Co-expression of Mesothelin and CA125/MUC16 Is a Prognostic Factor for Breast Cancer

Takahiro Einama (einama0722@ndmc.ac.jp)
National Defense Medical College: Boei Ika Daigakko https://orcid.org/0000-0002-4434-0803
Yoji Yamagishi
National Defense Medical College: Boei Ika Daigakko
Yasuhiro Takihata
National Defense Medical College: Boei Ika Daigakko
Takafumi Suzuki
National Defense Medical College: Boei Ika Daigakko
Tamio Yamasaki
National Defense Medical College: Boei Ika Daigakko
Yuichi Hirose
National Defense Medical College: Boei Ika Daigakko
Kazuki Kobayashi
National Defense Medical College: Boei Ika Daigakko
Naoto Yonamine
National Defense Medical College: Boei Ika Daigakko
Ibuki Fujinuma
National Defense Medical College: Boei Ika Daigakko
Takazumi Tsunenari
National Defense Medical College: Boei Ika Daigakko
Makiko Koga
National Defense Medical College: Boei Ika Daigakko
Yusuke Ishibashi
National Defense Medical College: Boei Ika Daigakko
Ken Nagata
National Defense Medical College: Boei Ika Daigakko
Takehiro Shiraishi
National Defense Medical College: Boei Ika Daigakko
Akiko Nakazawa
National Defense Medical College: Boei Ika Daigakko
Toshimitsu Iwasaki
National Defense Medical College: Boei Ika Daigakko
Eiji Shinto
National Defense Medical College: Boei Ika Daigakko
Kimi Kato
National Defense Medical College: Boei Ika Daigakko
Kimiya Sato
National Defense Medical College: Boei Ika Daigakko
Hideki Ueno
National Defense Medical College: Boei Ika Daigakko
Yoji Kishi
National Defense Medical College: Boei Ika Daigakko
Hitoshi Tsuda
National Defense Medical College: Boei Ika Daigakko

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Abstract

Background: The expression of mesothelin correlates with a poor prognosis in patients with breast cancer. Since mesothelin plays a role in cancer metastasis in association with CA125, we herein examined the clinicopathological significance and prognostic implications of the co-expression of mesothelin and CA125 in breast cancer.

Methods: The expression of mesothelin and CA125 was immunohistochemically examined in tissue samples collected from 478 breast cancer patients. The expression of these two molecules in more than 1% of tumor cells was defined as positive. The relationships between the co-expression of mesothelin and CA125, clinicopathological parameters, and clinical outcomes were analyzed by the chi-squared test and Cox's univariate and multivariate proportional hazard model analyses.

Results: Among 478 patients, mesothelin and CA125 were co-expressed in 48 (10%), mesothelin only in 75 (16%), CA125 only in 217 (45%), and neither in 234 (49%). A strong correlation was observed between the expression of mesothelin and CA125 (P =0.0004). The co-expression of mesothelin and CA125 correlated with unfavorable patient relapse-free survival (RFS) (P = 0.0001) and was identified as an independent predictor of RFS by Cox's multivariate analysis.

Conclusion: This is the first study to demonstrate the prognostic significance of the co-expression of mesothelin and CA125 in breast cancer. The co-expression of these two molecules may play a significant role in the acquisition of aggressive clinical behavior.

Introduction

Mesothelin (MSLN) is a 40-kDa cell surface glycoprotein that is expressed by normal mesothelial cells, which line the surface of the pleura, pericardium, and peritoneum(1, 2). It is also expressed in various types of malignant tumors, including malignant mesothelioma, ovarian cancer, and pancreatic cancer(3-6). The full-length human MSLN gene primarily encodes a 71-kDa precursor protein, which may be physiologically cleaved by several furin-like proteases into a membrane-bound 40-kDa C-terminal fragment and a 31-kDa N-terminal fragment that is secreted into the blood(2). The C-terminal 40-kDa fragment, named MSLN, is attached to the cell membrane through a glycosyl-phosphatidylinositol (GPI) anchor(2, 7).

CA125/MUC16 (CA125) is another cell surface glycoprotein that is present on the normal mesothelial cells lining body cavities(8, 9). An increase in the cell surface expression of CA125 has been reported in ovarian cancer and mesothelioma as well as in other cancers(8, 10-12). Due to its shedding into the blood circulation, serum CA125 is commonly measured to monitor disease progression in ovarian cancer patients and is also elevated in mesothelioma as well as under specific benign conditions(13-15). The gene encoding the peptide moiety of CA125 has been cloned and termed MUC16 because it shares characteristics associated with mucin proteins(16, 17).

MSLN may be one of the binding partners for CA125(18-21). Heterotypic adhesion through the high-affinity interaction between MSLN and CA125 may facilitate peritoneal metastasis from ovarian cancer(18, 20). However, the significance of the co-expression of MSLN and CA125 in breast cancer tissues currently remains unknown.

