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Molecular Profiling for Druggable Genetic Abnormalities in Carcinoma of Unknown Primary

Introduction

Carcinoma of unknown primary (CUP) represents a distinct disease entity encompassing 3% to 5% of all malignancies and is traditionally associated with poor prognosis with median survival of 6 to 9 months.[1] One of the key management challenges is identification of the origin of metastases, in order to facilitate rational treatment based on the primary tumor type. In this regard, tissue morphology and immunohistochemical profiles have become crucial discriminatory tools,[2] with the primary aim of identifying potentially curable or chemosensitive tumors, as well as those amenable to effective, minimally toxic therapies (eg, androgen deprivation therapy in prostate cancer or endocrine therapy for estrogen-positive breast cancer).[1,3] Herein we report a case of CUP, where molecular testing for a panel of somatic mutations discovered a druggable alteration that resulted in favorable outcome after instituting the corresponding targeted therapy.

Case Report

Our patient was a 50-year-old male, light-smoker, who presented with fatigue, weight loss, and neck swelling. Physical examination revealed no thyroid mass, but bulky neck lymphadenopathy. Staging work-up with computed tomography scan of the thorax, abdomen, and pelvis revealed mediastinal, neck, axillary lymphadenopathy, and no other distant metastases (Fig 1A). No pulmonary nodules or primary mass were seen. Initial fine-needle aspiration of the supraclavicular node was reported as poorly differentiated carcinoma. A further excision biopsy confirmed epithelial origin, and immunohistochemistry showed malignant cells strongly positive for cytokeratin 7 (CK7), weakly positive for CK5/6 and p63, but negative for CK20, CDX2, thyroid transcription factor-1, prostate-specific antigen, and prostatic acid phosphatase. Esophagastroduodenoscopy and colonoscopy did not reveal any abnormalities. As part of a research protocol, mutation profiling using the Sequenom massarray OncoCarta panel v1.0 (Sequenom, San Diego, CA) was performed to screen for 238 somatic mutations across 19 driver oncogenes that include *EGFR*, *KRAS*, and *BRAF*.

Our patient was initially commenced on gemcitabine-carboplatin chemotherapy and after two cycles, there was no significant reduction in size of lesions. At this juncture, the result from the Sequenom analysis revealed a thymine to guanine transversion in nucleotide position 2573 resulting in amino acid change at codon 858 from leucine to arginine (*L858R*) in exon 21 of *EGFR* gene, with mutant frequency of 71.3% (Fig 2A; lower panel), that was absent in the germline control (Fig 2A; upper panel). This was validated with Sanger sequencing in a College of American Pathologists–accredited laboratory, as seen in the electropherograms of the forward and reverse sequences, confirming the single nucleotide substitution at position 2573 from thymine to guanine (with a dominant mutant peak, red arrows) that resulted in the epidermal growth factor receptor tyrosine kinase inhibitor (EGFR TKI) –sensitizing *L858R* mutation. (Fig 2B). He was commenced on gefitinib monotherapy, with significant shrinkage of target lesions amounting to partial response (Fig 1B; indicated by red arrows), and experienced a progression-free survival (PFS) of 11 months.

Discussion

Poorly differentiated carcinomas of unknown primary origin are typically associated with a bleak prognosis. Much effort has been directed at improving our ability to delineate the site of primary tumor, for instance through application of high throughput genetic profiling using either mRNA[4] or more recently microRNA expression profiling.[5,6] Such studies have demonstrated high classification accuracies (> 90%), resulting in the development of reverse-transcription polymerase chain reaction (RT-PCR) based assays to determine organ-of-origin for directing treatment of CUP.[7] In a proof-of-concept study comparing physician choice versus gene expression–

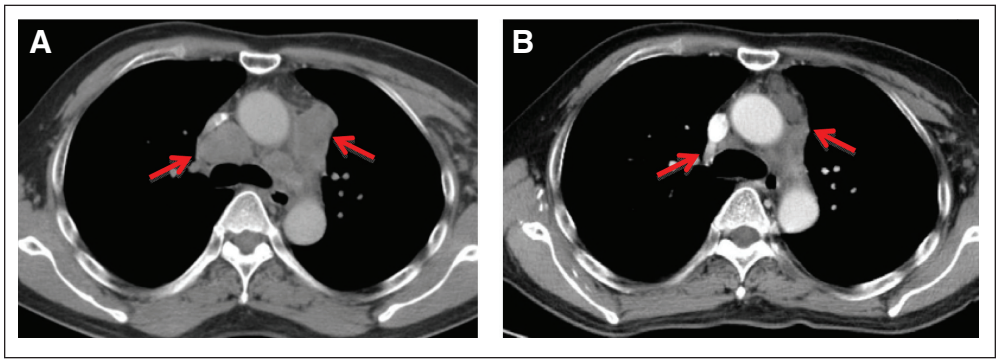

Fig 1.

