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ARTICLE

Genomic characterization and tumor evolution in paired samples of metaplastic breast carcinoma

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Metaplastic breast carcinomas are a rare and heterogeneous group of tumors (0.5–2%). They are mainly triple negative tumors but they present poorer chemotherapy responses and worse prognosis than other triple negative tumors. The aim of our study was to characterize the molecular profile and tumor evolution in matched (primary-relapse) tumor samples from patients with early-stage metaplastic breast carcinomas who had disease recurrence/progression. We performed genomic profiling of tumor biopsies at least from two different time points of their tumor evolution. Tumor samples were analyzed by DNA-Next Generation Sequencing (Illumina 2 x 75bp) using the Action OncoKitDX panel (Imegen-Health in Code group), which includes point mutations in 50 genes, CNVs, and fusion genes. Only pathogenic and likely pathogenic variants were considered for analysis and they were categorized following the ComPerMed criteria. We analyzed 21 matched tumor samples (8 primary and 13 relapse/progression samples). Genomic profiling of matched tumor samples revealed that mutations present in primary tumors are generally maintained in the relapse/disease progression. We did not find a significant increase in point mutations between primary and relapse/progression samples, although gene amplifications were found more frequently in relapse/progression samples. Tumor samples harbored high frequency of *TP53* (100%) and *TERT* promoter (29%) mutations, and of *MYC* amplifications (80% of which in relapse/progression samples). No *PI3KCA* mutations were found, but *PTEN* variations were enriched in 38% of samples (10% mutations and 28% deletions). *FGFR1* amplifications were identified in 13% of samples (primary tumor only). Neither *ERBB2* nor *EGFR* gene amplifications were detected. The most frequent pathogenic alterations occurred in cycle regulation's genes, including *TP53* and *TERT* promoter mutations, and *MYC* amplifications. Relapse/progression samples were highly enriched for *MYC* amplification. Larger studies are required to better characterize these tumors, and identify new strategies to improve the prognosis of these patients.

Modern Pathology (2022) 35:1066–1074; https://doi.org/10.1038/s41379-022-01017-7

INTRODUCTION

Metaplastic breast carcinoma is a heterogeneous and rare subtype that constitutes 0.25 to 2% of all breast cancers[1,2]. These tumors are histologically defined by differentiation of neoplastic epithelium into squamous or mesenchymal-like elements.

Clinicopathologic features of metaplastic breast carcinomas include larger size, higher histological grade, less lymph node involvement, and less vascular invasion than invasive carcinoma of no special type (ductal)[3,4]. Most metaplastic breast carcinomas (>90%) lack the expression of estrogen and progesterone receptors and HER2, which leads to their classification as triple negative breast cancers (TNBC). The 5th edition of WHO classification of breast tumors categorizes metaplastic breast carcinomas as mixed metaplastic carcinoma, low-grade adenosquamous carcinoma, fibromatosis-like metaplastic carcinoma, squamous cell carcinoma, spindle cell carcinoma, and metaplastic carcinoma with heterologous mesenchymal differentiation[5,6]. Unlike TNBC of no special type, metaplastic carcinomas are less responsive to standard chemotherapy treatments, and are associated with worse survival outcomes[7,8].

Some studies have suggested that metaplastic breast carcinomas may derive from undifferentiated pluripotent stem-cell-like cells[9]. Molecular analysis has shown that metaplastic breast carcinomas are enriched in stem cell and epithelial-mesenchymal transition (EMT) features, which has led to their classification within the claudin-low subgroup. This subgroup is characterized by being enriched in EMT, immune response and stem-cell process markers[10–12] involved in drug resistance, increased invasiveness, and the development of metastases. Furthermore, the co-occurrence of an EMT phenotype and alterations in *PI3KCA* would lead to increased aggressiveness of these tumors[10]. There are no specific pathognomonic mutations identified for metaplastic breast carcinomas. In several studies comparing metaplastic breast carcinomas to other TNBC, PI3K/AKT/mTOR,Wnt/β-catenin signaling pathway alterations, *MYC* and *TP53* alterations and *EGFR* amplifications were the most frequently

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Received: 28 October 2021 Revised: 20 January 2022 Accepted: 25 January 2022
Published online: 17 February 2022

detected variants[10,13,14]. Nonetheless, all of these studies were retrospective, based on small and heterogeneous populations with no paired tumor samples, and were carried out using different molecular techniques.

The aim of our study was to characterize the genomics and tumor evolution in matched (primary-relapse) samples of patients with metaplastic breast cancer.

MATERIALS AND METHODS

Study design and patient population

A search for patients with metaplastic breast carcinoma treated between 2009 and 2020 was conducted at Catalan Institute of Oncology-University Hospital of Bellvitge, L'Hospitalet (Barcelona). Only patients with at least two tumor samples available in the Pathology department (one of the primary tumor and other of the recurrence/progression) were selected. Data regarding histologic type, hormone receptor and HER2 status were collected from pathologic reports. Tumor stage and other clinical data, including treatment information were obtained from the electronic medical record. Study protocol was approved by the institutional review board at University Hospital of Bellvitge-IDIBELL (BB20-017). Informed written consent was obtained from all patients alive at the moment of the analysis.

