



Original contribution

Hepatocyte nuclear factor 4A expression discriminates gastric involvement by metastatic breast carcinomas from primary gastric adenocarcinomas

Taiga Koyama MD^{a,c}, Shigeki Sekine MD, PhD^{b,*}, Hirokazu Taniguchi MD, PhD^a, Hitoshi Tsuda MD, PhD^a, Masahiro Ikegami MD, PhD^c, Hiroshi Hano MD, PhD^c, Ryoji Kushima MD, PhD^a

^aPathology and Clinical Laboratory Division, National Cancer Center Hospital, 104-0045 Tokyo, Japan

^bPathology Division, National Cancer Center Research Institute, 104-0045 Tokyo, Japan

^cPathology Division, The Jikei University School of Medicine, 105-8461 Tokyo, Japan

Received 25 December 2010; revised 16 March 2011; accepted 6 April 2011

Keywords:

HNF4A;
Gastric carcinoma;
Lobular carcinoma;
Gastric metastasis

Summary Breast carcinomas sometimes metastasize to the stomach, and the histopathologic distinction of such metastases from primary gastric adenocarcinomas is often difficult. We characterized the clinicopathologic features of 21 breast carcinomas that had metastasized to the stomach and examined the use of a panel of antibodies, including hepatocyte nuclear factor 4A, for distinguishing the metastases from primary gastric diffuse-type adenocarcinomas. Histologically, all the metastatic breast carcinomas showed a poorly differentiated and/or signet ring cell morphology. Although most metastatic breast and primary gastric carcinomas contained signet ring cell components, the cases that were predominantly or exclusively composed of univacuolated-type signet ring cells were limited to metastatic breast carcinomas. Immunohistochemically, hepatocyte nuclear factor 4A was expressed in all 33 primary gastric carcinomas that were examined but was never expressed in metastatic breast carcinomas. Previously reported markers for breast and gastric carcinomas also showed a high specificity, but their sensitivities were quite variable. Estrogen receptor α , progesterone receptor, mammaglobin, and gross cystic disease fluid protein 15 were expressed in 76%, 33%, 52%, and 62%, respectively, of the metastatic breast carcinomas, whereas none of the primary gastric carcinomas expressed these antigens. CDX2, MUC5AC, MUC6, and CK20 were expressed in 36%, 85%, 27%, and 55%, respectively, of the primary gastric carcinomas. All the metastatic breast carcinomas were negative for these antibodies except for 1 case that expressed MUC5AC. Overall, the use of immunohistochemistry efficiently discriminated metastatic breast carcinomas from primary gastric carcinomas. In particular, the present study identified hepatocyte nuclear factor 4A as an excellent marker for differentiating the 2 lesions.

© 2011 Elsevier Inc. All rights reserved.

1. Introduction

In surgical pathology practice, we sometimes encounter breast cancer metastases to the gastrointestinal tract, especially to the stomach. Interestingly, most previous

* Corresponding author.

E-mail address: ssekine@ncc.go.jp (S. Sekine).

studies agree that invasive lobular carcinomas are more common than invasive ductal carcinomas among breast cancers that metastasize to the stomach [1-5]. The distinction of metastatic breast carcinomas from primary gastric carcinomas is clinically important because these lesions require different treatment strategies. Patients with metastatic breast carcinomas are usually treated with chemotherapy; on the other hand, if the lesion is a primary gastric carcinoma, further evaluation of the clinical stage is required before determining an appropriate therapeutic management. However, the histologic diagnosis of metastatic breast carcinomas is sometimes problematic because the typical histologic features of invasive lobular carcinomas, for example, linear, dissociated, and a single-file growth pattern, are similar to those of diffuse-type gastric carcinomas. In particular, invasive lobular carcinomas frequently show a signet ring morphology that can be easily confused with that of primary signet ring cell-type gastric carcinomas [2,5-8]. Furthermore, the stomach is the initial site of presentation of advanced breast cancers in some instances [1,5,9-11].

Immunohistochemistry using a panel of antibodies has been reported to be capable of differentiating metastatic breast carcinomas from primary gastric carcinomas [6,12,13]. Estrogen receptor (ER) α , progesterone receptor (PR), mammaglobin, and gross cystic disease fluid protein 15 (GCDFP-15) have been used as markers of breast carcinomas [6,8,12-20], whereas CDX2, MUC5AC, MUC6, and CK20 are highly specific to gastric carcinomas [6,13,15,21-27]. However, these antibodies have limitations in their sensitivities; thus, the use of multiple antibodies is often required.

Hepatocyte nuclear factor (HNF) 4A is a member of the nuclear receptor superfamily and is a critical developmental regulator of the visceral endoderm [28]. Recent studies have shown that HNF4A is expressed in an organ-specific manner in normal as well as neoplastic tissues [28-30]. Among nonneoplastic tissues, HNF4A is expressed in epithelial cells of the gastrointestinal tract, liver and pancreas, and the proximal tubules of the kidney but not in most other organs including mammary glands [28]. The expression of HNF4A in neoplastic lesions has not been extensively studied, but previous reports imply that tumors originating from HNF4A-positive organs generally retain HNF4A expression [28-30]. This suggests that HNF4A might be useful for determining the primary sites of metastatic tumors. Here, we tested the use of HNF4A, in addition to a previously tested panel of antibodies, for the diagnosis of metastatic breast carcinomas in the stomach.

