Molecular Classification of Metaplastic Carcinoma Using Surrogate Immunohistochemical Staining

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Key Words

Breast · Metaplastic carcinoma · Molecular subtypes · Immunohistochemistry

Abstract

Objective: The purpose of this study is to investigate molecular subtyping and its implications on metaplastic carcinoma according to surrogate immunohistochemical (IHC) staining. **Methods:** Following tissue microarray analysis of 34 cases of metaplastic carcinoma, IHC staining for cytokeratin (CK) 5/6, epidermal growth factor receptor (EGFR), claudin-3, claudin-4, claudin-7, E-cadherin, STAT-1, androgen receptor and GGT was performed and classified into basal-like, molecular apocrine, claudin-low, immune-related, mixed and null types. **Results:** Among the 34 cases of metaplastic carcinoma, 13 were of the basal-like type (35.2%), 9 of the mixed type (26.5%), 8 of the null type (23.5%), 3 of the claudin-low type (8.8%), and 1 was of the molecular apocrine type (2.9%). Depending on the cell type, there were differences between molecular subtypes, with the matrix-producing type occupying the largest proportion in the basal-like, null and mixed types. The spindle cell type represented the largest proportion in the claudin-low and molecular apocrine types, and the squamous cell type characterized the largest proportion in the basal-like type. **Conclusion:** Following molecular sub-

typing of metaplastic carcinomas using surrogate IHC markers, the largest number of cases was of the basal-like type, followed by the mixed, null, claudin-low and molecular apocrine types. There were differences between molecular subtypes according to the cell type.

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Introduction

Metaplastic carcinoma, which occurs in 0.2–5% of all breast cancers, represents a tumor with neoplastic epithelium differentiating into squamous cell and/or mesenchymal-appearing elements [1, 2]. It shows extensive variability in histologic features, and the World Health Organization (WHO) classification defines metaplastic carcinoma as a heterogeneous tumor that includes several different histologic subtypes, including low-grade adenosquamous carcinoma, fibromatosis-like metaplastic carcinoma, squamous cell carcinoma, carcinoma with mesenchymal differentiation and myoepithelial carcinoma [3]. Metaplastic carcinoma exhibits triple-negative breast cancer [TNBC; estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER-2) negative] in over 90% of cases [4].

Table 1. Clone, dilution and source of the antibodies used

Antibody	Clone	Dilution	Company
ER	SP1	1:100	Thermo Scientific, San Jose, Calif., USA
PR	PgR	1:50	DAKO, Glostrup, Denmark
HER-2	Polyclonal	1:1,500	DAKO, Glostrup, Denmark
Basal-like related			
CK5/6	D5/16B4	1:50	DAKO, Glostrup, Denmark
EGFR	EGFR.25	1:50	Novocastra, Newcastle, UK
Claudin-low related			
Claudin-3	Polyclonal	1:50	Abcam, Cambridge, UK
Claudin-4	Polyclonal	1:100	Abcam, Cambridge, UK
Claudin-7	Polyclonal	1:100	Abcam, Cambridge, UK
E-cadherin	36B5	1:100	Novocastra, Newcastle, UK
Molecular apocrine related			
AR	AR441	1:50	DAKO, Glostrup, Denmark
GGT-1	IgG2A	1:50	Abcam, Cambridge, UK
Immune related			
STAT1	807	1:50	Abcam, Cambridge, UK
CD20	L26	1:100	Abcam, Cambridge, UK
CD3	F 7.2.38	1:1,000	DAKO, Glostrup, Denmark
Proliferation related			
Ki-67	MIB-1	1:150	DAKO, Glostrup, Denmark

Table 2. IHC expression in patients with metaplastic carcinoma (n = 34)

