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Original contribution

Molecular characterization of metaplastic breast carcinoma via next-generation sequencing ☆

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Summary Metaplastic breast carcinoma (MBC) is a rare subtype of breast cancer with variable morphology. MBC is more often triple negative (ER−, PR−, HER2−) and is associated with poorer clinical outcome when compared with infiltrating ductal carcinoma. The purpose of our study is to identify molecular alterations in MBC using next-generation sequencing (NGS), which may aid chemotherapy selection and use of targeted therapy. A cohort of 18 patients with MBC yielded adequate DNA from microdissected formalin-fixed and paraffin-embedded tumor blocks. NGS was performed using the Ion AmpliSeq cancer hotspot mutation panel version 2 kit, which targets hotspot regions in 50 genes. Immunohistochemical stains for androgen receptor (AR), and programmed cell death ligand-1 were performed. A total of 23 genetic alterations were identified in 15 (83.3%) of 18 patients. Eleven genetic alterations in the PI3K signaling pathway were identified in 9 (50.0%) of 18 patients, including 7 *PIK3CA* mutations (38.9%), 3 *PTEN* genetic alterations (16.7%), and 1 *AKT1* mutation (5.6%). Ten (55.6%) of 18 patients each harbored 1 *TP53* genetic alteration. Additional genetic alterations identified were 1 *HRAS* mutation and 1 *ATM* mutation. AR immunoreactivity was identified in 2 (11.1%) of 18 patients. Programmed cell death ligand-1 was negative in all patients. NGS analysis demonstrated that PI3K pathway–related genetic alterations were detected in a high percentage of MBCs, suggesting that targeting the PI3K/mTOR pathway may be promising in patients with MBC. In addition, patients with AR expressing MBC may benefit from androgen antagonist treatment.

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1. Introduction

Metaplastic breast carcinoma (MBC) is a rare and histologically heterogeneous subtype of breast cancer [1-5]. It accounts for approximately 1% of breast cancer cases. The neoplastic epithelium shows differentiation into squamous- and/or mesenchymal-appearing elements made up of spindle, chondroid, osseous, or rhabdomyoid cells. More than 90% of MBCs are classified as "triple negative" and do not express estrogen receptor (ER), progesterone receptor (PR), or Her2 receptor. Patients with MBC usually present with higher clinical stage and have worse clinical outcomes when compared with patients with infiltrating ductal carcinoma, no special type (IDC NST). Even when compared specifically with other triple-negative breast cancers (TNBCs), MBC still has poorer cancer outcomes. Lymph node metastasis is less frequent in MBC than in IDC NST. Distant metastasis to the brain and lung, without nodal metastasis, has been well described in MBC. Based on National Cancer Database from years 2001 to 2003, patients with MBC, when compared with patients with IDC NST, present at an older age, with larger tumor size, with a lower likelihood of nodal involvement, and with higher AJCC stage [6].

The histopathogenesis of MBC is largely unknown. A popular hypothesis is the epithelial-mesenchymal transition (EMT) theory through which the epithelial cells lose their adhesion to other cells and acquire a fibroblast-like phenotype [4,5]. MBC displays an messenger RNA transcriptional profiling with enrichment for the EMT markers, Twist1 and Snai2/Slug [7]. EMT markers, such as Twist and E-cadherin, have been demonstrated in immunohistochemical studies to be overexpressed in MBC [8]. Breast cancer stem cell–like characteristics, as demonstrated in the overexpression of cancer stem cell marker proteins such as ALDH-1 and $CD44^{+}/CD24^{-}$, have also been demonstrated in MBC [7-9]. The enrichment in EMT and stem cell markers in MBC are features also seen in the mesenchymal and mesenchymal stem–like intrinsic subtypes of TNBC [10].

The treatment options for MBC include surgery, radiation, and chemotherapy [4,5]. Hormone and anti-Her2 therapies are usually nonviable options because most MBC cases do not express hormone or Her2 receptors. Surgical treatment of MBC has largely paralleled that of IDC NST. Simple or modified mastectomy is the mainstay of surgical options given the large size of the primary tumor. Radiation therapy, shown in retrospective studies to provide survival benefit, is typically recommended after surgery. The standard adjuvant and neoadjuvant chemotherapeutic regimens, derived from treatments for IDC NST, are offered to MBC patients, although there are limited data on the efficacy and benefits of specific chemotherapy agents and treatment combinations for this breast cancer subtype. Because of the rarity of MBC and the lack of prospective randomized treatment data specific to MBC, standardized treatment guidelines for MBC are very limited. Therefore, the optimal management of patients with MBC remains to be determined and current treatment presents a challenge to clinicians.

To capitalize on emerging targeted therapy and immunotherapy and to improve the outcomes for patients with MBC, there remains the need to better characterize the molecular and genomic makeup of MBC. For example, molecular analysis based on limited studies shows that PI3K pathway–related *PIK3CA* and *PTEN* mutations are detected in a high percentage in MBC [4,5]. If so, therapy directed at the PI3K pathway may show promise in the treatment of MBC.