Tumor samples

All samples of metaplastic breast carcinomas were provided by Biobank HUB-ICO-IDIBELL, integrated in the Spanish Biobank Network and funded by Instituto de Salud Carlos III (PT17/0015/0024) and by Xarxa de Bancs de Tumors de Catalunya sponsored by Pla Director d'Oncologia de Catalunya (XBTC). Tumor samples were reviewed by breast pathologists (TS, JB, and AP) and classified according to the latest WHO classification of Breast tumors[15]. Representative sections of formalin-fixed paraffin-embedded blocks of each metaplastic breast carcinoma were selected and used for DNA extraction and sequenced through Action OncoKitDx NGS gene panel (Imegen-Health in Code Group). A total of 21 paired samples from 8 patients (P1-P8) were included in the study. Gene panel sequencing was performed in 8 primary tumor samples, and their respective 13 recurrences (loco-regional or metastases).

Genomic DNA (gDNA) from the included samples was extracted with the RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE (Thermo Fisher Scientific) and the QIAamp DNA Investigator Kit (Qiagen). DNA concentration was quantified with the Qubit Fluorimeter (Thermo Fisher Scientific) with Qubit dsDNA BR Assay kit and Qubit dsDNA HS Assay kit (Invitrogen). The DNA Integrity Number (DIN) of the different samples was determined using the DNA ScreenTape assay (Agilent Technologies). Cases with a DIN value equal to or higher than 2.4 were considered despite the recommended cut-off DIN value of 3 by the Action OncoKitDx user guide. This cut-off value does not imply that there are no guarantees of a reliable sequencing result. The decision of including samples under DIN of 3 was made considering the limited number of samples available in the study. The samples finally included in the study obtained correct quality parameters in all sequencing steps.

NGS sequencing and bioinformatics pipeline

Tumor samples were analyzed by DNA Next Generation Sequencing (Illumina 2 x 75bp) using the Action OncoKitDX panel which studies point mutations in 50 genes, copy number variants (CNVs) throughout the genome, and fusion genes among 8 genes with any other partner of the genome. It also determined Microsatellite Instability (MSI) through 110 markers and identified pharmacogenetic SNPs associated with treatment toxicity or efficacy according to PharmGKB. All tumor suppressors or oncogenes currently within the standard of care of a high number of adult solid tumors were covered **(Supplementary material 1)**. The results were classified following the recommendations of the American College of Medical Genetics (ACMG)[15].

Bar-coded libraries were amplified and sequenced on the NextSeq 550 system (Illumina) for massive library sequencing in "Stand-alone" mode with 2 × 75 paired-end reads following the manufacturer's instructions. The Sample Sheet was generated using the Illumina Experiment Manager (IEM) software version 1.14.0 (Illumina). The monitorization of sequencing run quality was based on the Q30 value and cluster pass filter, setting a threshold at 80% and 70% respectively. The FASTQ files generated followed a quality evaluation applying the FastQC v0.11.5 software (Babraham Bioinformatics). QC metrics were also evaluated from the BAM file of each sample trough uniformity, average coverage and percentage of the region covered at 100x. Bioinformatic analysis, including the alignment to the reference sequence Genome Reference Consortium Human Build 37 (GRCh37), annotation and variant calling, followed a self-developed pipeline through the DataGenomics platform. The variant assessment and categorization were performed as previously reported by Martínez-Fernández, P[16].

RESULTS

Patient's and tumor characteristics

Between January 2009 and December 2020, 73 patients were diagnosed of metaplastic breast carcinoma in our institution, out of a total of 6000 breast cancers diagnosed (1, 2%). Among them, we selected tumor samples from those patients with both primary tumor and relapse/progression tissue available meeting the quality requirements for molecular study. We finally analyzed 21 matched primary-relapse tumor samples from eight patients that met those criteria: 8 primary tumor samples and 13 loco-regional/metastatic samples. In all patients' genomic characterization was performed at least at two different time points of their tumor evolution, but in three patients, genomic characterization was performed at three different time points, and in one patient at four time points. Baseline patient and tumor characteristics and first site of recurrence are listed in Table 1. The most common histology at diagnosis was spindle cell carcinoma in 3 patients (37%) (Fig. 1A). In two patients although the initial diagnosis was invasive carcinoma of no special type (ductal), the relapsed tumor was a metaplastic carcinoma, so that they were also included in this series. All except one case (in which progesterone receptor was 3%) had a triple negative profile and a high proliferation index measured by ki67 (between 30–80%). In the early setting, all patients received chemotherapy+/− radiotherapy per institutional guidelines. The histologic subtype of primary metaplastic breast carcinoma and their matched metastatic/recurrence were concordant in four cases whereas the morphology changed in four. Moreover in patient 2 (P2) the last recurrence (P2-R3) changed the morphologic subtype from squamous cell carcinoma to spindle cell carcinoma (Fig. 2). The most frequent histology of the first site of recurrence was metaplastic carcinoma with heterologous mesenchymal differentiation (chondroid) (Fig. 1B), found in 5 patients (62%). Tumor evolution and clinical outcome of the eight patients included in this study and pathogenic gene variants identified are summarized in Fig. 3.

