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ARTICLE

Invasive apocrine carcinoma of the breast: clinicopathologic features and comprehensive genomic profiling of 18 pure triple-negative apocrine carcinomas

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Abstract

Pure invasive apocrine carcinoma is a rare type of primary breast cancer, constituting ~1% of all breast cancers. Since most pure invasive apocrine carcinomas are triple negative, the lack of targeted therapies for triple-negative breast cancer has fostered efforts to discover actionable molecular targets in these tumors. In this study, we analyzed the clinicopathologic characteristics and comprehensive genomic profiling of 18 patients with pure triple-negative apocrine carcinomas (TNACs) using a 324-gene panel assay (FoundationOne CDx). The median age of these patients was 55.5 years, and the postmenopausal status rate was 77.8%. In total, 83.3% of patients were diagnosed with histological grade II, and 16.7% were diagnosed with grade III. The majority of patients presented at an early tumor-node-metastasis (TNM) stage (I: 38.9%; II: 50.0%; and III: 11.1%). The mean Ki-67 index was 9.7%, and the percent of PD-L1 positivity was 11.7%. With a median follow-up period of 76.5 months, one patient died, and two experienced distant metastases. There were 61 clinically relevant genomic alterations among all 18 pure TNACs, and the mean tumor mutation burden (TMB) was 3 Mut/Mb. The top ranked altered genes were *PIK3CA* (72.2%), *PTEN* (33.3%) and *TP53* (27.8%). There were four novel mutations found in *PTEN* and an actionable rearrangement involving *FGFR2-TACC2* that has not been reported in breast cancer before. In total, 88.9%, 50%, 44.4%, and 16.7% of TNACs had at least one clinically relevant genomic alteration in genes involved in the PI3K/mTOR, cell cycle, RAS/RAF/MEK and growth factor receptor-related pathways, respectively. All patients had at least one clinically relevant genomic alteration, and 94.4% had at least one actionable alteration. To the best of our knowledge, this study is the largest genomic sequencing cohort of pure TNACs. Incorporation of comprehensive genomic profiling into TNACs might shed light on potential therapeutic opportunities for both targeted drugs and immune checkpoint inhibitors.

Introduction

Invasive apocrine carcinoma is a rare type of primary breast cancer, constituting <4% of all breast cancers [1–3]. Pure invasive apocrine carcinoma is characterized by >90% of the tumor cells showing apocrine morphology (abundant eosinophilic and granular cytoplasm, with sharply defined cell borders, and with large nuclei containing prominent nucleoli) and a distinct hormone receptor profile: estrogen receptor/progesterone receptor negative and androgen receptor positive on immunohistochemistry [4]. Strictly defined as above, pure invasive apocrine carcinoma constitutes ~1% of all breast cancers [2].

The clinical features of pure invasive apocrine carcinomas are not yet fully understood, as most studies have recruited a small number of patients. Pure invasive apocrine carcinomas are categorized as either the HER2-enriched subtype or triple-negative breast cancer (TNBC). Several studies have reported that invasive apocrine carcinoma tends to occur in older women and might show a less aggressive course; however, data on the behavior of triple-negative

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apocrine carcinomas (TNACs) are still ambiguous [5]. In addition, there are no recommendations for standard treatments specific to pure invasive apocrine carcinomas. Therefore, TNAC may be grouped with and treated similarly to other hormone receptor-deficient, HER2-unamplified breast cancers, which typically involve broad-spectrum and multidrug chemotherapeutic regimens.

Although a few gene expression profiling studies have shown that pure invasive apocrine carcinoma forms a distinct molecular cluster compared with a variety of other breast cancer types, genomic landscape studies of TNAC are still lacking. It is also unknown whether the different genetic features of TNAC indicate specific clinical implications [6, 7]. Thus, there is potential utility in separating TNAC from the broad categorization of TNBCs since it does not fit either the clinical, morphologic or molecular mold of other TNBC types.

In this study, a systematic analysis of actionable gene aberrations was completed in patients with pure TNAC. We examined the clinicopathologic characteristics and comprehensive genomic profiles of 18 patients with pure TNAC to elucidate the potentially actionable genomic alterations in this population. The findings illuminate the molecular features of TNAC and highlight the underlying therapeutic targets of this specific type of TNBC.

Materials and methods

Patients

This study of human invasive apocrine carcinoma specimens was approved by the institutional review board of Fudan University Shanghai Cancer Center. From January 2010 to December 2014, all patients who were diagnosed with invasive apocrine carcinoma and underwent complete resection of the tumor at Fudan University Shanghai Cancer Center were reviewed. A total of 18 patients with pure TNAC were eligible for this study. Tumor-node-metastasis (TNM) stage was evaluated according to the eighth edition of the American Joint Committee on Cancer (AJCC) staging system [8].

