Incomplete inside-out growth pattern in invasive breast carcinoma: association with lymph vessel invasion and recurrence-free survival

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Abstract

Invasive micropapillary carcinoma (IMPC) is a rare subtype of epithelial tumor of the breast listed in the 2003 World Health Organization histologic classification of tumors of the breast. It is characterized by inside-out micropapillary morphology, frequent lymph vessel invasion (LVI), and lymph node metastasis; however, its etiology remains unknown. This study investigated the incomplete inside-out growth pattern (IGP) in invasive ductal carcinoma, not otherwise specified (NOS), and examined the association between incomplete IGP and clinicopathologic features, including the presence of intratumoral lymph vessels (ILV), LVI, nodal metastasis, and prognosis. Tumor tissues from 166 invasive duct carcinomas NOS and 10 IMPCs were immunostained using an anti-epithelial membrane antigen antibody to detect IGP, and with D2-40 antibody to determine the presence of ILV and LVI. Incomplete IGP was detected focally in 88 (53%) of 166 invasive duct carcinomas NOS. Transition areas between IMPC and invasive duct carcinoma NOS also showed prominent incomplete IGP in nine (90%) of 10 IMPCs. Incomplete IGP in invasive duct carcinomas NOS was associated with larger tumor size, higher frequencies of ILV, LVI, nodal metastasis, and poorer recurrence-free survival by univariate analysis. Incomplete IGP, ILV, and tumor size independently affected LVI by multivariate analysis. These findings indicate that incomplete IGP of tumor cell clusters is not uncommon, and is a useful tool for predicting LVI in invasive duct carcinoma NOS of the breast.

Key words: Breast cancer; Incomplete inside-out growth pattern; Lymph vessel invasion; Intratumoral lymph vessel; Invasive micropapillary carcinoma; Prognosis

Introduction

Several pathways mediate tumor dissemination, including local tissue invasion, direct seeding of body cavities or surface, and lymphatic or hematogenous spread [1]. Lymph node metastasis is known to be the most important independent prognostic factor in breast cancer. Invasion of tumor cells into lymphatic vessels is a critical step in metastasis, and lymph vessel invasion (LVI) is a predictor for axillary lymph node metastasis and a prognostic factor in node-negative cancer [2–8]. LVI can also be used to determine the appropriate indications for chemotherapy [7–9].

The mechanisms of lymphatic tumor spread have still not been clarified. Recent studies have indicated that lymphangiogenesis occurred in breast cancer, and that specific lymphangiogenic factors, such as vascular endothelial growth factors (VEGF)-C and D, were expressed [2–4]. However, other immunohistochemical studies have found no evidence of proliferation in lymphatic vessels [10, 11]. Thus the development of lymph vessels within breast tumors, and their relation to LVI and subsequent nodal metastasis, remain controversial [1, 12].

Invasive micropapillary carcinoma (IMPC) is a rare subtype of epithelial tumor of the breast listed in the 2003 World Health Organization (WHO) histologic classification of tumors of the breast [13]. It is characterized by aggregates of small clusters of tumor cells lying within clear stromal spaces resembling dilated vascular channels, reversed polarity, an “inside-out” micropapillary morphology, and frequent LVI and lymph node metastasis [13–17]. The term “inside-out growth pattern” and its clinicopathologic significance in breast cancer were first introduced by Peterse [18]. Further studies described the external surface of the tumor cell clusters as identical to a true luminal surface, and characterized by the presence of microvilli on electron microscopy and immumoreactivity to epithelial membrane antigen

(EMA) [19]. Gradual or abrupt transitions from typical invasive ductal carcinoma to the micropapillary components are observed in non-pure IMPC [13]. Routine pathologic examination has shown that these transition areas in IMPC frequently include tumor cell clusters with an incomplete inside-out growth pattern (IGP); “incomplete” IGP was defined in the present study as the presence of tumor cell clusters that were partly, but not completely fringed with strong EMA immunostaining facing the stroma. Incomplete IGP was also noted in invasive ductal carcinoma, not otherwise specified (NOS), and we therefore hypothesized that invasive duct carcinoma NOS with incomplete IGP would tend to develop LVI and nodal metastasis, simulating IMPC.

The purpose of this study was to investigate the correlation between incomplete IGP and the presence of intratumoral lymph vessels (ILV), LVI, and nodal metastasis in invasive duct carcinoma NOS of the breast. We also evaluated the prognostic value of incomplete IGP, and comment on a specific case that provides potential insight into the mechanisms of LVI.

Materials and methods

Patients

A total of 197 patients with invasive ductal carcinoma of the breast who underwent surgery from 2000 to 2004 were retrospectively analyzed. The study materials represented 67% (197/296) of all new breast carcinomas diagnosed in the same period. Complete axillary lymph node resection was performed in 98% (194/197), and no sentinel node biopsy was carried out in this period. Patients with bilateral breast cancer (synchronous, metachronous), clinically multifocal or multicentric tumors in the unilateral breast, distant metastasis, or malignancy at another site were excluded, together with patients who had received preoperative neoadjuvant chemotherapy. Special histologic types other than IMPC were excluded from the immunohistochemical analyses.

