A Versatile, Automated Assay For Same-Section Spatial Visualization Of Point Mutation, miRNA, mRNA, Protein and Protein-Protein Interactions to Understand Nonclinical Biodistribution (BD) and Persistence of Gene Therapy (GT) Products

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Introduction

Non-clinical biodistribution studies are increasingly sought by the FDA "to understand the *in vivo* distribution, persistence and clearance of gene therapy products at the site of administration and in target and non-target tissues". We report a one-of-kind spatial multiomics assay that integrates the visualization of miRNAs/ASOs/siRNAs, exonic regions, point mutations, mRNAs, proteins, and protein-protein interactions in the same section using a tyramide signal amplification (TSA)-based protease-free chemistry. To generate proof of concept, we used a modified RNAscope chemistry for robust detection sensitivity of exon junctions and point mutations to integrate findings from multiple studies that detail the malignant cancer progression mechanisms in *BRAF V600E* mutant cell lines and colon cancer tissues:

1. Increased mitogen activated protein kinase (MAPK) signaling in the presence of *BRAF* V600E mutant[1]
2. Increased MAPK signaling leads to increased *miR21* and decreased vascular endothelial growth factor (VEGF)[2]
3. *BRAF* V600E mutation leads to an increase in integrin heterodimerization, which leads to decrease in VEGF expression and increase in MAPK signaling[3,4,5]

The assay offers a powerful technique for multiomic analysis and accurate interrogation of complex tissues to obtain insights into novel biomarkers and therapeutic targets. In addition, the method can contextualize the biodistribution of CGTs with potential protein markers for cell types and further interrogate cell-cell/protein-protein interactions.

Method

Figure 1. Multiomic RNAscope Fluorescent Assay workflow. FFPE sections or cell pellets were first subjected to RNAscope pretreatment, and then oligo-conjugated antibodies were applied, followed by miRNA and RNA-specific probes for hybridization to target RNAs. After signal generation complexes were built, the signal was detected using TSA in a protease-free workflow. Single RNA transcripts for each target gene appear as punctate dots and specific antibody staining are visible using a fluorescent microscope or scanner.

Results

1. Modified BaseScope fluorescent assay using previously characterized melanoma cell lines and *BRAF* V600E point mutation probe shows signal in *BRAF* V600E in SK-MEL-28 and no signal in CHL-1.

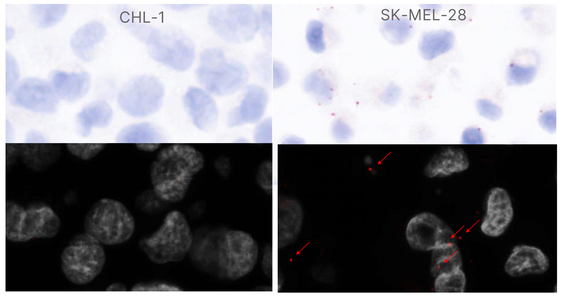

Figure 2. BaseScope assay using *BRAF* V600E mutation specific probes indicating *BRAF* V600E positive cells. Comparable signal in observed using new BaseScope fluorescent assay indicating *BRAF* V600E mutation in SK-MEL-28 cell pellet vs no signal in CHL-1 cell pellet.

2. Chromogenic and fluorescent assays to assess *VEGF* mRNA and protein proximity in melanoma cells with *BRAF* V600E mutant. Increased integrin heterodimerization coincided with decreased *VEGF* mRNA.

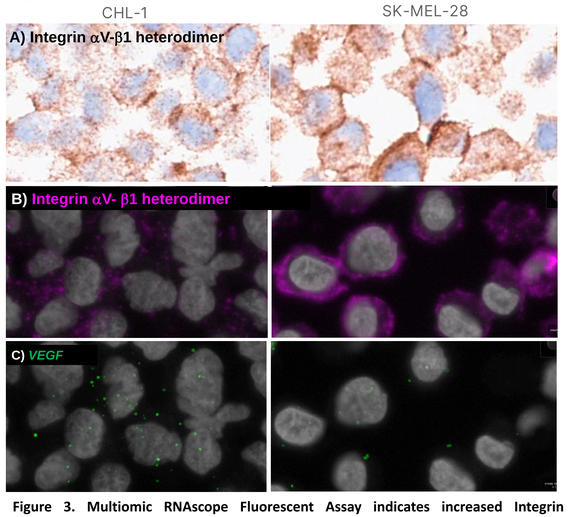

Figure 3. Multiomic RNAscope Fluorescent Assay indicates increased Integrin heterodimerization in *BRAF* V600E mutant cells and lower level of *VEGF* mRNA as compared to CHL-1 cells. A) Chromogenic assay indicating integrin αV-β1 heterodimerization B) Fluorescent multiomic assay indicating integrin αV-β1 heterodimerization C) Lower level of *VEGF* mRNA in *BRAF* V600E mutant cells.

