2018

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HHS Public Access

Author manuscript

Breast Cancer Res Treat. Author manuscript; available in PMC 2019 July 01.

Published in final edited form as:

Breast Cancer Res Treat. 2018 July ; 170(2): 425–430. doi:10.1007/s10549-018-4742-x.

A primary breast cancer with distinct foci of estrogen receptor alpha positive and negative cells derived from the same clonal origin as revealed by whole exome sequencing

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Abstract

Background/purpose—Tumor heterogeneity is a now well-recognized phenomenon that can affect the classification, prognosis and treatment of human cancers. Heterogeneity is often described in primary breast cancers based upon histologic subtypes, hormone- and HER2-receptor status, and immunolabeling for various markers, which can be seen within a single tumor as mixed cellular populations, or as separate discrete foci.

Experimental Design/Methods—Here we present a case report of a patient's primary breast cancer that had two separate but adjacent histologic components, one that was estrogen receptor (ER) positive, and the other ER negative. Each component was subjected to whole exome sequencing and compared for gene identity to determine clonal origin.

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Online Supplemental Materials are available for this article.

Disclosures: B.H.P. is a paid member of scientific advisory boards for Loxo Oncology, and has ownership interest in Loxo Oncology. B.H.P. has research contracts with Foundation Medicine, Inc. Under separate licensing agreements between Horizon Discovery, LTD and The Johns Hopkins University, B.H.P. is entitled to a share of royalties received by the University on sales of products. The terms of this arrangement are being managed by the Johns Hopkins University, in accordance with its conflict of interest policies. No potential conflicts of interest were disclosed by the other authors.

Results—Using prior bioinformatic tools, we demonstrated that both the ER positive and negative components shared many variants, including passenger and driver alterations. Copy number variations also supported the two components were derived from a single common clone.

Conclusions—These analyses strongly suggest that the two ER components of this patient's breast cancer were derived from the same clonal origin. Our results have implications for the evolution of breast cancers with mixed histologies, and how they might be best managed for optimal therapy.

Keywords

whole exome sequencing; breast cancer; estrogen receptor

Introduction

Breast cancer is not a single disease but a heterogeneous group of diseases with a broad range of clinical features, prognoses and treatment responses. Clinically, immunostaining remains the standard of care for assessing the presence of estrogen receptor-alpha (ER), progesterone receptor (PR) and HER2 within the primary tumor, with additional in situ hybridization testing for HER2 assessment when indicated [1]. The presence or absence of ER/PR and HER2 determines candicacy for endocrine therapies and HER2 directed therapies, respectively. Based on gene expression profiling, primary breast cancers can be further refined as luminal, HER2-like, or basal, which may have implications for treatment indications, progression and prognosis [2].

Recent evidence has demonstrated that tumor heterogeneity within a primary tumor is common [3] and may have clinical importance. For example, a fundamental question that often arises in clinical breast oncology is whether patients with discrete foci have polyclonal or monoclonal disease. This is often in the context of having multi-focal/multi-centric disease, or bilateral tumors, but with concordant receptor subtypes, leading to the quandary of whether these cancers share a common origin. With newer prognostic/predictive tests such as Oncotype DX, which analyzes a limited gene expression panel, heterogeneity among multiple foci could influence the decision to administer chemotherapy, depending if single vs. multiple sites of disease are tested [4]. On the other hand, when receptor subtypes are discordant, e.g. an ER positive tumor along with a separate focus of an ER negative tumor, it is generally thought that these tumors are from distinct clonal populations.