Histological criteria

Invasive apocrine carcinoma is defined by the WHO as invasive carcinoma characterized by large cells with abundant eosinophilic granular cytoplasm and enlarged nuclei with prominent nucleoli, resembling apocrine sweat glands [3]. Detailed cytological features that characterize invasive apocrine carcinoma are described by Rosen's Breast Pathology [9]. The nuclei are enlarged and pleomorphic compared with the nuclei of benign apocrine cells (Supplementary Fig. 1). Nuclear membranes tend to be hyperchromatic and irregular. Low-grade nuclei are usually slightly larger than nuclei in apocrine metaplasia, and nucleoli are usually present but inconspicuous. Some high-grade nuclei are strikingly enlarged and pleomorphic, with one or more macronucleoli that can be round, oval or teardrop shaped. Other high-grade apocrine nuclei have deeply basophilic and smudged chromatin in which little or no internal structure can be discerned. Binucleation is common. Variation in nuclear size is frequently observed, with adjacent nuclei showing threefold or greater differences in diameter. In most cases, the cytoplasm exhibits eosinophilia that is densely homogeneous or slightly granular. Cytoplasmic vacuolization or clearing is usually most prominent in apocrine carcinomas. The cytoplasm of some tumor cells occasionally has a light blue mucoid quality, and focal mucinous secretion may be present in the lumen of the neoplastic glands. Cell borders tend to be well defined.

To qualify as pure invasive apocrine carcinoma, we used the strict criteria defined by Vranic et al., in which apocrine cytological features were observed in >90% of the tumor cells together with estrogen receptor and progesterone receptor negativity and androgen receptor positivity on immunohistochemistry [4]. Patients showing partly apocrine morphology and those showing exclusively apocrine carcinoma in situ without an invasive apocrine component were excluded from further analysis.

Immunohistochemistry

Immunohistochemistry was performed on whole tissue sections using a Ventana Benchmark automated immunostainer (Ventana, Tucson, AZ) with the standard streptavidin-biotin complex method with 3,3′-diaminobenzidine as the chromogen. Antibody clones for estrogen receptor, progesterone receptor, HER2, Ki-67, androgen receptor and PD-L1 were SP1 (Roche), 1E2 (Roche), 4B5 (Roche), MIB1 (Roche), EPR1535 (Abcam), and SP142 (Roche), respectively.

Following the proposal by the 2010 American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines, strong nuclear staining observed in more than 1% of tumor cells was considered positive for estrogen receptor and progesterone receptor [10]. The percentage of Ki-67-positive cells and androgen receptor staining were assessed on the total number of cancer cell nuclei counted. PD-L1 expression in ≥1% of tumor-infiltrating immune cells was defined as PD-L1 positive.

The HER2 status was initially evaluated by immunohistochemistry [11]. For HER2 2+ tumors, a fluorescent in situ hybridization array was performed to determine the HER2 amplification status with the FDA-approved

PathVysion HER2/Neu DNA Probe Kit (Abbott Molecular, Abbott Park, IL).

Tumor-infiltrating lymphocytes (TILs) evaluation

Stromal TILs evaluation was followed by the international consensus scoring recommendations [12, 13]. Quantification was done on HE stained tissue sections at a magnification of ×20–40 with a ×10 ocular. TILs (lymphocytes and plasma cells) were scored in the stroma between the areas of carcinoma. Stromal TILs were presented as percentage of the stromal areas alone, while the areas of carcinoma cells were not included for assessment.

Comprehensive genomic profiling

All specimens were sequenced by FoundationOne CDx (F1CDx), an FDA-approved 324-gene panel assay conducted by DIAN (Hangzhou Lab) with licensed technologies. Sequence analysis methods and validation of the comprehensive genomic profiling platform used in this study have been described previously [14]. At least 50 ng of DNA per specimen was isolated and sequenced to high, uniform coverage. Base substitutions, short insertions and deletions (INDELs), focal gene amplifications and homozygous deletions, and select gene fusions were simultaneously detected by this assay.

Statistical methods

The GenVisR package was used to visualize the genome landscape [15]. R software or GraphPad Prism was used to plot other figures.

