

Evaluation of a Gene Expression Microarray Assay to Determine Tissue of Origin in Body Fluid Specimens

Stancel GA, MD; Coffey D, MD; Mody D, MD; Alvarez K, BS; Koen, T, MD; Fairley T, MT; Monzon FA, MD

Pathology, The Methodist Hospital and The Methodist Hospital Research Institute, Houston, TX. Pathology, Weill-Cornell Medical College, New York, NY.

INTRODUCTION

Carcinomas of unknown origin (CUP) represent 5% to 10% of reported cancer cases (1). Body fluid specimens are often the initial pathologic specimen received in CUP cases. Clinical, radiologic and pathologic workups can be expensive and sometimes are unsuccessful in identifying the primary tumor site, especially when limited tissue is available (2). Longer survival rates are reported for patients in whom a primary tumor is identified (3). Therefore, an alternative method for tissue of origin (TOO) determination in samples with limited tissue available is necessary. Using both Cellient and thrombin cell block methods from body fluid specimens, we evaluated the performance of the Pathwork Tissue of Origin (TOO) microarray test to determine correlation with reference diagnoses.

MATERIALS & METHODS

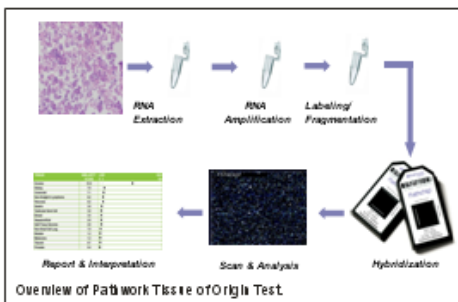
Samples: 17 body fluid specimens (9 metastases-positive and 8 negative) were collected and paraffin embedded by using both the thrombin (T) and Cellient (C) cell block methods for a total of 32 samples.

RNA Extraction: RNAs were extracted from five 10 µm tissue sections by using the Formapure Kit for isolation from formalin-fixed, paraffin-embedded specimens and the magnetic SPRIStand (Agencourt).

Pathchip™ microarrays: The RNAs were used for target preparation with a two-cycle amplification procedure followed by array hybridization to Pathchip™ microarrays. Arrays were washed and stained by using the Affymetrix GeneChip Fluidics Station FS450 and scanned by using the GeneChip Scanner 3000 (Figure 1).

Analysis: A TOO report was generated for each sample and compared to the primary tumor site. In addition, results from both the thrombin and Cellient cell block methods were compared. Student's test was used for statistical comparisons.

Figure 1



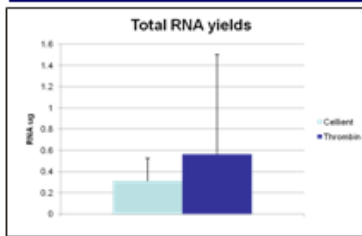
RESULTS

- 3 samples failed during amplification (all C) and 2 samples failed array data quality (both T), including both samples from an off-panel specimen (cholangiocarcinoma with abundant necrosis). So, 13/16 C (81.3%) and 14/16 T (87.5%) successfully yielded test results.
- 10 cases yielded TOO results for both C & T samples (62.5%). Of these, 7 were specimens containing neoplastic cells, of which 3 contained >60% tumor cells in the sample. TOO results for these specimens are concordant with the reference diagnosis (Table 1, Figure 2).
- One case with 30% tumor content showed successful determination of TOO (Ovary) while another case showed the correct TOO as the 2nd top score.
- All negative cases but one, showed an expression profile that was most similar to lymphoma, in agreement with the predominant presence of inflammatory cells in these samples (data not shown).
- Thrombin and Cellient block results were concordant in all cases with >60% tumor cells. For all 10 specimens, results were concordant in 8. Those with discordant results had low confidence scores.

