**High grade acinic cell carcinoma of the breast with clear cytoplasm mimics clear cell carcinoma in a BRCA1 mutation carrier: a case report and review of the literature on the molecular analysis**

**Authors:** Liu Min, Huang Qiao and Zhang Hongkai

DOI: 10.14670/HH-18-501
Article type: ORIGINAL ARTICLE
Accepted: 2022-07-27
Epub ahead of print: 2022-07-27
High grade acinic cell carcinoma of the breast with clear cytoplasm mimics clear cell carcinoma in a BRCA1 mutation carrier: a case report and review of the literature on the molecular analysis

Liu Min¹, Huang Qiao², Zhang Hongkai¹*

1. Department of Pathology, Beijing Hospital of Traditional Chinese Medicine, Capital Medical University, Beijing, 100010
2. Department of Breast Surgery, Beijing Hospital of Traditional Chinese Medicine, Capital Medical University, Beijing, 100010

*Corresponding author, E-mail: zhk0484@sina.com

Abstract

Acinic cell carcinoma of the breast is an extremely rare tumor. To the best of our knowledge, only one case is reported to have bilateral tumors and had both BRCA1 and TP53 mutation. Herein, we report another case of acinic cell carcinoma of the breast in a 29-years-old female carrying germline BRCA1 and TP53 mutation, and the tumor showed a complex combination of histological features which had not only the reported common features such as diffuse infiltrative small acinar or glandular structures mixed with solid nests, but also the uncommon widespread clear cells, high grade tumor cells. The immunohistochemical profile of the tumor cells was strongly positive for lysozyme and triple negative for ER, PR, HER2. Although she had bilateral high grade breast cancers, this patient refused postoperative adjuvant therapy this time and has been doing well in the past 12 months. As a rare form of triple-negative breast cancer with a relatively not so bad prognosis, more reports are needed to understand its biological characteristics.
KEYWORDS
Acinic cell carcinoma of breast, clear cell carcinoma, BRCA1 mutation

1 INTRODUCTION

Acinic cell carcinoma (ACC) is a rare subtype of malignant epithelial neoplasms of breast characterized by obvious serous acinar cell differentiation with zymogen-type cytoplasmic granules and immunohistochemical expression of amylase, lysozyme and alpha-1 anti-chymotrypsin, similar to those seen in salivary glands (Kravtsov et al., 2020). The first case in breast was reported by Roncaroli F in 1996 (Roncaroli et al., 1996) and is now classified as an exceptionally rare and salivary gland-type breast carcinoma entity (WHO Classification of Tumours Editorial Board, 2019).

Although ACC of the breast is similar to its salivary gland counterpart in the morphological, immunohistochemical and ultrastructural aspects (Damiani et al., 2000; Limite et al., 2014), some morphological features that have been reported frequently in ACC of parotid gland are not usually seen in the breast counterpart, including pushing borders, prominent intratumoral lymphoid infiltrate and variegated architectural growth patterns with solid and cystic areas. In fact, the secretory granules in breast and salivary gland ACCs are distinct: in the former, pink, eosinophilic granules are common, in the latter, the granules are predominantly basophilic. The molecular analysis of breast acinic cell carcinoma showed a DNA copy-number and mutation landscape similar to that of triple-negative breast carcinomas of conventional histology, but others had similar characteristics to micro glandular adenosis (Guerini-Rocco et al., 2015; Piscuoglio et al., 2015; Geyer et al., 2017). On the other hand, the mutation profiles of breast acinic cell carcinomas differed greatly from those of acinic cell carcinomas of the salivary gland-like tumors of the breast (Piscuoglio et al., 2015).

Herein, we present a case of acinic cell carcinoma of the breast in a 29-year-old female patient with BRCA1 and TP53 mutation and a family history of breast cancer, clear cell morphology with high-grade cells and microglandular adenosis
area. We also reviewed the reports concerning this rare entity which had the molecular test results.