Clinicopathologic features

Tumor size was measured as the largest dimension of the microscopic invasive component in pathologic sections. All tumors were graded according to a modified version of the Bloom-Richardson grading system [20]. Immunohistochemistry for hormone receptors (estrogen and progesterone; Dako, Tokyo, Japan) and Her2/new status (Dako) were evaluated at the time of diagnosis. Tumors with staining of >10% of tumor cell nuclei were defined as positive for hormone receptors. Her2/neu immunostaining was classified according to the Dako-HercepTest scoring system. All those cancers scoring equivocal (2+) were subjected to Her2/neu gene amplification analysis using fluorescence in-situ hybridization (FISH) (Path Vysion; Abbott, USA) at the time of diagnosis in three cases and at the time of study in nine

cases. When the fluorescent signal ratio of Her2/chromosome 17 exceeded 2.0, the sample was judged to be FISH-positive. Her2 status was divided into three categories: negative, 0/1+ and 2+ (FISH-negative); positive, 3+ and 2+ (FISH-positive); and not examined.

Immunohistochemical staining

Formalin-fixed, paraffin-embedded blocks from primary tumors were available for all patients. Three 4-μm thick serial sections were prepared from one representative block for each tumor. Sections from all cases were stained with hematoxylin and eosin (H&E), anti-epithelial membrane antigen (EMA) antibody, and D2-40 antibody. The streptavidin-biotin-immunoperoxidase method (Histofine SAB-PO kit; Nichirei, Tokyo, Japan) was used for immunohistochemistry. The epitope for D2-40 was retrieved by autoclaving the sections at 120°C for 30 min. No antigen retrieval was used for EMA immunostaining. Endogenous peroxidase was inhibited by 3% hydrogen peroxidase in methanol for 15 min. The sections were incubated with anti-EMA (prediluted; Nichirei) or D2-40 (1: 100 dilution; Dako) antibodies overnight at 4°C. After staining with 3,3’-diaminobenzidine, the sections were counterstained with hematoxylin for visualization of the nucleus. Interpretation of histologic and immunohistochemical specimens was made without knowledge of the other clinical or pathologic findings.

Criteria for IMPC and invasive duct carcinoma NOS with incomplete IGP

The luminal surface of invasive duct carcinomas NOS usually showed dense eosinophilic staining by H&E staining (Fig. 1a), and the surface consistently revealed strong membranous reactivity by EMA immunostaining (Fig. 1b). According to the WHO classification, we

defined IMPC by H&E staining as a carcinoma consisting of small clusters of tumor cells lying within clear stromal spaces resembling dilated vascular channels (Fig. 1c) [13]. In addition, IMPC in the present study was also restricted to an aggregate of three or more micropapillary clusters that were entirely fringed with strong EMA immunostaining (complete IGP) (Fig. 1d). An invasive carcinoma was classified as IMPC if it had at least one focal IMPC component.

Invasive duct carcinoma NOS with incomplete IGP was defined as the presence of tumor cell clusters that were partly, but not completely fringed with strong EMA immunostaining (incomplete IGP) facing the stroma (Fig. 1e–h). Single cell or single file infiltration of tumor cells was excluded from incomplete IGP. Simultaneous D2-40 immunostaining was used to exclude lymphatic tumor embolism (Fig. 1g). Tumors were considered to be incomplete IGP-positive if at least one incomplete IGP was found.

Definition of ILV and LVI

Obliterated lymph vessels were frequently detected within and beside the breast tumor by immunostaining with D2-40 antibody (Fig. 2a), while these vessels were rarely found in non-neoplastic breast tissue far from the tumor. Since these obliterated vessels might not be involved in lymphogenous metastasis, only opened lymph vessels were used to evaluate the presence or absence of intratumoral lymph vessels by D2-40 immunostaining (Fig. 2b). The term “intratumoral” included the borderline zone between the tumor and the surrounding non-neoplastic tissue in the present study. Tumors were considered to be ILV-positive when the endothelial cells in at least one opened vessel were immunostained with D2-40 antibody in the primary invasive tumor area.

LVI was defined as the presence of tumor cells surrounded by lymphatic endothelial cells that were immunostained with D2-40 antibody.

Statistical analysis

Differences between groups were analyzed using χ^2 tests and Student's *t*-tests. Multivariate analysis was conducted to investigate the factors influencing the presence of ILV, LVI, and nodal involvement. Survival curves for cancer-specific survival were constructed using the method of Kaplan and Meier, and the differences between the two groups were assessed using the log rank test. Disease-specific survival was calculated as the period from surgery to death of breast cancer. Probability (*p*) values were calculated using Stat View 5 software (SAS Institute, Cary, NC, USA). A p value of <0.05 was considered statistically significant.

Results

Clinicopathologic features

A total of 197 patients with invasive breast carcinoma were included in the present study. The median age at diagnosis was 54 years (range 32–96 years). Tumors ranged in size from 0.1–5.0 cm (median 1.6 cm). Of the patients with nodal resection (*n*=194), 61% (*n*=118) were node-negative and 39% (*n*=76) were node-positive. The frequencies of different histologic types were: invasive duct carcinoma NOS 84% (*n*=166), IMPC 5% (*n*=10), mucinous carcinoma 2% (*n*=4), invasive lobular carcinoma 4% (*n*=8), and others 5% (*n*=9). All IMPCs were mixed (non-pure) type, with the percentage of IMPC ranging from 5–75%. Among the patients with invasive duct carcinoma NOS, 162 (98%) of 166 were followed up until October 31, 2009, with a median follow-up period of 72 months (range, 8–111 months). The pTNM0 stages of the 166 invasive duct carcinomas NOS/10 IMPCs were as follows: pT1N0, 86/4; pT2N0, 12/2; pT4N0, 3/1; pT1N+, 33/3; pT2N+, 23/0; pT4N+, 7/0; unknown, 2/0.

