Localizing and quantifying tumor microenvironment (TME) contexture of human glioma with GeoMx® high-plex RNA profiling

nanoString®
NanoString Technologies 530 Fairview Avenue North, Seattle, WA 98109

Summary

The GeoMx™ Digital Spatial Profiler (GeoMx® DSP) is a novel, highly multiplexed-assay platform that digitally and spatially characterizes protein and RNA expression within intact tissue sections. Here, we present a proof-of-concept study on profiling the tumor microenvironment (TME) of glioma subsets which are defined by isocitrate dehydrogenase 1 (IDH1) mutation status.

Quantitative Single Cell Deconvolution (qSCD) development: scRNA-Seq has been a powerful tool for identifying cell types, but because of the dissociation required it loses all spatial information. We developed quantitative Single Cell Deconvolution (qSCD) to identify which tumor tissues and subregions are enriched with tumor-specific cell types of interests when using the GeoMx® system.

- Using publicly available scRNA-seq data sets[1,2], we were able to identify 15 tumor-specific cell types signatures of interest for non-small-cell-lung cancers and 8 for human gliomas.
- We demonstrated that the gene expression data from the many cell types within tumor sub regions can be deconvoluted to calculate cell type scores, which were validated using cell-type specific protein markers.

Key results: Using GeoMx® DSP data of comparing wtIDH1 and mutIDH1 gliomas, we were able to recapitulate differential tumor biology of human glioma subsets which are from The Cancer Genome Atlas Program (TCGA) data set, in addition to subregional heterogeneity of each tissue which is due to TME heterogeneity.

- Besides known dysregulated tumor signaling activations, leading factors for the differences are extracellular matrix organization and presence of bone-marrow derived immune cells and angiogenesis.
- GeoMx® DSP data showed that the presence of certain cell types of interest are either concordant, such as macrophage, vascular and neoplastic cells, or mutually exclusive to each other, for instance oligodendrocytes and neoplastic cells.
- VEGFA-high subregions are strongly associated with altered gene expression of hypoxia-related genes in contrast to high-vascular-score subregions, which supports that hypoxia promotes angiogenesis.

Introduction

GeoMx® DSP chemistry and workflow

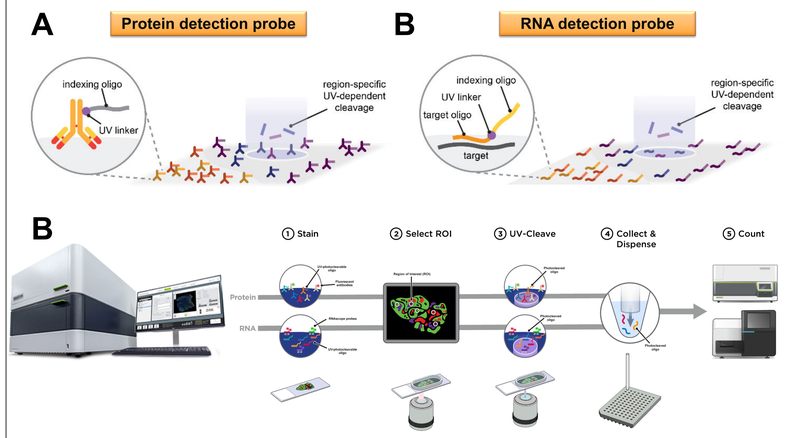


Fig 1. The GeoMx® Digital Spatial Profiler (GeoMx® DSP) is based on barcoding technology that enables spatially resolved, digital characterization of proteins or RNA in a highly multiplexed (over 2,000-plex) assay. The oligonucleotide tags cleaved from discrete regions are quantitated by NGS or nCounter, and counts are mapped back to tissue location, yielding a spatially-resolved digital profile of analyte abundance. Using a UV-cleavable linker, epitope-specific antibodies (A) or in-situ-hybridization probes (B) are conjugated with unique DNA-oligo tags. GeoMx® DSP shapes and illuminates UV lights over user-defined tissue subregions of interest only to cleave & collect DNA-oligo tags and records x,y coordinates of the subregions (C). Cleaved tags from each ROI are collected and counted using nCounter or an NGS sequencer.

