

## Immunohistochemical analysis of invasive micropapillary carcinoma pattern in four cases of gastric cancer

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**Abstract** Gastric cancer with the invasive micropapillary carcinoma (IMPC) pattern has been reported to be a variant with poor prognosis and rapid progression. To the best of our knowledge, only 4 cases of gastric cancer from Japan and 11 cases from Korea have been reported to contain the IMPC pattern. In the present study, 4 cases of gastric cancer containing the IMPC pattern from 2 Japanese men and 2 Japanese women are reported. The cancer tissues, including a recurrent lesion in 1 case and lymph node metastases in 2 other cases, were examined immunohistochemically to identify suitable markers for demonstrating the peculiar “inside out” pattern of IMPC and for analyzing HER2 expression. A characteristic IMPC pattern occupied more than 10 % of each cancer tissue in these 4 cases. Lymphatic invasions were very often detected; in fact, lymph node metastases were detected in 3 out of 4 cases. The unique “inside out” pattern in IMPC was clearly

revealed in all cases by staining with antibodies to both epithelial membrane antigen (EMA) and KL-6, but not with an antibody to CD10. HER2 was positive in 3 of 4 cases with the IMPC pattern, including cases with a recurrent lesion or lymph node metastases. Fluorescence in situ hybridization (FISH) analyses disclosed positive results in case 1, and case 3 including lymph node metastatic foci. Highest FISH titer was 6.8 in case 1, revealing marked amplification of HER-2 gene. Four cases of gastric cancer with the IMPC pattern were reported. EMA and KL-6, but not CD10, were particularly useful markers for visualizing the characteristic “inside out” pattern of the IMPC pattern in stomach cancers, similar to the markers for breast and urinary bladder cancers.

**Keywords** Gastric cancer · Invasive micropapillary carcinoma · Inside out pattern · Epithelial membrane antigen · KL-6 · Fluorescence in situ hybridization

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### Introduction

The invasive micropapillary carcinoma (IMPC) pattern was first reported as an entity associated with poor prognosis and rapid progression in cancers of the breast [1, 2], urinary bladder [3–7], colon [8–11], ovary [12], salivary glands [13], bile duct [14], etc. In IMPC pattern, cancer cells were proliferated in morula-like cell clusters without fibrovascular stroma, surrounded by a clear space. Then, epithelial membrane antigen (EMA) stained linearly only on the outside of these cell clusters, manifesting “inside out pattern”. It is extremely important to recognize this pattern in various kinds of cancers, when detected. Indeed, IMPC in breast cancer was recently classified as one of the special breast cancer subtypes in the 17th edition of

“General Rules for Clinical and Pathological Recording of Breast Cancer” by The Japanese Breast Cancer Society, 2012.

In total, 15 cases of stomach cancer with this variant have been reported in the literature, including 4 cases from Japan [15–18] and 11 cases from Korea [19].

In the present study, 4 cases of stomach cancer exhibiting an IMPC pattern are reported, including an autopsy case with widespread organ metastases; these cases were analyzed by immunohistochemistry with various antibodies. Epithelial membrane antigen (EMA) is typically used to visualize the characteristic “inside out” pattern of IMPC [6–8, 15, 20]. Recently, we have reported that KL-6, but not CD10, is also effective in demonstrating the peculiar “inside out” pattern of IMPC in addition to EMA in breast and urinary bladder cancers [20].

Moreover, we compared the IMPC pattern in the stomach with patterns in breast, urinary bladder, and colon cancers using published immunohistochemistry data [7, 13–16, 19–21]. Interestingly, the IMPC pattern in stomach cancer was similar to the patterns in breast and urinary bladder cancers, but different from the patterns in colon cancers. Then, fluorescence in situ hybridization (FISH) analyses have been done in these reported cases to examine the amplification of HER 2 gene.

## Materials and methods

The clinicopathological data for the 4 cases in this study are summarized in Table 1. A recurrent lesion, manifesting as dominant IMPC, was found 2 years later in case 2. Lymph node metastases were found in cases 1, 3, and 4. Metastatic foci in the lymph nodes exhibiting the IMPC pattern were also examined in cases 3 and 4.

