

# Intratumoral Heterogeneity in Breast Cancer: A Case Report and Molecular Discussion

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## Abstract

The emerging era of personalized medicine makes it increasingly important to consider intratumoral heterogeneity, which has been found in some breast cancer cases. However, its identification criteria, form of reporting, and subsequent effects on the clinical course of this disease remain controversial and not fully defined. Here, we report and discuss a case of breast invasive ductal adenocarcinoma with substantial intratumoral heterogeneity, discrepancy between Her2/neu immunostaining and *in situ* hybridization, and disparity between estrogen receptor status before and after neoadjuvant therapy.

**Keywords:** Breast, Estrogen receptor, ErbB-2, Genetic heterogeneity, Personalized medicine

## Introduction

Intertumoral and intratumoral heterogeneities are fundamental motives that began the emerging era of personalized cancer medicine. However, they are the most challenging and unresolved issues in cancer medicine.<sup>1,2</sup>

Intertumoral heterogeneity is well-described in breast cancer. Gene expression profiling techniques have resulted in segregation of breast tumors into four major molecular subtypes – luminal A, luminal B, Her2/neu, and triple negative (including basal-like). Subsequently,

efforts have been made to use immunohistochemical stains as surrogate markers that assign tumors to their corresponding molecular subtypes.<sup>1,3</sup>

Despite these achievements in intertumoral heterogeneity, the problem with intratumoral heterogeneity has not been sufficiently addressed and may be one of the most important reasons for unusual and unpredicted behaviors in a number of breast tumors. Current standards for breast biomarker assessment, such as the widely used 2013 American Society

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of Clinical Oncologists/College of American Pathologists (ASCO/CAP) guidelines, mainly focus on the overall status of hormone receptors and Her2/neu status. These guidelines do not state the problem of molecular heterogeneity, with the exception of Her2/neu heterogeneity in an *in situ* hybridization (ISH) study.<sup>4,5</sup>

Here, we present a case with significant intratumoral heterogeneity and discrepancy between Her2/neu immunostaining and ISH.

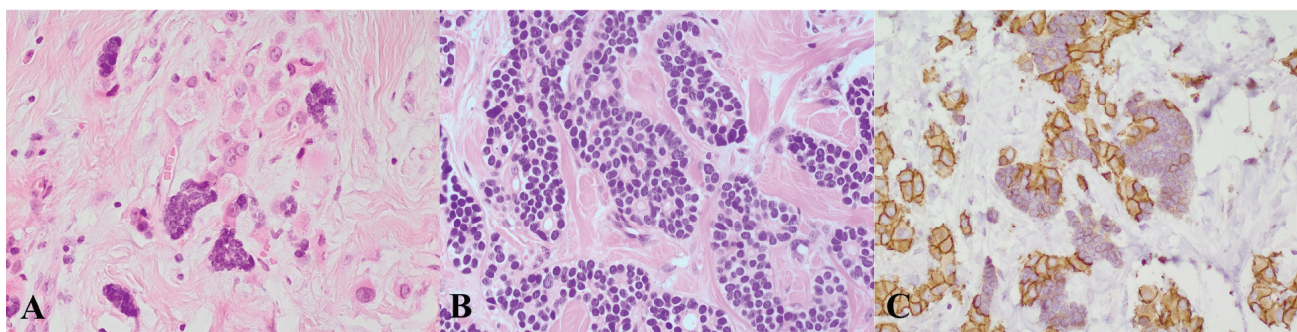
### Case Report

A 70-year-old Iranian woman was admitted to Shahid Faghihi Hospital, Shiraz, Iran in January 2017 to undergo breast tumor resection surgery. Her disease, invasive ductal carcinoma with bone metastases, was previously diagnosed through core needle biopsy (CNB) in another medical facility approximately six months before she referred to our hospital. She underwent preoperative radiation and chemotherapy that included trastuzumab and letrozole.

Initially, the patient presented with back pain. Spinal magnetic resonance imaging (MRI) showed multiple ill-defined signals in the thoracic, lumbar, and sacral vertebral bodies suggestive of bone metastases. A subsequent bone scan supported the MRI findings, and also revealed metastases in her ribs and skull. Chest and abdominal computed tomography (CT) scans did not detect any further metastases. In a search to find the primary site, a palpable mass with nipple retraction and discharge was discovered in the left breast. The subsequent

mammography showed an ill-defined density in the upper outer quadrant of the left breast that measured 5.5 cm in maximum diameter with accompanying prominent axillary lymphadenopathy. The right breast and axillae were normal per the imaging studies.

