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Imaging, Diagnosis, Prognosis

Clinical Cancer Research

Prospective Gene Signature Study Using microRNA to Identify the Tissue of Origin in Patients with Carcinoma of Unknown Primary

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Abstract

Purpose: Accurate identification of tissue of origin (ToO) for patients with carcinoma of unknown primary (CUP) may help customize therapy to the putative primary and thereby improve the clinical outcome. We prospectively studied the performance of a microRNA-based assay to identify the ToO in CUP patients.

Experimental Design: Formalin-fixed paraffin-embedded (FFPE) metastatic tissue from 104 patients was reviewed and 87 of these contained sufficient tumor for testing. The assay quantitates 48 microRNAs and assigns one of 25 tumor diagnoses by using a biologically motivated binary decision tree and a K-nearest neighbors (KNN). The assay predictions were compared with clinicopathologic features and, where suitable, to therapeutic response.

Results: Seventy-four of the 87 cases were processed successfully. The assay result was consistent or compatible with the clinicopathologic features in 84% of cases processed successfully (71% of all samples attempted). In 65 patients, pathology and immunohistochemistry (IHC) suggested a diagnosis or (more often) a differential diagnosis. Out of those, the assay was consistent or compatible with the clinicopathologic presentation in 55 (85%) cases. Of the 9 patients with noncontributory IHC, the assay provided a ToO prediction that was compatible with the clinical presentation in 7 cases.

Conclusions: In this prospective study, the microRNA diagnosis was compatible with the clinicopathologic picture in the majority of cases. Comparative effectiveness research trials evaluating the added benefit of molecular profiling in appropriate CUP subsets are warranted. MicroRNA profiling may be particularly helpful in patients in whom the IHC profile of the metastasis is nondiagnostic or leaves a large differential diagnosis. *Clin Cancer Res; 17(12); 4063–70. ©2011 AACR.*

Introduction

Carcinoma of unknown primary (CUP) patients poses a therapeutic challenge. When the putative site of origin cannot be assessed on the basis of clinicopathologic features, empiric treatment with "broad spectrum" doublet chemotherapies is usually the standard of care (1, 2). With the growing number of cytotoxic and targeted therapies shown to be effective against specific cancers (3–7), innovative methods to identify the tissue of origin (ToO) of CUP cancers may permit the use of more targeted therapies for CUP patients (8, 9). There is increasing evidence that CUP cancers, rather than being a distinct entity biologically and molecularly different from other cancers, are a group of unrelated site-specific tumors which happen to share the property of having a diminutive primary that escapes detection (10). Thus, accurate identification of the putative tumor of origin may be helpful in optimizing patient management.

Molecular profiling (MP) methods using various platforms including measuring mRNA, by DNA microarrays, or quantitative reverse transcriptase PCR (qRT-PCR), and more recently, microRNAs have been used to evaluate the ToO in metastatic samples. The data on known metastases have been validated by using independent blinded sets of tumor samples, in which the reference diagnosis is known, with an accuracy of about 80% to 90% (11–15). The study described in this article extends this to the more challenging group of true CUP patients. Unlike known cancers, a unique challenge to CUP is the inability to directly validate the accuracy of a profiling test given that there is typically no primary identified and the low rate of

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Translational Relevance

Carcinoma of unknown primary (CUP) is described as metastatic cancer without a detectable primary–there is increasing evidence that these cancers are unrelated groups of site-specific tumors which happen to share the property of having a diminutive primary that escapes detection. Our research evaluates the role of microRNA profiling in CUP to determine the primary cancer profile and compare the assay results to the clinicopathologic presentations. There is an unmet need to study molecular profiling assays in prospective trials with true CUP patients (currently most data is with known cancers). This research has direct application to the future of CUP treatments which has undergone a paradigm shift from empiric to individualized therapy–it may allow leverage of promising treatments available for known cancers to CUP. As novel therapies are developed for site-specific cancers, they may be evaluated in appropriate CUP subtypes on the basis of pathologic and profiling results in selected patients.

detection of latent primary cancers. One could argue that the clinical utility of profiling assays would ideally be evaluated in randomized trials comparing survival outcomes in patients receiving therapy on the basis of ToO prediction from MP versus those receiving standard empiric therapies. However, at this time, designing such a randomized trial is not feasible as an adequately powered trial would require more than 500 patients and still run the risk of ambiguous results because of the very heterogeneous presentations of CUP cancers.