Detection of IGP

All of the breast carcinomas showed EMA immunostaining in the cytoplasm and cytoplasmic membrane of the neoplastic cells. Staining intensity varied among the tumors, and occasionally differed within the same tumor. Since only the luminal surface of the ductal tumor cells consistently showed strong membranous EMA immunostaining at the cytoplasmic membrane, incomplete IGP could be detected even when typical micropapillary morphology was absent (Fig. 1h). Incomplete IGP was found focally in 88 (53%) of 166 invasive duct carcinomas NOS (Table 1). Each incomplete IGP was very limited, and occupied <5% of the

primary tumor area in the majority of cases (Fig. 1). The cluster with incomplete IGP was partly fringed with linear EMA immunostaining, which was partly surrounded by retraction cleft, or completely surrounded by clear stromal spaces (Fig. 1e–h). Among 10 IMPCs, one showed a nodular micropapillary component with abrupt transition to mucinous carcinoma. The remaining nine (90%) showed gradual transitions from micropapillary carcinoma to conventional ductal carcinoma, or a mixture of these components (Fig. 3a). These nine IMPCs consistently showed prominent incomplete IGP at transition areas, which were sometimes accompanied by adjacent tiny clusters or isolated tumor cells with inflammatory infiltrate (Fig. 3a–d). Incomplete IGP was also detected by H&E staining alone, which identified the presence of clusters with a sharp, dense eosinophilic surface accompanied by the retraction clefts (Fig. 3c).

Detection of ILV

In contrast to non-neoplastic breast tissue, D2-40 immunostaining of the primary tumor area revealed a marked decrease or disappearance of pre-existing lymph vessels, such as perivascular and periductal lymph vessels, in all patients. ILV was detected in only 73 patients with invasive duct carcinoma NOS (73/166, 44%) by D2-40 immunostaining (Table 1), and most of these were located at the periphery of the tumors (Fig. 2). Relatively abundant ILV, suggestive of lymphangiogenesis, was occasionally noted in granulation tissue after preoperative biopsy and in areas of inflammatory infiltration.

Relationship between incomplete IGP and clinicopathologic parameters

As shown in Table 1, patients with incomplete IGP-positive tumors had a high frequency of ILV (55/88, 63%) compared with incomplete IGP-negative patients (18/78, 23%; $p<0.0001$). Furthermore, incomplete IGP was significantly associated with higher frequencies of LVI (54/88, 61%; $p<0.0001$), and lymph node metastasis (46/87, 53%; $p<0.0001$). When pT1 tumors ($n=120$) were separately analyzed, incomplete IGP also influenced these factors; incomplete IGP-positive pT1 tumors showed higher frequencies of ILV (26/54, 48%; $p=0.006$), LVI (31/54, 57%; $p<0.0001$), and nodal metastasis (22/54, 41%; $p=0.001$) compared with incomplete IGP-negative pT1 tumors (not shown in Table 1). Pathologic tumor size was also related to the presence of incomplete IGP ($p<0.0001$). There were no significant correlations between incomplete IGP and patient age, histologic grade, hormone receptor status, or HER2 status (Table 1). The locations of incomplete IGP, ILV, and LVI were not always identical in the same tumor. However, one case revealed a close sequential association between incomplete IGP and LVI (Fig. 4a–d); a few tiny clusters with incomplete IGP were located in a clear stromal space. These clusters migrated via lymphatic drainage into an orifice of the narrow portion of a funnel-shaped lymph vessel, and a floating micropapillary cluster was formed in the dilated portion of the lymph vessel (Fig. 4a–d).

Comparison between invasive duct carcinoma NOS with incomplete IGP and IMPC

As shown in Table 1, the clinicopathologic features of IMPC were similar to those of invasive duct carcinoma NOS with incomplete IGP. There were no significant differences in clinicopathologic features between the two groups (age, $p=0.23$; tumor size, $p=0.56$; histologic grade, $p=0.34$; estrogen receptor, $p=0.27$; progesterone receptor, $p=0.32$; Her2 status, $p=0.25$; ILV, $p=0.74$; LVI, $p=0.56$; nodal metastasis, $p=0.17$; not shown in Table 1).

Multivariate analysis of ILV, LVI, and nodal metastasis

Multivariate analysis of factors associated with the presence of ILV, LVI, and nodal metastasis are shown in Table 2. Pathologic tumor size (p=0.002) and incomplete IGP (p<0.0001) were significant independent factors influencing the presence of ILV. Tumor size (p=0.049), incomplete IGP (p=0.0001), and the presence of ILV (p=0.0002) were significantly associated with LVI. Furthermore, LVI (p=0.004) and pathologic tumor size (p=0.005) were independent predictors for nodal involvement (Table 2).

Survival data for invasive duct carcinoma NOS with incomplete IGP and IMPC

Follow-up data were available for a total of 162 patients with invasive duct carcinoma NOS and 10 patients with IMPC. The 5-year and 8-year recurrence-free survival rates were 81% and 70% (p=0.0356), respectively, in patients with incomplete IGP-positive invasive duct carcinoma NOS, and 91% and 89%, respectively, in patients with incomplete IGP-negative invasive duct carcinoma NOS (Fig. 5a). There was no significant difference in disease-specific survival between patients with or without incomplete IGP (Fig. 5b). Only one of 10 patients with IMPC had recurrence and died of breast cancer; the remaining nine patients were alive and tumor-free at the end of follow-up.

