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Original Article

Aberrant expression of ALDH1, MMP9, Integrin αvβ3, and KiSS-1 in invasive ductal carcinoma and their clinical significance

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Abstract: Background: Aldehyde dehydrogenase 1 (ALDH1, a biomarker of cancer stem cells), matrix metalloproteinase 9 (MMP9, known as a matrilysin), Integrin αvβ3 (known as a biomarker of cell-matrix adhesion) and KiSS-1 (suppressor gene of tumor metastasis) are all related to cancer invasion and metastasis in many cancers. The purpose of this study was to investigate the expression of ALDH1, MMP9, Integrin αvβ3, and KiSS-1 in invasive ductal carcinoma (IDC), and their respective associations with clinical characteristics and survival in IDC. Methods: Immunohistochemical staining was used to detect the expression of ALDH1, MMP9, Integrin αvβ3, and KiSS-1 in 227 whole IDC tissue specimens. Patients' clinical and demographic data were both collected. Results: The expression of ALDH1, MMP9, and Integrin αvβ3 were significantly higher in IDC tissues than in the control tissues. The positive expressions of ALDH1, MMP9, and Integrin αvβ3 were positively associated with tumor grades, lymph node metastasis (LNM), tumor stages, and tumor node metastasis (TNM) stages, and inversely with overall survival (OS) and recurrence-free survival (RFS). Positive expression of KiSS-1 was negatively associated with tumor grades, LNM, tumor stages, and TNM stages, but positively with OS and RFS. A multivariate analysis demonstrated that the positive expression of ALDH1, MMP9, Integrin αvβ3, KiSS-1, ER, and HER-2, as well as TNM stages were independent prognostic factors for OS and RFS in IDC. Conclusions: The expression of ALDH1, MMP9, Integrin αvβ3, and KiSS-11 should represent promising biomarkers in predicting metastasis and prognosis, as well as being potential therapeutic targets for IDC.

Keywords: Invasive ductal carcinoma, ALDH1, MMP9, Integrin αvβ3, KiSS-1, prognosis

Introduction

In 2012, there were an estimated 1.7 million newly diagnosed breast cancer cases, and the disease caused 521,900 deaths, making it the most lethal cancer for women worldwide [1]. The most common type of breast cancer is invasive ductal carcinoma (IDC). As symptoms of breast cancer are usually not obvious at the early stages, more than half of the patients in China diagnosed with breast cancer have an advanced stage. Despite more advanced molecular and targeted treatment, overall 5-year survival rates are still unsatisfactory for patients with breast cancer.

Tumor recurrence and metastasis are the main reasons for anti-cancer treatment failure. This may be related to a small subpopulation tumor cells which are called cancer stem cells (CSCs). CSCs have the ability of self-renewal, multidirectional differentiation, quiescence, and natural resistance to chemotherapy or radiotherapy [2]. Moreover, CSCs are involved in the various biological processes of cancer, such as initiation, progression, metastasis, and recurrence [3]. Aldehyde dehydrogenase 1 (ALDH1) is a common member of the ALDH family of enzymes, which are located in the cytoplasm, mitochondria, and nucleus [4]. ALDH1 is also a common biomarker of CSCs in various human cancers. ALDH1 can detoxify and metabolize many exogenous and endogenous aldehydes and synthesize retinoic acid [5-7]. Previous studies have shown that ALDH1 should be considered as an effective biomarker for metastasis and recurrence in diverse cancers, such as colorectal, ovarian, lung, breast, and pancreatic cancer [5-9].

Table 1. Patients characteristics

Patients characteristics	Frequency (n)	Percentage (%)
Ages (years)		
≤60	108	47.6
>60	119	52.4
Location		
Left	113	49.8
Right	106	46.7
Bilateral	8	3.5
Smoking		
No	188	82.8
Yes	39	17.2
Alcohol		
No	172	75.8
Yes	55	24.2
Size (cm)		
≤2.0	66	29.1
2.0<S≤5.0	135	59.5
>5.0	26	11.5
T stage		
T1	69	30.4
T2	127	55.9
T3	21	9.3
T4a	10	4.4
Grade		
G1	55	24.2
G2	109	48.0
G3	63	27.8
Lymph node metastasis		
N0	118	52.0
N1	74	32.6
N2	30	13.2
N3	5	2.2
TNM stage		
I	37	16.3
II	139	61.2
III	51	22.5
ER expression		
Negative	104	45.8
Positive	123	54.2
PR expression		
Negative	119	52.4
Positive	108	47.6
HER2 expression		
Negative	160	70.5
Positive	67	29.5

The large family of matrix metalloproteinases (MMPs), which include collagenases, stromelysins, gelatinases, and matrilysins, have the ability to degrade the extracellular matrix (ECM) [10]. MMPs are involved in a series of normal processes, such as embryonic development and ECM remodeling, as well as abnormal processes, such as tumor metastasis [11]. MMP9, with the ability to digest gelatins or denatured collagens, is a common member of the MMPs [12]. MMP9 is involved in activating some signaling pathways during inflammation and cancer [10]. Studies have indicated that overexpression of MMP9 can facilitate the invasiveness and metastasis of cancer cells [11-13].