2. Materials and methods

2.1. Study group

Endoscopic biopsy specimens of 21 metastatic breast carcinomas were included in the present study. All the

cases were retrieved from the surgical pathology files of the National Cancer Center Hospital, Tokyo, Japan, between 1997 and 2010. Patients with metastatic breast carcinomas involving the stomach had been diagnosed in cases that (1) had a history of surgical treatment of primary breast cancer or concurrent gastric and breast tumors of identical histologic type and (2) were immunohistochemically consistent with metastatic breast carcinomas using 1 or more of the following antibodies: ER α , PR, GCDFP-15, mammaglobin, CK20, MUC5AC, MUC6, and CDX2. Paraffin-embedded specimens of the corresponding primary lesions were available in 12 cases of metastatic breast carcinomas, and these specimens were also subjected to immunohistochemical analysis for comparison with the metastatic lesions.

For histologic and immunohistochemical comparisons, endoscopic biopsy samples of primary gastric cancers were also examined. A consecutive series of 33 poorly differentiated and/or signet ring cell adenocarcinomas of the stomach were retrieved from our case files without taking age and sex into account. These patients were clinically confirmed not to have any breast tumors.

This study was approved by the Ethics Committee of the National Cancer Center, Tokyo, Japan.

2.2. Histologic evaluation

The histology of breast carcinomas involving the stomach was classified into poorly differentiated adenocarcinoma, signet ring cell carcinoma, or others. Both metastatic breast and primary gastric carcinomas were histologically examined for the presence of signet ring cell components. The cytologic features of signet ring cells, with either univacuolated or multivacuolated cytoplasm, were further classified as previously described [7]. Briefly, the univacuolated type is characterized by a single well-circumscribed intracytoplasmic lumen with/without a central eosinophilic inclusion. The multivacuolated type is characterized by foamy cytoplasm with an abundance of mucin-filled vesicles.

2.3. Immunohistochemistry

Deparaffinized 4- μ m-thick sections from each paraffin block were exposed to 0.3% hydrogen peroxide for 15 minutes to block endogenous peroxidase activity and then washed in deionized water for 2 to 3 minutes. For heat-induced epitope retrieval, the sections were subjected to citrate buffer (pH 6.0) at 121°C for 10 minutes. The primary antibodies that were used are monoclonal and listed in Table 1. For staining, we used an automated stainer (Dako, Glostrup, Denmark) according to the vendor's protocol. ChemMate EnVision (Dako) methods were used for detection. Appropriate positive and negative controls were used for each antibody.

Table 1 Antibodies used in the present study

Antibody	Clone	Dilution	Source
HNF4A	H1415	1:100	Perseus Proteomics (Tokyo, Japan)
ER α	1D5	1:100	Dako, Glostrup, Denmark
PR	PgR636	1:400	Dako
Mammaglobin	304-1A5	1:200	Dako
GCDFP-15	D6	1:200	Signet laboratories, Inc (Dedham, MA)
CDX2	CDX2-88	1:100	Bio Genex (San Ramon, CA)
MUC5AC	CLH2	1:200	Novocastra Laboratories, Ltd (Newcastle upon Tyne, UK)
MUC6	CLH5	1:100	
CK20	KS20.8	1:50	Dako

The extent of positive staining was graded semiquantitatively as follows: – (negative), \pm (1%-10% positive cells), + (11%-50% positive cells), and ++ (>51% positive cells). To determine the specificity and sensitivity of each antibody, cases with completely negative staining were regarded as negative, whereas cases with any positive staining were considered positive. Two observers (T. K. and S. S.) evaluated the immunohistochemical results. Discrepant

cases were reviewed using a multiheaded microscope to achieve consensus.

2.4. Statistical analysis

The Fisher exact test was used to analyze each 2-by-2 table of discrete data. $P < .05$ was considered statistically significant.

3. Results

3.1. Clinicopathologic features

The clinicopathologic features of metastatic breast carcinomas are summarized in Table 2. All the patients with metastatic breast carcinomas to the stomach were women, with a mean age of 59.6 years (range, 43-77 years). The mean interval between the diagnosis of primary breast carcinomas and the detection of gastric metastases was 65.8 months (range, 0-270 months). In 4 cases (cases 1, 8, 9, and 19), gastric metastasis was pointed out concurrently with or before the detection of the primary lesions.