Discussion

IMPC is quite rare in its pure form, but focal micropapillary growth has been reported in 3–6% of more common types of invasive carcinomas [13]. Based on the strict morphologic and immunohistochemical definitions of IMPC used in this series, IMPC accounted for 5% of invasive breast carcinomas, while incomplete IGP was detected by immunostaining in 53% of invasive duct carcinomas NOS. The microscopic differential diagnosis of incomplete IGP includes tumor embolism of lymph vessels and retraction artifacts with no evidence of micropapillary carcinoma [21]. Serial sections immunostained with anti-EMA and D2-40 antibodies can distinguish incomplete IGP from lymphatic tumor embolism and simple retraction artifacts.

IMPC generally shows frequent LVI and lymph node metastasis [13–17]; however, our 10 IMPC cases showed relatively low frequencies of LVI (50%) and nodal involvement (30%). The reason for this is unclear, but it may be related to the small number of cases and the histologic features [22]. Invasive duct carcinoma NOS with incomplete IGP, as expected, showed high frequencies of LVI (61%) and nodal metastasis (53%). This feature was retained even when only small tumors (pT1) were analyzed, resembling those of IMPC cases reported by Walsh and Bleiweiss [23]. Furthermore, nine of 10 IMPCs showed gradual transitions from invasive duct carcinomas NOS to micropapillary carcinomas, and these nine IMPCs consistently showed prominent incomplete IGP in the transition areas. This close relationship between IMPC and invasive duct carcinoma NOS with incomplete IGP suggests that both morphologic entities form a histologic spectrum characterized by varying degrees of a common inside-out morphology.

The biological significance and mechanisms of inside-out morphologic change have not been clarified. EMA is thought to include episialin (MUC-1), a glycoprotein normally located

on the apical cell surface of normal glandular epithelium [24]. In vitro studies have shown that increased MUC-1 expression was associated with decreased adhesion between adjacent cells, and between cells and the extracellular matrix [25–27]. The current study revealed that complete or incomplete IGP in either IMPC or invasive duct carcinoma NOS was consistently accompanied by clear spaces or retraction clefts between the cytoplasmic membrane and the surrounding stroma. This suggests that an inside-out morphologic change is at least partly responsible for the detachment of tumor cells from the stroma. As we identified an incidental case with a connection between a clear space and a D2-40 positive lymphatic vessel with draining tumor cells, it is possible that the clear spaces share the “pre-lymphatic channels” connecting to true lymphatics described by some investigators [28–30].

Although the reasons for the effects of incomplete IGP on the presence of ILV are unclear, tumor-stromal interactions appear to be involved. Invasive duct carcinoma NOS with incomplete IGP may potentially be less damaging to lymph vessels because of less remodeling of the extracellular matrix at the invasive front [31–33]. Reduced adhesiveness between the tumor cells and the stroma may contribute to the creation of this conservative microenvironment. The regulation of the expressions of cell-matrix adhesion molecules such as integrins may also be involved [32–34]. In contrast to IMPC and invasive duct carcinoma NOS with incomplete IGP, tubular carcinoma, a distinctive variant of ductal carcinoma, is characterized by well-formed neoplastic glands with good polarity, altered collagenous stroma, and virtually no lymphatic tumor emboli [35].

LVI in breast cancer potentially represents the divergence from local to systemic disease, and at the St Gallen meeting in 2009, extensive peritumoral vascular invasion was listed as one of the useful factors for identifying patients who required chemotherapy in addition to endocrine therapy [9]. We have previously identified a possible characteristic of local disease, which is histologically recognized as early invasive breast carcinoma without marginal

adipose tissue invasion or peritumoral LVI [8]. Several investigators have reported the occurrence of lymphangiogenesis in breast cancer and have suggested that it plays an important role in disease progression [2–4]. We speculated, however, that most lymph vessels within breast carcinoma tissue are pre-existing, entrapped vessels, rather than newly formed ones [10, 11]. This is based on several facts: 1) we found no apparent increase in lymph vessel number within tumor tissues, compared with normal breast tissue, except under special conditions, such as in granulation tissue after preoperative biopsy; 2) ILV were detected in only 44% of patients with invasive duct carcinoma NOS, and most of these were small in number and present at the periphery or border of the tumor, as shown by Mohammed et al [4]; and 3) in contrast to normal breast tissue, occluded lymph vessels were frequently found within or beside the tumor, suggesting obliteration, destruction, and subsequent disappearance of lymph vessels by the growing tumor. This fragile nature of lymph vessels, compared with blood vessels, might be explained by their simple structure, with fenestrated or absent basement membranes and no covering pericytes [36]. Consequently, lymphatic endothelial cells are in direct contact with the extracellular matrix at least in part, and are thus likely to be influenced by the microenvironment, such as the remodeling of the matrix accompanying the tumor invasion [31–33].

The majority of histologic LVI reported are tumor embolisms in lymph vessels, and the mechanism of LVI, including the entrance of tumor cells into the lymph vessels, has not been clearly demonstrated to date. In the current study, we incidentally observed an instantaneous picture of tumor cells entering a lymph vessel, which could help to elucidate the process of LVI associated with incomplete or complete IGP occurring in breast carcinomas. The suggested process is summarized as follows: a tumor cell cluster with IGP detaches itself from the stroma, then decomposes into tiny clusters or isolated cells, simulating epithelial-mesenchymal transition [34, 37], in a clear stromal space beside an opened lymph

vessel. The tumor clusters and cells then migrate via the lymphatic drainage into the narrow entrance of the lymph vessel, like immune cells homing to the lymphatics (drainage theory) [38–40]. Finally, they remain in the dilated lymph vessel in the peritumoral area to reconstruct a cluster with IGP. These intralymphatic tumor cells and clusters would provide a source of lymph node metastasis. The peritumoral LVI detected in conventional H&E specimens likely corresponded to this last event. The small clusters or isolated tumor cells with inflammatory infiltrate occasionally observed beside the clusters with incomplete IGP in invasive duct carcinoma NOS and IMPC also support this drainage theory. Interestingly, Guo et al [22] described lymphocytic infiltration of IMPC as one of the key factors influencing lymph node metastasis.