Tumor heterogeneity for many primary breast cancers is also seen within a single tumor mass, where varying percentage of cells and strength of labeling is seen by immunostaining for ER, PR and HER2. Here too, clinical management may be influenced by the amount and intensity of receptors present. For example, a breast cancer that has weak ER labeling in 5% of all cancer cells, may not derive as much benefit from endocrine therapies, and may influence the decision to recommend adjuvant chemotherapy. Oftentimes cases with variable receptor expression show cellular populations that are ER positive admixed with ER negative cells but do not demonstrate discrete focal areas of receptor positivity within the primary tumor mass. Here we present an unusual case report of a primary breast cancer containing two separate but adjacent ER positive and ER negative areas. We have previously

shown using microdissection and whole exome sequencing (WES) that primary breast cancers containing both an intraductal and metaplastic subtype were derived from the same clonal population [5]. Using a similar approach, we show here that the ER positive and ER negative components of this patient's primary tumor are derived from a common clonal origin. We discuss possible clinical implications of these findings below.

Materials and Methods

Case Report

Clinical History and Pathology Specimens—The patient is a 38 year old female who was diagnosed at an outside hospital with a T2N1 Grade 3 invasive ductal carcinoma (IDC) that was described as weakly ER positive on diagnostic biopsy, but was negative for ER/PR and HER2 receptors by immunohistochemistry staining on the surgical (lumpectomy) specimen. The pathology specimens were reviewed at Johns Hopkins Hospital and repeat stains on the surgical samples were performed and photographed. This study was carried out under an approved Institutional Review Board (IRB) protocol that allows for the use of remaining specimens for research purposes.

Tissue Processing and Nucleic Acid Extraction—Genomic DNA (gDNA) was isolated from formalin fixed paraffin embedded (FFPE) tissue using standard protocols. Briefly, H&E stained histology slides were examined by the study pathologist (P.A.) to identify areas of breast carcinoma. Ten to fifteen 5-micron thick unstained slides were deparaffinized and identified regions of interest were macrodissected using the Zymo pen and Pinpoint solution (Zymo Research, Irvine, CA), per the manufacturer's protocol. DNA was then purified and isolated from the paired tumor samples using a QIAamp® DNA FFPE tissue kit (Qiagen, Valencia, CA), per the manufacturer's protocol.

Whole Exome Sequencing and Analysis—Tumor DNA samples were submitted to Seqwright (Seqwright DNA Technology Services, Houston, TX) for next generation whole exome capture using the Agilent SureSelect 51 Mb Kit followed by next-generation sequencing using the Illumina Hi-Seq platform. The average read depths for the ER+ and ER− components were 86x and 90x, respectively.

A detailed description of the analysis pipeline is described elsewhere [5]. Briefly, we aligned paired end whole exome sequencing (WES) reads to the human reference genome (GRCh38) with BWA mem [6] with default parameters and called variants with GATK MuTect2 [7], using a panel of normals (PON) based on the ExAC [8] database. We required 10 supporting reads for each base change in both tumor components and at least 4 reads for the alternative allele, and allele frequencies greater than 10%. We further annotated and filtered the variants with SnEff and SnpSift [9].

For copy number variant analysis, we used CNVkit (version 0.8.3) [10] to detect copy number variants, with the following parameteres: cnvkit.py batch [sorted bam file] —normal –targets [exome regions bed file] –fasta [GRCh38] –split –annotate [ftp://hgdownload.cse.ucsc.edu/goldenPath//hg38/database/refFlat.txt.gz] –access [cnvkit-master/data/access-10kb.hg38.bed] –output-reference [SAMPLE].cnn.

Results

The breast specimen from the patient's surgery was used for standard hematoxylin and eosin staining, as well as ER labeling as previously described [11]. As seen in Fig. 1A, the tumor had two distinct morphologies, with an area that was glandular, and a separate region that had a "solid" or uniform area of tumor cells. A serial section was stained for ER, and this showed the glandular component had strong ER labeling, while the solid area was completely negative for ER labeling (Fig. 1B). This raised the intriguing possibility that these distinct ER expressing cells and histologic subtypes were either derived from a common precursor, or as an alternative explanation, they were derived from separate clones that happened to be adjacent to one another. To address these possibilities, we chose to separately analyze these tumors at a genetic level with WES, similar to our prior work with primary breast tumors containing both an invasive ductal and metaplastic component [5].

