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In situ single cell spatial multiomic profiling of 3D genome organization in fresh frozen (FF) colorectal carcinoma tissue in spatially resolved tissue micro-environments

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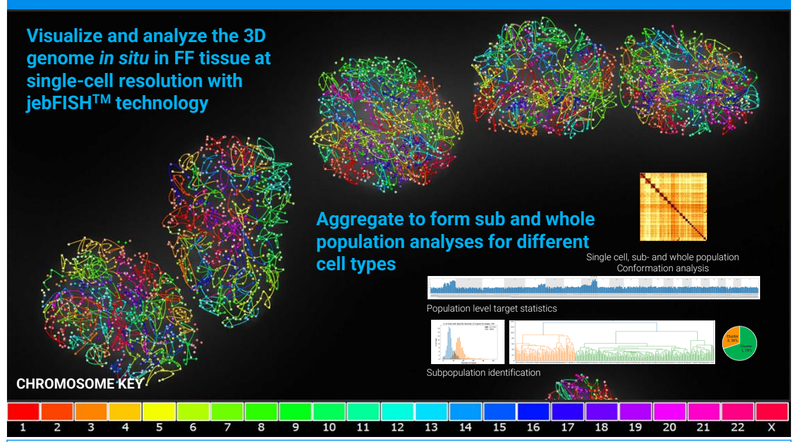
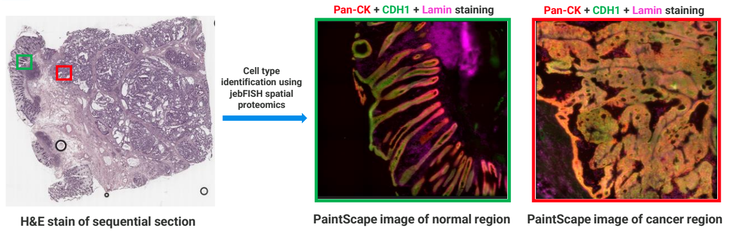
Introduction



- Here, we present a novel multiomic jebFISH™ protocol on the PaintScape™ platform that can be used to characterize intratumoral heterogeneity of 3D chromatin architecture of fresh frozen human colon tissue from colorectal cancer (CRC) patient samples in different tissue microenvironments across different cell types at single cell, sub-population and population level.
- CRC is one of the most common cancers worldwide and a second leading cause of cancer mortality.
- Tumor evolution and intra-tumor heterogeneity in CRC is fueled by chromosomal instability (CIN), 3D genome rewiring and epigenetic dysregulation.
- Many colon cancer cells show high CIN including gains in Chr8q, Chr 13q, Chr 20q and losses in Chr 8p, Chr17p, Chr14q.[1-6]
- Such selective copy gain/losses and rearrangement of those genomic regions cause dysregulation of oncogenic signaling pathways in a cell type dependent manner in the tumor microenvironment and increases cancer cell proliferation providing positive selection for cancer progression.

Technology and Methods

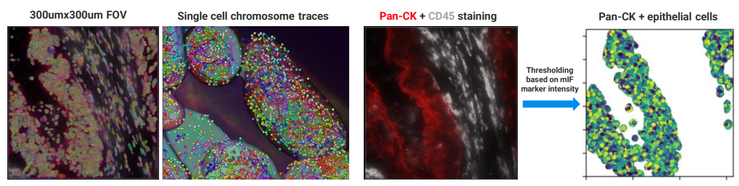
A Multiomic jebFISH™ workflow on PaintScape™ platform in 30 µm thick FF tissue sections



H&E stain of sequential section

PaintScape image of normal region

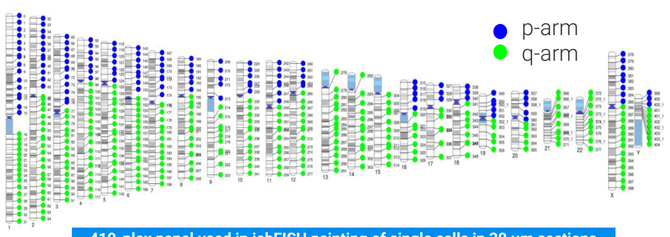
PaintScape image of cancer region



PaintScape enabled chromosome tracing in single 3D segmented cells in 30 µm thick CRC tissue section

