Personalized oncogenomics in the management of gastrointestinal carcinomas—early experiences from a pilot study

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ABSTRACT

Background Gastrointestinal carcinomas are genomically complex cancers that are lethal in the metastatic setting. Whole-genome and transcriptome sequencing allow for the simultaneous characterization of multiple oncogenic pathways.

Methods We report 3 cases of metastatic gastrointestinal carcinoma in patients enrolled in the Personalized Onco-Genomics program at the BC Cancer Agency. Real-time genomic profiling was combined with clinical expertise to diagnose a carcinoma of unknown primary, to explore treatment response to bevacizumab in a colorectal cancer, and to characterize an appendiceal adenocarcinoma.

Results In the first case, genomic profiling revealed an IDH1 somatic mutation, supporting the diagnosis of cholangiocarcinoma in a malignancy of unknown origin, and further guided therapy by identifying epidermal growth factor receptor amplification. In the second case, a BRAF V600E mutation and wild-type KRAS profile justified the use of targeted therapies to treat a colonic adenocarcinoma. The third case was an appendiceal adenocarcinoma defined by a p53 inactivation; Ras/RAF/MEK, Akt/mTOR, Wnt, and NOTCH pathway activation; and overexpression of RET, ERBB2 (HER2), ERBB3, MET, and cell cycle regulators.

Summary We show that whole-genome and transcriptome sequencing can be achieved within clinically effective timelines, yielding clinically useful and actionable information.

Key Words Oncogenomics, genomics, cholangiocarcinoma, colonic adenocarcinoma, appendiceal adenocarcinoma, targeted therapy, personalized medicine, bevacizumab

INTRODUCTION

Gastrointestinal (gi) carcinomas are molecularly heterogeneous and usually lethal at advanced stages.1,2 Although some predictive single-gene assays are available, approaches that are capable of simultaneously interrogating multiple genetic loci within finite biopsy samples will increasingly be required. Whole-genome sequencing (WGS) and RNA sequencing provide a comprehensive catalog of somatic mutations and gene expression measurements and can be of particular use in the clinical management of molecularly complex cancers such as GI carcinomas. We and others have reported on the real-time clinical use of sequencing in the diagnosis and treatment of advanced tumours3–7.

The interdisciplinary Personalized Onco-Genomics (POG) program at the BC Cancer Agency was conceived with the goal of using whole-genome analysis for clinical oncologic care. A pilot project aimed to address the frequency with which clinically informative results might be obtained through the application of whole-genome analysis. The POG program currently represents the largest precision medicine endeavor in Canada, and it has resulted in the first genomic definitions of rare cancer types such as peritoneal mesothelioma8 and, in the present report,
appendiceal adenocarcinoma. Patients with incurable advanced cancers, good performance status, and limited remaining conventional treatment options are eligible for enrolment. Whole-genome sequencing, RNA sequencing, and amplicon-based panel sequencing are performed on contemporaneous fresh-frozen biopsies, together with tumour DNA from archival formalin-fixed paraffin-embedded tissue and germline DNA from blood. A team of genome scientists, physicians, and computational biologists discusses each case and generates a treatment plan within a typical turnaround-time of 4–6 weeks from sample procurement.

Since 2012, more than 500 patients have been enrolled in the pog program. During that time, a number of challenges emerged—mostly related to fresh and fresh-frozen tissue acquisition, tumour genomic heterogeneity, and turnaround reporting time. We recently reported a detailed overview of our experiences of implementing WGS in clinical applications.

The case vignettes that follow address the application of the pog approach to common diagnostic and treatment problems in GI carcinomas and illustrate how genomic profiling yielded clinically important and biologically relevant information. These reports highlight the possibilities and potential applications of molecular technology in the future of routine cancer care.

METHODS

Ethics, Privacy, and Consent

Informed written consent for sequencing and publication of clinical and genomic data was obtained for each patient in the program. All protocols and procedures in the program, including the consent procedure, were approved by the University of British Columbia Research Ethics Committee (no. H12-00137). Raw sequencing data are maintained within a secure computing environment at Canada’s Michael Smith Genome Sciences Centre, and clinical data are maintained by physicians and a dedicated research team at the BC Cancer Agency.