In conclusion, incomplete IGP is common in invasive duct carcinoma NOS and frequently occurs in transitional areas of non-pure IMPC. Incomplete IGP is a useful tool for predicting LVI, and inside-out morphologic changes of tumor cell clusters may be an initial critical step in LVI in invasive ductal carcinomas. Further investigations, especially into the molecular mechanisms of LVI, are required to aid in the development of new therapeutic strategies.

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Conflict of interest statement

We declare that we have no conflicts of interest.

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Table 1 Clinicopathologic parameters of invasive duct carcinoma NOS with or without IGP (*n*=166) and IMPC (*n*=10)

Parameters	Invasive duct carcinoma NOS (*n*=166)			IMPC
	Incomplete IGP (+) (*n*=88)	Incomplete IGP (-) (*n*=78)	*p*a	
Age (years)[b]	55.6±11.3	56.8±13.5	0.56	60.3±13.6
Tumor size (cm)[b]	1.9±0.7	1.4±0.8	<0.0001	1.8±0.5
Histologic grade				
I	23	29	0.22	4
II	38	28		4
III	27	21		2
Estrogen receptor				
Negative	23	24	0.51	1
Positive	65	54		9
Progesterone receptor				
Negative	30	33	0.28	5
Positive	58	45		5
Her2 status				
Negative	54	59	0.14	6
Positive	12	6		4
Not examined[c]	22	13		0
ILV				
Absent	33	60	<0.0001	4
Present	55	18		6
LVI				
Negative	34	64	<0.0001	5
Positive	54	14		5
Nodal metastasis				
Negative	41	60	<0.0001	7
Positive	46	17		3
Not resected	1	1		0

NOS, not otherwise specified, *IMPC* invasive micropapillary carcinoma, *IGP* inside-out growth pattern, *ILV* intratumoral lymph vessel, *LVI* lymph vessel invasion. a*p* values show significant difference between incomplete IGP-positive and incomplete IGP-negative, [b]mean±standard deviation, [c]not examined cases were excluded from the statistical analyses

Table 2 Multivariate analysis of factors associated with the presence of ILV, LVI, and nodal metastasis in invasive ductal carcinoma NOS (*n*=164)

	p value (odds ratio)		
Factors	ILV	LVI	Nodal metastasis
Tumor size	0.002 (2.35)	0.049 (1.76)	0.005 (2.27)
Incomplete IGP	<0.0001 (4.37)	0.0001 (4.58)	0.08 (2.03)
ILV	na	0.0002 (3.67)	0.19 (1.57)
LVI	na	na	0.004 (2.35)

ILV intratumoral lymph vessel, *LVI* lymph vessel invasion, *IGP* inside-out growth pattern, *na* not adopted for each multivariate analysis

