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Comparative analysis of primary tumour and matched metastases in colorectal cancer patients: Evaluation of concordance between genomic and transcriptional profiles ☆

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Abstract ***Purpose:*** Focal and temporal tumour heterogeneity can represent a major challenge for biology-guided therapies. This study proposes to investigative molecular discrepancies between primary colorectal cancer (CRC) samples and matched metastases.
Experimental design: Surgical samples from primary and matched metastatic tissues from 13 CRC patients along with their adjacent normal tissue were evaluated. A mutational analysis was performed using a targeted Next Generation Sequencing assay (Foundation Medicine) with a focus on known recurrent somatic mutations as surrogate of key oncogenic events. Gene expression analysis was also performed to investigate transcriptional discrepancies.
Results: Among the 26 samples, 191 mutations were identified including mutations in *APC* (13 pts), *TP53* (11 pts), and *KRAS* (7 pts). Global concordance rate for mutations was 78% between primary and metastatic tumours and raised to 90% for 12 known recurrent mutations

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in CRC. Differential gene expression analysis revealed a low number of significantly variant transcripts between primary and metastatic tumours once the tissue effect was taken into account. Only two pathways (ST_ADRENERGIC, PID_REELINPATHWAY) were differentially up-regulated in metastases among 17 variant pathways. A common profile in primary and metastatic tumours revealed conserved pathways mostly involved in cell cycle regulation. Only two pathways were significantly down regulated compared to normal control, including regulation of autophagy (KEGG_REGULATION_OF_AUTOPHAGY).

Conclusion: These results suggest that profiles of primary tumour can identify key alterations present in matched CRC metastases at first metastatic progression. Gene expression analysis identified mainly conserved pathways between primary tumour and matched liver metastases.

1. Introduction

Metastatic progression is a multistep process involving a series of phenotypic changes in tumour cells, associated with the acquisition of new functions that are required for cell motility, dissemination and tissue invasion. These acquired genetic changes can be considered the result of selection during the metastatic process [1] in a context of high tumour instability, which is an intrinsic characteristic of tumour cells [2]. Recent studies have emphasised the heterogeneity of genomic profiles between the primary tumour and metastases but also between metastases and in different regions of the same tumour [3,4]. However, these discrepancies reflect a comprehensive molecular analysis. Intratumoural heterogeneity could be analysed by specifically considering some key changes known to be involved in oncogenesis in order to assess their level of conservation during the temporal and spatial evolution of the disease. We previously reported high concordance between primary tumour and first metastase for known recurrent genomic alterations compared to non-recurrent alterations in patients with non-small cell lung cancer who had not been treated with targeted therapies [5]. In colorectal cancer (CRC), high concordance between the primary tumour and liver metastases was concomitantly reported in a study focusing on five key genes (*KRAS*, *NRAS*, *BRAF*, *PIK3CA* and *TP53*) [6]. We proposed to extend the analysis in CRC patients to evaluate concordance in genomic profiles including a larger number of recurrent and non-recurrent genes, and to complete the study with a gene expression analysis in order to assess more precisely phenotypic heterogeneity between primary tumours and metastases.

2. Patients and methods

2.1. Inclusion criteria

Frozen surgical samples from primary tumours and matched metastase pairs and adjacent normal tissue were identified in the Pathology Department at Pitié Salpêtrière Hospital, Paris, France. Samples were archived from 2003 to 2010 and analysed concomitantly in 2012 at Gustave Roussy, Villejuif, France and Foundation Medicine, Cambridge, MA. The area in tumours was selected by the pathologist with an evaluation of cellularity on frozen sections. Tumour cellularity exceeding 50% was required for both the primary tumour and matched metastases. Complete clinical data were collected as well as informed consent.

2.2. Molecular analysis

Extraction was performed with the AllPrep DNA/RNA Mini Kit (QIAGEN) for simultaneous purification of genomic DNA and total RNA.

Next Generation Sequencing (NGS) was performed on 3230 exons in 182 cancer-related genes plus 37 introns from 14 genes that are often rearranged in cancer (189 genes in total, as seven genes were screened across both exons and introns). Paired-end sequencing (49×49 cycles) was performed using the HiSeq2000 (Illumina). Sequence data from gDNA were mapped to the reference human genome (hg19) using the BWA aligner [7] and processed using publicly available SAMtools [8], Picard (http://picard.sourceforge.net) and GATK [9]. Recurrent somatic alterations were defined as genomic alterations in genes that are mutated at $\geqslant 5\%$ in COSMIC or in the literature [10–12].

Gene expression analyses were performed with Agilent® SurePrint G3 Human GE 8×60 K Microarray (Agilent Technologies, AMADID 28004) using the one-colour Agilent labelling kit (Low Input Quick Amp Labeling Kit 5190-2306) adapted for small amount of total RNA. Microarray images were analysed by using Feature Extraction software version (10.7.3.1) from Agilent technologies. The microarray data related to gene expression assay have been submitted to the Array Express data repository at the European Bioinformatics Institute (http://www.ebi.ac.uk/arrayexpress/) under the accession number E-MTAB-2864.