Molecular alterations of metaplastic carcinomas

Tumor genomic characterization detected in each patient based on the different time points of their tumor evolution are listed in Table 2.

Cell cycle regulation. The most frequently mutated gene among both primary tumor and metastases samples identified was *TP53*. A pathogenic variant within *TP53* was identified in all samples (100%) but just 24% of sequenced samples also carried a *TP53* mono-allelic deletion (P1, P5, and P6). The allelic frequency of *TP53* variants was higher among metastases than primary tumor samples of these three cases (Fig. 3).

Mutations within the *TERT* promoter were detected in 2 (P3 and P8) out of 8 cases (25%) and they were observed in both primary tumor and metastatic/relapse samples. Both cases were a spindle cell carcinoma subtype at diagnosis but metastatic relapses of P3 changed and were classified as mixed metaplastic carcinoma (spindle cell and mesenchymal chondroid differentiation), keeping though the same mutational pattern.

MYC amplification was observed in 3 cases (P2, P6, and P7) out of 8 cases (37,5%) and in 24% of the 3 samples. Among these samples, 80% were samples from metastases and only 20% from primary tumors. Regarding tumor subtypes, no clear

Table 1. Baseline patient and tumor characteristics and first site of recurrence.

Patient ID	Age at diagnosis	Histology at diagnosis	TNM at diagnosis	Immunohistochemistry profile	Ki67 expression	1st site of recurrence	Histology of the 1st recurrence	Time to recurrence (months)
P1	45	Metaplastic carcinoma with heterologous mesenchymal differentiation (chondroid)	T2N1	ER-/PR-/HER2-	60%	Brain	Metaplastic carcinoma with heterologous mesenchymal differentiation (chondroid)	31
P2	57	Squamous cell carcinoma	T1cN0	ER-/PR-/HER2-	30%	Skull	Squamous cell carcinoma	18
P3	47	Spindle cell carcinoma	T2N0	ER-/PR-/HER2-	65%	Lung	Mixed spindle cell carcinoma and metaplastic carcinoma with heterologous mesenchymal differentiation (chondroid)	23
P4	59	Invasive carcinoma of no special type (ductal)	T2N0	ER-/PR + (3%)/HER2-	80%	Local + systemic recurrence	Mixed invasive carcinoma of no special type ductal and spindle cell carcinoma	21
P5	69	Mixed invasive carcinoma of no special type (ductal) and spindle cell carcinoma	T1cN0	ER-/PR-/HER2-	35%	Local recurrence	Mixed invasive carcinoma of no special type ductal and metaplastic carcinoma with heterologous mesenchymal differentiation (chondroid)	41
P6	47	Invasive carcinoma of no special type (ductal)	T2N0	ER-/PR-/HER2-	60%	Local recurrence	Metaplastic carcinoma with heterologous mesenchymal differentiation (chondroid)	22
P7	51	Metaplastic carcinoma with heterologous mesenchymal differentiation (chondroid)	T2N0	ER-/PR-/HER2-	47%	Local recurrence	Metaplastic carcinoma with heterologous mesenchymal differentiation (chondroid)	39
P8	65	Spindle cell carcinoma	T3N0	ER-/PR-/HER2-	60%	Lung	Spindle cell carcinoma	27

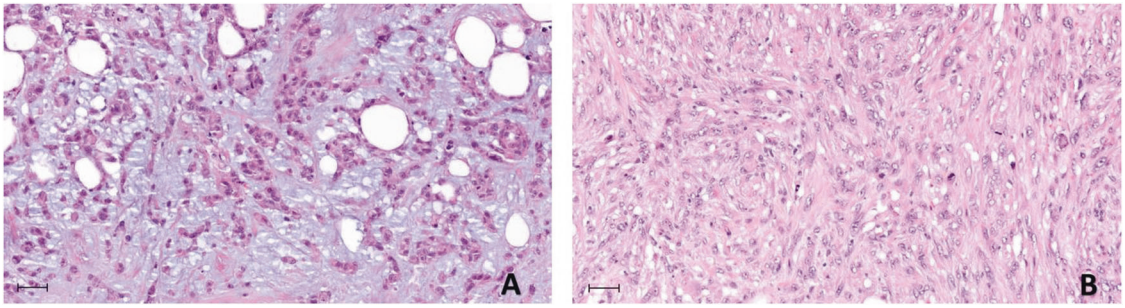

Fig. 1 Characteristic images of metaplastic carcinoma histological subtypes. A Metaplastic spindle cell carcinoma. **B** Heterologous mesenchymal differentiation (chondroid). Scale bars equal 50 µm.

relationship was observed; this alteration was found in mesenchymal chondroid differentiation (P6-R1, P7-PT, and P7-R1), squamous cell carcinoma (P2-R2), and spindle cell carcinoma (P2-R3).