Quantitative Single Cell Deconvolution (qSCD) algorithm and GeoMx® DSP RNA panel

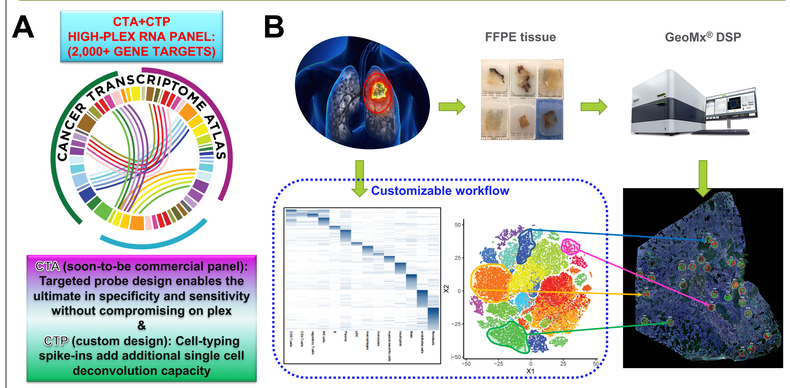


Fig 2. Cancer Transcriptome Atlas (CTA) and cell-typing spike-in panel (CTP) (A) and spatial mapping of cell types of interest using qSCD (B). scRNA-seq only reveals cell populations found within a tissue but is mute on roles in tumor microenvironment (TME) like the impact of one cell type on another's behavior. To gain insights inaccessible to single-cell methods, we demonstrate a harmonized analysis of scRNA-seq and NanoString GeoMx® data in tumors. This approach reveals the spatial distribution of cell populations defined via scRNA-seq, enabling detailed descriptions of cells' responses to each other and to their locations within the tumor. To identify gene signatures for cell types of interest, scRNA-seq data are employed[1], and then gene signatures are used to deconvolute mixed RNA expressions within a Region of Interest (ROI) to cell type scores. To measure spatial gene expression, we use CTA + CTP which includes over 2,000+ gene target-measuring in-situ hybridization probes, strongly associated with immunology, tumor biology and tumor microenvironment.

Validation prove that cell type scores from qSCD are quantitative and linearly correlated with cell-type specific protein markers.

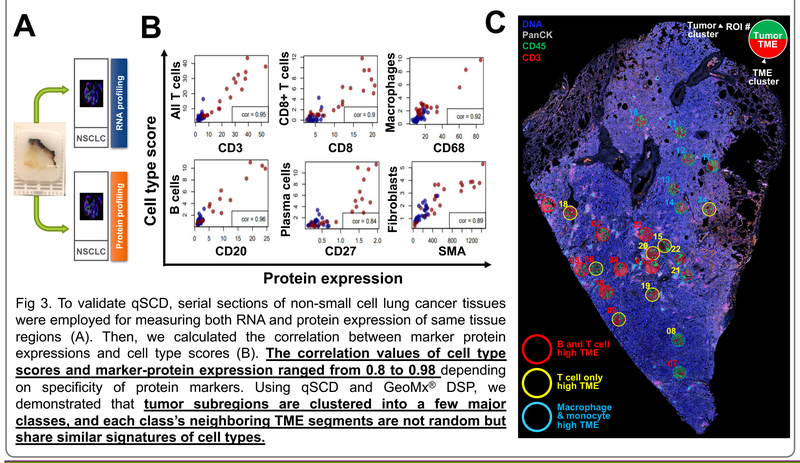
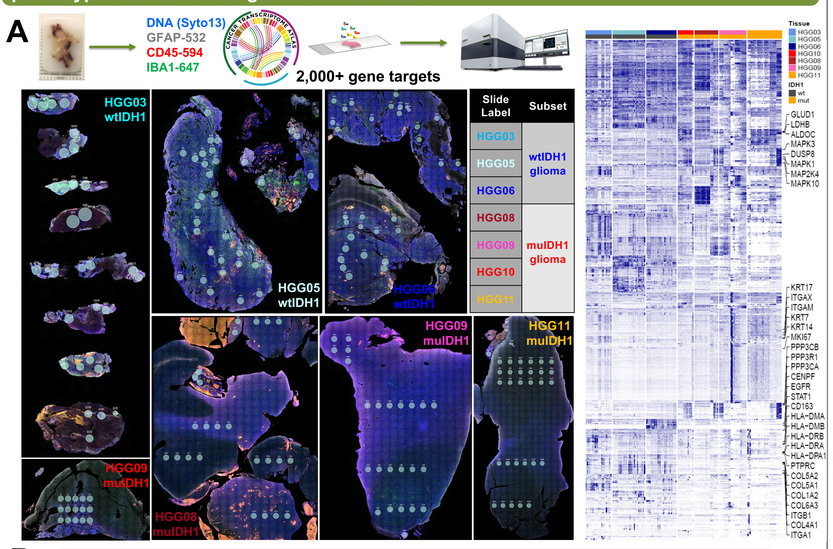


Fig 3. To validate qSCD, serial sections of non-small cell lung cancer tissues were employed for measuring both RNA and protein expression of same tissue regions (A). Then, we calculated the correlation between marker protein expressions and cell type scores (B). **The correlation values of cell type scores and marker-protein expression ranged from 0.8 to 0.98** depending on specificity of protein markers. Using qSCD and GeoMx® DSP, we demonstrated that **tumor subregions are clustered into a few major classes, and each class's neighboring TME segments are not random but share similar signatures of cell types.**

Results

GeoMx® DSP data support that IDH1 (R132H) mutation is a strong genotypic and phenotypic marker for the glioma classification.



