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Original article

Increased expression of CD146 and microvessel density (MVD) in invasive micropapillary carcinoma of the breast: Comparative study with invasive ductal carcinoma-not otherwise specified

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ABSTRACT

Invasive micropapillary carcinoma (IMPC) is a rare variant of ductal carcinoma of the breast, and is characterized by a high metastatic potential and an aggressive clinical course. Studies of CD146 expression and function in breast cancer remain scarce. The aim of this study was to evaluate the role of CD146 and microvessel density (MVD) in breast IMPC. CD146 mRNA expression and immunohistochemistry for CD146 and MVD measured by CD31 were assessed in 82 cases of IMPC and 137 cases of invasive ductal carcinoma, not otherwise specified (IDC-NOS). The mRNA level of CD146 in cancer specimens was higher in IMPC than in IDC-NOS. CD146 expression in tumor cells was up-regulated in IMPC as compared with that in IDC-NOS, and was positively correlated with histological grade, ER, PR status, and P53 expression in IMPC and IDC-NOS. CD146 expression in vascular endothelial cells was significantly higher than that in IDC, and was positively correlated with tumor progression in IMPC and IDC-NOS. MVD in IMPC was significantly higher than that in IDC. CD146 expression in tumor cells was positively correlated with that in vascular endothelial cells of IMPC and IDC-NOS. The association of CD146 expression with MVD and its correlation with progression in breast carcinoma indicated that CD146 is a potentially useful prognostic marker for breast cancer. CD146 could be a new drug target in the treatment of breast cancer.

Introduction

Invasive micropapillary carcinoma (IMPC) of the breast, a special subtype of breast cancer with a high incidence of axillary lymph node metastases, recurrence, distant metastasis, and poor clinical outcome [5,18], serves as a good model for the research of tumor invasion, metastasis, and relapse.

CD146, also referred to as MUC18, Mel-CAM, or MCAM, is a newly recognized cell adhesion molecule belonging to the immunoglobulin super-family [13,24]. It is initially identified as a progression marker of melanoma, and plays an important role in promoting melanoma progression and metastasis [23,24].

Angiogenesis, the outgrowth of new capillaries from preexisting vessels, is essential in a variety of physiological and pathological processes, such as embryo implantation, female menstrual cycle, tumor, and other diseases [8]. Numerous pieces of evidence have supported that the uncontrolled angiogenesis is a key point for tumor growth and metastasis [3,31], and thereby anti-angiogenesis has become one of the most promising strategies for cancer therapy [20]. CD146 is a key molecule in vascular endothelial cell activity and angiogenesis [19,21]. CD146 is associated with advanced tumor stage in ovarian cancers and could be a poor-prognosis factor that predicts early tumor relapse [1]. In pulmonary adenocarcinomas, CD146 expression is associated with shorter patient survival [12].

Recent findings [22] provided direct evidence that CD146 is a sensitive and specific immunocytochemical marker enabling the differential diagnosis of malignant pleural mesothelioma from reactive mesothelium. In addition, combined use of anti-CD146 and anti-Epithelial cell adhesion molecule (EpCAM) is likely to improve the detection of circulating tumor cells in breast cancer patients [17,30].

Evaluation of the expression of CD146 in IMPC has not been reported previously. In this study, we examined immunohistochemically the expression of CD146 and CD31 for microvessel

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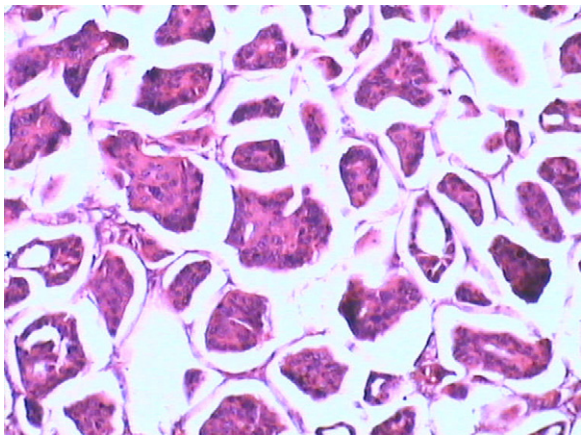

Fig. 1. Invasive micropapillary carcinoma (IMPC) (HE) with micropapillary differentiation, characterized by pseudopapillary or tubuloalveolar structures, often within clear spaces.

density (MVD) in 82 cases of IMPC in comparison with 137 cases of invasive ductal carcinoma, not otherwise specified (IDC-NOS).

Materials and methods

Patient selection and review

We analyzed paraffin-embedded tumor tissue of 82 patients with breast IMPC (Fig. 1) obtained from a total of 4211 cases of breast cancer diagnosed between January 2006 and December 2007. The cases were registered in the archives of the Department of Breast Cancer Pathology and Research Laboratory, Cancer Hospital of Tianjin Medical University, Tianjin, China.