Figure captions

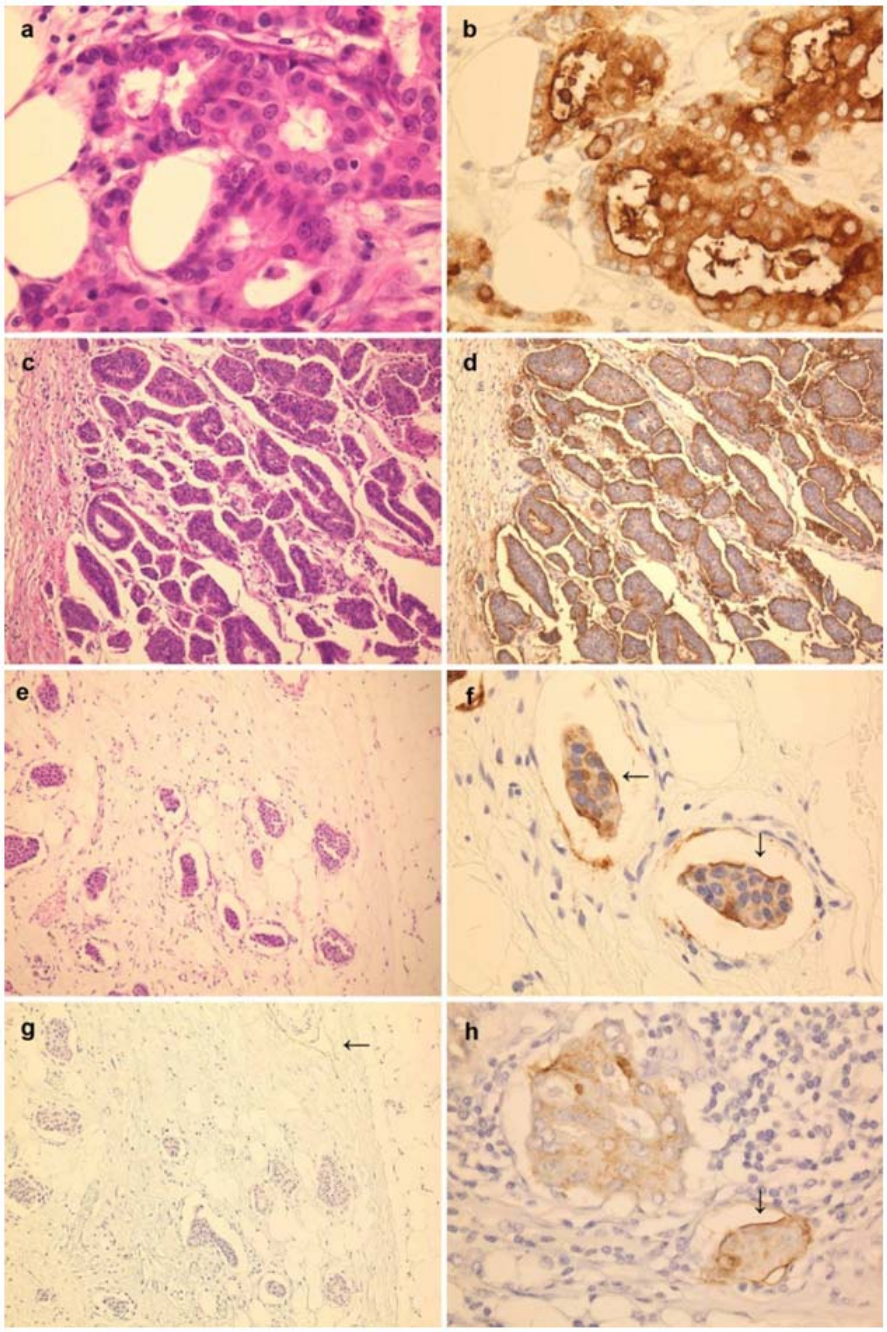

Fig. 1 a Invasive duct carcinoma NOS without IGP showing dense eosinophilic staining of the luminal surface (X 400). **b** Immunostaining for EMA showing membranous reactivity in the same part of the invasive duct carcinoma NOS (X 400). **c** IMPC consisting of aggregates of numerous micropapillary cell clusters within clear spaces (X 100). **d** Immunostaining for EMA clearly showing encircled membranous reactivity (complete IGP) in the majority of clusters (X 100). **e** Invasive duct carcinoma NOS with incomplete IGP showing isolated clusters surrounded by clear spaces (X 100). **f** Two of the clusters seen in Fig. 1e showing focal membranous reactivity (arrows) on their surface by EMA immunostaining (X 400). **g** Same area of Fig. 1e showing positive reactivity of the perivascular lymph vessel (arrow) but negative reactivity around the clusters by immunostaining with D2-40 (X 100). **h** Invasive duct carcinoma NOS with IGP showing a tiny cluster with focal membranous EMA immunostaining facing retraction cleft (arrow) (X 400)

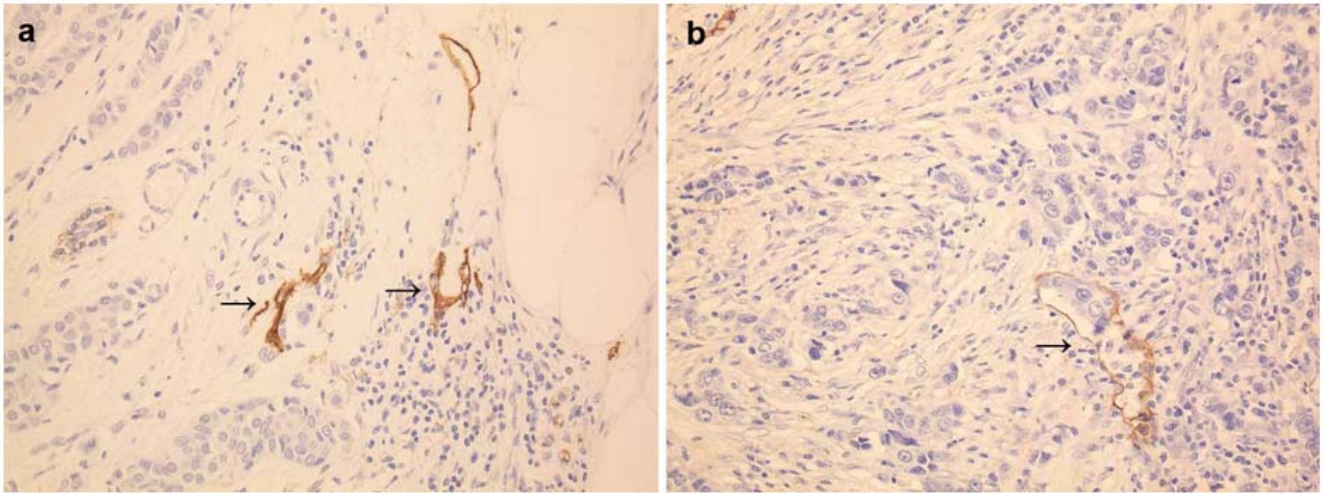

Fig. 2 a Absence of intratumoral lymph vessels. Immunostaining with D2-40 showing obliterated lymph vessels (arrows) in the borderline and peritumoral areas (X 200). **b** Presence of intratumoral lymph vessels. D2-40 immunostaining shows an opened lymph vessel containing some tumor cells in the tumor (arrow) (X 200)