Results

Clinicopathologic features of TNAC

The clinicopathologic features of TNACs examined in this study are shown in Table 1. All 18 TNAC patients were female, with a median age at diagnosis of 55.5 years (ranging from 44 to 83 years). The postmenopausal status rate was 77.8% (14/18). The largest diameter of these tumors ranged from 0.8 to 5 cm. A total of 83.3% (15/18) of patients were diagnosed with histological grade II, and 16.7% (3/18) were diagnosed with grade III. Fifty percent (9/18) of patients had nodal metastases at diagnosis, and the number of positive lymph nodes ranged from 1 to 10. Among these patients, 100% (9/9) had ipsilateral axillary lymph node metastases, while one patient had simultaneous infraclavicular lymph node metastases. The majority of patients included in this cohort presented at an early TNM stage according to eighth edition of AJCC staging system (IA: 38.9% [7/18]; IIA: 27.8% [5/18]; IIB: 22.2% [4/18]; and IIIC: 11.1% [2/18]) [8].

All patients had TNBC (estrogen receptor/progesterone receptor/HER2). The mean Ki-67 index was ~9.7% (ranging from 2 to 20%). All patients exhibited diffuse and at least moderate nuclear staining for androgen receptor (Supplementary Fig. 2). The percent of PD-L1 positivity was 11.7% (2/17). Due to the aggregation of hemosiderin-laden macrophages caused by obvious hemorrhage, one case showed difficulty for PD-L1 expression evaluation, thus we did not include this case in our final analysis. We also evaluated the stromal TILs for each case. The median value of stromal TILs was 20% (ranging from 5 to 30%) (Supplementary Table 1).

Seventeen patients underwent total mastectomy, while only one patient received breast-conserving surgery. Fourteen patients were treated with chemotherapy after surgery, four of whom received sequential radiation therapy.

After a median follow-up period of 76.5 months, 83.3% (15/18) of patients revealed no disease-related morbidity or mortality. Only one patient (5.6%) died, and two (11.1%) experienced distant metastases.

Comprehensive genomic profiling of TNAC

The maximum tumor mutation burden (TMB) of all specimens was 6 Mut/Mb, and the mean value was 3 Mut/Mb (Fig. 1a). All patients were microsatellite stable (MSS). There were 61 clinically relevant genomic alterations among all 18 TNAC patients (Table 2), and the mean count was ~3.4 alterations per sample (range 1–7) (Fig. 1b). The top ranked altered genes were *PIK3CA* (72.2%), *PTEN* (33.3%) and *TP53* (27.8%) (Fig. 1c). For all genes, the most common mutation types were single nucleotide variants (SNVs) or INDELs, which were the predominant alterations in *PIK3CA*, *PTEN*, *TP53*, *NF1*, *HRAS*, and *MAP3K* (Fig. 1c). In total, 88.9%, 50%, 44.4%, and 16.7% of TNAC patients had at least one clinically relevant genomic alteration in genes involved in the PI3K/mTOR, cell cycle, RAS/RAF/MEK and growth factor receptor signaling pathways, respectively (Fig. 1d). Furthermore, 100% of patients had at least one clinically relevant genomic alteration, and 94.4% had at least one actionable alteration.

All mutations in the *PIK3CA* gene were known pathogenic mutations, 61.5% (8/13) of which were p.H1047R, followed by p.C420R (2/13), p.E542K (2/13), p.E418K (1/13), p.E545K (1/13), and p.H1047L (1/13) (Fig. 2a). In addition, 8 mutations were detected in the *PTEN* gene. The *PTEN* p.K13E mutation has been reported in breast cancer, while p.L57fs*6 and p.P95L have been reported in other tumor types. Notably, three novel frameshift mutations and one missense mutation in the *PTEN* gene (I4fs*20,

Table 1 Clinicopathologic features of pure TNAC.

Case ID	Age (years)	Menopausal status	Histological grade	TNM stage	Tumor size (cm)	Lymph node metastases	Side	Quadrant	Operation	Adjuvant therapy
TNAC1	54	Post-	II	IIB	5.0	1/26	Right	Superior	Mastectomy	Chemothrapy + radiation
TNAC2	61	Post-	II	IIB	3.0	2/23	Right	Superior-external	Mastectomy	Chemotherapy
TNAC3	55	Post-	II	IA	1.8	0/15	Left	Internal	Mastectomy	Chemotherapy
TNAC4	63	Post-	III	IIA	4.0	0/20	Right	External	Mastectomy	Chemotherapy
TNAC5	45	Post-	II	IIIC	0.8	8/14	Right	Superior internal	Mastectomy	Chemothrapy+ radiation
TNAC6	47	Pre-	II	IIA	4.0	0/17	Right	Superior	Mastectomy	Chemotherapy
TNAC7	52	Pre-	II	IA	2.0	0/4	Left	External	Mastectomy	Chemotherapy
TNAC8	72	Post-	II	IA	1.2	0/15	Right	Internal	Mastectomy	N/A
TNAC9	75	Post-	II	IA	1.5	0/1	left	Superior-external	Breast-conserving surgery	N/A
TNAC10	55	Post-	II	IA	1.5	0/5	Left	Inferior	Mastectomy	Chemotherapy
TNAC11	83	Post-	III	IIA	2.0	1/15	Left	Superior-external	Mastectomy	N/A
TNAC12	56	Post-	II	IIA	2.0	1/18	Left	Superior internal	Mastectomy	N/A
TNAC13	54	Post-	II	IIIC	2.5	10/24	Right	Superior	Mastectomy	Chemotherapy
TNAC14	60	Post-	III	IA	1.7	0/4	Left	Superior-external	Mastectomy	Chemotherapy
TNAC15	64	Post-	II	IIA	2.0	1/17	Left	Superior internal	Mastectomy	Chemothrapy + radiation
TNAC16	47	Pre-	II	IIB	3.0	1/4	Right	Inferior-external	Mastectomy	Chemothrapy + radiation
TNAC17	68	Post-	II	IIB	4.2	1/14	Left	Superior	Mastectomy	Chemotherapy
TNAC18	44	Pre-	II	IA	1.0	0/8	Left	Superior-external	Mastectomy	Chemotherapy