Tumor invasiveness and metastasis are a complex and cyclic process mediated by adhesion molecules, including epithelial-cadherin, and integrin receptors. Integrins are composed of two subunits, α and β. Integrin αvβ3, which is able to regulate cell-matrix adhesion by binding to fibronectin, is an important member of the integrins [14]. Integrin αvβ3 is also able to regulate cell migration, growth, differentiation, and survival [15]. Integrin αvβ3 has been widely reported for its overexpression in diverse cancer cells [16] and should be considered as a useful biomarker for metastasis and prognosis in cancers.

KiSS-1 was originally identified in melanoma by the analysis of subtractive hybridization and was considered as a metastasis suppressor gene [17]. The KiSS-1 gene encodes a 145-amino-acid protein and is located on human chromosome 1q32. KiSS-1 can strengthen cell-cell adhesion by promoting E-cadherin expression and suppressing MMP expression [6, 18, 19]. KiSS-1 can also inhibit cell motility, proliferation, and invasion as well as metastasis [20]. It has been demonstrated that the down-regulation of KiSS-1 promotes the invasion and metastasis of cancer [6, 19, 21, 22]. KiSS-1 should also be considered as a useful biomarker for metastasis and prognosis in various cancers.

The involvement of ALDH1, MMP9, Integrin αvβ3, and KiSS-1 in the recurrence and metastasis of IDC indicates that they should be valuable biomarkers for measuring cancer progression and development. However, the associations of ALDH1, MMP9, Integrin αvβ3, and KiSS-1 in IDC have not been extensively reported. In this study, we explored the hypothesis that the above biomarkers were mutually correlated and associated with metastasis and prognosis in IDC.

Materials and methods

Patients and tissue samples

227 cases of IDC tissues from patients (median age: 55.3 years; range: 30-78 years) were collected at the Department of Pathology of the First Affiliated Hospital of Bengbu Medical University, from January 2011 to December 2012, along with 227 samples of the corresponding adjacent normal breast tissues. Patients who had received any anti-cancer therapy (chemotherapy, radiotherapy, or other anti-cancer therapy) were excluded. All specimens were obtained with the patients' written consent. This study was authorized by the Bengbu Medical University Ethics Committee before it started and was performed according to the guidelines of the Helsinki Declaration. All clinical and follow-up data was also collected (6 months intervals by phone or social media applications). The overall survival (OS) time was computed from the patient's surgery date to her death date or to December 2017 (mean OS: 55.9 months; range: 10-83 months). Tumor-node-metastasis (TNM) stages were appraised in accordance with the 7th edition of the guideline issued by the American Joint Committee on Cancer (AJCC). Tumor differentiation was graded in accordance with World Health Organization (WHO) standards. For other specific characteristics, see **Table 1**.

Immunohistochemistry

Immunohistochemical staining was conducted in accordance with the ElivisionTM Plus detection kit instructions (Lab Vision, USA). All IDC and control tissues were fixed in 10% buffered formalin and then embedded in paraffin. Continuous 4 μm-thick slices were cut. Subsequently, all slices were deparaffinized in xylene and dehydrated in graded alcohol. All slices were rinsed with phosphate buffer saline (PBS, pH 7.2) for 10 min, then incubated in methanol containing 3% H_2O_2 for 10 min at room temperature to inhibit endogenous peroxidase activity. All slices were placed in a citrate buffer (pH 6.0) and heated at 95°C for 30 min for retrieval antigen. Then they were rinsed several times in PBS, then all slices were quenched with goat serum for 30 min at room temperature and incubated with mouse monoclonal antibody against human ALDH1 (Abcam, USA), MMP9 (Abcam, USA), Integrin αvβ3 (Abcam, USA), and KiSS-1 (Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 37°C for 1 h. Lastly, all slices were counterstained with hematoxylin, dehydrated, air-dried, and mounted.

Assessment of immunostaining

Two independent pathologists who were blinded to the patients' clinical and follow-up data assessed the immunostaining results semi-quantitatively. To avoid any intratumoral heterogeneity of biomarker expression, ten high-power-field (HPF) representative fields from different areas of each IDC slice were evaluated. The results were scored in accordance with intensity scores (0: no staining; 1: weak staining; 2: moderate staining; 3: strong staining) and percentage scores (1: <11% positive cells; 2: 11-50% positive cells; 3: 51-75% positive cells; 4: >75% positive cells); the score was then multiplied using intensity scores and percentage scores which ranged from 0-12. Scores >2 were defined as positive results. For slices that were positive for all four of ALDH1, MMP9, Integrin αvβ3, and KiSS-1, an average of the final score of each slice was taken.