The initial CNB results confirmed the presence of an invasive ductal carcinoma with weakly positive estrogen receptor (ER) staining in 10% of the tumor cells, negative progesterone receptor (PgR), and positive Her2/neu (+3). However, the final resected specimen contained two intermingling distinct cell populations with different histomorphology and immunohistochemistry (IHC) features. The first cell population, a large cell component (LCC), comprised approximately 80% of the overall tumor cells and was composed of large cells with relatively abundant cytoplasm, vesicular chromatin, and conspicuous nucleoli. The second population consisted of a small cell component (SCC) that represented approximately 20% of the tumor cells comprised of basaloid cells with scant cytoplasm, dense chromatin, and no nucleoli (Figure 1A). Both components predominantly consisted of small to medium-sized clusters. However, the LCC also showed occasional sheets and frequent single cells. The SCC had prominent tubule formation, whereas the LCC lacked this feature (Figure 1B). Mitotic activity was moderate (6-10 mitoses per 10 high power fields) in the LCC, whereas the SCC showed exceptional mitoses. Overall, the LCC and SCC would have been



**Figure 1.** A) Two distinct intermingled tumor cell populations (original magnification, 400×; H&E stain). B) Tubule formation in the small cell component (SCC) (Original magnification: 400×; H&E stain). C) Her2/neu immunohistochemistry (IHC) showed that the large cell component (LCC) expressed Her2/neu protein (score: 3+), whereas the SCC did not express Her2/neu protein (score, 1+) (Original magnification: 400×).

considered as grades 2 and 1, respectively, based on the Nottingham modification of the Bloom-Richardson grading system.

Immunohistochemistry assessment indicated that both components were negative for ER and PgR. Her2/neu was 3+ in the LCC and 1+ in the SCC. Ki67 proliferation index was 20% in the LCC, but the SCC showed extremely low proliferation activity (less than 1%). The high molecular weight cytokeratin (CK5/6) and p53 were non-reactive in both components (Figure 1C).

Dual color Her2/neu chromogenic ISH (CISH) probes showed amplification of the Her2/neu gene in both components. The average number of HER2/neu signals per cell was 6, which made small clusters. The HER2/CEP17 ratio was approximately 3 (Figure 2).

Necrosis, lymphovascular invasion, and nipple or skin involvement were absent. However, all 33 dissected axillary lymph nodes had macrometastases involvement with substantial extranodal extension. Interestingly, the only type that metastasized was the LCC.

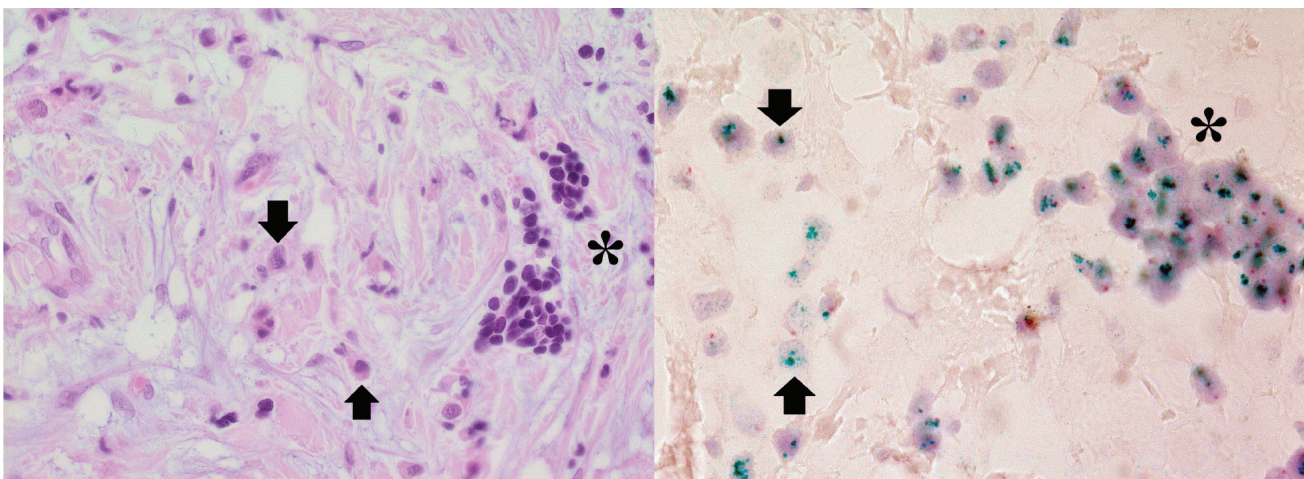
Unfortunately, the patient sustained a fall with a femur fracture three weeks after her discharge. Her condition deteriorated thereafter and she experienced bedsores, renal failure due to a urinary tract infection, and sepsis. She passed away in March, 2017.

## Discussion

Here, we reported a breast invasive ductal carcinoma with noticeable intratumoral heterogeneity comprised of two different tumor cell populations with distinctions in histomorphology, Her2/neu status, and molecular subtyping.

