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Targeted next-generation sequencing detects a high frequency of potentially actionable mutations in metastatic breast cancers

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ABSTRACT

Background: Metastatic breast cancer is a genetically heterogeneous disease and effective therapies for advanced stage disease are limited.
Methods: In this study, distant metastases of 22 formalin-fixed, paraffin-embedded (FFPE) breast cancer samples were sequenced using the Ion Torrent PGM and the 50 gene AmpliSeq Cancer Hotspot Panel v2 from 10 ng of extracted DNA using 318 chips. Data analysis was performed with the Ion Torrent Variant Caller Plugin (hg19) and Golden Helix's SVS software for annotation and prediction of the significance of the variants.
Results: All patients were female with a median age of 61 years (range 37–85 years). Metastatic sites included liver (n = 6, 27%), skin (n = 5, 23%), brain (n = 4, 18%), lymph node (n = 3, 14%), lung (n = 2, 9%), retroperitoneum (n = 1, 4.5%), and colon (n = 1, 4.5%). Overall, 28 variants in 11 genes were observed. Five (23%) samples showed no alterations and 17 (77%) showed at least one potentially biologically significant variant (BSV) defined as having FDA-approved drugs or clinical trials evaluating their significance. BSVs included mutations in the following genes: *TP53* (n = 8), *APC* (n = 4), *PIK3CA* (n = 5), *MET* (n = 2), *ERBB2* (n = 2), *AKT1* (n = 1), *CDKN2A* (n = 1), *KRAS* (n = 1), and *FGFR3* (n = 1).
Conclusions: Potentially actionable mutations were identified in the majority of breast cancer metastases. Evaluating metastatic breast tumors using a NGS approach provides a better understanding of the mechanisms behind tumor progression and evolution and also identifies additional potentially beneficial therapeutic targets for patient management or eligibility for clinical trials.

1. Introduction

Breast cancer is a diverse disease with discernable differences in presentation, clinical behavior, and response to therapy. Current routine testing for the biomarkers estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor (*ERBB2*/HER2) classifies breast cancer into various subtypes with an aim to predict behavior and employ targeted therapy. Gene expression profiling initially categorized breast cancer into four intrinsic subtypes with distinct clinical and molecular characteristics: luminal A (hormone receptor positive), luminal B (hormone receptor positive, HER2 positive/negative with high proliferation rate), HER2+ (hormone receptor negative and *ERBB2*/HER2 gene amplification with protein overexpression), and triple-negative/basal-like (Perou et al., 2000; Sorlie et al., 2001; De Abreu et al., 2014). In the last decade, microarray-based analysis and massively-parallel gene sequencing have provided insight into the molecular complexity of breast cancer and expanded our understanding of the prognostic and predictive genetic signatures of breast cancer (Weigelt et al., 2010). The intricate molecular heterogeneity of breast cancer challenges the reliability of a four-tier classification system. Furthermore, studies have shown that rarely are two breast tumors genetically identical, and genetic diversity exists even within the same tumor in the form of clonal subpopulations (Wang et al., 2013).

Current targeted treatment includes anti-endocrine and anti-HER2 therapy for some tumor types, however, not all patients respond equally or favorably. Despite current treatment regimens, patients may relapse with recurrent breast cancer and/or metastatic disease after initial treatment. A subset of breast cancers have mutations pre-disposing to resistance (primary resistance) or will acquire more complex genetic mutations which then lead to metastatic potential and/or treatment resistance (secondary resistance) (Navin et al., 2011; Bose et al., 2013). Although there have been significant advances in the treatment of primary breast cancer in the last 30 years with a significant decline in mortality rates, metastatic disease is still essentially incurable. Patients often exhaust standard treatment regimens and are left with few effective therapeutic options.

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To date, most research efforts have focused on defining the molecular characteristics of primary breast cancer. Large genomic databases harbor abundant data on the molecular makeup of primary breast cancer; however, the genetic landscape of breast tumor metastases is not well characterized. Like primary breast cancer, genomic profiling has demonstrated that metastases from breast cancer contain complex and patient-unique molecular landscapes (Wheler et al., 2014). Emerging initiatives such as AURORA, launched by the Breast International Group, stress the importance of collecting data on metastatic tumors and collating large datasets in order to map the clonal landscape of metastatic breast cancer (Zardavas et al., 2014).

In this study, we evaluate the mutational profile of breast cancer metastases through targeted next-generation sequencing with the aim to identify potential clinically actionable genetic aberrations.

2. Materials and methods

2.1. Case selection and sample preparation

In 2013, the molecular pathology laboratory at DHMC validated and incorporated sequencing as a routine clinical test for somatic mutational analysis in patients with solid tumors (Tsongalis et al., 2014). Some cases are referred to a multidisciplinary molecular tumor board to discuss and interpret the significance of the mutational profile to provide treatment recommendations (Tafe et al., 2015). For this study, we searched our pathology database to identify patients with metastatic breast cancer who underwent biopsy and subsequent sequencing of the metastatic tumor for clinical purposes. Biomarker status had been previously determined by immunohistochemistry for ER and PR and FISH (*ERBB2* amplification) for HER2 unless noted otherwise. Cases were reviewed to ensure appropriate tumor content (minimum of 10%). An appropriate formalin-fixed paraffin-embedded (FFPE) tissue block was selected for each case (except for one that only had frozen tissue available). Eight unstained FFPE tissue sections were cut at 4 μm each for DNA extraction. A pathologist identified the lesional area and the percent tumor cell content for each case which was the area macrodissected for extraction. Genomic DNA (gDNA) was obtained using the Gentra Pure Gene Kit (Qiagen) or the QiaCube (Qiagen; after August 2015), and quantified using the Quant-iT™ PicoGreen® dsDNA Assay Kit (Invitrogen) according to the manufacturer's recommendations. This study was approved by the Dartmouth College Committee for the Protection of Human Subjects (Study# 00029191).