Statistical analysis

Associations between clinical characteristics and ALDH1, MMP9, Integrin αvβ3, and KiSS-1 were compared using Chi-square or Fisher's exact test. The association between ALDH1, or MMP9, or Integrin αvβ3, or KiSS-1 was compared using Spearman's coefficient test. The effects of ALDH1, MMP9, Integrin αvβ3, and KiSS-1 on overall survival and recurrence-free survival were determined by the Kaplan-Meier method with a log-rank test. A multivariate analysis was done using the multivariate COX regression model test. SPSS 19.0 software (Chicago IL, USA) was used for statistical analysis. A value of $P<0.05$ was reckoned as statistically significant.

Results

Associations between ALDH1, MMP9, Integrin αvβ3, and KiSS-1 and clinical characteristics

To assess the contributions of ALDH1, MMP9, Integrin αvβ3, and KiSS-1 to IDC, the results of the immunohistochemical staining were assessed for both the IDC and the control tissue

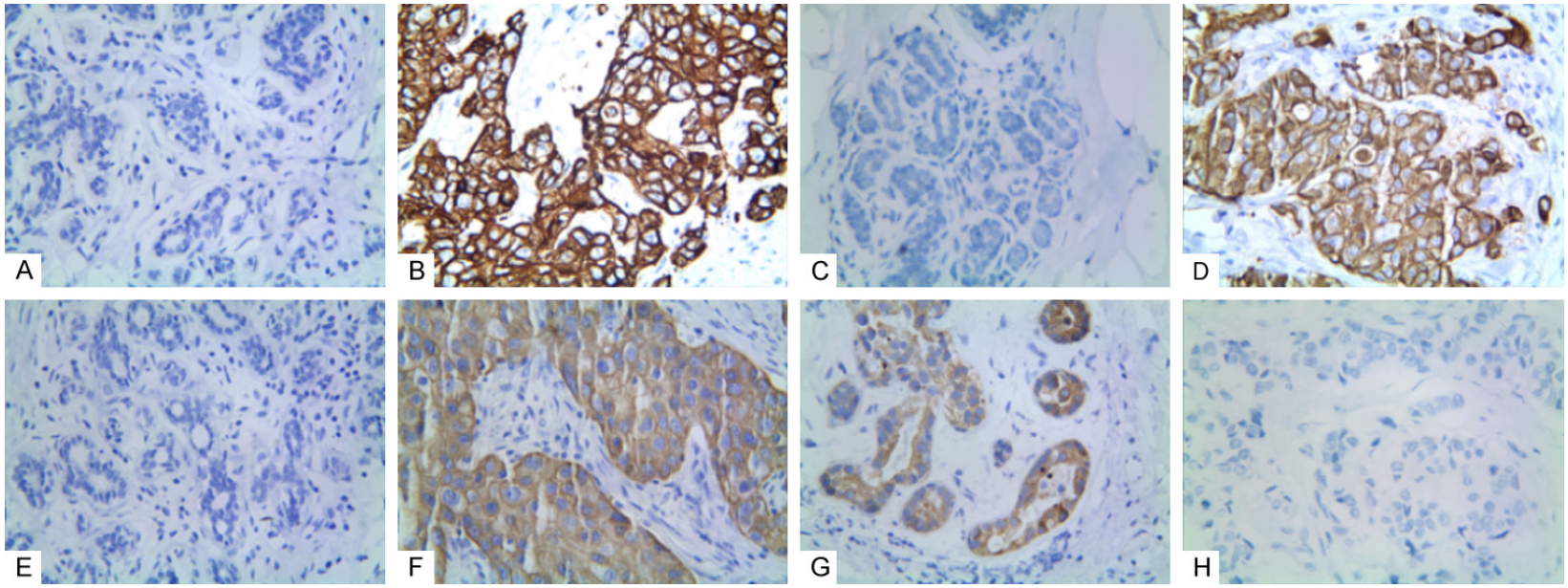

Figure 1. Immunostaining of ALDH1, or MMP9, or Integrinα vβ3, or KiSS-1 in invasive ductal carcinoma or the control tissue. A: Negative staining of ALDH1 in control tissue (100 magnification); B: Positive staining of ALDH1 in the membrane and cytoplasm of tumor cells (400 magnification); C: Negative staining of MMP9 in the control tissues (100 magnification); D: Positive staining of MMP9 in the cytoplasm of tumor cells (400 magnification); E: Negative staining of Integrin αvβ3 in the control tissues (100 magnification); F: Positive staining of Integrin αvβ3 in the cytoplasm of tumor cells (400 magnification; G: Positive staining of KiSS-1 in the cytoplasm of the control tissues (100 magnification); H: Negative staining of KiSS-1 in the tumor tissues (400 magnification).