Antibody		Patients, n (%)
ER	Negative	31 (91.2)
	Positive	3 (8.8)
PR	Negative	33 (97.1)
	Positive	1 (2.9)
HER-2	Negative (0, 1+)	33 (97.1)
	Equivocal (2+)	1
CK5/6	Negative	15 (44.1)
	Positive	19 (55.9)
EGFR	Negative	16 (47.1)
	Positive	18 (52.9)
Claudin-3	Negative	21 (61.8)
	Positive	13 (38.2)
Claudin-4	Negative	19 (55.9)
	Positive	15 (44.1)
Claudin-7	Negative	29 (85.3)
	Positive	5 (14.7)
E-cadherin	Negative	7 (20.6)
	Positive	27 (79.4)
AR	Negative	32 (94.1)
	Positive	2 (5.9)
GGT-1	Negative	33 (97.1)
	Positive	1 (2.9)
Stromal STAT-1	Negative	29 (85.3)
	Positive	5 (14.7)

TNBC serves as a breast cancer subtype with negative expression of ER, PR and HER-2 in approximately 20% of all breast cancers [5]. TNBC also represents a heterogeneous tumor with the following molecular subtypes: basal-like, claudin-low, molecular apocrine and immune-related (interferon-rich) types, for example [6]. Previous studies on tissue microarray (TMA)-based gene expression profiling reported that most metaplastic carcinomas correspond to the basal-like type [7, 8]. Within a subgroup of metaplastic carcinoma, spindle cell carcinoma reportedly corresponds to the claudin-low type [9, 10]. Based on metaplastic carcinoma subgroups, different molecular profiles are expected.

The purpose of this study is to investigate molecular subtyping and its clinical implications assessed by surrogate immunohistochemical (IHC) staining of metaplastic carcinoma samples.

Materials and Methods

Patient Selection and Clinicopathological Analysis

Patients with breast cancer who underwent surgery at the Severance Hospital from January 2000 to December 2012 and had a diagnosis of metaplastic carcinoma were included in the study. Patients who received hormonal therapy or chemoradiation prior to surgery were excluded. In total, 34 cases were recruited. All archival hematoxylin and eosin (HE)-stained slides for each case were reviewed by 2 pathologists (J.S.K. and W.H.J.). Histological grade was assessed based on the Nottingham grading system [11]. Tumor staging was based on the criteria of the 7th American Joint Committee on Cancer. Based on histopathological findings, the presence of the following parameters was examined: tumor margin (infiltrative or expanding), central necrotic zone, central fibrotic zone, lymphocytic infiltration and dominant cell type (matrix-producing, spindle and squamous cells). Clinical parameters included patient age at initial diagnosis, lymph node status, local/systemic recurrence and survival. This study was approved by the Institutional Review Board of the Severance Hospital.

Tissue Microarray

After reviewing HE-stained slides, the most suitable formalin-fixed paraffin-embedded tumor tissue samples were collected retrospectively. The most representative tumor area was marked on these tumor tissue samples, and 5-mm cores were extracted from the selected area using a punch machine and then placed onto a 5 × 4 recipient block. TMA was constructed with two tissue cores for all cases.

Immunohistochemistry

Antibodies used for IHC are shown in table 1. Formalin-fixed paraffin-embedded tissue sections were cut at 3 µm and mounted on silane-coated slides. Sections were then deparaffinized and rehydrated using xylene and alcohol solutions. Slides were preprocessed in a microwave oven for antigen retrieval and primary antibodies were added. Following incubation with the primary

Fig. 1. IHC expression in metaplastic carcinoma. The basal-like type shows squamous cell histology and intense expression of CK5/6, EGFR, claudin-4 and E-cadherin. The claudin-low type demonstrates spindle cell histology and no expression of CK5/6, EGFR, claudin-4 and E-cadherin. The null type exhibits negative

CK5/6, EGFR and AR expression along with positive expression of claudin-4 and E-cadherin, suggesting that this profile does not belong to any type. The mixed type was positive for CK5/6 and EGFR as well as negative for claudin-4 and E-cadherin, revealing characteristics of both the basal-like and claudin-low types.

antibodies, immunodetection was performed with biotinylated anti-mouse immunoglobulin, followed by peroxidase-labeled streptavidin using a labeled streptavidin biotin kit with 3,3'-diaminobenzidine chromogen as the substrate. Optimal primary antibody incubation time and concentration were determined via serial dilutions for each IHC assay with an identically fixed and embedded tissue block. Slides were counterstained with Harris hematoxylin. Staining was interpreted by 2 pathologists (J.S.K. and W.H.J.) under a multiview microscope.

