High-plex spatial RNA profiling reveals cell type-specific biomarker expression in early-stage melanoma

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INTRODUCTION

- Melanoma, the deadliest of the common skin cancers, develops through a gradual accumulation of mutations and overcomes environmental regulation[1]
- 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[2]
- 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[1,3]
- 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)[5] 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

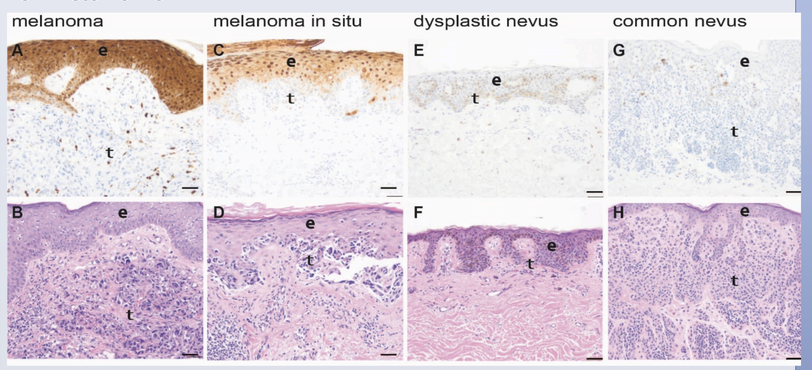

Table 1: Patient and tumor characteristics and S100A8 expression in a cohort of 252 tumors.

	Common nevus N (%)	Dysplastic nevus N (%)	Melanoma in situ N (%)	Invasive melanoma N (%)	Total N (%)
Total	68	66	69	49	252
Sex					
Male	28 (41.2)	35 (53.0)	39 (56.5)	33 (67.3)	135 (53.6)
Female	40 (58.8)	31 (47.0)	30 (43.5)	16 (32.7)	117 (46.4)
Average age (years)	44.1	52.8	62.5	62.2	55.0
Location of tumor					
Face	5 (7.4)	1 (1.5)	10 (14.5)	7 (14.3)	23 (9.1)
Scalp/neck	9 (13.2)	0 (0.0)	5 (7.2)	5 (10.2)	19 (7.5)
Trunk	39 (57.4)	29 (74.2)	22 (31.9)	12 (24.5)	122 (48.4)
Upper extremity	5 (7.4)	11 (16.7)	24 (34.8)	13 (26.5)	53 (21.0)
Lower extremity	10 (14.7)	5 (7.6)	7 (10.1)	12 (24.5)	34 (13.5)
Unknown	0 (0.0)	0 (0.0)	1 (1.4)	0 (0.0)	1 (0.4)
S100A8 IHC score					
Score 1 (0-4%)	51 (75.0)	48 (72.7)	21 (30.4)	3 (6.1)	123 (48.8)
Score 2 (5-25%)	15 (22.1)	13 (19.7)	13 (18.8)	7 (14.3)	48 (19.0)
Score 3 (26-50%)	1 (1.5)	2 (3.0)	8 (11.6)	10 (20.4)	21 (8.3)
Score 4 (51-75%)	0 (0.0)	2 (3.0)	19 (27.5)	12 (24.5)	33 (13.1)
Score 5 (>75%)	1 (1.5)	1 (1.5)	8 (11.6)	17 (34.7)	27 (10.7)

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[6], thought to be expressed by immune cells[7], 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.

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REFERENCES

1. Villanueva J, and Herlyn M. Melanoma and the tumor microenvironment. *Curr Oncol Rep*. 2008;10(5):439-46.

2. Finotello F, and Eduati F. Multi-Omics Profiling of the Tumor Microenvironment: Paving the Way to Precision Immuno-Oncology. *Frontiers in Oncology*. 2018;8(430).

3. Haass NK, Smalley KS, Li L, and Herlyn M. Adhesion, migration and communication in melanocytes and melanoma. *Pigment Cell Res*. 2005;18(3):150-9.

4. Passarelli A, Mannavola F, Stucci LS, Tucci M, and Silvestris F. Immune system and melanoma biology: a balance between immunosurveillance and immune escape. *Oncotarget*. 2017;8(62):106132-42.

5. Beechem JM. In: Thurin M, Cesano A, and Marincola FM eds. *Biomarkers for Immunotherapy of Cancer: Methods and Protocols*. New York, NY: Springer New York; 2020:563-83.

6. Clarke LE, Warf MB, Flake DD, 2nd, Hartman AR, Tahan S, Shea CR, et al. Clinical validation of a gene expression signature that differentiates benign nevi from malignant melanoma. *J Cutan Pathol*. 2015;42(4):244-52.

7. Wagner NB, Weide B, Gries M, Reith M, Tarnanidis K, Schuermans V, et al. Tumor microenvironment derived S100A8/A9 is a novel prognostic biomarker for advanced melanoma patients and during immunotherapy with anti-PD-1 antibodies. *J Immunother Cancer*. 2019;7(1):343.

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