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Cancer Subtypes of Breast Carcinoma with Micropapillary and Mucinous Component Based on Immunohistochemical Profile

Sun Young Min · Eun-Jung Jung
Hyesil Seol · In Ae Park

Department of Pathology, Seoul National University College of Medicine, Seoul, Korea

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Corresponding Author
In Ae Park, M.D.
Department of Pathology, Seoul National University College of Medicine, 28 Yeongeon-dong, Jongno-gu, Seoul 110-744, Korea
Tel: +82-2-2072-2930
Fax: +82-2-743-5530
E-mail: iapark@snu.ac.kr

Background: Micropapillary carcinoma (MPC) is known to have a worse prognosis than the other subtypes of breast cancer. Occasionally, MPC is observed in association with invasive ductal carcinoma not otherwise specified (IDC NOS), as well as mucinous carcinoma. **Methods:** We examined the immunohistochemical expression of an estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 (HER2) in 127 cases of surgically resected MPC or IDC NOS with MPC. Further, we classified these cases based on their immunohistochemical profile. **Results:** Among the IDC NOS with MPC cases, 47 were luminal A (62.7%), 10 were luminal B (13.3%), and 9 were HER2 (12.0%). The MPC cases included 4 luminal A (50.0%), 2 luminal B (25.0%) and 1 HER2 (12.5%) subtypes. Of the mucinous carcinomas with MPC, 4 were grouped as luminal A (57.1%), 1 as luminal B (14.3%), and 2 as HER2 (28.6%) subtypes. However, among the mucinous carcinomas, 33 were categorized as luminal A (89.2%), 3 as luminal B (8.1%), and 1 as HER2 (2.7%) subtype, indicating a low incidence of HER2 subtype as compared to the other subtypes. **Conclusions:** The luminal B and HER2 subtypes were prevalent in carcinomas with MPC. This result explains the poor prognosis of breast carcinomas with an MPC pattern.

Key Words: Breast carcinoma; Immunohistochemistry; HER2; Micropapillary carcinoma

Breast cancer is the most frequently diagnosed cancer in women, as well as the most common cause of cancer death in women of all ages.[1] Traditionally, breast carcinoma was classified by its histological appearance into various types such as ductal, lobular, tubular, mucinous, medullary, and metaplastic. Micropapillary carcinoma (MPC) is composed of small clusters of tumor cells within clear stromal spaces resembling dilated vascular channels and comprises < 2% of all invasive breast cancers. In a previous study, axillary lymph node metastases were found in 72-77% patients with MPC at initial diagnosis.[2]

Some histological types of carcinoma, mucinous or medullary, have good prognosis, while some histological types such as, MPC or metaplastic carcinoma, have a poor prognosis. The MPC pattern is known to be associated with aggressive behavior in breast cancer, but mucinous carcinoma, which was known to have an indolent behavior, frequently coexists with the MPC component. Besides, the prognosis of mixed mucinous carcinoma with an MPC pattern is not clear.[3,4]

After application of a new subclassification approach of invasive ductal carcinoma not otherwise specified (IDC NOS) based on the immunohistochemical (IHC) expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor (HER)2 into luminal A (ER+ and/or PR+, HER2-), luminal B (ER+ and/or PR+, HER2+), HER2 (ER-, PR-, HER2+), basal-like (ER-, PR-, HER2-, epidermal growth factor receptor [EGFR], or cytokeratin [CK]5/6+), and unclassified subtypes (ER-, PR-, HER2-, EGFR-, and CK5/6-),[5] an investigation was performed to determine whether a correlation exists between the histological type and new subtype based on IHC profile in other specific histological types of invasive carcinoma.[6] The new cancer classification system is derived primarily from typical IDC, and it is still debatable whether it could be applied to all histological subtypes.

The aim of this study was to identify the cancer subtypes based on the IHC profile in patients with breast carcinomas showing an MPC pattern and to investigate the difference of the cancer subtypes between the MPC and IDC portions.

