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Original Research

Prior systemic treatment increased the incidence of somatic mutations in metastatic breast cancer

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Abstract ***Background:*** Understanding the biology of breast cancer is important for guiding treatment strategies and revealing resistance mechanisms. Our objectives were to investigate the relationship between previous systemic therapy exposure and mutational spectrum in metastatic breast cancer and to identify clinicopathological factors associated with identified frequent somatic mutations.

Methods: Archival tissues of patients with metastatic breast cancer were subjected to hotspot molecular testing by next-generation sequencing. The variables that significantly differed ($P < 0.05$) in univariate analysis were selected to fit multivariate models. Logistic models were fit to estimate the association between mutation status and clinical variables of interest. Five-fold cross-validation was performed to estimate the prediction error of each model.

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Results: A total of 922 patients were included in the analysis. In multivariate analysis, previous systemic treatment before molecular testing (N = 186) was associated with a significantly higher rate of *TP53* and *PIK3CA* mutations compared with the lack of systemic treatment ($P < 0.001$ for both).
Conclusion: Systemic treatment exposure is an independent risk factor for high rates of *TP53* and *PIK3CA* mutation, which suggests the importance of testing samples after systemic therapy to accurately assess mutations. It is worth testing the gene profile when tumours become resistant to systemic treatments.

1. Introduction

The Cancer Genome Atlas project has provided a better understanding of the biology of breast cancer [1]. Recent clinical efforts have focused on understanding tumour characteristics based on genomic analysis and on developing models to identify patients who can benefit from molecular targeted therapies in clinical trials. Molecular analysis can reveal potential predictive markers of treatment response, especially to molecular targeted therapies, as well as prognostic markers [2–4]. A recent retrospective study demonstrated that *TP53* mutations were associated with shorter relapse-free survival and overall survival in breast cancer [3]. *PIK3CA* mutations have shown some association with better survival outcomes in some retrospective studies [3,5] and altered response to targeted therapies [6,7] but were associated with significantly worse survival in another retrospective study [8]. Although several molecular platforms have been used to understand genomic tumour characteristics, the cost of testing is still not affordable for all patients. In this regard, patient clinicopathological factors that predict which patients are more or less likely to have somatic mutations would be an important addition to clinical practice.

Furthermore, resistance mechanisms after exposure to systemic treatment constitute an emerging issue to be addressed. It has been proposed that previous treatment exposure potentially contributes to the development of new mutations in tumours [2,9,10]. However, in breast cancer, it is still unknown whether previous systemic treatment exposure is associated with high somatic mutation rate.

We hypothesised that previous exposure to systemic treatment is significantly associated with an increase in somatic mutations in breast cancer. In the present study, our objectives were to investigate the association between previous treatment exposure to systemic therapy and frequently mutated genes identified through hotspot molecular testing by next-generation sequencing and to identify clinicopathological factors associated with the identified somatic mutations.

2. Methods

2.1. Study design

Patients with metastatic breast cancer who were seen at MD Anderson Cancer Center from March 2012 to December 2014 were explained the study objective and those who signed informed consent were enrolled in the large-scale molecular testing protocol by using archival tumour samples. The protocol was approved by an institutional review board at the University of Texas MD Anderson Cancer (PA11-0852). All patients signed an informed consent form before molecular testing. Clinical data were collected retrospectively from electronic medical records. From the database, we extracted age, race, menopausal status, inflammatory breast cancer (IBC or non-IBC), hormone status (negative or positive), the human epidermal growth factor receptor 2 (HER2) status (negative or positive), subtype (HR−/HER2+, HR+/HER2−, HR+/HER2+ or triple-negative breast cancer [TNBC]), nuclear grade (I/II or III), histology (ductal, lobular or others) and previous systemic treatment exposure (previous treatment exposure or treatment naive). Systemic treatment was defined as either chemotherapy, hormonal therapy, targeted therapy or combinations of these.

2.2. Sample collection

After written informed consent was obtained, archived tumour samples were sent for molecular analysis. Archival specimens analysed in the present study were either primary or metastatic tumours and included formalin-fixed, paraffin-embedded core needle biopsies and tumour resection specimens. Although at the time of study enrolment all patients had metastatic breast cancer, some tumours sent for molecular analysis were treatment naive and the others have previous treatment exposure because we used archival tumour samples. Manual microdissection of tumour-rich areas was performed, and only cases with >20% tumour cellularity were included in this study.