Interpretation of IHC Staining

ER and PR IHC signals were deemed positive when >1% of invasive tumor cells were expressed [12]. HER-2 staining was scored according to the guidelines of the American Society of Clinical Oncology/College of American Pathologists into the following categories [13]: 0 (no immunostaining), 1+ (weak or incomplete

membranous staining on any portion of tumor cells), 2+ (complete membranous staining with either nonuniform or weak staining in $\geq 10\%$ of tumor cells) and 3+ (uniform intense membranous staining in $>30\%$ of tumor cells). Cases scored as 0–1+ were considered negative, cases scored as 2+ were deemed equivocal, and cases scored as 3+ were classified as positive.

Scoring of immunostained slides was performed according to the percentage of tumor cells exhibiting nuclear [androgen receptor (AR)], cytoplasmic [GGT-1, cytokeratin (CK) 5/6, IL-8 and STAT-1] and membranous [EGFR, claudin-3, claudin-4, claudin-7, CD3, CD20 and E-cadherin] staining. IHC results for CK5/6, AR, claudin-3, claudin-4, claudin-7, E-cadherin, IL-8, STAT-1 and GGT-1 were considered positive when $>10\%$ of tumor or stromal cells were stained. IHC results for EGFR were classified into negative, weak, moderate and intense expression groups. Cases with moderate or intense expression were classified as posi-

Table 3. Clinicopathological features according to the molecular subtype of metaplastic carcinoma

Parameters	Total (n = 34)	Basal-like (n = 13)	Claudin-low (n = 3)	Molecular apocrine (n = 1)	Null (n = 8)	Mixed (n = 9)	p value
Age, years	51.6±10.3	52.2±9.8	53.3±11.9	47.0±0.0	51.0±11.6	51.4±11.6	0.988
Histologic grade							0.472
II	10 (29.4)	4 (30.8)	1 (33.3)	0 (0.0)	4 (50.0)	1 (11.1)	
III	24 (70.6)	9 (69.2)	2 (66.7)	1 (100.0)	4 (50.0)	8 (88.9)	
Tumor stage							0.444
T1	10 (29.4)	4 (30.8)	2 (66.7)	0 (0.0)	3 (37.5)	1 (11.1)	
T2	16 (47.1)	7 (53.8)	0 (0.0)	1 (100.0)	4 (50.0)	4 (44.4)	
T3	8 (23.5)	2 (15.4)	1 (33.3)	0 (0.0)	1 (12.5)	4 (44.4)	
Nodal metastasis							0.975
Absent	26 (76.5)	10 (76.9)	2 (66.7)	1 (100.0)	6 (75.0)	7 (77.8)	
Present	8 (23.5)	3 (23.1)	1 (33.3)	0 (0.0)	2 (25.0)	2 (22.2)	
Cell type							0.033
Matrix producing	22 (64.7)	7 (53.8)	1 (33.3)	0 (0.0)	7 (87.5)	7 (77.8)	
Spindle cell	6 (17.6)	1 (7.7)	2 (66.7)	1 (100.0)	1 (0.0)	1 (11.1)	
Squamous cell	6 (17.6)	5 (38.5)	0 (0.0)	0 (0.0)	0 (0.0)	1 (11.1)	
Lymphocytic infiltration							0.608
Absent	11 (32.4)	3 (23.1)	1 (33.3)	1 (100.0)	3 (37.5)	3 (33.3)	
Present	23 (67.6)	10 (76.9)	2 (66.7)	0 (0.0)	5 (62.5)	6 (66.7)	
Tumor margin							0.347
Expanding	24 (70.6)	8 (61.5)	3 (100.0)	0 (0.0)	6 (75.0)	7 (77.8)	
Infiltrative	10 (29.4)	5 (38.5)	0 (0.0)	1 (100.0)	2 (25.0)	2 (22.2)	
Central fibrosis							0.866
Absent	32 (94.1)	12 (92.3)	3 (100.0)	1 (100.0)	8 (100.0)	8 (88.9)	
Present	2 (5.9)	1 (7.7)	0 (0.0)	0 (0.0)	0 (0.0)	1 (11.1)	
Central necrosis							0.074
Absent	26 (76.5)	11 (84.6)	2 (66.7)	1 (100.0)	8 (100.0)	4 (44.4)	
Present	8 (23.5)	2 (15.4)	1 (33.3)	0 (0.0)	0 (0.0)	5 (55.6)	
Distant metastasis							0.384
4 (11.8)	4 (11.8)	0 (0.0)	1 (33.3)	0 (0.0)	1 (12.5)	2 (22.2)	
Ki-67 LI, %	42.9±21.2	43.4±20.7	41.6±27.5	15.0±0.0	35.0±12.5	52.7±24.8	0.319
CD20 index	1.6±3.9	1.3±2.6	0.3±0.5	0.0±0.0	3.1±6.9	1.3±2.6	0.803
CD3 index	5.9±8.7	4.3±5.7	1.6±0.5	1.6±0.5	3.6±6.6	12.5±12.4	0.108