The purpose of our study is to better characterize MBC's genetic profile via next-generation sequencing (NGS). NGS is a powerful tool to detect genetic alterations and identify possible targetable mutations. Using Ion AmpliSeq cancer hotspot mutation panel version 2 kit by Thermo Fisher Scientific (Carlsbad, CA) on the extracted DNA from microdissected formalin-fixed and paraffin-embedded (FFPE) specimens, we identify potential mutations in hotspot regions in 50 oncogenes and tumor suppressor genes frequently mutated in human cancers. In addition, immunohistochemical studies determine the expression of androgen receptor (AR) and programmed cell death ligand-1 (PD-L1) to evaluate if androgen antagonist targeted therapy or immunotherapy (checkpoint inhibitors) may be potential treatment choices for MBC.

2. Materials and methods

A search was conducted of the clinically annotated tissue bank in the Department of Pathology at City of Hope for "metaplastic breast cancer." A patient was included in the study if she had a diagnosis of MBC (any type) and for whom pathologic material, demographic, clinicopathological, and treatment information could be procured. A cohort of 21 patients with MBC treated from 1995 to 2014 was identified. Histology, hormone receptor status, and pathologic stage were collected from pathologic reports. Demographic, clinical, and treatment information was abstracted from the medical records. This study was approved by institutional review board at City of Hope National Medical Center (No. 13274).

All slides harboring tumor were reviewed by a pathologist with experience in breast pathology to confirm the diagnosis, to determine the histologic components, and to identify FFPE representative blocks with MBC histologic component(s) of interest. The tumor area with the MBC histologic component (s) of interest was microdissected from two to four 5-μm-thick unstained paraffin slides. Genomic DNA was extracted from the tissue using the QIAcube (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The purified DNA was subsequently quantitated using the Qubit 2.0 Fluorometer (Carlsbad, CA). Of the 21 cases, 3 failed to yield adequate DNA for NGS, resulting in genetic analyses on 18 cases. NGS was performed on the Ion Torrent Personal Genome Machine (Thermo Fisher Scientific, Carlsbad, CA) using the Ion AmpliSeq Cancer Hotspot Panel version 2 kit (Thermo Fisher Scientific) according to the manufacturer's protocol. Briefly, a library of 207 amplicons was generated in a multiplex

polymerase chain reaction using 10 ng of purified genomic DNA. Unique Ion Xpress Barcodes were ligated to each sample. Barcoded libraries were purified, equalized, and combined for templating on Ion Sphere Particles. Templated Ion Sphere Particles were enriched, loaded onto the Ion 318 chip and sequenced on the Personal Genome Machine.

Eighteen cases had additional histologic material for immunohistochemical analysis. Immunohistochemistry studies were performed on 5-μm-thick unstained paraffin slides. Each case was stained for PTEN, MAPK, AR, and PD-L1. The PTEN antibody was the 11G8.1 clone from Cascade Bioscience (Winchester, MA) and was diluted to 1:250. Pretreatment and antigen retrieval were performed at high pH in a Leica Bond III (Leica Biosystems, Buffalo Grove, IL). The MAPK antibody was the Thr202/Tyr204 clone from Cell Signaling (Danvers, MA). It was diluted to 1:200 and pretreated at high pH in a Ventana Benchmark Ultra (Roche Diagnostics, Indianapolis, IN). The detection was performed with a Ventana Ultraview DAB. The AR antibody was the F39.4.1 clone from Biogenex (Fremont, CA), and was diluted to 1:100. It was pretreated in a Dako autostainer (Agilent, Santa Clara, CA) and detected in an Envision Mouse at High pH. The PD-L1 antibody, the SP142 clone from Spring Bioscience (Pleasanton, CA), was diluted to 1:150. It was pretreated in a Ventana Benchmark Ultra and detected in a Ventana Optiview DAB. The intensity of staining was graded from 0 to 3+, and the percent of tumor cells stained was estimated. H score was calculated for PTEN and MAPK antibodies [11,12]. H score = [fraction of cells with intensity grade 1 (%)] + [fraction of cells with intensity grade 2 (%) × 2] + [fraction of cells with intensity grade 3 (%) × 3].

Per conventional definitions, the cutoff for PTEN loss was defined as an H score of ≤10. The cutoff for MAPK positivity was defined as an H score of >10. The MAPK positivity is classified as weakly positive (H score 11-100), intermediately positive (H score 101-200), and strongly positive (H score 201-300). AR was considered positive if ≥1% of tumor cells were stained. PD-L1 was interpreted positive if ≥1% of tumor cells showed membrane staining.

