Comprehensive characterization of renal cell carcinomas identifies metabolic reprogramming of the tumor microenvironment associated with disease progression

Kirsteen H. Maclean, Lakshmi Chandramohan, Sergio Hernandez, Lisa Duncan, Brigitte Lovell, Qingyan Au, Harry Nunns, Erinn Parnell, Anna-Juncker-Jensen.

NeoGenomics Laboratories, Aliso Viejo, CA

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Introduction

Renal cell carcinoma (RCC) is one of the most frequent urological cancers with the most common histological subtype being clear cell renal cell carcinoma (ccRCC), an immunologically and histologically diverse tumor which is associated with a poor clinical outcome as up to 40% of ccRCC patients develop metastases1. While significant progress has been made in the development of immunotherapy for ccRCC, there are still many unanswered questions about the mechanisms of immune evasion and resistance, and the development of predictive biomarkers for optimal treatment strategies for individual patients. Recently, it has become clear that cellular metabolism may play a significant role in the immune response to ccRCC tumors, affecting both the ability of T-cells to function and the overall composition of the tumor microenvironment (TME). This remodeled TME leads to changes in nutrients and metabolites that can promote an immunosuppressive environment and prevent lymphocyte activation and recruitment, eventually promoting T-cell exhaustion

Methodology

Herein, we performed comprehensive transcriptomic profiling of ccRCC patient FFPE samples across all clinical TNM stages (Stages I-IV) to define the changes in metabolic processes and infiltrating immune cells with advancing disease. Specifically, the NanoString nCounter® metabolic pathways panel was used to investigate the complex mechanisms behind metabolic adaptation, metabolic switching, metabolic alterations, and study changes in mitochondrial respiration and glycolysis. The panel addresses 768 genes across 34 annotated pathways involved in the important themes for metabolism research (Figure 1).

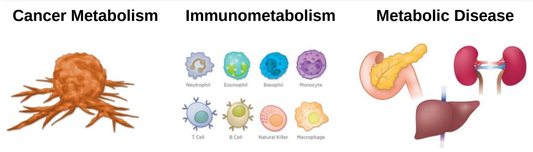

Figure 1: *Study key pathways involved in reprogrammed metabolism, profile immune cell types and understand disease pathogenesis*

Metabolic Panel Performance Specifications

Accuracy, precision, specificity, and RNA input range using the metabolic pathways Panel were assessed using a total of 50 FFPE samples across all clinical TNM stages (Stages I-IV) for ccRCC (Figure 2).

Specimen pathology review determined indication, specimen source, tumor percentage, and tumor surface area, and samples with less than 50% tumor were macro dissected for total RNA extraction and further evaluation.

A.

Client Specimen ID	RCC staging	Total No. of Cells	Total Tumor Percent	Percent of Tumor Circled	Tumor Surface Area (LxW, mm2)	Concentration (ng/µL)	Elution Volume (µL)	Yield (ng)	260/280	RIN	DV200 (%)
104727P	Stage III-male	More than 200	60	65	200	45.3	35	1585.5	1.81	1.7	48
104729P	Stage III-male	More than 100	65	70	100	42.5	35	1487.5	1.92	1.6	52
102268P	Stage III-Female	More than 200	70	75	550	174	35	6090	1.87	2.4	51
104750P	Stage I-male	More than 200	65	70	144	101.7	35	3559.5	1.96	2.2	67
104753P	Stage I-male	More than 200	50	55	160	81.8	35	2863	1.95	2.2	57
104803P	Stage I-female	More than 200	70	75	150	45.3	35	1585.5	1.89	1.4	44
104823P	Stage III-female	More than 200	60	65	150	49.7	35	1739.5	1.85	2.5	44
104945P	Stage II-male	More than 200	60	65	50	35	35	1225	1.85	1.5	54
104947P	Stage II-female	More than 200	65	70	100	16.8	35	588	1.68	1	52
RM24-00024-A13	Stage IV-male	More than 200	75	80	55	19.2	35	672	1.94	1.1	38
RM24-00024-A9	Stage IV-male	More than 200	60	65	100	21.6	35	756	1.78	1	52
105600P	Stage I-female	More than 200	55	60	180	44.9	35	1571.5	1.8	2.3	63
105885P	Stage II-male	More than 200	55	60	155	26.2	35	917	1.74	1.8	31
107510P	Stage IV-male	More than 200	50	60	360	105.7	35	3699.5	1.97	2.3	54
107737P	Stage III-female	More than 200	60	65	200	46.7	35	1634.5	1.91	2.4	45
114077P	Stage IV-male	More than 200	50	60	125	43.2	35	1512	1.89	2.4	44

B.

Test Specification	Test Results	Acceptable Criteria
Accuracy	100%	>95%
Repeatability	100%	>95%
Reproducibility	100%	>95%

C.

Sample	Av. Counts
Water (n=4)	28
E-Coli (n=4)	20
Tumors (n=50)	1100

D.

RNA input	Correlation (relative to 300ng)
300ng	1.0
150ng	0.997
50ng	0.988

E.

