CARCINOMA OF UNKNOWN PRIMARY – RARE BUT RIPE FOR HELP FROM GENOMICS?

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Abstract

The treatment of most cancer patients is based on first determining where the cancer arose anatomically – lung, breast, prostate and so on. For a group of patients with disseminated disease, this crucial starting point is missing. Carcinoma or adenocarcinoma of unknown primary is a diagnosis made where the likely site of origin cannot be detected. Here we describe the use of molecular genetic tests to improve both the identification of site of origin for carcinoma of unknown primary patients and possible novel treatment options based on the presence of specific mutations. We outline the Solving Unknown Primary Cancer study, which is aimed at determining the utility of molecular testing in carcinoma of unknown primary.

Carcinoma of unknown primary (CUP) accounts for approximately 3-5% of all cancer diagnoses,[1] is the sixth most common cause of cancer death in Australia,[2] and the fourth most common worldwide.[3] CUP encompasses a heterogeneous group of metastasised tumours for which, following extensive investigation, a primary anatomical site of origin cannot be identified. CUP has among the lowest 12-month survival rate of all cancers, with only 23% expected to survive beyond one year.[2] Because cancer treatment is predominantly based on site of origin, CUP poses significant challenges when applying conventional treatment paradigms. Identifying the primary tumour is also important to allow patients affordable access to drugs and to enter clinical trials. The lack of a definitive primary anatomical site often restricts treatment options to palliative chemotherapy, which lacks the effectiveness and precision of modern day cancer medicine,[4] and results in significant patient uncertainty and distress.

Can modern technologies improve CUP outcomes?

Cancer medicine is being transformed by the use of molecular analyses, including rapid and comprehensive DNA sequencing, to diagnose cancer with increased precision and predict the best therapeutic approaches for specific cancer types.[5-7] Targeted treatment approaches can be instituted when specific mutations are detected in a tumour sample for which specific small molecule inhibitors have been developed. The presence of so called actionable mutations in a sample may have implications for diagnosis, prognosis and prediction of therapeutic response.[8]

The recent development of advanced genomic tools presents a unique opportunity to improve the current management of CUP by implementing an approach that integrates molecular tests to both define the tissue of origin and also identify therapeutically actionable mutations. The use of molecular tumour profiling to identify tissue of origin and profiling for actionable mutations could form the basis of a new standard evaluation paradigm for CUP patient assessment.

Tissue of origin molecular profiling

Pattern of expression of the ~20,000 genes in the human genome is highly cell and tissue lineage-dependent, and individual cellular gene signatures are generally retained during cancer development, even in those cancers that metastasise from the primary tissue site.[9-13] The retention of tissue-specific expression by cancer forms the basis of a simple concept whereby a database of gene expression is developed from a range of solid cancers against which an unknown tumour, such as a CUP, can be bioinformatically referenced to predict the primary site of origin.[13,14] Current commercially available tests that implement molecular tumour profiling include CUPGuideTM and bioTheranostics Cancer Type ID. These assays use probability scores to predict the tissue of origin or at least reduce the options to a narrower list of differential diagnoses.[13,14] Evaluation of the tests is hampered by the fact that there is no definitive and widely-used standard for the diagnosis of CUP and therefore understanding the accuracy of a CUP prediction is problematic. Assay development typically relies on the ability to predict site of origin for a series of known cancers in a blinded fashion. For example, the CUPGuide assay can predict site of origin of known metastatic deposits with 89% accuracy.[8] Another approach is to test a cohort

of samples where site of origin was initially uncertain, but became apparent at some later stage through additional clinical or diagnostic information. In such cases, the use of a site of origin test could have reduced the time to definitive diagnosis and implementation of directed therapy.

Both CUPGuide and Cancer Type ID report findings as high, moderate, low or no significant match, based on the similarity levels of a test tumour to a known metastatic tumour. CUPGuide emphasises the additional use of detailed clinical, histological and radiological findings, together with analysis of gene expression signatures to accurately identify the site of origin of a tumour.[13,15] The use of similarity scores is not without its challenges and complexities, and results can be inaccurate or inconclusive.[16] Results may predict several likely sites of origin with only 'moderate' similarity, and may therefore fail to provide definitive information for clinicians and patients. As described below, we have recently used large-scale DNA sequencing to identify potentially actionable mutations.[17] This assay also yields information about carcinogen exposure, such as tobacco smoke or sunlight exposure, and this may also narrow the search of potential site of origin of a CUP sample.

Profiling for actionable mutations – next generation sequencing

DNA sequencing technologies have been important in identifying patients with inherited predisposition to cancer.[18] More recently, sequencing of the human genome has led to a personalised approach to oncology that is now being used clinically to predict the efficacy of drugs and to identify variants that guide therapeutic selection.[19-21] Examples include EGFR mutation or amplification in non-small cell lung cancer and colorectal cancer to determine patient suitability for EGFR tyrosine kinase inhibitors, or BRAF mutation detection to predict likely response of thyroid cancer or melanoma to BRAF inhibitors such as vemurafenib.[22-27] Compared with traditional Sanger sequencing, which has been limited to single gene 'hot spots', the recent development of massively parallel or next generation sequencing (NGS) has reduced cost and increased sequencing output enormously, allowing real-time assessment of hundreds to thousands of genes in individual patients, including those with CUP.

