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Discordant ERBB2 Status and Genome Wide DNA Copy Number Alterations in Breast Cancer and Synchronous Lymph Node Metastasis: A Case Report and Literature Review

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ABSTRACT

Erb-b2 receptor tyrosine kinase 2 (ERBB2) is a biomarker in the management of breast cancer (BC). Changes of ERBB2 status in primary and synchronous metastasis have been reported in approximately 5% of BC. Here we describe a 55-years-old female with a history of left-side BC and again diagnosed in 2019 with right-side BC tested ER-, PR-, ERBB2+ in breast tissue and triple negative in right axillary lymph node. The right BC and lymph node metastasis samples were tested by chromosomal microarray (CMA); the BC sample harbored more DNA copy number alterations than the lymph node specimen, suggesting that the lymph node metastasis was not directly originating from a dominant tumor clone in the right BC. This case highlights discordant ERBB2 status that can benefit from assessment of ERBB2 in both primary and metastatic specimens. This case also demonstrates the value of using CMA to help understand the clonal evolution of breast cancer metastasis especially when there are discordant biomarker results between primary and metastatic lesions.

KEYWORDS

Breast cancer; ERBB2; FISH; CMA; Primary tumor; Metastasis

1. INTRODUCTION

Erb-b2 receptor kinase 2 gene (ERBB2), also known as human epidermal growth factor receptor 2 (HER2), is located on chromosome 17q12 and encodes a receptor tyrosine kinase (OMIM: 164870). The status of ERBB2 expression and/or DNA amplification in breast cancer is an integral part of breast cancer evaluation to guide disease classification, prognostication, and treatment [1]. Immunohistochemistry (IHC) and in situ hybridization (ISH) are routinely used to examine ERBB2 protein expression and DNA amplification in primary and metastatic breast cancer specimens [2]. ERBB2 status is typically conserved during tumor progression; however, in a small subset of patients, there exists discordant ERBB2 status between primary and metastasized tumors [2-5]. For example, the total discordance of ERBB2 status in primary and metastatic breast cancers has been reported

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to be around 14%, with similar rates of switching to negatives or positives (e.g., ERBB2+ primary tumor/ERBB2- metastasis or ERBB2- primary tumor/ERBB2+ metastasis, both occur approximately 7%) [6]. Testing of both primary and metastatic samples should identify such discordant cases. The causes of discordancy can range from tumor heterogeneity to sampling biases [7]. In the following case, we report a patient presenting with discordant ERBB2 status in primary BC and axillary node metastasis with evidence of primary BC intro-tumor heterogeneity via chromosomal microarray (CMA).

2. CASE REPORT

A 55-years-old Caucasian female presented to the University of Texas Medical Branch (UTMB) in January 2019 with a chief complaint of painful lumpy right breast. She has a history of left side breast cancer, which was diagnosed in 2003 at a different institution as multifocal invasive ductal carcinoma, Black's nuclear grade 3, ER+, PR+, ERBB2-, Ki-67 90%. Her clinical stage was cT2 N0 M0, and she was treated with left lumpectomy, chemotherapy and radiation therapy followed by 5-year tamoxifen hormonal therapy. In January 2019, the patient began having a stabbing pain on her right lateral breast extending to the right axilla. Imaging studies demonstrated a 44 mm mass in the upper outer quadrant of her right breast and a high-density lymph node in the right axillary, both highly suggestive of malignancy. Core biopsy of the right breast lesion demonstrated invasive ductal carcinoma with apocrine features, poorly differentiated, Bloom-Richardson grade 3, ER 0%, PR 0%, ERBB2 IHC 2+, dual-probe fluorescence *in situ* hybridization (FISH) amplified with ERBB2/chromosome enumeration probe 17 (CEP17) ratio 2.5 and ERBB2 copy number/cell 4.9. Core biopsy of the positive right axillary lymph node demonstrated ER 0%, PR 0%, ERBB2 IHC 2+, dual-probe FISH non-amplified with ERBB2/CEP17 ratio 1.59 and *ERBB2* copy number/cell 2.95 (Figure 1). Repeat testing of the axillary lymph node was performed at another institution and resulted in concordance with the initial triple negative receptor status.

