KI REPORTS
KIReports.org
TRANSLATIONAL RESEARCH

Targeted Transcriptional Profiling of Kidney Transplant Biopsies

Tara K. Sigdel[1,4], Mark Nguyen[2,4], Dejan Dobi[3], Szu-Chuan Hsieh[1], Juliane M. Liberto[1], Flavio Vincenti[1,2,5], Minnie M. Sarwal[1,2,4,5] and Zoltan Laszik[3,4,5]

[1]Department of Surgery, University of California, San Francisco, San Francisco, California, USA; [2]Department of Nephrology, University of California, San Francisco, San Francisco, California, USA; and [3]Department of Pathology, University of California, San Francisco, San Francisco, California, USA

Introduction: Studies are needed to assess the quality of transcriptome analysis in paired human tissue samples preserved by different methods and different gene amplification platforms to enable data comparisons across experimenters.

Methods: RNA was extracted from kidney biopsies, either submerged in RNA-stabilizing solution (RSS) or stored in formalin-fixed, paraffin-embedded (FFPE) blocks. RNA quality and integrity were compared. Gene expression of the common rejection module and other immune cell genes were quantified for both tissue preservation methods in the same sample using conventional quantitative polymerase chain reaction (QPCR) by 2 different commercial platforms, (fluidigm [FD]) or barcoded-oligos (nanostring [NS]).

Results: RNA quality was inferior in FFPE tissues. Despite this, gene expression for 19 measured genes on the same sample, stored in FFPE or RSS, were strongly correlated on the FD ($r = 0.81$) or NS platforms ($r = 0.82$). For the same samples, interplatform gene expression correlations were excellent ($r = 0.80$) for RSS and moderate ($r = 0.66$) for FFPE. Significant differences in gene expression were confirmed on both platforms (FD: $P = 1.1E\text{-}03$; NS: $P = 2.5E\text{-}04$) for biopsy-confirmed acute rejection.

Conclusion: Our study provided supportive evidence that despite a low RNA quality of archival FFPE kidney transplantation tissue, small quantities of this tissue can be obtained from existing paraffin blocks to provide a viable and rich biospecimen source for focused gene expression assays. In addition, reliable and reproducible gene expression evaluation can be performed on these FFPE tissues using either a QPCR-based or a barcoded-oligo approach, which provides opportunities for collaborative analytics.

Kidney Int Rep (2018) **3,** 722–731; https://doi.org/10.1016/j.ekir.2018.01.014
KEYWORDS: FFPE; gene expression; kidney transplant; nanostring; QPCR; rejection

Massive data from high-throughput transcriptional profiling of almost 1.9 million samples is publically available in the Gene Expression Omnibus. Gene expression microarrays and RNA sequencing methods are usually used for this high-throughput discovery phase.[1] Although often criticized for the presence of false positives, the transcriptome data provides a snapshot or time-course spectrum of biological perturbations in human diseases.[2] Validation of genes discovered through these aforementioned methods for biomarker discovery and/or validation or mechanistic studies requires repeat measurements on the same tissue sample, as well as independent samples with the same phenotype. The validation studies are also important to control for demographic and clinical confounders that may have had a significant impact on gene-set perturbations. Due to a paucity of human tissue samples and the cost of experiments, these validation studies are often performed with low-throughput, but robust, assays such as quantitative polymerase chain reaction (QPCR).[3] Additional important considerations also include the quality of the tissue RNA, its adequacy for different platforms, and the depth and complexity of RNA interrogation technology, which highlights the critical importance of tissue mRNA preservation.[4–6] Addressing these questions are of paramount importance for the conduct of precision medicine in human diseases. Several approaches to preserve tissue samples have been tested.[4,7] Snap freezing in liquid nitrogen is not always

Correspondence: Minnie M. Sarwal, Department of Surgery, University of California San Francisco, 513 Parnassus Avenue, Med. Sci. Bldg., Room S-1268, San Francisco, CA 94143. E-mail: minnie.sarwal@ucsf.edu; or Zoltan Laszik, Department of Pathology, University of California San Francisco, 513 Parnassus Avenue, Med. Sci. Bldg., Room S-562, San Francisco, CA 94143. E-mail: zoltan.laszik@ucsf.edu

[4]These authors contributed equally to this work contribution.

[5]Senior authors.