Using targeted exome capture of more than 700 genes followed by NGS, we have identified potentially clinically actionable mutations in 75% (12 out of 16 cases) of CUP patients, where a likely site of origin could not be identified.[17] The strength of the prediction of a clinical approach in this small retrospective series varied and more cases need to be evaluated to determine the clinical applicability of mutation profiling in CUP, including identification of the most common mutations that are likely to be encountered in these patients.

Integrating genomics into the treatment of CUP

In 2013-14, Cancer Australia and the Victorian Cancer the Agency funded the 'Solving Unknown Primary Cancer' (SUPER) study. It is collecting clinical data and psychosocial experiential information as a foundation resource for future studies. It will also identify the unique psychosocial aspects of CUP, comparing quality of life, communication and supportive care needs of patients with CUP to matched control cases with advanced cancer of a known primary. Additionally, the study is integrating the two approaches of gene expression profiling and NGS DNA sequencing to investigate their utility in the optimal clinical assessment of CUP. When reporting real-time molecular evaluation of CUP tumours, using both the diagnostic genetic expression profiling and mutation profiling, four possible outcomes of the two tests are possible (table 1).

Table 1: *Possible outcomes of molecular tests for tissue of origin (CUPGuide) and a search for actionable mutations can yield results where neither are informative (outcome 1), tissue of origin is predicted but no actionable mutations are identified (outcome 2), actionable mutations are identified but tissue of origin yield no result (outcome 3); or both tests yield a positive result identifying both tissue of origin and actionable mutations (outcome 4).*

Outcomes	Tissue of origin test	Mutation profiling for actionable mutations
1	Tissue of origin predicted	No actionable mutation/s identified
2	No tissue of origin predicted	Actionable mutation/s identified
3	Tissue of origin predicted	Actionable mutation/s identified
4	No tissue of origin predicted	No actionable mutation/s identified

Determining the frequency of outcomes of the tests across a large patient cohort is critical in designing a future randomised clinical intervention trial based on test findings. SUPER will identify common mutations across a large series of CUP patients to inform trial design, particularly which drugs and industry relationships are likely to be most needed. In the meantime, SUPER will obtain information from clinicians following the provision of molecular information to measure the clinical impact of both assays in altering treatment plans and patient outcomes. SUPER will also provide practical information, including how often biopsies are able to provide sufficient material for successful application of the tests and approaches to assay development with the limited material often available for CUP patients. Where actionable mutations are found, we will record the circumstances where a suitable drug could be accessed, whether through an existing clinical

trial, compassionate access or through patient payment. We also expect to find germline mutations that are associated with increased genetic risk of cancer that may both explain the development of CUP in some patients and provide useful information to family members for cancer risk-reduction.

Future for CUP

Past studies have indicated that in a majority of CUP cases, a primary tumour is found in post-mortem autopsy,[28] suggesting that current diagnostic methods are not advanced enough to effectively manage or provide CUP patients with a targeted therapeutic approach. There is a clear need for the integration of genomics in the diagnosis and management of CUP, and more specifically molecular profiling for both site of origin and actionable mutations has much to contribute in delineating the many complexities of this diagnosis.

Governing ways to integrate this approach into current management will be essential in successfully advancing the diagnosis and treatment of patients with CUP. Within this setting, there are likely to be complexities that will need to be overcome. While actionable mutations are likely to be identified, accessibility to targeted drugs available may be problematic. Currently, it is not uncommon for oncologists to label a CUP patient as having a specific tumour type, even when diagnostic uncertainty remains, to facilitate provision of treatment with a drug where rebated access is limited to specific cancer types.[29]

The translation of potential targeted drugs from one setting to another is not always easily applied. For example, vemurafenib can successfully inhibit BRAF (V600E) oncoprotein in melanoma, but has little effect on colon cancer patients who have the same BRAFV600E mutation.[30] Molecular tumour boards, which involve scientists, bioinformaticians, molecular pathologists, and clinicians, are needed to interpret the findings of molecular tests and to establish a standardised approach in incorporating molecular profiling results into the management of CUP. Despite these challenges, it appears likely that incorporating molecular profiling will improve the quality of life and outcomes of CUP patients in the near future.