A correlation has been reported between the expression of MSLN and worse clinical outcomes in various cancer types(6, 21-23). We previously demonstrated that the expression of MSLN correlated with an aggressive tumor subtype and poor prognosis in patients with breast cancer(24). In the present study, we investigated the status of MSLN and CA125 expression in breast cancer by immunohistochemistry and examined the relationship between the co-expression of these two molecules and clinicopathological parameters, including patient relapse-free survival (RFS).

Materials And Methods

Patient Demographics and Tumor Specimens

The present study was performed with the approval of the Internal Review Board on ethical issues of the National Defense Medical College, Tokorozawa, Japan. Subjects comprised 478 patients who underwent surgery with curative intent for primary breast cancer between January 2002 and December 2012 at the Department of Surgery, National Defense Medical College Hospital. The clinicopathological parameters of these cases are summarized in Table 1.

Mean patient age was 59.2 (± 0.5 standard deviation [SD]) years. Four hundred and seventy-five patients (99.4%) were women, and the remaining 3 (0.6%) were men. The nuclear grade (NG) was classified according to General Rules for Clinical and Pathological Recording of Breast Cancer, 18th edition(25). Estrogen and progesterone receptors were assessed by immunohistochemistry and defined as positive based on immunoreactivity in 1% or more of constituent carcinoma cells(26). Judgments on human epidermal growth factor receptor 2 were made according to the American Society of Clinical Oncology/College of American Pathologists guidelines 2013(27). The Ki-67 labeling index (LI) was assessed according to the recommendations of the Breast Cancer Working Group(28), and was defined as high when 14% or more of constituent carcinoma cells were immunoreactive(29). Pathological T and N factors and stages were graded by the clinical and pathological findings of breast cancer according to UICC 8th edition. The median follow-up period was 94.0 months (range: 1.0 to 196.7 months). Fifty-three patients were lost to the follow-up, 71 relapsed, 59 had distant metastasis, and 32 died of breast cancer.

Immunohistochemistry

Formalin-fixed paraffin-embedded tissue blocks from 478 patients were collected from the archives of the Pathology Section, Department of Clinical Laboratories, National Defense Medical College Hospital. Tissue microarrays containing two representative tissue cores with a diameter of 2 mm for each case were constructed using a tissue microarrayer (Azumaya, Tokyo, Japan). Four-micrometer-thick sections were cut from these tissue microarray blocks and

mounted on charged glass slides, deparaffinized, and rehydrated through a graded series of ethanol. Dako Target Retrieval Solution pH 9.0 (catalog no. S2368; Dako, Carpinteria, CA, USA) was used for antigen retrieval, and slides were boiled in a pressure cooker (Pascal Pressure Cooker, model S2800; Dako) at 125℃ for 3 minutes. Sections were treated with 0.3% hydrogen peroxide for 5 minutes to block endogenous peroxidase activity. One slide for each section was then incubated with a mouse monoclonal antibody against MSLN (clone 5B2 diluted 1:50; Novocastra, Newcastle Upon Tyne, UK), and the other with a mouse monoclonal antibody against CA125 (clone M11 diluted 1:50; Dako) at room temperature for 30 minutes. They were reacted with a dextran polymer reagent combined with secondary antibodies and peroxidase (Envision/HRP; Dako) at room temperature for 30 minutes. Specific antigen-antibody reactions were visualized with 0.2% diaminobenzidine tetrahydrochloride and hydrogen peroxide. Slides were counterstained with hematoxylin for 10 minutes and then gently rinsed in reagent quality water.

Immunohistochemical Evaluation

All assessments were performed on the tumor region of the specimen (×200). Each slide was evaluated independently by 2 observers (T.E., Y.Y.) who were blinded to clinical outcomes. Immunostaining for MSLN and CA125 was assessed for both the staining proportion and intensity of tumor cells in each case. MSLN and CA125 expression levels were measured based on the percentage of cells showing the expression of each molecule as follows: <1%, 1% <10%, 10% <50%, and ≥50%. The intensities of the MSLN and CA125 immunoreactions were evaluated using the following scoring system: 1+, incomplete membrane staining and/or faint or barely perceptible cytoplasmic staining in tumor cells; 2+, the entire circumference of the cell membrane was stained and/or cytoplasmic staining exhibited moderate to strong staining. Cytoplasmic granular staining was also scored as 2+ (Fig. 1). The expression of MSLN and CA125 was positive when immunoreactivity was observed in 1% or more of tumor cells, irrespective of the intensity of immunoreactions, and negative when immunoreactivity was detected in less than 1% of cancer cells or was absent. Co-expression was positive when the expression of both MSLN and CA125 was detected, and was negative when the expression of MSLN, CA125, or both was absent.