Table 2. The correlation between ALDH1, or MMP9, or Integrin αvβ3, or KiSS-1 and clinical characteristics in invasive ductal carcinoma

Variable	ALDH1		P	MMP9		P	Integrin αvβ3		P	KiSS-1		P
	Negative	Positive		Negative	Positive		Negative	Positive		Negative	Positive	
Ages (years)			0.642			0.868			0.710			0.196
≤60	43	65		42	66		37	71		61	47	
>60	51	68		45	74		38	81		57	62	
Location			0.526			0.533			0.178			0.992
Left	51	62		46	67		42	71		59	54	
Right	40	66		37	69		29	77		55	51	
Bilateral	3	5		4	4		4	4		4	4	
Smoking			0.261			0.153			0.146			0.096
No	81	107		76	128		66	122		93	95	
Yes	13	26		11	28		9	30		25	14	
Alcohol			0.069			0.541			0.474			0.172
No	77	95		64	108		59	113		85	87	
Yes	17	38		23	32		16	39		33	22	
Size (cm)			<0.001			0.002			<0.001			0.002
≤2.0	42	24		36	30		37	29		26	40	
2.0<S≤5.0	49	86		46	89		31	104		71	64	
>5.0	3	23		5	21		7	19		21	5	
T stage			<0.001			0.006			<0.001			0.002
T1	45	24		37	32		39	30		25	44	
T2	44	83		44	83		29	98		69	58	
T3	3	18		4	17		5	16		16	5	
T4a	2	8		2	8		2	8		8	2	
Grade			<0.001			<0.001			<0.001			<0.001
G1	43	12		36	19		35	20		14	41	
G2	44	65		45	64		32	77		58	51	
G3	7	56		6	57		8	55		46	17	
Lymph node metastasis			<0.001			<0.001			0.001			<0.001
N0	64	54		60	58		53	65		39	79	
N1	23	51		24	50		16	58		49	25	
N2	7	23		2	28		6	24		25	5	
N3	0	5		1	4		0	5		5	0	
TNM stage			<0.001			<0.001			<0.001			<0.001
I	29	8		27	10		27	10		7	30	
II	55	84		54	85		39	100		69	70	
III	10	41		6	45		9	42		42	9	
ER expression			0.014			0.060			0.129			0.001
Negative	34	70		33	71		29	75		67	37	
Positive	60	63		54	69		46	77		51	72	
PR expression			0.154			0.125			0.019			0.171
Negative	44	75		40	79		31	88		67	52	
Positive	50	58		47	61		44	64		51	57	
HER2 expression			<0.001			<0.001			0.012			<0.001
Negative	79	81		73	87		61	99		63	97	
Positive	15	52		14	53		14	53		55	12	

specimens. ALDH1 positive staining was mainly confined in cancer cell cytoplasms. The positive rate of ALDH1 in the IDC specimens (58.6%, 133/227) was significantly higher than that in the control tissues (9.3%, 21/227; P<0.001; **Figure 1A** and **1B**). The expression of ALDH1 was positively correlated with tumor grades, size, T stages, lymph node metastasis (LNM) stages, TNM stages, expression of HER2 and inversely with ER expression, but not with the patients' age, smoking, alcohol, tumor location, and PR expression (**Table 2**).

Table 3. Correlation among ALDH1, MMP9, Integrin αvβ3, and KiSS-1 in IDC

Variable	ALDH1		r	P	MMP9		r	P	Integrin αvβ3		r	P
	Negative	Positive			Negative	Positive			Negative	Positive		
MMP9			0.423	<0.001*							0.467	<0.001*
Negative	59	28							53	34		
Positive	35	105							22	118		
KiSS-1			-0.499	<0.001@			-0.476	<0.001@			-0.375	<0.001@
Negative	21	97			19	99			19	99		
Positive	73	36			68	41			56	53		
ALDH1							0.423	<0.001*			0.455	<0.001*
Negative					59	35			55	39		
Positive					28	105			20	113		

*: Positive correlation; @: Negative correlation.

MMP9 positive staining was mainly confined to the cancer cell cytoplasm. Similar to ALDH1, MMP9 expression was significantly higher in IDC tissues (61.7%, 140/227) than in the control tissues (5.3%, 12/227; P<0.001; **Figure 1C** and **1D**). Furthermore, MMP9 expression was positively associated with tumor size, grades, T stages, LNM stages, TNM stages, as well as HER2 expression, but not with patients' age, smoking, alcohol, tumor location, and ER, PR expression (**Table 2**).

Integrin αvβ3 positive staining was mainly confined to the cancer cell cytoplasms. Integrin αvβ3 positive expression was significantly higher in the IDC group (67.0%, 152/227) than in the control group (2.2%, 5/227; P<0.001; **Figure 1E** and **1F**). Moreover, Integrin αvβ3 was significantly related to tumor size, grade, T stages, LNM stages, TNM stages, and HER2 expression, and negatively with PR expression, but not with patients' age, smoking, alcohol, tumor location, and ER expression (**Table 2**).

