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Human Immuno-Oncology Proteome Atlas enables a holistic proteomic approach for spatial biology discovery in FFPE tissues.

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Abstract

Background: The advancement of spatially resolved, multiplex proteomic and transcriptomic technologies has revolutionized and redefined the approaches to complex biological questions pertaining to tissue heterogeneity, tumor microenvironments, cellular interactions, cellular diversity, and therapeutic response. While spatial transcriptomics has traditionally led the way in plex, multiple studies have demonstrated a poor correlation between RNA expression and protein abundance, owing to transcriptional and translational regulation, target turnover, and most critically, post-translational protein modifications. Therefore, a more holistic proteomic atlas approach becomes critical to discovery biology. Previously, lack of successful detection of antibody-based probes well into the 100s served as a barrier to proteome-based interrogation of tissue while maintaining spatial context.

The GeoMx® Digital Spatial Profiler (DSP) platform is uniquely suited to support high-plex proteomics, allowing for the simultaneous analyses of proteins from discrete regions of interest (ROIs) in FFPE tissue sections while preserving spatial context. The assay relies upon abcam antibodies coupled to photocleavable DNA barcodes read out with NGS sequencing, allowing for theoretically unlimited plex. Here we present the Human GeoMx Immuno-Oncology Proteome Atlas (IPA), a >500-plex antibody-based proteomic discovery panel, compatible with immunohistochemistry on FFPE tissues with NGS readout. The panel content focuses on key areas of immuno-oncology, oncology, immunology, epigenetics, metabolism, cell death, and specific signaling pathway regulation.

Methods: We validated the specificity and sensitivity of the IPA on the GeoMx across >90 cell types and >50 human tissue types, normal and cancerous, representing FDA guidelines for antibody cross-reactivity testing. Using the validated IPA, we evaluated the proteomic landscape of various diseased colon tissue including adenocarcinoma, hyperplasia, and chronic inflammation (ulcerative colitis, Crohn's disease).

Results: Each of the individual antibodies in the IPA passed the specificity and sensitivity requirements which include exhibiting a maximum positive signal divided by the limit of detection, plus two standard deviations (SD) that is >5 in both cell pellet arrays and tissue microarrays; such a threshold gives a false positive rate of less than 10%. In addition, we compared the colonic diseased tissue to normal tissue and observed an upregulation of specific pathways associated with tumorigenesis and/or inflammation. Furthermore, we observed distinct differences in protein expression between several of the colonic diseases.

Conclusions: We demonstrate the power of the combination of the GeoMx DSP and curated Human GeoMx Immuno-Oncology Proteome Atlas to enable discovery biology by rapidly screening large numbers of tissues across critical potential therapeutic targets.

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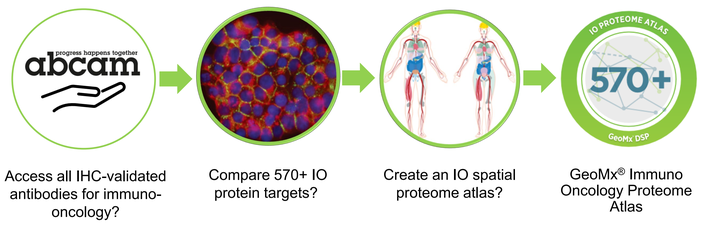
What if You Could…



Access all IHC-validated antibodies for immuno-oncology?

Compare 570+ IO protein targets?

Create an IO spatial proteome atlas?

GeoMx® Immuno Oncology Proteome Atlas

GeoMx Immuno Oncology Proteome Atlas Enables Detection of 100s of Proteins in Distinct Cell Populations

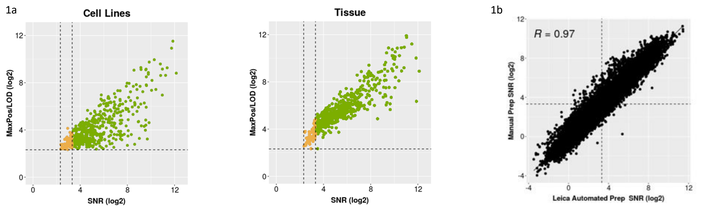


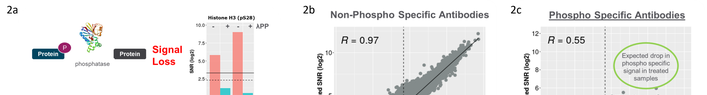
Figure 1: GeoMx Immuno Oncology Proteome Atlas (IPA) analytes demonstrate robust specificity and sensitivity and performs equivalently with either manual or automated sample prep. a) Target sensitivity and specificity performance measured across homogenous cell lines and cancer and normal tissues. **b)** GeoMx IPA analyte performance assessed on serial sections prepared with manual or automated (high throughput autostainer) workflows.

Designing an Immuno Oncology Proteome Atlas

Curated and validated IO Content

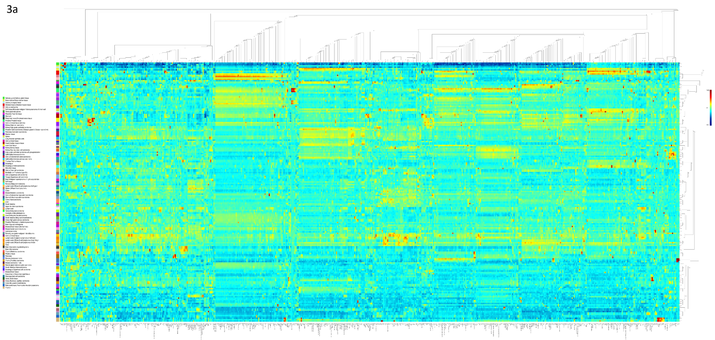
- Use Clinical Proteomic Tumor Analysis Consortium (CPTAC) data
- Focus on post-translational modifications
- Use high quality abcam antibodies
- Employ Immuno Oncology subject matter experts

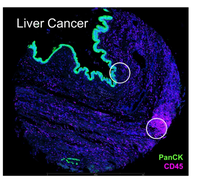
- Human Specific
- 570+ Proteins, Mapped to 556 Unique Genes
- 77 Functional Annotations
- All Hallmarks of Cancer



Post translational modifications often determine functionality, activity, or stability of a protein of interest.