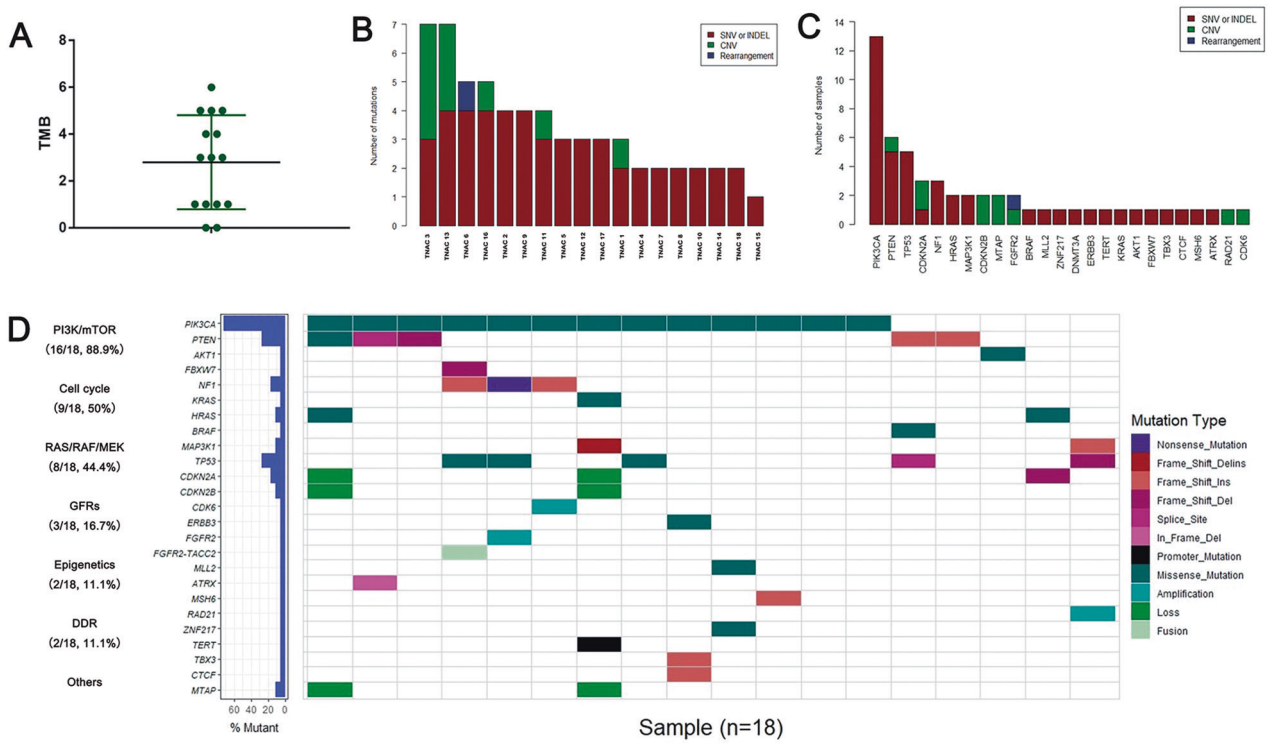

Fig. 1 Summary of somatic genomic alterations in 18 TNACs. **a** TMB in 18 TNAC samples. **b** Characteristic gene aberrations per sample. SNV/INDEL was the predominant mutation type. **c** Recurrent mutations and the top ranked altered genes. The top ranked altered genes were *PIK3CA* (72.2%), *PTEN* (33.3%) and *TP53* (27.8%). **d** The landscape of somatic genetic alterations in 18 TNAC patients. In total, 88.9%, 50.0%, 44.4% and 16.7% of TNAC patients had at least one clinically relevant genomic alteration in genes involved in the PI3K/mTOR, cell cycle, RAS/RAF/MEK and growth factor receptor (GFR) signaling pathways, respectively.