MATERIALS AND METHODS

Materials

We analyzed 127 cases diagnosed as MPC, IDC NOS with MPC, or mucinous carcinoma with MPC. Of the analyzed cases, 75 were IDC NOS with MPC, 8 were pure MPC, and 7 were

mucinous carcinomas with MPC. These 90 cases were diagnosed after performing a lumpectomy or total mastectomy between January 2004 and December 2009 at Seoul National University Hospital in Korea. Tumors with a predominant (>75%) micropapillary component were diagnosed as pure MPC.[6] If there was a small proportion of MPC, it was classified as IDC NOS with MPC (Fig. 1).[7] If there were a large amount of mucin and MPC with invasion into the stroma together (except tumor clusters floating in the mucin pool), these were diagnosed as mucinous carcinoma with MPC.

The remaining 37 cases were mucinous carcinoma diagnosed at Seoul National University Hospital between January 2000 and December 2004. According to the World Health Organization (WHO) classification, tumors with a large (at least 75%) mucinous component, were included in this group.

All tissue specimens were formalin-fixed and paraffin embedded. Representative sections were used for hematoxylin and eosin, IHC staining, and fluorescent *in situ* hybridization (FISH) study. In addition, age, sex, tumor size, and lymph node status were evaluated by reviewing medical records or the glass slides.

IHC staining

Four-μm sections were deparaffinized and rehydrated in graded alcohol. Antigen retrieval was achieved by heating in 0.01 mol/L citrate buffer for 5 minutes. Non-specific staining was blocked by treating sections with 1% horse serum in Tris-buffered saline (pH 6.0) for 5 minutes.

For the assessment of HER-2 expression, CB11 monoclonal antibody (Novocastra, Norwell, MA, USA) was used. Other antibodies were as follows: ER (1:50, clone 1D5, Dako, Carpinteria, CA, USA), PR (1:50, clone PgR636, Dako), HER2 (1:200, clone CB11, Dako), Ki-67 (1:800, clone MIB-1, Dako), and EGFR (1:50, clone H11, Dako). Tissue sections were incubated with each antibody for 70 minutes at room temperature. The slides were treated with biotinylated anti-mouse immunoglobulin antibody and streptavidin-peroxidase reagent, and incubated in diaminobenzidine followed by counterstaining with Mayer's hematoxylin.

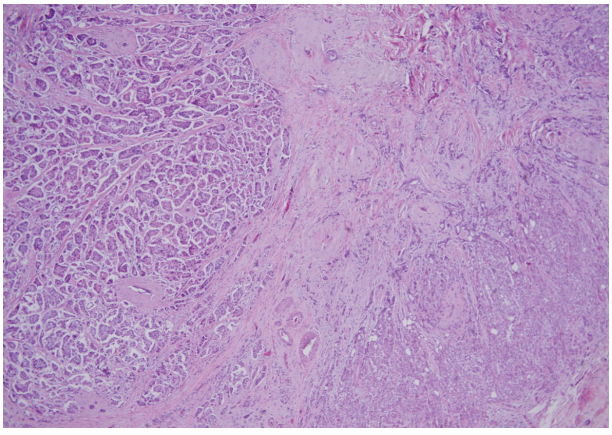

Fig. 1. A representative section of invasive ductal carcinoma not otherwise specified (IDC NOS) with micropapillary carcinoma (MPC), showing IDC NOS and the MPC portion in the same breast cancer (left, IDC NOS portion; right, MPC portion showing small clusters of tumor cells lying within clear stromal spaces).

For ER and PR, nuclear staining in >1% of tumor cells was classified as positive. For HER2, the *HER2* gene amplification or 3+ intense membranous IHC staining in at least 10% of tumor cells was determined as positive based on the American Society of Clinical Oncology/College of American Pathologists guideline.[8] The positive nuclear staining of Ki-67 was quantified as a percentage (positive tumor cells/100 tumor cells).

The IDC NOS and micropapillary portions were separately studied in 35 out of 75 cases of IDC NOS with MPC. In mucinous carcinomas with MPC, IHC staining was performed on the MPC component because the cellularity of the mucinous component was low.