Figure 2. Phospho specific antibodies demonstrate phospho specificity. a) Analyte performance assessed on serial sections treated with phosphatase or normal conditions. **b)** Non-phospho specific antibody signal to noise across phosphatase treated or normal conditions. **c)** Phospho specific antibody signal to noise across phosphatase treated or normal conditions.





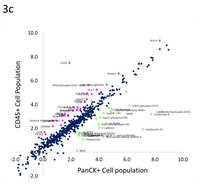
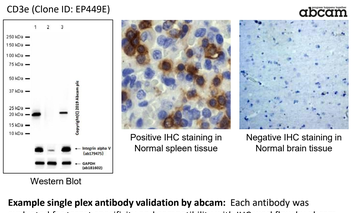


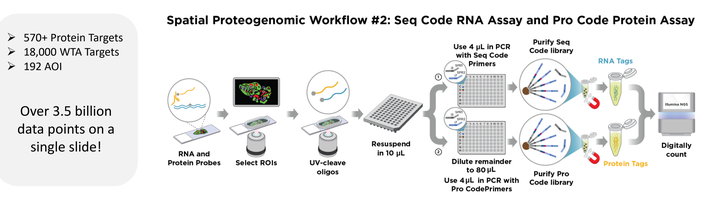
Figure 3. IPA enables detection of 100s of proteins in distinct cell populations. a) Unsupervised clustering of GeoMx Immuno Oncology Proteome Atlas 580 total targets to proteins of interest across 90+ normal and tumor tissues. **b)** Example liver cancer tissue was stained with additional morphology markers for CD45 (immune, magenta) and PanCK (tumor, green). **c)** Example proteome expression profile of 2 distinct regions of interest (ROI) on an individual liver cancer sample.

Single plex IHC validation by abcam

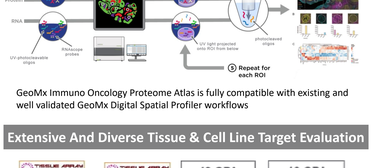


Example single plex antibody validation by abcam: Each antibody was evaluated for target specificity and compatibility with IHC workflow by abcam internal pathology and immunohistochemistry experts.

Spatial Proteogenomics with IPA: Novel GeoMx Pro Code Allows for Differential Amplification of Protein & RNA Analytes



GeoMx® Digital Spatial Profiler Workflow

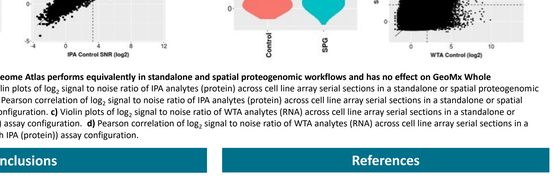


GeoMx Immuno Oncology Proteome Atlas is fully compatible with existing and well validated GeoMx Digital Spatial Profiler workflows

- 570+ Protein Targets
- 18,000 WTA Targets
- 192 AOI

Over 3.5 billion data points on a single slide!

Spatial Proteogenomic Workflow #2: Seq Code RNA Assay and Pro Code Protein Assay



Extensive And Diverse Tissue & Cell Line Target Evaluation

Multiplex validation of the GeoMx Immuno Oncology Proteome Atlas: Target specificity and sensitivity was measured on 90 different cell line CPA and FDA-approved screening arrays (TMA) with tumor and normal tissues.

Figure 4: GeoMx Immuno Oncology Proteome Atlas performs equivalently in standalone and spatial proteogenomic workflows and has no effect on GeoMx Whole Transcriptome Atlas performance. a) Violin plots of log_2 signal to noise ratio of IPA analytes (protein) across cell line array serial sections in a standalone or spatial proteogenomic (with WTA (RNA)) assay configuration. **b)** Pearson correlation of log_2 signal to noise ratio of IPA analytes (protein) across cell line array serial sections in a standalone or spatial proteogenomic (with WTA (RNA)) assay configuration. **c)** Violin plots of log_2 signal to noise ratio of WTA analytes (RNA) across cell line array serial sections in a standalone or spatial proteogenomic (with IPA (protein)) assay configuration. **d)** Pearson correlation of log_2 signal to noise ratio of WTA analytes (RNA) across cell line array serial sections in a standalone or spatial proteogenomic (with IPA (protein)) assay configuration.

Conclusions

- ✓ 570+ protein targets across cancer, metabolism, infectious disease, organ transplant, epigenetics, autoimmunity, and post-translational modifications
- ✓ Comprehensive coverage of the Hallmarks of Cancer, across 77 functional pathways
- ✓ High performance validated antibodies from the world's largest IHC-compatible antibody catalog - abcam
- ✓ Expanded plex of protein experiments allows for cost effective discovery of targets in disease tissue without additional workflow overhead.
- ✓ Spatial proteogenomics with Pro Code allows for differential amplification and analysis of Protein and RNA targets on a single slide with efficient sequence space usage
- ✓ Add 40 custom targets to further analyze targets of interest.

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GeoMx Immuno Oncology Proteome Atlas Public Dataset

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