Tissues obtained by surgical operation or autopsy were fixed in 10 % formalin solution, followed by dehydration in graded alcohols and embedding in paraffin. Dewaxed sections were stained with hematoxylin and eosin (HE).

Immunohistochemical staining was then performed using the labeled streptavidin–biotin (LSAB) 2 kit/HRP

(Dako, Kyoto, Japan) with diaminobenzidine as the substrate for horseradish peroxidase in accordance with the kit manual [7, 20]. The antibodies used are summarized in Table 2. The main focus of immunohistochemical staining was on the definitive demonstration of the “inside out” pattern of IMPC, particularly with the HER2 antibody. FISH analyses of HER2 gene were performed using 6 materials of case 1, case 2, recurrent focus of case 2, case 3 with lymph node metastases, and case 4, employing the method previously reported [22]. More than 2.0 of FISH titer is decided to be positive for amplification of HER 2 gene as reported [22].

All patients participating in the present study provided written informed consent, and the identities of all patients have been strictly protected.

## Results

In the IMPC pattern, cancer cells proliferated in micronests, which were embedded in a complicated network of connective tissues not associated with stromal component. The cancer cells in this peculiar pattern were highly invasive, and were typically found in front of the deep invasive part of the stomach, forming a characteristic tubular pattern in the surface areas (Fig. 1a–c). As shown in Fig. 1a–c, the tubular adenocarcinoma pattern was usually detected in the mucosal and submucosal layers, but the IMPC pattern was observed in much deeper parts of proper muscle and/or subserosal layers. The IMPC pattern was dominant in a recurrent lesion in case 2 and in the metastatic foci in the lymph nodes in cases 3 and 4, and was usually intermingled with tub1 or tub2.

The immunohistochemical results of the present study are summarized in Table 3. Both EMA (Fig. 2a) and KL-6 (Fig. 2b) were positive in all the examined cases, including the recurrent lesion in case 2 and the metastatic foci in cases 3 and 4, and clearly exhibited an “inside out” pattern. EMA stained mainly the outer surface of cancer cells in the IMPC pattern (Fig. 2a), and simultaneous positive staining was observed in the cytoplasm on occasion. In contrast, KL-6

**Table 1** Clinicopathological data of four gastric cancer cases, containing invasive micropapillary carcinoma (IMPC) pattern

Case	Age	Sex	Location	Size (cm)	Histology	Lymph nodes (+/total)	Depth
1	65	M	Pylorus, duodenum	8 × 7	tub2 > pap > mpc	16/19	pT3 (SE)
2	68	F	Pylorus	2.2 × 1.2	tub1 > mpc	0/37	pT2 (SM2)
Recurrence of Case 2	70	F	Corpus	3.7 × 3.5	mpc > tub1	0/1	pT3 (SS)
3	79	F	Corpus	10 × 12	tub2 > mpc	37/49	pT3 (SS)
4	67	M	Whole stomach	Massive <sup>a</sup>	por1 > tub2 > mpc	32/33	pT4 (SI)

*mpc* micropapillary carcinoma

<sup>a</sup> Cancer infiltrated widely in the stomach

stained the outer plasma membranes linearly, but not the cytoplasm (Fig. 2b). MUC5AC (Fig. 2c) and MUC 1 (Fig. 2d) positively stained both the cell surface and