R47fs*8, R55fs*44, and K197N) that have not been reported in either the cBioPortal or COSMIC database were detected (Fig. 2b). Furthermore, one cryptic splicing mutation in PTEN around exon 6 (c.493-2A > G) was detected. Two and three patients showed multiple simultaneous clinically relevant genomic alterations in the *PIK3CA* and *PTEN* genes, respectively.

TNAC had relatively low chromosomal copy number variants (CNVs) (Fig. 1b, c). Amplification of the *RAD21*, *FGFR2*, and *CDK6* genes was detected in 16.7% (3/18) of all patients. Furthermore, deletion of the *CDKN2A*, *CDKN2B*, *MTAP*, and *PTEN* genes was identified in 11.1% (2/18) of all patients. Importantly, apart from an amplification in *FGFR2*, a novel and actionable rearrangement involving *FGFR2-TACC2*, which has not been studied in breast cancer before, was detected in one patient (Fig. 3).

To explore the clinical relevance of the molecular features of TNAC patients, the alterations were categorized by levels of evidence supporting investigative therapies. We found that 17 patients in this study had potentially actionable alterations that corresponded to either an FDA-approved regimen or targeted therapy. Of them, with the mutations of *PIK3CA* gene, patients may benefit from PI3K/AKT/mTOR pathway inhibitors, such as the FDA-approved PI3K inhibitor Alpelisib (Supplementary Table 2). In addition to these gene aberrations, the immune checkpoint biomarker PD-L1 was also tested in all patients due to possible therapy from immune checkpoint blockade. Taken together, these results indicate a high proportion of actionable alterations with supportive clinical evidence for TNACs.

Discussion

Invasive breast carcinoma can show variable degrees of apocrine differentiation, described as 'invasive carcinoma with apocrine differentiation' or 'apocrine-like invasive carcinoma,' in which apocrine morphology is observed in between 10 and 90% of tumor cells [16]. The strict criteria used for all TNACs in our study were apocrine cytological features in >90% of the tumor cells together with a triple-negative phenotype and androgen receptor positivity on immunohistochemistry.

Compared with invasive ductal carcinoma of no special type, invasive apocrine carcinoma typically affects elderly women with a postmenopausal status [17]. TNAC often has a lower histological and nuclear grade than non-apocrine TNBC. In our study, 83.3% of TNACs were classified as histological grade II. Patients with TNAC also appeared to

Table 2 Clinically relevant genomic alterations of pure TNAC.

Case ID	TMB (Muts/Mb)	MSI	Genomic Findings
TNAC1	4	MSS	MAP3K1 E903fs*1; TP53 F385fs*37; RAD21 amplification
TNAC2	5	MSS	PTEN R47fs*8, R55fs*44; BRAF D594R; TP53 splice site 782 + 1G > A
TNAC3	1	MSS	PIK3CA H1047R; PTEN K13E; HRAS Q61K; CDKN2A deletion; CDKN2B deletion; MTAP deletion; PTEN exons 1–7 deletion
TNAC4	4	MSS	HRAS G13R; CDKN2A S7fs*21
TNAC5	NA	MSS	PIK3CA H1047R; MLL2 P3109H; ZNF217 A790V
TNAC6	1	MSS	FBXW7 K371fs*6; PIK3CA H1047R; NF1 S82fs*25; TP53 R175H; FGFR2-TACC2 fusion
TNAC7	1	MSS	PIK3CA E418K, C420R
TNAC8	6	NA	PIK3CA H1047R; DNMT3A R635Q
TNAC9	3	MSS	ERBB3 E332K; PIK3CA H1047R; CTCF T204fs*26; TBX3 E402fs*5
TNAC10	3	MSS	PIK3CA E542K; MSH6 W97fs*20
TNAC11	5	MSS	PIK3CA E545K; NF1 Y2285fs*1, splice site 204 + 1G > A; CDK6 amplification
TNAC12	5	MSS	PIK3CA E542K; PTEN splice site 493-2A > G; ATRX S1944del
TNAC13	1	MSS	PIK3CA H1047L; KRAS G12V; MAP3K1 V1331fs*9; TERT promoter −124C > T; MTAP exons2–8 deletion; CDKN2A deletion, CDKN2B deletion
TNAC14	NA	MSS	PIK3CA H1047R; PTEN I4fs*20
TNAC15	NA	NA	AKT1 E17K
TNAC16	3	MSS	PIK3CA C420R, H1047R; NF1 W571*; TP53 T125M; FGFR2 amplification
TNAC17	0	MSS	PIK3CA H1047R; TP53 R110P, R175H
TNAC18	0	MSS	PTEN L57fs*6, P95L

have a smaller tumor size, a lower rate of distant metastasis, and a lower rate of cancer-related death.