Fluorescent *in situ* hybridization

All cases with 2+ HER2 immunostain were further confirmed by FISH. The FISH experiments were performed according to the manufacturer's protocol (PathVysion kit, Vysis, Downers Grove, IL, USA) using formalin-fixed paraffin-embedded samples. Four-μm sections were deparaffinized in 3 changes of fresh xylene for 3 minutes each, dehydrated in 2 changes of 100% ethanol for 3 minutes each, and allowed to air dry. Slides were then placed in a preheated (80°C) pretreatment reagent (1 M sodium isothiocyanate; Vysis) for 13 minutes, rinsed in distilled water for 3 minutes, and allowed to air dry. Protease digestion was accomplished by placing the slides in prewarmed (37°C) protease solution (Vysis) for 13 minutes. Samples were then rinsed in distilled water for 3 minutes and then air-dried. The HER2 DNA probe kit (Novocastra) included 2 DNA probes directly labeled with different fluorescent dyes: the Spectrum-Orange fluorophore-labeled HER2 specific for the *HER2* gene locus on chromosome 17q11.2-q12, and the Spectrum-Green fluorophore-labeled chromosome enumerator probe targeting the alpha satellite DNA sequence located at the centromeric region of chromosome 17 (CEP17; 17p11.1- q11.1). At least 60 cells were scored in each preparation, and the copy numbers of

HER2 and CEP17 for each cell were recorded. *HER2* gene amplification was defined as a HER2 to CEP17 signal ratio of 2.2 or greater.

Cancer subtyping criteria based on immunohistochemical profile

The IHC-based definitions of breast cancer subtypes used in this study were as follows: luminal A (ER+ and/or PR+, HER-2-), luminal B (ER+ and/or PR+, HER2+), HER2 (ER-, PR-, and HER2+), basal-like (ER-, PR-, HER2-, EGFR, or CK5/6+), and unclassified (ER-, PR-, HER2-, EGFR-, and CK5/6-).[5]

Statistical analysis

Descriptive statistics were calculated. Comparisons between groups were performed using the paired t-test. p-values (two-sided test) of <0.05 were considered statistically significant. All statistical analyses were carried out using SPSS ver. 15.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Clinicopathological characteristics of 127 breast cancer patients

The clinicopathological characteristics of 127 breast carcinoma patients are summarized in Table 1. There were no significant differences in these features among the histological subtypes.

The immunohistochemistry-based subtype classification of breast cancer

The IHC expression rate for each histological subtype of cancer is summarized in Table 2 and Appendix 1. HER2 overexpression was observed in 37.5% of MPC, 42.9% of mucinous carcinomas with MPC pattern, and 25.3% of IDC NOS with MPC. On the other hand, only 10.8% of mucinous carcinoma showed HER2 overexpression.

Table 2. Immunohistochemical characteristics of the 127 breast cancer patients

	Pure MPC n (%)	IDC NOS with MPC n (%)	Mucinous carcinoma with MPC n (%)	Pure mucinous carcinoma n (%)
ER positive	6 (75.0)	57 (76.0)	5 (71.4)	36 (87.3)
PR positive	3 (37.5)	41 (54.7)	4 (57.1)	27 (73.0)
HER2 positive	3 (37.5)	19 (25.3)	3 (42.9)	4 (10.8)
EGFR positive	0 (0)	5 (6.7)	0 (0)	0 (0)
CK5/6 positive	0 (0)	4 (5.3)	0 (0)	0 (0)
Total	8 (100)	75 (100)	7 (100)	37 (100)

MPC, micropapillary carcinoma; IDC NOS, invasive ductal carcinoma not otherwise specified; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; EGFR, epidermal growth factor receptor; CK, cytokeratin.