For reverse transcriptase-polymerase chain reaction (RT-PCR), all breast cancer tissues were obtained from patients who had undergone surgical resection from January 2009 to December 2010. After the standard procedure for clinical diagnosis was completed, additional tissues were cut into small pieces, snap frozen in liquid nitrogen, and stored in a −80 °C freezer until use. The tumors which present over 50% the amount of the IMPC component were selected for PCR. None had received preoperative radiation or chemotherapy. Tissue used in this study was approved by the Ethics Committee of the Cancer Hospital of Tianjin Medical University.

The histopathology was reviewed, and in each case, the diagnosis was confirmed independently by three pathologists (LF, RL, and YF) using the WHO criteria [26]. IMPCs were divided into grades 1, 2 and 3 based on the Elston and Ellis grading system [6]. All of the IMPC were confirmed by immunohistochemical staining for epithelial membrane antigen (EMA). Our prior study [5] demonstrated that the amount of the IMPC component in a tumor had no significant association with the rate of lymph node metastasis and other prognostic factors, and therefore the cases were tabulated as a single group.

A hundred and thirty-seven patients with IDC-NOS were retrieved from the corresponding time period and randomized as control.

All patients were women ranging in age from 26 to 79 years (median 51 years).

Table 1
Comparison of clinicopathological characteristics of IMPC and IDC-NOS.

Characteristics	IDC-NOS	IMPC	*P*
Age (year), (mean ± SD)	51.6 ± 10.0	51.7 ± 9.2	0.926[a]
Tumor size (cm), (mean ± SD)	2.7 ± 1.7	3.6 ± 1.8	<0.001[a]
CD146 (vascular endothelial cells), (mean ± SD)	47.4 ± 19.4	55.2 ± 20.2	0.005[a]
CD31, (mean ± SD)	46.7 ± 19.2	55.1 ± 19.1	0.002[a]
Histological grade, no (%)			0.061[b]
Grade 1	18 (16.2)	8 (11.4)	
Grade 2	77 (69.4)	42 (60.0)	
Grade 3	16 (14.4)	20 (28.6)	
Lymph node metastasis, no (%)			<0.001[b]
Absent	77 (56.2)	13 (15.9)	
Present	60 (43.8)	69 (84.1)	
pTNM, no (%)			<0.001[b]
Stage 1	36 (26.3)	9 (11.0)	
Stage 2	69 (50.4)	26 (31.7)	
Stage 3/4	32 (23.3)	47 (57.3)	
ER, no (%)			0.363[b]
Negative	69 (51.5)	37 (45.1)	
Positive	65 (48.5)	45 (54.9)	
PR, no (%)			0.931[b]
Negative	58 (43.3)	35 (42.7)	
Positive	76 (56.7)	47 (57.3)	
HER-2, no (%)			0.165[b]
−/+	103 (76.9)	56 (68.3)	
++/+++	31 (23.1)	36 (31.7)	
Ki67, no (%)			0.393[b]
Negative	12 (9.6)	11 (13.4)	
Positive	113 (90.4)	71 (86.6)	
P53, no (%)			0.426[b]
Negative	83 (66.4)	50 (61.0)	
Positive	42 (33.6)	32 (39.0)	
VEGF, no (%)			0.015[b]
Negative	56 (44.8)	23 (29.1)	
Positive	69 (55.2)	59 (72.0)	
CD146 (tumor cells), no (%)			<0.001[b]
Negative	93 (67.9)	25 (30.5)	
Positive	44 (32.1)	57 (69.5)	

[a] Student's *t*-test.
[b] χ^2 test.