Despite the limited sample size in our study, the observed trends in the clinical outcome of TNAC also merit discussion, particularly compared with the clinical outcome of other aggressive subtypes of TNBC. Generally, 83.3% of patients showed no disease-related morbidity or mortality. None of the stage I patients experienced disease-related recurrence, metastases or death. This result is consistent with that reported by Mills et al. but inconsistent with that reported by Choi et al., who showed that androgen receptor expression correlated with a poor outcome among T1 TNBC patients [2, 18].

While the genomic landscape of TNBCs is heterogeneous and complex, we showed that many of the key somatic genetic alterations in TNAC can be detected using a targeted next-generation sequencing assay (F1CDx). TNAC displays a distinctive repertoire of somatic genetic alterations, characterized by less frequent *TP53* mutations, which are known to be predominant in ~80% of TNBCs [19, 20]. The most commonly mutated gene in our TNAC group was *PIK3CA*, followed by *PTEN* and *TP53*. Lehmann et al. also found *PIK3CA* mutations in 40% of luminal androgen receptor (LAR) tumors [19]. As a subtype of TNBC, LAR breast cancer is reported to comprise ~10% of TNBCs and shows high expression of androgen receptor mRNA and relatively higher levels of mutations involving the PI3K pathway (particularly *PIK3CA* gene mutations) than other TNBC subtypes [16, 21]. Although thought to be enriched in TNBCs with apocrine morphology, LAR tumors did not correlate with the histological analysis. The high frequency of PI3K pathway mutations in TNAC is more similar to that in luminal types of breast cancer [22].

As shown in our studies, all mutations in the *PIK3CA* gene were known pathogenic mutations, 61.5% of which were p.H1047R. As demonstrated by many other studies, the PI3K pathway is activated in 70% of breast cancers [23]. Several mechanisms may account for the activation of this pathway in breast cancer, including amplification and/or activating mutations of the *PIK3CA* gene, which encodes the p110a catalytic subunit of PI3K found in 20–40% of breast cancers [24, 25]. The most recurrent mutation, p.H1047R, which was also found in our study, leads to constitutive PI3K signaling and heterogeneous mammary tumors. Therefore, dozens of clinical trials have been designed to target *PIK3CA* or a related pathway [26, 27]. Yi et al. showed that the p.H1047R mutation might be a potential biomarker of sensitivity to everolimus, an mTOR inhibitor, in hormone receptor-positive metastatic breast cancer [28]. And it is noteworthy that the FDA-approved