2 MATERIAL AND METHODS

One case of breast ACC was identified from the pathologic department.

Immunohistochemistry was undertaken as part of the diagnostic workup in the case, and the antibodies and dilutions used are summarized in Table 1. The case underwent molecular analysis using her tumor tissues and peripheral blood samples tested for exons captured sequencing (all exons captured for 170 genes and partial exons captured for 851 genes which related to tumor genesis and development. More details are in S1) based on second-generation sequencing technology. Somatic mutations, copy number alterations, mutational signatures and fusion genes were determined using state-of-the-art bioinformatics methods. Also we reviewed the reports concerning this rare entity which had the molecular test results in the English language literature to date.

3 RESULTS

A 26-year old woman presented with a left breast mass. The core biopsy of the tumor in the left breast was invasive breast carcinoma of no special type, grade 2 and immunohistochemistry showed faint staining positive for estrogen receptors (ER), progesterone receptors (PR) and human epidermal growth factor receptor 2 (HER2) protein were negative, Ki-67 index 67%; epidermal growth factor receptor (EGFR) and GATA binding protein 3 (GATA3), TP53 were all strongly positive (Fig. 1A-1F). The core biopsy of left axillary lymph node showed cancer metastasis. The patient had neoadjuvant chemotherapy and subsequent total mastectomy. The final pathological report was ypT0N0. Her mother died of breast cancer at the age of about 40, and the mother’s younger sister and her grand-mother were still alive and well.

The patient had been accepting regular physical examination on the diseased side, then three and half years after surgery, the patient presented with a contralateral breast mass, which was a palpable, hard, immobile, irregular mass in the lower outer
quadrant of the right breast, approximately 4.0 cm in diameter. No skin retraction or
nipple discharge was noticed. Ultrasonography showed a heterogeneous hypoechoic/
anechoic cystic and solid nodule with an ill-defined border. Enhanced magnetic
resonance imaging (MRI) revealed an irregular lesion of high intensity. Both the
ultrasound and MRI classified it as BIRAD-S IV-b. Core needle biopsy of the lesion
revealed the tumor cells to be solid, glandular, clear cytoplasm. The tumor cells of the
ductal carcinoma in situ (DCIS) also had clear cytoplasm. Invasive carcinoma with
clear cytoplasm was diagnosed. Further thorough examinations, including computed
tomography of the thorax and abdomen and bone scintigraphy, showed no signs of
metastatic lesions. Laboratory examination, including cancer antigen 72-4 (CA72-4),
CA242, CA15-3, Carcinembryonic Antigen (CEA), CA125, CA19-9, Alpha
fetoprotein (AFP) and Prolactin were all within normal ranges.

Her mass in the right breast was diagnosed as cT2N0M0, stage IIA. She underwent
modified radical mastectomy. The intraoperative sentinel lymph nodes were negative.
Two sentinel lymph nodes in the ipsilateral axilla were examined; with a maximum
diameter of 1.5 cm and 0.3 cm. Frozen sections and serial sections of paraffin
embedded blocks were applied. Macroscopically, a friable white-yellow-colored
cystic and solid lesion measuring 4.2 x 3.0 x 2.0 cm was found, ill-defined and obvious
dilated ducts were seen, which was finally diagnosed as pT2N0 (sn) Mx. Histologically,
patterns of the arrangement of tumor cells included the presence of
solid, cribriform, micropapillary, papillary, and microglandular. Most of the cells (80%)
had clear cytoplasm, well to moderately differentiated cells with round or oval nuclei,
and hyaline balls in the center (Fig.2A). It was also noticed that some cells with
lightly eosinophilic bubbly cytoplasm contained large, coarse, and bright red
zymogene granules in the cytoplasm (Fig.2B). DCIS with or without necrosis were
around or in the invasive area. Most of the tumor cells were grade 2, however, there
were small areas corresponding to the dedifferentiation region (solid areas, grade 3,
mitotic activity 20 mitosis /10HPF) (Fig.2C). A small area of microglandular
adenosis was also noted at the periphery of the tumor (Fig2D).

