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Clonal evolution of colorectal cancer in a patient with serially resected metastases and liquid biopsies: a case report and discussion of the literature

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ABSTRACT

Background Metastatic colorectal cancer represents a striking example of clonal heterogeneity and tumour evolution, which generates acquired resistance to therapy. Once hard to perform, the study of clonal heterogeneity is now significantly aided by the use of liquid biopsies.

Method We herein report a case of a patient with colorectal cancer and serial development of multiple metastases which were all resected and genotyped. A rare point mutation was identified in the primary tumour (but not in any of the organ metastatic sites), as well as in the first and the last out of three consecutive liquid biopsies. The review of the literature offered some insight in the evolution of the patient's tumour and general directions on how to interpret liquid biopsy results.

Conclusions This patient case emphasises the need for large prospective studies designed to bridge liquid biopsy data with useful clinical endpoints, in order to optimally integrate this revolutionary tool in everyday practice.

Key questions

What is already known about this subject?

- Cancer is highly heterogeneous in both space and time, due to emergence of new clones with different metastatic potential throughout the course of the disease.
- Tumour heterogeneity is bidirectionally related to resistance to treatment through evolutionary selective pressure.
- The mutated gene described herein has been associated with a number of solid tumours, although rarely in somatic colorectal neoplasms.

What does this study add?

- This is a rare case of serially resected metastases which were tested for cancer-related mutations.
- Our data offer insight in tumour evolution, also giving a hint of how administration of targeted agents may interfere with it.
- The specific nucleotide substitution we describe has never been reported in any type of neoplasia.

How might this impact on clinical practice?

- Reporting rare cases is the only way to gain experience in how to deal with them.
- This case report also highlights the lack of evidence on actionability of markers studied by liquid biopsies and the need to prospectively validate their clinical utility.

INTRODUCTION

Clonal heterogeneity and clonal evolution, both very common features of colorectal neoplasia, refer to the emergence of distinct, subclonal tumour populations with differing genotypic abnormalities, either spontaneously or as a result of administered therapies that eliminate some vulnerable, and favour the growth of resistant, tumour cells. Conventionally, tumour clonal heterogeneity was difficult to study, as this required repeated biopsies associated with cost, morbidity and sampling errors. The technological breakthrough of liquid biopsies offered promise in this setting. 'Liquid biopsy' refers to the detection and characterisation of circulating cell-free nucleic acids (or cell-free DNA (cfDNA)) from peripheral venous blood. The characterisation of a DNA fragment as tumour derived (circulating tumour DNA (ctDNA)) is mainly based on the identification of a known genetic alteration or methylation pattern specific to the tumour and/or absent from normal tissue, by use of different techniques. The sensitivity of liquid biopsy depends on the abundance of tumour nucleic acids as represented by disease stage or overall tumour load. Here, we present a case of a patient with metastatic colorectal cancer (CRC) for whom multiple metastases and plasma were available throughout the disease course, thus enabling us to monitor cancer evolution.

CASE REPORT

A 44-year-old male patient presented to the emergency department of our University

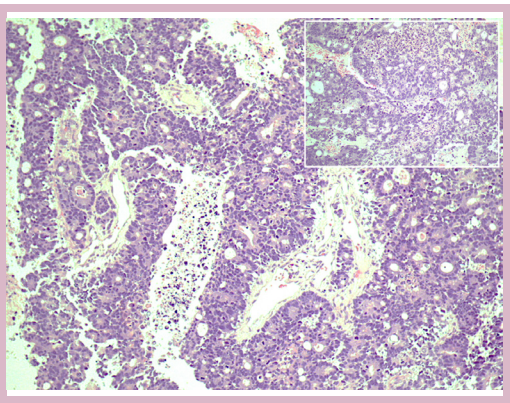

Figure 1 Infiltration of the cerebral cortex from a moderately differentiated adenocarcinoma (H&E ×100).

Hospital complaining of tenesmus and perineal pain in March 2012. He was an active smoker (60 pack-years) and his medical history consisting of hypertension and allergic rhinitis. His family history was significant and included a paternal grandmother with gynaecological cancer and three paternal aunts with undefined neoplasias.