Statistical Analysis

We used the X^2 test, Fisher's exact test, and the non-parametric Wilcoxon and Kruskal-Wallis tests to examine the relationships between the co-expression of MSLN and CA125 and clinicopathological parameters. Survival curves were drawn using the Kaplan-Meier method. Differences in survival curves were analyzed by the Log-rank test. The prognostic implications of the expression of MSLN and CA125 and other clinicopathological parameters were analyzed by Cox's univariate and multivariate proportional hazards models. All differences were considered to be significant at P <0.05. All statistical analyses were performed using JMP® 14 (SAS Institute Inc., Cary, NC, USA).

Results

Co-expression of MSLN and CA125 in Breast Cancer

The expression of MSLN was positive in carcinoma cells in 75 (15.7%) out of 478 breast cancer specimens, while the expression of CA125 was positive in 217 (45.4%) out of 478 specimens and in 48 (64.0%) out of 75 MSLN-positive specimens. Among 403 MSLN-negative cases, the expression of CA125 was positive in 169 cases and negative in 234. A correlation was observed between the expression of MSLN and CA125 (P = 0.0004) (Table 2).

The positive expression of MSLN correlated with the pathological T factor (P = 0.030), triple-negative subtype (P < 0.0001), NG3 (P < 0.0001), a higher Ki-67 LI (P = 0.0004), and higher relapse rate (P = 0.048). The positive expression of CA125 also correlated with the subtype (P = 0.0028) and a higher relapse rate (P = 0.045) (Table 3).

The co-expression of MSLN and CA125 was detected in 48 cases (10.0%) and correlated with the pathological T factor (P = 0.049), triple-negative subtype (P < 0.0001), NG3 (P < 0.0001), a higher Ki-67 LI (P = 0.0008), and higher relapse rate (P = 0.0022) (Table 3).

Clinical Analysis

Figure 2 shows the survival curves of 478 patients stratified by the MSLN and CA125 expression status of tumors. The RFS rate was significantly lower in patients with breast cancer expressing MSLN or CA125 than in those with breast cancer not expressing MSLN or CA125. Moreover, the prognosis of the group showing the co-expression of MSLN and CA125 was the poorest. Cox's univariate proportional hazards model analyses identified the pathological T factor, NG, lymphatic invasion, Ki-67 LI, and pathological N factor as significant risk factors for recurrence. Consistent with previous findings(24), the expression of MSLN was identified as a significant risk factor for recurrence (Hazard ratio (HR) 1.89, 95% confidence interval (CI) = 1.06-3.18, P = 0.0313). In the present study, the expression of CA125 was identified as a significant risk factor for recurrence (HR = 1.67, 95%CI 1.04-2.68, P = 0.0319), while its co-expression with MSLN was a significantly stronger risk factor (HR = 2.94, 95%CI 1.60-5.06, P = 0.0009).

In Cox's multivariate analyses, to exclude the possible effects of confounding factors, the multivariate analysis was performed including age, the pathological T factor, NG, lymphatic invasion, Ki-67 LI, and the pathological N factor with the expression of MSLN, CA125, or MSLN and CA125. The co-expression of MSLN and CA125 was identified as an independent predictor of RFS in breast cancer patients (HR =1 .92, 95%CI 1.01-3.46, P = 0.0483) as well as the pathological T factor (HR = 2.26, 95%CI 1.31-4.08, P = 0.0032) and pathological N factor (HR = 2.45, 95%CI 1.43-4.28, P = 0.0009) (Table 4).

Figure 3 shows the survival curves of 333 patients with hormone receptor (HR)-positive, HER2-negative breast cancer stratified by the MSLN and CA125 expression status of tumors. The RFS rate was significantly lower in patients with breast cancer expressing MSLN than in those with breast cancer not expressing MSLN (P = 0.0021). In contrast, the RFS rate was slightly lower in patients with breast cancer expressing CA125 than in those with breast cancer not expressing CA125 (P = 0.057). The prognosis of the group with the co-expression of MSLN and CA125 was the poorest. Cox's univariate and multivariate analyses were performed on 333 luminal-type cases (Table 5). The expression of MSLN was identified as a significant risk factor for recurrence (HR 3.16,

95%CI 1.36-6.54, *P* = 0.010). In luminal-type patients, the expression of CA125 was not a significant risk factor for recurrence (HR = 1.80, 95%CI 0.97-3.37, *P* = 0.0606); however, its co-expression with MSLN and CA125 was identified as a significant risk factor (HR = 5.00, 95%CI 1.87-11.2, *P* = 0.0027). In the multivariate analysis, the expression of MSLN and its co-expression with CA125 were independent predictors of RFS in luminal-type breast cancer patients (Table 5).

Discussion

The present study investigated the clinicopathological implications of the co-expression of MSLN and CA125 in patients with breast cancer. The co-expression of MSLN and CA125, detected in 10% of patients with breast cancer, correlated with the pT, subtypes, grades, and Ki67 LI of primary tumors and a poorer patient prognosis, which suggested that the co-expression of these two molecules plays a significant role in the acquisition of aggressive clinical behavior by breast cancer.