However, it is still important to evaluate the accuracy of MP in CUP in prospective trials because there is inadequate data showing that results from MP assays done on metastatic known cancer samples can be extrapolated to CUP. Hence, in the trial described herein, we used clinicopathologic presentations as a surrogate to evaluate MP predictions of ToO.

Multiple approaches have been used for molecular profiling. In this study, we describe a microRNA-based approach to determine the ToO in CUP patients. MicroRNAs, noncoding genes between 21 to 23 nucleotides in length, have been shown to control gene expression by regulating translation of mRNA into protein. Interestingly, microRNAs have been found to be important for tissue differentiation (16, 17) and tumorigenesis (18, 19) and seem to show highly tissue-specific expression (20–22). These features as well as their excellent preservation in formalin-fixed and paraffin-embedded tissues (FFPE; refs. 23, 24) suggest that microRNA expression profiles can serve as attractive markers for the molecular identification and characterization of tissues and tumors. The feasibility of using microRNA expression profiles from metastatic tumors to accurately identify ToO in patients with known primaries has been previously reported (13). Subsequently, a qRT-PCR assay which is based on the expression levels of 48 microRNAs was developed and was shown to be able to identify correctly the ToO in 85% of the cases in an independent validation set (14). This validation set was composed of tumor cases representing the 25 possible diagnoses corresponding to 17 distinct tissues and organs of origin in the tumor panel of the assay. By definition, the validation set was constructed to evaluate the performance of the assay, comparing the assay diagnosis with a "gold standard" reference diagnosis in patients with a known primary.

The aim of this study was to prospectively evaluate the clinical utility of ToO predictions generated by this microRNA-based assay for metastases in CUP patients in the context of currently available immunohistochemistry (IHC) and clinicopathologic "working diagnoses."

Materials and Methods

Patient and specimen inclusion criteria

Patients were prospectively enrolled in this study between July 2008 and June 2010 at the University of Texas, M D Anderson Cancer Center (MDACC). All patients were diagnosed with CUP at presentation, in that a primary cancer was not detected after a complete history and physical examination, detailed laboratory studies, imaging, and when indicated, invasive studies including endoscopy and colonoscopy as directed by symptoms, signs, and pathology. Patients with epithelial malignancies were eligible including patients with poorly differentiated carcinomas. Pathology data including morphology and immunohistochemical stains from MDACC or other referring institutions were available, as well as FFPE tissue sections of untreated or previously treated tumor biopsies or resection specimens. Patients with cytology-only specimens were not eligible. A unique study identifier was used to maintain patient anonymity. The study was approved by the Institutional Review Board of MDACC. The assays were conducted at Rosetta Genomics' laboratory in Philadelphia, PA as described below.

Sample preparation and microRNA assay

The assay was done on FFPE tissue. H&E slides were reviewed by a surgical pathologist (TBE) for suitability regarding tumor cell content, surrounding tissue, amount of necrosis, inflammation, hemorrhage, and fibrosis. Between 2 and 10 corresponding unstained sections were available for RNA extraction for each case. The method had been validated for a tumor cell content of at least 50%. When feasible, microdissection was done to increase the tumor cell content to beyond 50% on the basis of tumor size and histologic features.

Suitable samples were processed as previously described (14) to generate a putative ToO. Briefly, total RNA was extracted by using acid phenol–chloroform extraction, and RNA was reverse transcribed. The expression levels of 48 microRNAs that had been identified as informative during the development of the assay (14) were determined in duplicates by qRT-PCR. Samples were processed in batches

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starting with the extraction. The following external negative controls were used with each batch: a "no-sample" control with each extraction batch that did not contain any FFPE tissue, to control for contamination of the extraction process, and a "no RNA" sample to control for contamination of the reverse transcription and/or the quantitative PCR process. A well-characterized RNA sample was processed as an external positive control with each batch. In addition, internal quality parameters were monitored for the microRNA amplification of each patient specimen. qRT-PCR results for samples which passed the quality assessment criteria were analyzed by using 2 different classifiers: a K-nearest-neighbor (KNN) classifier and a binary decision tree (Tree), with 1 to 3 microRNAs at each node. The assay quantitates 48 miRNAs, and the 2 classifiers assign the ToO based on these expression levels. The assay was trained to identify 25 different tumors from 17 ToO that include the most common origins for CUP. A single diagnosis was reported when the KNN and Tree classifiers agreed, and both diagnoses were reported when the 2 classifiers disagreed. When the 2 classifiers agreed regarding the ToO but not the histologic subtype, the ToO was reported without a histologic subtype. Other tissues of origin have a lower probability but are not completely ruled out, as only the single best answer for each classifier (KNN and binary tree) is reported. Because the assay always reports ToO from the training database, origins of samples that are not represented in the tumor panel cannot be identified by the assay.