Table 1 Summary of patient's demographics, pathology, and clinical follow-up

Patient	Age (y)	Histology	Size (cm)	LN +/Toto	pTNM	ErPrHer2	ChemoTx	Radiation	Clinical follow-up
1	35	Mesenchymal (spindle)	3.0	1/41	T2 N1	−/−/−	N/A	N/A	Lost to follow-up
2	80	Mesenchymal (spindle)	3.4	2/26	T2 N1	−/−/−	N/A	N/A	Lost to follow-up
3	68	Mesenchymal (spindle)	4.1	0/2	T2 N0	−/−/−	Yes	Yes	Alive without Dz
4	62	Mesenchymal (spindle)	5.7	0/1	T3 N0	−/−/−	Yes	Yes	Death of cancer
5	68	Mesenchymal (osseous)	3.8	0/13	T2 N0	−/−/−	Yes	Yes	Death of cancer
6	73	Mesenchymal (spindle)	4.5	1/2	T2 N1	−/−/−	Yes	Yes	Death of cancer
7	50	Mesenchymal (spindle and chondroid)	1.9	1/21	T1cN1	−/−/−	Yes	No	Alive without Dz
8	84	Squamous and mesenchymal (spindle)	3.0	1mi/16	T2 N1mi	−/−/−	No	No	Alive without Dz
9	76	Mesenchymal (spindle)	1.7	0/4	T1 N0	−/−/−	Yes	Yes	Alive without Dz
10	82	Mesenchymal (spindle)	7.5	0/2	T3 N0	−/−/−	Yes	Yes	Death of cancer
11	39	Squamous	5.4	0/2	T3 N0	−/−/−	Yes	Yes	Alive without Dz
12	83	Squamous	3.0	14/16	T2 N3	−/−/−	N/A	N/A	Lost to follow-up
13	48	Squamous	0.8	0/1	T1 N0	−/−/−	Yes	Yes	Alive without Dz
14 [a]	50	Squamous	1.6	1/12	yT1N1	−/−/−	Yes	Yes	Alive without Dz
15 [b]	75	Mesenchymal (chondroid)	N/A	N/A	N/A	−/−/−	No	Yes	Death of unknown cause
16	80	Mesenchymal (spindle) and squamous	3.0	0/26	T2 N0	−/−/−	N/A	N/A	Lost to follow-up
17	50	Mesenchymal (chondroid)	3.5	0/5	T2 N0	−/+/−	Yes	No	Alive without Dz
18	76	Mesenchymal (spindle)	2.4	0/3	T2 N0	−/−/−	Yes	Yes	Alive without Dz
19	42	Mesenchymal (spindle) and squamous	1.5	0/16	T1 N0	−/−/−	Yes	Yes	Alive without Dz
20	50	Mesenchymal (spindle and chondroid)	3.3	0/7	T2 N0	−/−/−	Yes	Yes	Death of unknown cause
21	68	Mesenchymal (spindle)	3.3	ITC/1	T2 N0(i+)	−/−/−	Yes	Yes	Alive without Dz

Abbreviations: +, positive for ER, PR, or Her2; −, negative for ER, PR, or Her2; Dz, disease; ITC, isolated tumor cells; mi, micrometastasis; N/A, not applicable; Tx, therapy.

[a] History of neoadjuvant therapy.

[b] Only biopsy specimen is available.

3. Results

A total of 21 patients with MBC were identified from the institutional clinically annotated tissue database (Table 1). The average age of the patients was 63.4 years. The most common pathologic pattern was spindle cell mesenchymal differentiation in 14 patients, followed by squamous differentiation in 7 patients, chondroid differentiation in 4 patients, and osseous differentiation in 1 patient (Fig. 1). Five patients had mixed squamous and mesenchymal differentiation or mixed mesenchymal differentiation. All cases showed high-grade morphology. No low-grade adenosquamous carcinoma or fibromatosis-like metaplastic carcinoma was identified in our cohort. Twenty of 21 tumors demonstrated triple-negative receptor profile. Only one tumor showed ER negative, PR positive, and Her2-negative profile. The PR positive rate is 4.8% (1/21). The initial treatment was surgical resection in 19 of the 20 patients. One patient (patient 14) received neoadjuvant therapy, followed by surgical resection. Of the 19 patients who underwent surgical resection, the average size of the tumor was 3.4 cm. The pathologic T stages were pT2 in 12 patients, pT1 in 4, and pT3 in 3. Axillary lymph node metastasis was identified in 7 of the 19 patients, including pN1 in 5, pN1mi (micrometastasis) in 1, and pN3 in 1. The only patient who received neoadjuvant therapy before surgical resection was staged as ypT1N1. Four patients were lost to follow-up. There was a mean follow-up of 91 months in 17 patients. Of the 17 patients, 15 patients received adjuvant systemic cytotoxic chemotherapy, and 13 of the 15 patients also received adjuvant radiation therapy. Twelve of the 15 patients were treated with doxorubicin combined with other agents. One patient received only adjuvant radiation therapy, and another patient received no adjuvant therapy. Of the 17 patients, 4 patients died of breast cancer, 2 patients died of unknown causes, and 11 patients were alive without disease at last follow-up.