Rectosigmoidoscopy revealed two synchronous ulcerated lesions, extending 5 and 12 cm from the dentate line, respectively. A CT scan of the chest, abdomen and pelvis showed a hepatic metastasis in liver segment VI and subcentimetre lung nodules in the right upper and lower lobes, respectively, biopsied as non-malignant. The patient underwent rectosigmoidectomy by abdominoperineal resection. Pathology of the surgical specimens revealed adenocarcinoma in both sites and confirmed R0 resection. The two lesions were sized 4.4×2.5 cm and 2.1×2.1 cm; 18 out of the 27 excised lymph nodes were positive for carcinomatous metastasis. The tumour was therefore staged as IIIC (T3N2bMx) multicentric rectal adenocarcinoma and was found to be wild type in extended *RAS* testing (*KRAS* and *NRAS* exons 2, 3, 4 by Illumina MiSeq PCR platform) and microsatellite stable (by tumour PCR testing of the Bethesda panel of five dinucleotide repeats).

The patient was started on capecitabine-based concurrent external beam chemoradiotherapy (total dose 50.4 Gy in 19 fractions) followed by five cycles of chemotherapy (capecitabine orally at 1000 mg/m^2 twice daily on days 1–14 and oxaliplatin intravenously at 130 mg/m^2 on day 1 every 3 weeks, or CAPOX), which were well tolerated. In December 2012, he underwent resection of liver segment VI, which confirmed the synchronous metastasis, upstaging the carcinoma to stage IV disease. Four additional 3-week treatment courses of CAPOX with panitumumab (at 8 mg/kg body weight) were administered, with no CT evidence of residual disease. The patient was followed up for 6 months and subsequently quit the follow-up programme on his own volition.

In April 2016, CT and positron-emission tomography (PET)/CT depicted a 3×2.3 cm mass in the right lower pulmonary lobe, two paratracheal and one subcarinal lymph nodes (with maximal diameters at 2.0 and 2.3 cm, respectively) and a 12 mm right supraclavicular node. Recurrence was confirmed with resection of the supraclavicular lymph node, followed by lung lobectomy and resection of mediastinal nodes by an attending thoracic surgeon. The supraclavicular node and the lung deposit were positive for adenocarcinoma, with an immunohistochemical profile compatible with the known primary (cytokeratin 20 positive, CDX2 positive, cytokeratin 7 negative, thyroid transcription factor-1 negative, Napsin A negative). In September 2016, due to balance and gait problems, a brain CT and MRI revealed an enhancing right cerebellar 3.2×3×3 cm metastasis, managed with right suboccipital craniectomy followed by brain radiotherapy. Histology confirmed the presence of metastasis with morphology and immunophenotype consistent with gastrointestinal origin (figure 1).

Restaging CT scans of the abdomen and thorax showed no visible remaining disease. Before proceeding with further systemic therapy and in view of occurrence of multiple distant relapses which had been resected, we opted for study of clonal heterogeneity of the patient's malignancy in the context of a research protocol. Additionally, we needed to design a targeted therapeutic strategy. Next Generation Sequencing of 50 cancer-related genes was thus applied in all resected neoplastic material available (figure 2). The resected primary tumour was confirmed to be all *RAS* wild type; it, however, harboured a point mutation on the *STK11* gene exon 6 (STK11 c.759C>A). The mutation was somatic and tumour specific, as it was not present in germ line DNA

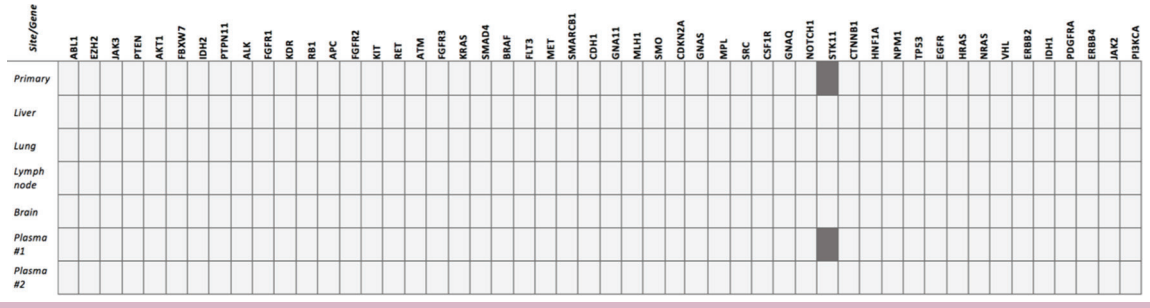

Figure 2 Tested genes per site of neoplastic material or cell-free DNA that was subjected to next-generation sequencing. Mutated genes are depicted in dark.