As a first step toward a standardized ToO reporting system, a "level of agreement" tool was created (Table 1). By using this tool, level 1 indicated that the MP results were either consistent with a specific IHC (e.g., test result of colon cancer in CDX-2 positive and cytokeratin 20 (CK20) positive metastasis) or an IHC differential, together with a specific clinical presentation or a latent primary. Level 2 agreement either indicated an MP result compatible with an IHC differential or with the clinical presentation (e.g., test result of biliary tract cancer in CK7 positive and CK20 negative tumor or a test result of renal cell carcinoma in a patient with lytic osseous metastases) or cases with noncontributory IHC (usually a large differential, e.g., undifferentiated carcinomas) in which the clinicopathologic presentation could not rule out the MP diagnosis. Level 3 indicated that the MP results either disagreed with the clinicopathologic features or it was uncertain whether the test result was likely in the context of an atypical presentation.

Table 1. "Level of agreement" designation to ToO MP results

Level of agreement	Criteria
1 "Consistent"	MicroRNA profile consistent with -histology and specific IHC profile OR -IHC differential and specific clinical presentation OR -latent primary
2 "Compatible"	MicroRNA profile compatible with -IHC differential OR -clinical presentation with IHC noncontributory
3 "Disagree" or "uncertain"	*Disagree:* microRNA profile not compatible with -latent primary cancer OR -histology and IHC *Uncertain:* difficulty in validating microRNA profile -noncontributory IHC AND -unusual clinical presentation

Results

A total of 104 patients (66 females) were enrolled in the study. Seventeen samples (16%) were excluded from further analysis because the tumor cell content in the block did not meet the criteria for the analysis. These samples typically consisted of very small biopsies with inadequate tissue left after extensive IHC work-up. The remaining 87 of 104 samples (84%) were considered suitable for analysis on the basis of the tumor cell content and underwent processing (of these, 39% were from small biopsies; Fig. 1) Microdissection was done in 43 samples. Seventy-four of the 87 samples (85%) passed all QA criteria and yielded a "putative primary" result. Table 2 depicts the demographics and tumor characteristics of these patients.

Level 1 or 2 agreement of the molecular profile with the clinicopathologic diagnosis was obtained in 62 of 74 (84%) successfully processed samples which amounts to 62 of 87 samples (71%) in which profiling was attempted. In 33 (45%) patient samples, the MP assay showed level 1 agreement. Two of these samples, lymph node metastases from the inguinal and pelvic areas, were correctly identified as squamous cell carcinoma by the assay, but the clinically most likely primary sites in the anogenital area were not assigned because the algorithm had not been trained to recognize primary squamous cell carcinoma locations in the anogenital area. In one patient, the metastases manifested as abdominal carcinomatosis showed concordant "colon" IHC and molecular profile. A primary tumor was later found in the terminal ileum suggesting a molecular signature overlap between small bowel and colorectal cancer (in practice, they also have similar therapies). In 29 patient samples (39%), the agreement level was 2. In 12 patients (16%) level 3 disagreement/uncertainty was reported.

Interestingly, pathology and IHC alone suggested a diagnosis or (more often) a number of differential diagnoses in 65 of the 74 cases (Fig. 1). Of these 65, 55 (85%) resulted in a microRNA profile that was consistent or compatible with the pathology diagnosis and/or the clinical data. Forty-six of these 55 cases matched the IHC data without taking into