PTEN/PI3K/AKT signaling pathway and MAPK pathway. *PTEN* alterations were frequently detected in our study of paired samples. Although *PTEN* mutations were present exclusively in 1 out of 8 patients (13%), a mono-allelic *PTEN* deletion was detected in 38% of the analyzed samples. Overall, a *PTEN* gene variation (mutation or deletion) was observed in 41% of the patients (P2, P4, P7, and P8). The samples corresponded to different tumor subtypes: squamous (P2-R2), spindle cell (P2-R3, P4-PT, P4-R1 and P8-R1, P8-R2), and, heterologous mesenchymal differentiation (chondroid) (P7-PT and P7-R1). It should be noted that in P4, this alteration was already present in the primary tumor (invasive carcinoma of no special type). Patient 7 samples showed co-existence of *PTEN* loss and *MYC* amplification. A *PIK3CA* amplification was detected exclusively in one patient (P7 carcinoma with heterologous mesenchymal differentiation, chondroid) and this alteration was identified in both the primary tumor and the relapse samples. None of the tumor samples analyzed harbored mutations in *PIK3CA*, AKT (*AKT1, AKT2, AKT3*) nor in the MAPK pathway genes included in the panel (*NRAS, HRAS, KRAS, MAP2K1*).

Tyrosine kinase receptors EGFR, ERBB2, and FGFR1. Among the sequenced samples, no *EGFR* or *ERBB2* gene amplifications were observed. *EGFR* gene gain (aneusomy) was observed in 25% of cases (P2, P3). An amplification of *FGFR1* was described in the primary tumor of one patient (P8), but it was lost in the metastatic sample.

Other gene alterations. In the tumor samples analyzed, other SNVs and CNVs have been detected in specific genes from particular cases. Among them, a *BRCA1* mutation was identified in one out of the eight patients (P6). The pathogenic mutation had a greater allelic frequency in the metastatic samples than in the primary tumor (VAF 3.85% in primary tumor vs 23.10% and 70.45% in paired metastases). No mutation was detected in *ARID1A* or *CTNNB1* genes. A *ZNF217* amplification (8–10 copies) was detected in both samples of one patient (P7). Additionally, a focal amplification of more than twenty copies was evidenced throughout 9p23-p22, including *NFIB* gene. It was observed in one patient, but solely in the most recent metastasis of this patient (P2-R3).

Structural events and Microsatellite instability. The gene panel used studies gene fusions and gains and losses of complete or part of chromosomes; both were reported as structural events. Overall, the sequenced samples were characterized by a high number of chromosomal copy number aberrations. This fact was proved in both primary tumor samples and their corresponding recurrence samples. Each sample carries an average number of eighteen large copy number variants, considering those of at least 6 kb in length (data not shown). No gene fusions of target genes were detected among the sequenced samples. Deletions, gains, and amplifications of single genes with clinical significance are reported in previous sections and complete data about mutations and CNVs to highlight is reported in Table 2.

Microsatellite stability was valued among all the samples. The majority of the analyzed samples (87%) presented values compatible with stable tumors (MSS) and only 13% of the cases presented an MSI consistent with low-grade unstable tumors (MSI-L).

Primary tumor vs recurrences. With regard to single nucleotide variants (SNV), few differences were found in terms of mutation-carrying genes between primary tumor and metastases samples. All genes mutated among metastasis samples were already mutated in their corresponding primary tumor. Overall, *TP53* mutations had greater allelic frequency among metastatic samples than among primary tumors. Concerning focal copy number variations (CNVs) involving genes previously implicated in metaplastic breast cancer, some disparities were detected between primary tumors and their corresponding metastases. In addition to an increased recurrence of *MYC* amplifications among metastatic samples (patients P2, P6, P8) reported above, a *BRCA1* deletion was identified exclusively in a metastatic tumor (P6).

DISCUSSION

Metaplastic breast carcinomas are characterized by histological and molecular heterogeneity, as well as poor survival outcomes. However, little is known about their molecular profile and tumor evolution. In the present study, genomic profiling of matched (primary-relapse) tumor biopsies of patients with metaplastic breast carcinoma revealed that mutations present in primary tumors are generally maintained in the relapse/disease progression samples, and only few tumor samples harbored new alterations, such as MYC amplification, which was enriched in the relapse/progression samples.

Importantly, all samples analyzed in this study, either from primary or relapsed paired tumors, carried *TP53* mutations. These findings suggest that *TP53* mutations play a prominent role in metaplastic breast carcinoma, and that dysregulation of this tumor suppressor gene occurs early during tumor evolution. However, despite the high prevalence of *TP53* mutations and the coexistence of *TP53* deletions, none *TP53* variant presented an allelic frequency compatible with a double hit affecting all tumor region analyzed in this study. In prior studies, *TP53* mutations have been identified in up to 70% of patients[13,17–20].

