CASE REPORT

Identification of E545k mutation in plasma from a PIK3CA wild-type metastatic breast cancer patient by array-based digital polymerase chain reaction

Circulating-free DNA a powerful tool for biomarker testing in advance disease

ATOCHA ROMERO, DANIEL ACOSTA-EYZAGUIRRE, JULIÁN SANZ, FERNANDO MORENO, GLORIA SERRANO, EDUARDO DÍAZ-RUBIO, TRINIDAD CALDÉS, and JOSÉ Á. GARCIA-SAENZ

MADRID, SPAIN

PIK3CA gene is frequently mutated in patients with breast cancer and it has been the focus of intense research. Inhibitors of PI3K pathway are being evaluated in ongoing clinical trials but the impact of PIKC3A mutation status on tumor response is yet uncertain. In the metastatic setting, several studies are evaluating the predictive value of PIK3CA mutations. However, results could be biased by biopsy localization. Digital polymerase chain reaction is a new technology that enables detection and quantification of cancer DNA molecules from peripheral blood and can potentially overcome such situation. As a proof of the concept, we present the case of a metastatic patient with a PIK3CA wild-type primary tumor in which the PIK3CA E545K mutation was identified in both the circulating-free DNA obtained from a peripheral blood sample and in the formalin-fixed, paraffin-embedded liver metastasis. (Translational Research 2015;166:783–787)

Abbreviations: cfDNA = circulating-free DNA; dPCR = digital polymerase chain reaction; ER = estrogen receptor; PR = progesterone receptor; wt = wild-type

In recent years, the predictive/prognostic value of PIK3CA has been the focus of research interest. However, the clinical relevance of genetic events occurring at this locus is still unclear.[5-8] Regarding the metastatic setting, several groups have been evaluating the potential prognostic value of PIK3CA mutations, assessed in the primary tumor, in large clinical trials such as BOLERO-2 and CLEOPATRA.[9,10] Nevertheless, it is important to point out that in many cases only the archival tumor is evaluated, and it is well established that metastatic cells may differ from the primary tumor cells.[11] Therefore, it cannot be ruled out that PIK3CA mutation status could be biased by biopsy localization.

The assessment of PIK3CA mutation status in circulating-free DNA (cfDNA) by digital polymerase

From the Medical Oncology Department, Hospital Clínico San Carlos, Madrid, Spain; Medical Oncology Department, Hospital Universitario Puerta de Hierro, Madrid, Spain; Medical Oncology Department, Centro Integral Oncológico Clara Campal, HM Hospitals, Madrid, Spain; Pathology Department, Hospital Clínico San Carlos, Madrid, Spain.

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Reprint requests: Atocha Romero, Medical Oncology Department, Hospital Universitario Puerta de Hierro Madrid, C/Hospital Universitario Puerta de Hierro, Calle Manuel de Falla, no. 1, Majadahonda, Madrid 28222, Spain; e-mail: atocha10@hotmail.com.

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AT A GLANCE COMMENTARY

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Background

The PIK3CA gene, which encodes the p110α catalytic subunit of PI3K, is mutated in more than one-third of breast cancer cases,[1] making this gene an attractive candidate for the development of targeted therapies. The mutation spectrum displays a nonrandom distribution, existing hotspot positions within the helical domain (exon 9, commonly E542K and E545K), and the kinase domain (exon 20, commonly H1047R), which account for 70% of all PIK3CA mutations in breast tumors.[1-4]

Translational Significance

PIK3CA gene is frequently mutated in breast cancer tumors. However, the clinical and pathologic relevance of PIK3CA mutations is far from clear. In this article, we report the case in which PIK3CA mutation that was not present in the primary tumor was detected in the patient's plasma by array-based digital polymerase chain reaction (dPCR) as an example of the clinical situation in which an estrogen receptor positive breast cancer gains a PIK3CA mutation in its distant metastasis, reflecting the heterogeneity of the metastatic disease and highlighting the importance that dPCR may have to overcome the biases that sample localization may have when studying predictive/prognostic factors. Remarkably, the methodology (array-based dPCR) used is novel and has been less explored than Droplet dPCR.

chain reaction (dPCR) can potentially overcome such limitation. As a proof of concept, we report here a case of a metastatic patient with a PIK3CA wild-type (wt) tumor in which the PIK3CA E545K mutation was identified in the cfDNA from a plasma sample. The postmortem analysis of the metastasis showed that this mutated DNA corresponded to a liver metastasis.

CLINICAL HISTORY

A 29-year-old woman, with no family history of cancer, was noted by imaging to have 2.5 cm left-sided breast mass. A computed tomography–guided fine-needle biopsy confirmed the presence of an estrogen receptor (ER) positive, progesterone receptor positive, and human epidermal growth factor receptor 2 negative invasive ductal carcinoma. Germline testing for mutations of the BRCA1 and BRCA2 genes discarded the presence of pathogenic mutations at these genes. Patient underwent radical left mastectomy and was staged as stage IIIA (pT2pN2 (7/16) M0) breast cancer. She sequentially received doxorubicin- and taxanes-based adjuvant chemotherapy, locoregional radiotherapy, and adjuvant tamoxifen plus ovarian suppression therapy. Bone recurrence occurred 2 years later. A computed tomography–guided biopsy of a rib mass confirmed the presence of a breast cancer metastasis with the same immunohistochemical pattern similar to that of primary tumor. The patient then started denosumab and first-line palliative letrozole plus goserelin hormone therapy. After 12 months of progression-free survival, liver and skin metastases were diagnosed. The patient received several palliative chemotherapy regimens, and she finally deceased on March 2014, 5 years after the primary diagnosis.

MATERIAL AND METHODS

This study was carried out according to the Code of Ethics of the World Medical Association (Declaration of Helsinki). A plasma sample was donated to the Hospital Clinico San Carlos Biobank after signing the appropriate informed consent. Once the patient deceased, her husband approved the sequence analysis of all available metastatic lesions and the publication of the present study.

cfDNA was extracted from 5 mL of plasma within 1 hour after blood extraction using the QIAamp Circulating Nucleic Acid Kit (Qiagen, Valencia, California) according to the manufacturer's instructions. cfDNA samples were analyzed by dPCR using rare mutation assays for E542K, E545K, and H1047R on QuantStudio 3-dimensional Digital PCR System (Life Technologies, South San Francisco, California). The result of the assay is reported as the ratio of mutant DNA molecules to wt DNA molecules.

For the analyses of formalin-fixed, paraffin-embedded (FFPE) tumor and metastasis samples, a hemotoxylin and eosin–stained slide was obtained and reviewed by a pathologist to confirm the presence of cancer cells within the section. Subsequently, DNA was isolated from four 5-mm-thick unstained FFPE sections using the QIAamp DNA FFPE Tissue Kit (Qiagen) following the manufacturer's protocol.

The presence of PIK3CA mutations on FFPE samples was determined using the cobas PIK3CA mutation test (Roche Molecular Systems, Branchburg, New Jersey) and was further confirmed by PCR amplification followed by Sanger sequencing. Primers and conditions used are available on request. PCR products were directly sequenced using the BigDye Terminator v1.1

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