MOLECULAR ONCOLOGY 8 (2014) 150–158

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Establishing the origin of metastatic deposits in the setting of multiple primary malignancies: The role of massively parallel sequencing

Leticia De Mattos-Arruda[a,b], *Francois-Clement Bidard*[c,d], *Helen H. Won*[c], *Javier Cortes*[a,e], *Charlotte K.Y. Ng*[c], *Vicente Peg*[b,f], *Paolo Nuciforo*[a], *Achim A. Jungbluth*[c], *Britta Weigelt*[c], *Michael F. Berger*[c,g], *Joan Seoane*[a,b,h,**], *Jorge S. Reis-Filho*[c,g,*]

[a]*Vall d'Hebron Institute of Oncology, Vall d'Hebron University Hospital, Barcelona, Spain*
[b]*Universitat Autònoma de Barcelona, Barcelona, Spain*
[c]*Department of Pathology, Memorial Sloan-Kettering Cancer Center, New York, NY, USA*
[d]*Department of Medical Oncology, Institut Curie, Paris, France*
[e]*Medica Scientia Innovation Research (MedSIR), Barcelona, Spain*
[f]*Pathology Department, Vall d'Hebron University Hospital, Barcelona, Spain*
[g]*Human Oncology and Pathogenesis Program, Memorial Sloan-Kettering Cancer Center, New York, NY, USA*
[h]*Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain*

ARTICLE INFO

Article history:
Received 22 August 2013
Received in revised form
8 October 2013
Accepted 11 October 2013
Available online 31 October 2013

Keywords:
Massively parallel sequencing
Clonality
Breast cancer
Ovarian cancer
Metastasis
BRCA1

ABSTRACT

In this proof-of-principle study, we sought to define whether targeted capture massively parallel sequencing can be employed to determine the origin of metastatic deposits in cases of synchronous primary malignancies and metastases in distinct anatomical sites. DNA samples extracted from synchronous tumor masses in the breast, adnexal, and pelvic-peritoneal regions from a 62-year-old *BRCA1* germline mutation carrier were subjected to targeted massively parallel sequencing using a platform comprising 300 cancer genes known to harbor actionable mutations. In addition to *BRCA1* germline mutations, all lesions harbored somatic loss of the *BRCA1* wild-type allele and *TP53* somatic mutations. The primary breast cancer displayed a *TP53* frameshift (p.Q317fs) mutation, whereas and the adnexal lesion harbored a *TP53* nonsense (p.R213*) mutation, consistent with a diagnosis of two independent primary tumors (i.e. breast and ovarian cancer). The adnexal tumor and all pelvic-peritoneal implants harbored identical *TP53* (p.R213*) and *NCOA2* (p.G952R) somatic mutations. Evidence of genetic heterogeneity within and between lesions was observed, both in terms of somatic mutations and copy number aberrations. The repertoires of somatic genetic aberrations found in the breast, ovarian, and pelvic-peritoneal lesions provided direct evidence in support of the distinct origin of the breast and ovarian cancers, and established that the pelvic-peritoneal implants were clonally related to the ovarian lesion. These observations were consistent with those obtained with immunohistochemical analyses employing markers to differentiate between

* *Corresponding author*. Department of Pathology, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10065, USA. Tel.: +1 212 639 8054; fax: +1 212 639 2502.
** *Corresponding author*. Translational Research Program, Vall d'Hebron Institut d'Oncologia, Pg Vall d'Hebron 119-129, 08035 Barcelona, Spain. Tel.: +34 93 4894167; fax: +34 93 489 40 15.
E-mail addresses: jseoane@vhio.net (J.S. Seoane), reisfilj@mskcc.org (J.S. Reis-Filho).

http://dx.doi.org/10.1016/j.molonc.2013.10.006

carcinomas of the breast and ovary, including WT1 and PAX8. Our results on this case of a patient with *BRCA1*-mutant breast and ovarian cancer demonstrate that massively parallel sequencing may constitute a useful tool to define the relationship, clonality and intra-tumor genetic heterogeneity between primary tumor masses and their metastatic deposits in patients with multiple primary malignancies and synchronous metastases.