With immunohistochemistry most of the tumor cells stained strongly for amylase,
lysozyme, α-1-antichymotrypsin (α1-ACT) (Fig 2E), epithelial membrane antigen (EMA), S-100(Fig 2F) and EGFR protein, SRY-Box transcription factor 10 (SOX10), CD117, cytokeratin (CK) 8/18 and membrane E-cadherin. Smooth muscle actin (SMA), P63, CK5/6, ER, HER2, and androgen receptor (AR) were all negative, but PR stained faintly positive in 15% cells. The Ki-67 index in the hot spots was 40%. GCDFP-15 was partially positive, but GATA-3 negative. TP53 had null expression. Mammaglobin, paired box gene 8 (PAX8), Placental-like alkaline phosphatase (PLAP), Spalt-like transcription factor 4 (SALL4), hepatocyte nuclear factor 1β (HNF1β), CK20, Chromogranin A (CgA) and synaptophysin (Syn) were negative. Considering all the morphology and immunohistochemistry results, we diagnosed the tumor as breast ACC. The two nodules histological features are compared in Tab.2.

Molecular analysis of exons captured sequencing based on the second-generation sequencing technology revealed that point mutations, small fragment insertions, or deletions affected genes including TP53, PRKAA1, FAS, ARID2, MET, FLT3, TP63 and PIK3CG. TP53 mutation frequency was 19.9% and BRCA1 pathogenic heterozygosity germline variant was found. Copy number analysis revealed the focal amplification of MYC and RECQL4. No fusion gene was identified in this patient including ETV6-NTRK3.

The patient refused any adjuvant treatment after surgery. She is disease-free up to 12 months after her last operation.

4 DISCUSSION

Despite a relatively small number of reported breast ACC, the genomic features of them had been studied thoroughly. It was found that breast ACC not only overlap with MGA in both the histologic characteristics and genomic features, but also overlap with TNBC (Guerini-Rocco et al., 2015; Tsang and Tse, 2016; Geyer et al., 2017). ACC mainly occurred in salivary glands, but also could be occasionally observed in other organs. Breast ACC was first described in 1996 by Roncaroli et al. (Roncaroli et al., 1996), and the first description breast ACC in a BRCA1 mutation
carrier was reported by Ripamonti et al. in 2013 (Ripamonti et al., 2013). Morphologically, breast ACC resembled the counterparts in salivary glands which have two distinct patterns of growth: the first is the tumor cells being poorly circumscribed, infiltrating in solid or nest pattern, often with focal necrosis; the second is the polygonal or round, containing coarse brightly eosinophilic granules in the amphiphilic cytoplasm tumor cells forming acinar, tubular, microglandular or microcystic structures (Chang et al., 2011; Beca et al., 2019). At present, it is generally accepted that microglandular adenosis and acinic cell carcinoma are part of the same spectrum of lesions and represent low-grade forms of triple-negative disease with no or minimal metastatic potential (Geyer et al., 2017). But occasionally, it was seen that a small subset could progress to high-grade triple-negative breast cancer. Areas composed of clear cells with hypernephroid appearance were also reported, which means breast ACC could have a wide morphologic spectrum of appearances (Conlon et al., 2016). Uniquely in our present case was the vast area of the striking clear tumor cells arranging in solid, cystic, tubular, papillary structures which led us to think it was a clear cell carcinoma of the ovary counterpart at first glance. Also, there was a small area of typical micro-glandular adenosis at the periphery of the tumor in our case, but it was also found that small part of the tumor had high grade morphology with brisk mitosis and obvious atypical tumor cells. In fact, the morphological diversity of ACC suggests that a considerable number of ACC might have been overlooked (Choh et al., 2012). It was advocated that microglandular adenosis-like areas at the periphery of a breast acinic cell carcinoma should be considered part of the carcinomatous process (Conlon et al., 2016).