The LCC cells were ER-/PgR-/Her2+ (3+)/20% Ki67, whereas the SCC cells were ER-/PgR-/Her2- (+1) with an extremely low proliferation index. These components were classified as Her2/neu and triple-negative (suspicious to basal-like), respectively, based on molecular subtyping of breast cancer that used IHC as a surrogate marker. Interestingly, SCC did not express CK5/6 and was not categorized as basal-like; unexpectedly, subsequent CISH revealed that SCC was Her2/neu gene amplified and Her2/neu in nature.<sup>3</sup>

Her2/neu gene amplification is present in approximately 1% of patients who lack Her2/neu protein expression (IHC 0/1+).<sup>4,6</sup> The ASCO/CAP states that this phenomenon is most likely due to an erroneous immunostaining technique. Here, we have performed the Her2/neu IHC twice, followed by a review by two independent molecular pathologists. Batch and internal controls also showed thorough staining patterns. Therefore, the possibility of a technical error seemed doubtful. Seol et al. have stated that this discrepancy is not



**Figure 2.** A) Another region of the tumor (H&E stain, original magnification: 400×). B) Its corresponding dual color chromogenic *in situ* hybridization (CISH) which shows amplification of the Her2/neu gene. Green and red dots represent the Her2/neu gene and chromosome 17 centromere (original magnification: 1000×). \*: Small cell component (SCC); Arrows: Large cell component (LCC).

attributed to technique and it represents true biological heterogeneity.<sup>7</sup> Varga et al. attributed this finding to variations in IHC results within Her2/neu gene amplified cases. Thus, they have encouraged the idea of performing ISH instead of IHC on all breast carcinoma specimens.<sup>6</sup> We believe that mechanisms underlying such findings are derived from differences in Her2/neu gene expression, at either the mRNA or protein levels that may prevent an amplified Her2/neu gene from being ultimately expressed as an Her2/neu protein on the cell's surface.

Of note, +1 or +3 Her2/neu tumors do not need reflex testing or further ISH study according to ASCO/CAP guidelines. Here, we performed the CISH assay for our own interests. It was clear that SCC would have been falsely categorized as triple negative (with no need for trastuzumab therapy) instead of the Her2/neu subtype (the main candidate for trastuzumab targeted therapy) if we had not performed ISH. In our opinion, this is a shortcoming of the ASCO/CAP guidelines that may overlook such patients.<sup>4</sup>

The LCC, as expected by its molecular characteristics, had an ominous behavior. In this patient, there was involvement in all dissected regional lymph nodes with extensive extranodal extension by the LCC. In contrast, the SCC did not show any aggressive behavior in the current specimen. However, in this age of personalized medicine, the clinical importance of heterogeneous areas such as SCC should not be ignored. Varga et al. have shown that Her2/neu amplified cases with an IHC score of 1+ had similar overall survival as IHC score 2+/3+ patients with concurrent gene amplification.<sup>6</sup> Seol et al. have stated that intratumoral heterogeneity is a sign of genetic instability across tumor cell populations; as with other solid tumors, it harbors a worse prognosis.<sup>7</sup> There is a lack of comprehensive evidence on whether patients with discordant IHC and ISH results would benefit from trastuzumab. Therefore, ASCO/CAP does not currently support this idea. Overall, we agree with Makroo et al. and advocate enrollment of these patients in future clinical trials.<sup>8</sup>

Low proliferative tumor populations, such as SCC, may not respond well to chemotherapy regimens and there is a greater chance for tolerance of primary treatments. In our opinion, if this case could have survived the LCC, then it was the SCC that could become a therapeutic challenge. Unfortunately, our patient passed away after about nine months.

There was a discrepancy between ER results from the preliminary CNB which indicated weakly positive ER (10%) and the post-neoadjuvant excisional specimen (ES) that was ER negative. It was not clear to us whether this finding was related to the type of specimen (CNB versus ES) or the time at which the specimen was obtained (before versus after neoadjuvant therapy). However, both options were reasonable. Previous studies have shown that the agreement in ER between CNB and EB is not complete. According to Tamaki et al., a discordancy rate of 4% is expected.<sup>9</sup> In addition, it has been well demonstrated that hormone receptors and Her2/neu status may change throughout neoadjuvant therapy. Niikura et al. have reported that 4.6% of ER-positive tumors become ER-negative after treatment.<sup>10</sup> Regardless of the discrepancy, it is wise to monitor molecular characteristics of a tumor during neoadjuvant therapy in order to appropriately guide the succeeding targeted therapy.

According to the 2013 ASCO/CAP guidelines, it is not required to report Ki67 staining, (which is necessary for molecular subtyping) and Her2 gene heterogeneity. The guidelines do not address the importance of molecular subtyping.<sup>5</sup> In our opinion, the exact underlying mechanisms and prognostic consequences of intratumoral molecular heterogeneity and discrepant Her2/neu assessments have yet to be fully realized. We recommend including these subjects in the next update of the ASCO/CAP guidelines in order to provide unified definitions for further clinical trials and to aid clinicians in the practice of personalized medicine.

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## Conflict of Interest

None declared.

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