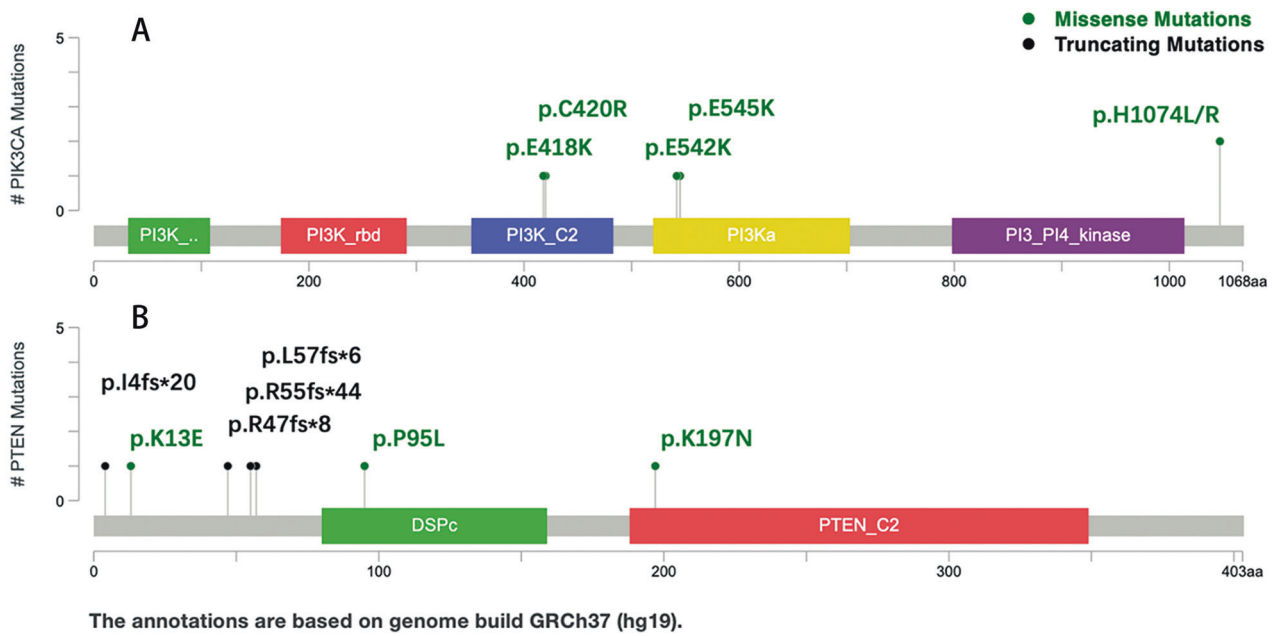

Fig. 2 Clinically relevant genomic alterations in *PIK3CA* and *PTEN* genes. a Location of mutations within *PIK3CA* gene. The most recurrent mutation was p.H1047R. **b** Location of mutations within *PTEN* gene. Three novel frameshift mutations and one missense mutation (I4fs*20, R47fs*8, R55fs*44, and K197N) that have not been reported in either the cBioPortal or COSMIC database were detected.

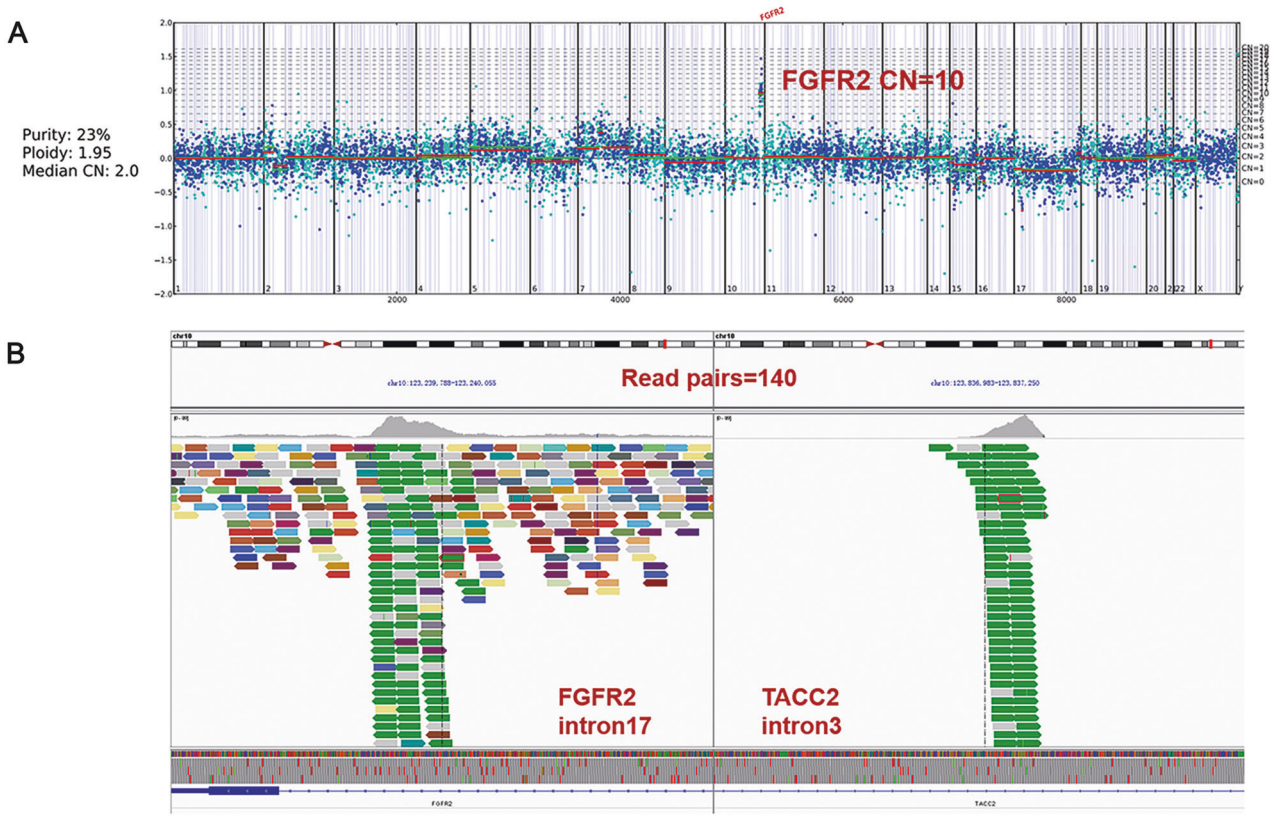

Fig. 3 Amplification of *FGFR2* and a novel and actionable rearrangement involving *FGFR2-TACC2* were identified. a *FGFR2* copy number (10 copies) was determined by modeling copy variation and aneuploidy across the genome and was compared to process-matched normal controls. **b** The *FGFR2-TACC2* fusion visualized using Integrated Genomic Viewers (IGV) software.