Immunohistochemically, most of the breast ACC cells displayed the characteristic triple-negative phenotype, expression of S100-protein, epithelial membrane antigen (EMA) and serous differentiation markers, including amylase, lysozyme and alpha L-antichymotrypsin (Limite et al., 2014). TP53 revealed strong nuclear expression in the cases harboring a p53 missense mutation, GATA3 was almost negative (Matoso et al., 2009). These features could be seen in our present case similar to the former reported paper, with the exception that PR was faintly expressed in 15% tumor cells
in our case, though ER was negative.

Approximately 10% of breast cancers have hereditary predisposition, among them nearly 20-30% are linked to germline mutations in BRCA1 and/or BRCA2 genes. The mutation carriers face a lifetime risk to develop breast or ovary cancer ranging from 57% to 65%, as well as prediction of contralateral breast cancer (Chen and Parmigiani, 2007; Mavaddat et al., 2013). BRCA1 mutation carriers frequently have mutations of the TP53 gene (Roncaroli et al., 1996; Chen S and Parmigiani, 2007). In BRCA1 mutation carriers, the pathological features of breast cancers are usually high grade invasive ductal cancer, not otherwise specified or with high proliferative index medullary type. They are also commonly triple negative (ER, PR, HER2 being negative). These features could all be seen in our present case which had P53 mutation, BRCA1 germline mutation and part high-grade differentiation. But these morphological features did not match the usually low grade ACC which means breast ACC perhaps has a diverse range of characteristics.

Breast ACC that occurred in a BRCA1 mutation carrier was first described by Ripamonti et al in 2013 year (Ripamonti et al., 2013). At present, among the 21 reported ACC with molecular tests (including our case) (Tab.3), we found BRCA1 mutation (germline or somatic) occurred in 6/21 patients, and with three germline mutations (including Ripamonti’s case and our present case), two cases were metachronous carcinomas in breast, in which the histological type of primary breast tumor on one side was invasive ductal carcinoma, with no special type. It seemed that the germline mutation of BCRA1 had a potential role in causing bilateral malignant breast tumors, including ACCs. This observation was consistent with what Shen TK et al. (Shen et al., 2014) observed in the salivary gland cancers in BRCA1 mutation-positive family members, they found out incidence rate of head and neck cancers was much higher in persons with the germline mutation of BCRA1 than the background incidence rate (0.052% vs. 0.003% per year).

TP53 was the most commonly mutated gene in ACCs, being present in 19/21 cases (Ripamonti et al., 2013; Guerini-Rocco et al., 2015; Geyer et al., 2017; Beca et al., 2019; Sarsiat et al., 2022). Conditional mouse models of BRCA1 and TP53 have been
shown to result in the development of rather heterogeneous tumors with the majority of lesions being of histologically high grade TNBCs (Liu et al., 2007; Molyneux et al., 2010; Ripamonti et al., 2013). Beca et al. (Beca et al., 2019) also thought the loss of function of BRCA1 and TP53 may not be sufficient to lead TNBC to high-grade TNBC since in their studies all the ACC were low grade and had indolent behavior. They speculated the MLH1 germline mutations may have relationship with breast ACCs (Beca et al., 2019), but our study and other studies did not find any MLH1 gene mutation. Some other reports showed that CTNNB1 N387K and K335T mutation may trigger the potential progression mechanism (Guerini-Rocco et al., 2015). Though our case did not display CTNNB1 mutation, we found some other mutations which include PRKAA1, FAS, ARID2, MET, FLT3, TP63, PIK3CG besides TP53 and BRCA1, which may exert a role in tumor dedifferentiation. More studies on the molecular aspect of these rare tumors are still warranted.