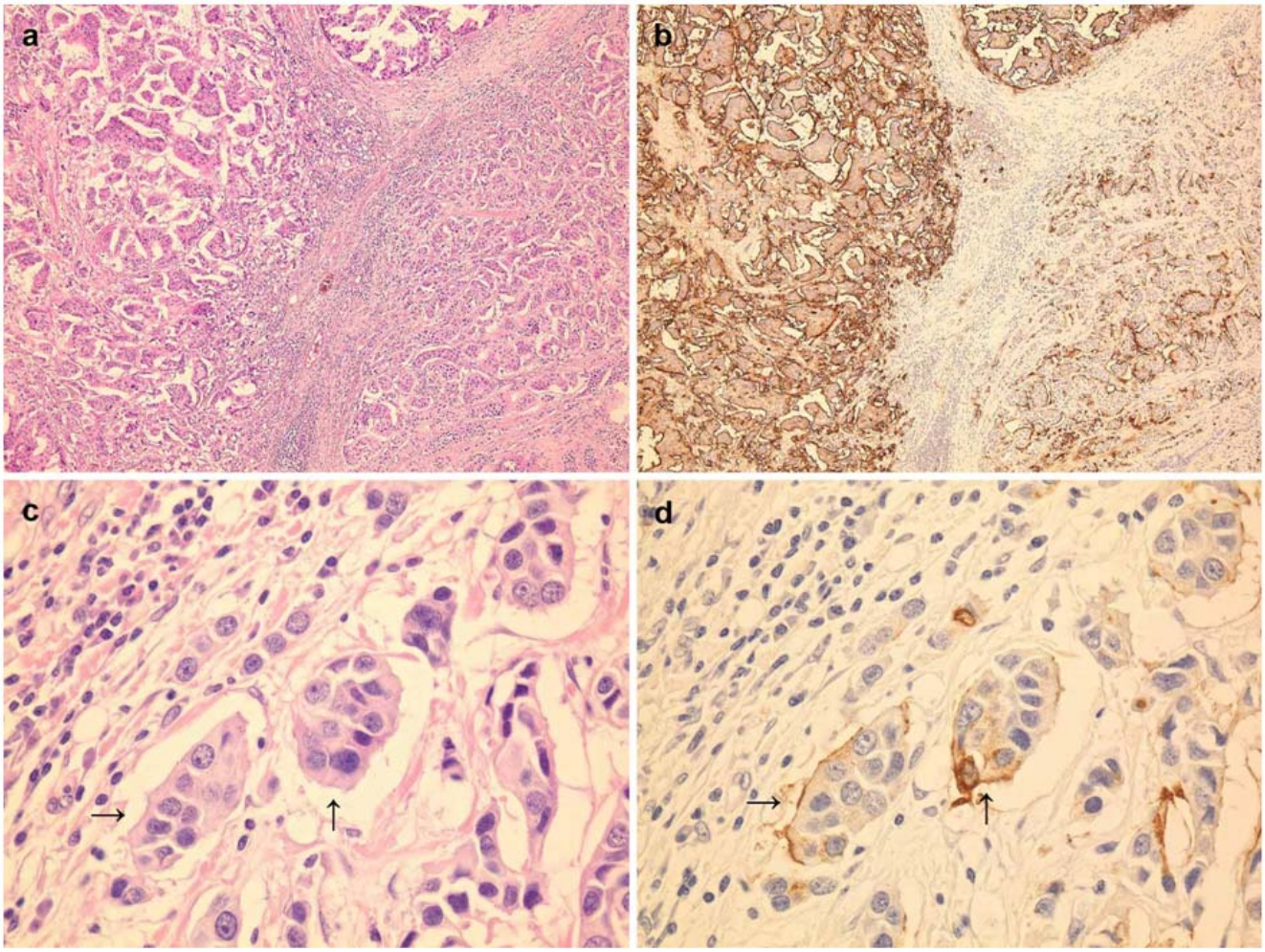

Fig. 3 IMPC of mixed type. **a** IMPC area (left half) and invasive duct carcinoma NOS with prominent incomplete IGP area (right half) are shown (X 40). **b** In contrast to IMPC area, invasive duct carcinoma NOS with incomplete IGP area showing incomplete hemming of EMA immunoreactivity in each cluster (X 40). **c** Incomplete IGP area containing tiny clusters and single tumor cells with inflammatory infiltration. Note a dense eosinophilic surface in part (arrows) (X 400). **d** The same surface showing strong EMA immunoreactivity (arrows) (X 400)