**Table 2** Antibodies used in the present study

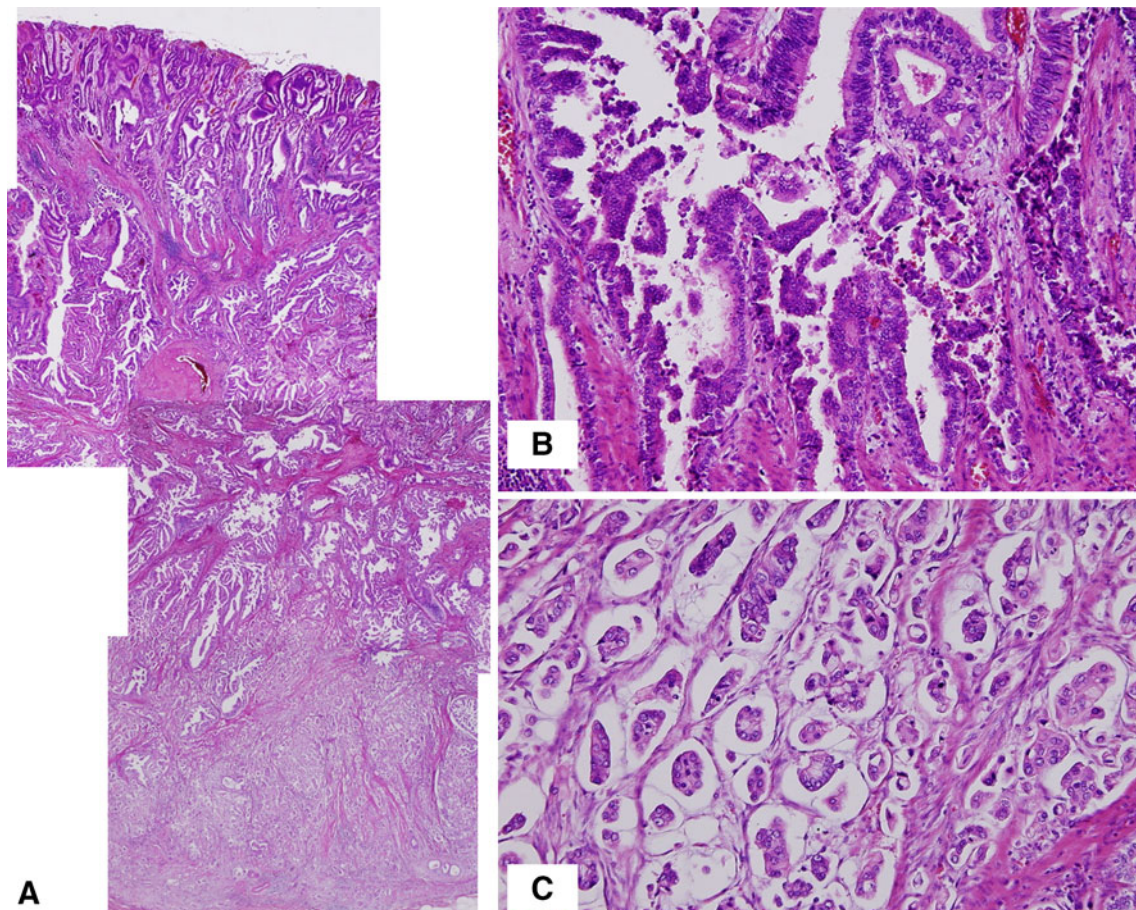
	Dilution		Pretreatment	Manufacturer
EMA	1:50	MW		Dako, Kyoto, Japan
MUC5AC	1:100	AC		Chemicon, CA, USA
MUC1	1:100	AC		BD Biosciences Pharmingen, San Jose, CA, USA
MUC2	1:50	AC		Novocastra, Newcastle, Upon Tyne, UK
KL-6	1:2560	NP		Provided by Eisai Co. LTD., Tokyo, Japan
CD10	Pre-diluted	AC		Novocastra, Newcastle, Upon Tyne, UK
HER-2	1:200	MW		Dako, Kyoto, Japan

EMA epithelial membrane antigen, MW microwave, AC autoclaving, NP no pretreatment

cytoplasm homogeneously in 3 of the 4 cases. CD10 staining was completely negative in all the examined cases (Fig. 2e).

