EBioMedicine xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Research Paper

Identifying Circulating Tumor DNA Mutation Profiles in Metastatic Breast Cancer Patients with Multiline Resistance

Zhe-Yu Hu [a,b,c,d], Ning Xie [a,b,c,1], Can Tian [a,b,c], Xiaohong Yang [a,b,c], Liping Liu [a,b,c], Jing Li [a,b,c], Huawu Xiao [a,b,c], Hui Wu [a,b,c], Jun Lu [a,b,c], Jianxiang Gao [a,b,c], Xuming Hu [a,b,c], Min Cao [a,b,c], Zhengrong Shui [a,b,c], Mengjia Xiao [a,b,c], Yu Tang [a,b,c], Qiongzhi He [e], Lianpeng Chang [e], Xuefeng Xia [e], Xin Yi [e], Qianjin Liao [d], Quchang Ouyang [a,b,c,*]

[a] Hunan Cancer Hospital, and the Affiliated Cancer Hospital of Xiangya Medical School, Central South University, Changsha 410013, China
[b] Department of Breast Cancer Medical Oncology, Hunan Cancer Hospital, Changsha 410013, China
[c] Department of Breast Cancer Medical Oncology, The Affiliated Cancer Hospital of Xiangya Medical School, Central South University, Changsha 410013, China
[d] Central Laboratory, The Affiliated Cancer Hospital of Xiangya Medical School, Central South University, Changsha 410013, China
[e] Geneplus-Beijing Institute, Beijing 102206, China

ARTICLE INFO

Article history:
Received 7 March 2018
Received in revised form 10 May 2018
Accepted 10 May 2018
Available online xxxx

Keywords:
Circulating tumor DNA (ctDNA)
Drug resistance
Gene mutation pattern
HR/HER2 subtype
Metastatic breast cancer
Progression-free survival

ABSTRACT

Purpose: In cancer patients, tumor gene mutations contribute to drug resistance and treatment failure. In patients with metastatic breast cancer (MBC), these mutations increase after multiline treatment, thereby decreasing treatment efficiency. The aim of this study was to evaluate gene mutation patterns in MBC patients to predict drug resistance and disease progression.

Method: A total of 68 MBC patients who had received multiline treatment were recruited. Circulating tumor DNA (ctDNA) mutations were evaluated and compared among hormone receptor (HR)/human epidermal growth factor receptor 2 (HER2) subgroups.

Results: The baseline gene mutation pattern (at the time of recruitment) varied among HR/HER2 subtypes. *BRCA1* and *MED12* were frequently mutated in triple negative breast cancer (TNBC) patients, *PIK3CA* and *FAT1* mutations were frequent in HR+ patients, and *PIK3CA* and *ERBB2* mutations were frequent in HER2+ patients. Gene mutation patterns also varied in patients who progressed within either 3 months or 3–6 months of chemotherapy treatment. For example, in HR+ patients who progressed within 3 months of treatment, the frequency of *TERT* mutations significantly increased. Other related mutations included *FAT1* and *NOTCH4*. In HR+ patients who progressed within 3–6 months, *PIK3CA*, *TP53*, *MLL3*, *ERBB2*, *NOTCH2*, and *ERS1* were the candidate mutations. This suggests that different mechanisms underlie disease progression at different times after treatment initiation. In the COX model, the ctDNA *TP53* + *PIK3CA* gene mutation pattern successfully predicted progression within 6 months.

Conclusion: ctDNA gene mutation profiles differed among HR/HER2 subtypes of MBC patients. By identifying mutations associated with treatment resistance, we hope to improve therapy selection for MBC patients who received multiline treatment.

1. Introduction

Breast cancer is the most prevalent cancer among females, with a relatively high survival rate. Indeed, the Surveillance Epidemiology and End Results (SEER) dataset (SEER 18, 2006–2012) in the United States indicates that the 5-year survival rate is 89.7%. This survival rate has actually improved in recent decades, in part due to the prevalence of population-based mammography screening and the systematic use of adjuvant therapies [1]. As a result, long-term tumor-bearing survival is now common among advanced breast cancer patients [2]. Importantly, many long-term surviving metastatic breast cancer (MBC) patients receive multi-line chemotherapy. Unfortunately, such treatment reduces the sensitivity of MBC tumor cells to most commonly used

Abbreviations: ctDNA, circulating tumor DNA; MBC, metastatic breast cancer; HR, hormone receptor; HER2, human epidermal growth factor receptor 2; TNBC, triple negative breast cancer; SEER, Surveillance Epidemiology and End Results; PFS, progression-free survival; ER, estrogen receptor; PR, progesterone receptor; IHC, immunohistochemistry; cfDNA, cell-free DNA; gDNA, genomic DNA; SNV, Single nucleotide variants; Indels, insertions and deletions; TMB, Tumor mutation burden; WES, whole exome sequencing; ROC, receiver operating characteristic; AUC, area under the curve; HR, hazard ratio; CI, confidence interval; GX, gemcitabine+capecitabine; AI, aromatase inhibitor.