Table 1. Clinicopathological characteristics of 127 breast cancer patients

Characteristics		Micropapillary carcinoma		Mucinous carcinoma	
		Pure MPC n (%)	IDC NOS with MPC n (%)	Mucinous carcinoma with MPC n (%)	Pure mucinous carcinoma n (%)
Age (yr)	< 50	5 (62.5)	40 (53.3)	6 (85.7)	13 (45.9)
	≥ 50	3 (37.5)	35 (46.7)	1 (14.3)	24 (64.8)
Gender	Male	0 (0)	0 (0)	0 (0)	0 (0)
	Female	8 (100)	75 (100)	7 (100)	37 (100)
Nuclear grade	1	0 (0)	0 (0)	0 (0)	-
	2	3 (37.5)	17 (22.7)	3 (42.9)	-
	3	5 (62.5)	58 (77.3)	4 (57.1)	-
Histological grade	1	0 (0)	0 (0)	0 (0)	-
	2	4 (50.0)	21 (28.0)	4 (57.1)	-
	3	4 (50.0)	54 (72.0)	3 (42.9)	-
Tumor size	T1a	0 (0)	0 (0)	0 (0)	1 (2.7)
	T1b	1 (12.5)	3 (4.0)	0 (0)	2 (5.4)
	T1c	2 (25.0)	19 (25.3)	1 (14.3)	18 (48.6)
	T2	4 (50.0)	51 (68.0)	6 (85.7)	13 (35.1)
	T3	1 (12.5)	2 (2.7)	0 (0)	3 (8.1)
LN metastasis	Absent	3 (37.5)	26 (34.7)	5 (71.4)	35 (94.6)
	Present	5 (62.5)	49 (65.3)	2 (28.6)	2 (5.4)

MPC, micropapillary carcinoma; IDC NOS, invasive ductal carcinoma not otherwise specified; LN, lymph node; -, not available.

The cancer phenotype according to each histological subtype is summarized in Table 3. In MPC, the cancer subtypes based on immunohistochemistry were as follows: 4 (50.0%) were luminal A, 2 (25.0%) were luminal B, 1 (12.5%) was HER2 type, and 1 was (12.5%) unclassified type. In cases of IDC NOS with MPC, 47 (62.7%) were luminal A, 10 (13.3%) were luminal B, 9 (12.0%) were HER2, 2 (2.7%) were basal-like type, and 7 (9.3%) were unclassified type.

Mucinous carcinoma cases with an MPC pattern consisted of 4 luminal A (57.1%), 1 luminal B (14.3%) and 2 HER2 (28.6%) subtypes. Mucinous carcinomas were mostly luminal A (89.2%) or B subtypes (8.1%), except for 1 HER2 type (2.7%).

Upon comparison of pure MPC and IDC NOS with MPC, luminal B or HER2 subtype was more frequent in pure MPC (3/8) than in IDC NOS with MPC (19/75) (37.5% vs 25.3%, $p = 0.026$). The comparison of mucinous carcinoma with an MPC pattern and pure mucinous carcinoma showed that luminal B or HER2 subtypes were more prevalent in the former (3/7) than in mucinous carcinoma (4/37) (42.9% vs 10.8%, $p < 0.001$). In general, HER2 expression was higher in carcinomas with an MPC pattern than other histological subtypes of breast carcinoma. And the luminal A subtype decreased in mucinous carcinoma with an MPC pattern (4/7, 57.1%) than in mucinous carcinoma (33/37, 89.2%).

Differences in the immunohistochemical profile between each component of IDC NOS with MPC

In 35 cases of IDC NOS with MPC, there was no significant difference in the subtypes based on the IHC profile between IDC and MPC (Table 4), except for 1 case in which IDC NOS portion was unclassified subtype and MPC portion exhibited luminal A features.

Ki-67 labeling index differed between 2 portions. The IDC NOS portion tended to have a slightly higher Ki-67 labeling index than MPC portion (mean, 5.2% vs 3.83%) ($p = 0.126$) (Fig. 2).

Table 3. Cancer subtypes of 127 breast cancer patients according to immunohistochemical profile

	Pure MPC n (%)	IDC NOS with MPC n (%)	Mucinous carcinoma with MPC n (%)	Pure mucinous carcinoma n (%)
Luminal A	4 (50.0)	47 (62.7)	4 (57.1)	33 (89.2)
Luminal B	2 (25.0)	10 (13.3)	1 (14.3)	3 (8.1)
HER2	1 (12.5)	9 (12.0)	2 (28.6)	1 (2.7)
Basal-like	0 (0)	2 (2.7)	0 (0)	0 (0)
Unclassified	1 (12.5)	7 (9.3)	0 (0)	0 (0)
Total	8 (100)	75 (100)	7 (100)	37 (100)

MPC, micropapillary carcinoma; IDC NOS, invasive ductal carcinoma not otherwise specified; HER2, human epidermal growth factor receptor 2.

