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High-plex spatial transcriptomic characterization of canine tumor tissue

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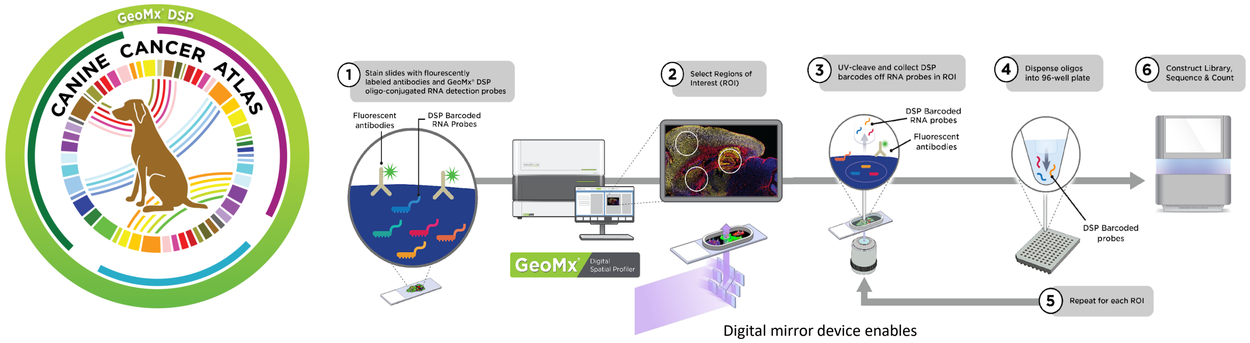
Background

Combination therapy to treat hematological and solid malignancies including chemotherapy, radiation, targeted and immunotherapy all hold huge potential for eliciting clinical responses. Informative pre-clinical testing of these approaches can be greatly facilitated using immune competent animals with spontaneous tumors. Pet dogs are immunologically outbred, immune competent and develop spontaneous tumors such as non Hodgkin's lymphoma, glioblastoma, osteosarcoma, urothelial carcinoma and melanoma that share remarkable clinical, biological and genetic features with their human counterparts. As such, pre clinical testing of therapeutic approaches in dogs with cancer promises to accurately inform human clinical trial design. For this comparative approach to provide maximum information to accelerate human clinical translation of novel combination therapies and identify correlative biomarkers of therapeutic response, it is necessary to develop research tools for deep interrogation of the canine tumor microenvironment (TME). Here we present spatial transcriptomic analysis of multiple canine tumor and tissue types using GeoMx® digital spatial profiler (DSP) Canine Cancer Atlas (CCA) panel.

Methods for Profiling using the GeoMx Canine Cancer Atlas

FFPE slides or tissue microarrays were used to profile tumor and normal tissue from canines. Each slide was stained with tissue specific immunofluorescent antibodies, including PanCK, CD45, Vimentin, IBA1, CD3, and CD45.. Regions of interest were selected to assess the TME and normal tissue as possible. Slides were then run on the DSP using the CCA panel that contains 1962-canine specific genes using standard DSP methods.

GeoMx Digital Spatial Profiling with the Canine Cancer Atlas enables quantification of 1962 protein-coding genes in precisely defined regions of interest