We previously reported that the expression of MSLN correlated with tumor size, the subtype of breast cancer, higher NG and Ki-67 LI, and a higher relapse rate(24), which support findings on the expression of MSLN in pancreatic ductal adenocarcinoma, gastric cancer, extrahepatic bile duct cancer, and colorectal adenocarcinoma(6, 21-23, 30, 31). The expression of MSLN has potential as a prognostic marker in patients with invasive breast cancer(32, 33). Furthermore, among breast cancer subtypes, the expression of MSLN was found to be significantly more frequent in triple-negative breast cancer (TNBC) than in non-TNBC(34-37). Therefore, MSLN has potential as a novel immunotherapy target for TNBC; however, the relationship between its expression and patient prognosis remains controversial(34, 35, 37).

The present results demonstrated that the expression of MSLN correlated with that of CA125 in breast cancer. It is important to note that the patient group with breast cancer showing the co-expression of these two molecules had poorer RFS than those with high expression levels of MSLN or CA125 alone or low expression levels of MSLN and CA125. In the present study, the co-expression group more frequently had larger tumors, a higher NG, and higher Ki-67 LI, similar to the group with high MSLN expression levels, which correlated with recurrence. In ovarian and pancreatic cancers, the co-expression of MSLN and CA125 was previously found to be more frequent in the patient group with a more advanced clinical stage, higher histological grade, and worse clinical outcome(20, 21, 38). These findings indicate that the co-expression of these two molecules promotes tumor development and metastasis, leading to a poorer prognosis.

The functional properties of the co-expression signaling pathway have not yet been elucidated in detail. CA125 was identified as an MSLN ligand, and the binding of CA125 to MSLN has been shown to induce cell-to-cell adhesion in these cancer cells(18). Mizukami reported that sherbet-like aggregates of cancer cells that are biologically active, but have not formed a gross tumor mass, were only present in the patient group treated with amatuximab, a chimeric anti-MSLN monoclonal antibody(39). This effect is consistent with the anti-tumor activity of amatuximab, which utilizes immune-effector cells to kill antibody-bound cells via antibody-dependent cellular cytotoxicity and suppresses heterotypic cell formation to its cognate receptor on neighboring cells via the blockade of MSLN (39). Co-expression has been linked to the activation of both the AKT and ERK1/2 signaling pathways and has also been implicated in the metastatic growth and dissemination of cancer cells(40-43). Similar to other GPI-anchored proteins, MSLN requires CA125 and/or utilizes other receptors for intracellular signaling.

The binding interaction between MSLN and CA125 also selectively stimulates matrix metalloproteinase (MMP)-7, but not MMP-2 or MMP-9, in pancreatic cancer cells, which offers a novel perspective into the poor prognosis associated with their co-expression and the activation of MMP-7(44). MMP-7 is overexpressed in many tumors and may play an important role in tumor pathogenesis (45, 46). MMP-7 is a biomarker of fibrosis in the liver(47, 48) and the lungs(49). Koyama et al. reported the attenuation of cholestatic liver fibrosis in mice homozygous for the mesothelin gene mutation, mesothelin (-/-) mice, and/or CA125 (-/-) mice, indicating the potential of MSLN as a target for antifibrotic therapy(50).

In conclusion, we herein demonstrated the clinicopathological significance of the co-expression of MSLN and CA125 in breast cancer, particularly in the luminal type, as an independent prognostic factor. An immunohistochemical examination of the expression of MSLN and CA125 in surgically resected tumor specimens may be clinically useful for prognostication after surgical therapy in patients with breast cancer.

Declarations

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Competing interests: The authors declare that they have no competing interests.

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Authors' contributions: TE, YY, and HT performed the planning, acquisition of data, analysis of data, and writing of the manuscript. YT, TS, TY, YH, KK, NY, IF, TT, MK, YI, and YK acquired clinical data, KN, TS and ES acquired pathological data, and AN, TI, KK and KS conducted tumoral mesothelin and CA125 data

acquisition and data analysis. HU substantively revised the draft. All authors substantively revised the draft. All authors read and approved the final manuscript.

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Tables

Table 1 Clinicopathological parameters of 478 patients with breast cancer in the present study

Parameter		N=478 (%)
Age	≤50	115 (24.2)
	>50	363 (75.8)
Pathological T factor	pTis	1(0.2)
	pT1	263 (55.0)
	pT2	194 (40.6)
	pT3	20 (4.2)
Pathological N factor	N0	300(63.0)
	N1	123(25.6)
	N2	34(7.0)
	N3	21(4.4)
Pathological Stage	0	1(0.2)
	I	200(41.8)
	II	221(46.2)
	III	56(11.7)
Subtype	ER/PgR+ and HER2-	333 (70.0)
	ER/PgR+ and HER2+	30 (6.1)
	HER2+	34 (7.1)
	TNBC	81 (16.8)
Lymphatic invasion	Positive	284(59.4)
	Negative	194(40.6)
Nuclear grade	1	117(24.5)
	2	140(29.3)
	3	221(46.2)
Ki-67 labeling index	≥14	298(62.3)
	<14	180(37.7)
Recurrence	Yes	71 (13.0)
	No	407 (87.0)