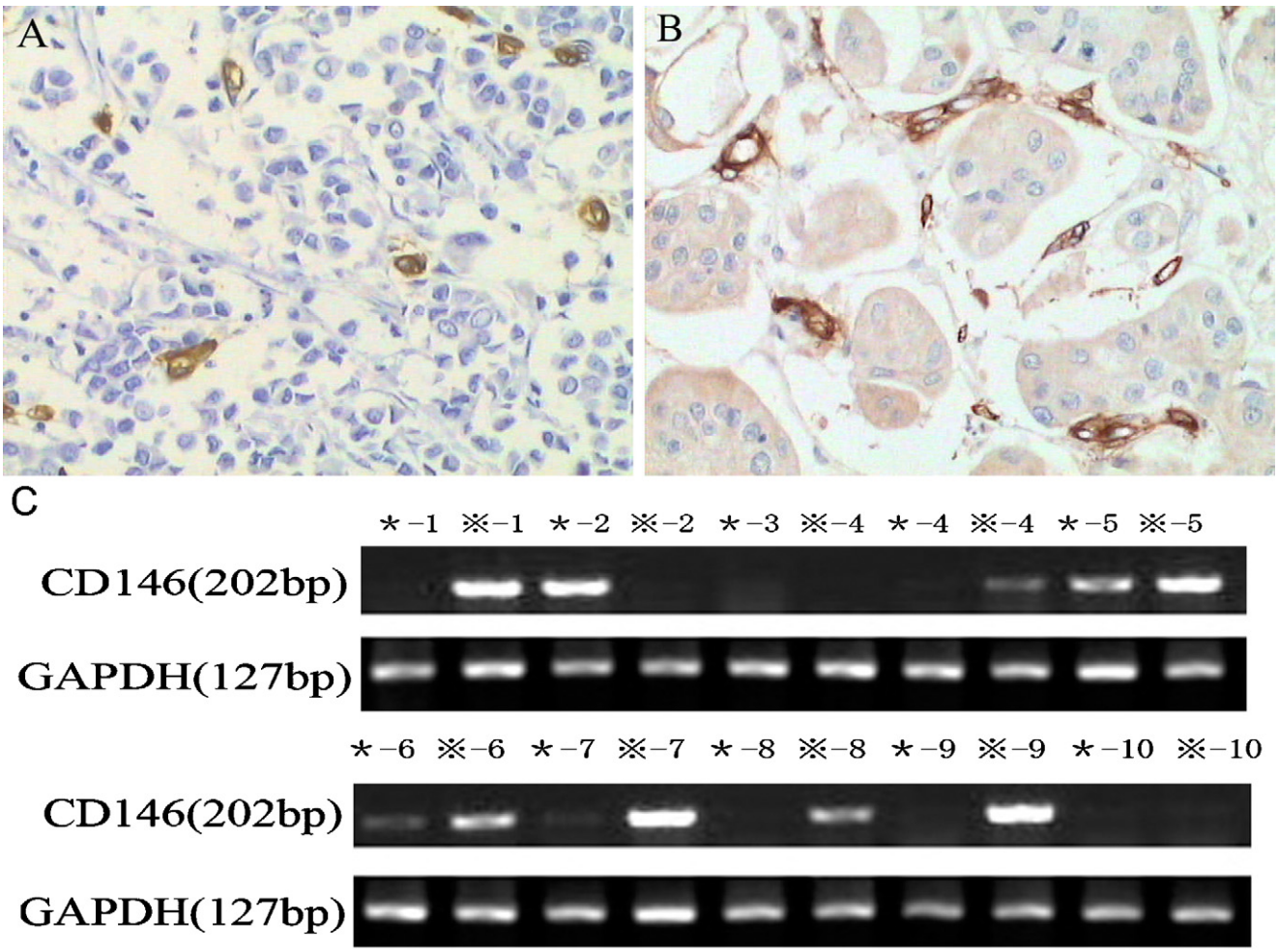

Fig. 2. Immunohistochemical staining and RT-PCR of human breast tumors for CD146. (A) Invasive ductal carcinoma, not otherwise specified (IDC-NOS) tumor cells negative for CD146. (B) IMPC was positive for CD146. (C) The relative ratio of CD146 signal in IMPC was higher than IDC-NOS in 7 pairs, and lower in 1 pair (*, IDC-NOS; ※, IMPC).

Twenty cases of normal tissues from IMPC and IDC-NOS patients, respectively, were also stained for CD146 and CD31. The normal tissues were obtained >5 cm from the tumor site and confirmed by HE staining.

Reverse transcriptase-polymerase chain reaction and polymerase chain reaction

CD146 mRNA expression was assessed in 10 pairs of IDC-NOS and IMPC by semi-quantitative RT-PCR, with TRIzol Reagent (Invitrogen) according to the manufacturer's instructions to extract RNA. The first-strand complementary DNA was synthesized with oligo(dT) primer by using the Reverse Transcription System (Promega). PCR amplification was performed in a 25-μl reaction with Taq polymerase (TaKaRa) and 2 μl of the first-strand cDNA synthesis mixture as template. The primer sequences for CD146 were as follows: FP, 5′-CTGCTGAGTGAACCACAGGA-3′; RP, 5′-TCAGGTCATGCAACTGAAGC-3′ (202 bp). To avoid contamination with genomic DNA, PCR primers spanned at least one intron of the CD146 gene. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control (127 bp), and its primer sequences were as follows: FP, 5′-GGTGGTCTCCTCTGACTTCAACA-3′; and RP, 5′-GTTGCTGTAGCCAAATTCGTTGT-3′. Twenty-eight and 25 cycles of PCR amplification were used for CD146 and GAPDH, respectively. Each RT-PCR assay was repeated at least twice for confirmation. The intensity of a band was quantitated with image analysis software (Gel-Pro 4400 Image System, Beijing, China), and the ratio of CD146 signal to GAPDH signal calculated for each IMPC and IDC-NOS sample, increased expression was defined when the ratio of IMPC to IDC-NOS was higher than 2.

Immunohistochemical staining

Sections (4-m thick) were dewaxed, hydrated, and heated for 2 min for antigen retrieval in a conventional pressure cooker. They were then treated with 3% H_2O_2 for 10 min to reduce endogenous activity and incubated with normal goat serum for 10 min to eliminate nonspecific staining. Thereafter, the primary antibodies were applied at 37 °C for 2 h including ER (mouse IgG, Zymed, USA, 1:150), PR (mouse IgG, Zymed, USA, 1:150), HER-2 (mouse IgG, Newmarkers, USA, 1:800), P53 (mouse IgG, Zymed, USA, 1:100), Ki67 (mouse IgG, Zymed, USA, 1:100), VEGF (mouse IgG, Zymed, USA, 1:100), CD146 (mouse IgG, ascites generated in our lab, 1:200), and CD31 (mouse IgG, Zymed, USA, 1:100). After washing, biotin-labeled secondary antibody against mouse immunoglobulin was applied for 20 min at room temperature. The slides were rinsed and covered with streptavidin–biotin–peroxidase for 20 min. All sections were counterstained with 3, 3′-diaminobenzidine tetrahydrochloride. Slides were counterstained with hematoxylin and mounted for light microscopy. Sections were incubated with goat serum for negative control of immunoreaction.

