TRASCRIPTOME NEXT GENERATION SEQUENCING (NGS) FROM FORMALIN-FIXED, PARAFFIN-EMBEDDED (FFPE) KIDNEY BIOPSIES IS FEASIBLE

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OBJECTIVES

Archival, formalin-fixed, paraffin-embedded (FFPE) kidney biopsies are widely available although an underused resource for systems medicine. The present study aimed to establish next generation sequencing (NGS) of the transcriptome from FFPE renal biopsies.

METHODS

Core biopsies were obtained with a 16g needle from 16 patients undergoing (partial) nephrectomy at the time of surgery in the urological operating room. Paired biopsies from each patient with histologically-confirmed clear cell renal cell carcinoma (ccRCC) and non-tumorous ("normal") tissue were either formalin-fixed or stored in an RNA-stabilizing agent (RNAlater®, Qiagen) for up to one year until analyses. Total RNA was extracted utilizing the miRNeasy FFPE kit or the miRNeasy micro kit (Qiagen), respectively. Transcriptome sequencing libraries were prepared using the TruSeq RNA Access Library Prep Kit® (Illumina, CA; US). Sequencing was performed at an Illumina HiSeq 2500 instrument. Alignment of reads to the GRCh38 reference genome was guided by Tophat and Bowtie, respectively. Comparative analysis was done using voom/Limma R-package (www.bioconductor.org). Pathway analysis was performed with Ingenuity Pathway Analysis (IPA, www.ingenuity.com). The expression of three selected genes was confirmed by immunohistochemistry.

RESULTS

Analysis of the FFPE and the RNAlater® datasets yielded similar numbers of detected RNA species, differentially expressed transcripts and significantly affected pathways. Among the transcripts with the highest fold changes in both datasets were NPTX2 and CA9, both higher expressed in tumor, and UMOD, that was much more abundant in non-tumor tissue. **Figure1:** Immunohistochemistry confirmed the up-regulation of CA9 and NPTX2 as well as down-regulation of uromodulin in ccRCC. These three genes are known to be differentially regulated in ccRCC. **Figure 2:** In multidimensional scaling plot (MDS), samples segregated by disease status, but not by storage condition. In both datasets, pathway analyses revealed the presence of gene signatures of cancer and nephrotoxicity, renal damage and immune response. **Figure 3:** Tumor-overexpressed TGFb1 was identified as an important upstream regulator of target gene expression in both data sets of ccRCC, while tumor-underexpressed ERBB2 appears to have tumor suppressor role with many of its target genes overexpressed in ccRCC. In essence, we have obtained a cancer signature in accordance with the literature and with biological concordance between FFPE and RNAlater data sets.

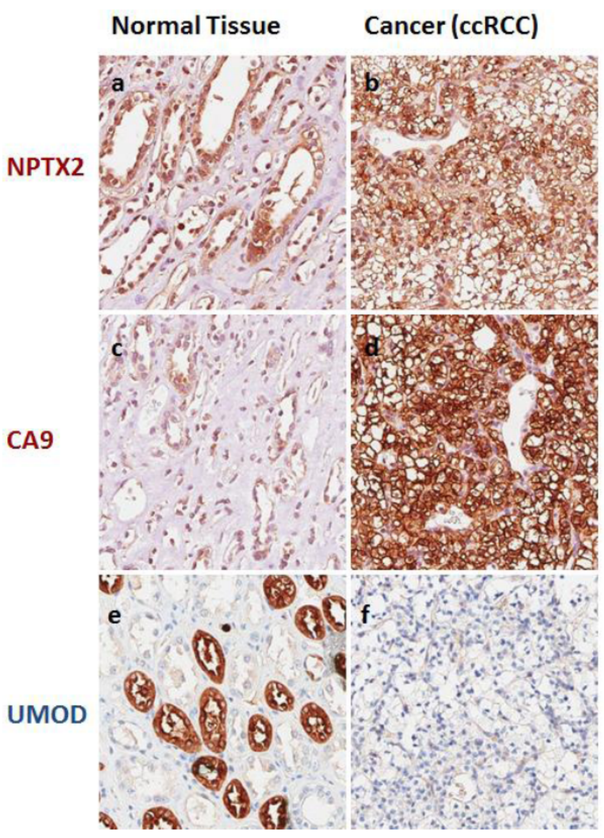

Figure 1: Immunohistochemistry of **a,b:** Neuronal Pentraxin 2 (*NPTX2*), **c,d:** Carbonic Anhydrase IX (*CA9*) and **e,f:** Uromodulin (*UMOD*)