Tumour Sampling

Metastatic or recurrent tumours were sampled under imaging guidance. The samples were frozen and embedded in optimal-cutting-temperature compound for DNA and RNA extraction and were also prepared as frozen sections for histologic correlation. In addition, tumour DNA and RNA were extracted from formalin-fixed paraffin-embedded tissue from earlier (usually primary) lesions. Matching normal DNA was extracted from peripheral blood leukocytes. Paired-end DNA and RNA sequencing libraries were generated at the Genome Sciences Centre, and sequencing was performed using the HiSeq platform (version 3: Illumina, San Diego, CA, U.S.A.). Simultaneously, targeted deep sequencing was performed using the Ion AmpliSeq oncogene panel platform (Life Technologies, Carlsbad, CA, U.S.A.) and the Ion Torrent PGM sequencing platform (Life Technologies). Coverage for WGS was 80–100× on frozen tumour tissue and 40× for DNA from archival formalin-fixed paraffin-embedded tissue and germline DNA from blood. A minimum of 500× coverage was required for the targeted amplicon reads.

Bioinformatic Analysis

Reads were aligned to the human genome (reference: GRCh37-lite) using the BWA software application (version 0.5.7h). Reads from multiple lanes were merged and duplicate-marked using the Picard application (version 1.38, http://sourceforge.net/projects/picard/). Variants were called using mpileup and subsequently filtered with varfilter (SAMtools, version 0.1.17^11). The tumour sample was compared with the normal sample to identify somatic copy-number variants [CNAnvar (version 0.0.6, http://www.bcgsc.ca/platform/bioinfo/software/cnaseq)], loss of heterozygosity events [APOLLO (version 0.1.1^12)], single nucleotide variants [SAMtools (version 0.1.17), MutationSeq (version 1.0.2^13), Strelka (version 0.4.6.2^14)], and small insertions and deletions (Strelka). The RNA sequencing reads were analyzed with JAGuaR to include alignments to a database of exon junction sequences and subsequent repositioning onto the genomic reference. The RNA sequencing data were processed using the Genome Sciences Centre’s wrss (whole-transcriptome shotgun sequencing) pipeline coverage analysis (version 1.1) with the “stranded” option to determine gene and exon read counts and normalized expression level. Expressed variants were called with SNVMix2 (version 12.1-r1c16) and SAMtools (version 0.1.13). Gene expression in the tumour was compared with a compendium of normal tissues and with one or more normal libraries of the same tissue type to identify upregulated and downregulated genes. Genomic and RNA sequencing tumour data were also both assembled using Trans-ABySS (version 1.4.3^17) to identify structural variants and fusion genes. Variants were annotated to genes using the Ensembl database (version 59.69^18).

Genes were linked to cancer pathways using cosmic (Wellcome Trust Sanger Institute, Genome Campus, Hinxton, U.K.), kegg (Kanehisa Laboratories, University of Tokyo, Tokyo, Japan), and Ingenuity Pathway Analysis (Qiagen, Redwood City, CA, U.S.A.), and linked to drugs using DrugBank and the Therapeutic Target Database. Literature review for drug–target combinations and pharmacogenetics was integrated to identify potential therapeutic recommendations. For the analysis of germline variants predisposing to GI cancers, genes with germline variants were compared against a compiled list of GI cancer predisposition genes (Table i); the list of genes was compiled from a parallel study of next-generation sequencing of germline DNA in hereditary GI tumours.

RESULTS AND DISCUSSION

In Cases of Diagnostic Uncertainty, Molecular Studies Can Aid in Diagnosis and Guide Targeted Treatment

Patient 1, a previously well 33-year-old woman, presented with increasing back pain and new-onset leg weakness. Computed tomography imaging showed multiple vertebral and pelvic lytic lesions, together with extensive intra-abdominal and retroperitoneal lymphadenopathy. The only visceral mass seen on imaging was a large hepatic lesion.

Biopsies of the liver and vertebral lesions showed a moderately differentiated adenocarcinoma (Figure 1)
that lacked a site-specific immunohistochemical expression profile. Amplicon-based panel sequencing, WGS, RNA sequencing, and Sanger sequencing independently confirmed the presence of a somatic heterozygous mutation in the IDH1 gene, resulting in a p.Arg132Cys amino acid change. Such IDH1 mutations have been identified in up to a quarter of intrahepatic cholangiocarcinomas, but are rare elsewhere.20

Clinical, pathologic, and genetic correlation thus yielded a diagnosis of cholangiocarcinoma. Genomic profiling provided a rationale for treatment of the patient with a cholangiocarcinoma-specific chemotherapy regimen of gemcitabine and cisplatin. Erlotinib was also prescribed based on the detection of epidermal growth factor receptor copy-number gain and overexpression. Erlotinib has been shown to provide benefit in the treatment of biliary tract cancers.21

Real-Time Genomic Profiling Characterizes the Molecular Basis of Treatment Resistance and Predicts Treatment Response

Patient 2, a 30-year-old woman, presented with worsening abdominal pain secondary to a colonic obstruction. She underwent a right hemicolectomy for a microsatellite-stable pT3N2b low-grade colonic adenocarcinoma and was subsequently found to have synchronous hepatic and para-aortic lymph node metastases.

