

A comparative analysis of cancer hotspot mutation profiles in circulating tumour cells, circulating tumour DNA and matched primary lung tumour

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Background

The increased knowledge in molecular biology of cancer has resulted in the development of a number of successful targeted therapies. These targeted therapies show dramatic response in a small proportion of patients who possess specific genetic aberrations.

Blood based mutation profile analysis is becoming an increasingly important non-invasive form of mutation screening in cancer. Whilst many have reported on single mutation comparisons between blood based and primary tumour tissue, limited information is available on multiplex comparisons between the DNA extracted from circulating tumour cells (CTC) and circulating free tumour DNA in the plasma (ctDNA) against the current standard of FFPE analysis of primary tumour and no information exists comparing next generation sequencing (NGS) profiles between the 3 different substrates within the same patient.

The aim of our work is to report the concordances between CTCs and ctDNA versus the primary FFPE tumour mutations using a NGS hotspot panel.

Methods

Pre-operative whole blood samples were collected from 30 patients who underwent thoracic surgery at the Royal Brompton Hospital. CTCs were isolated using ScreenCell MB devices from 6ml of whole blood, and ctDNA from 1ml aliquots of plasma removed from matching 9ml of EDTA blood samples. Matching FFPE samples were retrieved from post-resection primary tumour tissue and corresponding DNA extracted from three 10µm PCR rolls.

DNA was extracted from the CTCs, ctDNA and matched FFPE tissues using Qiagen kits (QIAamp DNA Micro kit, QIAamp DNA blood mini kit and QIAamp FFPE tissue kit, respectively). The 90 (30 matched triplicates) DNA samples were sequenced by Illumina HiSeq using Z3 cancer panel (Illumina, San Diego). Agreements of variant calls were compared between the three DNA substrates and a kappa statistic was reported using Stata 13.

Results

Between 2011 and 2013, samples from 30 consenting patients were obtained. In total, 10 had primary lung cancer, 19 had secondary lung cancer, and 1 (intentionally included) had no evidence of cancer. From the 90 samples, a total of 18,821 variant calls were identified after the removal of known 1,048 germline variants (table 1). Within the hotspot panel alone, the mean (SD) number of variant calls per patient was 151 (44) on FFPE samples, 136 (49) on CTC samples and 463(108) on ctDNA samples.

There was good agreement between CTCs and FFPE of 79.8% with a Kappa statistic of 0.42 (P<0.001) (table 2). Agreement between ctDNA and FFPE was much poorer at 12.7% with a Kappa statistic of -0.40 (P=1.000) (table 3). The results also suggested poor agreement between CTC and ctDNA of 16.1% with a Kappa statistic of -0.32 (P=1.000).

Focusing on single gene comparisons on the multiplex platform, agreement was considerably better for KRAS and EGFR for CTCs compared to ctDNA at 44% versus 11% for KRAS and 92% versus 9% for EGFR respectively. Discordances were largely due to an increased number of variants that were identified in ctDNA and not in CTC or FFPE tissue.

Table 1. Total variant calls per substrate

	Number of variant calls
CTC	5,029
FFPE	5,513
Plasma	14,559
Total number of variant calls	19,869
Total number of variants call excluding known germline variants	18,821

Table 2. Concordance between FFPE and CTC

		CTC		
		Positive	Negative	Total
FFPE	Abnormal	2,396	2,129	4,525
	Normal	1,676	12,620	14,296
	Total	4,072	14,749	18,821

Kappa statistic of 0.42 (P<0.001)

Table 3. Concordance between FFPE and ctDNA

		ctDNA		
		Positive	Negative	Total
FFPE	Abnormal	981	3,544	4,525
	Normal	12,896	1,400	14,296
	Total	4,072	14,749	18,821

Kappa statistic of -0.40 (P=1.000)

Discussion

Currently the standard method of obtaining cancer mutation profiles is through solid tumour tissue biopsy in lung cancer. However, sample acquisition is invasive and associated with complications. The information obtained is not always proportional to the risk, given that only a small proportion of patients who would benefit from targeted therapy. In addition, through treatment and the development of the cancer, resistance may occur, and it might result in a further biopsy to understand the progression of the mutation profile.

Blood based mutation profiling is increasingly important within the cancer community, but little information has been generated on increasingly large amount of information obtained through next generation sequencing. Currently we are not able to harness the vast amount of information obtained. Furthermore, it remains a challenge to identify the correct substrate in the blood as a surrogate for mutation profiling.

Our results suggest a close concordance between FFPE primary tumour and CTCs, but we are not certain if this may be more reflective of germline DNA variants between the two as opposed to somatic mutations that may be higher in the ctDNA. This may also explain the poor concordance between CTC and ctDNA. The observed increase in the number of variants detected on single gene analysis due to processing, sample or analytic difficulties with ctDNA as a limitation of our work.

Ultimately, the correct substrate would be reflective of the underlying purposes to which the information will be used. Currently, a limited number of targeted therapies are available suggesting that a single gene approach is likely to be the appropriate method of detection.

Conclusions

Our results suggest on a next generation sequencing platform that the global genetic variant profile between DNA extracted from CTC had good agreement with FFPE primary tumour tissue, and the agreement between ctDNA and FFPE was much poorer.

Acknowledgements

We gratefully acknowledge funding from The Peter and Mary Fu Foundation (Singapore), Point Hope Group (Singapore) and ScreenCell (Paris, France). This project was also supported by Illumina (San Diego, USA), and the National Institute of Health Research Respiratory Disease Biomedical Research Unit at the Royal Brompton and Harefield NHS Foundation Trust and Imperial College London.