RNA/Protein Multiomics Imaging Using RNAscope Technologies to Understand Nonclinical Biodistribution (BD) and Persistence of Gene Therapy (GT) Products

Sonali A. Deshpande, Anushka Dikshit, Manvir Sambhi, Li-Chong Wang, **Maithreyan Srinivasan**
7707 Gateway Blvd, Newark, CA

Introduction

With over ten approved gene therapies (GT) and over 1500 ongoing GT clinical trials, the FDA has issued recommendations to generate nonclinical biodistribution (BD) data for "the evaluation and interpretation of nonclinical pharmacology and toxicology findings prior to initiation of human clinical trials". While several technologies to assess BD exist, RNA *in situ* hybridization is increasingly used to spatially visualize the delivered therapeutic, target gene, transgene and/or cell markers and complement information gathered from molecular technologies such as quantitative polymerase chain reaction (qPCR). Recently, RNAscope technologies have been widely used to co-detect the presence of vector and transgene expression as well as ASO/siRNAs and their intended mRNA targets using Tyramide Signal Amplification (TSA)-based fluorescent readouts on fixed, fresh frozen and formalin fixed paraffin embedded (FFPE) tissues, supported by manual and automated workflows using the Leica Bond Rx system. To enhance the information gained from these technologies and to satisfy FDA recommendations in understanding BD in the context of on and off-target cell types/tissues, we have developed a novel method that enables the detection of protein targets along with miRNAs/ASOs/siRNAs and mRNAs. We have leveraged this technology to investigate the spatial expression profile of miRNA and associated RNA targets across different tissue type and contextualize the RNA information to specific cell types using antibodies to visualize cell-type specific markers. This assay was used to demonstrate expression of miRNAs and target genes implicated in tumor initiation, progression, and angiogenesis. Expression of miR-205, associated tumor target genes such as *PTEN* and tumor suppressor *TP53* was visualized in head and neck cancers. Downregulation of tumor-suppressor, *TP53* resulted in up-regulation of miR-205, which has been shown to down-regulate *PTEN* expression (PMID: 32854238). PanCK antibodies stained the tumor region in the tissue. This novel platform will enable researchers to simultaneously visualize regulatory RNA with target RNAs, protein, cell type and morphology markers in intact cells/tissues with single cell resolution. This technology can provide meaningful insights into disease pathology driven by miRNAs as well as assess biodistribution and efficacy of oligonucleotide therapeutics.

Method

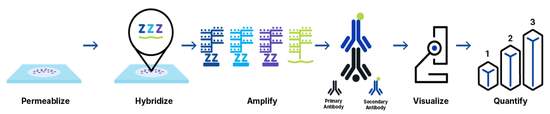

Figure 1. RNAscope Plus smRNA-RNA-Protein Fluorescent Assay workflow. FFPE sections were first subjected to pretreatment and then smRNA and RNA-specific probes were hybridized to target RNAs. After signal generation complexes were built, the signal was detected using TSA. Protein targets were detected using sequential immunofluorescence. Single RNA transcripts for each target gene appear as punctate dots that are visible using a fluorescent microscope.

1. Multiomic co-detection of small RNAs, mRNAs and protein in cervical cancer and Head/Neck cancer tissues

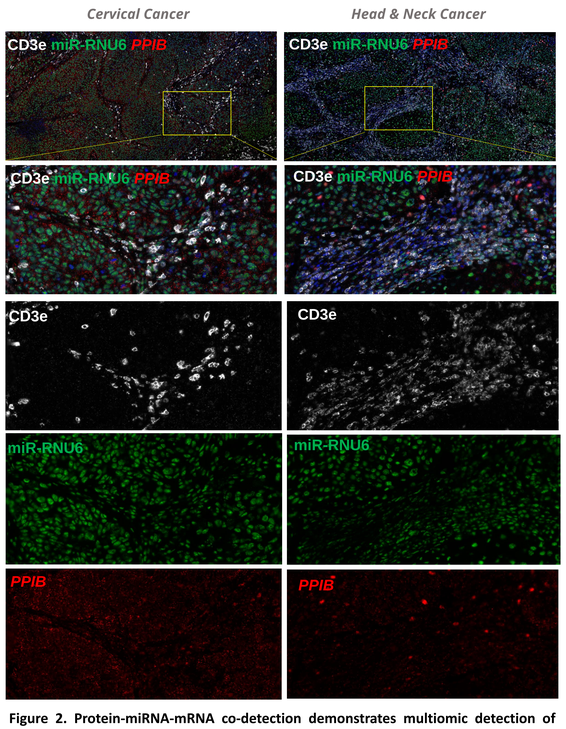

Figure 2. Protein-miRNA-mRNA co-detection demonstrates multiomic detection of smRNA and mRNA targets in cancer tissues. Immunofluorescence staining of CD3e antibody along with miR-RNU6 and *PPIB* positive control in cervical cancer and Head/Neck cancer tissues.