In our series, mutations within the *TERT* promoter were detected in two patients (6 of out 21 samples: 28%). Interestingly and similar to which occurred with *TP53* mutations, *TERT* promoter mutations were observed in all tumor samples analyzed from these two patients (primary tumors and two additional time points of their tumor evolution). Of note, both cases were spindle cell carcinoma subtype (67% of all spindle tumor cases). In contrast to other types of tumors, *TERT* promoter mutations have been rarely

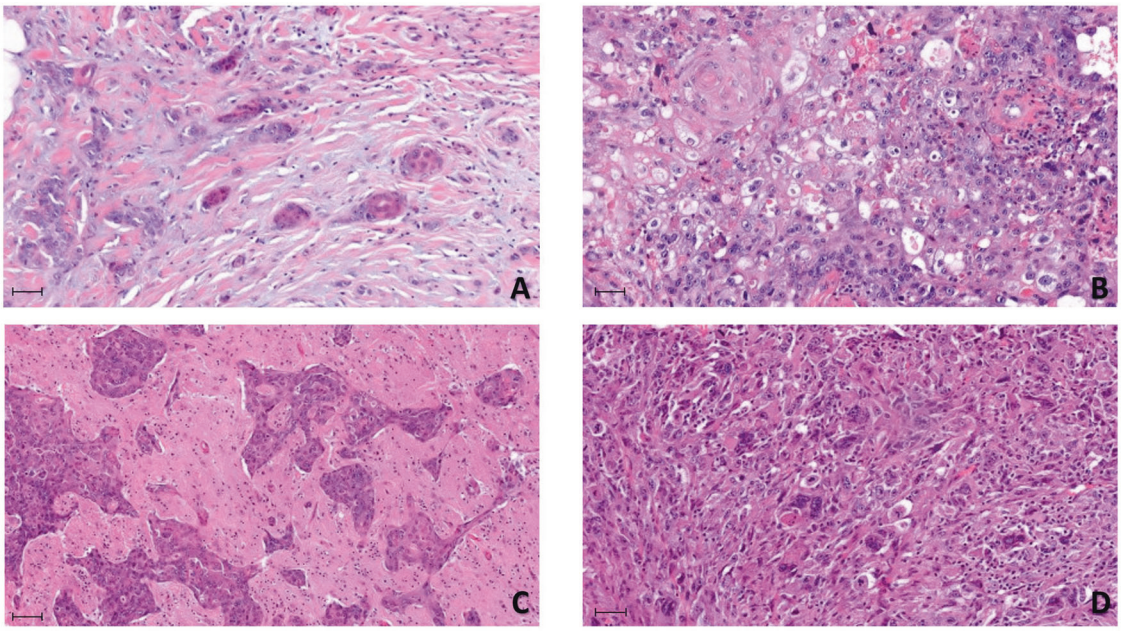

Fig. 2 Representative histopathological images of primary metaplastic carcinoma and subsequent metastases in patient 2. Primary metaplastic carcinoma with squamous differentiation (**A**). Local recurrence of metaplastic squamous cell carcinoma with abundant keratinization and marked nuclear atypia (**B**). Metastatic metaplastic squamous cell carcinoma in cranial vault infiltrating cerebral tissue (**C**). Metastatic metaplastic spindle cell carcinoma of the skull featuring high-grade spindled cells and abundant mitoses (**D**). Scale bars equal 50 μm on (**A**, **B**, and **D**), and 100 μm on (**C**).

observed in invasive breast cancers (<1%)[21]. However, Krings G et al. also described a 25% incidence of *TERT* alterations in their series of metaplastic breast carcinomas, specifically in tumors with spindle (47%) and/or squamous differentiation, but not matrix-producing carcinomas[17]. These mutations are also considered to be an earliest genetic event in tumorigenesis[22]. *PIK3CA* mutations have been previously described as one of the most prevalent alterations in metaplastic breast carcinomas, being identified in 30–50% of cases[10,15,22,23]. Data from 19 metaplastic breast carcinoma included in c-BioPortal, showed a 26.3% of *PI3KCA* mutated samples. Most of the *PIK3CA* mutations detected were activating, causing deregulation of the pathway, and they have been associated with worse prognosis than non-carriers. However, in our study a *PIK3CA* amplification was detected only in one patient but none of the tumor samples analyzed harbored mutations in *PIK3CA*, *AKT* or in the MAPK pathway, despite an adequate gene coverage by the panel[16]. This difference in incidence of *PI3KCA* alterations could possibly be due to the small number of samples in our and others published series, which could lead to sample selection bias. Detecting *PIK3CA* mutations in metaplastic breast carcinoma may lead to PIK3a inhibitors recommendation within a clinical trial[24] although the evidence is still scarce.