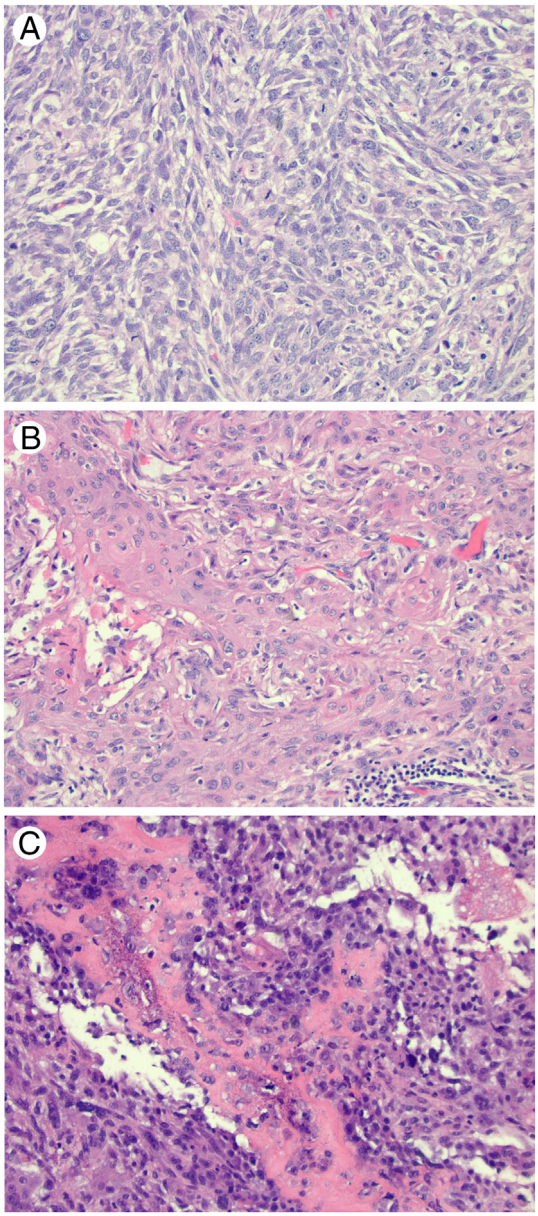

Fig. 1 Representative histologic phenotypes of MBC. A, MBC with spindle cell differentiation. B, MBC with squamous differentiation. C, MBC with osseous differentiation (osteoid matrix). Original magnification ×20.

Table 2 Summary of NGS

Patient	Genetic alterations		
	Gene	Base	Amino acid
1	*TP53*	686_687del	Cys229Tyrfs*10
2	*PIK3CA*	3140A>G	His1047Arg
3	*TP53*	818G>A	Arg273His
4	*PIK3CA*	1633G>A	Glu545Lys
	HRAS	183G>C	Gln61His
5	*TP53*	916C>T	Arg306*
6	*PIK3CA*	1633G>A	Glu545Lys
7	*TP53*	642_643del	His214Glnfs*7
	PIK3CA	1637A>G	Gln546Arg
	PTEN	720_723delCTTT	Tyr240*
8	*TP53*	796G>A	Gly266Arg
	AKT1	49G>A	Glu17Lys
9	*TP53*	734G>A	Gly245Asp
	ATM	1009C>T	Arg337Cys
10	*PIK3CA*	3140A>T	His1047Leu
11	*TP53*	742C>T	Arg248Trp
	PTEN	202 T>C	Tyr68His
12	*TP53*	742C>T	Arg248Trp
	PIK3CA	3140A>G	His1047Arg
	PTEN	955dupA	Thr319Asnfs*6
13	*TP53*	454_466del	Pro152Alafs*14
14	*PIK3CA*	3140A>T	His1047Leu
15	*TP53*	377A>G	Tyr126Cys
16	None	None	None
17	None	None	None
18	None	None	None

The FFPE tumor material from 3 patients was unable to yield adequate DNA extraction for NGS. Eighteen patients had adequate material for NGS evaluation (Table 2). A total of 23 genetic alterations were identified in 15 (83.3%) of 18 tumors. Eleven genetic alterations involved in the PI3K signaling pathway were identified in 9 (50.0%) of 18 tumors. They included 7 *PIK3CA* point mutations (38.9%, all missense mutations), 3 *PTEN* genetic alterations (16.7%, including 1 missense mutation, 1 deletion resulting in stop codon, and 1 duplication with frameshift mutation), and 1 *AKT1* missense mutation (5.6%). Ten (55.6%) of 18 tumors harbored *TP53* genetic alterations (including 6 missense mutations, 3 deletions with frameshift mutations, and 1 nonsense mutation). Additional genetic alterations identified were 1 *HRAS* missense mutation and 1 *ATM* missense mutation. Three tumors did not reveal significant genetic alterations. Most genetic alterations were missense mutations resulting in amino acid alterations (17/23; 73.9%). Three (13%) of 23 deletions with frameshift mutations were identified. One duplication with frameshift mutation, 1 nonsense point mutation resulting in a stop codon, and 1 deletion resulting in a stop codon were identified. Both *TP53* genetic alterations and genetic alterations involving in the PI3K pathway were identified in both squamous and mesenchymal differentiations. The histologic phenotypes harboring the 10 *TP53* genetic alterations were spindle (3), squamous (3), chondroid (1), osseous (1), mixed spindle and chondroid (1), and mixed squamous and spindle differentiation (1). The histologic phenotypes demonstrating the 7 *PIK3CA* mutations were spindle (4), squamous (2), and mixed spindle and chondroid differentiation (1).