The differential diagnosis includes glycogen-rich clear cell carcinoma (GRCCC), lipid-rich carcinoma, secretory carcinoma, clear cell myoepithelial carcinoma or carcinoma in situ. In this case, there were eosinophilic granules in the cytoplasm of tumor cells, which were positive of lysozyme and alpha1-antichymotrypsin by immunohistochemical staining and were not completely transparent, so GRCCC was excluded. Also, as we did not find the solid tumor nest surrounded by glassy basement membrane like material and immunohistochemical staining results did not show positive of myoepithelial markers, so clear cell myoepithelial carcinoma were not be considered. Furthermore, Molecular analysis results did not find the ETV6-NTRK3 translocation in tumor, secretary carcinoma was excluded too.

Most acinar cell carcinoma had a favorable outcome based on early case series (Choh et al., 2012; Shingu et al., 2013; Limite et al., 2014), but there were cases with poor prognosis (Chang et al., 2011; Sarsiat et al., 2022) and usually related to the presence of a poorly differentiated invasive component. In fact, one-third of reported cases of breast acinic cell carcinoma have been associated with the presence of a ductal carcinoma (not the acinar cell carcinoma component), and which is frequently poorly differentiated (Conlon et al., 2016) as showing in our case. But the exact
prognosis was hard to know because of the rarity of this subtype. The patient in our case rejected the adjuvant therapies including the chemotherapy and had an uneventful process during the past 12 months.

Our study had several limitations. First, we did not compare the microglandular adenosis with the clear tumor cell area and the high-grade area; we could not know the relation or disparities among these cells. Second, we did not have a comparative sequencing of her previous tumor, and could not know the difference between the two tumors in her bilateral breast. However, it was the second germline BRCA1 mutation patient with ACC of the breast in our case and it was the first one which had obvious clear cell differentiation and high-grade tumor cell area, neither was usual feature observed in the other reported ACC cases. We are sure our case would consummate useful information about this rare entity in its histology, molecular changes and prognosis.

In general, our case showed some similar genomic profiles (like the BRCA1 and TP53 mutations) as reported ACCs in breasts in the literature, but also our case had some unique features including very young age, bilateral tumors, relatively favorable prognosis despite the high grade tumor morphology and rejecting the adjuvant treatment. Further studies are warranted to elucidate the true face of ACCs in breast.

**Ethical approval**

This study was approved by Beijing Hospital of Traditional Chinese Medicine, Capital Medical University. A written consent was obtained from the patient.

**References**

(2019). WHO Classification of Tumours Editorial Board. Breast Tumours. 5th ed. WHO Classification of Tumours Editorial Board. IARC. Lyon, France. pp 139-141.