ER, ER, Estrogen receptor;

HER2, Human epidermal growth factor receptor 2;

PgR, Progesterone receptor;

TNBC, Triple-negative breast cancer

Table 2 Mesothelin and CA125 immunostaining in breast cancer

	No. of cases (%)		
	CA125 expression		
	Positive	Negative	Total
Mesothelin expression			
Positive	48 (64.0)	27 (36.0)	75
Negative	169 (41.7)	234 (58.3)	403
Total	217	261	478

χ^2 test $P = 0.0004$

Table 3. Clinicopathological parameters according to mesothelin and CA125 expression levels

		Number of cases (%)								
		MSLN			CA125			MSLN and CA125 co-expression		
Parameter	Total N=478 (%)	Positive N=75 (%)	Negative N=403 (%)	*P*-value	Positive N=217 (%)	Negative N=261 (%)	*P*-value	Positive N=48 (%)	Negative N=430 (%)	*P*-v
Age, years (mean±SD)		60.3 (±11.6)	58.9 (±11.3)	0.33	57.7(±11.2)	60.2 (±11.4)	**0.012**	59.6(±11.6)	60.3 (±11.3)	
Pathological T factor										
T1 (<2 cm)	264	31 (11.8)	233 (88.2)	**0.030**	125 (47.3)	139 (52.7)	0.054	19 (7.2)	245 (92.8)	**0.0**
T2 (2-5 cm)	194	37 (19.1)	157 (80.9)		87 (44.8)	107 (55.2)		24 (12.4)	170 (87.6)	
T3 (>5 cm)	20	7 (35.0)	13 (65.0)		5 (25.0)	15 (75.0)		5 (25.0)	15 (75.0)	
Pathological N factor										
pN1, pN2, pN3	178	29 (16.3)	149 (83.7)	0.78	79 (44.3)	99 (55.6)	0.73	23 (12.9)	155 (87.1)	0.1
pN0	300	46 (15.3)	254 (84.6)		138 (46.0)	162 (54.0)		25 (8.3)	275 (91.7)	
Pathological Stage										
0-I	201	27 (13.4)	174 (86.6)	0.13	100 (49.8)	101 (50.2)	0.26	16 (8.0)	185 (92.0)	0.1
II	221	34 (15.4)	187 (84.6)		94 (42.5)	127 (57.5)		22 (10.0)	199 (90.0)	
III	56	14 (25.0)	42 (75.0)		23 (41.1)	33 (58.9)		10 (17.9)	46 (82.1)	
Subtype										
ER/PgR+ and HER2-	333	31 (9.3)	302 (90.7)	**<0.0001**	137 (41.1)	196 (58.9)	**0.0028**	17 (5.1)	316 (94.9)	**< 0.0**
ER/PgR+ and HER2+	30	1 (3.3)	29 (96.7)		15 (50.0)	15 (50.0)		0 (0.0)	30 (100.0)	
HER2+	34	7 (20.6)	27 (79.4)		16 (47.0)	18 (53.0)		4 (11.8)	30 (88.2)	
TNBC	81	36 (44.4)	45 (55.6)		49 (60.5)	32 (39.5)		27 (33.3)	54 (66.7)	
Lymphatic permeation										
Positive	284	46 (16.2)	238 (83.8)	0.71	123 (43.3)	161 (56.6)	0.27	24 (8.5)	260 (91.5)	0.1
Negative	194	29 (14.9)	165 (85.1)		94 (48.5)	100 (51.5)		24 (12.4)	170 (87.6)	
Nuclear grade										
1	117	12 (10.3)	105 (89.7)	**< 0.0001**	55 (47.0)	62 (53.0)	0.22	7 (6.0)	110 (94.0)	**< 0.0**
2	140	4 (2.9)	136 (97.1)		55 (39.3)	85 (60.7)		3 (2.1)	137 (97.9)	
3	221	59 (42.1)	162 (57.9)		107 (48.4)	114 (51.6)		38 (17.2)	183 (82.8)	
Ki-67 labeling index (%)										
≥14	298	33 (11.1)	265 (88.9)	**0.0004**	134 (45.0)	164 (55.0)	0.81	19 (6.4)	279 (93.6)	**0.0**
<14	180	42 (23.3)	138 (66.7)		83 (46.1)	97 (53.4)		29 (16.1)	151 (83.9)	
Relapse										
Yes	71	17	54 (76.1)	**0.048**	40 (56.3)	31 (43.7)	**0.045**	15 (21.1)	56 (78.9)	**0.0**

		(23.9)					
No	407	58 (14.3)	349 (85.7)	177 (43.5)	230 (56.5)	33 (8.1)	374 (91.9)

SD, standard deviation; ER, Estrogen receptor; PgR, Progesterone receptor; HER2, Human epidermal growth factor receptor 2; TNBC, Triple-negative breast cancer; MSLN; mesothelin

X^2 test.