MYC is the most frequently amplified gene in metaplastic breast cancer. *MYC* copy number variations have been reported recurrently among different patient series, being identified in more than 17% of patients[14]. The role of *MYC* in metaplastic breast carcinoma is poorly understood and the evolutions of *MYC* alterations between diagnosis and tumor relapse have not been well defined. We detected *MYC* amplifications in 24% of the total samples analyzed. Interestingly, most of *MYC* amplification cases corresponded to relapsed tumor samples (80%). These findings suggest that *MYC* amplification would not be a recurrent driver at diagnosis but rather an oncogenic promoter during tumor development[25]. Additionally, *MYC* amplifications have been described more frequently in squamous or spindle cell differentiation[26] however, such morphologic correspondence was not found in our series except for one case with MYC amplification that corresponded to squamous cell carcinoma (P1).

No *EGFR* gene amplification was observed among the sequenced samples. However, *EGFR* gene gain (aneusomy) was detected in 25% of cases. These results are in line with previously published data; although it may be over-expressed in one-third of metaplastic breast carcinomas, no activating mutations have been commonly found in *EGFR*[26,27].

NFIB (Nuclear Factor IB; 9p23-p22) amplification was detected in the most recent relapse sample analyzed from patient number 2 (P2). *NFIB* has been repeatedly amplified or over-expressed in TNBC. This last relapse does not present a squamous differentiation as the previous ones, but acquires spindle cell traits. Therefore, and at least in this patient, *NFIB* gene amplification is associated with an advanced disease stage and might be related to the histological evolution identified, since it represents the main molecular difference with respect to previous samples.

ZNF217 (Zinc Finger Protein 217) amplification was detected only in patient 7, which falls within a mesenchymal subtype with chondroid differentiation. The involvement of *ZNF217* overexpression in the epithelial-mesenchymal transition has been previously described in breast cancer[28] and it is associated with poor prognosis[29]. In fact, it may promote bone metastases in breast cancer[30]. However, the involvement of *ZNF217* in metaplastic breast carcinoma has not been well established. In our series, it is detected in 13% of cases (P7) and it was observed both in the primary and relapsed tumor samples. The patient did not present bone metastatic relapses during the tumor evolution. Despite *ZNF217* may be implicated in the tumor development and the possible association of this biomarker with a poor prognosis, more data are required to be able to transfer clinical information in patients with metaplastic breast cancer when *ZNF217* amplification is detected. Wnt/β-catenin pathway has been proposed as one of the pathways most implicated in MBC oncogenesis. Different series observed alterations in this pathway in around 50–90% of metaplastic breast carcinomas[13,31]. Unfortunately,