Table 4. Cancer subtypes of 35 IDC NOS with MPC based on the immunohistochemical profile

	IDC NOS portion n (%)	MPC portion n (%)
Luminal A	25 (71.4)	26 (74.2)
Luminal B	3 (8.6)	3 (8.6)
HER2	2 (5.7)	2 (5.7)
Unclassified	5 (14.3)	4 (11.4)
Basal-like	0 (0)	0 (0)
Total	35 (100)	35 (100)

IDC NOS, invasive ductal carcinoma not otherwise specified; MPC, micropapillary carcinoma; HER2, human epidermal growth factor receptor 2.

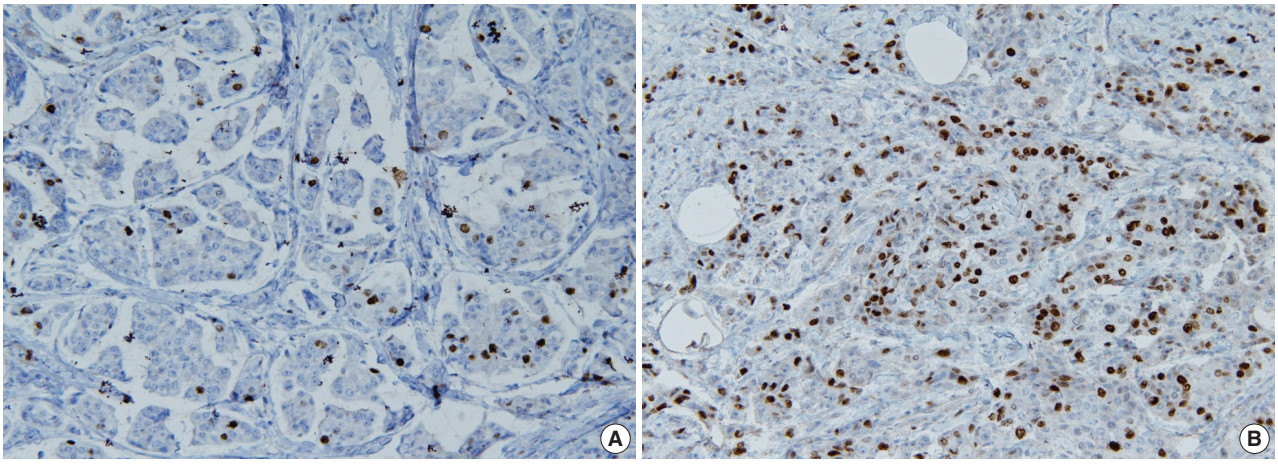

Fig. 2. Ki-67 labeling index in invasive ductal carcinoma not otherwise specified (IDC NOS) with micropapillary carcinoma (MPC) showing a difference in expression between MPC and IDC NOS components in the same tumor (A, IDC NOS portion; B, MPC portion).

Table 5. Cancer subtypes of MPC and IDC based on immunohistochemical profile in the previous literature

	MPC			IDC NOS		
	ER+	PR+	HER2+	ER+	PR+	HER2+
Zekioglu *et al.*[6]	21/31 (46)	19/31 (61)	17/31 (54)	23/60 (38)	19/60 (32)	31/60 (51)
Kim *et al.*[23]	7/36 (19.4)	7/36 (19.4)	14/36 (38.9)	7/32 (21.9)	7/32 (21.9)	9/32 (31.3)
De la Cruz *et al.*[21]	8/16 (50.0)	5/16 (31.2)	8/16 (50.0)	112/150 (74.3)	97/150 (64.7)	33/150 (20.3)
Marchiò *et al.*[24]		20/24 (83.3)	3/24 (12.5)		42/48 (87.5)	9/48 (18.8)
Varga *et al.*[25]	11/11 (100.0)	9/11 (81.8)	8/11 (72)			
Luna-Moré *et al.*[26]	33 (72.7)	(45.4)	(36.3)			
Weigelt *et al.*[20]	8 (81)	(64)	(59)			

Values are presented as number (%).
MPC, micropapillary carcinoma; IDC NOS, invasive ductal carcinoma not otherwise specified; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2.