Values in bold are significantly different.

Table 4 Cox's univariate and multivariate analyses for relapse in breast cancer patients

Parameter (Favorable vs. Unfavorable)	Univariate		Multivariate					
			Including mesothelin expression		Including CA125 expression		Including MSLN and CA125 co-expression	
	Hazard ratio (95% CI)	*P*-value	Hazard ratio (95% CI)	*P*-value	Hazard ratio (95% CI)	*P*-value	Hazard ratio (95% CI)	*P*-value
Age (>50 vs ≤50)	1.04 (0.59-1.73)	0.0897						
Pathological T factor (pT2, pT3 vs pTis, pT1)	3.69 (2.22-6.41)	**< 0.0001**	2.33 (1.35-4.18)	**0.0020**	2.35 (1.36-4.22)	**0.0020**	2.26 (1.31-4.08)	**0.0032**
Nuclear grade (3 vs. 1, 2)	2.31 (1.43-3.82)	**0.0005**	1.43 (0.739-2.35)	0.197	1.50 (0.892-2.58)	0.128	1.39 (0.81-2.42)	0.228
Lymphatic invasion (Positive vs. Negative)	2.39 (1.38-4.40)	**0.0013**	1.34 (0.747-2.56)	0.197	1.34 (0.744-2.56)	0.336	1.42 (0.79-2.73)	0.247
Ki-67 labeling index (%) (≥14.0 vs. <14.0)	1.62 (1.00-2.59)	**0.0481**	1.25 (0.753-2.07)	0.380	1.28 (0.774-2.11)	0.332	1.19 (0.71-1.98)	0.507
Pathological N factor (pN1, pN2, pN3 vs. pN0)	3.76 (2.31-6.29)	**< 0.0001**	2.61 (1.54-4.54)	**0.0003**	2.54 (1.50-4.43)	**0.0005**	2.45 (1.43-4.28)	**0.0009**
Estrogen receptor (Positive vs Negative)	2.43 (1.28-3.39)	**0.0037**	1.26 (0.59-2.83)	0.548	1.24 (0.60-2.78)	0.566	1.17 (0.55-2.62)	0.697
Progesterone receptor (Positive vs Negative)	2.42 (1.26-3.21)	**0.0038**	1.54 (0.72-3.09)	0.256	1.53 (0.71-3.07)	0.267	1.49 (0.69-2.99)	0.296
HER2 receptor (Negative vs Positive)	0.96 (0.50-1.93)	0.91						
Mesothelin expression (Positive vs. Negative)	1.89 (1.06-3.18)	**0.0313**	1.34 (7.38-2.35)	0.319				
CA125 expression (Positive vs. Negative)	1.67 (1.04-2.68)	**0.0319**			1.60 (1.00-2.58)	**0.0494**		
MSLN and CA125 co-expression (Positive vs. Negative)	2.94 (1.60-5.06)	**0.0009**					1.92 (1.01-3.46)	**0.0483**

CI, confidence interval

Table 5 Cox's univariate and multivariate analyses of relapse in 333 patients with luminal-type breast cancer

Parameter (Favorable vs. Unfavorable)	Univariate		Multivariate			
			Including mesothelin expression		Including MSLN and CA125 co-expression	
	Hazard ratio (95% CI)	*P*-value	Hazard ratio (95% CI)	*P*-value	Hazard ratio (95% CI)	*P*-value
Age (≤50 vs >50)	1.04 (0.50-2.01)	0.919				
Pathological T factor (pT2, pT3 vs pTis, pT1)	4.85 (2.46-10.4)	**< 0.0001**	2.93 (1.44-6.47)	**0.0027**	2.95 (1.45-6.51)	**0.0023**
Nuclear grade (3 vs. 1, 2)	1.61 (0.78-3.75)	0.205				
Lymphatic invasion (Positive vs. Negative)	1.55 (0.84-2.92)	0.160				
Ki-67 labeling index (%) (≥14.0 vs. <14.0)	1.73 (0.87-3.26)	0.110				
Pathological N factor (pN1, pN2, pN3 vs. pN0)	6.65 (3.30-14.9)	**< 0.0001**	5.05 (2.43-11.57)	**< 0.0001**	4.79 (2.32-11.0)	**< 0.0001**
Mesothelin expression (Positive vs. Negative)	3.16 (1.36-6.54)	**0.010**	2.96 (1.25-6.26)	**0.016**		
CA125 expression (Positive vs. Negative)	1.80 (0.97-3.37)	0.061				
MSLN and CA125 co-expression (Positive vs. Negative)	5.00 (1.87-11.2)	**0.0027**			3.46 (1.28-7.90)	**0.017**