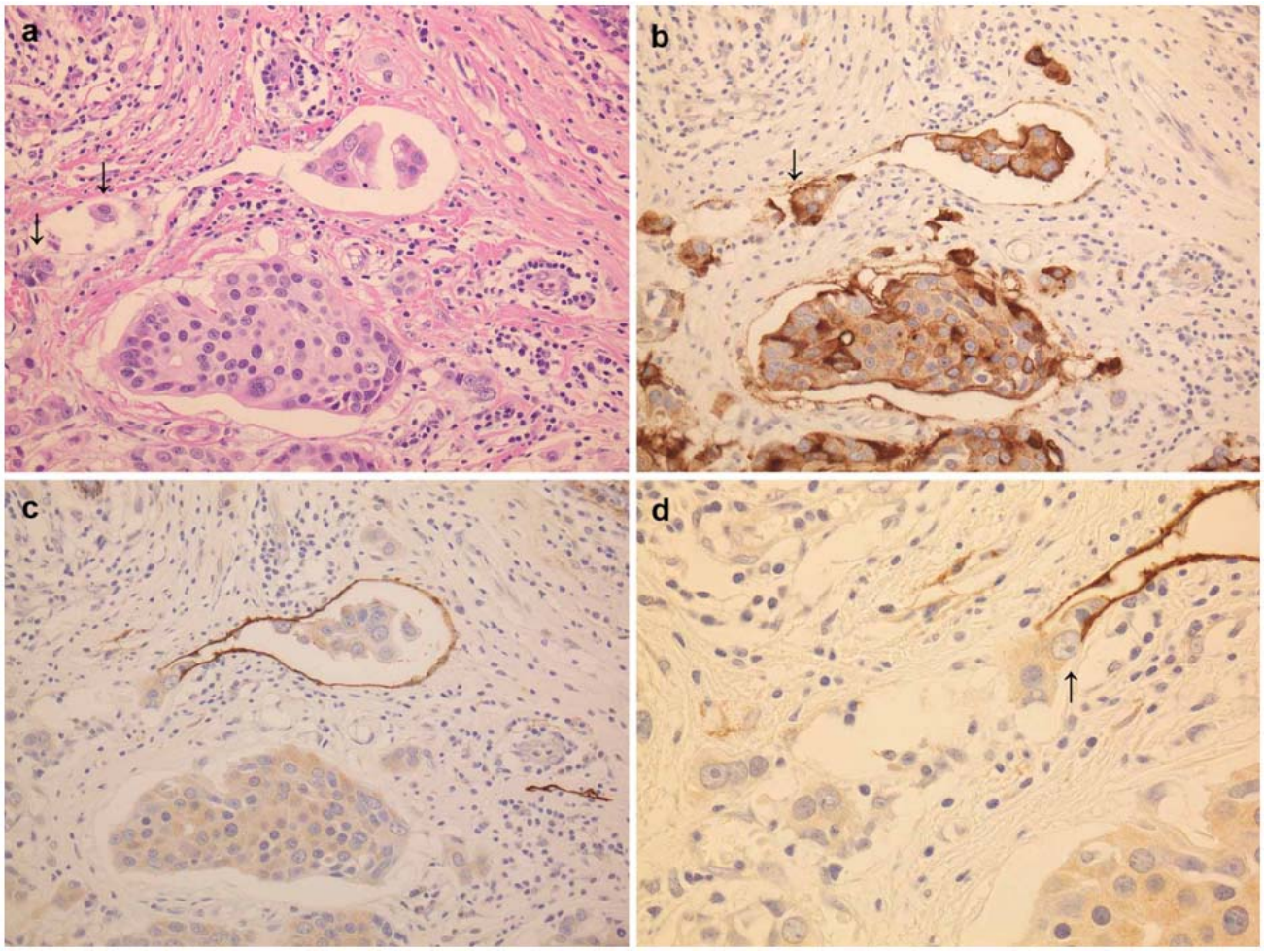

Fig. 4 Lymph vessel invasion in invasive duct carcinoma NOS with incomplete IGP. **a** A few tiny clusters (arrows) are present in a clear stromal cavity in front of the opening of a lymph vessel (see Fig. 4c) (X 200). **b** One of the clusters (arrow) and an adjacent larger cluster showing incomplete IGP by EMA immunostaining. A micropapillary floating cluster is also noted within a dilated lymph vessel (see Fig. 4c) (X 200). **c** Immunostaining with D2-40 clearly demonstrates the funnel-shaped lymph vessel containing the loosely arranged micropapillary cluster at the dilated portion (X 200). **d** High-power view of D2-40 immunostaining highlights a tiny tumor cluster just entering into the narrow portion of the lymph vessel (arrow) (X 400)

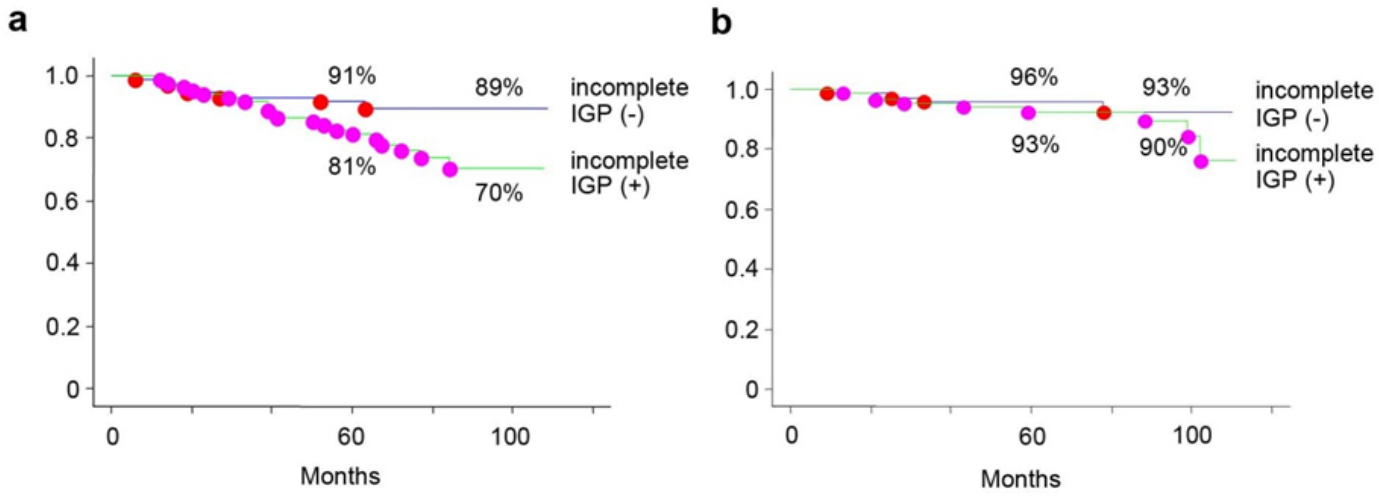

Fig. 5 Recurrence-free (**a**) and disease-specific (**b**) survival of 86 incomplete IGP-positive and 76 incomplete IGP-negative patients with invasive duct carcinoma NOS. The 5- and 8-year survivals (%) are shown (**a**, p=0.0356; **b**, p=0.3564)