B **GeoMx® DSP data recapitulate differential tumor biology between wtIDH1 and mulDH1 gliomas**

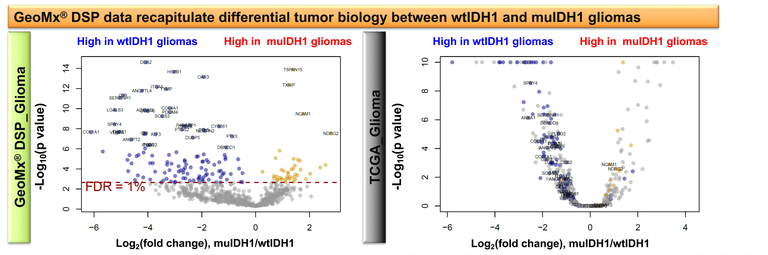


Fig 4. To identify differentially expressed (DE) genes of gliomas, three wtIDH1 (HGG03, 05 and 06) and four mulDH1 (HGG08, 09, 10, 11) glioma tissues were measured by GeoMx® DSP (A). To visualize tissues and select ROIs, DNA-Syto13 (blue), GFAP-532 (grey), CD45-594 (red), and IBA1-647 (green) antibody were used, and fifteen to thirty-two ROIs/slide were selected for GeoMx® DSP measurement, and the results are shown as a heatmap. **DE gene analysis shows that numerous genes are differentially expressed between wtIDH1 and mulDH1 gliomas, and DE analysis of TCGA human glioma data supports such finding (B)[2].**

GeoMx® DSP suggests that TME heterogeneity is the leading factor of intratumoral heterogeneity

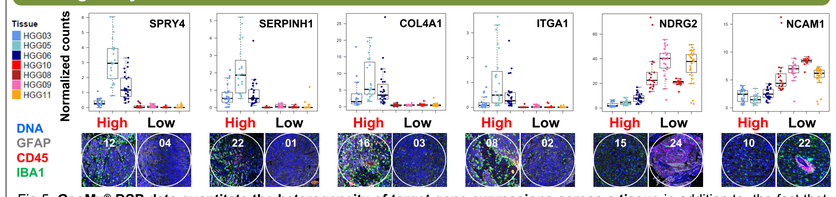


Fig 5. **GeoMx® DSP data quantitate the heterogeneity of target gene expressions across a tissue** in addition to the fact that ECM-related genes are globally high in wtIDH1 gliomas. Summary of ROI image analysis suggests that such variation is the product of TME heterogeneity.

GeoMx® DSP data show that ECM-pathway genes are globally up-regulated in wtIDH1 gliomas but heterogeneously expressed visualized by the spatial pathway viewer.

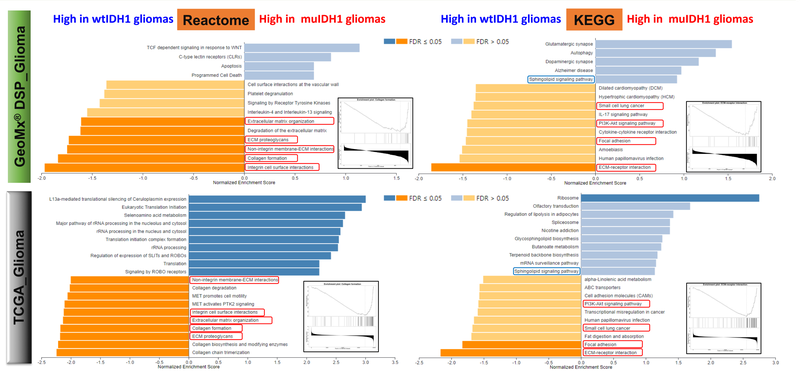


Fig 6. Differential pathway activation between wtIDH1 and mulDH1 gliomas. DE data were projected to the pathway analysis platforms such as Reactome and KEGG (http://www.webgestalt.org/). **We found that the up-regulated genes in wtIDH1 gliomas are associated with ECM-related pathways. The finding was cross-checked with the TCGA glioma data set.** Among the top 10 pathways, four to five of them among the top ten DE pathways are overlapped across GeoMx® DSP and TCGA data sets.