Many definitive lymphatic invasions were found in the mucosal and submucosal layers, and cancer cells proliferated in the IMPC pattern within the lymphatics (Fig. 3a). D2-40 antibody staining was positive in the endothelial cells of these lymph vessels. In the IMPC component detected in the lymphatic invasion (Fig. 3b), the characteristic “inside out” pattern of IMPC was confirmed by staining with EMA and KL-6 antibodies. Peculiar linear positivity was detected on the outside of cancer cell micronests in the IMPC pattern with the EMA antibody (Fig. 3c), but more distinct linear positivity was observed in the KL-6 antibody (Fig. 3d).

Although MUC1 and MUC5AC were strongly positive in 3 of the 4 cases, they were not suitable for demonstrating the “inside out” pattern of IMPC because of their strong, but diffuse, staining pattern. MUC2 staining was completely negative in the IMPC pattern in all of the gastric cancer cases.



**Fig. 1** In a general view of the recurrent lesion in case 2 (a), an irregular tubular pattern was observed in the mucosal and submucosal layers (b), and the invasive micropapillary carcinoma (IMPC) pattern

was detected in the muscular and subserosal layers (c) in the gastric wall. a HE,  $\times 20$ ; b, c HE,  $\times 200$ . HE hematoxylin and eosin

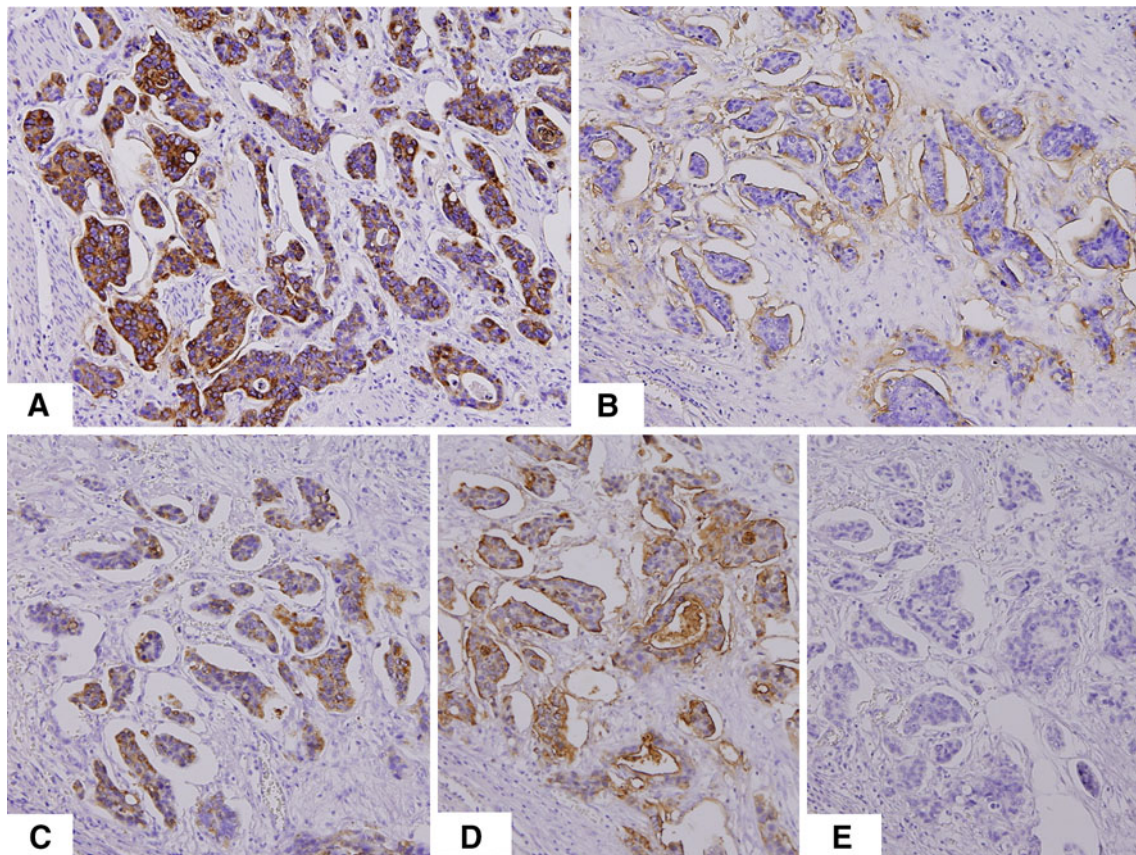
**Table 3** Immunohistochemical characteristics of the invasive micropapillary carcinoma pattern in gastric cancer