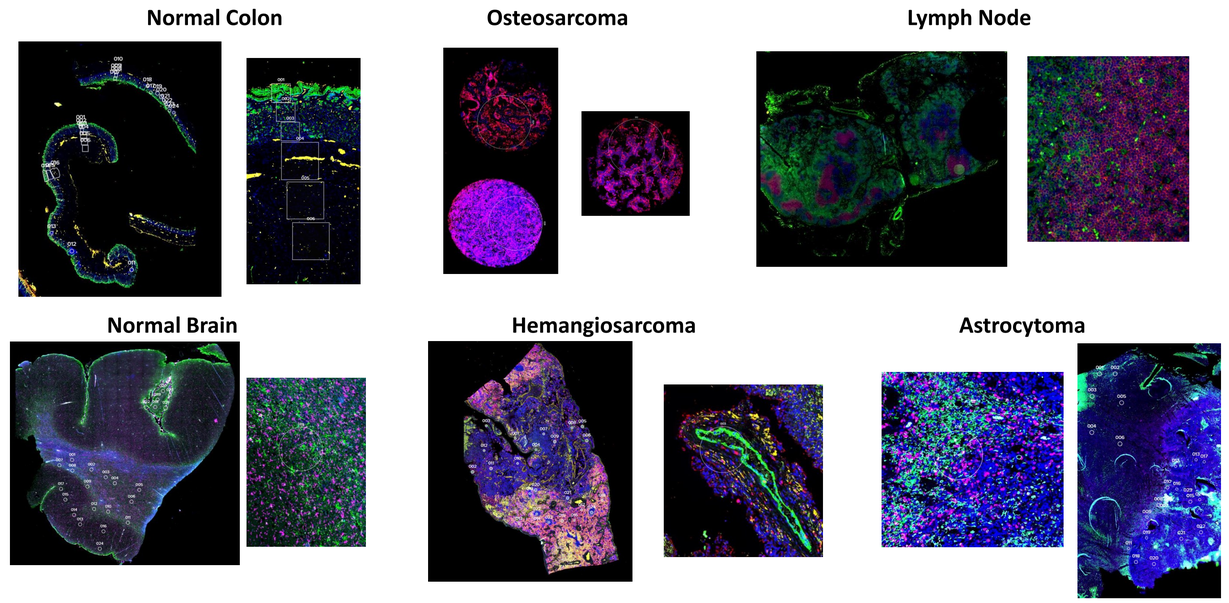


Digital mirror device enables specific barcode cleavage in ROIs of any shape

Gene coverage and pathways in the GeoMx CCA

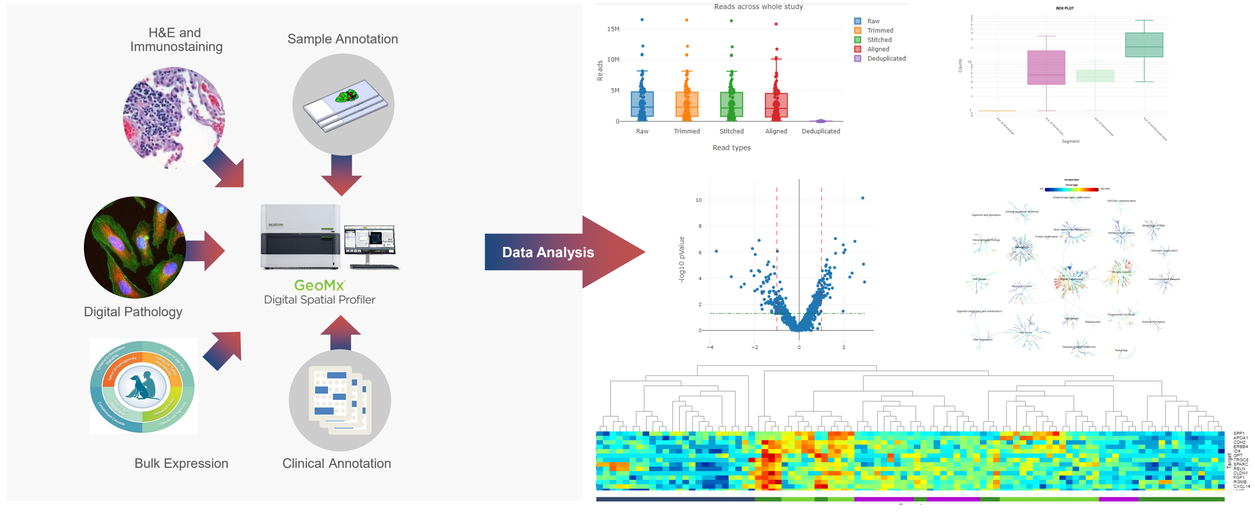
Adaptive Immunity		Cell Function		Immune Response		Innate Immunity		Metabolism		Physiology & Disease		Signaling Pathways			
B Cells	35	Apoptosis	121	Chemokine Signaling	121	Complement System	48	Amino Acid Synthesis & Transport	46	Angiotensin System	5	AMPK	48	NO	9
BCR Signaling	78	Autophagy	64	Cytotoxicity	6	Dendritic Cells	22	Arginine Metabolism	14	Cancer Type Relevant	208	Androgen	34	Notch	83
Cancer Antigens	3	Cell Adhesion & Motility	207	IL-1 Signaling	63	DNA Sensing	46	Fatty Acid Oxidation	7	Circadian Clock	26	EGFR	20	p53	76
MHC Class I Antigen Presentation	61	Cell Cycle	167	IL-17 Signaling	50	Glycan Sensing	59	Fatty Acid Synthesis	5	Drug Resistance	6	ERBB2	24	PDGF	33
MHC Class II Antigen Presentation	18	Cilium Assembly	8	IL-2 Signaling	39	Host Defense Peptides	19	Glutamine Metabolism	9	Matrix Remodeling and Metastasis	61	Estrogen	89	PI3K-Akt	252
T Cells	90	Differentiation	250	IL-6 Signaling	19	Inflammasomes	11	Glycolysis & Glucose Transport	28	Neuroendocrine Function	7	FGFR	42	PPAR	20
T-cell Checkpoints	27	DNA Damage Repair	92	Immune Exhaustion	20	Myeloid Inflammation	104	Glycosylation	12			FoxO	84	Purinergic	5
TCR Signaling	109	EMT	108	Interferon Response Genes	29	Neutrophil degranulation	120	IDH1/2	10			GPCR	168	Retinoic Acid	5
TH1 Differentiation	23	Endocytosis	58	Lymphocyte Regulation	89	NK Activity	93	Lipid Metabolism	95			Hedgehog	46	TGF-beta	107