The endoscopic appearance of gastric lesions was linitis plastica-like in 8 cases (38%), multiple erosions in 6 cases

Table 2 Clinicopathologic features of metastatic breast carcinomas

Case no.	Age/sex	Primary histology	Interval ^d (mo)	Endoscopic finding	Histology	Signet ring cells
1	43/F	ILC ^a	0 ^c	SMT with ulceration	POR > SIG	UNI
2	45/F	NA	76	SMT	SIG > POR	UNI > MULTI
3	46/F	IDC	45	Linitis plastica	POR	–
4	49/F	ILC	18	SMT with erosion	SIG = POR	UNI > MULTI
5	51/F	ILC	87	SMT	POR	–
6	52/F	ILC	62	Linitis plastica	SIG = POR	MULTI
7	53/F	ILC	54	Multiple erosions	POR > SIG	UNI > MULTI
8	54/F	ILC	0 ^c	Multiple SMTs	SIG > POR	MULTI > UNI
9	54/F	ILC	0	Multiple erosions	SIG > POR	MULTI > UNI
10	55/F	NA	17	Multiple erosions	POR	–
11	55/F	ILC	7	Multiple SMTs	POR > SIG	MULTI > UNI
12	55/F	ILC	145	Linitis plastica	SIG > POR	UNI > MULTI
13	59/F	ILC	1	Linitis plastica	SIG = POR	UNI
14	62/F	ILC	107	Linitis plastica	SIG > POR	MULTI > UNI
15	70/F	IDC	270	SMT with erosion	POR	–
16	71/F	Mixed ^b	183	Multiple erosions	POR > SIG	MULTI > UNI
17	73/F	IDC	30	Linitis plastica	SIG > POR	MULTI
18	73/F	ILC	121	Multiple erosions	POR > SIG	MULTI
19	76/F	IDC	0	Multiple erosions	POR > SIG	MULTI
20	77/F	ILC + IDC ^c	109	Linitis plastica	SIG	UNI
21	77/F	ILC	39	Linitis plastica	SIG	MULTI

Abbreviations: F indicates female; ILC, invasive lobular carcinoma; IDC, invasive ductal carcinoma; SMT, submucosal tumor; POR, poorly differentiated; SIG, signet ring cell; UNI, univacuolated; MULTI, multivacuolated; NA, not available.

^a Diagnosis was made by axillary lymph node biopsy.

^b Mixed invasive ductal lobular carcinoma.

^c Bilateral tumors, invasive lobular in the right breast, and invasive ductal in the left breast.

^d Interval between diagnosis of primary breast carcinomas and the detection of gastric metastases.

^e Gastric lesions were detected before the primary lesions.

(28%), a submucosal tumor with a central ulceration/erosion in 3 cases (14%), solitary submucosal tumor in 2 cases (10%), and multiple submucosal tumors in 2 cases (10%). The histologic subtypes of the primary lesions of the metastatic breast carcinoma cases were invasive lobular in 13 cases (62%), invasive ductal in 4 cases (19%), and mixed invasive ductal lobular in 1 case (5%). One case had bilateral tumors, with an invasive lobular carcinoma in the right breast and an invasive ductal carcinoma in the left breast (case 20). Detailed information on the histology of the primary lesions was not available in 2 cases (cases 2 and 10).

Histologically, all the gastric involvements by metastatic breast carcinomas showed a poorly differentiated morphology. None of the cases showed gland formation. Signet ring

cell components were identified in 17 cases (81%). In 11 cases, signet ring cell components were predominant or equal to the poorly differentiated components; and in the other 6 cases, the signet ring cell components were only minor. In 4 cases (19%), the entire biopsy specimen was composed of poorly differentiated components.

Among the 17 cases with signet ring cells, all the signet ring cells were a univacuolated type in 3 cases (Fig. 1A), whereas all the signet ring cells were a multivacuolated type in 5 cases. The other cases had both univacuolated and multivacuolated signet ring cell components.

With regard to the primary gastric carcinoma cases used as a control, the male-female ratio of the patients was 19:14; and their mean patient age was 65.5 years (range, 42-85 years). Histologically, signet ring cells were present in 31 of the 33 cases. In all the patients, signet ring cells were predominantly or exclusively the multivacuolated type (Fig. 1B), whereas minor components of univacuolated signet ring cells were observed in 13 (39%) of the 31 cases with signet ring cells.