Table 2
Association of CD146 expression in tumor cells with clinicopathological characteristics of IMPC and IDC-NOS.

Characteristics	IMPC			IDC-NOS		
	CD146, − no (%)	CD146, + no (%)	*P*	CD146, − no (%)	CD146, + no (%)	*P*
Age			0.407			0.671
≤50 y	10(25.0)	30(75.0)		45(66.2)	23(33.8)	
>50 y	14(33.3)	28(66.7)		48(69.6)	21(30.4)	
Tumor size			0.801			0.814
≤2 cm	6(31.6)	13(68.4)		34(66.7)	17(33.3)	
>2 cm	18(28.6)	45(71.4)		59(68.6)	27(31.4)	
Histological grade			0.025			0.001
Grade 1	6(75.0)	2(25.0)		17(94.4)	1(5.6)	
Grade 2	12(28.6)	30(71.4)		48(62.3)	29(37.7)	
Grade 3	5(25.0)	15(75.0)		5(31.2)	11(68.8)	
Lymph node metastasis			0.005			0.081
Absent	8(61.5)	5(38.5)		57(74.0)	20(26.0)	
Present	16(23.2)	53(76.8)		36(60.0)	34(40.0)	
pTNM			0.343			0.691
Stage 1	4(44.4)	5(55.6)		26(72.2)	10(27.8)	
Stage 2	9(34.6)	17(65.4)		47(68.1)	22(31.9)	
Stage 3/4	11(23.4)	36(76.6)		20(62.5)	12(37.5)	
ER			0.004			<0.001
Negative	5(13.5)	32(86.5)		36(52.2)	33(47.8)	
Positive	19(42.2)	26(57.8)		54(83.1)	11(16.9)	
PR			0.037			0.027
Negative	6(17.1)	29(82.9)		33(56.9)	25(43.1)	
Positive	18(38.3)	29(61.7)		57(75.0)	19(25.0)	
HER-2			0.839			0.427
−/+	16(28.6)	40(71.4)		71(68.9)	32(31.1)	
++/+++	8(30.8)	18(69.2)		19(61.3)	12(38.7)	
Ki67			0.007			0.231
Negative	7(63.6)	4(36.4)		10(83.3)	2(16.7)	
Positive	17(23.9)	54(76.1)		75(66.4)	38(33.6)	
P53			0.002			0.024
Negative	21(42.0)	29(58.0)		62(74.7)	21(25.3)	
Positive	3(9.4)	29(90.6)		23(54.8)	19(45.2)	
VEGF			0.885			0.723
Negative	7(30.4)	16(69.6)		39(69.6)	17(30.4)	
Positive	17(28.8)	42(71.2)		46(66.7)	23(33.3)	

Immunohistochemical evaluation

Using light microscopy, stained tissue sections were reviewed by two pathologists in a blinded fashion. All unclear cases were discussed with another pathologist.

Tumors with no reactivity, weak reactivity, or moderate to strong reactivity in <10% of tumor cells were graded as negative, whereas those with weak reactivity, moderate to strong degree of reactivity in ≥10% tumor cells were considered positive.

Cases were considered as positive for ER or PR if nuclear immunoreactivity was present in ≥1% of tumor cells. For HER-2, the immunohistochemical score was assigned according to the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) Guideline [28]. No staining (0); weak, incomplete membrane staining in any percentage of cells (1+); weak or moderate heterogeneous complete membrane staining in at least 10% of cells (2+); strong, complete homogeneous membrane staining in >30% of cells (3+). For p53, Ki67 and VEGF the cut off value was 10%.

MVD was determined by the number of microvessels positive for CD31, including only the venuelae and lymphatic vessels in the tumor stroma. Labeling was observed in the cytoplasm of endothelial cells. Any endothelial cells or endothelial cell clusters with immunopositivity and clearly separated from adjacent clusters and background, with or without a lumen, were recorded as an individual blood vessel. Vessels that had a thick muscular layer were excluded from the count. The MVD was quantified by two pathologists independently, according to the method of Weider [7,14]. The slides were first examined at low-power magnification (×40) to identify the areas with a high concentration of vessels within the tumor. Three areas where the most concentrated microvessels existed were then selected in each case. A ×200 field in each of these three areas was counted, and the average counts of the three fields were recorded. Discrepant cases were reviewed by the group, and the consensus results were used for the analysis.

CD146 expressed in the vascular endothelial cells of tumor was quantified similar to CD31. Vessels that had a thick muscular layer or small breast ductal with positive immunoreaction were excluded from the count.