TH17 Differentiation	42	Epigenetic Modification	177	Lymphocyte Trafficking	47	NLR Signaling	82	Mitochondrial Metabolism / TCA	55			HIF1	79	VEGF	71
TH2 Differentiation	21	Immortality & Stemness	33	NF-kB Signaling	115	RAGE Signaling	8	Nucleotide Synthesis	8			Insulin	84	Wnt	137
TH9 Differentiation	11	Ion Transport	42	Other Interleukin Signaling	183	RNA Sensing	60	Pentose Phosphate Pathway	7			JAK-STAT	123	Hippo	8
Treg Differentiation	15	Lysosome	16	Prostaglandin Inflammation	4	TLR Signaling	136	Tryptophan & Kynurenine Metabolism	8			MAPK	266		
		Oxidative Stress	164	TNF Signaling	94			Vitamin & Cofactor Metabolism	23			MET	36		
		Phagocytosis	100	Type I Interferon Signaling	47							mTOR	122		
		Proteotoxic Stress	19	Type II Interferon Signaling	42							Myc	27		
		RNA Processing	34	Type III Interferon Signaling	8										
		Senescence	131												

GeoMx Profiling of Multiple Normal and Cancer Canine Tissues



Spatial analysis based on tissue morphology, tumor microenvironment and immune infiltrated hotspots

Integrated Individual Gene and Pathway Analysis Built In



Summary and Conclusions

We were able to spatially detect over 1700 genes across multiple tissue types from canines, including osteosarcoma, glioblastoma, melanoma and normal tissues. Genes were detected in spatial compartments including malignant tumor, tumor stroma and normal tissue.

Together the GeoMx CCA allow for interrogation of the TME of multiple tumor types and has the potential to inform spatial biomarkers for response to therapy, as well as translate the effectiveness of these therapies to humans.

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