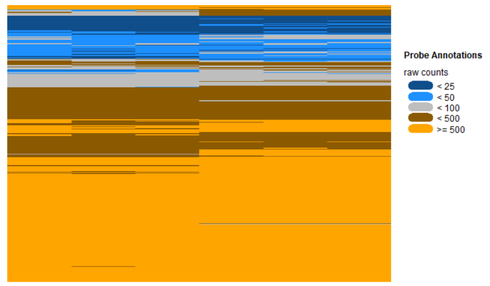

Figure 2: *Analytical validation of the nCounter metabolic pathways panel.* ***A.*** *Sample specifications.* ***B-D****. Accuracy, Repeatability, Reproducibility, Specificity and correlations related to RNA input amounts.* ***E.*** *Repeatability on sequential sections from a stage III female and stage I male*

Differential expression analysis comparisons and cell type profiling across ccRCC stages 1-IV

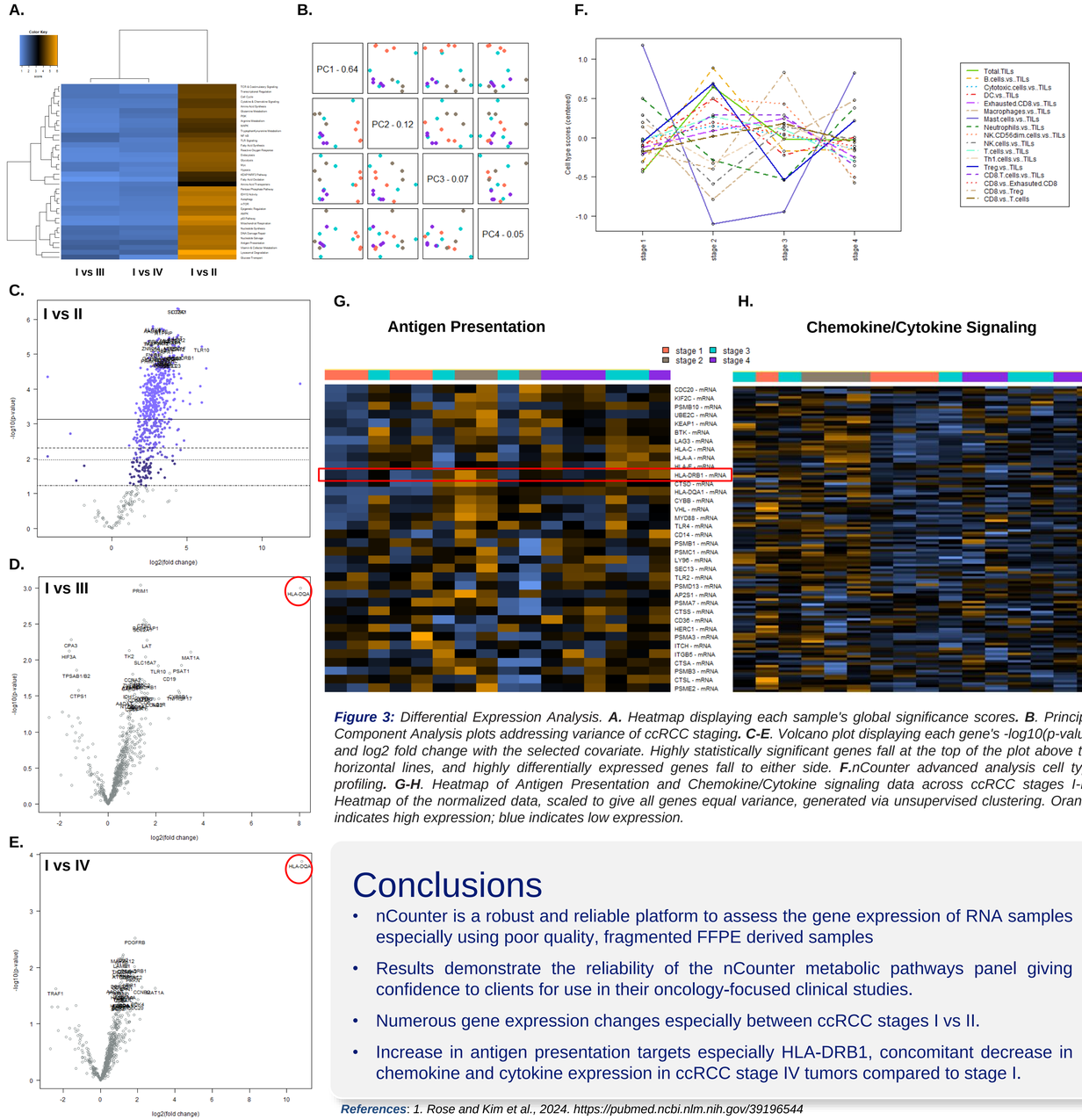

Figure 3: *Differential Expression Analysis.* ***A.*** *Heatmap displaying each sample's global significance scores.* ***B****. Principal Component Analysis plots addressing variance of ccRCC staging.* ***C-E****. Volcano plot displaying each gene's -log10(p-value) and log2 fold change with the selected covariate. Highly statistically significant genes fall at the top of the plot above the horizontal lines, and highly differentially expressed genes fall to either side.* ***F.****nCounter advanced analysis cell type profiling.* ***G-H****. Heatmap of Antigen Presentation and Chemokine/Cytokine signaling data across ccRCC stages I-IV. Heatmap of the normalized data, scaled to give all genes equal variance, generated via unsupervised clustering. Orange indicates high expression; blue indicates low expression.*

Conclusions

- nCounter is a robust and reliable platform to assess the gene expression of RNA samples especially using poor quality, fragmented FFPE derived samples
- Results demonstrate the reliability of the nCounter metabolic pathways panel giving confidence to clients for use in their oncology-focused clinical studies.
- Numerous gene expression changes especially between ccRCC stages I vs II.
- Increase in antigen presentation targets especially HLA-DRB1, concomitant decrease in chemokine and cytokine expression in ccRCC stage IV tumors compared to stage I.

References: *1. Rose and Kim et al., 2024. https://pubmed.ncbi.nlm.nih.gov/39196544*

Kirsteen.maclean@neogenomics.com