**INTRODUCTION**

- Melanoma, the deadliest of the common skin cancers, develops through a gradual accumulation of mutations and overcomes environmental regulation
- Markers of early melanoma evolution and predictors of durable treatment response remain largely undiscovered
- Spatially resolved techniques are likely to outperform bulk molecular profiling for discovery of early stage and predictive biomarkers
- Previous studies revealed the importance of keratinocyte-derived growth factors and cell adhesion molecules in limiting melanocyte proliferation and elucidated mechanisms by which malignant melanocytes escape this regulation
- However, prior studies did not capture the spatial element of melanocyte-keratinocyte interactions in situ in patient-derived primary melanomas and benign melanocytic tumors

**AIM**

- To better elucidate tumor-microenvironment interactions during melanoma evolution using spatial transcript profiling
- To validate potential biomarker by immunohistochemistry (IHC)

**MATERIALS AND METHODS**

- Expression of over 1,000 genes in 134 regions of interest (ROIs) in patient-derived formalin-fixed, paraffin-embedded (FFPE) tissue sections of benign and malignant melanocytic tumors were examined
- NanoString GeoMx® Digital Spatial Profiler (DSP) was used to profile 200µm circular ROIs enriched for melanocytes, or neighboring keratinocytes or immune cells
- S100A8 and S100A9 expression was analyzed by IHC

**RESULTS**

- Pairwise correlation coefficients revealed that cell type and tumor type both affect similarity between ROIs
- Linear regression identified genes that were significantly enriched in melanocyte-rich and immune-rich ROIs
- S100A8 expression was enriched in the keratinocyte-rich ROIs of melanoma in situ
- Binary logistic regression model showed increased S100A8 IHC score significantly associated with invasive melanoma tumor type (OR=2.49, 95%CI 1.93-3.21), and it remained significant after adjusting for sex, anatomic site, and age (OR=2.54, 95%CI 1.92-3.36) (Figure 1; Table 1)

**Figure 1:** S100A8 is detected in the keratinocyte microenvironment of melanoma

**Table 1:** Patient characteristics and S100A8 expression in a cohort of 252 tumors.

<table>
<thead>
<tr>
<th>Score 0 (4%)</th>
<th>Score 1 (0-45%)</th>
<th>Score 2 (5-25%)</th>
<th>Score 3 (26-50%)</th>
<th>Score 4 (51-75%)</th>
<th>Score 5 (&gt;75%)</th>
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<tr>
<td>N (%)</td>
<td>N (%)</td>
<td>N (%)</td>
<td>N (%)</td>
<td>N (%)</td>
<td>N (%)</td>
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<tr>
<td>Total 68</td>
<td>66</td>
<td>69</td>
<td>49</td>
<td>252</td>
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<td>Location</td>
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<td></td>
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<tr>
<td>Face</td>
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<td>1 (1.5)</td>
<td>10 (14.5)</td>
<td>7 (14.3)</td>
<td>23 (31.0)</td>
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<tr>
<td>Scalp/neck</td>
<td>39 (57.4)</td>
<td>29 (47.0)</td>
<td>22 (31.9)</td>
<td>12 (24.5)</td>
<td>122 (48.4)</td>
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<tr>
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<tr>
<td>Extremity</td>
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<td>11 (16.7)</td>
<td>24 (34.8)</td>
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<tr>
<td>Extremity</td>
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<td>7 (10.1)</td>
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<td>0 (0.0)</td>
<td>1 (1.4)</td>
<td>0 (0.0)</td>
<td>1 (0.4)</td>
</tr>
</tbody>
</table>

**CONCLUSIONS**

- Our results demonstrate a framework for high-throughput, spatial and cell type-specific resolution of gene expression in archival tissue of primary tumors
- The framework is applicable to the development of biomarkers during tumor evolution, including in the overlooked epidermal microenvironment of melanoma
- We discovered that the damage-associated molecular pattern (DAMP) S100A8, which is a known melanoma marker, thought to be expressed by immune cells, is keratinocyte-derived in melanoma
- Future DSP studies profiling a larger number of patients and ROIs are warranted to further resolve the interplay between keratinocytes and melanocytes during melanomagenesis.

**ACKNOWLEDGEMENTS**

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**REFERENCES**