The fundamental rationale of our approach is that tumors derived from the same clonal origin would share many genetic/genomic alterations including single nucleotide variants (SNV), and copy number variants (CNV) and that these would be shared at the same level for both "driver" and "passenger" mutations, i.e. alterations that would or would not, respectively, be expected to impart any functional consequence. Conversely, if clones were separately derived, it would be highly unlikely for the two components to share passenger mutations as well as driver mutations, as passenger mutations would be quickly gained separately once the clones diverged. For the purposes of distinguishing driver from passenger variants, we categorized SNVs as low (likely passenger mutations), moderate or high (likely driver mutations) impact based upon established criteria as described in our past studies [5, 12]. As shown in Fig. 2, the two separate ER positive and ER negative components shared a large number of SNVs, most of which were in the low impact category, i.e. passenger alterations. Although normal germline DNA was unavailable for controls, we employed a "panel of normal" encompassing > 60,000 normal genomes from the ExAC project, which we have used in past studies to filter out likely benign single nucleotide polymorphisms and variants [12]. Even after filtering with our panel of normals, the vast majority of variants were shared between the two distinct components. While shared SNVs after filtering with our panel of normals generally did not have the same allelic frequencies (Supplemental Fig. 1), this could be the result of contamination from normal gDNA within the tissue specimens. Given the number of shared SNVs, our data strongly suggest that the ER positive and negative components were derived from the same clonal origin.

Somatic copy number changes can also be assessed to infer clonality, similarly to SNVs. As shown in Fig. 3, circus plots demonstrated that the majority of copy number changes, whether gains or losses, were found in both ER components of the tumor. These data further support the notion that a common clone gave rise to both ER positive and negative components of the primary tumor.

Discussion

Systemic treatment for ER positive tumors generally involves the use of endocrine therapies, which are not used for ER negative tumors. Therefore, it is critical to assess receptor status

for all breast cancers as expression of ER greatly influences clinical management of the disease. The case report presented here has a number of clinical implications. First, we show that discrete separate regions of ER expression can exist within the same primary tumor. This could lead to sampling error where only a single component is assayed for ER expression, potentially missing an opportunity for using endocrine therapies if the ER positive component was not assessed. Second, the existence of a completely triple negative component (ER/PR/HER2 negative) generally mandates consideration for chemotherapy in the adjuvant and metastatic settings. Had the ER positive component been the only area assessed, one may have considered omitting adjuvant chemotherapy depending on other clinical and pathologic features. Third, from our molecular analysis, it is clear that ER negative tumors can be derived from ER positive cancer cells. Although this is likely to be an uncommon event based upon the now known mutational landscape of ER positive versus ER negative tumors [13], the findings here support that this is a possibility. Interestingly, we did not find any genetic alterations in ER (supplemental Table S1) that might explain the loss of ER expression, and opine that epigenetic silencing is likely the cause of this finding based upon past preclinical studies [14]. Whether the gene expression changes of these tumors reflect “classic” intrinsic subtypes for ER and TNBC, i.e. luminal and basal, remains uncertain and unfortunately, we did not have additional adequate sample material to perform these analyses. One could speculate however, that the ER positive component may be a more aggressive luminal B phenotype, while the receptor negative component may still give a “luminal” signature as has been described in the past for TNBC, which interestingly have a mutation profile more similar to ER positive tumors than classic basal TNBC [15]. Nonetheless, our study demonstrates the need for vigilance when assessing receptor status and that surveying multiple components of a primary tumor may be warranted in certain circumstances.