KiSS-1 positive staining was mainly located at the cytoplasms of the cancer cells. KiSS-1 expression was significantly lower in the IDC group (48.0%, 109/227) than in the control group (96.9%, 220/227; P<0.001; **Figure 1G** and **1H**). In addition, the expression of KiSS-1 was inversely associated with tumor size, grade, T stages, LNM stages, TNM stages, as well as HER2 expression, and positively with ER expression, but not with patients' age, smoking, alcohol, tumor location, and PR expression (**Table 2**).

A Spearman correlation coefficient analysis showed inverse associations between KiSS-1 + expression and that of ALDH1 (r=-0.499, P<0.001), MMP9 (r=-0.476, P<0.001), or Integrin αvβ3 (r=-0.375, P<0.001) expression. The expression of ALDH1 was positively associated with the expression of MMP9 (r=0.423, P<0.001), or Integrin αvβ3 (r=0.455, P<0.001). The Expression of MMP9 was positively associated with Integrin αvβ3 expression (r=0.467, P<0.001; **Table 3**).

OS and RFS analyzes

Follow-up data indicated that OS was significantly lower in IDC patients in the ALDH1 + group (48.2±15.7 months) when compared with those in the ALDH1 - group (67.0±11.8 months; log-rank =55.955, P<0.001, **Figure 2A**). Similarly, the OS of the MMP9 + group (49.0±15.3 months) was significantly shorter than those in the MMP9 - group (67.2±13.1 months; log-rank =57.810, P<0.001; **Figure 2B**); The OS of Integrin αvβ3 + group (50.0± 14.7 months) was significantly lower than that of the Integrin αvβ3 - patients (68.0±14.6 months; log-rank =62.549, P<0.001; **Figure 2C**). Inversely, the OS of KiSS-1 + patients (66.8± 11.7 months) was significantly longer than that of the KiSS-1 - patients (45.9±14.8 months; log-rank =72.669, P<0.001; **Figure 2D**).

RFS data showed that the positive expression of the ALDH1 group (43.5±14.9 months) was significantly shorter than that of the ALDH1 - negative group (62.0±12.5 months; log-rank =56.949, P<0.001; **Figure 3A**). The RFS of the MMP9 - positive patients (44.2±14.6 months) was significantly shorter that of the MMP9 - negative patients (62.3±13.3 months; log-rank =55.727; P<0.001; **Figure 3B**). Similarly, the RFS of the Integrin αvβ3 - positive patients (45.2±14.3 months) was significantly lower th-