Statistical analysis

The SPSS 15.0 software package (SPSS, Chicago, IL, USA) was used for statistical analysis. Student's *t*-test and ANOVA test were performed for continuous variable comparisons, and correlations between two variables were evaluated by χ^2 test. A two-sided $P < 0.05$ was considered statistically significant in all the statistical analyses.

Results

Clinical information

Compared with the control group of IDC-NOS, IMPC (Fig. 1) were larger in size ($P < 0.001$), had a higher stage ($P < 0.001$), and a higher lymph node metastasis rate ($P < 0.001$) (Table 1).

VEGF expression in tumor cells was up-regulated in IMPC as compared with that in IDC-NOS ($P = 0.015$, Table 1).

Table 3
Association of CD146 expression in tumor vascular endothelial cells and MVD with clinicopathological characteristics of IMPC and IDC-NOS.

Characteristics	IMPC ($n=82$)				IDC-NOS ($n=137$)			
	CD146 (vascular endothelial cells)		CD31 (MVD)		CD146 (vascular endothelial cells)		CD31 (MVD)	
	Mean ± SD	*P*	Mean ± SD	*P*	Mean ± SD	*P*	Mean ± SD	*P*
Age		0.254		0.112		0.778		0.907
≤50 y	52.6 ± 20.3		51.7 ± 19.4		47.9 ± 19.7		47.2 ± 19.3	
>50 y	57.7 ± 20.0		58.4 ± 18.5		46.9 ± 19.2		46.8 ± 18.6	
Tumor size		0.703		0.757		0.196		0.257
≤2 cm	53.7 ± 18.0		54.0 ± 17.4		44.6 ± 19.5		44.7 ± 18.8	
>2 cm	55.7 ± 20.9		55.5 ± 19.7		49.1 ± 19.3		48.4 ± 18.9	
Histological grade		0.001		0.001		0.003		0.004
Grade 1	44.4 ± 11.8		44.9 ± 11.9		38.3 ± 19.7		38.8 ± 19.2	
Grade 2	53.0 ± 20.5		53.5 ± 18.9		48.7 ± 18.2		48.1 ± 17.7	
Grade 3	71.0 ± 20.9		69.0 ± 17.9		61.3 ± 23.1		60.0 ± 19.3	
LN metastasis		0.038		0.038		0.022		0.019
Absent	44.6 ± 17.6		45.1 ± 17.9		44.1 ± 19.1		43.7 ± 18.7	
Present	57.3 ± 20.1		57.0 ± 18.9		51.7 ± 19.1		51.3 ± 18.5	
pTNM stage		0.007		0.004		0.009		0.008
Stage 1	37.8 ± 9.7		38.0 ± 9.8		42.1 ± 19.0		42.1 ± 18.1	
Stage 2	52.9 ± 17.7		52.4 ± 16.7		46.2 ± 18.3		45.6 ± 18.4	
Stage 3/4	59.9 ± 20.2		60.0 ± 19.8		56.0 ± 20.0		55.6 ± 18.6	
ER		0.136		0.210		0.002		0.002
Negative	58.9 ± 22.3		58.1 ± 20.9		52.6 ± 19.3		52.1 ± 18.7	
Positive	52.2 ± 17.9		52.7 ± 17.4		42.5 ± 18.5		42.4 ± 17.9	
PR		0.536		0.730		0.003		0.005
Negative	56.9 ± 19.8		56.0 ± 19.1		53.4 ± 19.3		52.6 ± 19.1	
Positive	54.0 ± 20.6		54.5 ± 19.3		43.3 ± 18.6		43.5 ± 18.0	
HER-2		0.022		0.017		0.451		0.436
−/+	51.8 ± 19.7		51.8 ± 18.4		46.9 ± 20.0		46.7 ± 19.5	
++/+++	62.7 ± 19.5		62.5 ± 19.0		50.0 ± 17.7		49.7 ± 17.0	
Ki67		0.358		0.341		0.098		0.059
Negative	50.0 ± 12.6		50.0 ± 12.5		37.9 ± 17.4		36.7 ± 18.3	
Positive	56.1 ± 21.1		55.9 ± 19.9		47.7 ± 19.5		47.6 ± 18.9	
P53		0.360		0.457		0.127		0.180
Negative	53.6 ± 19.0		53.9 ± 18.3		44.9 ± 19.1		44.9 ± 18.7	
Positive	57.8 ± 22.0		57.1 ± 20.4		50.5 ± 19.8		49.7 ± 19.5	
VEGF		0.027		0.036		0.346		0.420
Negative	47.4 ± 15.4		48.1 ± 14.5		48.6 ± 18.4		48.1 ± 18.4	
Positive	58.3 ± 21.1		57.9 ± 20.1		45.3 ± 20.3		45.3 ± 19.6	
CD146 (tumor cells)		0.034		0.048		0.008		0.004
Negative	47.9 ± 19.7		48.6 ± 19.4		44.4 ± 18.4		43.9 ± 18.1	
Positive	58.3 ± 19.7		57.8 ± 18.5		53.8 ± 20.2		53.6 ± 19.1	

CD146 expression in tumor cells of IMPC and IDC-NOS by RT-PCR

The mRNA level of CD146 in cancer specimens was elevated in 10 pairs of IMPC and IDC-NOS (Fig. 2C). The relative ratio of CD146 signal of IMPC was higher than in IDC-NOS in seven pairs.