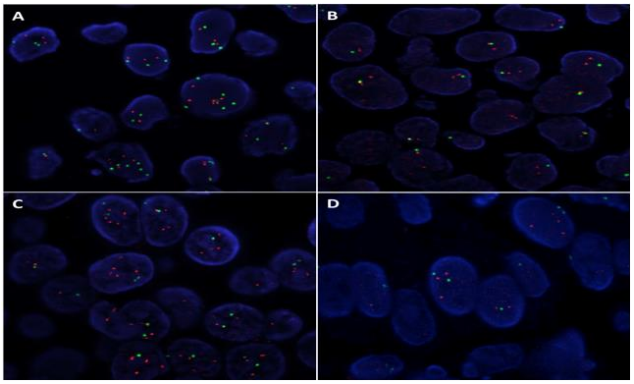

Figure 1: Representative dual-probe ERBB2/CEP17 FISH results. Red and green represent ERBB2 and CEP17 signals, respectively. (A) Normal control; (B) Cutoff control; (C) Right breast cancer tissue; (D) Right axillary lymph node metastasis. The average ERBB2/CEP17 ratios were (A) 1.05; (B) 1.96; (C) 2.5; and (D) 1.59. The average ERBB2 copy numbers/cell were (A) 3.00; (B) 2.55; (C) 4.9; and (D) 2.95. Hematoxylin and eosin slides from both primary and metastatic sites were reviewed and circled by a breast pathologist (JH) for FISH analyses. Dual-probe FISH was performed using PathVysion HER-2 DNA Probe Kit according to manufacturer's instructions (Abbott Laboratories).

The patient was staged as cT2 N1 M0 and completed 6 rounds of neoadjuvant therapy consisting of Carboplatin and Taxotere followed by bilateral skin-sparing total mastectomy, and autologous lymph node transplant; she declined radiation therapy. Post-surgery, the patient's surgically resected tissues demonstrated complete pathologic response with 24 lymph nodes negative for metastatic carcinoma and no residual carcinoma identified in bilateral breast tissues.

The patient denied a family history of breast cancer but sought genetic testing due to her history of early onset and recurrent breast cancers. A 14 gene testing (Invitae Breast Cancer Panel, https://www.invitae.com/en/physician/tests/01202/) of peripheral blood revealed a heterozygous BRCA1 mutation, c.1961dup (p.Tyr655fs, NM_007294.3, rs80357853), predicted to result in nonsense mediated decay of BRCA1 products. This pathogenic variant has been reported in tumor samples of breast, ovary, and

stomach (COSV58789969, https://cancer.sanger.ac.uk/cosmic/mutation/overview?id=114902644) [8]. The result supported a diagnosis of hereditary breast and ovarian cancer syndrome [1]. Chromosomal microarray (CMA) was performed on both primary and lymph node metastatic biopsies and demonstrated discordance with respect to genome-wide DNA copy number alterations (CNAs), with the breast cancer specimen harboring more genomic changes than the lymph node (Figure 2).

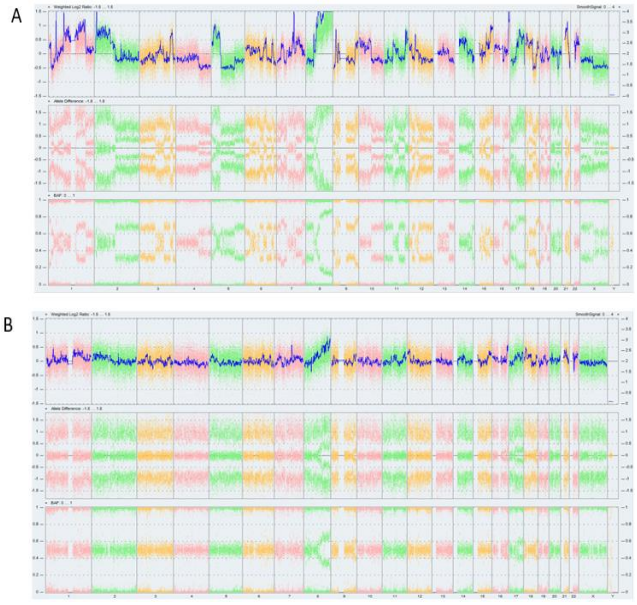

Figure 2: Whole genome view of CMA results. (A) Primary breast cancer; (B) Lymph node metastasis. Shown are weighted log2 ratio (top), allele difference (middle), and B allele frequency (bottom). Formalin-fixed paraffin-embedded tumor specimens were tested. Hematoxylin and eosin slides from both primary and metastatic sites were reviewed and circled by a breast pathologist (JH). Macrodissection was performed on both the primary and lymph node metastatic samples prior to DNA extraction for CMA analyses. CMA was performed using Affymetrix OncoScan FFPE Assay Kit according to manufacturer's instructions (Thermo Fisher Scientific Inc.).