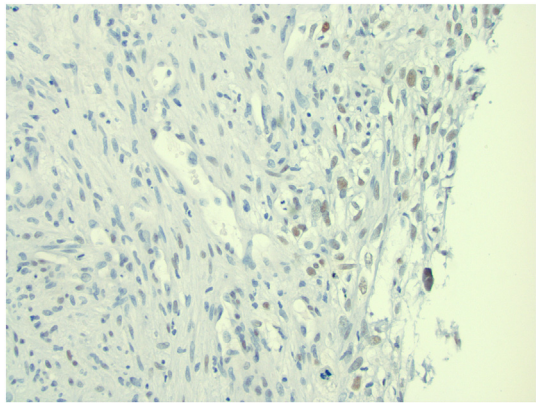

Fig. 2 Representative immunohistochemical analysis of PR in MBC with spindle cell differentiation (40%, 2+). Original magnification ×20.

Eighteen patients had material available for immunohistochemical analysis (Table 3). MAPK immunoreactivity was present in 7 (38.9%) of 18 tumors. PTEN loss was identified in 12 (66.7%) of 18 tumors. PTEN and/or MAPK abnormality were demonstrated in 14 (77.5%) of 18 tumors. PTEN and/or MAPK abnormalities, as demonstrated by immunohistochemistry, did not seem to be closely correlated with the genetic alterations in PI3K pathway detected by NGS. PD-L1 was negative in all tumors. AR immunoreactivity was seen in 2

Table 3 Summary of immunohistochemistry

Patient	AR	PD-L1	MAPK	PTEN
1	Neg (0%)	Neg (0%)	Neg (1%, 1+, H:1)	Loss (0%, H:0)
2	Neg (0%)	Neg (0%)	Neg (0%, H:0)	Loss (5%, 1+, H:5)
3	Pos (40%, 2+)	Neg (0%)	Pos ++ (90%, 2+, H:180)	Loss (0%, H:0)
4	Neg (0%)	Neg (0%)	Neg (0%, H:0)	Intact ++ (5%, 40%, 35%; 3+, 2+, 1+; H:130)
5	Neg (0%)	Neg (0%)	Pos + (15%, 2+, H:30)	Loss (5%, 1+, H:5)
6	Neg (0%)	Neg (0%)	Pos ++ (40%, 3+, H:120)	Intact + (5%, 20%; 2+, 1+; H:30)
7	Neg (0%)	Neg (0%)	Neg (3%, 2+, H:6)	Loss (0%, H:0)
8	Neg (0%)	Neg (0%)	Neg (0%, H:0)	Intact + (10%, 20%; 2+, 1+; H:40)
9	Neg (0%)	Neg (0%)	Neg (0%, H:0)	Loss (10%, 1+, H:10)
10	Neg (0%)	Neg (0%)	Neg (0%, H:0)	Loss (1%, 5%; 2+, 1+; H:7)
11	Neg (0%)	Neg (0%)	Neg (0%, H:0)	Intact + (40%, 2+, H:80)
12	Neg (0%)	Neg (0%)	Pos + (10%, 20%;3+, 2+; H:70)	Intact + (20%, 1+, H:20)
13	Pos (5%, 2+)	Neg (0%)	Pos +++ (60%, 20%;3+, 2+; H:220)	Loss (0%, H:0)
14	N/A			
15	N/A			
16	Neg (0%)	Neg (0%)	Neg (0%, H:0)	Loss (5%, 1+, H:5)
17	Neg (0%)	Neg (0%)	Pos + (30%, 2+, H:60)	Loss (0%, H:0)
18	Neg (0%)	Neg (0%)	Pos ++ (80%, 2+, H:160)	Intact + (20%, 1+, H:20)
19	Neg (0%)	Neg (0%)	Neg (0%, H:0)	Loss (0%, H:0)
20	Neg (0%)	Neg (0%)	Neg (0%, H:0)	Loss (5%, 1+, H:5)
21	N/A			

NOTE. The numbers in the parentheses for MAPK and PTEN refer to percentage of tumor cells stained; the intensity of staining; H score. MAPK positivity: +, weakly positive (H score 11-100); ++, intermediately positive (H score 101-200); +++, strongly positive (H score 201-300). Loss of PTEN: H score ≤ 10.
Abbreviations: H, H score; N/A, no material available for immunohistochemistry; Neg, negative; Pos, positive.

(11.1%) of 18 patients, one exhibiting spindle cell morphology with 40% tumor cells staining and the other demonstrating squamous morphology with 5% tumor cells staining (Fig. 2).

4. Discussion

MBC is a rare tumor with diverse morphology. The different morphologic elements in MBC are shown to be clonally related and share single lineage based on genomic characterization [2,4,7]. Using comparative genomic hybridization method in a cohort of 28 MBC patients, *PIK3CA* mutations are detected in 47% of MBC patients [7]. Six of 7 PI3K/Akt pathway phosphorylation sites are also highly phosphorylated in MBC. NGS has been applied on MBC material recently. The frequent genetic alterations identified by NGS are summarized in Table 4 [13-16]. In a study by Ross et al [13], 65% of the genetic alterations are involved in the PI3K/Akt/mTOR pathway and 60% are involved in the cell-cycle regulatory genes. A study by Edenfield et al [14] identifies 3 genetic alterations with possible therapeutic targets, *FLT3* (60%), *PIK3CA* (48%), and *ERBB4* (36%). A study by Ng et al [16] concludes that somatic mutations involved in the PI3K/AKT/mTOR pathway and Wnt pathway are enriched in MBC compared with TNBC (data from The Cancer Genome Atlas; 57% versus 22%, 51% versus 28%). PI3K/AKT/mTOR pathway and Wnt pathway activations are confirmed by RT profiler polymerase chain reaction assays in this study. In our cohort of 18 MBC patients, the most frequently mutated genes are *TP53* (56%), *PIK3CA* (39%), and *PTEN* (17%). The genetic alterations involved in the PI3K signaling pathway are identified in 50% of MBC patients. The frequency of genetic alterations in the PI3K/AKT/mTOR signaling pathway in our study is similar to that reported in the literature (Table 4). The frequent genetic alterations in TNBC reported in the literature are *TP53* mutations (62% in basal-like TNBC and 43% in non–basal-like TNBC) and mutations involved in the PI3K/AKT/mTOR signaling pathway, including *PIK3CA* mutations (10.2%) and *PTEN* mutations (7.7%). MBC seems to harbor more frequent mutations involved in the PI3K/AKT/mTOR signaling pathway compared with that of TNBC [17,18].