CD146 expression in tumor cells of IMPC and IDC-NOS by immunohistochemistory

CD146 expression in tumor cells was up-regulated in IMPC as compared with that in IDC-NOS ($P<0.001$, Table 1), and was positively correlated with histological grade and P53 expression, and negatively with ER, PR status in IMPC and IDC-NOS (Table 2). In addition, CD146 expression in tumor cells was positively correlated with lymph node metastasis ($P=0.005$) and Ki67 expression in IMPC ($P=0.007$) (Table 2).

CD146 expression in vascular endothelial cells of IMPC and IDC-NOS by immunohistochemistry

CD146 expression in vascular endothelial cells was significantly higher than that in IDC ($P=0.005$, Table 1) and was positively correlated with lymph node metastasis, histological grade, pTNM stage in IMPC, and IDC-NOS (Table 3).

In addition, CD146 expression in vascular endothelial cells was higher in the group where the tumor cells positively expressed HER-2 and VEGF ($P=0.022$, $P=0.027$, Table 3) in IMPC.

CD146 expression in vascular endothelial cells was negatively correlated with ER, PR expression ($P=0.002$, $P=0.003$, Table 3) in IDC-NOS.

No correlation was found between the vascular endothelial cell expression of CD146 and patient year, tumor size, Ki67, and P53 status (Table 3) in IMPC and IDC-NOS.

Microvessel density in IMPC and IDC-NOS

Microvessels were located predominantly in the stroma surrounding the tumor nests (Figs. 2 and 3). MVD identified by CD31 immunohistochemistry in IMPC was significantly higher than that in IDC ($t=-3.140$, $P=0.002$) (Table 1), and was positively correlated with lymph node metastasis, histological grade, pTNM stage, and HER-2 status in IMPC and IDC-NOS (Table 3). In addition, MVD was higher in the group where the tumor cells positively expressed VEGF ($P=0.036$) (Table 3) in IMPC.

MVD was negatively correlated with ER, PR expression ($P=0.002$, $P=0.005$) (Table 3) in IDC-NOS. No correlation was found between the expression of MVD and patient age, tumor size, Ki67, and P53 status (Table 3) in IMPC and IDC-NOS.

Correlation of CD146 expression and MVD in IMPC and IDC-NOS

CD146 expression in tumor cells was positively correlated with that in tumor vascular endothelial cells of IMPC ($P=0.034$) and

