

Gene Expression Profiles Help Identify the Tissue of Origin for Metastatic Brain Cancers



Wu AHB¹, Drees JC¹, Wang HP², VandenBerg SV³, Lal A⁴, Henner WD⁴ and Pillai R⁴
¹Department of Laboratory Medicine, University of California, San Francisco, CA, ²Guangzhou First Municipal People's Hospital and Guangzhou Medical College, China, ³Department of Pathology, University of California, San Diego, CA and ⁴Pathwork Diagnostics, Redwood City, CA



BACKGROUND

- Metastatic brain cancers are the most common intracranial tumor and occur in about 15% of all cancer patients.
- The Pathwork[®] Tissue of Origin Test is the first FDA-cleared gene expression test to aid in the diagnosis of metastatic, poorly differentiated and undifferentiated tumors, using frozen specimens.
- Here we report on the performance of the test in diagnosing the primary site for metastatic brain cancer patients.

STUDY DESIGN

- Specimens for this study were brain metastases of known primary sites, representing 11 independent tissue types.
- Specimens were processed at laboratories which were blinded to the available diagnosis of primary site using the Pathwork[®] Tissue of Origin Test.
- Results were compared with the known tissue of origin (available diagnosis) and the agreement between test results and available diagnoses was assessed.
- Results were analyzed both overall and for the specimens with primary sites on the Pathwork Tissue of Origin Test Panel. These include: bladder (BL), breast (BR), colorectal (CO), gastric (GA), testicular germ cell (GC), kidney (KI), hepatocellular (LI), non-small cell lung (LU), non-Hodgkin's lymphoma (LY), melanoma (ME), ovarian (OV), pancreas (PA), prostate (PR), thyroid (TH) and soft tissue sarcoma (SC).

MATERIALS AND METHODS

Specimen Requirements for the Tissue of Origin Test

- The specimens were derived from excisional biopsies of brain metastases.
- Specimens were fresh frozen and stored at -80°C
- Specimens used for the Tissue of Origin Test contained ≥60% tumor content and <20% necrosis.
- Specimens were obtained under an IRB-approved protocol.
- Specimens were obtained from UCSF Neurological Surgery Tissue Bank (16), Cytomyx (1) and data files from GeneLogic (7) and Gene Expression Omnibus (1).

Sample Processing

- Specimens were processed as described in Monzon *et. al.* and Dumur *et. al.*
- Total RNA was isolated from the specimens used for this study using the Qiagen RNeasy method.
- The RNA was used for target preparation with the Affymetrix GeneChip Expression assay followed by array hybridization to Pathchip microarrays.
- The arrays were washed and scanned using Affymetrix fluidics stations and scanners respectively.
- Positive/negative controls (total RNA) were run with every target preparation batch.
- Microarray CEL files were processed through the Tissue of Origin Test to generate a Tissue of Origin Test Report.

RESULTS

- When available, H&E sections adjacent to the tumor sample were reviewed by a pathologist to determine the percentage of tumor cells and necrosis.
- Twenty-five specimens were reviewed for test specimen requirements and entry into the study.
- Fifteen specimens met all criteria and were entered.
- Sample processing: Of the 15 specimens that passed specimen requirements, 14 generated qualified data files.
- Among the 14 specimens that passed specimen requirements, 1 had originated from an 'off-panel' tissue type.
 - The off-panel generated 13 tissue rule-outs.
- The Tissue of Origin Test accurately predicted the Available Diagnoses in 12/13 (92.3%) cases.
 - An additional sample that did not pass data quality nonetheless gave a result that agreed with the available diagnosis.

Table 1: Patient Demographics

Primary Site (n=15)	
Tissue Type	No. of Cases
Lung	2
Breast	3
Melanoma	2
Lymphoma	3
Sarcoma	1
Colon	1
Head & Neck	1
Gastric	1
Kidney	1
Patient Age Years (n=6)	
Median	41
Range	21-56
Patient Gender (n=6)	
Male	3
Female	3

The brain metastases in this study had originated from nine independent tissue types. The frequencies of these tissues were representative of the incidence of the origins of brain metastases.

Table 2: Clinical Characteristics of Brain Metastases Cases

Case ID	Available Diagnosis	Specimen Morphology	Location within Brain
A	Sarcoma	Sarcoma	Temporal-Parietal
B	Kidney	Renal cell carcinoma	Temporal-Occipital
C	Melanoma	Malignant Melanoma	Parietal Lobe
D	Melanoma	Malignant Melanoma	Frontal Lobe
E	Breast	N/A	Cerebellum
F	Breast	Carcinoma	N/A
G	Lung	Poorly Differentiated Carcinoma	Frontal Lobe
H	Lymphoma	Diffuse large B-cell lymphoma	Temporal Lobe
I	Lymphoma	Diffuse large B-cell lymphoma	N/A
L	Colon	Adenocarcinoma	Temporal Lobe
K	Breast	Adenocarcinoma	Cerebellum
L	Head & Neck	Squamous Cell Carcinoma	N/A
M	Gastric	Adenocarcinoma	N/A
N	Lung	Adenocarcinoma	N/A
O	Lymphoma	Diffuse large B-cell lymphoma	N/A

77.8% of the brain metastases were located in the cerebral hemispheres; the other 22.2% were located in the cerebellum. (N/A = Not Available)