Table 1

Sample	% Tumor	Sample Site	Reference Diagnosis	Top Score	Top TOO	2nd Score	2nd TOO	3rd Score	3rd TOO	Prediction	Agreement
2716C	95	Reural	Ovarian	97	Ov	0.6	N	0.4	BL	Ovarian	Yes
2716T	95	Reural	Ovarian	97	Ov	0.6	NA	0.5	N	Ovarian	Yes
2213C	80	Peritoneal	Gastric	36.5	GA	27	NA	9.3	LU	Gastric	Yes
2213T	80	Peritoneal	Gastric	46.7	GA	1.9	NA	14.7	Ov	Gastric	Yes
2216C	70	Alveolar	Ovarian	76.9	Ov	4.4	N	3.3	CO	Ovarian	Yes
2216T	80	Alveolar	Ovarian	70.5	Ov	0.7	N	2.7	CO	Ovarian	Yes
2279C	30	Relic Wash	Ovarian	30.4	Ov	15	CO	9.4	LV	Ovarian	Yes
2279T	30	Relic Wash	Ovarian	51.8	Ov	7.9	N	7.1	CO	Ovarian	Yes
2060C	5	Reural	Breast	45.7	LV	15.1	N	6.6	LI	Lymphoma	No
2060T	30	Reural	Breast	17.9	LV	17.6	BR	12	SC	Lymphoma	No
366C	5	Reural	Ovarian	18.4	GA	17.4	LV	13.2	SC	Gastric	No
366T	5	Reural	Ovarian	29.2	LV	7.2	GA	6.3	Ov	Lymphoma	No
330C	<5	Reural	Prostate	25.1	LV	16.2	Ov	12.8	GA	Lymphoma	No
330T	<5	Reural	Prostate	38.4	LV	13.1	Ov	11.2	N	Lymphoma	No

Figure 3



- There was no statistical difference between total RNA yields between Cellient and thrombin blocks (Figures 3 & 4).
- In paired analysis, top scores were significantly higher in thrombin vs. Cellient blocks (P = 0.047, Ttest). However when comparing only blocks with >60% tumor, the difference was not significant. No other measure of performance was significantly different between the two blocks.

Figure 2

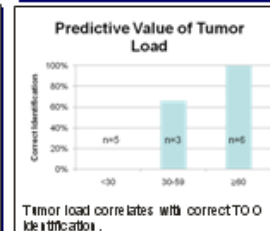


Figure 4

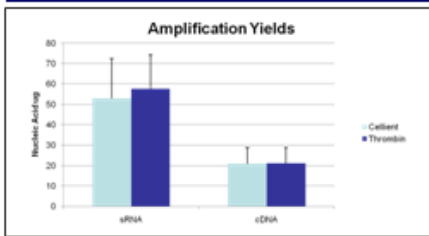
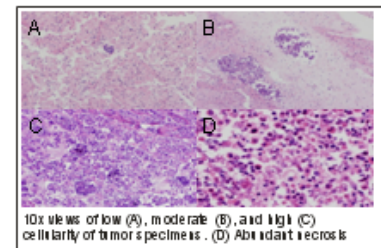


Figure 5



DISCUSSION & CONCLUSION

Gene expression profiling with microarrays have been shown to be useful in identifying the tissue of origin in solid tumor specimens (4). Therefore, we evaluated whether we could apply this tool to fluid cytology specimens.

We initially attempted to extract RNA from fluids preserved in RNAlater solution, however, salt contamination from RNAlater was nearly impossible to remove. Since the Pathwork Tissue of Origin Test can now be done on formalin-fixed paraffin-embedded tissues, we decided to evaluate this approach in cell blocks of body fluids.

Although one would have expected that alcohol fixation would provide better results for this molecular pathology application, we determined that the thrombin and Cellient cell block methods were equally efficient for isolating RNA from samples with sufficient cellularity. In addition, both block types showed similar yields in intermediate products during sample preparation (sRNA and cDNA). Interestingly, after hybridization, top scores for TOO identification were statistically higher in thrombin when compared to Cellient blocks. However when comparing only blocks with >60% tumor, the difference was not significant.

All specimens that met current testing requirements (>60% tumor) showed correct TOO identification. Most specimens with low tumor load or negative specimens showed profiles most similar to lymphoma, which is consistent with the predominant lymphocytic background. However, this issue could be a problem when lymphoma is being considered as part of the differential diagnosis (which is not a frequent occurrence in effusions). In this preliminary study, the FFPE version of the Pathwork TOO shows adequate performance for the workup of body fluid specimens harboring metastatic carcinoma. We found that the % tumor in a specimen and a non-necrotic specimen are important parameters for successful performance.

References

1. Pavlidis N, Fizazi K. Carcinoma of unknown primary (CUP). *Crit Rev Oncol Hematol* 2009;69(3):271-278.
2. Schraga DV, Jamet AR. The need to consider survival, outcome, and expense when evaluating and treating patients with unknown primary carcinoma. *Arch Intern Med* 1992;152(19):2093-2094.
3. Bishop JF, Tracey E, Glass P, Jaffe P, Pater D. Progress of sub-types of cancer of unknown primary (CUP) compared to metastatic cancer. *J Clin Oncol* 2007;25(suppl 18): 210-10.
4. Monzon FA, et al. Multicenter Validation of a 1,500-Gene Expression Profile for Identification of Tumor Tissues of Origin. *J Clin Oncol* 2009; 27:2953-2958.