Molecularly guided highly multiplexed digital spatial analysis reveals differential gene expression profiles in the WNT-β-catenin pathway between melanoma and prostate tumors

Anushka Dikshit[1], Daniel Zollinger[2], Chris Merritt[2], Karen Nguyen[2], Jill McKay-Fleisch[2], Courtney Anderson[1] and Xiao-Jun Ma[1]

[1]Advanced Cell Diagnostics, a Bio-Techne brand, Newark, CA, USA , [2]NanoString Technologies, Seattle, WA, USA

#2707

Introduction

The canonical WNT-β-catenin signaling pathway is vital for development and tissue homeostasis but becomes strongly tumorigenic when dysregulated. This pathway can alter the transcriptional signature of a cell to promote malignant transformation, growth and metastasis. However, thorough characterization of these transcriptomic signatures has been challenging because traditional methods lack either spatial information, multiplexing, or sensitivity/specificity. To overcome these challenges, we developed a novel workflow combining the single molecule and single cell visualization capabilities of the RNAscope *in situ* hybridization (ISH) assay with the highly multiplexed spatial profiling capabilities of the GeoMx® Digital Spatial Profiler (DSP) RNA assays. Using these methods, we sought to spatially profile and compare gene expression signatures of tumor niches with high and low *CTNNB1* expression.

Experimental design

❖ After examining the expression of *CTNNB1* and *WNT7B* in 120 core multi-tumor TMA using the **RNAscope Duplex assay** we determined that melanoma tumors had significantly higher expression of *CTNNB1* compared to other tumors. Interestingly, prostate tumors had the weakest *CTNNB1* expression. High *CTNNB1* expression was also associated with moderate-high *WNT7B* expression.

❖ **Objective:** To spatially profile and compare gene expression signatures of tumor niches with high and low *CTNNB1* expression.

❖ **Samples used:** 3 Melanoma FFPE tissues (Mel23, Mel27, Mel33)
3 Prostate cancer FFPE tissues (Pros01, Pros02, Pros05)

❖ **Joint Workflow:**
Probe hybridization for both RNAscope and DSP GeoMx was performed on the Leica automated stainer. ROI selection and profiling was performed on the NanoString GeoMx DSP and the DSP quantification was performed using the nCounter.

- **ROI selection:** *CTNNB1* and *WNT7B* expression detected using RNAscope Multiplex Fluorescent Assay V2.
- **GeoMx DSP analysis:** High and low CTNNB1 expressing ROIs were profiled using the 78 oncology target gene panel
- **Confirmation:** Targets were further interrogated and visualized with single cell resolution using the RNAscope assay.

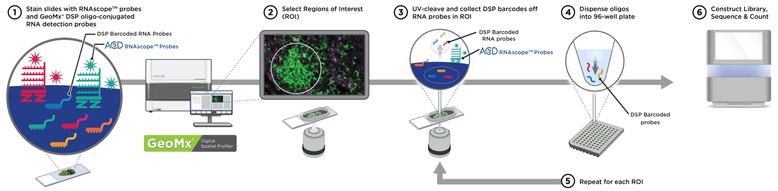


Figure 1: Step-wise depiction of the RNAscope-GeoMx DSP workflow for spatial gene expression analysis.

Results

RNAscope analysis indicated significantly increased expression of *CTNNB1* in melanoma tumors compared to prostate cancer tumors.

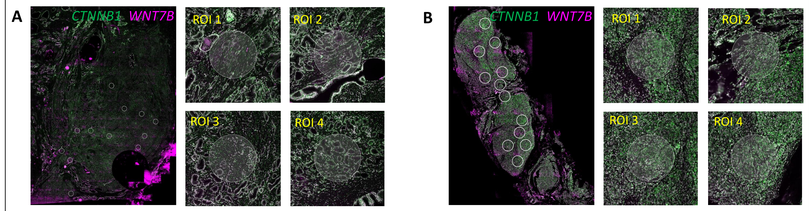


Figure 2: RNAscope ISH as a molecular guide for GeoMx DSP to identify *CTNNB1*-rich and -poor regions in tumors. *CTNNB1* and *WNT7B* mRNA expression was visualized using the RNAscope Multiplex Fluorescent V2 assay. **A**, prostate tumor showed moderate-low expression for both *CTNNB1* and *WNT7B*, while, **B** melanoma tumor demonstrated very high expression for *CTNNB1* and moderate expression for *WNT7B*.

GeoMx Digital Spatial Profiling of genes implicated in cancer indicated a strong correlation between *CTNNB1* and targets related to tumor growth and immune evasion