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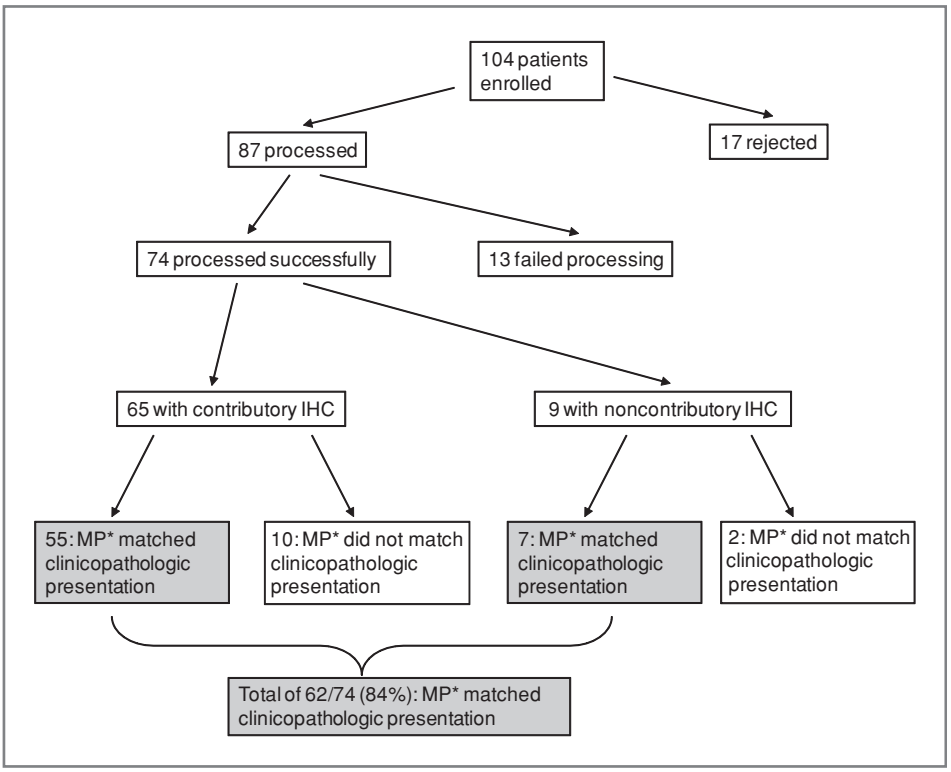

Figure 1. Algorithm illustrating patient flow through the study. *KNN or Tree.

account clinical information. The remaining 9 of 74 cases were not classifiable with conventional pathology and exhaustive IHC. In these diagnostically most challenging cases, the assay results were compatible with the clinical presentation in 7 cases (78%).

Table 2. Patient and tumor characteristics (for patients with assay results)

Patient or tumor characteristics	No. of patients ($n = 74$, %)
Male	29 (39)
Female	45 (61)
Median age (y)	58 (range 20–83)
Metastatic sites at presentation[a]	
Lymph nodes	44 (59)
Liver	31 (42)
Lung	24 (32)
Bone	17 (23)
Pelvic mass/adnexae	16 (22)
Skin/subcutaneous	9 (12)
Omentum/peritoneum	25 (34)
Adrenal	5 (7)
Other	18 (24)
Tumor differentiation	
Well differentiated	0 (0)
Moderately differentiated	30 (41)
Poorly differentiated	41 (55)
Unavailable	3 (4)

[a]Most patients presented with more than 2 sites of disease.

As the assay can return either 1 or 2 answers, depending on the agreement between the 2 classifiers, we turned to studying the 2 classifiers separately. Overall, the KNN classifier provided a level 1 or 2 agreement with clinicopathologic findings in 58 cases (78%) and the Tree provided a level 1 or 2 agreement in 46 cases (62%). For 34 samples (46%), the histologic diagnosis rendered by the 2 classifiers (KNN and Tree) agreed. Out of these, in 28 cases a single overall diagnosis was generated by the test. In the remaining 6 cases, the KNN and Tree diagnoses were squamous cell carcinoma of head and neck and squamous cell carcinoma of the lung. The assignments were treated as squamous cell carcinoma of head and neck/lung. We looked to see whether the concordance with the clinicopathologic diagnosis was greater in patients in whom KNN and Tree gave the same ToO assignment versus those in whom they provided different results. Twenty-seven (79%) of these 34 single histology results as rendered by the assay were consistent or compatible with the clinicopathologic findings. Thirty-five (88%) of the 40 tumors in which the ToO prediction differed between the 2 classifiers showed a match of the clinicopathologic findings, with at least 1 of the 2 assay predictions from KNN and Tree. When analyzing KNN and Tree separately in these 40 cases in which the 2 classifiers differed, the KNN provided a better match with the clinicopathologic diagnosis than the tree in 17 of 40 cases (43%), whereas the tree alone provided a better match than the KNN in 10 of 40 cases (25%). In 8

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Table 3. MicroRNA results and correlation with IHC and therapy (patients with Level 1 and 2 agreement)