* Corresponding author at: Department of Breast Cancer Medical Oncology, Hunan Cancer Hospital and The Affiliated Cancer Hospital of Xiangya Medical School, Central South University, No. 283, Tongzipo Road, Changsha 410013, China.
E-mail address: oyqc1969@126.com (Q. Ouyang).

[1] Zheyu Hu and Ning Xie contributed equally to this manuscript

https://doi.org/10.1016/j.ebiom.2018.05.015

drugs. This complicates the process of selecting effective drugs for progressed MBC patients. One way to streamline this drug selection process is by identifying potential drug-sensitive gene mutations in circulating tumor DNA (ctDNA).

ctDNA can be derived from liquid biopsies (minimally-invasive blood from cancer patients). While traditional imaging tools or serum biomarkers (e.g., CEA and CA153) are not timely nor sensitive enough to reflect small changes in tumor mutations, monitoring ctDNA provides valuable and sensitive blood-based biomarkers in advanced cancers. Indeed, ctDNA levels correspond to the tumor burden [3]; thus, ctDNA screening can help monitor the tumor response to treatment [4–7]. ctDNA mutations can also indicate tumor sensitivity to specific chemotherapy drugs [8–12]. For example, *PIK3CA* and *BRCA1* are common mutations in MBC [13,14]. While *PIK3CA* mutations suggest sensitivity to the mTOR inhibitor Everolimus [15], *BRCA1* mutations suggest sensitivity to the PARP inhibitor Olaparib [16]. Thus, screening for ctDNA mutations provides a minimally-invasive tool for doctors to identify effective drug-based therapies in MBC patients.

Conversely, monitoring ctDNA can also aid early detection of genetic events underlying drug resistance and inform potential combination therapy approaches [17]. For example, increased *PIK3CA* mutations following treatment initiation suggest tumor progression and poor progression-free survival (PFS) [18]. In estrogen receptor (ER)-positive patients, *ESR1* mutations following endocrine therapy (except Fulvestrant) indicate treatment resistance [19–23], while in HER2+ patients, mutations in *TP53* and *PIK3CA* are associated with anti-HER2 therapy resistance [24]. According to NCCN guidelines, breast cancer patients are recommended to be diagnosed and treated based on their hormone receptor (HR)/human epidermal growth factor receptor 2 (HER2) status. In general, triple negative (HR-/HER2-), HR-positive and HER2-positive are three main subtypes. However, previous studies did not fully and systematically characterize ctDNA gene mutation patterns in MBC patients related to HR/HER2 status and treatment. Such information is critical for both efficient surveillance of tumor gene mutations and accurate treatment. In this study, we recruited 68 MBC patients to systematically investigate their ctDNA mutation profiles relative to HR/HER2 status. Potential ctDNA markers for monitoring tumor progression, drug resistance, and treatment response were analyzed.

2. Methods

2.1. Patient Cohort and Clinical Data Collection

This study was approved by the Ethics Committee at the Affiliated Cancer Hospital of Xiangya School of Medicine at Central South University. A total of 68 metastatic breast cancer patients were enrolled in this study who were treated from January 2016 to November 2017 at the Department of Breast Oncology in the Affiliated Cancer Hospital of Xiangya School of Medicine. Informed consent was obtained from each patient prior to study onset. According to the American Joint Committee on Cancer staging system, patients diagnosed with stage III/IV primary breast malignant tumors (site: C50.0–C50.9; histology type: invasive ductal carcinomas or lobular carcinomas) were recruited. Other inclusion criteria included: 1) pathologically confirmed triple negative MBC patients, HER2+ positive recurrent or MBC patients, and ER+/progesterone receptor (PR)+recurrent or MBC patients; 2) according to RECIST 1.1 standards, patients had at least one measurable lesion; 3) aged between 18 and 70 years; 4) liver, renal, and blood tests showed a neutrophil count > 2.0 g/l, Hb > 9 g/l, platelet count > 100 g/l, AST and ALT >0.5 ULN, TBIL <1.5 ULN, and Cr < 1.0 ULN. The exclusion criteria included: 1) multiple primary cancers; 2) patients with immunodeficiency or organ transplantation history; 3) patients with heart disease or heart abnormalities such as cardiac infarction and severe cardiac arrhythmia (Fig. S1). Basic demographic and clinical information including age, pathology, laterality, stage, metastatic sites, HR/HER status, imaging records, and treatment history were collected [25].