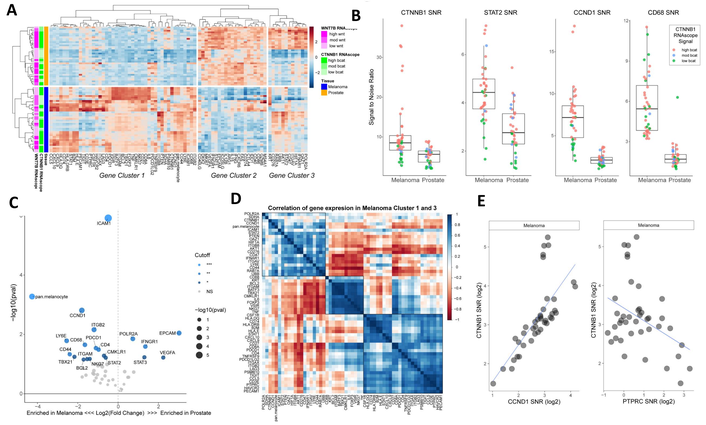


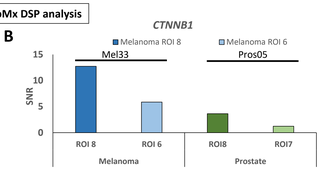
Figure 3. Gene expression analysis by GeoMx DSP on CTNNB1-high and CTNNB1-low expressing ROIs in melanoma and prostate tumors. **A**, Unsupervised hierarchical clustering of all GeoMx data showed ROIs from melanoma and prostate samples cluster separately. Gene cluster 1 consisted primarily of targets expressed higher in melanoma tumors. Gene cluster 2 consisted primarily of genes expressed higher in prostate, while Gene cluster 3 consisted of genes shared between prostate and melanoma. **B**, Quantitation of signal to noise ratios showed *CTNNB1*, *STAT2*, *CNND1*, and *CD68* expressed at a higher level in melanoma samples. **C**, A linear mixed effects model controlling for individual samples showed significant differential expression of many genes between prostate and melanoma samples, including *CTNNB1*, *STAT2*, *CNND1*, and *CD68*. **D**, A heatmap of correlation coefficients between each gene in cluster 1 and 3 in (3A) ordered by hierarchical clustering showed three distinct groups of genes with *CTNNB1* showing strong correlation with *CCND1*, *ICAM1*, and *STAT2* among other targets. The strongest inverse correlations were with immune cell (*PTPRC*) and T-cells (*CD3E* and *CD4*) markers illustrating the role for *CTNNB1* in immune suppression. **E**, SNR ratios for *CTNNB1* and *CCND1* illustrate a significant positive correlation (left; R= 0.71) with a non-significant negative correlation with *PTPRC* (right; R= -0.40).

Targets with strong correlation to *CTNNB1* expression were significantly upregulated in melanoma tissues compared to prostate cancer

A

SNR from GeoMx DSP analysis

GENES	MELANOMA		PROSTATE	
	High CTNNB1 ROI 8	Low CTNNB1 ROI 6	High CTNNB1 ROI 8	Low CTNNB1 ROI 7
STAT2	7.019	3.15	1.81	0.69
CD68	11.54	4.26	1.45	1.67
CCND1	8.54	4	2.15	2.67
CTNNB1	12.73	5.87	3.64	1.23



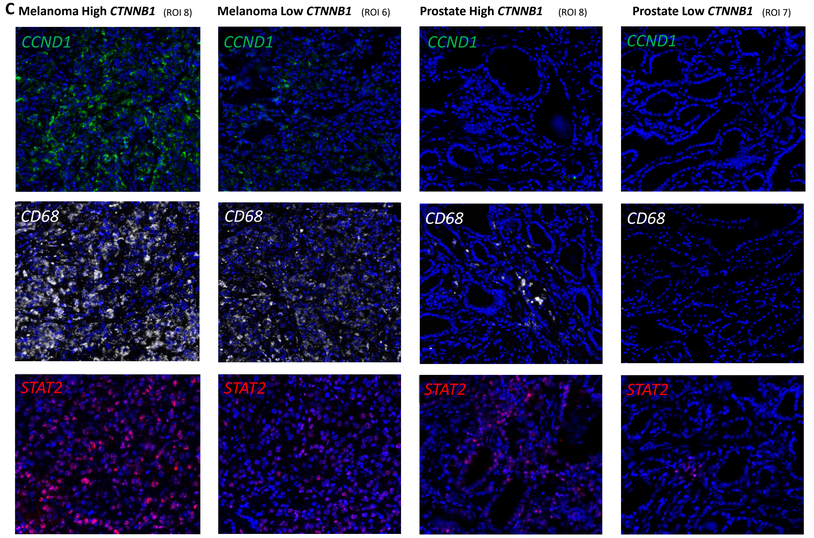


Figure 4: Validation revealed that expression of target genes changed proportionally to *CTNNB1* expression in tumors niches **A**, Signal to noise ratio (SNR) values for CTNNB1 and other target genes with GeoMX DSP nCounter analysis. **B**, Graph depicts differences in *CTNNB1* SNR expression in relatively high and low ROIs of melanoma and prostate cancer tissues. **C**, Validation of GeoMx DSP analysis using RNAscope Multiplex Fluorescent v2 assay showed significantly higher expression of *STAT2*, *CCND1* and *CD68* in the melanoma tumor compared to prostate cancer tissue indicating a direct correlation to *CTNNB1* expression levels.

Summary

- Combining the RNAscope ISH assay and the GeoMx DSP RNA assay into one joint workflow allowed transcriptional profiling of regions with high and low *CTNNB1* expression within melanoma and prostate tumors and helped identify genes potentially regulated by the WNT- β-catenin pathway.
- This novel workflow can be fully automated and is well suited for interrogating the tumor , stroma and their interactions.

Contact: anushka.dikshit@bio-techne.com