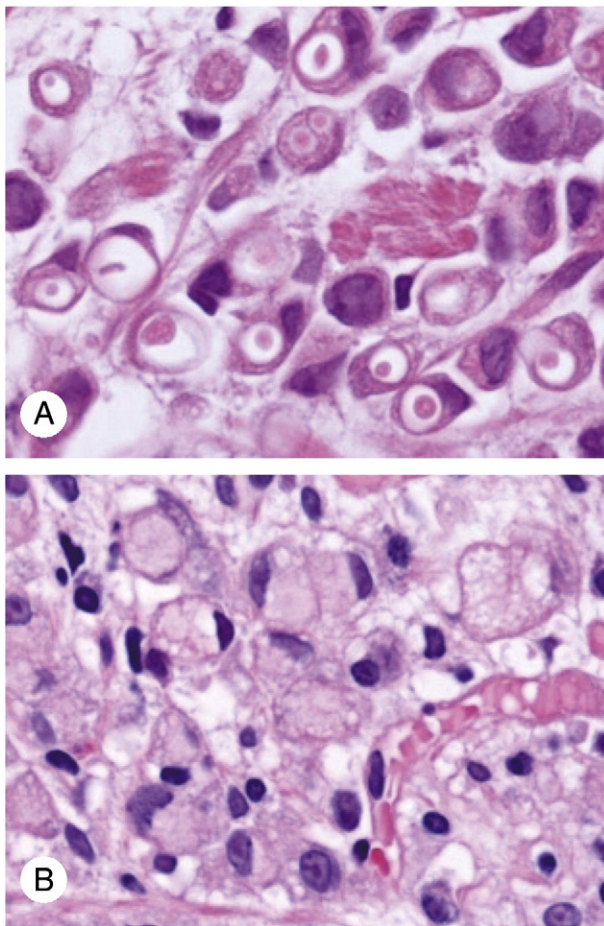


Fig. 1 Histologic features of gastric involvement by metastatic breast and primary gastric carcinomas of signet ring cell type. A, Metastatic breast carcinoma to the stomach. The tumor cells are mainly composed of univacuolated signet ring cells that are characterized by sharply demarcated intracytoplasmic lumina with central eosinophilic inclusions. B, Primary gastric adenocarcinoma. The signet ring cells are a multivacuolated type with predominantly foamy and abundant mucin-filled cytoplasm that pushes the nuclei against the cell membrane (hematoxylin and eosin, original magnification $\times 400$).

3.2. Immunohistochemistry

The results of the immunohistochemical analysis are summarized in Tables 3 and 4. In 21 gastric lesions of metastatic breast cancers as well as 12 primary sites of the breast cancers, HNF4A was consistently negative (Fig. 2A). In contrast, in the 33 primary gastric cancers, HNF4A was diffusely positive in 31 cases and focally positive in 2 cases (Fig. 3A). HNF4A was consistently expressed in nonneoplastic gastric epithelium but was negative in stromal cells in all the cases that were examined. For the discrimination of these 2 entities, the sensitivity and specificity of HNF4A were both 100%.

In gastric lesions of metastatic breast carcinomas, ER α , PR, mammaglobin, and GCDFP-15 were expressed in 16 (76%), 7 (33%), 11 (52%), and 13 (62%) cases, respectively (Fig. 2B-E). One case (case 15) was negative for all these antibodies. None of the primary gastric carcinomas expressed any of these 4 antibodies.

Primary lesions of metastatic breast carcinomas were also stained for the same panel of antibodies in 12 cases. ER α , PR, mammaglobin, and GCDFP-15 were positive in 10 (83%), 9 (75%), 6 (50%), and 4 (33%) cases, respectively. The immunophenotypes were entirely concordant between the primary and metastatic lesions in only 1 of the 12 cases, but the other cases showed some differences. In 3 cases, ER α was positive in the primary lesions but negative at metastatic sites. Similarly, the loss of PR expression at the metastatic sites was observed in 6 cases. Mammaglobin expression was lost in the metastatic lesions in 3 cases. On the other hand, mammaglobin expression was observed only in the metastatic lesions in 3 cases. In 4 cases, GCDFP-15 was negative in the primary lesions but positive in metastatic lesions of the stomach.

Table 3 Results of immunohistochemical staining

Antibody	Breast carcinoma								Primary gastric carcinoma			
	Metastasis to the stomach				Primary lesion							
	–	±	+	++	–	±	+	++	–	±	+	++
HNF4A	21	0	0	0	12	0	0	0	0	0	2	31
ER α	5	1	7	8	2	2	0	8	33	0	0	0
PR	14	1	3	3	3	4	3	2	33	0	0	0
Mammaglobin	10	3	3	5	6	3	3	0	33	0	0	0
GCDFP-15	8	1	4	8	8	2	2	0	33	0	0	0
CDX2	21	0	0	0	12	0	0	0	21	5	4	3
MUC5AC	20	0	1	0	12	0	0	0	5	1	9	18
MUC6	21	0	0	0	11	1	0	0	24	3	5	1
CK20	21	0	0	0	12	0	0	0	15	4	7	7

NOTE. –, negative; ±, 1% to 10% positive cells; +, 11% to 50% positive cells; and ++, more than 51% positive cells.

In primary gastric carcinomas, CDX2, MUC5AC, MUC6, and CK20 were expressed in 12 (36%), 28 (85%), 9 (27%), and 18 (55%) cases, respectively (Fig. 3B-E). These 4 markers were negative in all the metastatic and primary breast carcinomas except for 1 metastatic breast carcinoma (case 2) that stained positive for MUC5AC and 1 primary breast carcinoma (case 6) that stained positive for MUC6.

With regard to the reactivity of these antibodies in nonneoplastic gastric mucosa, CDX2 was expressed in metaplastic epithelium, MUC5AC was expressed in foveolar epithelium, MUC6 was expressed in pyloric glands and mucous neck cells of the fundic glands, and CK20 was expressed in metaplastic and foveolar epithelium. The expressions of ER α , PR, mammaglobin, and GCDFP-15 were completely absent in nonneoplastic gastric mucosa.

The sensitivity and specificity of each antibody for metastatic breast and primary gastric carcinomas are summarized in Table 4. ER α , PR, mammaglobin, and GCDFP-15 were 100% specific to metastatic breast carcinomas; but the sensitivities were variable, ranging from 33% to 76%. CDX2, MUC5AC, MUC6, and CK20 were highly specific to primary gastric carcinomas. Their sensitivities varied from 27% for MUC6 to 85% for MUC5AC.