Conclusion

We describe a case report of an early stage breast cancer with two histologic components, one that is ER positive and the other ER negative. Genetic analyses using whole exome sequencing demonstrates that these two components were derived from the same clonal origin, suggesting that despite the differing histologies and receptor status, the patient did not have two independent primary tumors, which may have broader implications for therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank various members of the Park and Wheelan lab for helpful thoughts and discussions. This work was supported by NIH R01CA194024, NIH P30CA006973, Susan G. Komen, the Commonwealth Foundation, the Breast Cancer Research Foundation, the Marcie Ellen Foundation, The Helen Golde Trust, and The Canney Foundation. None of the funding sources influenced the design, interpretation or submission of this manuscript.

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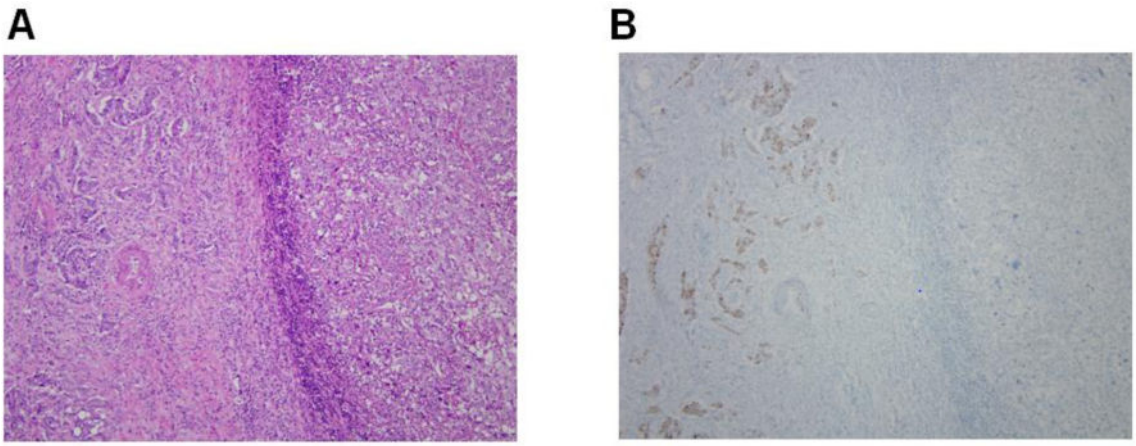

Figure 1. A primary breast tumor with separate regions of ER positive and negative expression

A) Hematoxylin and eosin staining demonstrating distinct histologies with a glandular pattern on the left and a more solid phenotype on the right. **B)** Serial section of the same tumor showing ER labeling in the glandular component, but no labeling in the solid region to the right. 100x magnification.