Numbers (%) are given except for the labeling index (LI) and age (means ± SD).

tive. The CD20 and CD3 indices were defined as the percent area with CD20-/CD3-positive lymphocyte infiltration in the tumor area and surrounding stroma. We examined the entire TMA core field when evaluating the CD20 and CD3 indices. IHC staining for Ki-67 was scored by counting the number of positively stained nuclei and then expressed as a percentage of total tumor cells (Ki-67 labeling index).

Molecular Classification of Metaplastic Carcinoma according to IHC

According to IHC results, TNBCs were subclassified into 6 subtypes: basal-like type (CK5/6 positive and/or EGFR positive), molecular apocrine type (AR positive and/or GGT-1 positive), claudin-low type (claudin-3, claudin-4 and claudin-7 negative and/or E-cadherin negative), immune-related type (stromal STAT positive), mixed type (>2 types mixed) and null type (not belonging to any other subtype).

Statistical Analysis

Data were analyzed using SPSS for Windows, version 12.0 (SPSS Inc., Chicago, Ill., USA). To determine statistical significance, Student's *t* and χ^2 tests were used for continuous and categorical variables, respectively. Statistical significance was defined as *p* < 0.05.

Results

Clinicopathological Characteristics of Metaplastic Carcinoma

In total, 34 cases of metaplastic carcinoma were included in the study, and 24 of the 34 cases (70.6%) demonstrated histologic grade III, and 8 cases (23.5%) pre-

Table 4. Clinicopathological features between basal-like-mixed types and other types of metaplastic carcinoma

