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Molecular Tumor Profiling in the Diagnosis of Patients with Carcinoma of Unknown Primary (CUP): Retrospective Evaluation of the Tissue of Origin Test (Pathwork Diagnostics)

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BACKGROUND AND RATIONALE

- For most patients with carcinoma of unknown primary site (CUP), standard pathologic evaluation with histologic examination and IHC staining cannot define the tissue of origin. Most of these patients receive a trial of empiric chemotherapy, often with a taxane/platinum regimen. Reliable identification of the tissue of origin would probably improve the treatment of CUP patients by allowing site-specific therapy to be administered.
- Molecular tumor profiling is a new diagnostic technique that may accurately identify the tissue of origin in most patients with CUP. Several available assays have shown high accuracy levels (80-89%) when tested on metastatic tissue in patients with known primary sites.
- The Tissue of Origin Test (Pathwork Diagnostics; Redwood City, CA) uses gene microarray technology to assay 2000 gene markers. The assay has been FDA-cleared and validated in detecting tumors from 15 tissues of origin representing 58 morphologies (JMD, in press).
- In this study, we retrospectively performed the Tissue of Origin Test on biopsy specimens from 48 patients with CUP. Assay results were correlated with clinical features, results of routine pathologic examination, and response to treatment. In addition, results were compared to those given by the Veridex 10-gene CUP assay which had been previously performed on all of these tumor specimens (JCO 26: 4442, 2008).

STUDY OBJECTIVES

- To evaluate the ability of the Tissue of Origin Test to predict the primary site in patients with CUP.
- To correlate the predicted site of origin with clinical features, results of routine pathologic exam, and response to empiric treatment.
- To compare the tissue of origin predictions of 2 molecular assays, the Tissue of Origin Test and the Veridex 10-gene assay.

STUDY DESIGN

- Archived formalin-fixed, paraffin-embedded tumor biopsy specimens from 48 CUP patients were identified. Complete clinical and pathologic information was available on all patients.
- Specimens were sent to Pathwork Diagnostics. 45 specimens contained adequate amounts of tumor, and the Tissue of Origin Test was performed. Clinical information provided included only the site biopsied and the sex of the patient.
- Assay results were provided to investigators at SCRI, and correlated with clinicopathologic features, treatment response, and results of the previously performed Veridex 10-gene CUP assay.

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MOLECULAR ASSAY RESULTS (N = 45)

Predicted Primary Site	Tissue of Origin Test	Veridex 10-Gene CUP Assay
Lung (non-small cell)	11	7
Pancreas	6	7
Sarcoma	6	0
Ovary	5	3
Colorectum	4	4
Kidney	3	0
Liver	3	0
Breast	2	0
Gastric	2	0
Urinary bladder	1	0
Lung (small cell)	0	3
"Other"*	0	19
Non-diagnostic	2	0
Unsuccessful	0	2

* The Veridex assay was designed to detect 6 primary sites (lung, colon, pancreas, ovary, prostate, breast). Molecular profiles not corresponding to any of these 6 sites were categorized as "other."

COMPARISON OF STANDARD PATHOLOGY RESULTS WITH TISSUE OF ORIGIN TEST PREDICTIONS

- 23 of 45 patients had 1 or more primary sites suggested by standard pathology techniques (histology + IHC staining)

Comparison of Standard Pathology Results with Tissue of Origin Test Predictions	Number of Patients	Tissue of Origin Test Results: Correlates with One of Suspected Sites	New Site	No Diagnosis
1	12	7	5	0
2	7	3	3	1
3	3	1	2	0
4	1	0	1	0

COMPARISON OF TISSUE OF ORIGIN TEST AND VERIDEX 10-GENE ASSAY

- When predictions were made by both assays (N = 23):
  - Identical predictions in 13 patients (57%)
  - Disparate predictions in 10 patients (43%)
- In 19 patients categorized as "Other" by the Veridex assay, 18 had specific predictions by the Tissue of Origin Test (sarcoma – 4, ovary – 2, NSCLC – 2, kidney – 2, liver – 2, gastric – 2, breast – 2, colorectal – 1, pancreas – 1)

CORRELATION OF MOLECULAR ASSAY RESULTS AND CLINICOPATHOLOGIC FEATURES

Tissue of Origin Test Prediction	Sites of Metastasis	Histology	Best Response to First-Line Treatment	Veridex Assay Result
NSCLC	Bone, soft tissue	Adenocarcinoma	PD	Lung
NSCLC	Hilar and supraclavicular nodes	Non-small cell carcinoma	CR	Lung
NSCLC	Lymph nodes, lung, liver	Squamous carcinoma	PR	Lung
NSCLC	Lymph nodes, lung, pancreas	Large cell carcinoma	SD	Lung
NSCLC	Lymph nodes, lung, liver, kidney	Carcinoma, epithelioid	PR	Lung
NSCLC	Kidney, spleen	Poorly differentiated carcinoma	PD	Lung
NSCLC	Bone, lymph nodes, liver, lung	Squamous carcinoma	PR	"Other"
NSCLC	Lung	Adenocarcinoma	PD	"Other"
NSCLC	Lymph nodes, jugular vein mass	Squamous carcinoma	PD	Colon
NSCLC	Lymph nodes, lung	Mucinous adenocarcinoma	UE	Pancreas
NSCLC	Lymph nodes	Adenocarcinoma	PR	Pancreas
Sarcoma	Lymph nodes, lung	Carcinoma	PD	"Other"
Sarcoma	Liver, adrenal gland, pericardial effusion	Poorly differentiated carcinoma	SD	"Other"
Sarcoma	Lymph nodes, lung	Adenocarcinoma	SD	"Other"
Sarcoma	Lymph nodes, lung, liver	Non-small cell carcinoma	PR	"Other"
Sarcoma	Lung, liver, pelvis	Poorly differentiated carcinoma	PD	Ovarian
Sarcoma	Liver	Adenocarcinoma	SD	Unsuccessful
Ovary	Lymph nodes, lung, liver	Poorly differentiated adenocarcinoma	PD	Lung
Ovary	Lung	Adenocarcinoma	PR	"Other"
Ovary	Lung, liver	Poorly differentiated adenocarcinoma/ endometrioid and serous papillary	SD	"Other"
Ovary	Lymph nodes, liver, lung, bone, abdomen	Carcinoma	SD	Ovary
Ovary	Lymph nodes, pleura, liver, abdomen	Poorly differentiated carcinoma	PR	Ovary
Colorectum	Pleura, adrenal	Adenocarcinoma	SD	Colorectum
Colorectum	Abdomen, soft tissue neck	Poorly differentiated adenocarcinoma	SD	Lung, small cell
Colorectum	Liver, omentum, peritoneum, pancreas	Adenocarcinoma	UE	"Other"
Colorectum	Liver	Adenocarcinoma	UE	Pancreas

SUMMARY AND CONCLUSIONS

- The Tissue of Origin Test was able to predict the site of origin in 43 of 45 tissue biopsies (96%) from patients with CUP.
- In general, predictions were consistent with clinical features, histology, and response to empiric therapy. The 5 patients identified as "ovarian" had atypical distribution of metastases. The number of sarcomas (6) was unusual; sarcoma was considered as a possibility by histologic exam in only 1 case.
- The Tissue of Origin Test was able to predict a primary site in a higher percentage of biopsies than the Veridex 10-Gene Assay (96% vs 53%). The substantial discrepancy between the assay results may be related to the limited number of genes assessed by the Veridex assay.
- Molecular tumor profiling appears to make a valuable contribution to the diagnosis of patients with CUP. Further studies are necessary to confirm the value of these assays in patients management.