Use of a 92-gene Molecular Classifier to Predict the Site of Origin for Primary and Metastatic Tumors with Neuroendocrine Differentiation

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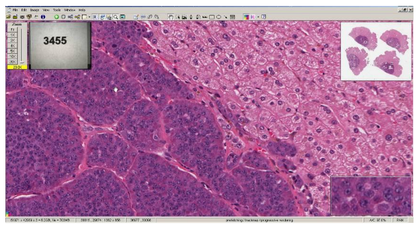
INTRODUCTION

- A diagnosis of neuroendocrine carcinoma (NEC) is often morphologically straight-forward, however, the tumor site of origin may remain elusive in a metastatic presentation.
- Neuroendocrine tumor subtyping has important implications for grading, staging, and management, especially as target therapies (everolimus, sunitinib) have been recently approved for use in pancreatic neuroendocrine tumors.
- This study describes the use of a 92-gene molecular cancer classifier (CancerTYPE ID, bioTheranostics Inc.) for predicting neuroendocrine tumor site of origin.

STUDY DESIGN

- Seventy-five (44 metastatic and 31 primary) formalin-fixed, paraffin-embedded tumor samples with neuroendocrine differentiation that were part of a larger validation study of the 92-gene molecular cancer classifier were selected for study.
- All cases submitted for the 92-gene assay were adjudicated between Mayo Clinic, UCLA, and Massachusetts General Hospital by the originating site pathologist and a second institutional pathologist by online whole slide imaging. Clinical and pathologic information was available.
- Clinicopathologic diagnoses were classified as follows:
  - **Intestinal NEC** (n=12)
  - **High-grade pulmonary NEC** (small cell or large cell, n=11)
  - **Low-grade pulmonary NEC** (pulmonary carcinoid, n=11)
  - **Merkel cell carcinoma** (n=10)
  - **Pancreatic NEC** (n=10)
  - **Pheochromocytoma/paraganglioma** (n=10)
  - **Medullary thyroid carcinoma** (n=11).
- Blinded FFPE tumor sections were submitted and tested using the 92-gene RT-PCR assay.
- Cases were tested using a prespecified classification model that reports computational algorithm results as rank probabilities. The top-ranking tumor type predicted by the classifier was compared to the adjudicated reference diagnosis.



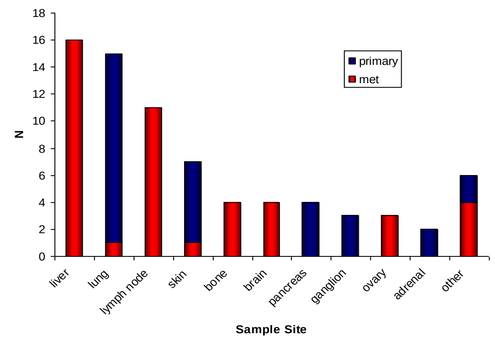
Shown is a whole slide image of a metastatic carcinoid tumor in the liver originating from the small bowel.

RESULTS

Case Characteristics

		Sample		Tumor Grade (of 3)			
	n	Primary	Metastatic	n/a	1	2	3
Intestinal	12	1	11	0	8	4	0
Merkel Cell	10	7	3	0	0	0	10
Pancreatic	10	4	6	0	2	6	2
Pheo/ Paraganglioma	10	5	5	10	0	0	0
Pulmonary	22	14	8	0	11	0	11
Thyroid Medullary	11	0	11	10	0	0	0
Total	75	31 (41%)	44 (59%)	20 (27%)	21 (28%)	10 (13%)	23 (31%)

Biopsy Sites



Assay Performance by Tumor Subtype

Neuroendocrine Subtype	n	Matches	Sens	Spec	PPV	NPV
Intestinal	12	12	1.00	1.00	1.00	1.00
Pulmonary High Grade	11	10	0.91	1.00	1.00	0.98
Pulmonary Low Grade	11	10	0.91	1.00	1.00	0.98
Merkel Cell	10	10	1.00	0.97	0.83	1.00
Pancreatic	10	8	0.80	0.98	0.91	0.97
Pheo/paraganglioma	10	10	1.00	1.00	1.00	1.00
Thyroid Medullary	11	11	1.00	1.00	1.00	1.00
TOTAL	75	71	0.95			

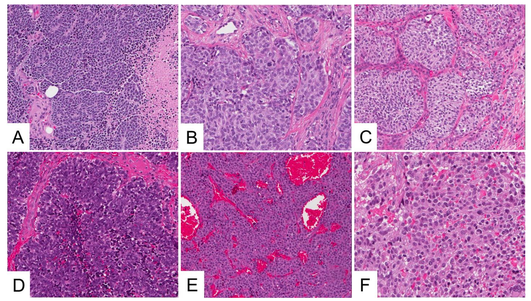
- The 92-gene classifier correctly predicted the reference subtype diagnosis in 71 of 75 neuroendocrine cases (95%).

92-Gene Assay Predictions vs. Reference Diagnosis

Reference diagnosis: Neuroendocrine subtype	Germ cell non-seminoma	Neuroendocrine-GI Carcinoid	Neuroendocrine-Merkel Cell	Neuroendocrine-Pancreatic islet cell	Adrenal-pheo	Neuroendocrine-Lung Carcinoid	Neuroendocrine-lung-small/large cell	Thyroid-medullary	Grand Total
Intestinal		12							12
Merkel cell			10						10
Pancreatic	1		1	8					10
Pheo/paraganglioma					10				10
Pulmonary low-grade				1		10			11
Pulmonary high-grade			1				10		11
Thyroid Medullary								11	11
Grand Total	1	12	12	9	10	10	10	11	75

- Three of the 4 incorrectly predicted cases were correctly predicted to the neuroendocrine carcinoma level, but were assigned an incorrect subtype

Example Cases



H&E examples of cases correctly classified by the 92-gene assay.

- **A:** Primary Merkel cell carcinoma.
- **B:** Metastatic medullary thyroid carcinoma causing pathologic hip fracture.
- **C:** Pancreatic islet cell tumor metastatic to liver .
- **D:** Pulmonary small cell carcinoma metastatic to brain.
- **E:** Ileal carcinoid metastatic to liver.
- **F:** Pheochromocytoma metastatic to liver

RESULTS

- All 75 neuroendocrine tumors met quality control parameters and were classified by the assay.
- The cohort was comprised of 59% metastatic tumors and 41% primary tumors. The most common biopsy site was liver, followed by lung and lymph node.
- The 92-gene classifier correctly predicted the reference subtype diagnosis in 71 of 75 neuroendocrine cases and had an overall concordance rate of 95%.
- Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for predicting individual subtypes are shown.
- Three of the 4 incorrectly predicted cases were correctly predicted to the neuroendocrine carcinoma level, but were assigned an incorrect subtype.

CONCLUSION

- The 92-gene classifier demonstrated excellent accuracy for prediction of subtype in a heterogeneous cohort of both primary and metastatic neuroendocrine tumors.
- While these results were part of a larger parent study designed to characterize performance of the classifier in a broad range of tumor histologies and did not adjust for neuroendocrine tumor primary site prevalence, these findings show promise for use of the 92-gene assay in classifying neuroendocrine tumors of uncertain primary site.