The patient underwent whole-genome profiling performed on her primary tumour (archival) and liver metastasis (fresh-frozen) after disease progression on treatment with FOLFIRI (fluorouracil–leucovorin–irinotecan) and bevacizumab. In both the archival primary and frozen metastatic tumours, a BRAF V600E mutation and wildtype KRAS alleles were detected, which provided a rationale for the use of sorafenib and cetuximab.22

Of particular interest, disease progression during bevacizumab treatment had occurred in her hepatic lesions, while her residual extrahepatic disease regressed (Figure 2). Sequencing showed high-level amplification and overexpression of vascular endothelial growth factor A (VEGFA) in the hepatic lesions but not elsewhere. The VEGFA overexpression likely contributed to treatment resistance in the hepatic lesions and might have arisen because of selection for and expansion of a pre-existing clone with amplified VEGFA. Results from animal models have also suggested that bevacizumab resistance can arise as a result of VEGFA overexpression and activation of other oncogenic pathways.23,24

In addition to suggesting initial treatments, genomic profiling could be used to monitor VEGFA gene amplification and activation of other resistance pathways, allowing for optimization of systemic treatment with bevacizumab and other agents in patients with colorectal cancer—a concept that could be achieved by targeting synergistic pathways or by using alternative therapeutic targets when resistance develops.
Genomic Profiling Provides a Comprehensive Understanding of Poorly Characterized Malignancies

Bona fide non-mucinous high-grade appendiceal adenocarcinomas are associated with poor prognosis. These rare neoplasms are neither neuroendocrine tumours nor low-grade appendiceal mucinous neoplasms. Little is known about their molecular abnormalities beyond the low frequency of both KRAS mutations and microsatellite instability, and some differences relative both to low-grade mucinous carcinomas and to colorectal carcinomas.

Patient 3, a 38-year-old woman, presented with pelvic pain. Imaging showed bilateral ovarian masses with diffuse omental nodules and ascites, and an appendiceal mass was noted on laparoscopy. Although a primary gynecologic malignancy was initially suspected, a poorly differentiated non-mucinous adenocarcinoma was found to be originating in the appendix (Figure 3).

The patient was treated with the FOLFOX and FOLFIRI (fluorouracil–leucovorin–oxaliplatin) regimens with good response and underwent cytoreductive surgery and hyperthermic intraperitoneal chemotherapy. Genomic profiling was used to explore her cancer after liver metastases developed.

The tumour had broad areas of copy-number change and loss of heterozygosity. As observed in other high-grade tumours, the tumour was KRAS wild-type and microsatellite stable. Inactivation of p53 was evident, as was activation of the Ras/raf/MEK, Akt/mTOR, Wnt, and notch pathways. From a therapeutic standpoint, the tumour showed overexpression of several targetable receptor tyrosine kinases, including RET, ERBB2 (HER2), ERBB3, and MET. Other abnormalities included overexpression of several cell cycle regulators that could render the tumour amenable to cell cycle inhibitors, and overexpression of histone deacetylases and topoisomerases that could warrant use of histone deacetylase and topoisomerase IIa inhibitors respectively.

Our analysis confirms that although poorly-differentiated appendiceal adenocarcinomas are complex at a molecular level, they could present several opportunities for targeted treatments.

Summary

A complete set of genomic findings from the preceding 3 cases, as well as previously published cases from the POG program, can be viewed online at the Web site of the International Cancer Genome Consortium (https://www.ebi.ac.uk/ega/studies; studies EGAD00001001308, EGAD00001001307, and EGAD00001001309).

CONCLUSIONS

With improvements in sequencing technology, sample procurement, and interpretation of genomic variants, we are in the process of learning how to apply genomic data to the treatment of individual patients. These early experiences in our pilot project have shown that real-time genomic profiling of gastrointestinal tumours can yield a wealth of biologic and clinically important information, and hence improve the understanding and management of these cancers at the level of the individual patient. As we learn from these experiences and interrogate further tumours at earlier stages, we expect that significant improvements in outcomes will result from sequencing and collaborations within multidisciplinary teams. The Personalized Onco-Genomics program continues to explore the utility and clinical integration of novel molecular technologies, with the ultimate goal of providing precision cancer treatment in Canada.

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CONFLICT OF INTEREST DISCLOSURES
We have read and understood Current Oncology’s policy on disclosing conflicts of interest, and we declare that we have none.

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