3. BaseScope *BRAF* V600E mutant signal in colon tumor tissues

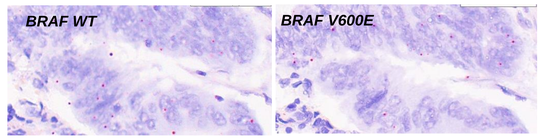

Figure 4. Commercial BaseScope assay using *BRAF* V600 WT and *BRAF* V600E probes indicating *BRAF* V600E positive tissue. Human colon cancer tissue stained using chromogenic BaseScope assay indicating BRAF V600E mutation.

4. Interaction between miR-21, BaseScope : *BRAF* V600E, mRNA: *VEGF and MAP2K1,* and Integrin in cancer tissue

1. *BRAF V600E* mutation increases MAPK pathway, which is associated with increase in miR-21 expression and leads to malignant proliferation.
2. Decrease in *VEGF* expression has been observed in *BRAF* V600E mutants.
3. Increase in integrin heterodimerization observed in *BRAF* V600E mutants which leads to decrease in *VEGF* expression.

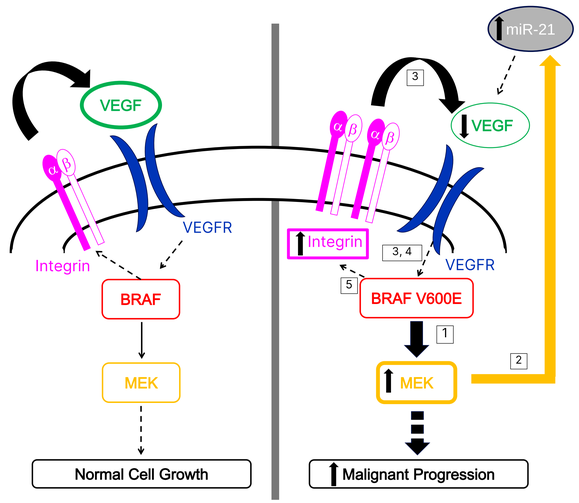

Figure 5. *BRAF* V600E mutation is associated with changes in levels of miRNA, mRNA and proteins. *BRAF* V600E mutation is known to increase miR-21, integrin heterodimerization and MAP kinase pathway downstream while decreasing VEGF. Bold numbers in squares refer to the respective references, solid arrows and dashed arrows indicate direct and multi-step interactions, respectively

Conclusions

- The same-section, 6-plex assay uses newly improved RNAscope chemistry with robust sensitivity of detection for exon junctions and point mutations while retaining the sensitivity and specificity for mRNA and small RNA targets.
- Here we present a proof-of-concept study to assess the impact of *BRAF* V600E mutation on miRNA, mRNA and protein targets in the MAPK signaling pathway.
- The assay offers a powerful and flexible technique to conduct non-clinical biodistribution studies using multiomic readouts.

5. Multiomic co-detection of miRNA: miR-21, BaseScope: *BRAF* V600E point mutation, mRNA: *VEGF and MAP2K1,* protein: PanCK and protein proximity assay: Integrin αV−β1 heterodimer in human colon tumor tissue using Multiomic RNAscope assay.

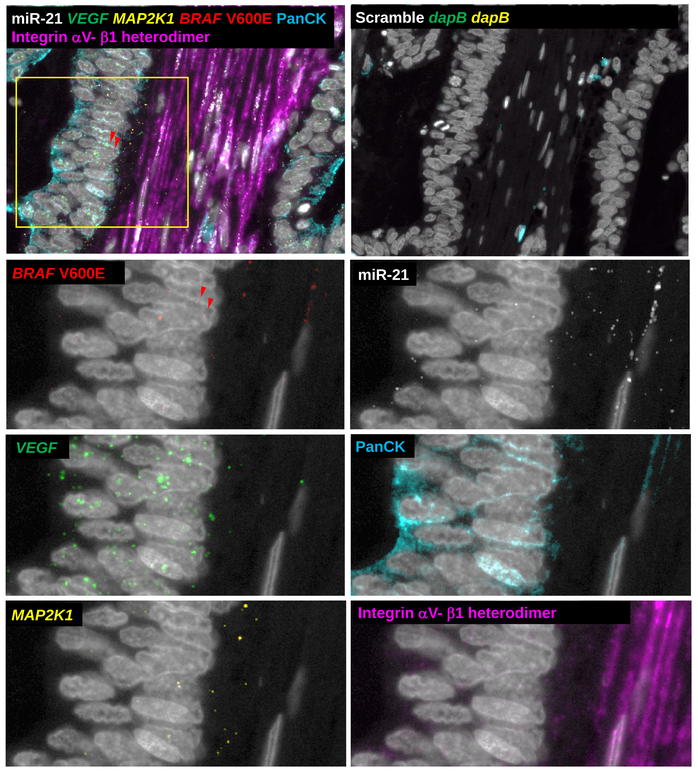

Figure 6. Expression of *BRAF* V600E mutant and associated tumor target genes in colon cancer tissue. Modified multiomic RNAscope assay utilized to detect point mutation, mRNA, miRNA, protein and protein-protein proximity in same section to validate a complex biological pathway for cancer progression. Nuclei were counterstained with DAPI.

References

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5. Mautone et al., *Cancers (Basel)*, 13, 12, 2937, 2021.