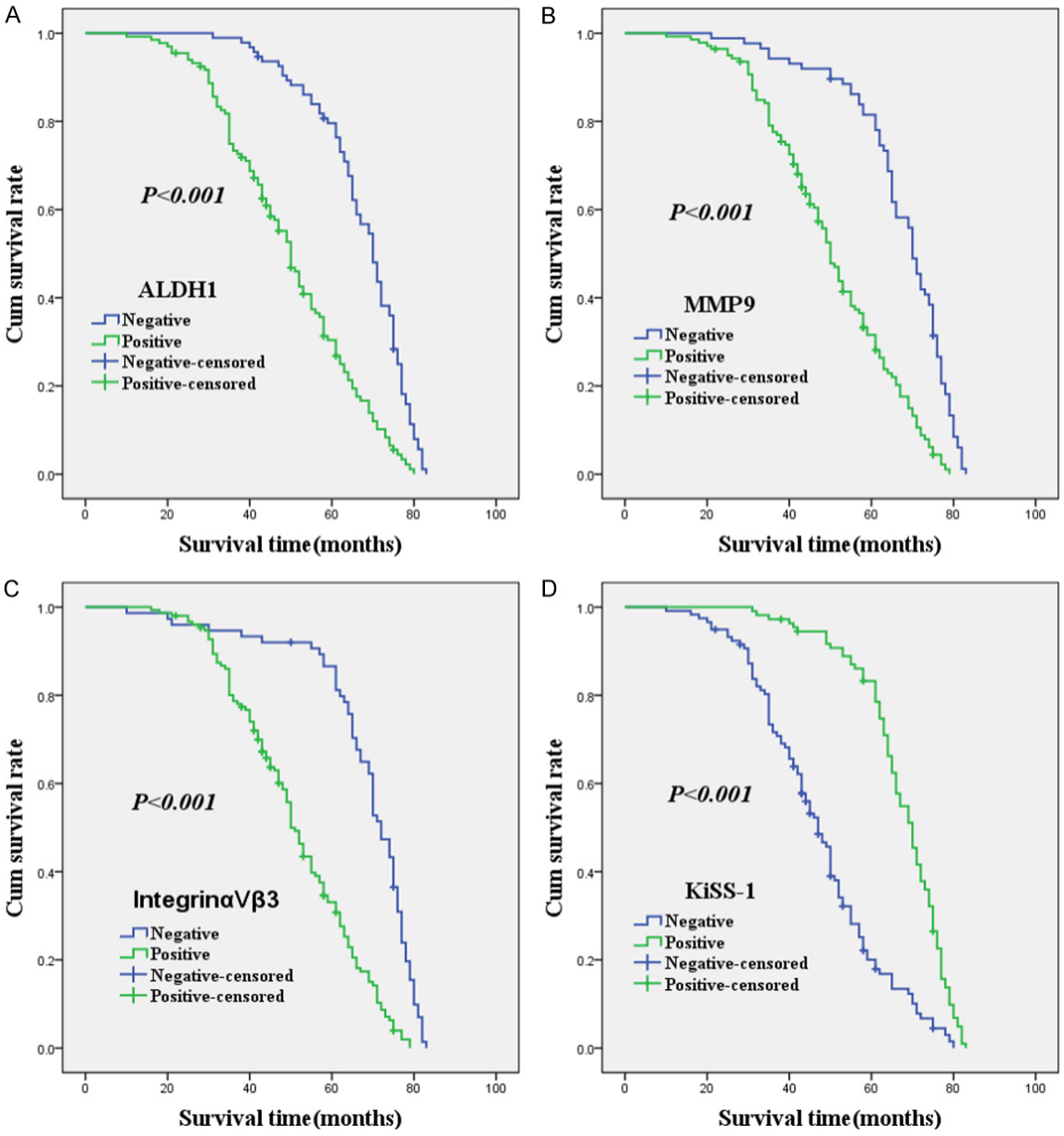

Figure 2. Overall survival of patients with IDC. The y-axis represents the percentage of patients; the x-axis, their survival in months. (A) Overall survival of all patients in relation to ALDH1 (log-rank =55.955, P<0.001); (B) Overall survival of all patients in relation to MMP9 expression (log-rank =57.810, P<0.001); (C) Overall survival of all patients in relation to Integrin αvβ3 expression (log-rank =62.549, P<0.001); (D) Overall survival of all in relation to KiSS-1 expression (log-rank =72.669, P<0.001); In (A-D) analyses, the green line represents patients with positive ALDH1, or MMP9, or Integrin αvβ3, or KiSS-1 and the blue line representing the negative ALDH1, or MMP9, or Integrin αvβ3, or KiSS-1 group.

an that of the Integrin αvβ3 - negative patients (63.2±14.5 months; log-rank =60.805, P< 0.001; **Figure 3C**). Inversely, the RFS of the KiSS-1 - positive group (61.7±12.3 months) was significantly higher than that of the KiSS-1 - negative group (41.4±13.9 months; log-rank =73.851, P<0.001; **Figure 3D**).

Multivariate analysis

A multivariate analysis suggested that positive expressions of ALDH1, MMP9, Integrin αvβ3, KiSS-1, ER, as well as HER2, and the TNM stages were independent prognostic factors for OS in IDC (**Table 4**). The multivariate analysis also