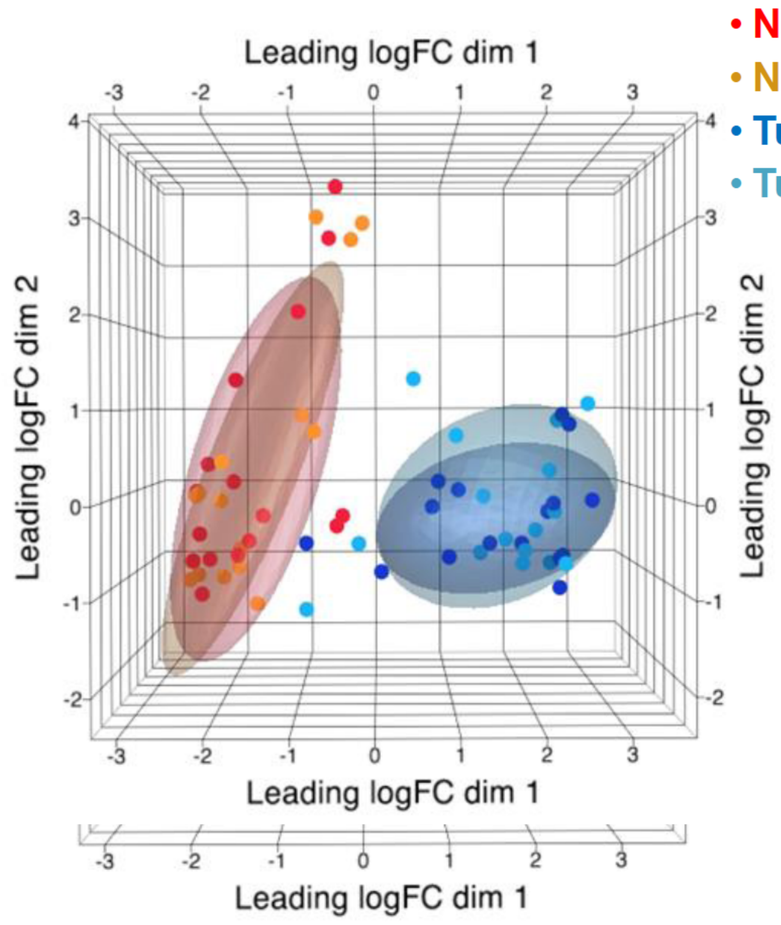

Figure 2: Multidimensional scaling plot of filtered list of genes in all samples; n = 16

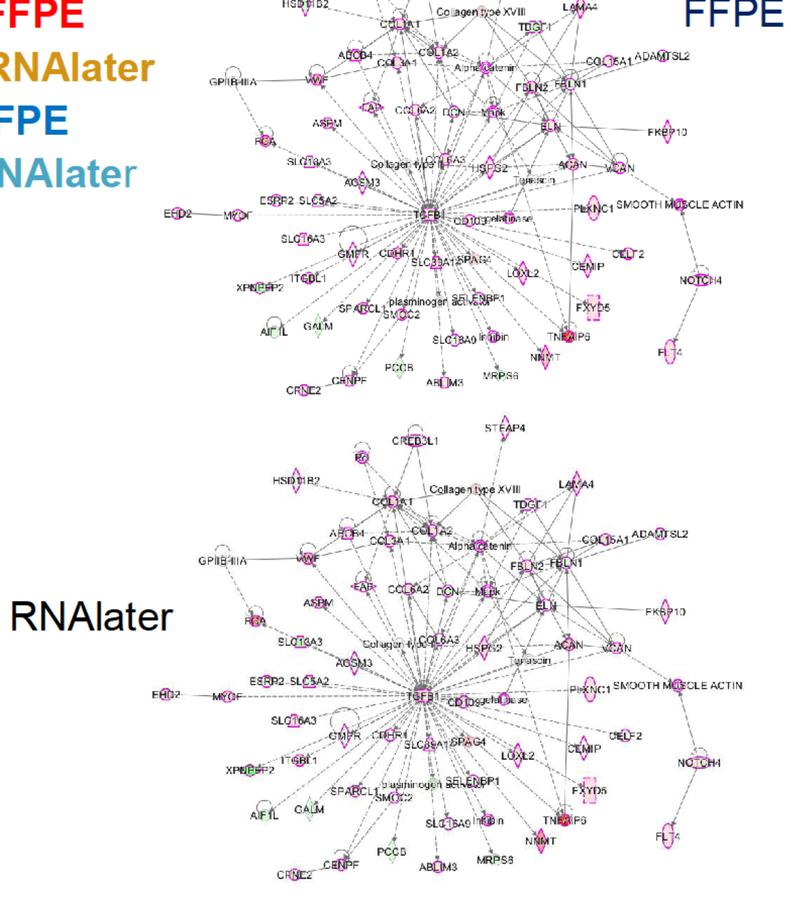

Figure 3: Proteins with red outline are involved in cancer. Red fill indicates overrepresentation of the gene in ccRCC, green indicates underrepresentastion. Colour intensity reflects range of fold change.

CONCLUSIONS

NGS of the transcriptome is feasible in archival FFPE kidney biopsies with biological correlation to RNAlater® stored material. Thus, NGS greatly expands the utility of renal tissue specimens.

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