4. Discussion

Gastric metastases of breast carcinomas can be clinically confused with primary gastric carcinomas, particularly linitis plastica, because of the similarity of their endoscopic and histologic findings. In agreement with previous studies, a linitis plastica-like gross appearance was the most common endoscopic finding for metastatic breast carcinoma [1,2,6]. However, metastatic breast carcinomas can also present with various gross appearances, including submucosal tumor-like, erosive, and ulcerated lesions [1,5,10]. In the present study, we confirmed these results and showed that metastatic tumors frequently occur as multiple lesions.

The histologic subtypes of the primary breast lesions were predominantly invasive lobular carcinomas, as previously reported [1-5]. All metastatic breast carcinomas showed a poorly differentiated morphology; and none of the cases exhibited gland formation. Signet ring cells were identified in most of the metastatic breast carcinomas, including some of the metastatic invasive ductal carcinomas.

Previous studies have suggested that metastatic breast and primary gastric carcinomas can potentially be differentiated based on morphology using hematoxylin and eosin-stained sections [7,26,31]. These previous studies suggested that the signet ring cells of lobular carcinoma are of a univacuolated type, which is characterized by a single, well-circumscribed intracytoplasmic lumina. On the other hand, signet ring cells of gastric carcinomas usually have multivacuolated cytoplasm with foamy and abundant mucin-filled vesicles.

In the present study, the cases in which all or most of the signet ring cells were univacuolated were limited to metastatic breast carcinomas. However, significant proportions of metastatic breast and primary gastric carcinomas contained both univacuolated and multivacuolated signet ring cells. Thus, a definitive diagnosis of metastatic breast carcinomas based solely on their histologic features may be difficult, whereas lesions predominantly composed of univacuolated signet ring cells are suggestive of metastatic breast carcinomas, rather than primary gastric carcinomas.

Immunohistochemically, all the primary gastric carcinomas but none of the metastatic breast carcinomas were positive for HNF4A. Thus, the use of this antibody alone allowed metastatic breast carcinomas to be discriminated from primary gastric carcinomas. The expression of HNF4A has not been extensively studied in tumors. Remarkably, however, all the primary gastric carcinomas that have been previously examined (total of 49 cases) were uniformly positive for HNF4A [28,29]. Moreover, the staining for HNF4A was mostly diffuse and strong, which is a major advantage for its use in the diagnosis of biopsy specimens, where only a limited amount of tissue is available.

Table 4 Summary of immunohistochemical results

Antibody	Metastatic breast carcinoma	Primary gastric carcinoma	<i>P</i>	Sensitivity	Specificity
Markers for breast carcinoma					
ER α	16/21 (76%)	0/33 (0%)	9.6×10^{-10}	76%	100%
PR	7/21 (33%)	0/33 (0%)	6.6×10^{-4}	33%	100%
Mammaglobin	11/21 (52%)	0/33 (0%)	3.7×10^{-6}	52%	100%
GCDFP-15	13/21 (62%)	0/33 (0%)	1.8×10^{-7}	62%	100%
Markers for gastric carcinoma					
HNF4A	0/21 (0%)	33/33 (100%)	1.9×10^{-15}	100%	100%
CDX2	0/21 (0%)	12/33 (36%)	1.6×10^{-3}	36%	100%
MUC5AC	1/21 (5%)	28/33 (85%)	3.0×10^{-9}	85%	95%
MUC6	0/21 (0%)	9/33 (27%)	2.0×10^{-2}	27%	100%
CK20	0/21 (0%)	18/33 (55%)	6.0×10^{-5}	55%	100%

NOTE. *P* values indicate the significance of the difference between gastric involvement by metastatic breast cancer and primary gastric carcinoma.

Our study also confirmed that previously reported markers could differentiate metastatic breast carcinomas and primary gastric carcinomas with a high specificity. ER α , PR, mammaglobin, and GCDFP-15 were specific markers of breast carcinomas. Although several older studies have reported that up to 28% of gastric carcinomas expressed ER [32,33], these findings were based upon studies using a particular antibody against ER, clone H222, which is no longer used in standard practice. Similarly, 1 study reported that mammaglobin was expressed in 13% of gastric carcinomas that had metastasized to the lymph nodes; however, they used a polyclonal mammaglobin antibody,

which is not widely used [18]. Overall, these previous reports and the present study indicate that these 4 markers can specifically detect metastatic breast carcinomas when appropriate antibodies are used. On the other hand, their sensitivities for the identification of metastatic breast carcinomas were variable. ER α was the most sensitive marker for breast carcinomas, followed by GCDFP-15, mammaglobin, and PR.