DISCUSSION

The micropapillary patterns are found in adenocarcinomas of many organs. In stomach cancers, a micropapillary pattern was associated with an advanced cancer stage.[9] However, the Ki-67 labeling index was significantly higher in control adenocarcinoma than the micropapillary component found in ovaries,[10] lungs,[11] stomach,[9] colon,[12,13] and urinary bladder.[14] Our study also showed that the IDC NOS portion had a tendency to have a slightly higher Ki-67 labeling index than MPC portion ($p = 0.126$).

Invasive micropapillary urothelial carcinomas of the bladder were reported to be associated with advanced stages and lymph node metastasis, and a positive result for HER2.[15] The *HER2* gene is a member of the gene family encoding transmembrane receptors for growth factors, including EGFR, HER2, HER3, and HER4. HER2 overexpression was found in approximately 25-30% of invasive breast cancers and is a well-known prognostic factor associated with poor survival in breast carcinoma.[16] In an early analysis of 68 cases of invasive MPC of the breast, the disease-free group had ER-positive (73%) and HER2-negative tumors.[17]

In our study, HER2 expression in MPC was higher (36.4%) than in previous results on common breast cancer (Table 5). Additionally, 42.9% of mucinous carcinoma with MPC cases showed HER2 overexpression, while only 10.8% of mucinous carcinoma showed HER2 overexpression.

According to the recent subtyping of breast cancers by IHC profile, IDC NOS and invasive lobular carcinoma showed luminal A (54-59%), luminal B (18-21%), basal-like (14-16%), and HER2 (6-9%) types.[5] Another study on breast cancer subtype that included a large group of IDC NOS cases only ($n = 1,550$) reported the prevalence of cancer subtypes as luminal A, 1,053 cases (67.9%); luminal B, 90 cases (5.8%); HER2, 107 cases (6.9%); basal-like, 223 cases (14.4%); and unclassified, 77 cases (5.0%) type.[18] In a Japanese breast cancer study including 4,266 subjects, the subtype distribution was as follows: luminal A type, 3,046 cases (71%); luminal B type, 321 cases (8%); HER2 type, 398 cases (9%); and triple negative type, 501 cases (12%).[19]

In this study, luminal B type and HER2 type were more frequent in MPC or IDC NOS with MPC than in IDC NOS of other reports.

It is unknown whether this classification approach can be applied to all histological subtypes. Weigelt et al.[20] reported that IDC NOS and invasive lobular carcinoma contained all cancer subtypes, but special type cancers belonged to one molecular subtype. Another study on 16 invasive MPC revealed lower frequency of positive ER/PR ($p < 0.05$, $p < 0.01$) and a higher frequency of HER2 overexpression ($p < 0.025$) (Table 5).[21] On the other hand, in a study on 671 primary breast carcinomas (including 27 cases of IDC with pure or partial MPC component), the rate of HER2 protein overexpression and ER/PR status in IDC with MPC was similar to those of common breast cancer.[22]

In summary, breast carcinomas showing an MPC pattern were primarily composed of luminal A, luminal B, and HER2 subtypes. MPC patterns were related with frequent HER2 positivity in IDC NOS or mucinous carcinoma. In previous studies, the HER2 positivity of IDC NOS and MPC have been variably reported (Table 5).[23-26] The clinical implication of HER2 positivity in MPC could not elucidated in this study due to the lack of follow-up data.

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Appendix 1. HER2 expression and amplification in 127 breast cancer patients determined by immunohistochemistry and FISH

	MPC	IDC NOS with MPC	Mucinous carcinoma with MPC	Mucinous carcinoma
HER2 IHC				
Negative	3	27	1	16
1+	1	22	3	15
2+	3	15	2	3
3+	1	11	1	3
Total	8	75	7	37
HER2 FISH[a]				
Amplification	2	8	2	1
No amplification	1	7	0	2
Total	3	15	2	3

[a]HER2 FISH study was performed in all cases with 2+ HER2 immunostain.
HER2, human epidermal growth factor receptor 2; FISH, fluorescent *in situ* hybridization; MPC, micropapillary carcinoma; IDC NOS, invasive ductal carcinoma not otherwise specified; IHC, immunochemistry.