References

1. Fizazi K, Greco FA, Pavlidis N, et al. Cancers of unknown primary site: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2011;22 Suppl 6:vi64-8.
2. Australian Institute of Health and Welfare. Cancer in Australia 2010: in brief. Canberra: Australian Institute of Health and Welfare, 2010.
3. Pavlidis N, Pentheroudakis G. Cancer of unknown primary site. The Lancet 2012;379(9824):1428-35.
4. Greco FA, Oien K, Erlander M, et al. Cancer of unknown primary: progress in the search for improved and rapid diagnosis leading toward superior patient outcomes. Ann Oncol 2011.
5. Meric-Bernstam F, Mills GB. Overcoming implementation challenges of personalized cancer therapy. Nature Reviews Clinical Oncology 2012;9(9):542-48.
6. Schilsky RL. Personalized medicine in oncology: the future is now. Nature reviews Drug discovery 2010;9(5):363-66.
7. Ross JS, Schenkein DP, Pietrusko R, et al. Targeted therapies for cancer 2004. Am J Clin Pathol 2004;122(4):598-609.
8. Dancey JE, Bedard PL, Onetto N, et al. The genetic basis for cancer treatment decisions. Cell 2012;148(3):409-20.
9. Dennis JL, Hvidsten TR, Wit EC, et al. Markers of adenocarcinoma characteristic of the site of origin: development of a diagnostic algorithm. Clin Cancer Res 2005;11(10):3766-72.
10. Ramaswamy S, Tamayo P, Rifkin R, et al. Multiclass cancer diagnosis using tumor gene expression signatures. Proceedings of the National Academy of Sciences 2001;98(26):15149-54.
11. Shedden KA, Taylor JM, Giordano TJ, et al. Accurate molecular classification of human cancers based on gene expression using a simple classifier with a pathological tree-based framework. Am J Pathol 2003;163(5):1985-95.
12. Su AI, Welsh JB, Sapinoso LM, et al. Molecular classification of human carcinomas by use of gene expression signatures. Cancer Res 2001;61(20):7388-93.
13. Tothill RW, Kowalczyk A, Rischin D, et al. An expression-based site of origin diagnostic method designed for clinical application to cancer of unknown origin. Cancer Res 2005;65(10):4031-40.
14. Erlander MG, Ma X-J, Kesty NC, et al. Performance and clinical evaluation of the 92-gene real-time PCR assay for tumor classification. J Mol Diagn 2011;13(5):493-503.
15. Hainsworth JD. Molecular tumor profiling in the diagnosis of patients with carcinoma of unknown primary site: retrospective evaluation of gene microarray assay. Journal of Molecular Biomarkers & Diagnosis 2011.
16. Beck AH, Rodriguez-Paris J, Zehnder J, et al. Evaluation of a gene expression microarray-based assay to determine tissue type of origin on a diverse set of 49 malignancies. Am J Surg Pathol 2011;35(7):1030-37.
17. Tothill RW, Li J, Mileshkin L, et al. Massivelyφparallel sequencing assists the diagnosis and guided treatment of cancers of unknown primary. J Pathol 2013;231(4):413-23.
18. Robson M, Offit K. Inherited predisposition to cancer: introduction and overview. Hematology/oncology clinics of North America 2010;24(5):793-97.
19. Ku CS, Naidoo N, Hartman M, et al. Cancer Genome Sequencing. eLS.
20. Swanton C, Caldas C. From genomic landscapes to personalized cancer management—is there a roadmap? Ann N Y Acad Sci 2010;1210(1):34-44.
21. Stratton MR, Campbell PJ, Futreal PA. The cancer genome. Nature 2009;458(7239):719-24.
22. Kobayashi S, Boggon TJ, Dayaram T, et al. EGFR mutation and resistance of non–small-cell lung cancer to gefitinib. N Engl J Med 2005;352(8):786-92.
23. Sequist LV, Joshi VA, Jänne PA, et al. Response to treatment and survival of patients with non-small cell lung cancer undergoing somatic EGFR mutation testing. Oncologist 2007;12(1):90-98.
24. Cooper WA, O'Toole S, Boyer M, et al. What's new in non-small cell lung cancer for pathologists: the importance of accurate subtyping, EGFR mutations and ALK rearrangements. Pathology-Journal of the RCPA 2011;43(2):103-15.
25. Monzon FA, Ogino S, Hammond MEH, et al. The role of KRAS mutation testing in the management of patients with metastatic colorectal cancer. Archives of pathology & laboratory medicine 2009;133(10):1600-06.
26. Ross JS, Torres-Mora J, Wagle N, et al. Biomarker-Based Prediction of Response to Therapy for Colorectal Cancer Current Perspective. Am J Clin Pathol 2010;134(3):478-90.
27. Chapman PB, Hauschild A, Robert C, et al. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. N Engl J Med 2011;364(26):2507-16.
28. Pentheroudakis G, Golfinopoulos V, Pavlidis N. Switching benchmarks in cancer of unknown primary: from autopsy to microarray. Eur J Cancer 2007;43(14):2026-36.
29. Karapetis C. Perceptions of CUP - Results and Analysis of a National Survey of Australian Oncologists. Clinical Oncological Society of Australia Annual Scientific Meeting. Brisbane, 2012.
30. Prahallad A, Sun C, Huang S, et al. Unresponsiveness of colon cancer to BRAF (V600E) inhibition through feedback activation of EGFR. Nature 2012;483(7388):100-03.