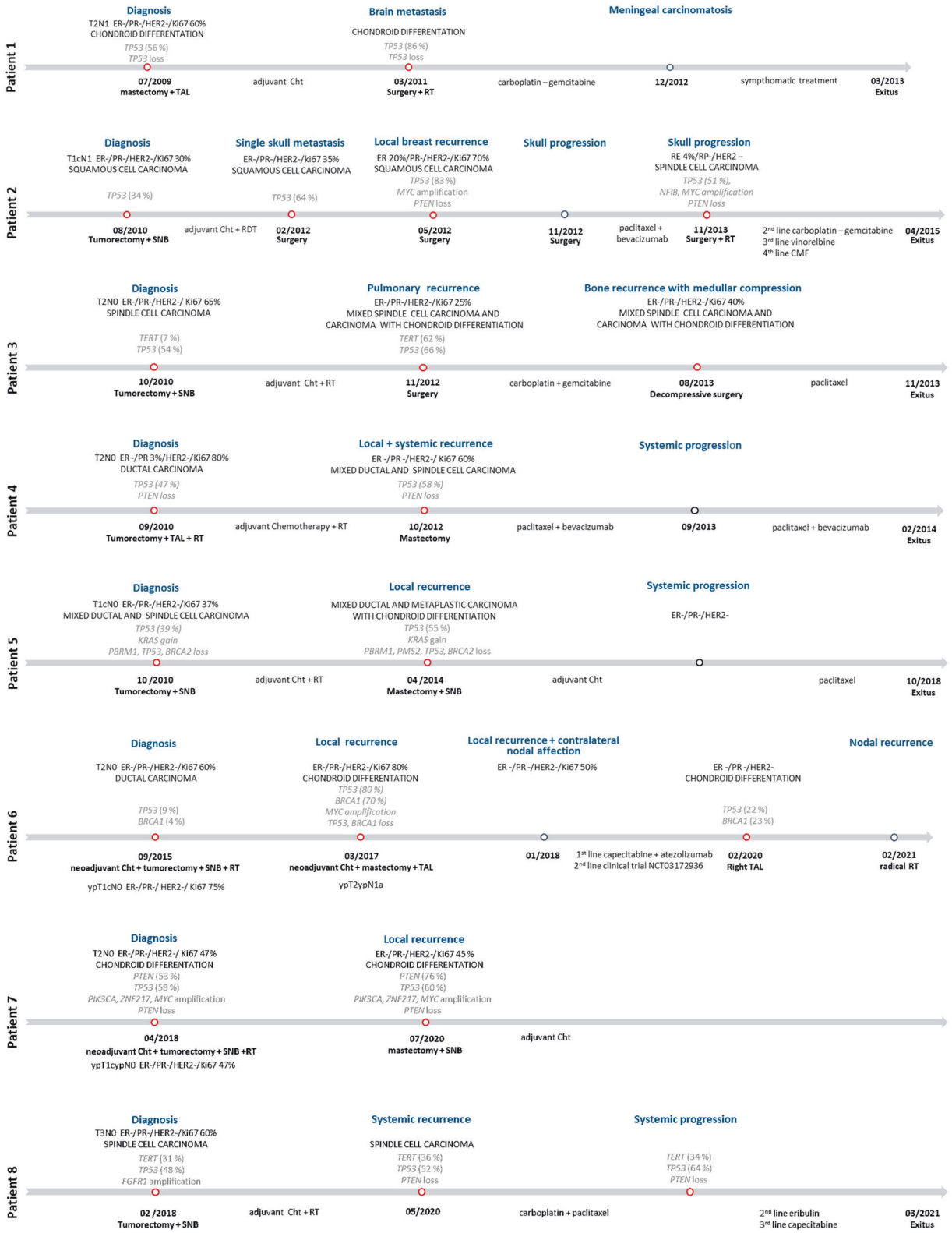

Fig. 3 Summary of genomic characterization and tumor evolution in paired samples of patients with metaplastic breast carcinoma. Cht chemotherapy, RT radiotherapy, ER estrogen receptor, PR progesterone receptor, SNB sentinel node biopsy, TAL total axillary lymphadenectomy, CMF cyclophosphamide, methotrexate, 5 – fluorouracil.

genes involved in this pathway are not the target of the panel, and therefore, we do not have information regarding them.

Relationship between different genetic alterations and histological subtypes is poorly understood and the evolution of these mutations during tumor progression is unknown. Due to the small size of our series, we could not establish any relationship between mutational profile and pathological subtypes of metaplastic breast carcinomas.

Table 2. Pathogenic gene variants identified in each patient based on the different time points of their tumor evolution.

Patient ID	Primary tumor (PT)	First tumor recurrence (R1)	Second tumor recurrence/ progression (R2)	Third tumor recurrence/ progression (R3)
P1	**MCHMD**	**MCHMD**	–	–
	TP53 (c.625C > T; p.Arg209Trp) ***TP53*** loss	***TP53*** (c.625C > T; p.Arg209Trp) ***TP53*** loss	–	–
P2	**SQCC**	**SQCC**	**SQCC**	**SPCC**
	TP53 (c.594G > T; p.Met198Ile)	***TP53*** (c.594G > T; p.Met198Ile)	***TP53*** (c.594G > T; p.Met198Ile) ***MYC*** amplification ***PTEN*** loss	***TP53*** (c.594G > T; p.Met198Ile) ***MYC*** amplification ***PTEN*** loss ***NFIB*** amplification
P3	**SPCC**	**SPCC + MCHMD**	**SPCC + MCHMD**	–
	TERT (Chr: 5, Position: 1295228C > T) ***TP53*** (c.528_530delinsGCA; p. Cys176_Pro177delinsTrpHis)	***TERT*** (Chr: 5, Position: 1295228C > T) ***TP53*** (c.528_530delinsGCA; p. Cys176_Pro177delinsTrpHis)	***TERT*** (Chr: 5, Position: 1295228C > T) ***TP53*** (c.528_530delinsGCA; p. Cys176_Pro177delinsTrpHis)	–
P4	**ICNST**	**ICNST + SPCC**	–	–
	TP53 (c.597_599dup; p.Asn200dup) ***PTEN*** loss	***TP53*** (c.597_599dup; p.Asn200dup) ***PTEN*** loss	–	–
P5	**ICNST + SPCC**	**ICNST + MCHMD**	–	–
	TP53 (c.206_212dup; p.Leu72PhefsTer40) ***KRAS*** gain ***PBRM1*** loss ***TP53*** loss ***BRCA2*** loss	***TP53*** (c.206_212dup; p.Leu72PhefsTer40) ***KRAS*** gain ***PBRM1*** loss ***TP53*** loss ***BRCA2*** loss ***PMS2*** loss	–	–
P6	**ICNST**	**MCHMD**	**MCHMD**	–
	BRCA1 (c.1884_1885insCAGG; p.Arg629GlnfsTer8) ***TP53*** (c.407G > A; p.Arg136His)	***BRCA1*** (c.1884_1885insCAGG; p.Arg629GlnfsTer8) ***TP53*** (c.407G > A; p.Arg136His) ***TP53*** loss ***MYC*** amplification ***BRCA1*** loss	***BRCA1*** (c.1884_1885insCAGG; p. Arg629GlnfsTer8) ***TP53*** (c.407G > A; p.Arg136His)	–
P7	**MCHMD**	**MCHMD**	–	–
	PTEN (c.259C> T; p.Gln87Ter) ***TP53*** (c.902_911del; p.Met301SerfsTer2) ***PIK3CA*** amplification ***ZNF217*** amplification ***MYC*** amplification ***PTEN*** loss	***PTEN*** (c.259C> T; p.Gln87Ter) ***TP53*** (c.902_911del; p.Met301SerfsTer2) ***PIK3CA*** amplification ***ZNF217*** amplification ***MYC*** amplification ***PTEN*** loss	-	–
P8	**SPCC**	**SPCC**	**SPCC**	–
	TERT (Chr: 5, Position: 1295228C > T) ***TP53*** (c.542A > G; p.Tyr181Cys) ***FGFR1*** amplification	***TERT*** (Chr: 5, Position: 1295228C > T) ***TP53*** (c.542A > G; p.Tyr181Cys) ***PTEN*** loss	***TERT*** (Chr: 5, Position: 1295228C > T) ***TP53*** (c.542A > G; p.Tyr181Cys) ***PTEN*** loss	-

