Case Reports in Oncology

Case Rep Oncol 2023;16:778–784
DOI: 10.1159/000533376
Received: April 13, 2023
Accepted: July 26, 2023
Published online: September 1, 2023

Case Report

Utility of RNA Expression to Determine the Tissue of Origin of Malignancies with an Inconclusive Histopathology

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Keywords
RNA sequencing · Tissue of origin · Carcinoma of unknown primary · Genomic data

Abstract
We present 2 cases of cancer of unknown origin in which RNA-based cancer classification testing provided vital insight and directed treatment management. The tissue of origin could not be determined in both of these patients utilizing morphology and immunohistochemical analysis of the tissue samples. Next-generation sequencing and tumor-of-origin testing using an RNA-based molecular cancer classifier were performed to elucidate the possible tissue of origin. A 61-year-old male with a history of localized basal cell carcinoma presented with a 4.4-cm axillary lymph node in addition to upper extremity edema and supraclavicular lymphadenopathy. RNA-based tumor origin testing revealed skin basal or squamous cell carcinoma as the likely tissue of origin, with a probability of 97%. He received vismodegib, a hedgehog inhibitor, after progression on cemiplimab and experienced a partial response by RECIST criteria, which is currently ongoing for over a year. A 74-year-old female patient with a remote history of ovarian cancer for which she underwent resection and adjuvant chemotherapy presented 15 years later with abdominal pain. The diagnostic workup revealed a 2-cm pancreatic mass and enlarged peritoneal lymph nodes. RNA sequencing revealed a 99% likelihood of the tissue of origin being serous ovarian carcinoma. Subsequently, she underwent surgery and adjuvant chemotherapy and is currently in remission with letrozole maintenance. Genomic data already plays a crucial role in therapeutic decision-making for individuals with cancer. These cases highlight the complementary role of genomic data in the diagnostic workup of cancer, leading to favorable patient outcomes.

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DOI: 10.1159/000533376

Introduction

Carcinoma of unknown primary (CUP) is a heterogeneous group of cases for which the tissue of origin cannot be determined [1]. CUPs comprise approximately 2–3% of all cancers diagnosed globally and are generally treated with empiric chemotherapy. Unfortunately, the overall prognosis is generally poor, as only a minority of these patients respond well to empiric treatment. At approximately 8 months, the median overall survival for these individuals with CUP is dismal [2, 3].

Physical examination, serum tumor markers, imaging modalities such as CT, MRI, PET, mammograms, and endoscopic evaluation are utilized to identify the site of origin when morphology and immunohistochemical (IHC) staining are inconclusive [3]. This workup tends to be intensive, often inconclusive, and leads to delays in the delivery of care. In three studies involving patients with metastatic disease, IHC findings failed to concur with the site of origin in nearly 33% of cases [4].

In this era of cancer genomics, gene expression assays to identify the site of origin are becoming widely available. Tumor-of-origin testing platforms use tumor RNA expression patterns to predict the most probable cancer type(s). The origin testing workflow is shown in Figure 1. Microarrays or real-time reverse transcriptase polymerase chain reaction techniques are utilized to measure mRNA transcripts [5]. Most commercial genomic testing platforms have developed large databases of clinical data and RNA expression profiles of patients whose genomic data have been sequenced by them, and these libraries are currently used as reference for determining tissue of origin in individuals with CUP [4, 6]. Here, we describe two fascinating cases where a commercially available RNA-based molecular cancer classifier was successfully used to determine the site of origin after an exhaustive initial workup failed to do so.