(2010). BRCA1 basal-like breast cancers originate from luminal epithelial progenitors and not from basal stem cells. Cell Stem Cell. 7, 403-417.
<table>
<thead>
<tr>
<th>Antibody</th>
<th>Clone</th>
<th>Vendor</th>
<th>Working dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER</td>
<td>SP1 (Rabbit)</td>
<td>Ventana</td>
<td>RTU</td>
</tr>
<tr>
<td>PR</td>
<td>1E2 (Rabbit)</td>
<td>Ventana</td>
<td>RTU</td>
</tr>
<tr>
<td>HER2</td>
<td>4B5(Rabbit)</td>
<td>Ventana</td>
<td>RTU</td>
</tr>
<tr>
<td>Ki-67</td>
<td>UMBAB107 (Mouse)</td>
<td>Sino Biological</td>
<td>RTU</td>
</tr>
<tr>
<td>EGFR</td>
<td>UMBAB95 (Mouse)</td>
<td>Sino Biological</td>
<td>RTU</td>
</tr>
<tr>
<td>TP53</td>
<td>DO-7 (Mouse)</td>
<td>Sino Biological</td>
<td>RTU</td>
</tr>
<tr>
<td>Amylase</td>
<td>OTI6D4 (Mouse)</td>
<td>Origene</td>
<td>1:50</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>polyclonal (Rabbit)</td>
<td>Sino Biological</td>
<td>RTU</td>
</tr>
<tr>
<td>α1-ACT</td>
<td>polyclonal (Rabbit)</td>
<td>Sino Biological</td>
<td>RTU</td>
</tr>
<tr>
<td>EMA</td>
<td>UMBAB57 (Mouse)</td>
<td>Sino Biological</td>
<td>RTU</td>
</tr>
<tr>
<td>S100</td>
<td>15E2E2+4C4.9 (Mouse)</td>
<td>Sino Biological</td>
<td>RTU</td>
</tr>
<tr>
<td>SOX10</td>
<td>EP268 (Rabbit)</td>
<td>Sino Biological</td>
<td>RTU</td>
</tr>
<tr>
<td>CD117</td>
<td>YR145 (Mouse)</td>
<td>Sino Biological</td>
<td>RTU</td>
</tr>
<tr>
<td>CK8/18</td>
<td>B22.1&amp;B23.1 (Mouse)</td>
<td>Sino Biological</td>
<td>RTU</td>
</tr>
<tr>
<td>E-cadherin</td>
<td>UMBAB184 (Mouse)</td>
<td>Sino Biological</td>
<td>RTU</td>
</tr>
<tr>
<td>SMA</td>
<td>UMBAB237 (Mouse)</td>
<td>Sino Biological</td>
<td>RTU</td>
</tr>
<tr>
<td>p63</td>
<td>4A4+UMAB4 (Mouse)</td>
<td>Sino Biological</td>
<td>RTU</td>
</tr>
<tr>
<td>CK5/6</td>
<td>OTI1C7 (Mouse)</td>
<td>Sino Biological</td>
<td>RTU</td>
</tr>
<tr>
<td>AR</td>
<td>EP120 (Rabbit)</td>
<td>Sino Biological</td>
<td>RTU</td>
</tr>
<tr>
<td>GCDFP-15</td>
<td>EP95 (Rabbit)</td>
<td>Sino Biological</td>
<td>RTU</td>
</tr>
<tr>
<td>GATA-3</td>
<td>EP368 (Rabbit)</td>
<td>Sino Biological</td>
<td>RTU</td>
</tr>
<tr>
<td>Mammmaglobin</td>
<td>304-1A5 (Mouse)</td>
<td>Sino Biological</td>
<td>RTU</td>
</tr>
<tr>
<td>PAX8</td>
<td>OTI6H8 (Mouse)</td>
<td>Sino Biological</td>
<td>RTU</td>
</tr>
<tr>
<td>PLAP</td>
<td>EP194(Rabbit)</td>
<td>Sino Biological</td>
<td>RTU</td>
</tr>
<tr>
<td>SALL4</td>
<td>6E3 (Mouse)</td>
<td>Sino Biological</td>
<td>RTU</td>
</tr>
<tr>
<td>HNF1β</td>
<td>polyclonal (Rabbit)</td>
<td>Sino Biological</td>
<td>RTU</td>
</tr>
<tr>
<td>CK20</td>
<td>EP23 (Rabbit)</td>
<td>Sino Biological</td>
<td>RTU</td>
</tr>
<tr>
<td>Synaptophysin</td>
<td>UMBAB112 (Mouse)</td>
<td>Sino Biological</td>
<td>RTU</td>
</tr>
<tr>
<td>Chromogranin</td>
<td>LK2H10 (Mouse)</td>
<td>Sino Biological</td>
<td>RTU</td>
</tr>
</tbody>
</table>

Abbreviations: ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; EGFR, epidermal growth factor receptor; α1-ACT, α-1-antichymotrypsin; EMA, epithelial membrane antigen; SOX10, SRY-Box transcription factor 10; SMA, smooth muscle actin; AR, androgen receptor; GCDFP-15, gross cystic disease fluid protein 15; GATA3, GATA binding protein 3; PAX8, paired box gene 8; PLAP, Placental-like alkaline phosphatase; SALL4, Spalt-like transcription factor 4; HNF1β, hepatocyte nuclear factor 1β; RTU, ready to use.
### Tab.2 Comparing the heterochrony nodules histological features in bilateral breast.