Case	Age	Sex	EMA	KL-6	MUC1	MUC5AC	CD10	MUC2	HER2
1	65	M	+	+	+	+	-	-	2+(2+) <sup>a</sup>
2	68	F	+	+	+	+	-	-	-(-)
Recurrence of Case 2	70	F	+	+	+	+	-	-	1+(2+)
3	79	F	+	+	-	+	-	-	2+(2+)
	LN meta		+	+	-	+	-	-	2+(2+)
4	67	M	+	+	+	-	-	-	-(-)
	LN meta		+	+	+	-	-	-	-(-)

Parentheses indicate the HER2 score in tubular patterns

*LN meta* Lymph node metastases

<sup>a</sup> HER2 score in the invasive micropapillary carcinoma (IMPC) pattern



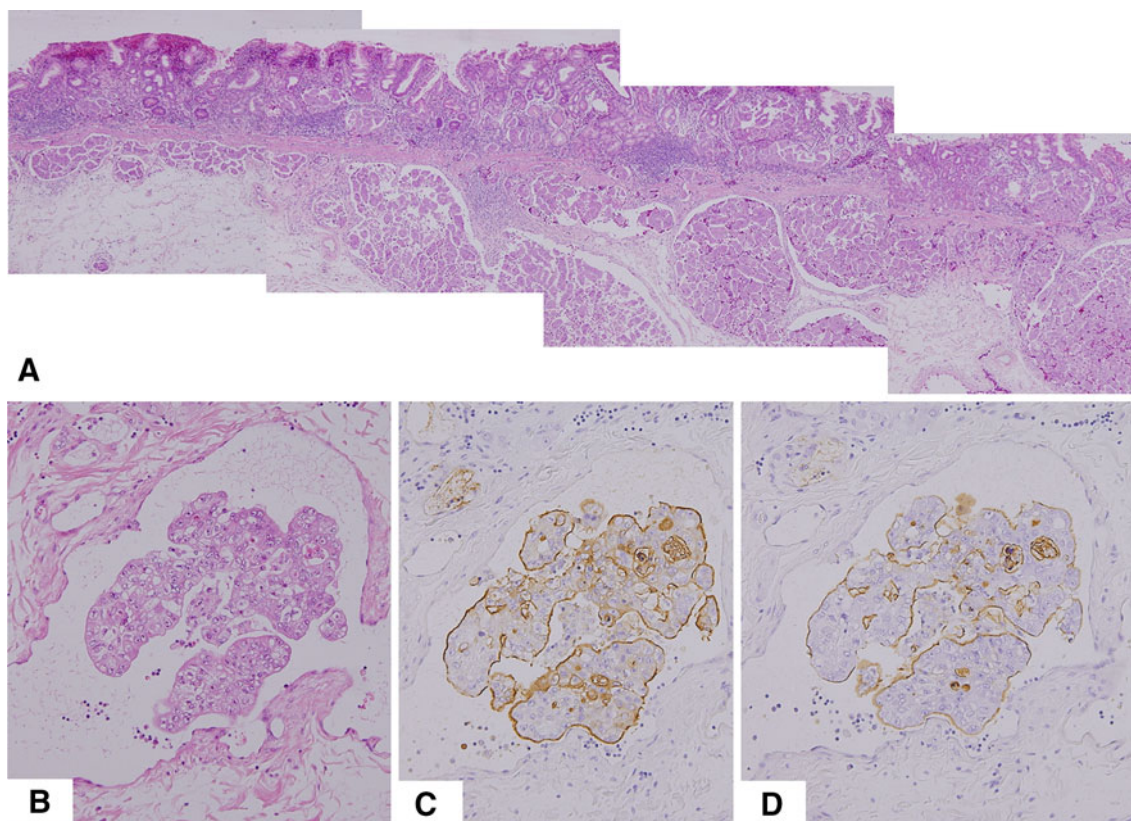
**Fig. 2** Immunohistochemical analysis of a serial section from case 1 demonstrated that the cancer cells in the invasive micropapillary carcinoma (IMPC) pattern were positive for EMA (a), KL-6 (b),