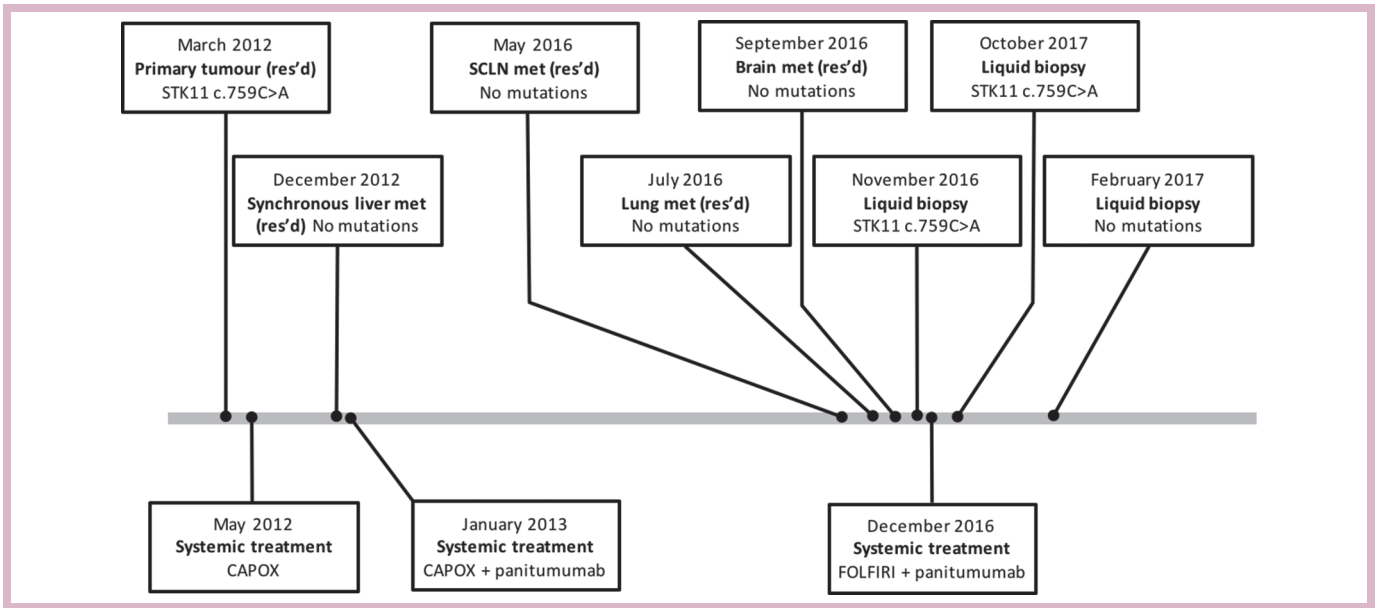

Figure 3 Mutational evolution of the tumour in the various sites over time. CAPOX, capecitabine/oxaliplatin; FOLFIRI, folinic acid/5-fluorouracil/irinotecan; met, metastasis; res'd, resected; SCLN, subclavicular lymph node.

isolated from peripheral blood white blood cells at baseline. The other metastatic sites (from the liver, supraclavicular lymph node, lung and brain) tested negative for mutations in all the examined genes, including *STK11*.

Chemotherapy (intravenous infusions of irinotecan at 180 mg/m^2, leucovorin at 400 mg/m^2, 5-fluorouracil at 400 mg/m^2 on day 1, then at 4800 mg/m^2 over 2 days, FOLFIRI) plus panitumumab (intravenously at 480 mg/kg on day 1) every 2 weeks were initiated in order to eradicate potential micrometastatic disease and a peripheral venous blood sample was drawn in November 2016 before the initiation of systemic therapy. Peripheral blood cfDNA in November 2016 yielded the presence of the *STK11* mutation that was detected in the primary tumour resected in March 2012. A repeat cfDNA sample was drawn in February 2017 (after one cycle of FOLFIRI/panitumumab) with no evidence of any mutation. In the patient's last follow-up visit in October 2017 (4 months after the completion of 11 cycles of FOLFIRI/panitumumab), he was clinically and radiologically disease free. During the same visit, a third cfDNA sample was acquired, in which the *STK11* mutation was again identified. The clonal make-up of the malignancy over time is summarised in figure 3.