<table>
<thead>
<tr>
<th>Side</th>
<th>Histological Subtype</th>
<th>Gross morphology (pattern)</th>
<th>Arrangement of the tumor cells</th>
<th>Peripheral of the tumor</th>
<th>ER</th>
<th>PR</th>
<th>HER-2</th>
<th>Ki-67</th>
<th>TP53</th>
<th>GATA-3</th>
<th>Axillary lymph node (ipsilateral)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left</td>
<td>invasive carcinoma of no special type, Grade 2</td>
<td>6cm (solid)</td>
<td>glands and acini</td>
<td>unknown</td>
<td>faint staining</td>
<td>negative</td>
<td>0</td>
<td>67%</td>
<td>strong positive</td>
<td>positive</td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>breast ACC, Grade 2&amp;3</td>
<td>4cm (solid and cystic)</td>
<td>solid, cribriform, micropapillary, papillary, and microglandular with clear cytoplasm</td>
<td>DCIS with clear cytoplasm, including a small area of microglandular adenosis</td>
<td>negative weakly positive</td>
<td>0</td>
<td>40% null expression</td>
<td>negative</td>
<td>negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of cases</td>
<td>Bilateral</td>
<td>Grade</td>
<td>Family history</td>
<td>Methods of gene tests</td>
<td>HRCA1</td>
<td>TP53</td>
<td>Other mutations</td>
<td>Ref.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------</td>
<td>-----------</td>
<td>-------</td>
<td>----------------</td>
<td>-----------------------</td>
<td>-------</td>
<td>------</td>
<td>-----------------</td>
<td>------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>YES</td>
<td>NA</td>
<td>YES*</td>
<td>LOH and sequencing</td>
<td>constitutional mutation; somatic loss of the wild-type allele</td>
<td>c.654_655insGTG mutation</td>
<td>Not available</td>
<td>(Ripamonti et al., 2013)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 pure and 6 mixed</td>
<td>NA</td>
<td>G1-2</td>
<td>NA</td>
<td>sequencing</td>
<td>1 somatic mutation; 1 germline mutation</td>
<td>7/8 Mutations (one pure and six mixed cases)</td>
<td>PIK3CA, M TOR, CTNNB1, ERBB4, ERBB3, INPP4B and FGFR2</td>
<td>(Guerini-Rocco et al., 2015)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 pure and 6 mixed</td>
<td>NA</td>
<td>G1-2</td>
<td>NA</td>
<td>sequencing</td>
<td>1 somatic mutation (E1419* coupled with loss of heterozygosity)</td>
<td>7/8 Mutations</td>
<td>somatic mutations, ERBB4 (2/8; G6V and c.2203-1G-T) FGFR2 (S252W), ERBB3 (R667S), INPP4B (N223Y) and PIK3CA (E542Q) genes; an amplicon spanning MYC, SLA and COL14A1(1/8)</td>
<td>(Geyer et al., 2017)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 (1 reported in 2015)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>sequencing</td>
<td>1 somatic homozygous deletion</td>
<td>2/3 hotspot mutations</td>
<td>DNA repair-related genes (2/3), MLH1 pathogenic germline variant (1/3), focal amplification of 12q14.3–12q21.1 (encompassing MDM2, HMG12, FRS2 and PTPRB)</td>
<td>(Beca et al., 2019)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>NO</td>
<td>G3</td>
<td>NA</td>
<td>sequencing</td>
<td>NO germline/somatic mutation</td>
<td>mutation (c.747G&gt;T)</td>
<td>RET (c.2899G&gt;A)</td>
<td>(Weaver et al., 2021)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Our case</td>
<td>YES</td>
<td>G3</td>
<td>Yes**</td>
<td>sequencing</td>
<td>heterozygosis germline mutation (p.R1751*)</td>
<td>mutation (c.323_329dupGTTC CG)</td>
<td>PRKAA1, FAS, ARID2, MET, FLT3, TP63, PIK3CG; focal amplification of MYC and RECQL4</td>
<td>Our case</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Mother (ovarian cancer), maternal aunt (breast cancer and ovarian cancer); **Mother (breast cancer)

Abbreviation: NA, Not Available; LOH, loss of heterozygosity; H&E, hematoxylin and eosin stain
S1. Material and methods of exons captured sequencing.