Case 1

A 61-year-old male with hypertension, diabetes, and a history of chest wall basal cell carcinoma (BCC) resection presented 2 years later with edema of the right upper extremity. Venous Doppler revealed subclavian and brachial vein thrombosis, and he was initiated on apixaban. A 4.4-cm firm, nonmobile right axillary mass was noted upon physical examination. CT chest revealed lymphadenopathy in the right axillary and supraclavicular regions and several pulmonary nodules. No extrathoracic disease was noted on CT imaging of the abdomen and pelvis. A subsequent PET scan showed hypermetabolic right axillary and supraclavicular adenopathy, but no primary malignancy was identified. Ultrasound-guided core needle biopsy of the right axillary lymph node revealed metastatic carcinoma. The IHC revealed that stains were negative for TTF-1 and positive for CAM 5.2, p40, and p63. The staining pattern and morphology did not suggest a definite tissue of origin. Next-generation sequencing testing was performed on tissue obtained from the axillary lymphadenopathy, and the following pathogenic genetic alterations were discovered (Table 1):

Table 1. Pathogenic genetic alterations

ATM c652C>T stop gain	*NF1 c.3610C>T missense variant*
ARID1A c.4609C>T stop gain	*TP53 c.530_531delinsTT missense variant*
PTCH1 c.2362+1G>A splice region variant	*SPOP c.79-1G>A splice region variant*
TERT c.146C>T variant – promoter mutation	*PTEN c.116C>T missense variant*
PTPRD c.807G>A stop gain	*CEBPA c.247C>T stop gain*
NSD1 c.6328C>T stop gain	*CREBBP c.4078C>T stop gain*

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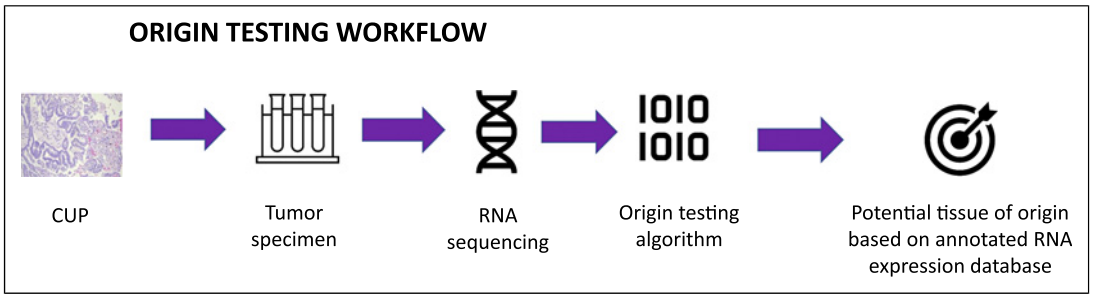

Fig. 1. Tumor origin testing workflow diagram. (i) Tissue or blood sample is collected; (ii) RNA sequencing is performed on the sample; (iii) data are compared with large internal database of annotated molecular data; and (iv) site of origin with high probability is predicted for guiding site-specific management.

The tumor mutational burden (TMB) was 20.1 mutations/mB, PD-L1 expression testing was performed by IHC assay utilizing monoclonal mouse anti-PD-L1, clone 22C3, and came back negative (<1%). The tumor proportion score (TPS) for PD-L1 was <1%. The combined proportion score (CPS) for PD-L1 was <1%. RNA-based tumor origin testing predicted this to be squamous cell or BCC in origin with an estimated probability of 97%. Given the patient's history of BCC, metastatic recurrence of BCC was favored as the most likely diagnosis. The patient was initiated on cemiplimab, an anti-PD-1 monoclonal antibody.

The patient tolerated cemiplimab well, experiencing only a grade 1 maculopapular rash. CT chest with contrast after four cycles of cemiplimab revealed stable disease by RECIST criteria. Six cycles and approximately 4 months after treatment initiation with cemiplimab, a PET/CT revealed increase in the size of the supraclavicular lymph node, consistent with a progressive disease by RECIST criteria. The patient was initiated on vismodegib, an oral hedgehog signaling pathway inhibitor approved for use in advanced BCC [7]. Chest and neck CT performed a month after initiation of vismodegib revealed a decrease in the right supraclavicular lymph node size from 2.4 × 2.0 cm to 0.8 × 0.7 cm, in addition to the resolution of right axillary lymphadenopathy. No new lesions were identified, consistent with partial treatment response by RECIST criteria. The patient remains on vismodegib therapy over a year after treatment initiation, with continued treatment response seen on subsequent scans.