RESULTS (continued)

Figure 1: Sample Tissue of Origin Test Report (Case ID K in this study)

Data Quality: Acceptable

TISSUE	SIMILARITY SCORE	LOW	HIGH
		0 5	100
Breast	90.6	◆	◆
Non-Small Cell Lung	5.8	◆	
Colorectal	1.5	◆	
Pancreas	1.1	◆	
Bladder	0.2	◆	
Prostate	0.1	◆	
Thyroid	0.1	◆	
Kidney	0.1	◆	
Hepatocellular	0.1	◆	
Gastric	0.1	◆	
Testicular Germ Cell	0.0	◆	
Soft Tissue Sarcoma	0.0	◆	
Melanoma	0.0	◆	
Non-Hodgkin's Lymphoma	0.0	◆	

- The Tissue of Origin Test Report presents a Similarity Score to each of the 15 tissue types included in the Tissue of Origin Test panel.
- The Similarity Score is a measure of the similarity of the gene expression profile of the specimen to the profile of the indicated tissue, ranging from 0 (very low similarity) to 100 (very high similarity).
- Similarity Score for all 15 tissues sum to 100.
- Rule-outs: Any tissue type with a Similarity Score less than or equal to 5 had a 99.8% probability of not being the Tissue of Origin. Thus, the Tissue of Origin Test can be used to exclude origins from specific tissues on the Test Panel.

Table 3: Tissue of Origin Test result for individual brain metastases cases

Case ID	Qualified Data File	Available Diagnosis	Tissue of Origin Test Results	Highest Similarity Score	Agreement with Available Diagnosis
E	Pass	Breast	Breast	90.6	Yes
F	Pass	Breast	Breast	81	Yes
K	Pass	Breast	Breast	90.5	Yes
H	Pass	Lymphoma	Lymphoma	90.4	Yes
I	Pass	Lymphoma	Lymphoma	87.7	Yes
O	Pass	Lymphoma	Lymphoma	94.4	Yes
G	Pass	Lung	Lung	62.6	Yes
N	Pass	Lung	Ovarian	89.4	No
C	Pass	Melanoma	Melanoma	77	Yes
D	Pass	Melanoma	Melanoma	75.9	Yes
J	Pass	Colon	Colon	82.4	Yes
M	Pass	Gastric	Gastric	35	Yes
B	Failed	Kidney	Kidney	45.9	Yes*
A	Pass	Sarcoma	Sarcoma	65.2	Yes
L	Pass	Head & Neck	Lung	77.3	Off-Panel

- Fourteen of the 15 specimens entered into the study were processed to yield qualified data files and Tissue of Origin Test Results.
- Fourteen of the 15 specimens (93%) entered into the study were covered by the Tissue of Origin Test panel. One specimen was head and neck which is off panel.
- For the on-panel specimens the Tissue of Origin Test accurately predicted the primary in 92.3% of the cases.
- The average numbers of tissues that were ruled out for each test was 13.

RESULTS (continued)

Figure 2A: Distribution of available diagnoses and Tissue of Origin Test Results by tissue

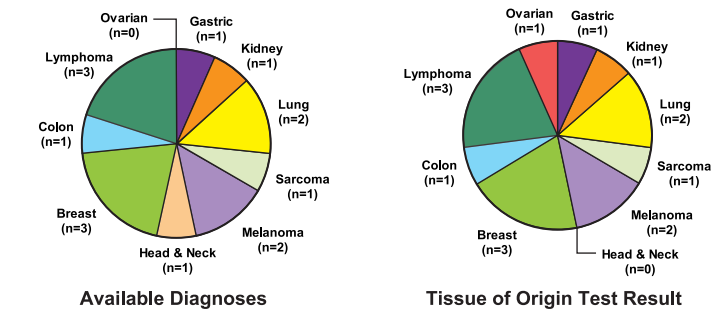


Figure 2B: Confusion Matrix of Tissue of Origin Test Results of Brain Metastasis

Clinically Available Diagnosis of Primary Site	Tissue of Origin Test Result										
	Result/Specimen	Number of Specimens	BR	CO	GA	LU	LY	KI	ME	SC	OV
BR	3	3									
CO	1	1		1							
GA	1	1			1						
LU	2	2				1					1
LY	3	3					3				
KI	1	1						1			
ME	2	2							2		
SC	1	1								1	

SUMMARY AND CONCLUSIONS

- The Pathwork Tissue of Origin Test shows a high degree of agreement with the available diagnosis for brain metastases (92.3% positive percent agreement). In addition, the test provided an average number of 13 tissues that could be definitively ruled out (>99%).
- These cases were not specifically selected for primaries on the Tissue of Origin Test panel. Nonetheless, 93% of the specimens' primary sites were available on the Tissue of Origin Test panel. For the off panel specimen, the Test generated 13 tissue rule outs.
- The Pathwork Tissue of Origin Test could be a very useful tool for pathologists as they classify metastatic brain cancers. Accurate identification of primary tumors could lead to improved clinical management, and consequently, better prognosis for these patients.

REFERENCES

- Monzon *et al.*, (2009) Journal of Clinical Oncology, 27, 1-13.
- Dumur *et al.*, (2008) Journal of Molecular Diagnostics, 10, 67-77