PI3K inhibitor Alpelisib is highly recommended in *PIK3CA* mutated hormone receptor-positive breast cancer according to the most recently updated guidelines [29]. Although the pure apocrine carcinomas in our study were negative for estrogen receptor and progesterone receptor, 72.2% of the patients still harbored *PIK3CA* mutations, thus it is rational to assume the potential effect of Alpelisib in the treatment strategy to TNAC.

The tumor suppressor gene *PTEN* is also a high-penetrance cancer predisposition gene that encodes a phosphatidylinositol phosphatase. Germline *PTEN* mutations have been detected in patients with autosomal dominant cancer predisposition syndromes, such as Cowden syndrome, Lhermitte–Duclos syndrome, and Bannayan–Zonana syndrome [30, 31]. Somatic mutations and deletions of this gene have been reported in many types of sporadic tumors, including endometrial cancer, glioblastoma, breast cancer, and prostate cancer. Most tumor-derived point mutations of *PTEN* in breast cancer causes a truncation of the *PTEN* protein and consequently induce loss of function [32]. It is noteworthy that germline *PTEN* mutations have been described in patients with Cowden syndrome that are prone to develop breast cancer with apocrine differentiation [33].

In our study, eight mutations were detected in the *PTEN* gene. The p.K13E mutation has been reported in glioblastoma and breast cancer and is considered pathogenic [32, 34, 35]. In contrast to wild-type *PTEN*, the K13E mutant form fails either to prevent protein kinase B/Akt phosphorylation or inhibit cell proliferation when expressed in *PTEN*-null U87MG cells [35]. The P95L mutation is considered oncogenic in several cancer types [36–38]. There are limited data on the L57fs*6 mutation, which was previously reported in metastatic colorectal tumors [39]. Although the four novel mutations in the *PTEN* gene (I4fs*20, R47fs*8, R55fs*44, and K197N) were speculated to be oncogenic according to the cBioPortal database, the detailed effects of these mutations have not been carefully analyzed. Interestingly, one cryptic splicing mutation in *PTEN* around exon 6 (c.493-2A > G) was detected. This mutation might cause exon 6 skipping and result in a frameshift at glycine 165 that terminates after eight codons [40]. A study showed that the locations of *PTEN* mutations, especially those outside exons 5–7, could act as significant and independent positive prognostic indicators for survival in endometrial carcinomas [41]. Thus, *PTEN* mutation screening in breast cancer might also provide useful prognostic information.

TNAC had a low level of genetic instability, with few amplifications and deletions, and no recurrent copy number alterations were identified in our group. However, we found a novel actionable rearrangement involving *FGFR2-TACC2* in 1 of 18 patients. These chromosomal translocations joining in-frame members of the fibroblast growth factor receptor-transforming acidic coiled-coil gene families (*FGFR-TACC* gene fusions) were first discovered in human glioblastoma multiforme. The most common fusion type is *FGFR3-TACC3*, which has been found in many other cancer types, such as lung cancer and bladder cancer, but the rearrangement involving *FGFR2-TACC2* has never been reported in breast cancer. Early clinical data have shown promising effects in patients with malignant tumors harboring *FGFR-TACC* fusions and treated with FGFR inhibitors [42, 43].

As well demonstrated in prostate cancer, several hotspot mutations of androgen receptor gene could be detected in castration-resistant prostate cancer. And the mutations of androgen receptor gene can convert the effect of androgen receptor antagonist into an agonist [44, 45]. However, we did not identify any mutations of androgen receptor gene in our study. In spite of this, strong expression of androgen receptor in TNAC also implies a potential clinical benefit from androgen receptor inhibitors, such as enzalutamide or abiraterone [46, 47]. In one of the phase II trials, enzalutamide showed clinical activity and was well tolerated in patients with advanced androgen receptor-positive TNBC [47]. Thus, androgen receptor antagonist may also become a candidate in the treatment selections of TNAC.

To the best of our knowledge, this study is the largest genomic sequencing cohort of pure TNACs. We demonstrated that most pure TNACs had at least one actionable alteration, and the characteristics of TNACs were similar to those of luminal phenotype breast carcinomas. Incorporation of comprehensive genomic profiling into TNAC may shed light on the potential therapeutic opportunities for both targeted drugs and immune checkpoint inhibitors.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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