Parameters	Total (n = 34)	Basal-like + mixed type (n = 22)	Other types (n = 12)	p value
Age, years	51.6±10.3	51.9±10.3	51.2±10.7	0.862
Histological grade				0.271
II	10 (29.4)	5 (22.7)	5 (41.7)	
III	24 (70.6)	17 (77.3)	7 (58.3)	
Tumor stage				0.489
T1	10 (29.4)	5 (22.7)	5 (41.7)	
T2	16 (47.1)	11 (50.0)	5 (41.7)	
T3	8 (23.5)	6 (27.3)	2 (16.7)	
Nodal metastasis				1.000
Absent	26 (76.5)	17 (77.3)	9 (75.0)	
Present	8 (23.5)	5 (22.7)	3 (25.0)	
Cell type				0.053
Matrix producing	23 (67.6)	14 (63.6)	8 (66.7)	
Spindle cell	5 (14.7)	2 (9.1)	4 (33.3)	
Squamous cell	6 (17.6)	6 (27.3)	0 (0.0)	
Lymphocytic infiltration				0.459
Absent	11 (32.4)	6 (27.3)	5 (41.7)	
Present	23 (67.6)	16 (72.7)	7 (58.3)	
Tumor margin				1.000
Expanding	24 (70.6)	15 (68.2)	9 (75.0)	
Infiltrative	10 (29.4)	7 (31.8)	3 (25.0)	
Central fibrosis				0.529
Absent	32 (94.1)	20 (90.9)	12 (100.0)	
Present	2 (5.9)	2 (9.1)	0 (0.0)	
Central necrosis				0.210
Absent	26 (76.5)	15 (68.2)	11 (91.7)	
Present	8 (23.5)	7 (31.8)	1 (8.3)	
Distant metastasis	4 (11.8)	2 (9.1)	2 (16.7)	0.602
Ki-67 LI, %	42.9±21.2	47.2±22.4	35.0±16.9	0.109
CD20 index	1.6±3.9	1.3±2.6	2.1±5.7	0.578
CD3 index	5.9±8.7	7.6±9.7	2.8±5.4	0.123

Numbers (%) are given except for the labeling index (LI) and age (means ± SD).

sented with lymph node metastases. Cell type distribution showed the matrix-producing type in 22 cases (64.7%), spindle cell type in 6 cases (17.6%) and squamous cell type in 6 cases (17.6%). Distant metastases were observed in 4 cases (11.8%).

Molecular Subclassification of Metaplastic Carcinoma by Surrogate IHC

Results of surrogate IHC staining for molecular subtyping of metaplastic carcinomas are shown in figure 1, and tables 2 and 3. Based on the metaplastic carcinoma classification with surrogate IHC markers, 13 were of the

Table 5. Correlation between cell type and IHC markers

Markers	Cell type			p value
	Matrix producing (n = 23)	Spindle cell (n = 5)	Squamous cell (n = 6)	
CK5/6				0.024
Negative	11 (47.8)	4 (80.0)	0 (0.0)	
Positive	12 (52.2)	1 (20.0)	6 (100.0)	
EGFR				0.039
Negative	13 (56.5)	3 (60.0)	0 (0.0)	
Positive	10 (43.5)	2 (40.0)	6 (100.0)	
Claudin-3				0.554
Negative	15 (65.2)	2 (40.0)	4 (66.7)	
Positive	8 (34.8)	3 (60.0)	2 (33.3)	
Claudin-4				0.101
Negative	15 (65.2)	3 (60.0)	1 (16.7)	
Positive	8 (34.8)	2 (40.0)	5 (83.3)	
Claudin-7				0.018
Negative	22 (95.7)	4 (80.0)	3 (50.0)	
Positive	1 (4.3)	1 (20.0)	3 (50.0)	
E-cadherin				0.256
Negative	5 (21.7)	2 (40.0)	0 (0.0)	
Positive	18 (78.3)	3 (60.0)	6 (100.0)	
AR				0.106
Negative	23 (100.0)	4 (80.0)	5 (83.3)	
Positive	0 (0.0)	1 (20.0)	1 (16.7)	
GGT-1				0.090
Negative	23 (100.0)	5 (100.0)	5 (83.3)	
Positive	0 (0.0)	0 (0.0)	1 (16.7)	
Stromal STAT-1				0.603
Negative	19 (82.6)	5 (100.0)	5 (83.3)	
Positive	4 (17.4)	0 (0.0)	1 (16.7)	

Numbers (%) are shown.