CK20, MUC5AC, MUC6, and CDX2 were confirmed to be useful for identifying primary gastric carcinomas. In our study, 1 case of metastatic breast carcinoma expressed MUC5AC. Indeed, O'Connell et al [6] also reported a similar

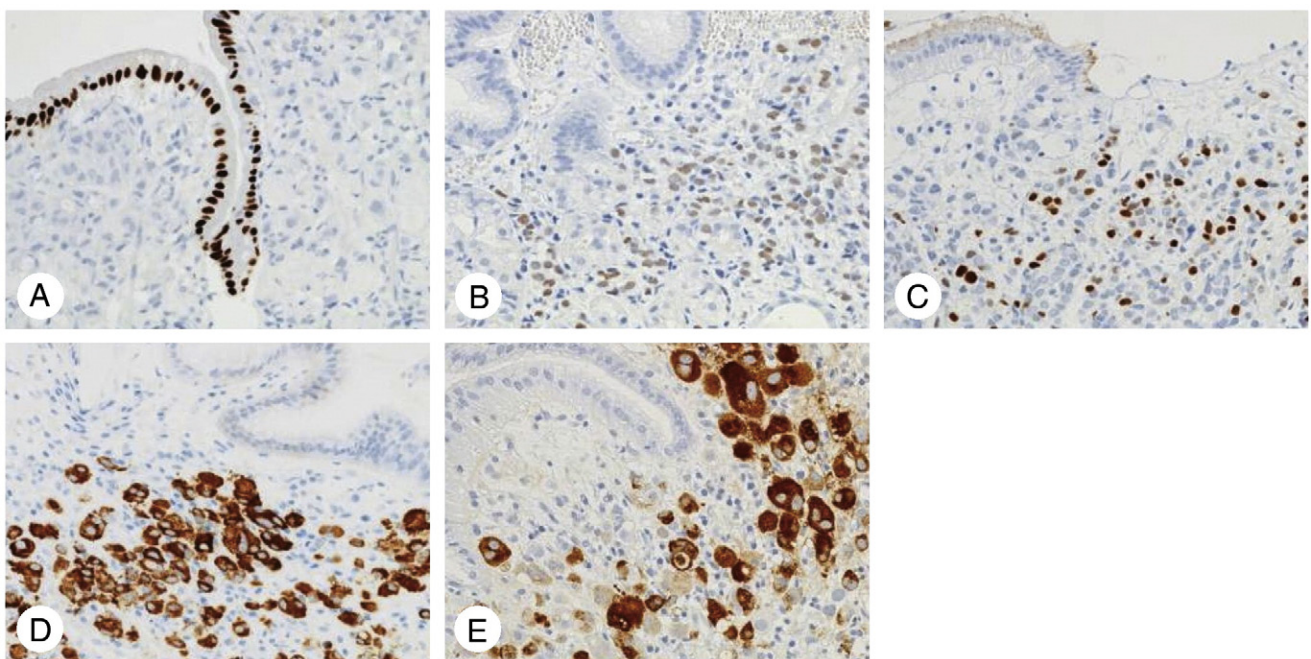


Fig. 2 Immunohistochemical staining of metastatic breast carcinoma to the stomach. HNF4A is expressed in nonneoplastic gastric epithelium but is negative in metastatic breast carcinoma cells infiltrating the lamina propria (A). A metastatic breast carcinoma to the stomach shows the nuclear expression of ER α (B) and PR (C) and the cytoplasmic expression of mammaglobin (D) and GCDFP-15 (E) (original magnification $\times 200$).

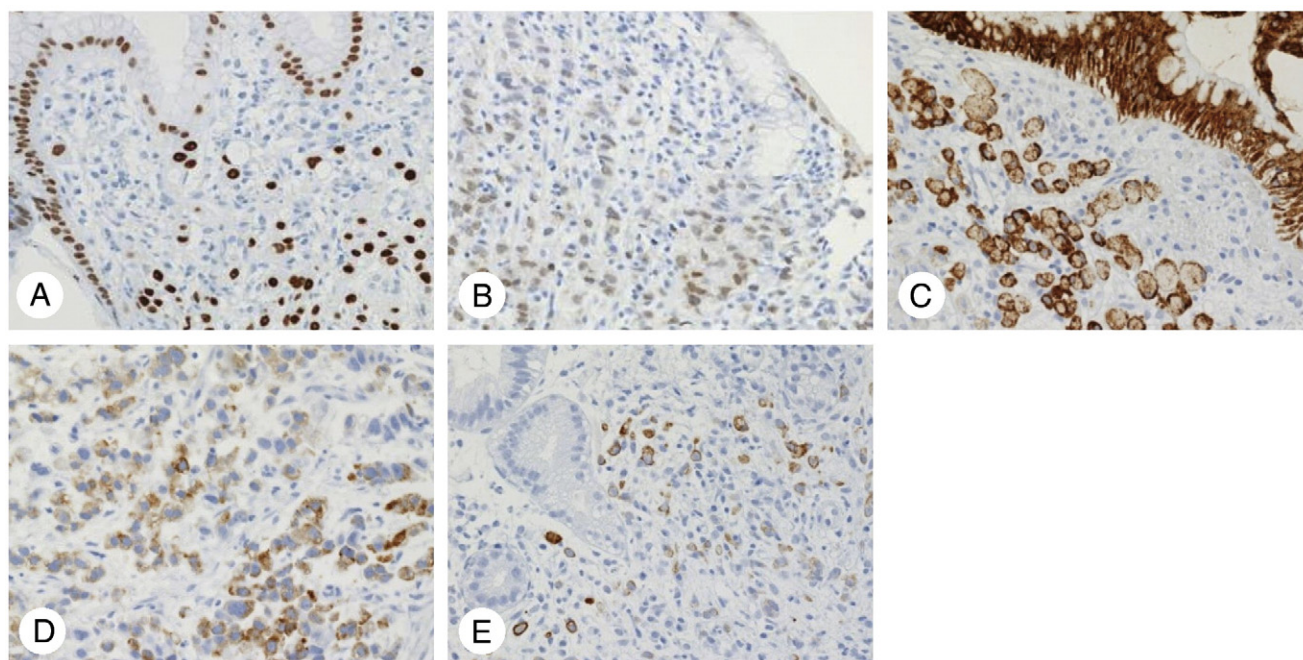


Fig. 3 Immunohistochemical staining of primary gastric carcinoma. Primary gastric carcinoma showing the nuclear expression of HNF4A (A) and CDX2 (B) and the cytoplasmic expression of MUC5AC (C), MUC6 (D), and CK20 (E). The nonneoplastic gastric epithelium is strongly positive for HNF4A (A) and MUC5AC (C) (original magnification $\times 200$).