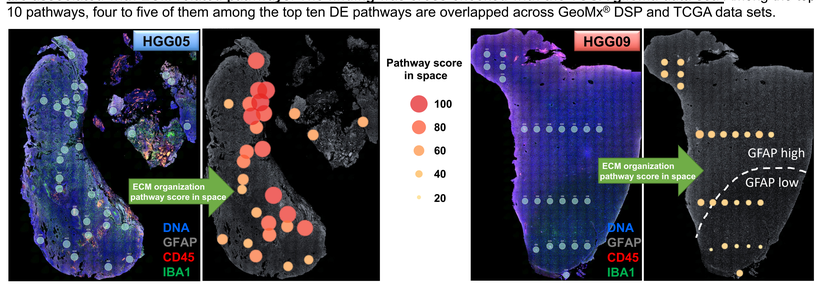


Fig 7. Spatial pathway viewer and heterogeneity of pathway activation. We mapped the ECM organization pathway outputs back to the original ROI coordinates. **The result shows that ROIs of wtIDH1 gliomas generally have high ECM-related pathway scores but show strong intratumoral heterogeneity.** mulDH1 gliomas generally show low ECM-related pathway scores, but we found that high GFAP regions show relatively higher ECM-related pathway scores, which might indicate that those regions with high GFAP are more malignant.

Quantitative Single Cell Deconvolution (qSCD) analysis, which harmonizes GeoMx® DSP and scRNA-seq data, identifies spatial abundances of glioma-specific cell types.

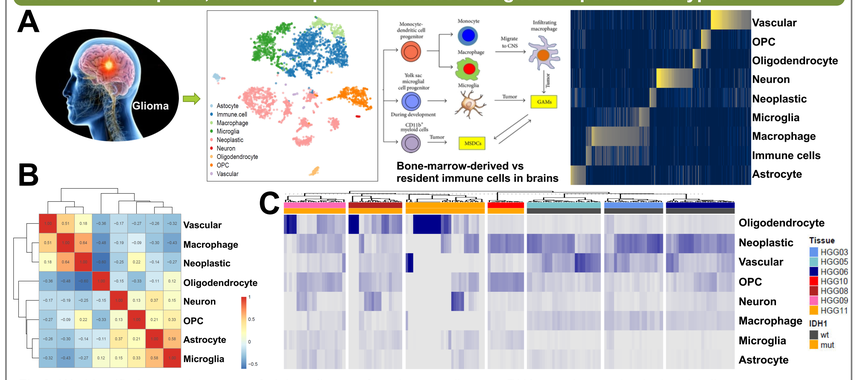


Fig 8. We identified gene signatures of eight cell types from human glioma scRNA-seq data and calculated cell type scores across GeoMx® DSP samples using qSCD analysis (A). Most subregions of wtIDH1 gliomas had high scores in neoplastic cells as scRNA-seq data set employed wtIDH1 glioma samples. **GeoMx® DSP data showed that the presence of certain cell types are either concordant, such as macrophage, vascular and neoplastic cells, or mutually exclusive to each other, for instance oligodendrocytes and neoplastic cells (B and C).**

qSCD successfully quantitates macrophages and microglias in the space and suggests that macrophage infiltration is strongly associated with chemotaxis and wtIDH1 gliomas.

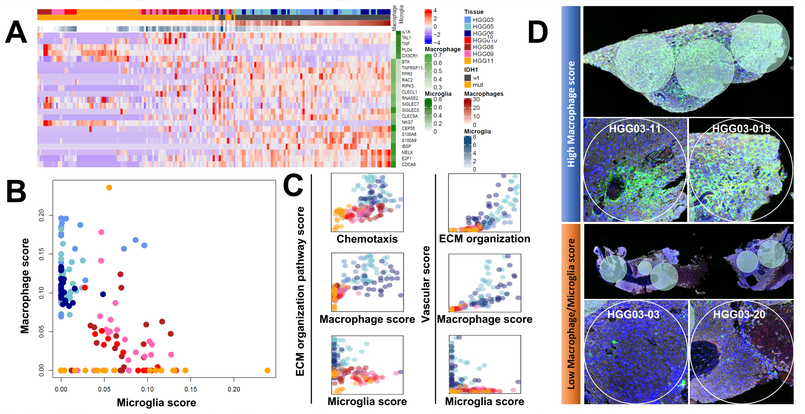


Fig 9. qSCD analysis shows that wtIH1 gliomas have high macrophage presence in contrast to mulDH1 gliomas (A and B). To understand the causality of this difference, **we ran qSCD, spatial pathway and image analysis and found that Macrophage-high ROIs are strongly associated with higher chemotaxis scores in addition to ECM organization and vascular scores (C and D).** As tumor-associated macrophage/microglia promotes tumor growth and survival, we speculate that wtIDH1 gliomas are more capable in reshaping tumor microenvironments (TME), resulting in TME supporting tumor growth rather than tumor death.