3. DISCUSSION

We described a case with discordant ERBB2 status that was positive in primary breast cancer and negative in lymph node metastasis. Current ERBB2 testing guideline in breast cancer by the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) designates that all metastatic breast cancer patients have ERBB2 test performed at metastatic site, if such sample is available [2]. Limited information is provided for the workup in patients presenting with evidence of tumor heterogeneity between primary and metastatic sites [2,8]. In this case, CMA was performed on both tumor specimens following discordant ERBB2 results and showed less genomic alterations in the lymph node than primary breast cancer (Figure 2), which does not support clonal evolution of lymph node metastasis from a dominant clone in the primary breast cancer. This circumstance suggests that CMA or other genomic tests may aid in determining extent of clonal divergence and tumor progression in metastatic cases. The determination of breast metastasis heterogeneity is crucial as it has been reported that a significant change in treatment occurred in breast cancer patients with discordant ERBB2 status with bone metastasis compared to those with concordant ERBB2 status [9]. While the evidence of clinical outcome is limited in the efficacy of treatment changes in ERBB2 discordant metastatic breast cancer patients, CMA testing of tumor specimens may aid in optimizing patient care with regards to treatment options.

ERBB2 overabundance by IHC or/and ERBB2 amplification by ISH have been used as predictive biomarker for anti-ERBB2 therapies [1,2]. However, there are breast cancer cases with indeterminant ERBB2 results requiring additional studies to determine a final category [2]. CMA testing may help such equivocal cases. Haskell et al. (2018) performed CMA and FISH on four breast cancer cases in order to develop an integrated approach to determine ERBB2 DNA amplification [10]. They found that while FISH was able to provide essential ERBB2 data, CMA was able to utilize multiple genetic regions as copy number normalization controls in addition to ploidy determination of cell populations. CMA can provide a higher resolution view of the genome including ploidy effect on ERBB2 normalization, copy number alterations (CNAs) affecting chromosome 17 control probes, CNAs of other oncogenes and tumor suppressor

genes, and prognostic outcomes/clinical correlations of certain CNAs in breast cancer [10]. Future investigations should consider the utility of integrating CMA testing in order to accurately genotype breast cancers.

Differences in assay positive cut-offs can be a contributing factor to discordant ERBB2 ISH results. For example, different versions of ASCO/CAP ERBB2 testing guidelines in breast cancer have differences in ERBB2/CEP17 and ERBB2 cut-offs to designate ISH positive status [2,11,12]. Over the years, outcome data with regards to anti-ERBB2 trials lead to amendments and recategorization of ERBB2 status based on IHC and ISH results [2,11,12]. CMA may be able to address these incongruencies by adding additional information to current testing algorithms. Chen et al. (2017) explored the concordance of ERBB2 in breast tissues utilizing CMA in 20 ERBB2 positive and 21 negative breast cancer tissues defined by IHC and FISH [8]. While amplifications are typically associated with 2 or more copies, the group found that utilizing a threshold of 4 or more copies for tumor cells yielded 90% (18/20) concordance with IHC/FISH, with 1 case demonstrating intra-tumoral heterogeneity via FISH and the other case reported as borderline ERBB2 positive via FISH [8]. All the 21 ERBB2 negative cases were tested negative via CMA, demonstrating an overall concordance rate of 95% (39/41) when compared to conventional FISH/IHC ERBB2 testing, suggesting comparable utility of CMA with current ERBB2 methods [8].

Multiple mechanisms of pathogenesis have been explored to explain breast cancer tumor heterogeneity. This patient's tumor cells might have acquired a second hit to the normal allele of BRCA1 resulting in accelerated chromosomal instability and heterogeneity as BRCA1 normally functions to maintain genomic stability (OMIM: 113705). However, breast tissue BRCA1 status was not tested; as a result, contribution of BRCA1 mutation to tumor heterogeneity in this patient is uncertain. Investigations have demonstrated that patients harboring BRCA1 mutations are associated with a lower prevalence of ERBB2 positivity in breast cancers [13,14]. It is conceivable that a somatic mutation of BRCA1 might have occurred in a right breast cancer subclone that metastasized to lymph node. Alternatively, Vogel et al. (2019) noted that multiple studies have detected the presence of independent cell clones in primary tumors that can be positive or negative for ERBB2, tumor progression and metastasis from different subclones can be one factor contributing discordant ERBB2 status between primary and metastatic tumors [7]. Further investigations in mechanisms of ERBB2 receptor discordance should be explored as Leni et al. (2014) reported that losses of ERBB2 amplification result in worse post-relapse survival and overall survival in BC patients [15]. In contrast, ERBB2 positive expression in metastatic lymph nodes of BC may allow the possible addition of ERBB2 targeted therapy [16]. A key question worth exploring in understanding ERBB2 discordancy mechanisms is whether changes in tumor heterogeneity are caused primarily by selective pressures resulting in conversion towards a dominant cell-dependent pathway (e.g. oncogene addiction) or whether tumor heterogeneity is independent of selection and can somewhat randomly diverge into multiple cell clones. Determination of these mechanisms would presumably result in the utilization of different therapeutic strategies in patients, as a conversion theory suggests the utilization of more conservative treatments for the emerging dominant clone, while a multiple clone theory suggests addition of more broad-spectrum treatments. Consequentially, understanding mechanisms of discordance may result in novel therapeutics and improved outcomes in breast cancer patients.

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