result: 1 of the 19 breast carcinomas that had metastasized to the gastrointestinal tract expressed MUC5AC in their study. In addition, studies on primary breast carcinomas have shown that minor subsets of breast carcinomas express CK20, MUC5AC, or MUC6 [15,26,27,34,35]. Thus, although our study showed that these gastric carcinoma markers are quite specific, the expression of CK20, MUC5AC, or MUC6 may not completely exclude the possibility of a metastatic breast carcinoma. The expression of CDX2 has never been reported in breast carcinomas, but its sensitivity for the identification of gastric carcinomas was relatively low in the present study.

In summary, breast carcinomas that metastasize to the stomach consistently show a poorly differentiated or signet ring cell morphology, regardless of the histology of the primary lesion. A predominance of univacuolated signet ring cells favors a diagnosis of metastatic breast carcinomas, but it may be difficult to conclusively differentiate these lesions from primary gastric carcinomas based solely on morphology. The use of immunohistochemistry is effective for the diagnosis of gastric metastases of breast carcinomas. ER α , PR, mammaglobin, and GCDFP-15 are specific markers for breast carcinomas, whereas HNF4A, CK20, MUC5AC, MUC6, and CDX2 are useful for identifying primary gastric carcinomas. Remarkably, HNF4A was able to distinguish all primary gastric carcinomas from metastatic breast carcinomas. We suggest that HNF4A may be a highly useful marker for excluding metastatic breast carcinomas in the diagnosis of gastric biopsy specimens.

Acknowledgment

The authors thank Mr Shigeru Tamura for photographic assistance and Ms Sachiko Miura and Ms Chizu Kina for skillful technical assistance in immunohistochemistry.

References

- [1] Taal BG, Peterse H, Boot H. Clinical presentation, endoscopic features, and treatment of gastric metastases from breast carcinoma. *Cancer* 2000;89:2214-21.
- [2] Harris M, Howell A, Chrissohou M, et al. A comparison of the metastatic pattern of infiltrating lobular carcinoma and infiltrating duct carcinoma of the breast. *Br J Cancer* 1984;50:23-30.
- [3] Lamovec J, Bracko M. Metastatic pattern of infiltrating lobular carcinoma of the breast: an autopsy study. *J Surg Oncol* 1991;48:28-33.
- [4] Borst MJ, Ingold JA. Metastatic patterns of invasive lobular versus invasive ductal carcinoma of the breast. *Surgery* 1993;114:637-41.
- [5] Schwarz RE, Klimstra DS, Turnbull AD. Metastatic breast cancer masquerading as gastrointestinal primary. *Am J Gastroenterol* 1998;93:111-4.
- [6] O'Connell FP, Wang HH, Odze RD. Utility of immunohistochemistry in distinguishing primary adenocarcinomas from metastatic breast carcinomas in the gastrointestinal tract. *Arch Pathol Lab Med* 2005;129:338-47.
- [7] Battifora H. Metastatic breast carcinoma to the stomach simulating linitis plastica. *Appl Immunohistochemistry* 1994;2:225-8.
- [8] Raju U, Ma CK, Shaw A. Signet ring variant of lobular carcinoma of the breast: a clinicopathologic and immunohistochemical study. *Mod Pathol* 1993;6:516-20.