basal-like type (35.2%), 9 were of the mixed type (26.5%), 8 were of the null type (23.5%), 3 were of the claudin-low type (8.8%) and 1 was of the molecular apocrine type (2.9%). Among the 9 cases of the mixed type, we observed basal-like + claudin-low types in 4 cases, basal-like + immune types in 4 cases and basal-like + immune + apocrine types in 1 case. Depending on the cell type, there were significant differences between molecular subtypes, with the matrix-producing type constituting the largest proportion in basal-like, null and mixed types. The spindle cell type occupied the largest proportion in the claudin-low and molecular apocrine types. The squamous cell type exhibited the largest proportion in the basal-like type ($p = 0.033$). Classifying metaplastic carcinomas into basal-like + mixed types and other types, there was no significant difference between the two types with respect to clinicopathological parameters (table 4).

Table 6. Correlation between clinicopathological factors and the expression of E-cadherin/claudin proteins

Parameters	E-cadherin			Claudin-3			Claudin-4			Claudin-7		
	negative (n = 7)	positive (n = 27)	p value	negative (n = 21)	positive (n = 13)	p value	negative (n = 19)	positive (n = 15)	p value	negative (n = 29)	positive (n = 5)	p value
Histological grade			0.324			0.524			0.656			0.617
II	1 (14.3)	9 (33.3)		7 (33.3)	3 (23.1)		5 (26.3)	5 (33.3)		9 (31.0)	1 (20.0)	
III	6 (85.7)	18 (66.7)		14 (66.7)	10 (76.9)		14 (73.7)	10 (66.7)		20 (69.0)	4 (80.0)	
Tumor stage			0.044			0.403			0.913			0.271
T1	2 (28.6)	8 (29.6)		7 (33.3)	3 (23.1)		6 (31.6)	4 (26.7)		10 (34.5)	0 (0.0)	
T2	1 (14.3)	15 (55.6)		8 (38.1)	8 (61.5)		9 (47.4)	7 (46.7)		13 (44.8)	3 (60.0)	
T3	4 (57.1)	4 (14.8)		6 (28.6)	2 (15.4)		4 (21.1)	4 (26.7)		6 (20.7)	2 (40.0)	
Nodal metastasis			0.724			0.378			0.039			0.179
Absent	5 (71.4)	21 (77.8)		15 (71.4)	11 (84.6)		12 (63.2)	14 (93.3)		21 (72.4)	5 (100.0)	
Present	2 (28.6)	6 (22.2)		6 (28.6)	2 (15.4)		7 (36.8)	1 (6.7)		8 (27.6)	0 (0.0)	
Lymphocytic infiltration			0.116			0.549			0.914			0.692
Absent	4 (57.1)	7 (25.9)		6 (28.6)	5 (38.5)		6 (31.6)	5 (33.3)		9 (31.0)	2 (40.0)	
Present	3 (42.9)	20 (74.1)		15 (71.4)	8 (61.5)		13 (68.4)	10 (66.7)		20 (69.0)	3 (60.0)	
Tumor margin			0.956			0.891			0.656			0.104
Expanding	5 (71.4)	19 (70.4)		15 (71.4)	9 (69.2)		14 (73.7)	10 (66.7)		22 (75.9)	2 (40.0)	
Infiltrative	2 (28.6)	8 (29.6)		6 (28.6)	4 (30.8)		5 (26.3)	5 (33.3)		7 (24.1)	3 (60.0)	
Central fibrosis			0.458			0.724			0.863			0.545
Absent	7 (100.0)	25 (92.6)		20 (95.2)	12 (92.3)		18 (94.7)	14 (93.3)		27 (93.1)	5 (100.0)	
Present	0 (0.0)	2 (7.4)		1 (4.8)	1 (7.7)		1 (5.3)	1 (6.7)		2 (6.9)	0 (0.0)	
Central necrosis			0.019			0.087			0.039			0.179
Absent	3 (42.9)	23 (85.2)		14 (66.7)	12 (92.3)		12 (63.2)	14 (93.3)		21 (72.4)	5 (100.0)	
Present	4 (57.1)	4 (14.8)		7 (33.3)	1 (7.7)		7 (36.8)	1 (6.7)		8 (27.6)	0 (0.0)	
Distant metastasis			0.004			0.562			0.412			0.377
Absent	4 (57.1)	26 (96.3)		18 (85.7)	12 (92.3)		16 (84.2)	14 (93.3)		25 (86.2)	5 (100.0)	
Present	3 (42.9)	1 (3.7)		3 (14.3)	1 (7.7)		3 (15.8)	1 (6.7)		4 (13.8)	0 (0.0)	