MUC5AC (c), and MUC1 (d), but negative for CD10 (e). a–e labeled streptavidin biotin complex (LSABC) method,  $\times 200$

HER2 staining was partially positive in the tubular pattern of cancer cells as well as in the IMPC pattern in case 1 (Fig. 4a–c), the recurrent lesion in case 2, and the lymph node metastases in case 3, in which the HER2 scores were 1+ or 2+. However, HER2 staining was negative in the original lesion in case 2 and in case 4. Interestingly, both positive and negative results were obtained in the IMPC pattern in 1 section from case 3, as shown in Fig. 4d,

e. Diffusely positive HER2 staining was not observed in all cases examined.

Thus, both EMA and KL-6 were effective in demonstrating the peculiar “inside out” feature in the IMPC pattern in all 4 cases of stomach cancer, including the recurrent lesion in case 2 and the lymph node metastases in cases 3 and 4; CD10 was not an effective marker of the “inside out” pattern in stomach cancer.



**Fig. 3** Marked lymphatic invasion of cancer cells was detected in the mucosal and submucosal layers in case 3, which proliferated in the invasive micropapillary carcinoma (IMPC) pattern (a). In the serial sections, the same region stained with HE (b), EMA (c), and

KL-6 (d) are shown. Note the linear staining of both EMA (c) and KL-6 (d), revealing the “inside out” pattern. **a** HE,  $\times 20$ ; **b** HE,  $\times 400$ ; **c**, **d** LSABC method,  $\times 400$

HER2 was positive in the IMPC patterns of the stomach in 3 out of 4 cases, including the metastatic foci of the lymph nodes of case 3 as shown in Table 3. FISH analyses disclosed the amplification of HER-2 gene in case 1 and both primary and lymph node metastatic foci of case 3, obtaining FISH titers 6.8 (Fig. 5), 2.4 and 2.5, respectively. Immunohistochemically, their HER-2 scores were 2+, as shown in Table 3. As for the primary and recurrent foci of case 2 and case 4 were negative for HER 2 gene amplification exhibiting FISH titers 1.4, 0.9 and 0.8, respectively. Thus, in total HER 2 gene amplification with FISH was positive in 3 out of 6 materials examined.