2.2. Next-generation sequencing and data analysis

Next-generation sequencing was performed using the Ion AmpliSeq™ Cancer Hotspot Panel v2, which consists of 50 oncogenes and tumor suppressor genes covering approximately 2800 Catalog of Somatic Mutations in Cancer (COSMIC) mutations as described previously (Tsongalis et al., 2014). In brief, barcoded libraries were prepared from 10 ng of extracted DNA and multiplexed for sequencing using 318v2 chips. Tumor samples were evaluated for genomic alterations including single nucleotide variants, insertions and deletions. Data analysis was performed using the Ion Torrent Variant Caller Plugin (v4.0) and reference genome hg19. Golden Helix's SVS software and medical literature were used for annotation and prediction of the significance of variants. For the purposes of this study, an alteration was considered potentially biologically significant if an approved or investigational therapy in breast cancer or other solid tumor was available.

3. Results

3.1. Specimens, sites, histopathologic data

Patient information and corresponding individual mutation profiles are summarized in Table 1. Twenty-four patient samples were submitted for next-generation sequencing as part of routine practice over a 22-month period as requested by the treating oncologist. Two samples did not have sufficient DNA for sequencing and thus were excluded from this data set. Twenty-two metastatic tumors from 22 patients (average age 61 years, range 37–85 years) were successfully sequenced. Surgical specimens were obtained from biopsies and small excisions (n = 17, 77%), and cytologic specimens were obtained from fine needle aspirates (FNA) (n = 5, 23%). Metastatic sites included liver (n = 6, 27%), skin (n = 5, 23%), brain (n = 4, 18%), lymph node (n = 3, 14%), lung (n = 2, 9%), retroperitoneum (n = 1, 4.5%), and colon (n = 1, 4.5%). Histologic types of the corresponding primary tumors were predominantly invasive ductal carcinoma of no special type (n = 12, 63%). The remainder of the primary breast tumors were invasive lobular carcinoma (n = 3, 16%), invasive carcinoma with ductal and lobular features (n = 2, 11%), invasive mucinous carcinoma (n = 1, 5%), and metaplastic carcinoma (n = 1, 5%). Histologic grades were available for eighteen primary tumors: all were intermediate (n = 7) or high grade (n = 11). Histologic data from primary tumors were not available for three patients; data was either unavailable from records or the patient presented with metastatic disease and did not have their primary tumor examined. Full biomarker (ER, PR, and HER2) profiles were available for 17 primary tumors. Eight patients had ER+, PR+, HER2− tumors, three patients had ER+, and/or PR+, HER2+ tumors, one patient had an ER−, PR−, HER2+ tumor, and five patients had triple-negative (ER−, PR−, HER2−) tumors. Full biomarker profiles were available for 21 metastatic tumors, 23.8% of which were discordant from the primary profile. Thirteen patients had ER+, PR+, HER2− tumors, one patient had an ER+, and/or PR+, HER2+ tumor, two patients had ER−, PR−, HER2+ tumors, and five patients had triple-negative (ER−, PR−, HER2-) tumors. Five cases showed a change in one or more biomarkers from the primary to metastatic tumor, which included loss of ER (n = 1), PR (n = 2), and HER2 (n = 2) expression, and gain of PR (n = 1) and HER2 (n = 1) expression. All patients were treated with standard regimens including anti-estrogen therapy, anti-HER2 therapy, chemotherapy, and/or radiotherapy. At the time of this publication, 12 patients are alive with metastatic disease (two of whom are currently without any measureable evidence of disease (cases 7 and 8), 10 died of metastatic disease. Four of the five patients with no mutation identified lived more than 3 years with metastatic disease.

3.2. Somatic mutation analysis

Twenty-two of 24 samples were successfully sequenced. A maximum of 10 samples were sequenced on 318v2 chips with a mean depth of coverage of 2800× (minimum 2065×; maximum 4800×). Mutations are summarized in Fig. 1. Overall, 28 variants in 11 of the 50 genes analyzed were observed. Five samples showed no alterations and 17 (77%) samples showed at least one potentially biologically significant variant (BSV). Nine tumors showed multiple variants (up to four), and eight tumors showed only a single variant. Amongst cases with at least one variant, the average number of variants was 1.7 per tumor. The 28 genomic variant aberrations included 23 base substitutions and five short insertions/deletions. Small insertions and deletions led to frame shifts in all five cases. The most commonly altered genes included *TP53* (n = 8, 36%), *PIK3CA* (n = 5, 23%), *APC* (n = 4, 18%), *MET* (n = 2, 9%), and *ERBB2* (n = 2, 9%). Aberrations in the following genes were also found, however less frequently: *AKT1* (n = 1), *CDKN2A* (n = 1), *KRAS* (n = 1), *FGFR3* (n = 1), *SRC* (n = 1), and *CDH1* (n = 1). The majority of variant mutations were considered potentially biologically significant with the exception of *CDH1*. No two metastatic tumors had the same molecular profile. Two cases showed common mutations in *MET*; however, these cases had distinct overall molecular make-ups.

Genetic mutations according to ER, PR, and HER2 status are summarized in Fig. 1. Triple-negative tumors showed the highest frequency of