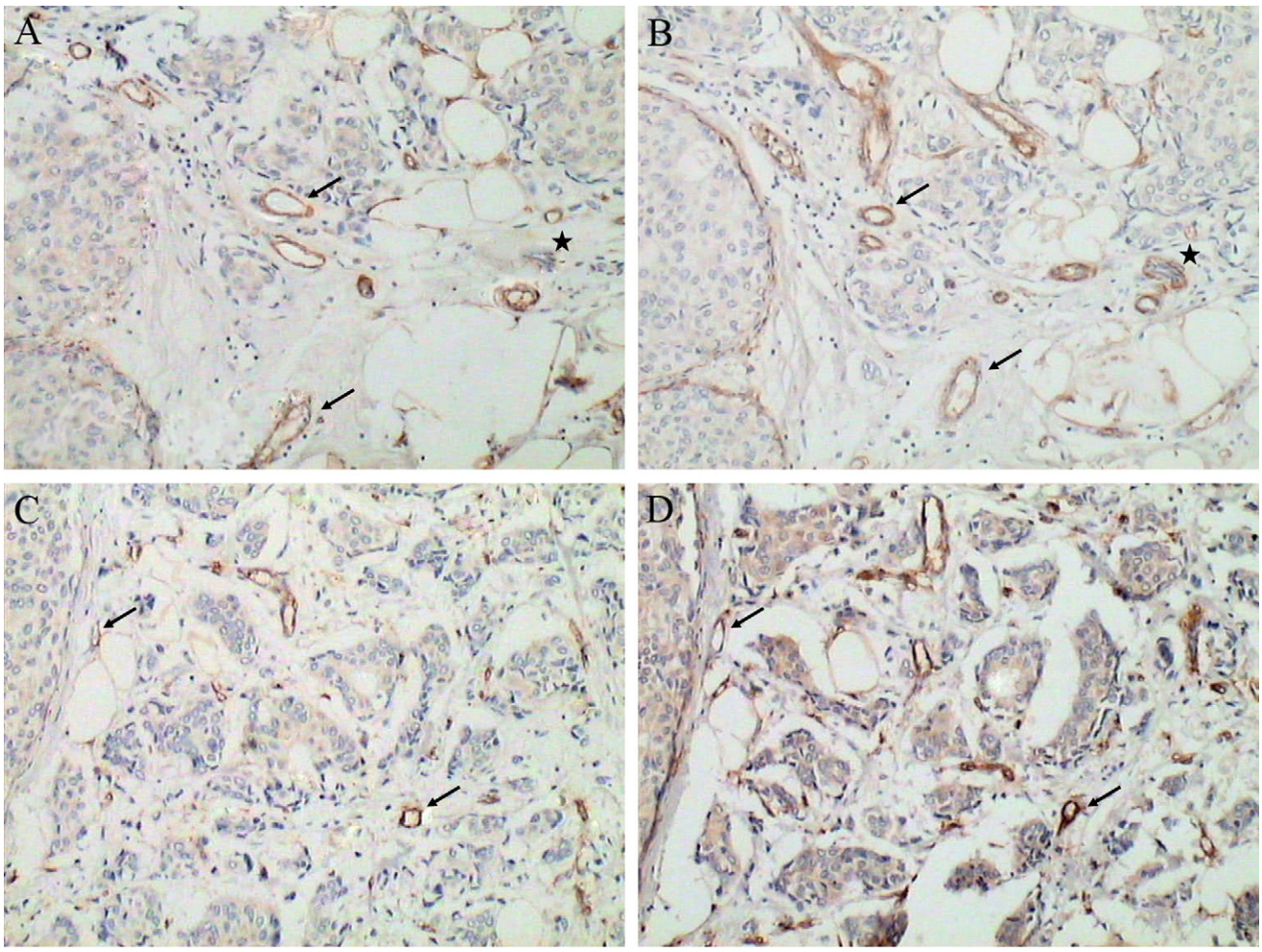

Fig. 3. Immunohistochemical staining of human breast tumors for CD31 and CD146. (A and B) IDC-NOS was positive for CD31 and CD146 in the stroma. (C and D) IMPC was positive for CD31 and CD146 in the stroma and also tumor cells were positive for CD146. Arrows indicate vessels positive for CD31 and CD146 in the same tumor in continuous sections. Star indicates a small duct positive for CD146 (B), while in the same tumor in continuous sections CD31 (A) was negative.

IDC-NOS ($P = 0.008$). CD146 expression in tumor cells was positively correlated with MVD in IMPC ($P = 0.048$) and IDC-NOS ($P = 0.004$).

MVD and CD146 expression in adjacent normal tissue from IMPC and IDC-NOS patients

CD146 was positively expressed in myoepithelial cells of the normal tissue. There was no statistically significant with MVD by CD31-stained and CD146 expression in the vascular endothelial cells of adjacent normal tissue of IMPC and IDC-NOS (Fig. 4, data not shown).

Discussion

Invasive micropapillary carcinoma (IMPC) is a distinct, unusual variant of ductal carcinoma of the breast, and is often associated with lymph-vascular invasion and lymph node metastasis [18]. It serves as a good system in analyzing the mechanism underlying the difference in tumor biological behavior, especially metastasis. Histologically, it is characterized by pseudopapillary or tubuloalveolar structures, often within clear spaces [15].

To understand what may be important in the process of tumor metastasis and aggressive behavior, we evaluated the expression of adhesion molecules CD146 and vascular marker CD31 in IMPC and compared it with that in IDC-NOS, a very frequent tumor of breast. The expression of CD146 by immunohistochemistry was significantly up-regulated in IMPC compared to IDC-NOS, and microvessel density (CD31) was significantly higher as compared with the control group.

The term IMPC of the breast was first used by Siriaunkgul and Tavassoli [25]. They described nine examples of this lesion. Invasive micropapillary carcinoma is characterized by small papillary structures that lack true central fibrovascular cores. It is known for its high incidence of axillary lymph node metastasis, recurrence, and distant metastasis [16,26], and is listed as an independent subtype of invasive breast carcinoma in the 2003 World Health Organization (WHO) histological classification of tumors of the breast. In the present study, IMPC were of larger size, of higher stage, had a higher lymph node metastasis rate, and exhibited increased lymphovascular invasion, as compared with the control group of IDC-NOS.

CD146, also known as melanoma cell adhesion molecule or MCAM, is a key cell adhesion protein in vascular endothelial cell activity and angiogenesis. CD146 promotes tumor progression of many cancers including melanoma, ovarian, lung, and prostate [12,18,23,29]. Although the biological role of CD146 in normal tissue remains unclear, CD146 has been suggested to play an important role in cancer, angiogenesis, cardiovascular diseases, implantation, and placentation [21].