Case 2

We present the case of a 73-year-old female with prediabetes, insomnia, hypertension, and stage III adenocarcinoma of the ovary. For treatment of ovarian carcinoma, she underwent a hysterectomy with bilateral salpingo-oophorectomy and omentectomy followed by adjuvant carboplatin/paclitaxel. Fifteen years later, she presented to the emergency department with a chief complaint of severe abdominal pain and ongoing nausea and vomiting for a few days before the presentation. A CT scan of the abdomen and pelvis revealed a lobulated and exophytic hypoattenuating lesion with internal septations and peripheral calcifications arising from the pancreatic tail. MRCP revealed a mixed cystic and solid mass involving the pancreatic tail, which favored pancreatic cystadenoma or cystadenocarcinoma. A CT chest with contrast was unremarkable. A subsequent PET/CT revealed metabolic activity within the periphery of the known pancreatic tail lesion with max SUV 4.4, in addition to partly calcified upper abdominal and retroperitoneal lymph nodes with a max SUV of 9. Fine-needle aspirate biopsy of the pancreatic mass revealed a serous neoplasm, with IHC stains positive for estrogen receptor (ER). The tissue of origin could not be determined by morphology. CA 125 level was normal. Given ER positivity, a mammogram and breast MRI were performed to identify a possible breast primary and revealed no abnormalities. Given the

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rarity of ovarian cancer recurrence 15 years after initial resection, tumor-of-origin testing via a commercially available next-generation sequencing assay, which included RNA sequencing for tumor of origin, was performed. PD-L1 IHC testing was performed using the Dako PD-L1 22C3 clone. Genomic analysis of the tumor specimen revealed PD-L1 TPS less than 1, CPS 3, and TMB 4.2 mutations/mB. The tumor sample was mismatch repair proficient and microsatellite stable. RNA sequencing-based origin testing predicted the malignancy to be serous ovarian carcinoma with an estimated probability of 99%.

She underwent a distal pancreatectomy with splenectomy. Pathology from surgical resection revealed a low-grade serous carcinoma involving the peripancreatic soft tissue and lymph nodes. IHC staining was positive for CK7, WT-1, PAX-8, and ER was strongly positive (95%) and negative for CK20 and CDX-2. She subsequently received adjuvant carboplatin and paclitaxel, followed by letrozole maintenance. She continues to be on letrozole maintenance, and imaging reveals remission over a year after the surgical resection of the recurrent malignancy.

Methods

We utilized Tempus Tumor Origin (TO) testing. The Tempus TO test uses tumor RNA expression to estimate the patient's most probable cancer type from 68 possible cancer types. The Tempus TO test was created using an extensive internal database of clinical and annotated molecular tumor data. The probable site of origin is determined by comparing the tumor specimen to this reference database. The PD-L1 IHC was performed to determine the TPS and combined positive score using the DAKO PD-L1 22C3 clone. The TPS is calculated as a percentage of viable tumor cells staining for PD-L1 relative to all viable tumor cells. The CPS is calculated as the number of PD-L1 staining cells in the tumor microenvironment divided by the total viable tumor cells multiplied by 100. The authors have completed the CARE Checklist for this case report, which is attached as online supplementary material (for all online suppl. material, see https://doi.org/10.1159/000533376).