MCHMD metaplastic carcinoma with heterologous mesenchymal differentiation, *SQCC* squamous cell carcinoma, *SPCC* spindle cell carcinoma, *ICNST* invasive carcinoma of no special type.

While genetic biomarkers are scarce in metaplastic breast cancer, some of the reported alterations may transfer clinical useful information. None diagnostic, prognostic, therapeutic or resistance to therapy tier I variants were identified but several Tier II alterations were detected. More precisely, tier II variants were identified among 63% of patients (38% of total samples). *MYC* amplification has been reported as a therapy resistance marker[25]. *PTEN* loss of function mutations and gene deletions has been described as a marker of resistance to PI3K-p110-alpha selective inhibitors such as alpelisib. Therefore, and although clinical evidence is still lacking, the search for actionable markers in this tumor type seems essential[24].

The major limitation of our series is the small number of patients who can carry out unrepresentative results. In addition, it must be taken into account that only patients with accessible disease for biopsy at the time of relapse/progression have been included, which may condition the results.

To our knowledge, this is the first series to compare the molecular profile of matched tumor samples of metaplastic breast carcinomas. These data suggest that mutations present in primary tumors are maintained in disease relapse/progression and only a few new alterations emerged in the tumor evolution of metaplastic breast carcinomas, such as *MYC* amplification. Due to the small sample size of the series, no relationship between the mutational profile and the pathological subtypes of metaplastic breast carcinomas was established. A better understanding of the pathogenesis of this group of tumors may lead to individualized treatment approaches in order to improve the prognosis of these patients.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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ACKNOWLEDGEMENTS

We want to particularly acknowledge the patients and the Biobank HUB-ICO- IDIBELL (PT17/0015/0024) integrated in the Spanish Biobank Network for their collaboration, and also Elvira Purqueras for the management of the tumor samples. Also, we are indebted to the "Biobanc de l'Hospital Infantil Sant Joan de Déu per a la Investigació" Integrated in the Spanish Biobank Network of ISCIII for the sample and data procurement. This manuscript did not receive specific funding.

AUTHOR CONTRIBUTIONS

P.G., P.C., and I.C. performed genetic tests. A.S., M.C., A.P., J.B.-S., T.S., and S.P. collected and analyzed clinical and anatomo-pathologic information. A.S., P.G., M.C., A.P., J.B.-S., P.C., S.R., A.V., A.F.O., C.F., M.G.G., S.V., R.V.V., T.S., IC, and S.P. integrated and analyzed the clinical data together with molecular data. A.S., P.G., and S.P. wrote the manuscript. All authors reviewed and accepted the manuscript.

COMPETING INTERESTS

A.S. has served as advisor/consultant role for Novartis and Seagen. Payment or honoraria for lectures, presentations, speakers' bureaus, manuscript writing, or educational events: Eisai, Novartis, Pierre-Fabre. Support for attending meetings and/or travel: Pfizer, Novartis. M.G.G has served as advisor/consultant role for Agendia and Astra-Zeneca. Payment or honoraria for lectures, presentations, speaker's bureaus, manuscript writing, or educational events: Pfizer, Novartis, Daiichi-Sankyo, and Eisai. Support for attending meetings and/or travel: Roche, Pfizer, Novartis, Lilly, and Daiichi-Sankyo. R.V.V has served as advisor/consultant role for Novartis and Lilly. Payment or honoraria for lectures, presentations, speaker's bureaus, manuscript writing, or educational events: Novartis, Eisai. Support for attending meetings and/or travel: Novartis, Eisai, and Lilly. S.P. has served as

advisor/consultant role for AstraZeneca, Daiichi-Sankyo, Polyphor, Novartis, Seattle Genetics, Roche, Eisai, and Pierre-Fabre.

ETHICS APPROVAL/CONSENT TO PARTICIPATE

Study protocol was approved by the institutional review board at University Hospital of Bellvitge-IDIBELL (Reference: BB20-017). Informed written consent was obtained from all patients alive at the moment of the analysis. All samples were properly coded and anonymized. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41379-022-01017-7.

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