2.2. Receptor Status Evaluation

ER and PR status were evaluated by immunohistochemistry (IHC) based on the American Society of Clinical Oncology guidelines [26,27]. The steps for IHC evaluation were as follows: 1) calculate the percent of ER/PR positively stained invasive tumor cells; 2) record the intensity of staining; 3) interpret that a minimum of 1% of invasive tumor cells staining positive for ER/PR in a specimen is HR+, while specimens exhibiting <1% of tumor cells staining for ER or PR of any intensity is HR-. For HER2 status, HER2+ was defined as a tumor area >10% with contiguous and homogeneous tumor cells indicated as HER2+ via gene amplification or IHC (HER2 copy number or HER2:CEP17 quantified by fluorescent in situ hybridization based on counting at least 20 cells within the area). The latest record of HR/HER2 status from recurrence biopsies was applied in this study.

2.3. Blood Sample Collection and DNA Extraction

Peripheral blood samples were collected 7 days before treatment, at 2–3 cycles of treatment when the first evaluation was performed, and at disease progression (image evaluation based on RECIST 1.1 standards). Peripheral blood samples were collected in Streck tubes (Streck, Omaha, NE, USA) and centrifuged within 72 h to separate the plasma from peripheral blood cells. Cell-free DNA (cfDNA) was extracted from plasma usinga QIAamp Circulating Nucleic Acid Kit (Qiagen, Hilden, Germany). Genomic DNA (gDNA) was extracted from peripheral blood cells using a QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). Both DNA extractions were performed according to the manufacturer's instructions. gDNA was sequenced as the normal control sample. Volumes of blood collected and of circulating-free DNA extracted (concentrations and total amounts) for all patients are listed in Table S1.

2.4. Target Capture and Next-Generation Sequencing

Both cfDNA and gDNA libraries were constructed with the KAPA DNA Library Preparation Kit (Kapa Biosystems, Wilmington, MA, USA) using the manufacturer's protocol. Capture probes were designed to cover coding sequences and hot exons of 1021 genes that are frequently mutated in solid tumors. A detailed description of the capture experiments has been reported previously [28]. Libraries were hybridized to custom-designed biotinylated oligonucleotide probes (Integrated DNA Technologies, Iowa, IA, USA). DNA sequencing was performed using the HiSeq 3000 Sequencing System (Illumina, San Diego, CA) with 2 × 101-bp paired-end reads. In Table S2, all genes included in our panel are listed. Clonal hematopoietic mutations were filtered as previously described [29], including those in *DNMT3A*, *IDH1*, and *IDH2* and specific alterations within *ATM*, *GNAS*, and *JAK2*.

2.5. Sequencing Data Analysis

From raw data, terminal adaptor sequences and low-quality reads were removed. The BWA (version 0.7.12-r1039) tool aligned clean reads to the reference human genome (hg19), and Picard (version 1.98) marked PCR duplicates. Realignment and recalibration was performed using GATK (version 3.4–46-gbc02625). Single nucleotide variants (SNV) were called using MuTect (version 1.1.4) and NChot, a software developed in-house to review hotspot variants [28]. Small insertions and deletions (Indels) were called using GATK. Somatic copy number alterations were identified with CONTRA (v2.0.8). Significant copy number variation was expressed as the ratio of adjusted depth between ctDNA and control gDNA. The final candidate variants were all manually verified in the Integrative Genomics Viewer. Sequencing stats of all samples are shown in Table S1. This sequencing method was previously proven credible with simulated cfDNA [28], so we did not validate the mutations found in ctDNA by sequencing tumor biopsies.

Please cite this article as: Hu Z-Y, et al, Identifying Circulating Tumor DNA Mutation Profiles in Metastatic Breast Cancer Patients with Multiline Resistance, EBioMedicine (2018), https://doi.org/10.1016/j.ebiom.2018.05.015