Joint spatial pathway and qSCD analysis of GeoMx® DSP data supports the hypothesis that high-VEGFA subregions of wtIDH1 gliomas are low in angiogenesis and thus activate hypoxia-mediated angiogenesis, resulting in high VEGFA expression.

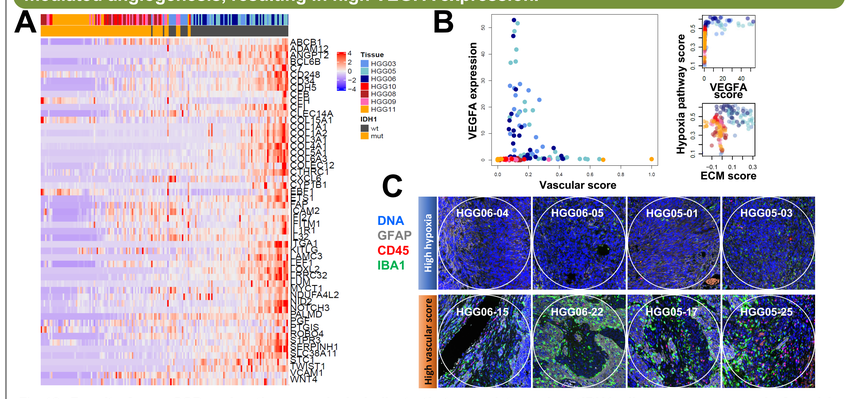


Fig 10. **Results from qSCD and pathway analysis indicate that vasculatures in wtIDH1 gliomas are commonly found in contrast to those of mulDH1 gliomas (A), and we also observed the bipolar distribution of ROIs from wtIDH1 gliomas when VEGFA and vascular scores are plotted (B).** We hypothesized that tumor regions with low vasculature secrete VEGFA to promote angiogenesis. To test the hypothesis, we correlated the hypoxia pathway to VEGFA expression in space. The results support the hypothesis that highly hypoxic subregions of wtIDH1 gliomas are high in VEGFA expression, indicating that wtIDH1 gliomas use hypoxia-mediated angiogenesis for vascularization.

Conclusion

- The Cancer Transcriptome Atlas (CTA) allows you to focus your sequencing power on the targets that matter without compromising the coverage of relevant targets from tumor to immune biology.
- GeoMx® DSP data using the Cancer Transcriptome Atlas (CTA) + cell-typing spike-ins (CTP) panel successfully recapitulates genotype/phenotype-associated biology within glioma subsets found from the TCGA RNA-seq glioma data.
- Quantitative Single Cell Deconvolution (qSCD) analysis allows GeoMx® DSP to use cell types generated from scRNA-seq data to identify spatial abundances of tumor-specific cell types.
- GeoMx® DSP generates spatially annotated data sets with a single run, such as spatial gene-expression quantitation, pathway analysis, quantitative Single Cell Deconvolution (qSCD) analysis and fluorescent tissue images with visualization markers of user's choice.
- Such multi-dimensional GeoMx® DSP capacity provides more spatially enriched information than traditional methods alone, such as tissue imagers, flow cytometry (FACS), scRNA-seq and RNA-seq, can do.

Reference

1. Zilionis R, et al, Single-Cell Transcriptomics of Human and Mouse Lung Cancers Reveals Conserved Myeloid Populations across Individuals and Species, Immunity. 2019 May 21;50(5):1317-1334.e10.
2. Darmanis S, et al, Single-Cell RNA-Seq Analysis of Infiltrating Neoplastic Cells at the Migrating Front of Human Glioblastoma, Cell Rep. 2017 Oct 31;21(5):1399-1410.
3. Amankulor N, Mutant IDH1 regulates the tumor-associated immune system in gliomas, Genes Dev. 2017 Apr 15; 31(8): 774–786

Researchers interested in participating in NanoString's technology access program for its Digital Spatial Profiling technology should contact us at TAP@nanostring.com.

Troy McEachron[1], Youngmi Kim[2], Patrick Danaher[2], Sarah Warren[2], Gabriel Zada[1], Lee Gibbs[1], Brenn Nelson[2], Joseph M Beechem[2]*
1. University of Southern California
2. NanoString Technologies, Inc.
*Correspondence: jbeechem@nanostring.com

AGBT, 02-23-2020 ~02-26-2020