Molecular classification (profile)	IHC (*n*, %)[a]	First-line treatment (*n*, %)	Response
Colon ($n = 13$)	CK7 + (6/13, 46%) CK20 + (11/13, 85%) CDX-2 + (11/13, 85%)	5-FU + oxaliplatin (10/13, 77%)	PR (4), SD (2)[b], NA (2)
Ovarian ($n = 6$)	CK7 + (6/6, 100%) CK20 – (6/6, 100%) WT1+ (3/6, 50%) ER + (3/6, 50%)	Paclitaxel +carboplatin (6/6, 100%)	PR (5) NA (1)
Pancreaticobiliary ($n = 10$)	CK7+ (9/10, 90%) CK20 – (7/10, 70%) TTF-1 – (7/10, 70%) CDX-2 + (4/10, 40%)	Gemcitabine based (7/8), FOLFOX (1/8)[c]	PR (4), SD (3) PD (3)
Lung adeno ($n = 6$)	CK7 + (5/6, 83%) CK20 – (3/6, 50%) TTF-1 – (5/6, 83%)	Paclitaxel or gemcitabine + carboplatin (5/6, 83%)	PR (2), PD (3)
Head and neck vs. lung squamous ($n = 6$)	CK7 + (5/6, 83%) CK20 – (5/6, 83%) TTF-1 – (5/6, 83%)	Paclitaxel + carboplatin (4/6, 67%) surgery, radiation in (2/6)	PR (2), PD (2)

Abbreviations: NA, not available; PR, partial response; PD, progressive disease; SD, stable disease.

[a]IHC data including CK7, CK20, TTF-1, CDX-2, WT-1/ER (where applicable) available on most patients.

[b]Two additional patients received FOLFOX in the stage IV adjuvant setting after bilateral oophorectomy for ovarian metastases, currently with no evidence of disease.

[c]–patient died with rapidly progressive disease with no therapy.

cases, the answers from both classifiers, even though different, were both equally consistent and compatible with the clinicopathologic presentation. The remaining 5 cases (12% of the 40 cases in which the 2 classifiers provided different results) were discordant with the clinicopathologic presentation for both answers rendered by the assay.

Pertinent clinical data, pathology/IHC, molecular results for all patients whose specimens were processed successfully are detailed in the Supplementary Table. Table 3 highlights and summarizes IHC features and treatment responses for patients in selected diagnostic categories whose molecular profiles were in agreement with the clinicopathologic cancer diagnosis.

Discussion

Until recently, despite the large numbers of patients diagnosed with CUP, innovative research and individualized approaches to managing these patients have lagged behind many other solid tumors. The availability of ToO MP assays hold promise for the increasing individualization of therapy for CUP patients (12, 14, 25, 26). Our study confirms that microRNA profiling can be successfully done on CUP patients with clinical FFPE tissue samples, including decalcified bone specimens. Small sample size and extensive IHC analysis sometimes precludes successful MP analysis; approximately 15% of the blocks may be exhausted and unacceptable for further testing. In addition, an assay failure rate of 15%, mostly because of insufficient RNA is not uncommon in clinical practice. Even under the more favorable conditions of a clinical test validation, in which primary tumors and metastases from known origins are tested, similar failure rates have been reported (27). Enrichment of the specimen for tumor cells by using microdissection allows inclusion of biopsies and resection specimens that contain a high percentage of nontumor cells. However, adequate sample acquisition remains a challenge in CUP patients.

MicroRNA profiling, using the assay described, results in an agreement with the clinical and/or pathologic presentation in 62 of 74 samples (84%) that were processed successfully. In the cohort presented here, performance of the KNN was better than of the binary decision tree. However, both classifiers are important as they jointly provide a better match with the clinicopathologic diagnosis. In clinical practice, the assay is reported as 2 ToO predictions whenever the 2 classifiers disagree. The KNN result is reported as the "most likely tumor of origin" and the Tree result is reported as the "second most likely tumor of origin," reflecting the observation that the KNN is a more likely match but that the Tree result should also be considered in the differential diagnosis.

IHC is helpful in CUP (28, 29) but is not without limitations—most importantly, IHC may not show a staining pattern that results in a specific diagnosis. This might be

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due to the nature of the tumors, for example, tumor locations for which no specific markers are available, dedifferentiated tumors which have lost expression of characteristic markers, technical factors, or selection of markers that are unsuitable to make the correct diagnosis. In this study, IHC in conjunction with histology, imaging, and clinical presentation gave a strong working diagnosis in 27 patients (36%) and IHC was noncontributory in 9 cases (12%). In the remaining cases, the IHC provided a large differential diagnosis, for example, in the case of CK7+ adenocarcinoma, which may include upper GI, pancreaticobiliary, breast, lung, gynecologic, and other carcinomas. In addition, CUP tumors might have a different tumor biology that is reflected in IHC results that are different from their "non-CUP" counterparts, for example, CUP cancers may have a higher rate of TTF-1 negative adenocarcinoma (Table 3).