DISCUSSION

The gene that was found mutated in our patient (*STK11*, also called *LKB1*) encodes a serine/threonine kinase that coordinates cell growth, polarity, motility and metabolism.[1] STK11 is a well-established tumour suppressor and a promoter of apoptosis[1] whose actions are partly mediated by AMPK.[2] Besides its function against tumour growth, it appears to be required for normal development in utero. Germ line mutations in the STK11 gene are frequently associated with Peutz-Jeghers syndrome and related neoplasias, while somatic mutations have been reported in various malignancies among which non-small cell lung carcinoma, cervical cancers and cutaneous melanomas.[1 2]

STK11 is only rarely mutated in somatic CRC (in 5/653 or 0.8%).[3] Of note, the specific single nucleotide substitution in the STK11 gene we discovered, namely, c.759C>A (p.Y253), is previously unreported in any type of neoplasia; however, the importance of this case lies elsewhere. It is unique in the sense that all four metastatic sites were resected and genetically analysed—a strategy difficult to implement in clinical practice which, however, may offer exceptional insight in the spatiotemporal genetic evolution of the tumour.

RAS mutation status being more than 90% concordant between primary tumour and metastases,[4 5] 'private' mutations are rare (accounting for 18 out of 434 mutations in tumour tissue samples from 69 patients),[5] often associated with more than one independent primaries.[4] Synchronous primary CRCs occur in 3.4%–6.2% of patients with CRC, with 9/13 pairs of synchronous primary CRCs exhibiting discordant *KRAS* mutational profiles.[4] Interestingly, our patient harboured two distinct rectal primaries. It is thus possible that the metastases originated from the *STK11*-wild type rectal primary, whereas cfDNA harbouring the *STK11* mutation originated from the other, supposedly *STK11*-mutated rectal primary. The fact that the patient's mutation was 'primary specific' is an even rarer finding. This could be a result of onsite heterogeneity and associated random sampling error (as genetic analysis of the metastases was performed on block sections rather than the whole of the surgical specimens), although the examination of more than one metastatic sites weakens this hypothesis. Finally, one could hypothesise that the absence of the *STK11* mutation from the metastatic tissue is owing to the administered treatment, as a direct result

of the lowered tumour burden achieved by therapy. *KRAS*-mutant allele levels have also been reported to gradually drop following anti-estimated glomerular filtration rate (EGFR) therapy withdrawal.[6 7] However, the effect of EGFR inhibitors on malignant clonal evolution[5] mainly refers to the emergence of de novo mutations[8 9] rather than the evanescence of existing ones.

In our patient case, the true clinically relevant question lies in the appearance, disappearance and subsequent reappearance of the *STK11* mutation in the bloodstream. The use of ctDNA for the detection of *KRAS* mutations in patients with CRC is associated with high specificity (0.96 and 0.98[10 11] in two recent meta-analyses), the detection of a mutation being impossible outside the context of residual disease[12] and trustworthy for diagnosing recurrence, while negative results cannot assure the complete elimination of tumour cells. In light of this data, the identification of the *STK11* mutation in the patient's circulation points to an occult niche of tumour cells shedding mutant DNA.

In conclusion, liquid biopsies are rapidly being integrated as useful adjuncts in clinical practice. Among their various emerging applications, they illustrate molecular heterogeneity over time and space, track tumour dynamics, spontaneous and therapy induced and help monitor response to therapy and minimal residual disease.[13] However, only rationally designed prospective studies that couple liquid biopsy data to clinically relevant endpoints will identify the clinical utility and actionableness of this powerful tool, along with its contribution to studying clonal heterogeneity.

Contributors MK, GZ, EK and GP had full access to all of the data of the case and take the responsibility for the integrity and interpretation of the data. AG kindly provided and edited Figure 1. MK performed the literature search, designed and edited the text, Figures 2 and 3 and their legends. GP conceived and finally reviewed the manuscript. All the authors contributed to the writing of the manuscript.

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