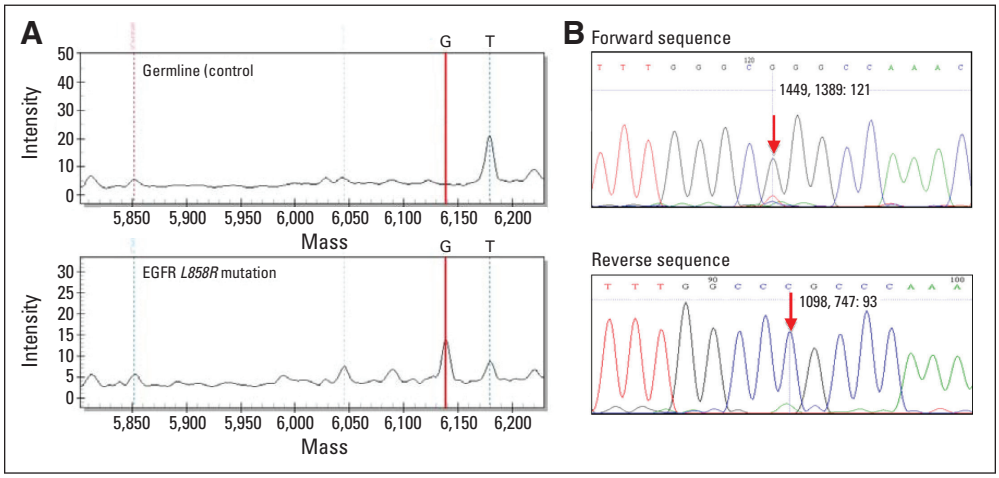

Fig 2.

based treatment allocation, overall response rate was an encouraging 32%, and PFS at 7.4 months.[8]

More recently, the advent of targeted therapeutics and companion diagnostics have led to a paradigm shift in the treatment of solid tumors.[9] Increasingly, tissue of origin and histological subtype are insufficient, and further evaluation for genetic alterations that are therapeutically amenable to targeted therapies mandated. Following on the successes of EGFR and ALK inhibitors, *EGFR* mutations and *ALK* translocations are now routinely evaluated in lung adenocarcinomas.[10] BRAF targeting therapies have also recently entered the clinic. In the pivotal phase III trial of vemurafenib, a drug optimized through fragment-based design, survival benefit was observed in a specific molecularly selected group of metastatic melanoma harboring $BRAF^{V600E}$ – rendering a disease traditionally associated with high aggressiveness and poor outcomes eminently treatable.[11,12]

Moreover, such mutations can occur in diverse tumor types. A literature review of all tumor types harboring EGFR TKI–sensitizing mutations include triple-negative breast cancers (7%),[13] esophageal cancers (12%),[14] and up to 30% in papillary thyroid cancers.[15] Of note, both *EGFR* and *BRAF* mutations have been reported in anaplastic thyroid cancers, representing potentially druggable targets in a typically chemotherapy-refractory poorly differentiated tumor.[16,17] Furthermore, emerging literature is beginning to reveal the molecular taxonomy of various solid tumors, where commonly implicated driver alterations such as *KRAS*, *PI3KCA* and *BRAF*,[17-19] are now prime targets for novel therapeutic agents. While these studies highlight the potential applicability of pathway-driven therapeutics in the treatment of poorly differentiated epithelial cancers, one caveat that has yet to be fully unraveled is the relevance of the cellular context of such alterations.

For example, despite response rates as high as 48% to 81% in $BRAF^{V600E}$ cutaneous melanoma,[12,20] only one out of 19 patients with colon cancers (5%) harboring the same mutation treated with vemurafenib responded.[21] Possible reasons for this discrepancy include the presence co-occurring mutations in parallel oncogenic pathways conferring resistance (eg, *PTEN* or *PI3KCA* mutations),[22] as well as discordance in *BRAF* mutant status between primary and metastatic sites (38.5%).[23] More recently, high EGFR expression in colon cancer was identified as a potential resistance mechanism to vemurafenib through elegant RNA interference experiments that revealed strong synergy with EGFR inhibition in colon cancer models. Furthermore, both gefitinib or cetuximab could circumvent this feedback activation—thereby providing a rational basis for future combinatorial approaches.[24]

Such studies highlight the complexity and multidimensionality of developing precise predictive biomarkers and underscore the importance of applying tools that can interrogate cancer genomes, transcriptomes and proteomes with sufficient resolution. Moving forward, greater depth and breadth of genomic analysis can be achieved with next-generation sequencing platforms (eg, Ion Torrent or whole exome sequencing), potentially even down to single cell resolution,[25] and will no doubt provide new insights to the interactions between individual genes and cell types. Further advances are anticipated with state-of-the-art avenues for minimally invasive tissue acquisition such as circulating tumor cells, that will enable depiction of evolving genetic alterations and resistance mechanisms while on treatment.[26]

In conclusion, our case illustrates the role of molecular testing for a druggable somatic alteration (*EGFR L858R* mutation) in a patient with CUP that predicted for response to a currently available approved drug (gefitinib). Other examples include *KIT* mutations (imatinib), *BRAF* mutations (vemurafenib), *HER2* amplification (lapatinib), and *ALK*, *ROS1* translocations (crizotinib)—and this list will no doubt continue to expand.[27] Given the myriad of possible presentations in a disease that portends an otherwise poor prognosis with combination chemotherapy approaches, screening CUP for druggable alterations should be considered—based on availability of validated molecular diagnostic assays and access to drugs. With the advent of high-throughput platforms that allow multiplexed analysis of drug-response biomarkers, a critical challenge remains in implementing such technology to the clinic in a rational, cost-effective and robust manner. Expedient identification of molecular subgroups of patients will ultimately facilitate prospective clinical validation of pathway-driven therapeutics—such as VE-Basket study of vemurafenib in *V600* mutation positive cancers (NCT 01524978)—and promise to further improve stratified management of solid tumors including CUP.

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