The results reported here suggest that in the near term, microRNA assay may be most helpful in guiding management when IHC studies are nondiagnostic or provides a large differential diagnosis. Therefore, where applicable, MP tests may complement instead of compete against IHC in selected patients.

This study identifies some limitations to the current microRNA-based profiling methods. A disagreement with the clinical and pathologic findings of 16% may probably be acceptable in the context of CUP because of the lack of a true gold standard in most cases, and it certainly lies within the performance of other MP assays for CUP. Second, because the assay may report 2 diagnoses, the clinician may be confronted with 2 different ToO predictions, including some in which the therapeutic management may differ significantly. Even though the KNN results, which are reported as "more likely," have been found to have a higher level of concordance, clinical judgment and integration of other clinical and pathologic data is necessary for determination of the most appropriate patient management. Third, because the assay can only report ToOs that it has been trained to recognize, samples with origins that are not in the tumor panel by definition cannot be classified correctly with the assay. This was noted with 2 samples from the pelvic area, where squamous cell cancer metastases were correctly identified as squamous cell cancers, but the clinically most likely primary location in the genital or anorectal area was not suggested by the algorithms because they had not been trained to recognize these primary tumor locations. Finally, 6 patients presented with a renal carcinoma profile which poses a challenge and an opportunity for MP because IHC can be noncontributory in renal cancers (RCC). Given that RCC has specific therapeutic needs, this CUP subset warrants additional study. These observations highlight the fact that it is important to put assay results in context of clinicopathologic presentation of the patient.

CUP physicians are excited about the emerging role MP is assuming in the identification of ToO. The microRNA assay reported herein does not replace any of the traditional diagnostic procedures done in CUP patients, but does offer additional information in cases in which the clinical and pathologic impressions remain ambiguous. Moreover, MP may provide further clarity when all other clinicopathologic efforts have failed. Given the continued growth and evolution of the field, it is important that investigators gain better understanding of 2 key questions: (i) how consistent is a diagnosis derived from an MP assay with other data that suggest a specific ToO (e.g., are profiling results concordant or discordant with IHC or the diagnosis of a primary that emerges during the

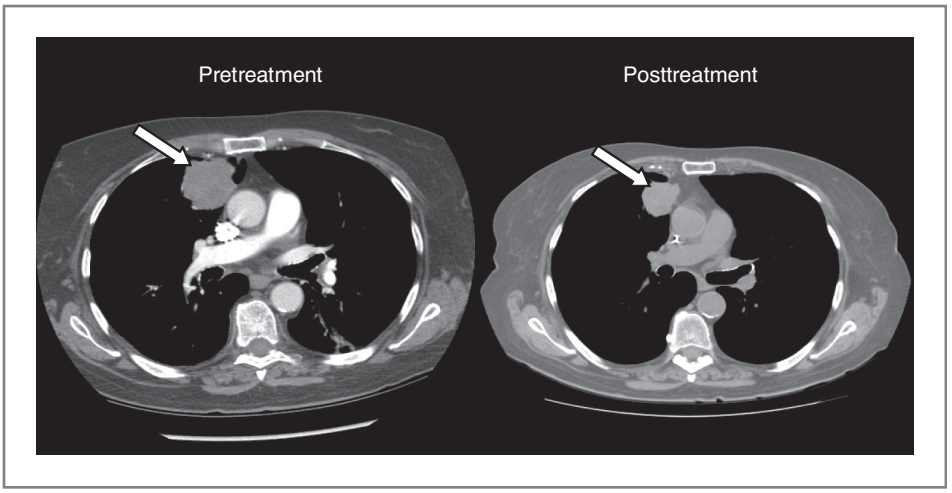

Figure 2. Pre- and posttreatment CT scan of a 73-year-old woman, nonsmoker, who presented with a solitary lung mass (white arrow) with no other evidence of disease. Biopsy-proven moderately differentiated adenocarcinoma with an IHC profile suggestive of metastatic colon cancer–CK20 and CDX-2 were positive, and CK7 and TTF-1 were negative. Two colonoscopies have failed to show a tumor. The microRNA assay diagnosis was colon adenocarcinoma. Patient was treated with 5FU- and oxaliplatin-based therapy followed surgery. The final pathology conducted on the metastatectomy specimen again confirmed the metastatic colon cancer profile.