CI, confidence interval

Figures

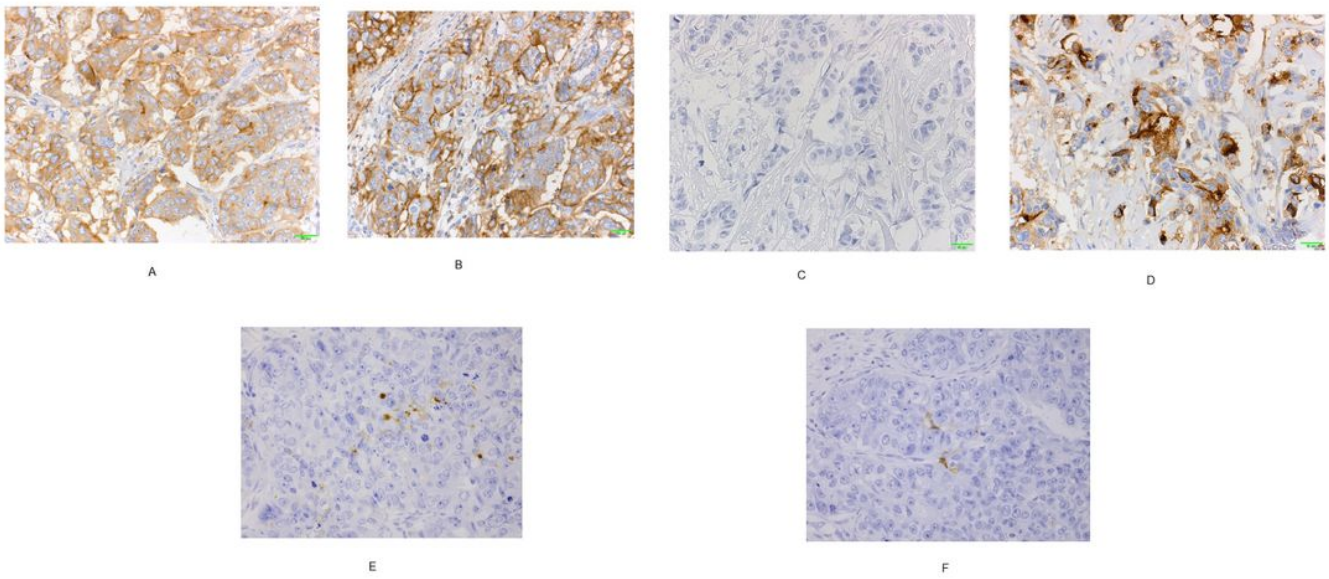

Figure 1

Representative cases of mesothelin and CA125 expression in breast cancer (A-F). Patient 1: Mesothelin (A) and CA125 (B) is diffusely positive in triple-negative breast cancer. Patient 2: Mesothelin was not expressed (C), whereas CA125 (D) was moderately to strongly expressed. Patient 3: The expression of mesothelin (E) and CA125 (F) was detected in the cytoplasm only in <10% of cancer cells. Immunoperoxidase stain, original magnification ×400.