Tumor tissue biopsies and peripheral blood samples were obtained from patients with informed consent for biomarker analyses. DNA from the sampled FFPE was isolated using a commercially available kit (QIAamp DNA Mini Kit, Qiagen, Hilden, Germany, catalog:51306). The total DNA yield must be greater than or equal to 1 ug, while 260/280 and 260/230 is greater than or equal to 1.8 and 2, respectively. gDNA were sheared into 200-250bp fragments using a Covaris S2 instrument (Woburn, MA, USA), and indexed NGS libraries were prepared. For gDNA, after end-repairing and A-tailing reactions, targeted adapters were ligated to both ends of the double-stranded gDNA fragments, followed by PCR to generate sufficient numbers of fragments prior to hybridization. Afterward, all libraries were hybridized to self-built probes (IDT, Coralville, IA, USA) which covers 1021 genes (Table S1), and DNA sequencing was performed using the MGISEq-2000 Sequencing System (BGI, Shenzhen, China) per the manufacturer’s guidelines.

For raw sequencing data, individual adaptor sequences, reads with more than 50% low-quality or N bases and their mate pair were removed. Remaining reads were mapped to the reference human genome (hg19) using the Burrows-Wheel Aligner (http://bio-bwa.sourceforge.net/) with default parameters. Duplicate reads were identified and marked with Picard’s Mark Duplicates tool (https://software.broadinstitute.org/gatk/documentation/toddocs/4.0.3.0/picard_sam_ markduplicates_MarkDuplicates.php). Local realignment and base quality recalibration were performed using The Gene Analysis Toolkit (https://www.broadinstitute.org/gatk/). Somatic single-nucleotide variations (SNVs) and insertions or deletions of small fragments (Indels) were called using the MuTect2 algorithm (https://software.broadinstitute.org/gatk/documentation/toddocs/3.8-0/org_broadinstitute_gatk_tools_walkers_cancer_m2_MuTect2.php). Several filtrations were performed: (1) synonymous variants were ignored; (2) variants with frequencies higher than 1% in several SNP databases (ExAC, ESP6500, dbSNP, 1000G) were removed; (3) variants with less than 0.5% allele frequency were removed; (4) variants detected in matched PBL were defined as germline variations.
and removed. Remain were high credible somatic mutations.

**Figure Legends**

Fig.1A: Patterns of the tumor cells in the left breast mass were arranged in solid and diagnosed as invasive breast carcinoma of no special type, grade2.

Fig.1B-1F: Immunohistochemistry showed faint positive staining for estrogen receptors (ER), negative staining for progesterone receptors (PR) and positive for GATA-3 and EGFR, Ki-67 index was high (67%).

Fig.2A: Patterns of the tumor cells in the right breast mass were arranged in solid, cribriform, micropapillary, papillary, and microglandular. Most of the cells (80%) had clear cytoplasm, well to moderately differentiated cells with round or oval nuclei, and hyaline balls in the center; the Insertion Fig. show clear cytoplasm of the tumor cells.

Fig.2B: Some cells with lightly eosinophilic zymogen granules in the cytoplasm;

Fig.2C: The high-grade dedifferentiation region with brisk mitotic activity (showing transition of conventional ACC and high-grade dedifferentiation area);

Fig 2D: Microglandular adenosis was also noted at the periphery of the tumor (CK5/6 staining);

Fig 2E: Tumor cells stained strongly positive for lysozyme;

Fig 2F: Tumor cells stained strongly positive for S-100 staining.