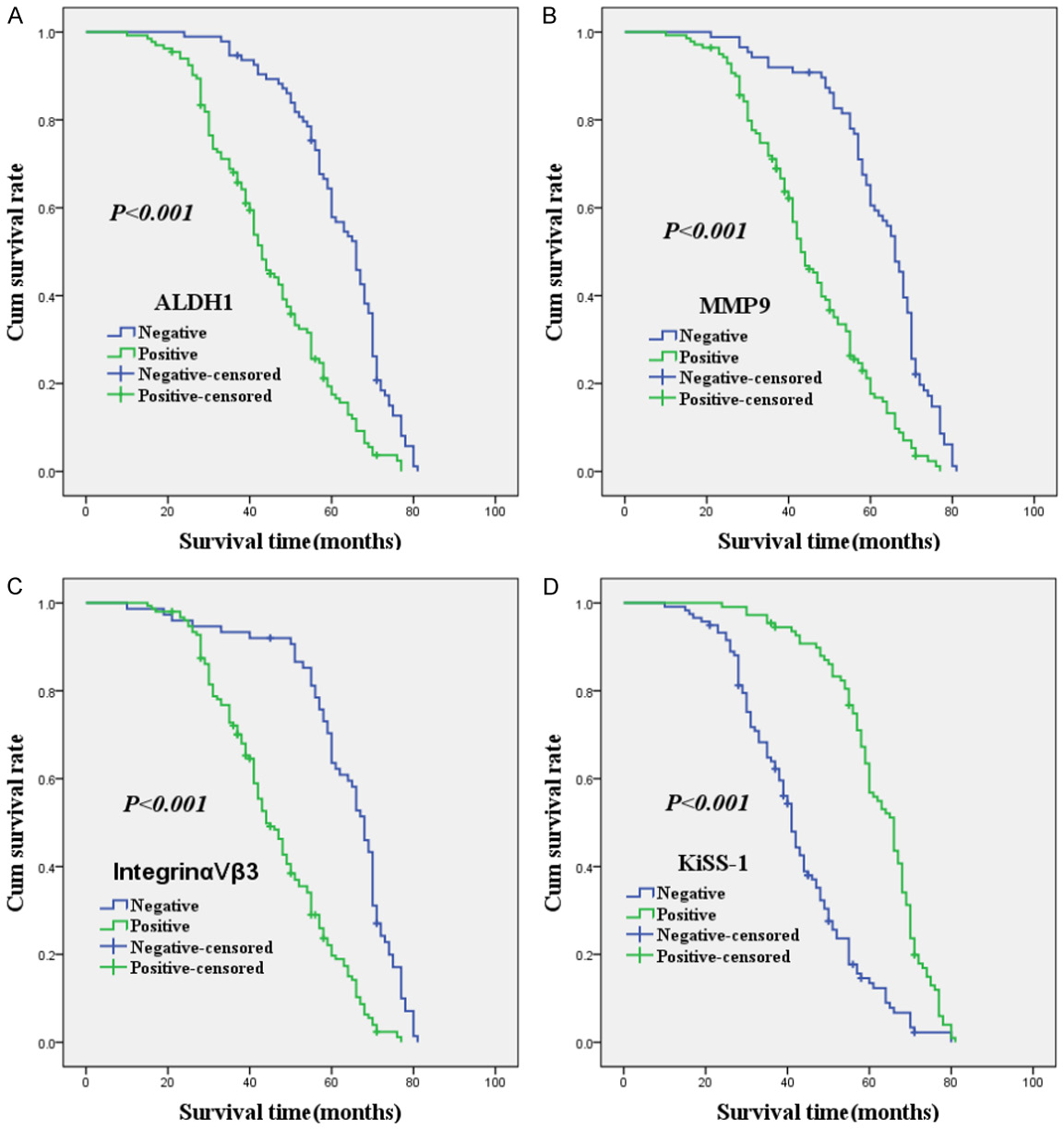

Figure 3. Recurrence-free survival of patients with IDC. The y-axis represents the percentage of patients; the x-axis, their survival in months. (A) Recurrence-free survival of all patients in relation to ALDH1 (log-rank =56.949, P<0.001); (B) Recurrence-free survival of all patients in relation to MMP9 expression (log-rank =55.727, P<0.001); (C) Recurrence-free survival of all patients in relation to Integrin αvβ3 expression (log-rank =60.805, P<0.001); (D) Recurrence-free survival of all in relation to KiSS-1 expression (log-rank =73.851, P<0.001); In (A-D) analyses, the green line represents patients with positive ALDH1, or MMP9, or Integrin αvβ3, or KiSS-1 and the blue line representing the negative ALDH1, or MMP9, or Integrin αvβ3, or KiSS-1 group.

suggested that the positive expressions of ALDH1, MMP9, Integrin αvβ3, KiSS-1, ER, as well as HER2, and TNM stages were independent prognostic factors for RFS in IDC (**Table 5**).

Discussion

IDC is a highly aggressive and heterogeneous cancer, which may affect the productivity of biomarker appraisal. Therefore, the prognostic and metastatic value of biomarkers should be thoroughly evaluated to ensure their validity. ALDH1, which can degrade many endogenous and exogenous aldehydes, is a common biomarker of CSCs in many cancers [4-7, 23, 24]. In this study, we found that ALDH1 was positively associated with tumor size, grade, T stages, LNM stages, and TNM stages. Furthermore,

Table 4. Multivariate analysis of overall survival for 227 patients with IDC

Covariate	B	SE	*P* value	Exp (B)	95% CI
ALDH1	0.586	0.188	0.002	1.790	1.238-2.588
MMP9	0.480	0.188	0.011	1.616	1.117-2.337
Integrin αvβ3	0.825	0.203	<0.001	2.282	1.534-3.396
KiSS-1	-0.671	0.178	<0.001	0.511	0.361-0.724
ER	-0.377	0.177	0.033	0.686	0.485-0.970
HER2	0.363	0.182	0.046	1.438	1.007-2.052
TNM stages	0.632	0.250	0.008	1.941	1.189-3.169

Table 5. Multivariate analysis of recurrence-free survival for 227 patients with IDC

Covariate	B	SE	*P* value	Exp (B)	95% CI
ALDH1	0.488	0.190	0.010	1.628	1.122-2.362
MMP9	0.393	0.184	0.032	1.482	1.034-2.124
Integrin αvβ3	0.798	0.203	<0.001	2.220	1.491-3.307
KiSS-1	-0.713	0.177	<0.001	0.490	0.347-0.694
ER	-0.379	0.180	0.035	0.685	0.481-0.974
HER2	0.362	0.181	0.045	1.436	1.008-2.045
TNM stages	0.509	0.246	0.038	1.664	1.028-2.693

OS and RFS analyzes indicated that the ALDH1 + IDC patients had significantly lower OS time than did the ALDH1 - patients. These results demonstrate that ALDH1 played an important role in IDC development and metastasis, and can be considered as a useful biomarker for IDC. These results were consistent with previous studies, including those of breast cancer and other cancers [4-7, 23, 24].