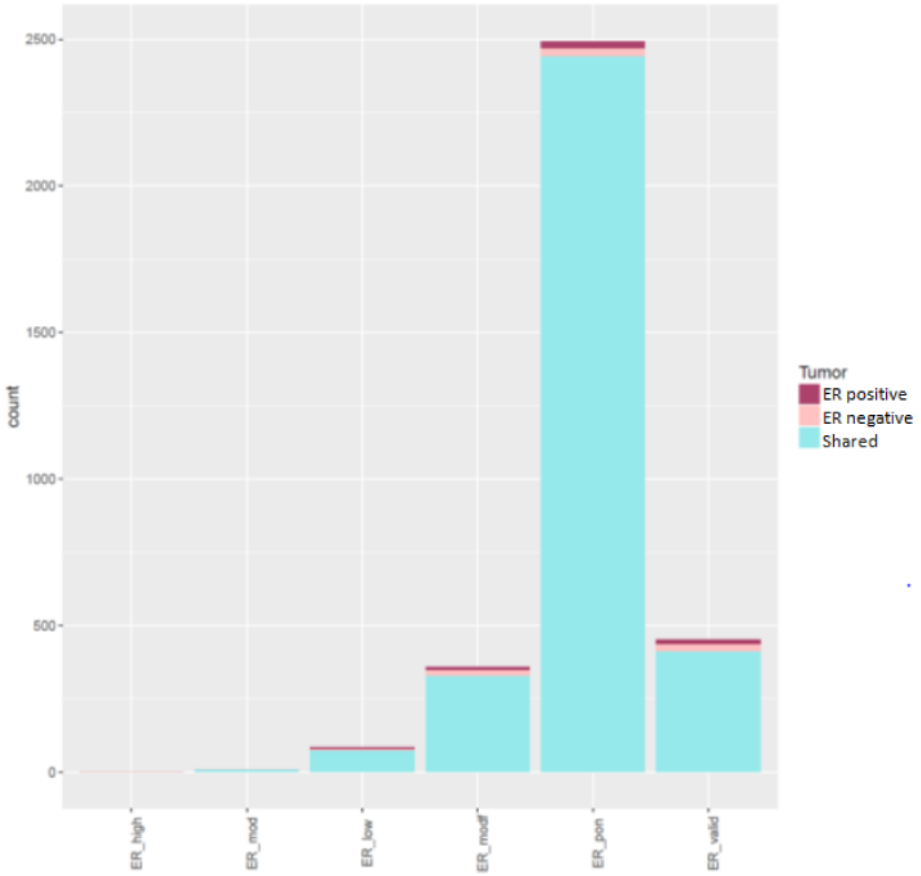

Figure 2. Variants shared between the ER positive and ER negative components of a primary breast tumor

Each stacked bar represents the percent allele sharing in the high, moderate (mod), low, modifier (modf), panel of normal (pon) and valid categories (where valid represents all categories not excluded by the panel of normals). Variants shared between the two components are represented in light blue. Y axis represent the number of variants.

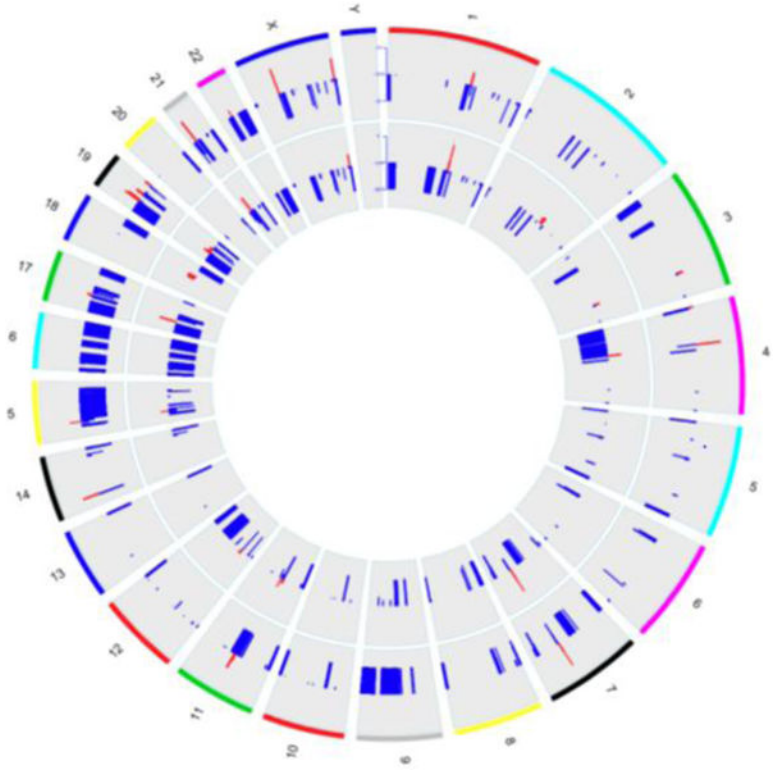

Figure 3. Copy number variation (CNV) segments in the ER positive and ER negative components of a primary breast tumor are similar

Circus plot depicting CNV from the two ER components in concentric rings. The ER positive component is depicted in the outer ring, while the ER negative component is shown the inner ring. Copy number gains are displayed in red and losses are displayed in blue. Each chromosome is denoted on the outer ring.