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Original Research

High-depth sequencing of paired primary and metastatic tumours: Implications for personalised medicine

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Abstract ***Background:*** Next-generation sequencing of large panel of genes had been associated with clinical benefit in a significant proportion of patients with advanced cancer. However, the molecular profile of the primary tumour from the initial surgical specimen might significantly differ from the molecular profile in a tumour sample obtained from a biopsy of a metastatic site.
Patients and methods: We compare the genetic profile of primary tumours and paired metastases by using a large panel of cancer genes. Training and validation set including a total of 152 primary and metastatic tumour pairs were sequenced (up to 429 genes) focussing on variants described in the Catalogue of Somatic Mutations in Cancer (COSMIC).
Results: Training and validation set including a total of 152 primary and metastatic tumour pairs were sequenced focussing on variants described in COSMIC. Agreement rate between the couples of primary and metastasis on COSMIC variants was 65% (24/37) and 43% (49/115) in the training and validation cohort, respectively. That rose to 74% (20/27) and 58% (42/73) when focussing on targetable mutations. In five cases, the discordance was related to appearance of secondary resistance mutation, giving a targetable refined agreement rate of 67% (67/100).
Conclusion: Up to 40% of paired primary tumour/metastases have discordant molecular profile. Liquid biopsies may overcome, in the near future, the limits of tumour tissue genotyping.

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1. Background

Cancer is the result of cumulative genetic alterations that lead normal cells to become cancer cells. Many cellular and molecular mechanisms of tumour development, growth and metastasis have been elucidated, leading to the identification of new cancer-specific molecular targets. As a result of these efforts, a myriad of new agents that directly or indirectly target these putative factors have been developed and approved, with encouraging anti-tumour activity: epidermal growth factor receptor (EGFR) inhibitors in non-small cell lung cancer (NSCLC), human epidermal receptor-2 (HER2) inhibitors in breast or gastric cancer and BRAF inhibitors in melanoma, for instance. These molecular targeted therapies have been developed as cytotoxic agents based on tumour location and/or histology. However, oncogenic molecular alterations can exist independent of the tumour location, with only a potential change in their frequency. With increasing knowledge of the biology of human tumours, most tumours are known to be heterogeneous, suggesting that the 'one-size-fits-all' approach or single-drug regimens for patients with the same tumour type/histology is suboptimal. Therefore, the need to 'personalise' cancer therapy has been recognised, requiring as a critical step for the comprehensive assessment of the biological characteristics of tumours from each individual.

The increasing availability of next-generation sequencing (NGS) technologies has created a growing interest in physicians and patients for the use of these technologies in routine cancer care and, in particular, for directing patients to relevant clinical trials [1]. The recently published MOSCATO 01 study has shown that tumour sequencing improves outcome in 33% of patients with advanced cancers [2], confirming results from previous reports [3,4].

Almost all past or ongoing molecular profiling studies have implemented the collection of fresh specimens through tumour biopsy to perform molecular screening [2,5,6]. However, archived formalin-fixed, paraffin-embedded (FFPE) tissue of the primary tumour represents the most readily available resource. Several single-gene-profiling studies performed in breast, lung or colorectal cancer revealed a high concordance rate of the status of crucial genes such as *KRAS* or *HER2* between the primary tumour and paired metastasis suggesting that invasive, resource-consuming and expensive metastasis biopsies are unnecessary [7–9]. However, to date, no studies have compared the mutational profile of primary tumours and paired metastasis by using NGS of a large panel of genes (>100) in the context of molecular screening programs. The aim of this study is to compare the rate of targetable alterations in primary and paired metastasis-coupled samples on various and frequent cancer to assess if the type of tissue may impact the results of molecular screening of cancer patients.

2. Methods

2.1. Patient selection

The training cohort was based on patients selected to have genomic screening after written informed consent as part of our institutional early-phase trial unit, and they were screened between January 2014 and July 2015 (Institut Bergonié, Comprehensive Cancer Center, Bordeaux, France). Only patients with available matched primary and metastasis samples were selected to enter the present study.

Materials were retrieved from the Department of Pathology. Samples were analysed at Institut Bergonié Cancer Center, Bordeaux, France or by Foundation Medicine, Cambridge, MA.

The validation cohort was independently selected from the international data-sharing project known as the American Association for Cancer Research (AACR) Project, Genomics Evidence Neoplasia Information Exchange (GENIE) [10]. The analysis was based on sample and mutation annotation tables available in GENIE via Synapse data portal (synapseid: syn7222066, at https://www.synapse.org/#!Synapse:syn7222066/files/).

All data available in GENIE were screened for identification of matched primary and metastasis samples. Couples were manually curated to ensure that the location of the metastatic sample was consistent with the primary tumour cancer type.

Clinical information such as sex, age at sequencing of primary and metastasis sites was extracted from the clinical database of the Institut Bergonié for the training cohort and the GENIE database.

The protocol was approved by the Institutional Review Board of Institut Bergonié National Ethics Committee and was performed in accordance to the Good Clinical Practice Guidelines of the International Conference on Harmonisation.

2.2. Next-generation sequencing

For the training cohort, sequencing techniques are described in Supp data 1. Tumoural DNA was isolated from an FFPE-archived sample. Massive parallel DNA sequencing was performed as previously described [3].

For GENIE validation cohort, 93% of the samples were originated from the MSK studies IMPACT410 and IMPACT341. Sequencing characteristics are described in the document http://www.aacr.org/Documents/GENIEDataGuide.pdf. For most of the investigated samples, sequencing was performed on FFPE material via hybridisation capture of the coding regions of a panel of 341–410 genes, using Illumina platforms.