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observation of the patient (a latent primary) or further detailed work-up; and (ii) will the profile results allow us to abandon empiric first-line cytotoxic chemotherapy and replace it with more specific and effective therapy? Currently, first-line therapies overlap in several cancers, though with emerging newer agents, there will be a growing number of opportunities to use therapies known to be effective against specific cancers. Depending on their perceived robustness, profiling assays could help direct additional lines of therapies. A profiling assay result may also direct further search for the ToO and the use of specific molecular tests that may inform the therapy of specific CUP subsets (e.g., Her-2 IHC/FISH, *EGFR*, or *ALK* mutational analysis; refs.30–32). Finally, CUP has no gold standard, thus in selected patients, independent confirmation of IHC and other diagnostic results with completely independent diagnostic modalities can assist the treating physician in discussing prognosis and the best management with a patient. As an example, Figure 2 illustrates the essence of a CUP cancer–the radiology of this patient depicts a typical primary lung cancer; IHC suggested a GI tract cancer with no primary identified on endoscopy and colonoscopies. The microRNA assay complemented the IHC data and increases the confidence to pursue colorectal therapy options for this patient.

Our ultimate goal is to provide a helpful framework in which profiling and pathology are integrated in a cost and clinically effective algorithm with a positive impact on patient survival and quality of life. This study provides encouraging indications of the value of MP in CUP and suggests that this modality should be evaluated further.

Disclosure of Potential Conflicts of Interest

S. Rosenwald, Y. Spector, A. Chajut, and R. Aharonov are employees of Rosetta Genomics Ltd., Israel, and T.B. Edmonston, J. Macinskas, and D. Cohen are employees of Rosetta Genomics Inc., Philadelphia, PA. G. R. Varadhachary has received a commercial research grant.