Discussion

CUPs are characterized by metastatic disease with no identifiable primary tumor at presentation [3]. Identifying the site of origin is paramount, as the tissue of origin governs the treatment of cancers [2, 3]. Treatments based on site of origin lead to improved outcomes, such as progression-free and overall survival, as seen in numerous randomized controlled trials [8]. It has been long assumed that CUP treatment response would be comparable to the response of predicted primary tumor [9]. A meta-analysis using a random-effects model and inverse variance method found that site-specific treatment had a trend toward improved OS compared to empiric treatment for CUP (HR = 0.73; 95% confidence interval: 0.52–1.02) [9]. A large prospective trial used tumor genomic profiling results to guide site-specific therapy and revealed a 98% rate for successfully predicting the site of origin. The study revealed an improvement in median overall survival compared to historical comparison data of patients with CUP who received empiric chemotherapy [10].

IHC markers help determine the tumor lineage. Cytokeratin intermediate filaments are often used to classify tumors according to the site of origin [11]. However, these are not completely specific. Even PSA can be elevated in cancers other than the prostate, such as salivary gland carcinoma [12]. A retrospective study evaluated six serum biomarkers,

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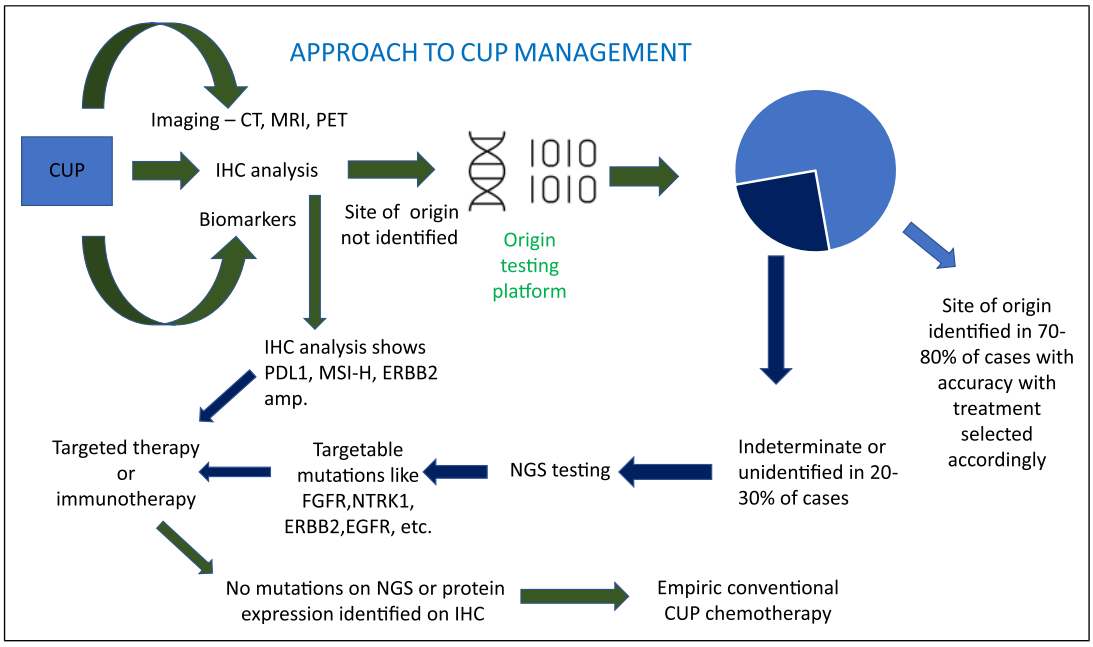

Fig. 2. Approach to CUP management. In cases where diagnosis is not established with imaging, biomarker, and immunohistochemistry analysis, origin testing platform may be used which helps identify site in most cases leading to site-specific management. In remaining few cases, NGS testing and IHC may help find targets for therapy; if unsuccessful, then conventional empiric CUP chemotherapy is used. NGS, next-generation sequencing.

including CEA, CA 19-9, CA 15-3, CA 125, beta-HCG, and alpha-fetoprotein in 85 CUP patients and showed that none of these markers were predictive of response to chemotherapy or survival; there was a general nonspecific overexpression of these markers in CUP [13].