1. Introduction

Massively parallel sequencing analysis is reshaping the way the genomic, transcriptomic and epigenomic analyses of human tumors are performed (McDermott et al., 2011; Natrajan and Reis-Filho, 2011; Sandoval and Esteller, 2012; Yates and Campbell, 2012). This technology, when applied to the study of germline DNA, has resulted in the identification of genes whose mutations result in increased cancer predisposition (Natrajan and Reis-Filho, 2011; Yates and Campbell, 2012) or are causative of hereditary diseases (Poduri et al., 2013). In the context of somatic genetics and cancer transcriptomics, massively parallel sequencing analyses have not only led to the characterization of the repertoire of somatic genetic aberrations in human cancers, but also to the unraveling of intra-tumor genetic heterogeneity, mutational signatures in human cancer, identification of mutations that can explain response or resistance to specific therapeutic agents, and characterization of novel fusion genes and 'read-throughs' in epithelial malignancies (Alexandrov et al., 2013; Aparicio and Caldas, 2013; Castellarin et al., 2013; Crockford et al., 2013; Li and Durbin, 2009; McDermott et al., 2011; Natrajan and Reis-Filho, 2011; Turner and Reis-Filho, 2012; Yap et al., 2012).

Massively parallel sequencing approaches have recently been successfully employed in the characterization of the repertoire of mutations in biological materials other than primary or metastatic tumor tissue, including circulating cell-free plasma DNA and cervical cytological preparations, where the genetic information provided by these analyses may constitute a way of monitoring disease progression and response to specific therapies (De Mattos-Arruda et al., 2013; Kinde et al., 2013). Furthermore, this technology is rapidly being incorporated into the diagnostic armamentarium of oncologists and pathologists, in particular in the form of targeted sequencing approaches (i.e. massively parallel sequencing approaches based on the sequencing of a limited number of clinically relevant or biologically important genes or mutations) (Domchek et al., 2013; Wagle et al., 2012).

One of the potential applications of massively parallel sequencing is establishing the clonality between neoplastic lesions. The establishment of clonality between different tumor masses is particularly important in cases where patients present with synchronous primary disease and metastatic deposits, and there is uncertainty as to whether some of the metastatic deposits may constitute a second primary cancer. For example, in *BRCA1* and *BRCA2* germline mutation carriers, patients may present with both breast, abdominal and pelvic disease, and establishing whether the patient has two primaries (breast and ovarian) or metastatic disease is of clinical importance, given the therapeutic implications these diagnoses would have. Although histopathological and immunohistochemical analysis of primary tumors and metastatic deposits remain the lynchpins of diagnostic pathology and the usual means to define the primary site of a carcinoma of unknown primary site or whether a metastatic deposit stemmed from a given primary tumor, these approaches are by no means infallible, given potential changes in immunohistochemical markers as disease progresses from primary to metastatic (Amir et al., 2012; Niikura et al., 2012), overlap in the distribution of markers between metastatic tumors from different sites (Amir et al., 2012), and technical issues (Pusztai et al., 2010).

In this proof-of-principle study, we describe the use of targeted massively parallel sequencing as a means to define the site of origin of multiple metastases in a patient with two primary malignancies. Using a combination of massively parallel sequencing and bioinformatic methods, we analyzed a set of paraffin-embedded tumor tissue samples from a patient with synchronous breast and adnexal masses and pelvic-peritoneal metastases, whose histopathological and immunohistochemical features were insufficient to determine the origin of the metastatic deposits. Based on the repertoire of mutations found in each sample analyzed, not only the site of origin of the metastatic deposits could be defined, but also the clonal relatedness of the lesions biopsied, demonstrating the potential of this approach to provide answers for challenging clinical scenarios.

2. Material and methods

This study was approved by the IRB of Vall d'Hebron Institute of Oncology (Barcelona, Spain).

2.1. *Clinical history*

A 62-year-old Caucasian woman was referred in early 2012 to the Vall d'Hebron Institute of Oncology with history of a palpable 6 cm mass (largest diameter) in the right breast and a pelvic mass, which have grown in size over the last 12 months. The patient had no personal history of cancer. Family history was investigated and an unconfirmed history of pelvic cancer of the patient's mother was inferred.

Mammography revealed abnormal radiological features (Figure 1A and B), and a core biopsy of the breast mass was performed. Histological examination revealed a high-grade invasive ductal carcinoma of no special type of the breast. Immunohistochemical analysis was performed using antibodies against estrogen receptor (ER) (Novocastra, Leica Biosystems Newcastle Ltd, UK, 6F11, 7 ml Bond ready-to-use,