

Abstract Number: 4423

Title: Optimized RNA extraction and RT-PCR assays provide successful molecular analysis on a wide variety of archival fixed tissues

Ming Zhou, Mary Bronner, Cristina Magi-Galluzz, Ralph Tuthill, Frederick Baehner, Mei-Lan Liu, Debjani Dutta, Jennie Jeong, Ya-Tien Chen, James R. Hackett, Maureen Cronin. Cleveland Clinic, Cleveland, OH, Genomic Health, Inc., Redwood City, CA

Background: Molecular study of archival fixed tissues is of considerable interest as samples with clinical and pathological annotations are readily available. Key elements for molecular profiling archival tissues are an RNA extraction method that performs efficiently with different tissue types and fixation protocols, and an assay platform that detects expression of gene(s) of interest with high fidelity in the fragmented RNA typically recovered from these samples. We have optimized RNA extraction and multi-gene quantitative RT-PCR (QRT-PCR) assays to profile gene expression in fixed breast tissues. A commercial assay (Oncotype DX™) in clinical use predicts prognosis and therapeutic response. In this study, we demonstrate this RNA extraction and RT-PCR methodology apply to a variety of archival tissues fixed using different protocols.

Methods: 5 archival renal cell carcinoma (RCC), 13 colon, 36 prostate, 4 primary and 15 metastatic melanoma samples fixed in formalin, Hollandes or Zenkers fixative and stored for variable lengths of time (0.5-25 years) were studied. From each specimen, RNA was extracted from 3 10- μ sections. RNA yield and size distribution were analyzed. QRT-PCR was used to profile multiple house-keeping and organ-specific genes, with cycle threshold (CT) reflecting gene expression level.

Results: (1) Effect of tissue fixatives on RNA yield, quality and gene expression. 5 RCC specimens were divided and part of each specimen was fixed in formalin and part in Hollandes fixative. Hollandes fixation yielded less, more fragmented RNA (average 48.4% less, with majority <100 bases) than formalin fixation. However, after normalization against 5 house-keeping genes, mean (SD) expression (\log_2 CT) of the 48 genes was similar in formalin and Hollandes fixed tissues (7.8 (3.3) vs 7.7 (3.3), $p=0.893$) and expression levels across samples and genes was highly concordant ($r = 0.99$, $p<0.001$). (2) Effect of archival age and fixatives on RNA yield, quality and gene expression. QRT-PCR was performed on RNA extracted from 3 colon cancer specimens fixed in Zenkers in 1982, 2 specimens fixed in Hollandes in 1985, and 8 specimens fixed in formalin in 2004. Average RNA yields were 3755, 8648 and 12244 ng for the 3 fixation methods. RNA was fragmented to a significantly greater degree with Zenkers and Hollandes-fixed samples. Though significant ($p=0.046$), differences in mean normalized expression levels for 48 genes were small: formalin 7.2 (2.8), Hollandes 7.7 (2.8), and Zenkers 7.9 (3.1). Using the same techniques, RNA extracted from archival prostate and melanoma tissues was profiled for up to 384 genes with similar success.

Conclusion: Tissue fixation and storage significantly affect RNA yield and quality in archival fixed tissues. However, we have developed RNA extraction methods and RT-

PCR expression assays that apply to a variety of tissues fixed and stored using different protocols.