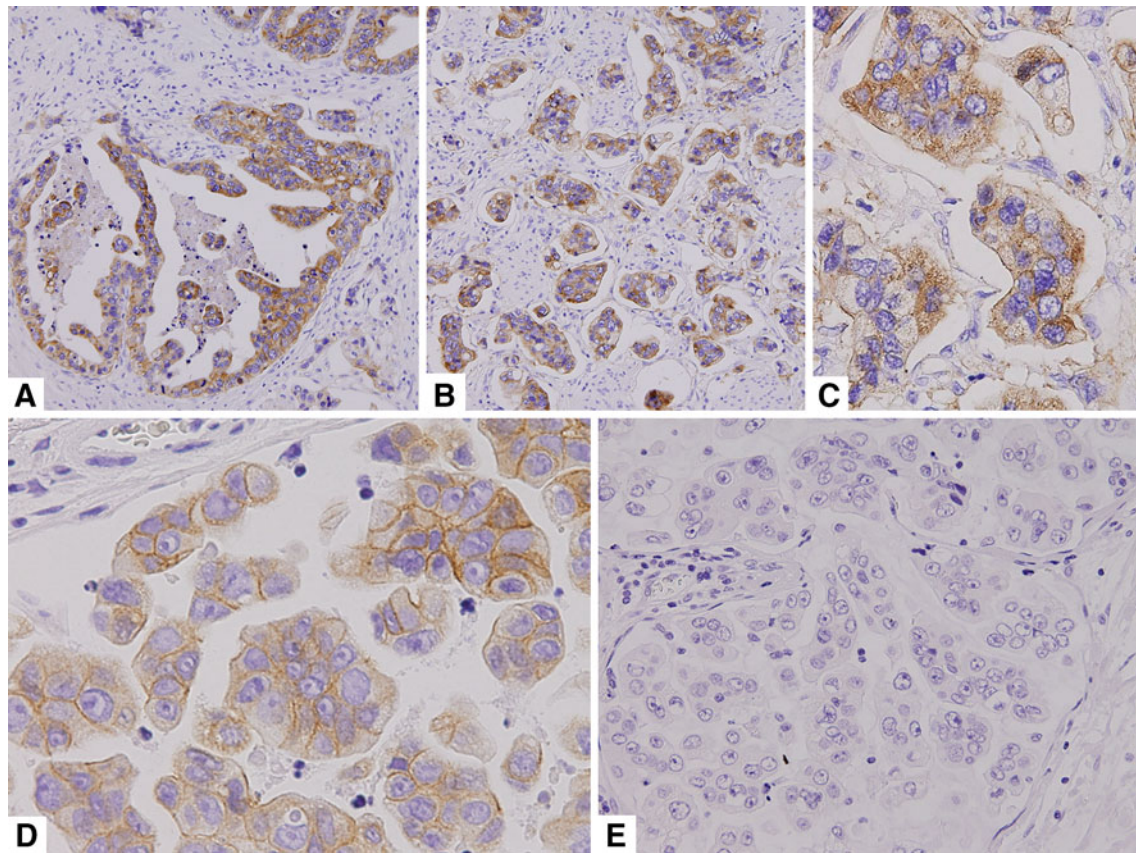
## Discussion

Cancers exhibiting the IMPC pattern have been reported not only in the stomach [15–18], but also in the breast [1, 2], urinary bladder [3–7], colon [8–11], salivary glands [13], etc. The IMPC pattern was generally thought to be associated with poor prognosis and aggressive behavior [4–6, 8, 9, 13, 14, 21]. The IMPC pattern is believed to exhibit an even poorer prognosis in the stomach, as shown in the

present autopsy case 4, but the evidence is inconclusive according to one report [19].

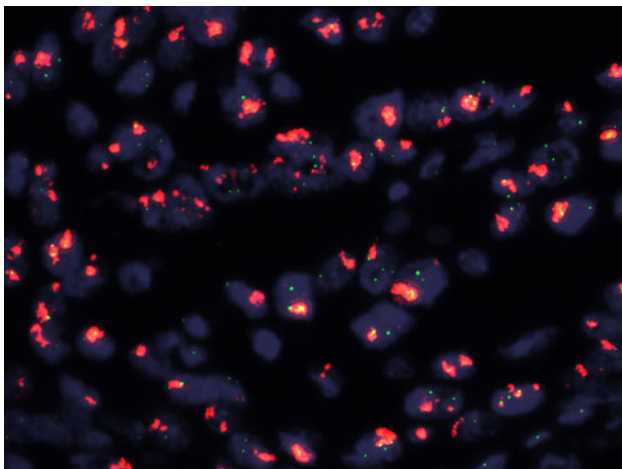
Extensive lymphatic invasions were detected in cases 3 and 4 in the present study, resulting in lymph node metastases in 3 out of 4 cases, as shown in Table 1. It was reported that all 14 cases of salivary gland carcinoma containing the IMPC pattern exhibited lymph node metastases, and were associated with poor prognosis and rapid clinical course [13]. Bile duct carcinoma containing the IMPC pattern was reported to exhibit similar behavior [14]. The proportion of IMPC in cancer tissues varies from case to case, from less than 10 % to almost 100 % [6, 9]. The IMPC pattern is typically detected in the leading front of cancer invasion [19, 23], was shown in case 2 (Fig. 1a).