Numbers (%) are shown.

Clinicopathological Characteristics according to the Metaplastic Carcinoma Subtype

First, we correlated cell types with surrogate IHC marker status. CK5/6, EGFR and claudin-7 expression are associated with the cell type (table 5). Expression of CK5/6 and EGFR in the squamous cell type is higher than in the spindle cell type ($p = 0.024$ and $p = 0.039$, respectively); however, claudin-7 expression in the squamous cell type is higher than in the matrix-producing type or spindle cell type ($p = 0.018$). We analyzed clinicopathological parameters according to surrogate IHC marker status. Results showed significant correlations between the following parameters: T stage and E-cadherin status ($p = 0.044$), central necrosis and E-cadherin status ($p = 0.019$), distant metastases and E-cadherin status ($p = 0.004$), lymph node metastases and claudin-4 status ($p = 0.039$), and central necrosis and claudin-4 status ($p = 0.039$; table 6).

Discussion

In this study, we investigated the molecular subtype of metaplastic carcinoma of the breast using surrogate IHC. In our sample, 64.7% of metaplastic carcinoma samples were of the basal-like type. However, a previous study has reported up to 91% of metaplastic carcinoma samples belong to the basal-like type based on surrogate IHC, demonstrating that the basal-like type was less frequent in this study [14]. One of the most important reasons for inconsistent proportions is that there is no consensus on the definition of the basal-like type. Strictly, the basal-like type should be defined using gene profiling analysis, which is complicated to apply in clinical practice. Therefore, the surrogate IHC marker panels are recommended since they in turn result in various definitions of basal-like type [15–17]. These include: (1) lack of ER, PR and HER2 expression ('triple-negative' immunophenotype);

Fig. 2. Representative histology of the matrix-producing type in basal-like and null types of metaplastic carcinoma, which is mainly composed of the matrix and cell components, respectively.

(2) expression of one or more high-molecular-weight/basal CKs (CK5/6, CK14 and CK17); (3) lack of expression of ER and HER2 in conjunction with expression of CK5/6 and/or EGFR, and (4) lack of expression of ER, PR and HER2 in conjunction with expression of CK5/6 and/or EGFR. Among these, there is no consensus on positive criteria for each basal marker. If our study had also applied the basal marker CK14 and CK17, the proportion of the basal-like type would have increased. Compared to prior studies, which analyzed whole tissue slides, this study evaluated TMA composed of limited tumor tissues; the difference in the size of tissues may have resulted in the lower proportion of the basal-like type [14]. This is one of the limitations of this study.

Molecular subtyping of metaplastic carcinomas demonstrated the following distribution: basal-like type (38.2%), mixed type (26.5%), null type (23.5%), claudin-low type (8.8%) and molecular apocrine type (2.9%). In

earlier reports that used surrogate IHC of TNBC, 26.2% were of the null type, 23.0% of the claudin-low type, 22.1% of the basal-like type, 18.9% of the mixed type and 9.8% of the molecular apocrine type, with significant differences between molecular subtype distributions [18]. The ratio of the basal-like and mixed types was higher in metaplastic carcinoma samples, and the proportion of the claudin-low and molecular apocrine types were higher in TNBC. As of now, >90% metaplastic carcinoma samples have been identified as basal-like type using gene profiling and surrogate IHC. Such data correspond with the results from this study [8, 14]. Higher proportions of the mixed type, a combination of several molecular subtypes, may be explained by the variable histological components of metaplastic carcinoma, but their exact classification requires further study. For example, the molecular apocrine type with apocrine morphology is characterized by ER negativity, AR positivity and HER-2

overexpression/amplification [19], features that are distinct from metaplastic carcinoma. Therefore, it would be expected that the molecular apocrine type would not often be seen with metaplastic carcinoma, which was confirmed by our results.