The morphologic diversity of MBC and the distinct clinical characteristics that distinguish this subtype of breast cancer underlie the need to study the molecular subtyping and genomic heterogeneity in MBC. The molecular subtyping mainly uses microarray-based copy number profiling and microarray-based and RNA sequencing–based gene expression profiling methods on frozen specimens [19,20]. Intrinsic molecular subtyping reveals that MBCs with spindle cell differentiation are of claudin-low subtype; MBCs with squamous and chondroid differentiation are mainly of basal-like subtype [19]. In addition, MBCs with spindle cell differentiation reveal downregulation of epithelial-to-mesenchymal transition–related genes, including reduced expression of *CDH1* and *EPCAM* genes. These findings are consistent with claudin-low intrinsic subtype [20]. TNBC molecular subtyping shows that MBCs with chondroid differentiation are of mesenchymal-like subtype. MBCs with spindle differentiation are of unstable or mesenchymal stem–like subtype. Finally, MBCs with squamous differentiation are of multiple subtypes [19]. Mutations in the

Table 4 Literature review of frequent genetic alterations in MBC [a]

Study	N	Method	*TP53*	PI3K/Akt pathway	Cell-cycle regulatory genes	Wnt pathway	Other genetic alterations
Ross et al [13]	20	NGS hybridization capture (19 genes)	75%	*PIK3CA* (40%) *PTEN* (25%)	*CDKN2A/B* (20%) *CCND3* (15%) *CCNE1* (10%)		*MYC* (30%) *MLL2* (30%) *EGFR* (10%) *KDM6A* (10%)
Edenfield et al [14]	25	Ion AmpliSeq Cancer Hotspot Panel v2 kit (50 genes)	64%	*PIK3CA* (48%)			*FLT3* (60%) *ERBB4* (36%) *KIT* (28%) *KDR* (28%) *SMAD4* (24%) *JAK3* (20%) *STK11* (20%) *CSFIR* (16%) *ATM* (12%)
Joneja et al [15]	57	Illumina TruSeq Amplicon cancer hotspot panel (45 genes)	56%	*PIK3CA* (23%)			
Ng et al [16]	35	SureSelect Human All Exo v4 platform (whole-exome sequencing)	69%	*PIK3CA* (29%) *PIK3R1* (11%) *PTEN* (11%).		*FAT1* (11%)	*ARID1A* (11%) *KMT2C* (11%)
Our data	18	Ion AmpliSeq Cancer Hotspot Panel v2 kit (50 genes)	56%	*PIK3CA* (39%) *PTEN* (17%)			

[a] Only genetic alterations with >10% frequency are included in the table.

TP53 gene are identified in all MBCs with squamous differentiation, compared with 33% of MBCs with other phenotypes. Furthermore, at the genomic level, using whole-exome sequencing, Ng et al [16] demonstrate that the different morphologic subtypes of MBC show similar mutational signature, but differ in mutational frequency of specific genes, notably the absence of *PIK3CA* mutation in MBC with chondroid differentiation. In our study, *TP53* mutations and *PIK3CA* mutations are identified in MBC with both squamous and spindle mesenchymal differentiation, the 2 frequent phenotypes in our cohort. The small number of MBC with chondroid and osseous differentiation precludes definitive conclusions about the frequency of specific mutations. More studies with a larger cohort of patients are needed to further address the heterogeneity of different subtypes of MBC at the genomic level.