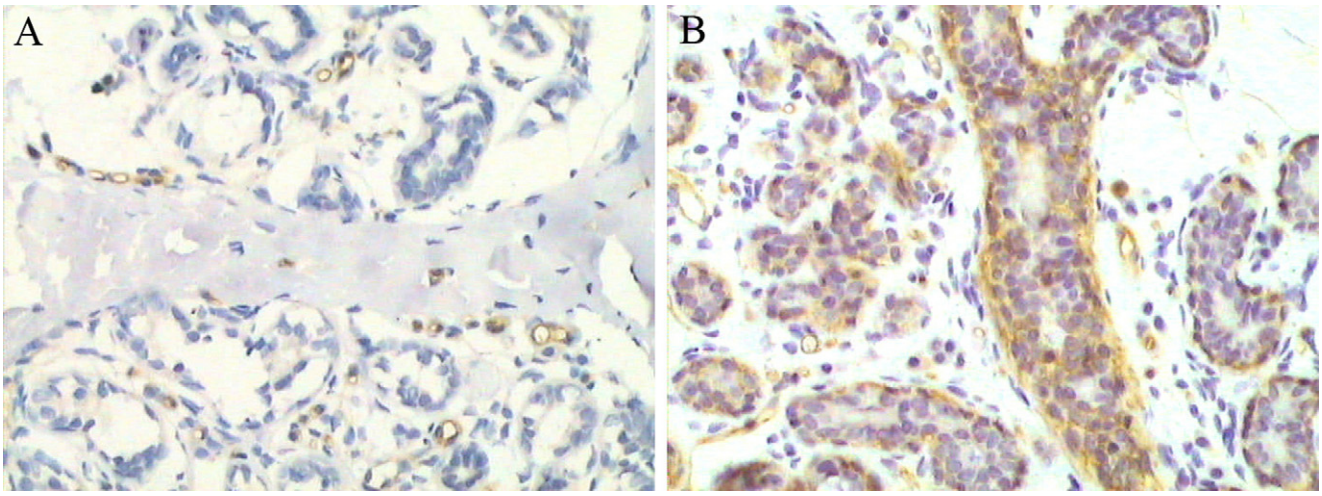

Fig. 4. Immunohistochemical staining of human breast normal tissues for CD31 (A) and CD146 (B). All of the breast normal tissues were positive for CD146.

It has been reported that CD146 expression is associated with a poor prognosis in human breast tumors and with enhanced motility in breast cancer cell lines [30]. Recent findings from a study by Garcia et al. [9] demonstrate that CD146 was correlated with patient death. However, it is clearly evident from their immunohistochemical analysis of the breast tumor that CD146 expression was limited to the endothelium only (CD146 is a well known marker of endothelial cells), while breast carcinoma cells were clearly negative for CD146. In our study, CD146 expression in tumor cells was up-regulated in IMPC as compared with that in IDC-NOS and was positively correlated with histological grade, ER, PR status, and P53 expression in IMPC and IDC-NOS. In addition, CD146 expression in tumor cells was positively correlated with lymph node metastasis and Ki67 expression in IMPC. CD146 expression in tumor vascular endothelial cells was also significantly higher than that in IDC. These results indicate that the increased expression of CD146 in IMPC might play an important role in breast cancer progression.

Angiogenesis is a prerequisite for tumor growth and progression. It has been found to correlate with the metastatic potential in breast, prostate, and bladder carcinomas, and in melanomas [3,27]. The intra-tumor MVD is a direct reflection of tumor angiogenesis, and can be visualized by immunohistochemical staining with antibodies against vascular endothelium such as anti-CD31 [2,4].

The current study demonstrates a significantly higher density of microvessel vessels in IMPC than in IDC-NOS. Similarly, Gong et al. [10] have reported that MVD was significantly higher in IMPC than in tubular carcinoma of breast. In addition, our data show that MVD in the group of IMPC with CD146 positivity was significantly higher as compared with the group without CD146 immunoreaction. It has been suggested that an increase in tumor vessel density may enhance the chance of tumor cells entering the circulation and reaching the lymph nodes. The newly formed vessels have leaky and weak basement membranes, which tumor cells can penetrate more easily than the mature vessels. Therefore, large numbers of small vessels facilitate metastasis to lymph nodes and distant metastasis.

Angiogenesis is essential to tumor growth and metastasis. Studies have shown that the intensity of angiogenesis measured by MVD correlates with tumor aggressiveness and metastasis [3]. In our study, MVD identified by CD31 immunohistochemistry was positively correlated with histological grade, pTNM stage, and HER-2 status in IMPC and IDC-NOS.

In addition, MVD was higher in the group where the tumor cells positively expressed CD146 and VEGF in IMPC. The importance of VEGF as a regulator of normal and tumor blood vessel growth has been increasingly characterized. VEGF increases vascular permeability and has a well established role in stimulating angiogenesis, a prerequisite of tumor growth [11].

Recent analysis discovered that CD146 was indispensible for the activation of p38/IKK/NFκB signaling cascade and subsequent up-regulation of pro-angiogenic genes, including at least IL-8, VEGF, ICAM-1, andMMP-9, in response to tumor secretions [32]. CD146 dimerization plays an important role in tumor-induced angiogenesis, and its dimerization was required for VEGF-induced tube-formation, migration, and actin rearrangement [33]. Given these observations, we concluded that CD146 and VEGF may synergetically induce angiogenesis in breast cancer.

In conclusion, these results indicate that the increased expression of CD146 in IMPC might play an important role in angiogenesis and tumor progression. CD146 is also associated with MVD, lymph node metastasis, and VEGF. We suggest that increased expression of CD146 plays an important role in promoting angiogenesis in breast cancer, and that in addition to being used as a prognostic marker for breast carcinoma, could emerge as an attractive new drug target in the treatment of breast cancer.

Conflict of interest

The authors declare that they have no competing interests.

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