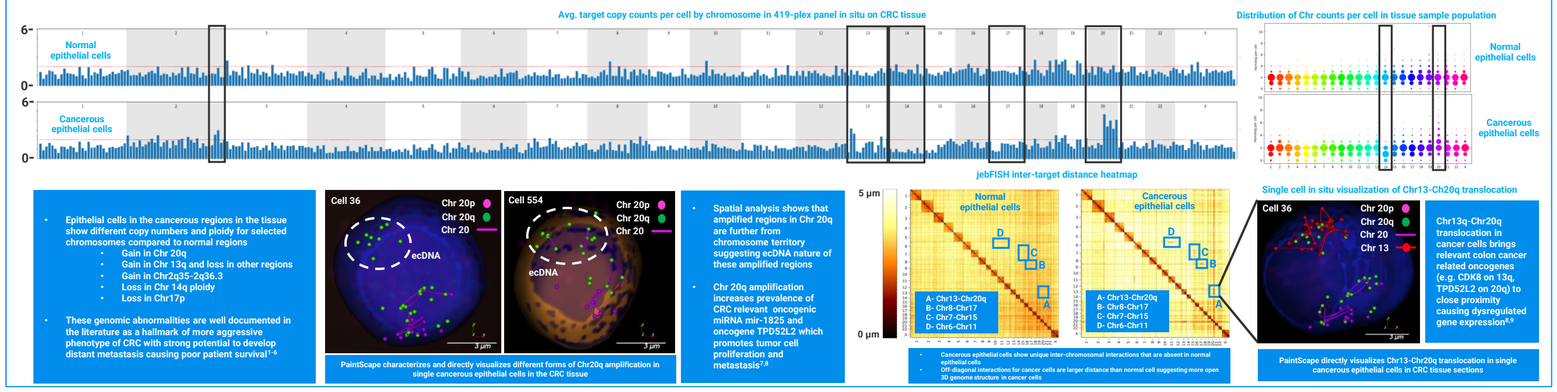
PaintScape demonstrates automated cell type identification *in situ* in 30 µm thick CRC tissue section

B ChromoPaint™ FF PanChromoMPX Panel Loci



419-plex panel used in jebFISH painting of single cells in 30 µm sections of FF CRC tissue

PaintScape™ directly visualizes and characterizes *in situ*, single cell 3D genome structural genotype and conformational changes of normal and cancerous epithelial cells in 30 µm thick FF CRC tissue section



- Epithelial cells in the cancerous regions in the tissue show different copy numbers and ploidy for selected chromosomes compared to normal regions
  - Gain in Chr 20q
  - Gain in Chr 13q and loss in other regions
  - Gain in Chr2q35-2q36.3
  - Loss in Chr 14q ploidy
  - Loss in Chr17p
- These genomic abnormalities are well documented in the literature as a hallmark of more aggressive phenotype of CRC with strong potential to develop distant metastasis causing poor patient survival[1-6]

PaintScape characterizes and directly visualizes different forms of Chr20q amplification in single cancerous epithelial cells in the CRC tissue

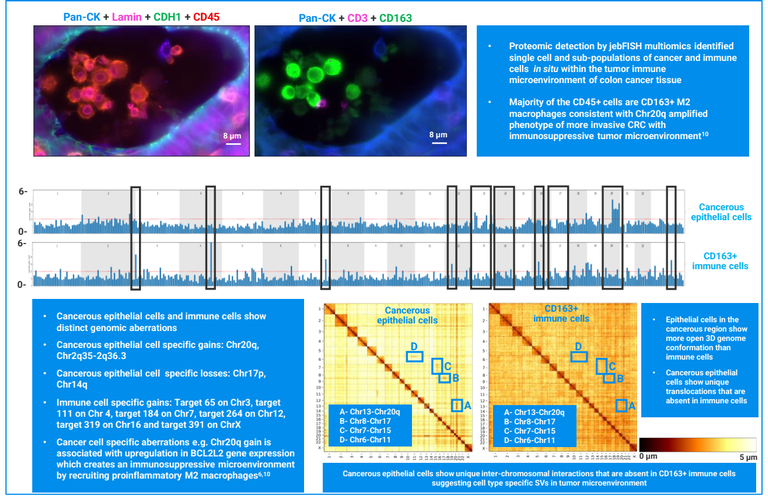
- Spatial analysis shows that amplified regions in Chr 20q are further from chromosome territory suggesting ecDNA nature of these amplified regions
- Chr 20q amplification increases prevalence of CRC relevant oncogenic miRNA mir-1825 and oncogene TPD52L2 which promotes tumor cell proliferation and metastasis[7,8]

Cancerous epithelial cells show unique inter-chromosomal interactions that are absent in normal epithelial cells
Off-diagonal interactions for cancer cells are larger distance than normal cell suggesting more open 3D genome structure in cancer cells

Chr13q-Chr20q translocation in cancer cells brings relevant colon cancer related oncogenes (e.g. CDK8 on 13q, TPD52L2 on 20q) to close proximity causing dysregulated gene expression[8,9]

PaintScape directly visualizes Chr13-Chr20q translocation in single cancerous epithelial cells in CRC tissue sections

PaintScape characterizes distinct 3D genome profiles across different cell types in tumor immune microenvironment (TIME)