The most common and consistently identified targetable genetic alterations with high frequency in MBC are involved in the PI3K/AKT/mTOR signaling pathway, which plays an important role in regulating the cell cycle and tumor development. Inhibitors of the PI3K/AKT/mTOR signaling pathway are ideal candidates for targeted therapy for MBC [5]. Currently, the most often used targeted therapies for PI3K/AKT/mTOR signaling pathway are the mTOR inhibitors such as temsirolimus and everolimus. These agents are usually given in combination therapy with bevacizumab (vascular endothelial growth factor inhibitor) and liposomal doxorubicin. In a phase I clinical trial with 12 advanced MBC patients, temsirolimus in combination with bevacizumab and liposomal doxorubicin resulted in a response rate of 42% and a clinical benefit rate of 50% [21]. In another clinical trial of a cohort of 23 metastatic MBC patients, various temsirolimus-containing regimens had a response rate of 25% and a clinical benefit rate of 33% [22]. Although only patients who received regimens with either liposomal doxorubicin plus bevacizumab (n = 18) or liposomal doxorubicin (n = 1) demonstrated response, the regimens that included temsirolimus and liposomal doxorubicin achieved better outcome with a response rate of 32% (n = 19). A larger phase I clinical trial treated 52 patients with liposomal doxorubicin, bevacizumab, and temsirolimus (n = 39) or liposomal doxorubicin, bevacizumab, and everolimus (n = 13), with an overall response rate of 21% and a clinical benefit rate of 40% [23]. In this study, 32 (74%) of 43 cancers studied using NGS harbored PI3K pathway molecular aberrations. Furthermore, clinical response was limited to patients with PI3K pathway aberrations (31% versus 0%, P = .04). Finally, clinical benefit seemed to be associated with response to the temsirolimus regimens, although the finding was not statistically significant (5.1 months versus 2.9 months, P = .35). Overall, mTOR inhibitors seem to be a promising targeted therapy for MBC, especially when PI3K pathway aberrations are identified. A larger, prospective, randomized study is necessary to further confirm treatment benefit.

In addition to PI3K inhibitors, cyclin-dependent kinase inhibitors may be potential targeted therapies for MBC based on NGS findings from the MBC specimens. Recently, CDK4/6 inhibitors have obtained accelerated Food and Drug Administration approval for the treatment of advanced ER-positive and Her2-negative luminal breast cancer [24]. ER expression remains the best marker to select patients for CDK4/6 inhibition. Furthermore, inhibitors of Wnt/β-catenin may provide potential benefit for MBC patients and are currently in preclinical development. A Wnt pathway small-molecule inhibitor, CWP232228, which antagonizes binding of β-catenin to T-cell factor in the nucleus, is demonstrated to inhibit the growth of breast cancer cell lines with stem-like cells [25].

The tumor immune microenvironment has been evaluated in MBC via immunohistochemistry for PD-L1 and programmed cell death 1 (PD-1) expression in a previous study in which the PD-L1 antibody used in this study is clone SP142 (Spring Bioscience), and the PD-1 antibody used is clone EH12.1 (BD Biosciences/Pharmingen) [15]. The PD-L1 positivity cutoff is ≥2+ intensity staining in ≥5% tumor cells. PD-1 expression (membranous staining at any intensity) in tumor-infiltrating lymphocytes is assessed by counting positively stained lymphocytes in 10 consecutive high-power fields in the tumor. PD-L1 expression is detected in 46% of MBC cases (33/72 patients), significantly higher than that of other breast cancer subtypes (6%-9% for hormone-positive, HER2-positive breast cancers and TNBC). PD-1–positive tumor-infiltrating lymphocytes vary greatly in MBC cases. However, our study fails to confirm the findings of the previous study. We did not identify PD-L1 immunoreactivity in MBC using the same antibody clone and analysis platform as described in the study led by Joneja et al [15]. It is well known that antibody clone, analysis platform, interpretation, and cutoff values are factors leading to variability of PD-L1 immunohistochemistry. The difference between the 2 studies may be attributed to the validation process and the antibody dilution, which was not specified in the published study. Approximately 20% of TNBCs have PD-L1 and PD-1 expression. Checkpoint inhibitor immunotherapy has been tested in TNBC [18]. It shows a moderate degree of activity and overall tolerability in patients with heavily pretreated metastatic TNBC. Further studies are needed to determine expression levels of PD-1 and PD-L1 in MBC as well as possible development of immunotherapy in this patient population.

Many breast cancers express AR. AR expression frequency in TNBC shows a wide range of 6.6% to 75% in the reported studies, primarily due to variability in the number of patients included and the cutoff used for AR positivity (≥1% or >10%) [26]. Antiandrogen therapy in advanced TNBC has produced promising results in clinical study. However, studies on AR expression in MBC are scarce. One previous study reported 0% AR expression in MBC, but the size of the sample group was unclear [27]. In our small cohort of 18 MBC patients, AR is detected in 2 patients (11%). MBC patients with AR expression may benefit from antiandrogen therapy.

In summary, NGS genetic profiling analysis demonstrates that PI3K pathway–related genetic alterations are detected in a high percentage of MBCs. These findings suggest that targeted therapy directed at the PI3K/mTOR pathway may be

promising for the treatment of MBC. Further prospective studies are needed to determine the role of PI3K/mTOR-targeted therapies in MBC.

References

[1] Gibson GR, Qian D, Ku JK, Lai L. Metaplastic breast cancer: clinical features and outcomes. Am Surg 2005;71:725-30.

[2] Reis-Filho JS, Lakhani SR, Gobbi H, Sneige N. Metaplastic carcinoma. In: Lakhani SR, Ellis IO, Schnitt SJ, Tan PH, van de Vijver MJ, editors. WHO Classification of Tumors of the Breast. Lyon: IARC; 2012. p. 48-52.

[3] Nelson RA, Guye ML, Luu T, Lai LL. Survival outcomes of metaplastic breast cancer patients: results from a US population–based analysis. Ann Surg Oncol 2015;22:24-31.

