC1954. SKBR3 shows a subpopulation of low expressors.

B Genome structure and DNA copy number distribution of FOXM1 in cell subpopulations



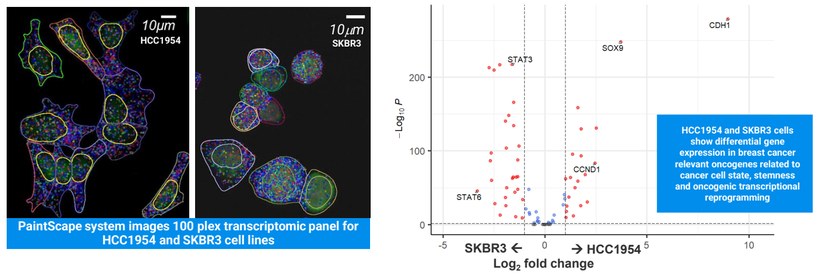
- HCC1954 is dominated by high FOXM1 expression cells and shows a defined TAD structure (positions 4-12) with increased enhancer-promoter interactions (FOXM1 gene at position 8)
- SKBR3 cell subpopulation with high FOXM1 expression shows a similar defined TAD structure
- SKBR3 cell subpopulation with low FOXM1 expression has a less organized TAD structure with reduced enhancer-promoter interactions



- FOXM1 DNA copy number shows nearly identical distributions between the high and low expressing sub-populations in SKBR3, suggesting that the higher gene expression is not driven by DNA copy number
- These observations suggest proper TAD 3D organization is required for higher gene expression due to increased interactions between gene promoter and associated regulatory elements[9]

PaintScape platform correlates transcriptomics with 3D genome structure in different breast cancer cells

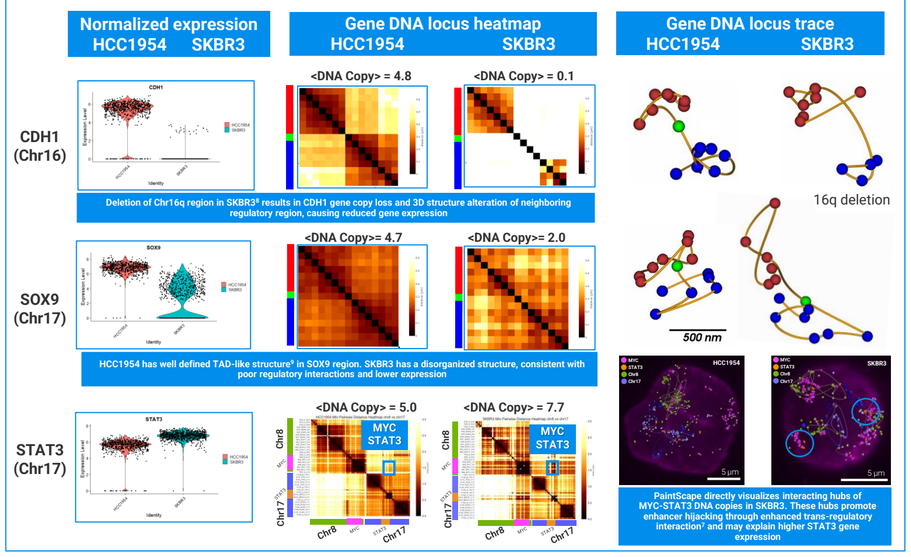
A PaintScape transcriptomics measures differential expression between HCC1954 and SKBR3 cell lines



PaintScape system images 100 plex transcriptomic panel for HCC1954 and SKBR3 cell lines

HCC1954 and SKBR3 cells show differential gene expression in breast cancer relevant oncogenes related to cancer cell state, stemness and oncogenic transcriptional reprogramming

B PaintScape system characterizes both the DNA copy number and structural genome differences at the single cell level between cell lines showing differential gene expression



Deletion of Chr16q region in SKBR3[8] results in CDH1 gene copy loss and 3D structure alteration of neighboring regulatory region, causing reduced gene expression

HCC1954 has well defined TAD-like structure[9] in SOX9 region. SKBR3 has a disorganized structure, consistent with poor regulatory interactions and lower expression

PaintScape directly visualizes interacting hubs of MYC-STAT3 DNA copies in SKBR3. These hubs promote enhancer hijacking through enhanced trans-regulatory interaction[7] and may explain higher STAT3 gene expression

Conclusions

The PaintScape system enables high-resolution identification, characterization, and direct *in situ* visualization of single-cell 3D genome architecture and spatial gene expression in single cells revealing disease state specific oncogenic function. The novel same-cell multiomic approach on the PaintScape platform shows how disruptions in genome organization drive disease mechanisms in HER2⁺ breast cancer by linking altered chromatin topology to dysregulated expression of key oncogenic pathway genes. Distinct structure-function correlations are revealed:

- **Copy number variation including amplification and deletions *in situ* in single cells and sub-population**
  - Focal amplifications of important oncogenes: MYC, CCND1, SNAI1 in HCC1954; MET, MYC and SNAI1 in SKBR3
  - Loss in CDH1 DNA copy in SKBR3
- **Single cell ploidy variation of whole chromosomes and sub-chromosomal regions**
  - Higher variation in sub-population of cells with variable chromosome ploidy in HCC1954 than SKBR3
- **ecDNA copy number, structure, nuclear locations and *in situ* interactions**
  - ecDNA type amplification of MYC and CCND1 genes in HCC1954 cells; MET, MYC and SNAI1 genes in SKBr3 cells
  - These ecDNAs show simultaneous proximity of multi-loci interactions involving key oncogenes in individual single BC cell sub-populations
  - Interacting hubs of MYC and CCND1 in HCC1954
  - Interacting hubs of MYC, STAT3 and SNAI1 in SKBR3
- **3D genome structure, spatial gene expression and same cell multiomic structure-function correlations *in situ***
  - PaintScape spatial transcriptomics identified differential expression of key breast cancer related genes CDH1, SOX9, STAT3, FOXM1 that are associated with cell state, stemness and oncogenic transcriptional reprogramming between HCC1954 and SKBR3
    - This is driven by underlying copy number variations (CNVs), genomic rearrangements, structural alterations
    - PaintScape 3D genome data shows increased enhancer-promoter interactions and strengthened intra-TAD interactions for highly expressed genes revealing mechanism of dysregulated expression
  - Same cell multiomic ability of PaintScape system identified sub-population of cancer cells with distinct 3D genome structure and spatial RNA expression profile
    - PaintScape transcriptomics identified high and low FOXM1 RNA expressing cells in SKBR3
    - High FOXM1 expressing cells show well defined TAD structure with increased enhancer-promoter interactions
    - These findings establish a framework for linking spatial transcriptomic signatures to structural genomic events, with implications for understanding intratumoral heterogeneity and clonal evolution in breast cancer cell lines

PaintScape System directly visualizes *in situ* 3D genome changes and associated RNA expression in the same cells

References

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