Depending on the metaplastic carcinoma cell type, differences between molecular subtypes were observed. The matrix-producing cell type occupied the largest proportion in the basal-like, null and mixed types. The spindle cell type constituted the largest proportion in the claudin-low and molecular apocrine types. The squamous cell type exhibited the largest proportion in the basal-like type. Since no prior study has classified metaplastic carcinoma according to the TNBC subtype, comparison is challenging. The high proportion of the claudin-low type in spindle cell morphology may be due to the characteristically low expression of claudin-3, claudin-4, claudin-7 and E-cadherin, which are involved in epithelial cell adhesion and EMT (epithelial-to-mesenchymal transition)-related molecule expression [10]. The relationship between squamous cell and basal-like types has been demonstrated previously, and evidence indicates that breast cancer with squamous metaplasia resides on a tumor spectrum of basal/myoepithelial differentiation. First, histological, IHC and ultrastructural transitions exist between myoepithelial and squamous cells [20, 21]. Second, when cultured in specific media with squamous cells, myoepithelial cells but not luminal cells demonstrate squamous cell metaplasia [22]. In our study, the squamous cell type was not present in the non-basal-like type, so the relationship between squamous cell and basal-like types appears high.

Several markers were associated with the cell type, which was investigated in this study using surrogate IHC. CK5/6 and EGFR were expressed in the squamous cell type, and the basal-like and squamous cell types are related since CK5/6 and EGFR are basal markers. Additionally, in most instances, claudin-7 expression did not appear in the spindle cell or matrix-producing types. Prior studies reported low claudin expression in the mesenchymal component of metaplastic carcinoma. Our study showed that matrix-producing and spindle cells had lower expression of claudin than squamous cells, a finding that corresponds with earlier work [23]. The low expression of claudin in matrix-producing and spindle cells, which are of nonepithelial morphology, can be explained the participation of claudin in epithelial cell adhesion. Loss of E-cadherin in this study was associated with distant metastases, which is in agreement with prior studies that demonstrated an associa-

tion between low E-cadherin expression and metastases in basal-like carcinoma [24]. Our results revealed that a low claudin-4 expression correlated with a high rate of lymph node metastases. However, this is in contrast to other studies which found that high claudin-4 expression was associated with a poor prognosis in breast cancer [25]. Differences in study findings may be due to subject selection, as in our study only patients with metaplastic carcinoma were investigated.

The null type amounted to 23.5% in this study, which is in accord with a prior study on the molecular subtypes of TNBC, which reported 26.2% [18]. The first possible interpretation for the null type is that other subtypes, such as the basal-like type, are incorrectly categorized due to insufficient tissue evaluation and surrogate IHC staining. Second, the authentic null type does not belong to any other subtype already specified. In this study, there are no differences in the clinicopathological characteristics among the null and other types except for the cell type. Compared with the 14 matrix-producing cases of basal-like and null types, the 7 cases of the basal-like type and the 3 cases of the null-like type (42.9%) contained more cell components than the matrix component. However, the 4 cases of the null type (57.1%) contained more matrix than cell components (fig. 2; $p = 0.018$). To clarify the reason for this discrepancy, further studies are needed.

In summary, through the classification of metaplastic carcinoma into molecular subtypes with surrogate IHC markers, the basal-like type had the largest number of cases, followed by the mixed, null, claudin-low and molecular apocrine types. There were significant differences between molecular subtypes according to the cell type.

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Disclosure Statement

The authors declare no conflict of interest.

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