[4] Abouharb S, Moulder S. Metaplastic breast cancer: clinical overview and molecular aberrations for potential targeted therapy. Curr Oncol Rep 2015;17:431.

[5] Tzanninis IG, Kotteas EA, Ntanasis-Stathopoulos I, Kontogianni P, Fotopoulos G. Management and outcomes in metaplastic breast cancer. Clin Breast Cancer 2016;16:437-43.

[6] Pezzi CM, Patel-Parekh L, Cole K, Franko J, Klimberg VS, Bland K. Characteristics and treatment of metaplastic breast cancer: analysis of 892 cases from the National Cancer Data Base. Ann Surg Oncol 2007; 14:166-73.

[7] Hennessy BT, Gonzalez-Angulo AM, Stemke-Hale K, et al. Characterization of a naturally occurring breast cancer subset enriched in epithelial-to-mesenchymal transition and stem cell characteristics. Cancer Res 2009;69:4116-24.

[8] Oon ML, Thike AA, Tan SY, Tan PH. Cancer stem cell and epithelial-mesenchymal transition markers predict worse outcome in metaplastic carcinoma of the breast. Breast Cancer Res Treat 2015;150:31-41.

[9] de Beça FF, Caetano P, Gerhard R, et al. Cancer stem cells markers CD44, CD24 and ALDH1 in breast cancer special histological types. J Clin Pathol 2013;66:187-91.

[10] Lehmann BD, Bauer JA, Chen X, et al. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. J Clin Invest 2011;121:2750-67.

[11] Dupont Jensen J, Laenkholm AV, Knoop A, et al. PIK3CA mutations may be discordant between primary and corresponding metastatic disease in breast cancer. Clin Cancer Res 2011;17:667-77.

[12] Cossu-Rocca P, Orrù S, Muroni MR, et al. Analysis of PIK3CA mutations and activation pathways in triple negative breast cancer. PLoS One 2015;10:e0141763.

[13] Ross JS, Badve S, Wang K, et al. Genomic profiling of advanced-stage, metaplastic breast carcinoma by next-generation sequencing reveals frequent, targetable genomic abnormalities and potential new treatment options. Arch Pathol Lab Med 2015;139:642-9.

[14] Edenfield J, Schammel C, Collins J, Schammel D, Edenfield WJ. Metaplastic breast bancer: molecular typing and identification of potential targeted therapies at a single institution. Clin Breast Cancer 2017;17:e1-10.

[15] Joneja U, Vranic S, Swensen J, et al. Comprehensive profiling of metaplastic breast carcinomas reveals frequent overexpression of programmed death-ligand 1. J Clin Pathol 2017;70:255-9.

[16] Ng CKY, Piscuoglio S, Geyer FC, et al. The landscape of somatic genetic alterations in metaplastic breast carcinomas. Clin Cancer Res 2017;23: 3859-70.

[17] Shah SP, Roth A, Goya R, et al. The clonal and mutational evolution spectrum of primary triple-negative breast cancers. Nature 2012;486: 395-9.

[18] Hurvitz S, Mead M. Triple-negative breast cancer: advancements in characterization and treatment approach. Curr Opin Obstet Gynecol 2016;28: 59-69.

[19] Weigelt B, Ng CK, Shen R, et al. Metaplastic breast carcinomas display genomic and transcriptomic heterogeneity. Mod Pathol 2015;28:340-51.

[20] Piscuoglio S, Ng CKY, Geyer FC, et al. Genomic and transcriptomic heterogeneity in metaplastic carcinomas of the breast. NPJ Breast Cancer 2017;3:48.

[21] Moroney J, Fu S, Moulder S, et al. Phase I study of the antiangiogenic antibody bevacizumab and the mTOR/hypoxia-inducible factor inhibitor temsirolimus combined with liposomal doxorubicin: tolerance and biological activity. Clin Cancer Res 2012;18:5796-805.

[22] Moulder S, Helgason T, Janku F, et al. Inhibition of the phosphoinositide 3-kinase pathway for the treatment of patients with metastatic metaplastic breast cancer. Ann Oncol 2015;26:1346-52.

[23] Basho RK, Gilcrease M, Murthy RK, et al. Targeting the PI3K/AKT/mTOR pathway for the treatment of mesenchymal triple-negative breast cancer: evidence from a phase 1 trial of mTOR inhibition in combination with liposomal doxorubicin and bevacizumab. JAMA Oncol 2017;3: 509-15.

[24] Echavarria I, Jerez Y, Martin M, López-Tarruella S. Incorporating CDK4/6 inhibitors in the treatment of advanced luminal breast cancer. Breast Care (Basel) 2017;12:296-302.

[25] Jang GB, Hong IS, Kim RJ, et al. Wnt/β-catenin small-molecule inhibitor CWP232228 preferentially inhibits the growth of breast cancer stem–like cells. Cancer Res 2015;75:1691-702.

[26] Rampurwala M, Wisinski KB, O'Regan R. Role of the androgen receptor in triple-negative breast cancer. Clin Adv Hematol Oncol 2016;14: 186-93.

[27] Park S, Koo J, Park HS, et al. Expression of androgen receptors in primary breast cancer. Ann Oncol 2010;21:488-92.