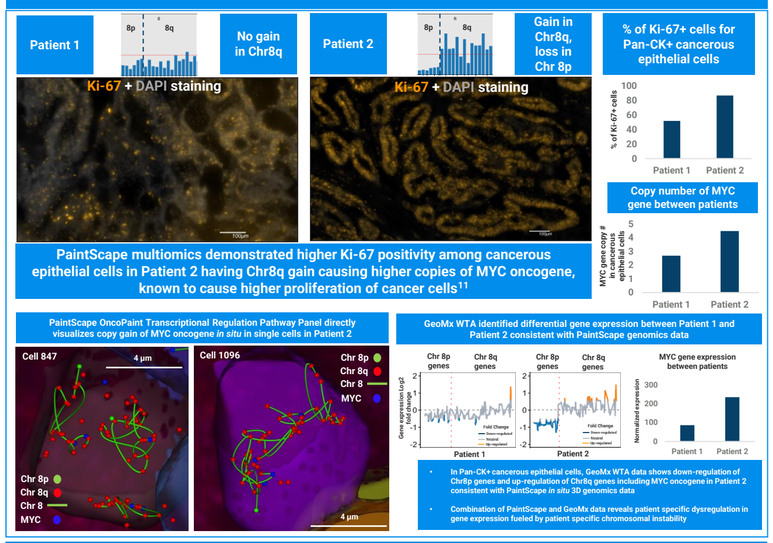
- Proteomic detection by jebFISH multiomics identified single cell and sub-populations of cancer and immune cells *in situ* within the tumor immune microenvironment of colon cancer tissue
- Majority of the CD45+ cells are CD163+ M2 macrophages consistent with Chr20q amplified phenotype of more invasive CRC with immunosuppressive tumor microenvironment[10]

- Cancerous epithelial cells and immune cells show distinct genomic aberrations
- Cancerous epithelial cell specific gains: Chr20q, Chr2q35-2q36.3
- Cancerous epithelial cell specific losses: Chr17p, Chr14q
- Immune cell specific gains: Target 65 on Chr3, target 111 on Chr 4, target 184 on Chr7, target 264 on Chr12, target 319 on Chr16 and target 391 on ChrX
- Cancer cell specific aberrations e.g. Chr20q gain is associated with upregulation in BCL2L2 gene expression which creates an immunosuppressive microenvironment by recruiting proinflammatory M2 macrophages[8,10]

- Epithelial cells in the cancerous region show more open 3D genome conformation than immune cells
- Cancerous epithelial cells show unique translocations that are absent in immune cells

Cancerous epithelial cells show unique inter-chromosomal interactions that are absent in CD163+ immune cells suggesting cell type specific SVs in tumor microenvironment

PaintScape multiomics and GeoMx WTA correlated chromosomal aberration with cancer cell proliferation

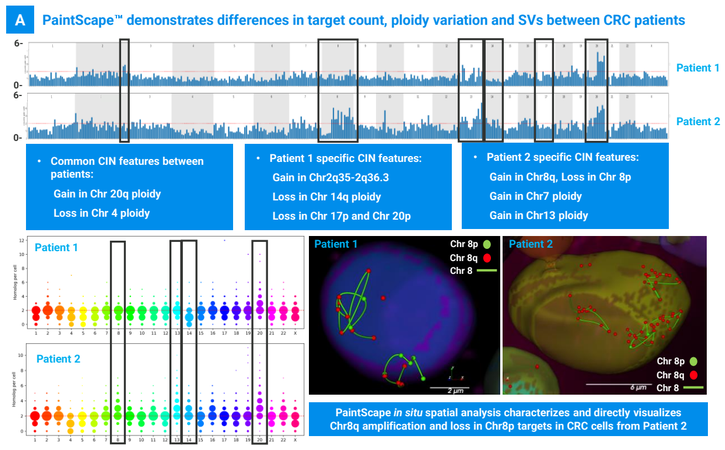


PaintScape multiomics demonstrated higher Ki-67 positivity among cancerous epithelial cells in Patient 2 having Chr8q gain causing higher copies of MYC oncogene, known to cause higher proliferation of cancer cells[11]

- In Pan-CK+ cancerous epithelial cells, GeoMx WTA data shows down-regulation of Chr8p genes and up-regulation of Chr8q genes including MYC oncogene in Patient 2 consistent with PaintScape *in situ* 3D genomics data
- Combination of PaintScape and GeoMx data reveals patient specific dysregulation in gene expression fueled by patient specific chromosomal instability

PaintScape characterizes chromosomal instability (CIN) of cancerous epithelial cells in CRC tissue from different patients