Immunohistochemically, cancer cells in the IMPC component have been reported to be similar to the original cancer cells [6, 9]. A peculiar “inside out” pattern of IMPC was clearly demonstrated by staining with an EMA antibody. This finding is of great importance for identifying the IMPC component, as demonstrated previously [8, 15, 20]. In the present study, KL-6 was linearly positive along the surface of the IMPC pattern, as previously reported [20]. KL-6 was also a good marker for demonstrating the “inside



**Fig. 4** HER2 staining exhibited irregular tubular (a) and invasive micropapillary carcinoma (IMPC) (b, c) patterns in case 1. In the same section from case 3, HER2 staining revealed distinctly positive

(d) and completely negative (e) regions. a, b LSABC method,  $\times 200$ ; c, d, e LSABC method,  $\times 400$



**Fig. 5** In case 1, FISH titer of HER 2 gene were 6.8, demonstrating strong amplification of HER-2 gene, in which tubular and micropapillary patterns were both HER-2 score 2+ by immunohistochemistry as shown in Fig. 4a–c

out” pattern of IMPC in breast and urinary bladder cancers [20], similar to EMA, and also demonstrated this peculiar pattern in the stomach, effectively. As observed in the present cases, EMA was able to stain the IMPC pattern and

occasionally exhibited cytoplasmic positivity in cancer cells [20].

In the present cases of gastric cancer with IMPC pattern, both MUC-1 and MUC5AC were positive in 3 out of 4 cases, but MUC-2 was negative in all cases (Table 3). Previous reports of the IMPC pattern in stomach cancers indicated that MUC-1 was positive [16], which reveals reverse polarity, but negative in another case [15]. MUC-2 was negative in all previously reported cases [16, 19], while MUC5AC was mostly positive [15, 16, 19]. Although CD10 was reported to be positive in colon cancers with the IMPC pattern [11, 20], none of the present 4 cases in the stomach were positive for this antibody, as previously reported [19]. Therefore, CD10 was useful for demonstrating the “inside out” pattern in colon cancers [11, 20]. In this sense, the IMPC pattern of stomach cancer was similar in morphology, but different in nature, to that of colon cancer, and immunohistochemically similar to breast and urinary bladder cancers, as reported [20].

In the present study, HER2 staining in both primary and metastatic foci was identical in cases 1 and 3 (Table 3), but the primary focus was negative and only the recurrent focus was positive in case 2. This suggests that the

recurrent lesion expressing HER2 may have been more aggressive in its biological behavior. The negative results of the autopsy case (case 4) may be due to the inhibition of HER2 staining as a result of the tissue processing techniques used. In previous reports, none of 13 cases were positive for HER2 staining in stomach cancers with the IMPC pattern [10, 16], while 7 out of 14 cases of salivary gland cancer were positive [13]. From these results, HER2 positivity might be different in each organ that is examined. Although positive results for HER2 staining were obtained in the IMPC pattern in 3 out of 4 cases, more accumulated cases should be examined. At present, targeted therapy with HER2 antibody for stomach cancer, as reported recently [21], is proving difficult.

In the literature, it was suggested that diminished  $\beta$ -catenin expression might be related to tumor growth of IMPC pattern and progression [16], that aberrant expression of HER-2 and p53 was reported in IMPC pattern in salivary gland cancers [13], and that MLH-1, MSH-2 and p53 expressed in colon cancers with no statistical significance between IMPC-positive and negative groups [9]. Moreover, it was also reported that E-cadherin was downregulated in relation with tumor proliferation in stomach cancer [15], but it was denied in another report [18].

Although HER-2 gene has been amplified in cases 1 and 3 including lymph node metastatic foci by FISH method, it is also of interest that IMPC patterns in these cases were both HER-2 score 2+ by immunohistochemistry. The present report is the first one, demonstrating HER2 gene amplification in stomach cancers with IMPC pattern by FISH. We need to confirm these facts using more accumulated cases in future.

The IMPC component should be recognized as one of the histological markers of poor prognosis and aggressive clinical course in stomach cancers. Clinicians should be aware of the presence of the IMPC component in gastric cancers.

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