McMillan et al. [14] retrospectively assessed the role of abdominal CT in CUP patients and found that it identified the primary tumor in less than 50% of cases. FDG PET is commonly used in CUP; however, retrospective studies have shown that it identifies the primary tumor in only a fraction of patients [15, 16]. In clinical scenarios where a breast primary is suspected, such as adenocarcinoma discovered in an axillary lymph node, a primary breast lesion can be discovered by imaging modalities such as a breast MRI. Still, the breast MRI may fail to discover the primary lesion in approximately a quarter of the cases [17]. Thus, while imaging and tumor markers are useful for identifying the site of origin in certain clinical scenarios, there is an unmet need to determine the tissue of origin when traditional modalities are unrevealing. The approach to CUP management is delineated in Figure 2.

The origin testing platform used in the cases above utilizes a machine learning RNA-based molecular cancer classifier to identify the most likely cancer type from multiple possible cancer types with an accuracy of over 90% [18]. A retrospective study analyzed 289 patients with CUP diagnosis and revealed that cholangiocarcinoma, lung adenocarcinoma, and pancreatic adenocarcinoma were the most common cancer types, as predicted by the origin testing algorithm, and this led to an alteration in therapeutic decision-making in over 80% of the patients [19].

Although high TMB is considered tumor-agnostic and predictive of response to immunotherapy in several malignancies, it may not be useful across all malignancies [20]. The first patient was initially treated with an immune checkpoint inhibitor, a site-specific therapy for BCC. However, the patient progressed after a short duration of 4 months.

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Furthermore, he was initiated on another site-specific therapy for BCC, vismodegib, and experienced a partial response sustained for over a year. While BCC is the most common malignancy in Caucasians, metastatic BCC is relatively rare, with an incidence of less than 1% [21]. In the second case, tumor-of-origin testing led to the diagnosis of ovarian carcinoma, which was treated, and the patient has been in remission for over a year on letrozole maintenance therapy. Ovarian cancer recurrence presenting as ER-positive pancreatic mass 15 years after initial resection is a rare clinical scenario. Furthermore, studies have shown that letrozole maintenance therapy has superior relapse-free survival for patients with ovarian cancer compared to placebo [22, 23]. The patient would have experienced a significantly higher likelihood of relapse after surgery and chemotherapy without letrozole maintenance treatment. These diagnoses were unlikely to have been considered before the availability of tumor-of-origin testing utilizing RNA expression. Thus, genomic data may help determine the tissue of origin when other modalities fail and lead to site-specific management, which may enhance patient outcomes.

Conclusion

When the site of origin is not established with advanced imaging tools, biomarker studies, and IHC analysis, validated RNA-based molecular testing may be performed to help determine the site of origin with high probability. Such testing lends diagnosis of the site of origin in most cases and enables the provision of site-specific therapy. Thus, RNA-based tumor-of-origin testing may ultimately culminate in more favorable clinical outcomes for patients.

Statement of Ethics

Written informed consent was obtained from the patients for the publication of this case report and any accompanying images. Ethical approval is not required for this study in accordance with local guidelines of Saint Luke's Hospital.

Conflict of Interest Statement

There is no conflict of interest for Himil Mahadevia, Lara Kujtan, Marc Roth, Parth Sharma, Eric Ewing, Jennifer R. Buckley, or Dhruv Bansal.

Funding Sources

There was no funding source for study design, data collection and analysis, or manuscript preparation.

Author Contributions

Conception and design: Dhruv Bansal and Himil Mahadevia; collection and assembly of data: Himil Mahadevia, Lara Kujtan, and Marc Roth; and data analysis and interpretation: Himil Mahadevia, Dhruv Bansal, Parth Sharma, Eric Ewing, Jennifer R. Buckley, Lara Kujtan, and Marc Roth.

Data Availability Statement

The patient data are available through Saint Luke's Hospital's electronic medical records. Further inquiries can be directed to the corresponding author.

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