RESEARCH

Identification of Germline Pharmacogenomic Variants Using a Clinical Targeted Sequencing Panel for Formalin-fixed Paraffin-embedded Tumours

Shyong Quin Yap[1] and Aly Karsan[1,2*]

*Correspondence: akarsan@bcgsc.ca
[1]British Columbia Cancer Research Centre, 675 West 10th Ave, Vancouver, BC, Canada
Full list of author information is available at the end of the article

Abstract

Background: Because the tumour genome contains germline information, clinical laboratories can leverage tumour genomic testing to perform initial screening for clinically relevant germline variants such as variants in pharmacogenomic (PGx) genes. Subsequently, only patients with potential germline PGx variants would be subjected to downstream germline testing. While this procedure would be more cost-effective than sequencing and analyzing normal DNA for every patient, the concordance of germline PGx variants between tumour and blood specimens has not been evaluated; hence, the feasibility of detecting germline PGx variants in tumour DNA remains uncertain.

Results: Address tumours are often FFPE and formalin induces artifacts

Conclusions: Text for this section.

Keywords: Tumour sequencing; Germline pharmacogenomics testing; Formalin artifacts

Background

Tumour profiling using next-generation sequencing (NGS) technologies has been increasingly integrated into oncologic care to detect targetable somatic mutations and personalize treatments for cancer patients. Although analysis of tumour-normal paired samples is required to accurately discriminate between somatic and germline variants, most clinical laboratories only sequence tumour samples to minimize cost and turnaround time. However, genomic analyses of tumours can also reveal secondary genomic findings, which are germline information that may have clinical implications for patients and their family members. In fact, several studies demonstrated that a germline cancer-predisposing variant is present in 3-10% of patients undergoing tumour-normal sequencing. Therefore, clinical laboratories providing tumour genomic testing must be equipped to perform germline confirmatory testing on potential germline variants or be prepared to refer such cases to external services.

Because the tumour genome contains germline information, clinical laboratories can leverage tumour genomic testing to perform initial screening for clinically relevant germline variants such as variants in pharmacogenomic (PGx) genes. Subsequently, a similar framework for validating secondary germline findings can be

applied, in which only patients with potential germline PGx variants are subjected to downstream germline testing. This procedure for germline PGx testing is more cost-effective because sample processing, sequencing, and analysis of normal DNA are not required for every patient. The ability to implement germline PGx testing at a reduced cost can significantly benefit patient care because these variants cause functional changes in drug targets and drug disposition proteins (proteins involved drug metabolism and transport), thereby contributing to inter-patient differences in chemotherapeutic response. Hence, such genomic information can be used to guide the selection of chemotherapeutic drugs and optimization of drug dosage for cancer patients, leading to improved safety and efficacy of treatment and reduced risk of toxicity.

Detection of genomic alterations in tumour DNA is also faced with technical challenges conferred by formalin-fixed paraffin-embedded (FFPE) tumour specimens. Tumour biopsies are often formalin-fixed to preserve tissue morphology for histological examination and to enable storage at room temperature; however, formalin fixation causes DNA fragmentation and base modifications, which pose difficulties in using DNA extracted from FFPE tumours for clinical genomic testing. Fragmentation damage caused by formalin fixation leads to reduced template DNA for PCR amplification, thereby affecting the efficiency of amplicon-based NGS testing. Furthermore, the degree of DNA fragmentation was shown to be higher in tissues from older FFPE blocks and tissues fixed with formalin of lower pH. Formalin fixation is also problematic because it gives rise to depurination, which generates abasic sites, and cytosine deamination resulting in C>T/G>A transitions. These forms of formalin-induced DNA damage contributes to the presence of sequence artifacts in FFPE specimens, which can be inaccurately identified as real genomic alterations.

In this study, we analyzed amplicon-based NGS sequencing data from 213 patients with tumour-normal paired samples to investigate whether germline PGx testing can be performed using FFPE tumours. To evaluate the effects of DNA damage caused by formalin fixation, we compared the coverage and quality of sequencing data between tumour and matched normal (blood) specimens and assessed the prevalence of formalin-induced sequence artifacts in FFPE tumour specimens. Finally, we measured the concordance of germline PGx variants between tumour and blood specimens to determine the feasibility of using a clinical tumour sequencing panel to screen for germline PGx variants in FFPE tumours.

Methods

Patient Samples

Blood and FFPE tumour specimens were acquired from 213 patients recruited for The OncoPanel Pilot (TOP) study, a pilot study to optimize the OncoPanel, which is an amplicon-based tumour sequencing panel, and assess its application for guiding clinical decision-making. Patients in the TOP study are those with advanced cancers and the distribution of cancer types are listed in Table 1. The age of paraffin block for tumour specimens ranges from 18 to 5356 days with a median of 274 days.

Library Construction and Sequencing

DNA extractions were carried out manually using a QIAGEN AllPrep kit (OCT samples) or QIAGEN FFPE DNA extraction kit (all fixative samples). Equal

amounts of genomic DNA (250ng per sample, determined by fluorometric quantitation, were used for library input. Subsequent details of the sequencing process are similar to those already described [7]: Inputs were sheared to obtain fragment size distributions centered on 3000bp prior to direct amplicon generation and library generation using a RainDance Thunderstorm instrument. Barcoded amplicons were sequenced on an Illumina MiSeq using v2 chemistry with Paired-end 250bp reads, at 16 libraries per pool (typically 1.5-2M reads per library). Samples from this project were sequenced at random across a total of 10 pools.

Variant Calling and Curation

Statistical Analysis

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Results

Assessment of DNA Input and Amplicon Yield

Comparison of Sequencing Metrics Between Blood and FFPE Tumours

Evaluation of Sequencing Artifacts in FFPE Specimens

Sensitivity and Positive Predictive Value of Detecting Germline Variants in FFPE Specimens

Discordant Germline Variants

Discussion

Conclusions

Competing interests
The authors declare that they have no competing interests.

Author's contributions
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Author details
[1]British Columbia Cancer Research Centre, 675 West 10th Ave, Vancouver, BC, Canada. [2]Department of Pathology and Laboratory Medicine, University of British Columbia, Random Street, Random Post Code Vancouver, BC, Canada.

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Figures

Figure 1 Workflow for Library Construction and Sequencing. A short description of the figure content should go here.

Figure 2 Sample figure title. Figure legend text.

Tables

Table 1 Distribution of cancer types in the TOP cohort.

Cancer Type	Number of Cases	Percentage (%)
Colorectal	97	46
Lung	59	28
Melanoma	18	8
Other*	17	8
GIST	7	3
Sarcoma	4	2
Neuroendocrine	4	2
Cervical	2	0.9
Ovarian	2	0.9
Breast	2	0.9
Unknown	1	0.5

*This category includes thyroid, peritoneum, sarcomatoid carcinoma of lung, Fallopian tube, gastric, endometrial, anal, salivary gland, pancreas, and small bowel cancers.

Additional Files

Additional file 1 — Sample additional file title
Additional file descriptions text (including details of how to view the file, if it is in a non-standard format or the file extension). This might refer to a multi-page table or a figure.

Additional file 2 — Sample additional file title
Additional file descriptions text.