Spatially Resolving RNA Biomarkers using GeoMx™ Digital Spatial Profiler for Early Diagnosis and Prognosis of Melanoma

Summary

Our study provides an experimental framework for identifying early biomarkers of melanoma development and immune predictors of treatment response.

Rationale

Diagnosis of melanoma is currently based on histological examination. However, in some cases melanomas are difficult to differentiate from common, benign nevi (mole). Therefore, a diagnostic tool needed to facilitate early diagnosis and accurate prognosis of melanomas.

Experimental Design

Patient-derived formalin-fixed, paraffin-embedded (FFPE) tissue sections from four case classes were evaluated: common melanocytic nevic, dysplastic melanocytic nevic, melanoma, and melanoma. Using the Digital Spatial Profiler™, a high-resolution scanner, we simultaneously acquired 1,412 immune-oncology-related gene expression profiles within discrete 200µm diameter circular regions of interest (ROIs) in each tissue section. ROIs were chosen based on enrichment for melanocytes, inflammatory infiltrates, or keratinocytes, thus facilitating direct comparison across both cell types and case types.

Key Results

- High reproducibility between replications (R² = 0.96 for DSP replicates, R² = 0.98 for sequencing replicates)
- High-purity when probing for 1,412 genes simultaneously, on average 275 genes per ROI (range 60-775) were above the limit of detection (LOD) in melanoma tissues.
- ROIs clustered strongly by dominant cell type and secondarily by case type, providing molecular support for pathological case classifications.
- Differential expression analysis identified known biomarkers of melanoma, including loss of BAP1 and up-regulation of PTEN, as well as case- and ROI-type-specific expression differences in melanogenesis-associated genes and other immune-oncology-related genes.

Results

Phase I experiment with one patient per case class (6-16 ROIs per patient, 108-gene subset of probe panel) demonstrated excellent correlation between serial sections (DSP replicates performed on different days; each ROI = gene in one ROI) and technical replicates (duplicate library prep & sequencing from same DSP run; each ROI = one probe in one ROI).

Differential Expression Analysis

A) Volcano plots comparing gene expression in ROIs from melanoma cases vs. common nevus cases. Counts from all ROIs of each type (epidermis, immune-rich or melanocyte-rich) were averaged. Horizontal black line represents p = 0.05. B) Boxplots showing expression of selected genes in different ROI types from each case class. Counts were housekeeper-normalized in both panels and filtered to >LOD in panel A only.

ROI Clustering

Phase II ROIs cluster primarily by dominant cell type (top color bar) and secondarily by case class (bottom color bar). Only genes with counts greater than the LOD were included in the analysis and counts were normalized to the geometric mean of 12 housekeeper genes included in the panel. Clustering was performed in R using the Pearson distance measure & complete linkage clustering.

Strong correlation of Phase I & Phase II data for the 108 genes present in both panels.

Sensitivity & Scality

- Average # of genes/ROI (LOD)
- Phases I & II: 1 patient per case type, 108-gene RNA panel. Phase II: 3 patients per case type, 1,412-gene RNA panel. LOD = mean + 2 standard deviations of the 80 negative probes

References

1. University of California (Davis) School of Medicine, Department of Dermatology & Pathology and Laboratory Medicine
2. Nanostri technologies, Inc.
3. University of California (Davis) School of Medicine, Department of Biochemistry & Molecular Medicine

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