Received 9 January 2018; accepted 30 January 2018; published online 3 February 2018

convenient and is costly to maintain. Several RNA stabilizing solutions (RSSs) retain RNA integrity once the biosample is submerged.[8] Formalin-fixed, paraffin-embedded (FFPE) tissues capture biology and have been extensively used for immunohistochemistry and *in situ* hybridization.[9] They are a rich source of biological information, although the degradative nature of formalin fixation on nucleic acids has been a major barrier to widespread adoption for transcriptomic analysis.[10] However, modern molecular techniques with improved fixation, extraction, amplification, and quantification of genetic materials have made DNA and RNA analysis possible on biospecimens previously believed to be unsatisfactory or unavailable.[11–13] QPCR is the conventional approach and workhorse for low-throughput gene expression validation because it is robust and has ease of experimental setup and data analysis.[14,15] Recently, a new platform for low-throughput gene expression based on molecular barcoding to quantify mRNA that does not require amplification has become available.[16–18] Previous studies have favorably compared the barcoded-oligo assay to QPCR in other clinical settings[19,20] Recently, a study by Adam *et al.* quantified the expression of a literature-derived, antibody-mediated rejection 34-gene panel in fresh-preserved and FFPE kidney tissues with QPCR and the barcoded-oligo assay, respectively.[19] Their findings demonstrated reasonable correlation ($r = 0.487$; $P < 0.001$) between the 2 assays.[19] However, there has not been a true 2 × 2 (4-way) study that compares the preservation method (FFPE and fresh-preserved) and mRNA quantification platform (QPCR and barcoded-oligo assay).

In this carefully planned and executed National Institutes of Health–funded study (U01 AI113362-01), we evaluated the integrity of RNA in kidney transplant (tx) biopsies (bx) preserved in RSS or FFPE tissue blocks. The amplification performance of selected target genes by the QPCR platform (fluidigm [FD]) and the platform that uses barcoded-oligos (nanostring [NS]) on both types of tissues was assessed. Finally, we examined the usefulness of the 2 types of tissues and the 2 platforms on a gene biomarker panel for inflammation and acute rejection (AR) of kidney transplantation. Although this study focused on kidney transplant biopsy analysis, the data presented and the strategies are applicable to any organ or tissue source of interest that is handled similarly.

METHODS

Patient Enrollment and Study Design

Twenty renal tx recipients were enrolled into the study (Table 1), divided into 2 phases. First, 10 consecutive for-cause kidney transplant biopsy were selected, in which matched tissues in RNA preservative and routinely processed FFPE blocks were available from the same patient at the same time point, regardless of the histological diagnosis. In addition to processing tissue for FFPE, approximately one-quarter of each bx from these patients was submerged in RNAlater. These 10 bx were evaluated for the cross-biospecimen (RSS vs. FFPE) quality and RNA amplification, with the latter being examined between the Fluidigm Biomark system (South San Francisco, CA) and nCounter system (NanoString Technologies, Seattle, WA) platforms for selected gene expression for 19 target genes. Second, 10 additional bx with a diagnosis of AR (n = 5; determined by either cause or 6-month protocol bx) and with normal morphology (n = 5; 6-month protocol bx) were selected to test the performance of the individual and combined common rejection module (CRM) score expression of selected genes. FFPE tissue was used only because matching RNAlater preserved tissue did not exist for these samples, based on their previously noted ability to discriminate organ transplant biopsy with AR[21] (Table 1).

Total RNA Extraction From FFPE Embedded and RNAlater Submerged Tissue

We used 4- × 10-μm-thick sections from 1 core of a 16-gauge needle biopsy to extract total RNA from FFPE samples. We initially evaluated the minimal input RNA needed by assessing the RNA quantity from 3 different approaches of 4, 7, and 9 sections, and determined that using 4 FFPE sections was sufficient for obtaining sufficient RNA for QPCR (data not shown), using the PureLink FFPE Total RNA Isolation Kit (Thermo Fisher, Catalog no. K1560-02, Thermo Fisher Scientific, Foster City, CA). RNAlater submerged tissue was obtained from one-quarter to one-half of a 16-gauge needle bx (Qiagen, Valencia, CA) and stored at −80°C; total RNA was extracted using a master mix of 790-μl TRIzol and 10-μl glycogen. Tissue samples were homogenized, incubated at 15°C to 25°C for 5 minutes, and 160-μl chloroform was added for phase separation. The mixture was incubated again at 25°C for 2 minutes, followed by centrifugation at 4°C and used for RNA extraction using the RNeasy Micro Kit (Qiagen Catalog no. 4004). RNA quantity and integrity were determined with the Thermo Scientific NanoDrop ND-2000 UV-Vis Spectrophotometer (Thermo Fisher Scientific) and Agilent Bioanalyzer (Agilent Technologies, Santa Clara, CA), respectively.

cDNA Synthesis and QPCR for the FD Platform

A total of 50-ng RNA was reversed transcribed into complementary DNA using Superscript II (Invitrogen,