The MMP9 gene, an adhesion molecule, is closely related to invasion and metastasis. The knockout of MMP9 has been showed to suppress carcinogenesis and metastasis and delay tumor.

vascularization [25, 26]. In this study, MMP9 expression was found to be closely associated with tumor size, grade, T stages, LNM stages, and TNM stages. Moreover, OS and RFS analyzes suggested that IDC patients with MMP9 + specimens had significantly shorter OS time than did the MMP9 - patients. These results indicated that MMP9 overexpression promotes IDC invasion and metastasis, as shown by previous studies [10-13, 26].

Tumor cell migration is a key step in cancer progression and metastasis. Cell invasion and metastasis is a complex process mediated by cell-cell adhesion molecules (such as E-cadhesion) and cell-matrix adhesion (such as Integrins). Integrin αvβ3 can promote tumor cell invasion and metastasis by regulating cell growth, differentiation, and migration [14, 15]. In the current study, we found that Integrin αvβ3 expression was significantly higher in IDC tissues than in the control tissues, and its expression was positively associated with tumor size, grade, T stages, LNM stages, and TNM stages. In addition, a Kaplan-Meier analysis of OS and RFS indicated that IDC patients with Integrin αvβ3 + specimens had a significantly lower survival time than did the Integrin αvβ3 - patients. These findings suggest that Integrin αvβ3 overexpression should promote IDC invasion and metastasis, which is consistent with the findings of other studies [14, 15, 27, 28].

KiSS-1, which can suppress tumor cells proliferation, migration, and metastasis, is known as a suppressor of cancer metastasis [6, 17-22, 29]. In this study, we found that KiSS-1 expression was significantly lower in IDC tissues than that in the control tissues, and its expression was inversely correlated with tumor size, grade, T stages, LNM stages, and TNM stages. Furthermore, OS and RFS analysis demonstrated that KiSS-1 positive expression patients had significantly longer survival than the KiSS-1 negative patients. These results suggest that the down-expression of KiSS-1 should promote tumor cells invasion and metastasis, and it implies a poor prognosis. This finding is similar to those of other studies [6, 17-19, 21, 22, 29].

Invasive ductal carcinoma (IDC) is the most common type of breast cancer. ALDH1 is a common biomarker of CSCs; therefore, its overexpression should be involved in the initiation and progression of IDC [30]. CSCs should promote cancer cells' epithelial-mesenchymal transition (EMT) to cause invasion and metastasis. EMT is defined as the loss of epithelial features and the acquisition of a mesenchymal phenotype [31, 32]. EMT also induces obvious morphological changes of cancer cells and cell-cell or cell-matrix adhesion molecule expression in order to induce cancer cells into further invasion and metastasis [33]. MMP9 overex-

pression often degrades the extracellular matrix and remodels the cytoskeleton. This may promote cancer cell migration, invasion, and metastasis [34]. Integrin αvβ3 is a member of the family of cell surface adhesion receptors which can regulate cell migration, growth, and differentiation. Overexpression of Integrin αvβ3 promotes cancer cells' aggressiveness and implies a poorer prognosis [15]. Overexpression of Integrin αvβ3 also promotes tumor angiogenesis and angiogenesis of the surrounding tissues, which can cause further tumor cell invasion and metastasis [35]. Normally, KiSS-1 inhibits cancer cell metastasis by the enhancement of E-cadherin expression and the inhibition of MMPs expression [18, 19]. So, aberrant expression of KiSS-1 should further promote cancer cell invasion and metastasis [6, 19]. In this study, OS and RFS analysis indicated that the expression of ALDH1, MMP9, Integrin αvβ3, and KiSS-1 were significantly associated with the survival time of patients with IDC; a COX multivariate regression analysis showed that positive expression of ALDH1, MMP9, Integrin αvβ3, KiSS-1, ER, and HER2, as well as TNM stages were independent prognostic factors for IDC patients. These results demonstrate that the expression of ALDH1, MMP9, Integrin αvβ3, and KiSS-1 should be considered as useful biomarkers for IDC, especially in predicting the metastasis and prognosis of patients with IDC.

Conclusions

Our results indicate that ALDH1, MMP9, Integrin αvβ3, and KiSS-1 may be involved in IDC development, and the combined detection of ALDH1, MMP9, Integrin αvβ3, and KiSS-1 should be considered valuable biomarkers for metastasis and prognosis in IDC.

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Disclosure of conflict of interest

None.

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