2. RNAscope Plus smRNA-RNA assay shows co-detection of one miRNA (miR-205) and 3 mRNA (*TP53, PTEN* and *PanCK*) in Head/Neck cancer tissue

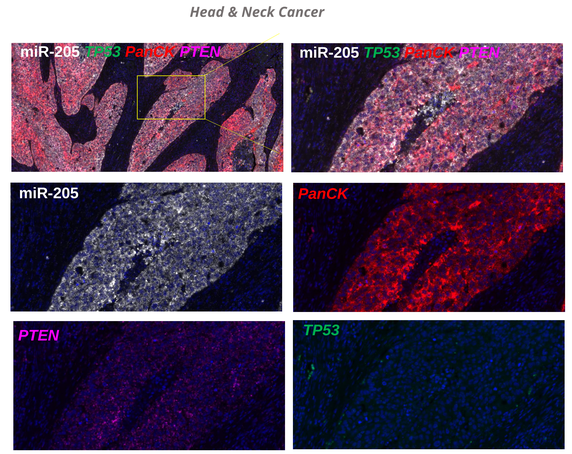

Figure 3. Expression of miR-205 and associated tumor target genes in head/neck cancer tissue 4-plex smRNA-RNA assay using 1 miRNA (miR-205) and 3 mRNAs targets (*PanCK, PTEN and TP53*) in tumor. miRNA-205 was widely expressed in cancer cells across the tissue. Low expression of tumor-suppressor, TP53 corresponds to upregulation of miR-205, which downregulates PTEN expression. Nuclei were counterstained with DAPI.

Conclusions

- Simultaneous detection of different target types including 1 smRNA target (ASO/siRNA), up to 3 mRNA targets and protein using immunofluorescence enables biodistribution studies to include mRNA and/or protein as cell-type markers.
- Expression of miRNAs and target genes/proteins implicated in tumor initiation, progression, and angiogenesis was demonstrated in FFPE cancer tissues.
- Meaningful insights into disease pathology driven by miRNAs as well as biodistribution and efficacy of oligonucleotide therapeutics can be evaluated using this novel multiomic assay.

3. Multiomic co-detection of miRNA: miR-205, mRNA: *TP53 and PTEN* and protein: PanCK in cervical cancer and Head/Neck cancer tissue using an improved RNAscope Plus smRNA-RNA assay and sequential immunofluorescence. PanCK protein staining pattern in Head/Neck cancer is observed in the same region as *PanCK* mRNA detection shown in Figure 3.

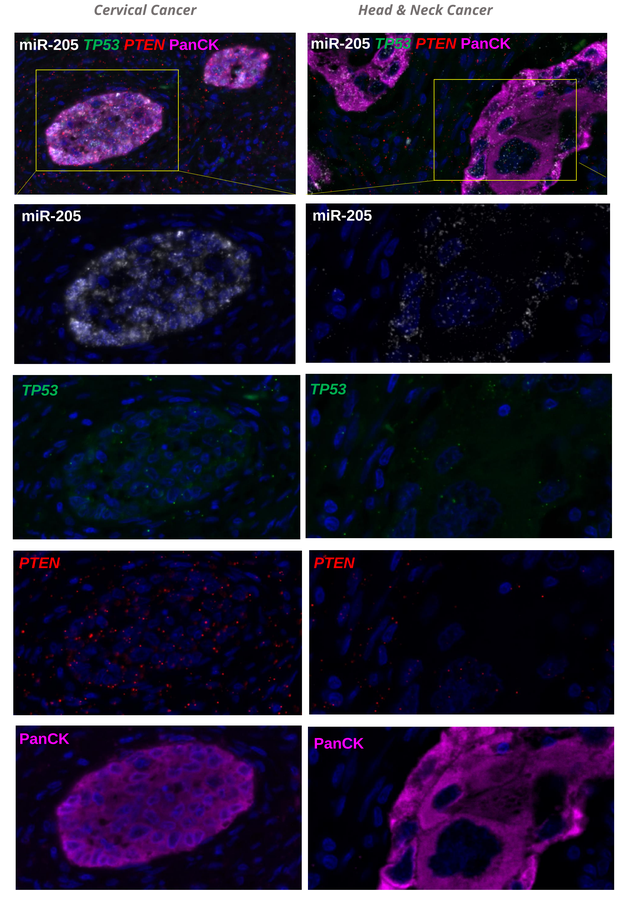

Figure 4. Expression of miR-205 and associated tumor target genes in head and neck cancer tissue 3-plex smRNA-RNA assay using 1 miRNA (miR-205), 2 mRNAs targets (*PTEN and TP53*) and 1 protein (PanCK) using IF in tumor. miRNA-205 was expressed in cancer cells across the tissue. Nuclei were counterstained with DAPI.