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References

1. Culine S, Lortholary A, Voigt JJ, Bugat R, Theodore C, Priou F, et al. Cisplatin in combination with either gemcitabine or irinotecan in carcinomas of unknown primary site: results of a randomized phase II study–trial for the French Study Group on Carcinomas of Unknown Primary (GEFCAPI 01). J Clin Oncol 2003;21:3479–82.
2. Hainsworth JD, Spigel DR, Litchy S, Greco FA. Phase II trial of paclitaxel, carboplatin, and etoposide in advanced poorly differentiated neuroendocrine carcinoma: a Minnie Pearl Cancer Research Network Study. J Clin Oncol 2006;24:3548–54.
3. Brugarolas J. Renal-cell carcinoma–molecular pathways and therapies. N Engl J Med 2007;356:185–7.
4. Hudis CA. Trastuzumab–mechanism of action and use in clinical practice. N Engl J Med 2007;357:39–51.
5. Miller K, Wang M, Gralow J, Dickler M, Cobleigh M, Perez EA, et al. Paclitaxel plus bevacizumab versus paclitaxel alone for metastatic breast cancer. N Engl J Med 2007;357:2666–76.
6. Tsao MS, Sakurada A, Cutz JC, Zhu CQ, Kamel-Reid S, Squire J, et al. Erlotinib in lung cancer–molecular and clinical predictors of outcome. N Engl J Med 2005;353:133–44.
7. Varadhachary GR, Raber MN, Matamoros A, Abbruzzese JL. Carcinoma of unknown primary with a colon-cancer profile-changing paradigm and emerging definitions. Lancet Oncol 2008;9:596–9.
8. Greco FA. Therapy of adenocarcinoma of unknown primary: are we making progress? J Natl Compr Canc Netw 2008;6:1061–7.
9. Pavlidis N, Briasoulis E, Hainsworth J, Greco FA. Diagnostic and therapeutic management of cancer of an unknown primary. Eur J Cancer 2003;39:1990–2005.
10. Pentheroudakis G, Briasoulis E, Pavlidis N. Cancer of unknown primary site: missing primary or missing biology? Oncologist 2007;12:418–25.
11. Ma XJ, Patel R, Wang X, Salunga R, Murage J, Desai R, et al. Molecular classification of human cancers using a 92-gene real-time quantitative polymerase chain reaction assay. Arch Pathol Lab Med 2006;130:465–73.
12. Monzon FA, Lyons-Weiler M, Buturovic LJ, Rigl CT, Henner WD, Sciulli C, et al. Multicenter validation of a 1,550-gene expression profile for identification of tumor tissue of origin. J Clin Oncol 2009;27:2503–8.
13. Rosenfeld N, Aharonov R, Meiri E, Rosenwald S, Spector Y, Zepeniuk M, et al. MicroRNAs accurately identify cancer tissue origin. Nat Biotechnol 2008;26:462–9.
14. Rosenwald S, Gilad S, Benjamin S, Lebanony D, Dromi N, Faerman A, et al. Validation of a microRNA-based qRT-PCR test for accurate identification of tumor tissue origin. Mod Pathol 2010;23:814–23.
15. Tothill RW, Kowalczyk A, Rischin D, Bousioutas A, Haviv I, van Laar RK, et al. An expression-based site of origin diagnostic method designed for clinical application to cancer of unknown origin. Cancer Res 2005;65:4031–40.
16. Gregory PA, Bert AG, Paterson EL, Barry SC, Tsykin A, Farshid G, et al. The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1. Nat Cell Biol 2008;10:593–601.
17. Park SM, Gaur AB, Lengyel E, Peter ME. The miR-200 family determines the epithelial phenotype of cancer cells by targeting the E-cadherin repressors ZEB1 and ZEB2. Genes Dev 2008;22:894–907.
18. He L, Thomson JM, Hemann MT, Hernando-Monge E, Mu D, Goodson S, et al. A microRNA polycistron as a potential human oncogene. Nature 2005;435:828–33.
19. Raver-Shapira N, Marciano E, Meiri E, Spector Y, Rosenfeld N, Moskovits N, et al. Transcriptional activation of miR-34a contributes to p53-mediated apoptosis. Mol Cell 2007;26:731–43.
20. Landgraf P, Rusu M, Sheridan R, Sewer A, Iovino N, Aravin A, et al. A mammalian microRNA expression atlas based on small RNA library sequencing. Cell 2007;129:1401–14.
21. Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, et al. MicroRNA expression profiles classify human cancers. Nature 2005;435:834–8.
22. Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, et al. A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci U S A 2006;103:2257–61.
23. Li J, Smyth P, Flavin R, Cahill S, Denning K, Aherne S, et al. Comparison of miRNA expression patterns using total RNA extracted from matched samples of formalin-fixed paraffin-embedded (FFPE) cells and snap frozen cells. BMC Biotechnol 2007;7:36.
24. Liu A, Tetzlaff MT, Vanbelle P, Elder D, Feldman M, Tobias JW, et al. MicroRNA expression profiling outperforms mRNA expression

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profiling in formalin-fixed paraffin-embedded tissues. Int J Clin Exp Pathol 2009;2:519–27.
25. Greco FA, Spigel DR, Yardley DA, Erlander MG, Ma XJ, Hainsworth JD. Molecular profiling in unknown primary cancer: accuracy of tissue of origin prediction. Oncologist 2011;15:500–6.
26. Varadhachary GR, Talantov D, Raber MN, Meng C, Hess KR, Jatkoe T, et al. Molecular profiling of carcinoma of unknown primary and correlation with clinical evaluation. J Clin Oncol 2008;26:4442–8.
27. Pillai R, Deeter R, Rigl CT, Nystrom JS, Miller MH, Buturovic L, et al. Validation and reproducibility of a microarray-based gene expression test for tumor identification in formalin-fixed, paraffin-embedded specimens. J Mol Diagn 2011;13:48–56.
28. Dennis JL, Hvidsten TR, Wit EC, Komorowski J, Bell AK, Downie I, et al. Markers of adenocarcinoma characteristic of the site of origin: development of a diagnostic algorithm. Clin Cancer Res 2005;11: 3766–72.
29. Krishna M. Diagnosis of metastatic neoplasms: an immunohistochemical approach. Arch Pathol Lab Med 2010;134:207–15.
30. Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science 2004;304:1497–500.
31. Shaw AT, Yeap BY, Mino-Kenudson M, Digumarthy SR, Costa DB, Heist RS, et al. Clinical features and outcome of patients with non-small-cell lung cancer who harbor EML4-ALK. J Clin Oncol 2009;27:4247–53.
32. Tanizaki J, Okamoto I, Takezawa K, Tsukioka S, Uchida J, Kiniwa M, et al. Synergistic antitumor effect of S-1 and HER2-targeting agents in gastric cancer with HER2 amplification. Mol Cancer Ther. 2010;9: 1198–207.

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