- [9] Oda I, Kondo H, Yamao T, et al. Metastatic tumors to the stomach: analysis of 54 patients diagnosed at endoscopy and 347 autopsy cases. *Endoscopy* 2001;33:507-10.
- [10] Karamlou TB, Vetto JT, Corless C, et al. Metastatic breast cancer manifested as refractory anemia and gastric polyps. *South Med J* 2002;95:922-5.
- [11] Ferri LE, Onerheim R, Emond C. Linitis plastica as the first indication of metastatic lobular carcinoma of the breast: case report and literature review. *Can J Surg* 1999;42:466-9.
- [12] Brown RW, Campagna LB, Dunn JK, et al. Immunohistochemical identification of tumor markers in metastatic adenocarcinoma. A diagnostic adjunct in the determination of primary site. *Am J Clin Pathol* 1997;107:12-9.
- [13] Park SY, Kim BH, Kim JH, et al. Panels of immunohistochemical markers help determine primary sites of metastatic adenocarcinoma. *Arch Pathol Lab Med* 2007;131:1561-7.
- [14] Nadji M, Gomez-Fernandez C, Ganjei-Azar P, et al. Immunohistochemistry of estrogen and progesterone receptors reconsidered: experience with 5,993 breast cancers. *Am J Clin Pathol* 2005;123:21-7.
- [15] Tot T. The role of cytokeratins 20 and 7 and estrogen receptor analysis in separation of metastatic lobular carcinoma of the breast and metastatic signet ring cell carcinoma of the gastrointestinal tract. *APMIS* 2000;108:467-72.
- [16] Takeda Y, Tsuta K, Shibuki Y, et al. Analysis of expression patterns of breast cancer-specific markers (mammaglobin and gross cystic disease fluid protein 15) in lung and pleural tumors. *Arch Pathol Lab Med* 2008;132:239-43.
- [17] Wick MR, Lillemoe TJ, Copland GT, et al. Gross cystic disease fluid protein-15 as a marker for breast cancer: immunohistochemical analysis of 690 human neoplasms and comparison with alpha-lactalbumin. *HUM PATHOL* 1989;20:281-7.
- [18] Han JH, Kang Y, Shin HC, et al. Mammaglobin expression in lymph nodes is an important marker of metastatic breast carcinoma. *Arch Pathol Lab Med* 2003;127:1330-4.
- [19] Sasaki E, Tsunoda N, Hatanaka Y, et al. Breast-specific expression of MGB1/mammaglobin: an examination of 480 tumors from various organs and clinicopathological analysis of MGB1-positive breast cancers. *Mod Pathol* 2007;20:208-14.
- [20] Wang Z, Spaulding B, Sienko A, et al. Mammaglobin, a valuable diagnostic marker for metastatic breast carcinoma. *Int J Clin Exp Pathol* 2009;2:384-9.
- [21] Werling RW, Yaziji H, Bacchi CE, et al. CDX2, a highly sensitive and specific marker of adenocarcinomas of intestinal origin: an immunohistochemical survey of 476 primary and metastatic carcinomas. *Am J Surg Pathol* 2003;27:303-10.
- [22] Lau SK, Weiss LM, Chu PG. Differential expression of MUC1, MUC2, and MUC5AC in carcinomas of various sites: an immunohistochemical study. *Am J Clin Pathol* 2004;122:61-9.
- [23] Pinto-de-Sousa J, David L, Reis CA, et al. Mucins MUC1, MUC2, MUC5AC and MUC6 expression in the evaluation of differentiation and clinico-biological behaviour of gastric carcinoma. *Virchows Arch* 2002;440:304-10.
- [24] Reis CA, David L, Nielsen PA, et al. Immunohistochemical study of MUC5AC expression in human gastric carcinomas using a novel monoclonal antibody. *Int J Cancer* 1997;74:112-21.
- [25] Reis CA, David L, Carvalho F, et al. Immunohistochemical study of the expression of MUC6 mucin and co-expression of other secreted mucins (MUC5AC and MUC2) in human gastric carcinomas. *J Histochem Cytochem* 2000;48:377-88.
- [26] Chu PG, Weiss LM. Immunohistochemical characterization of signet-ring cell carcinomas of the stomach, breast, and colon. *Am J Clin Pathol* 2004;121:884-92.
- [27] Moll R, Lowe A, Laufer J, et al. Cytokeratin 20 in human carcinomas. A new histodiagnostic marker detected by monoclonal antibodies. *Am J Pathol* 1992;140:427-47.
- [28] Kojima K, Kishimoto T, Nagai Y, et al. The expression of hepatocyte nuclear factor-4alpha, a developmental regulator of visceral endoderm, correlates with the intestinal phenotype of gastric adenocarcinomas. *Pathology* 2006;38:548-54.
- [29] Tanaka T, Jiang S, Hotta H, et al. Dysregulated expression of P1 and P2 promoter-driven hepatocyte nuclear factor-4alpha in the pathogenesis of human cancer. *J Pathol* 2006;208:662-72.
- [30] Takano K, Hasegawa G, Jiang S, et al. Immunohistochemical staining for P1 and P2 promoter-driven hepatocyte nuclear factor-4alpha may complement mucin phenotype of differentiated-type early gastric carcinoma. *Pathol Int* 2009;59:462-70.
- [31] Gad A, Azzopardi JG. Lobular carcinoma of the breast: a special variant of mucin-secreting carcinoma. *J Clin Pathol* 1975;28:711-6.
- [32] Kojima O, Takahashi T, Kawakami S, et al. Localization of estrogen receptors in gastric cancer using immunohistochemical staining of monoclonal antibody. *Cancer* 1991;67:2401-6.
- [33] Yokozaki H, Takekura N, Takanashi A, et al. Estrogen receptors in gastric adenocarcinoma: a retrospective immunohistochemical analysis. *Virchows Arch A Pathol Anat Histopathol* 1988;413:297-302.
- [34] Tot T. Patterns of distribution of cytokeratins 20 and 7 in special types of invasive breast carcinoma: a study of 123 cases. *Ann Diagn Pathol* 1999;3:350-6.
- [35] Matsukita S, Nomoto M, Kitajima S, et al. Expression of mucins (MUC1, MUC2, MUC5AC and MUC6) in mucinous carcinoma of the breast: comparison with invasive ductal carcinoma. *Histopathology* 2003;42:26-36.