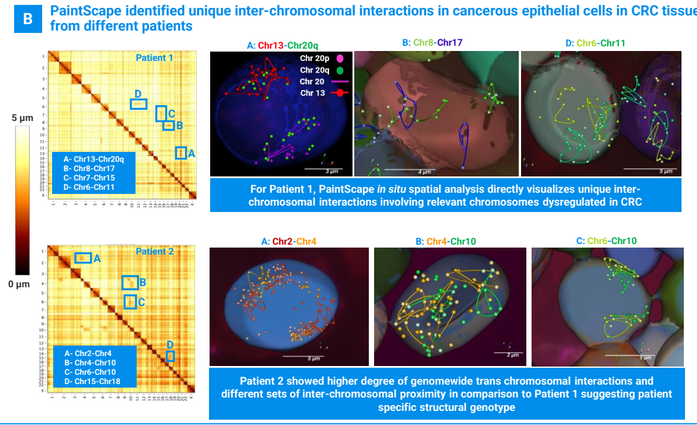
A PaintScape™ demonstrates differences in target count, ploidy variation and SVs between CRC patients



- Common CIN features between patients:
  - Gain in Chr 20q ploidy
  - Loss in Chr 4 ploidy
- Patient 1 specific CIN features:
  - Gain in Chr2q35-2q36.3
  - Loss in Chr 14q ploidy
  - Loss in Chr 17p and Chr 20p
- Patient 2 specific CIN features:
  - Gain in Chr8q, Loss in Chr 8p
  - Gain in Chr7 ploidy
  - Gain in Chr13 ploidy

PaintScape *in situ* spatial analysis characterizes and directly visualizes Chr8q amplification and loss in Chr8p targets in CRC cells from Patient 2

B PaintScape identified unique inter-chromosomal interactions in cancerous epithelial cells in CRC tissue from different patients



For Patient 1, PaintScape *in situ* spatial analysis directly visualizes unique inter-chromosomal interactions involving relevant chromosomes dysregulated in CRC

Patient 2 showed higher degree of genomewide trans chromosomal interactions and different sets of inter-chromosomal proximity in comparison to Patient 1 suggesting patient specific structural genotype

Conclusions

PaintScape™ enables identification, characterization and direct *in situ* visualization of single cell 3D genome features of different cell types in FF Colorectal Cancer tissue across different patients. Distinct structural genotypes are revealed:

- **Copy number variation including amplification and deletions *in situ* in single cells and sub-populations**
  - Identified cancer vs normal cell sub-populations with
    - gain in Chr 8q, Chr 13q and Chr 20q in cancer cells
    - loss in Chr 8p, Chr 14q, Chr 17p and Chr 20p in cancer cells
  - These CIN patterns are suggestive of more aggressive phenotype of CRC with metastatic potential
- ***In situ* single cell ploidy variation of whole chromosomes and sub-chromosomal regions**
  - Higher variation in sub-population of cells with variable chromosome ploidy in cancer cells compared to normal cells
  - Chr8, Ch13q and Chr20 show much higher chromosome ploidy in cancer cell sub-populations
  - LOH of Chr14q and Chr4 in cancer cell sub-populations
- **Inter-chromosomal interactions, potential translocations and simultaneous proximity of multi-loci interactions *in situ* in single cells**
  - For patient 1: Chr13q-Chr20q, Chr8-Chr17, Chr7-Chr15 and Chr6-Chr11interactions
    - Chr13q-Chr20q translocation in cancer cells brings relevant CRC related oncogenes (e.g. CDK8 on 13q, TPD52L2 on 20q) to close proximity causing dysregulated gene expression
  - For patient 2: Chr2-Chr4, Chr4-Chr10, Chr6-Chr10 and Chr15-Chr18 interactions.
- **ecDNA copy number, nuclear locations and *in situ* interactions in single cells**
  - Unique cancer cell sub-population having a fraction of amplified targets of Chr20q and Chr8q regions further from Chr20 or Chr8 territory, suggesting possible ecDNA nature of those amplified targets
    - Amplified Chr20q targets interact genomewide with all other targets with specific short-distance interaction with Chr13q
- **Cell type specific differences in 3D genome structure *in situ* in single cells in tissue immune microenvironment**
  - Identified and visualized unique 3D genome aberration of individual chromosomes in Pan-CK+ epithelial cells and CD45+CD163+ immune cells
  - Immune cells show relatively more compact 3D genome structure than cancerous epithelial cells
  - Cancer cells show sub-populations with unique sets of inter-chromosomal interactions that are absent in immune cells
- **Multiomic correlations of cancer cell states between patients**
  - Patient 2 with Chr8q gain shows much higher Ki-67 positivity rate for cancerous epithelial cell sub-populations compared to Patient 1 lacking Chr8q gain
  - Chr8q gain increases prevalence of key oncogene MYC which upregulates Ki-67 leading to higher proliferation and greater genome wide instability

PaintScape™ enables *in situ* direct visualization of 3D genome in FF Colorectal Cancer tissue in spatially resolved tumor microenvironments

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