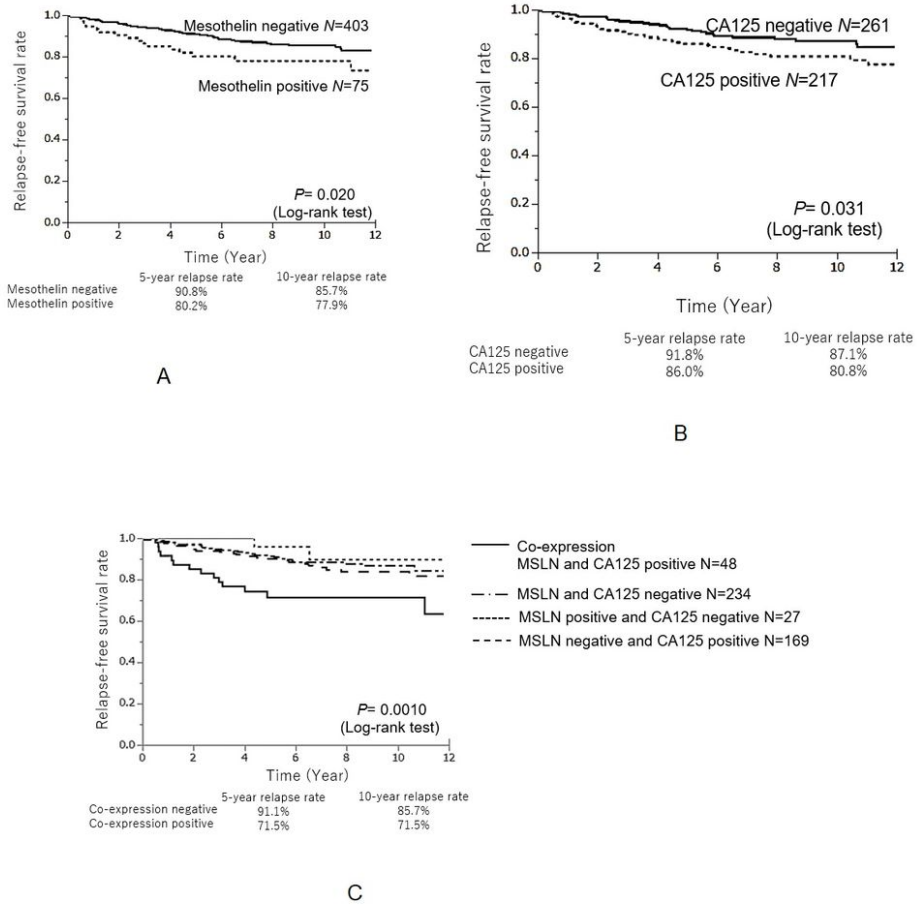

Figure 2

Relapse-free survival in patients with breast cancer after surgical therapy stratified by the status of the expression of mesothelin (A), CA125 (B), and mesothelin and CA125 (C). (A) The curve for the patient group with mesothelin-positive tumors was significantly different from that for the patient group with mesothelin-negative tumors (median RFS, 10.8 vs 12.0 years; P = 0.020). (B) The curve for the patient group with CA125-positive tumors was significantly different from that for the patient group with CA125-negative tumors (median RFS was 11.3 vs 12.2 years in the CA125-negative expression group; P = 0.031). (C) The curve for the patient group with tumors co-expressing mesothelin and CA125 was significantly different from that for the patient group with tumors not co-expressing these two molecules (MSLN- and CA125-negative N=234, MSLN-positive and CA125-negative N=27, MSLN-negative and CA125-positive N=169) (median RFS was 9.9 vs 12.1 years; P = 0.0001).

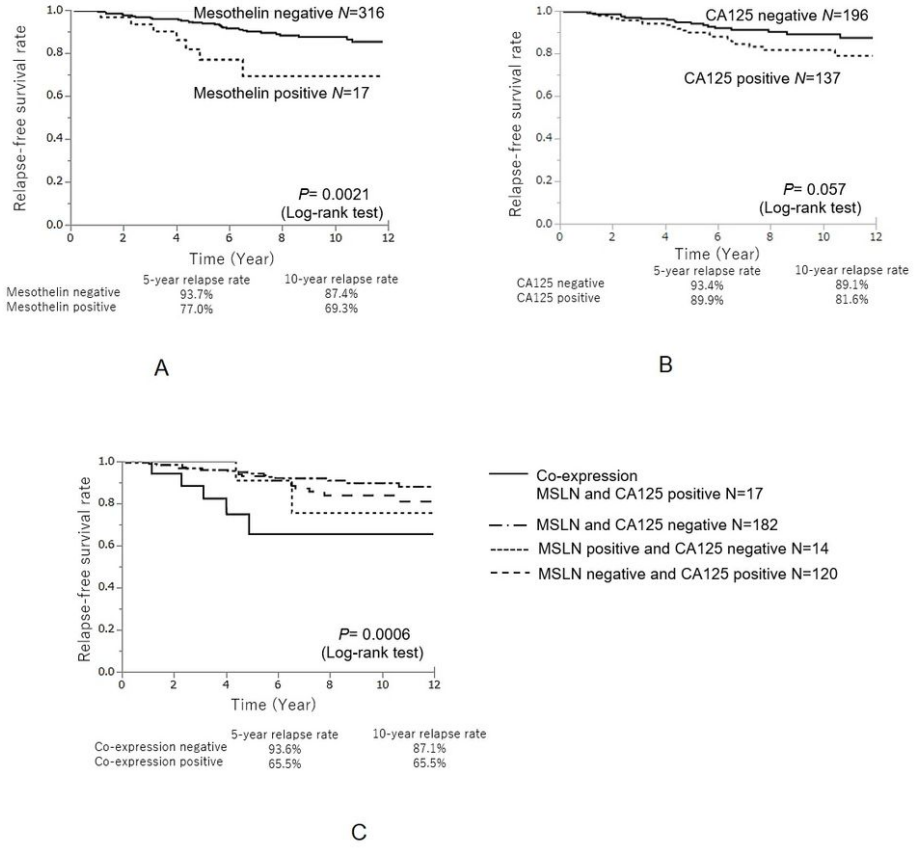

Figure 3

Relapse-free survival in luminal-type breast cancer patients after surgical therapy stratified by the status of the expression of mesothelin (A), CA125 (B), and mesothelin and CA125 (C). (A) The curve for the patient group with mesothelin-positive tumors was significantly different from that for the patient group with mesothelin-negative tumors (median RFS, 10.4 vs 12.3 years; P = 0.0021). (B) The curve for the patient group with CA125-positive tumors was significantly different from that for the patient group with CA125-negative tumors (median RFS, 11.5 vs 12.5 years in the CA125-negative expression group; P = 0.057). (C) The curve for the patient group with tumors co-expressing mesothelin and CA125 was significantly different from that for the patient group with tumors not co-expressing these two molecules (MSLN- and CA125-negative N=182, MSLN-positive and CA125-negative N=14, MSLN-negative and CA125-positive N=120) (median RFS, 9.8 vs 12.2 years; P = 0.0006).