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 immunoncology, 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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