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Combined caveolin-1 and epidermal growth factor receptor expression as a prognostic marker for breast cancer

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Abstract. Previous studies have indicated that caveolin-1 (Cav-1) is able to bind the signal transduction factor epidermal growth factor receptor (EGFR) to regulate its tyrosine kinase activity. The aim of the present study was to evaluate the clinical significance of Cav-1 gene expression in association with the expression of EGFR in patients with breast cancer. Primary breast cancer samples from 306 patients were analyzed for Cav-1 and EGFR expression using immunohistochemistry, and clinical significance was assessed using multivariate Cox regression analysis, Kaplan-Meier estimator curves and the log-rank test. Stromal Cav-1 was downregulated in 38.56% (118/306) of tumor tissues, whereas cytoplasmic EGFR and Cav-1 were overexpressed in 53.92% (165/306) and 44.12% (135/306) of breast cancer tissues, respectively. EGFR expression was positively associated with cytoplasmic Cav-1 and not associated with stromal Cav-1 expression in breast cancer samples; however, low expression of stromal Cav-1 was negatively associated with cytoplasmic Cav-1 expression in total tumor tissues, and analogous results were identified in the chemotherapy group. Multivariate Cox's proportional hazards model analysis revealed that, for patients in the estrogen receptor (ER)(+) group, the expression of stromal Cav-1 alone was a significant prognostic marker of breast cancer. However, in the chemotherapy, human epidermal growth factor receptor 2 (HER-2)(-), HER-2(+) and ER(-) groups, the use of combined markers was more effective prognostic marker. Stromal Cav-1 has a tumor suppressor function, and the combined marker stromal Cav-1/EGFR expression was identified as an improved prognostic marker in the diagnosis of breast cancer. Parenchymal expression of Cav-1 is able to promote EGFR signaling in breast cancer, potentially being required for EGFR-mediated initiation of mitosis.

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Key words: breast cancer, caveolin-1, epidermal growth factor receptor, prognosis

Introduction

Breast cancer is the most common female malignancy worldwide and its incidence increases annually (1,2). The caveolin-1 (Cav-1) gene is a member of the caveolin gene family, which is located at chromosomal locus 7q31.1 (3,4), and encodes the Cav-1 protein, which is a principal component of plasma membrane caveolae (5,6). Cav-1 is expressed in various breast cell types, including mammary gland epithelial cells, fibroblasts, adipocytes, endothelial cells and smooth muscle cells (7-9), and functions in tumorigenesis primarily through lipid transport, membrane transport, gene regulation and signal transduction (10). Its abnormal expression in breast cancer, where it is a putative tumor suppressor (11), is associated with the occurrence, progression and poor prognosis of breast cancer (12-14). Epidermal growth factor receptor (EGFR) interacts directly with the caveolin-scaffolding domain through a caveolin-binding sequence motif located in the intracellular kinase domain of the receptor (5,15), and this interaction has been demonstrated to modulate EGFR-mediated signaling (16,17).

Cav-1 mutation or abnormal expression is able to positively regulate the expression of EGFR (15,18,19), which is overexpressed in ~33% of breast cancers (20). Abnormal activation of EGFR often indicates poor prognosis and has a marked association with differentiation and metastasis of tumor cells (21-27). Importantly, EGFR is highly concentrated in caveolae membrane fractions and binds Cav-1 via a caveolin-binding motif of the kinase domain (15,28,29).

Considering the prognostic capacity of EGFR in breast cancer, the wide expression of Cav-1 in numerous breast cancers and the association between them, the present study

sought to use a combination approach analyzing two proteins as a combined prognostic marker for breast cancer, something which, to the best of our knowledge, has not previously been reported.

Materials and methods

Tissue microarray (TMA). Archived paraffin blocks of 306 female breast cancer tumor tissues and 50 adjacent normal tissues were obtained from The Third Affiliated Hospital of Harbin Medical University (Harbin, China) between January 2007 and December 2007 (age range, 27-82 years; median age, 49 years; operated). Tissue samples used in the present study were approved by The Hospital Ethics Committee for Ethical Review of Research Involving Human Subjects at Harbin Medical University. None of the patients in the present study received radiation or chemotherapy prior to the surgery that produced the paraffin-embedded tissues. Breast cancer TMAs created from each of the 356 total tissue samples were used for immunohistochemistry (IHC). Primary cancers were evaluated in accordance with the 7th edition of the American Joint Committee on Cancer tumor-node-metastasis (TNM) staging system (30). Estrogen receptor (ER) (+), progesterone receptor (PR)(+), human epidermal growth factor receptor 2 (HER-2) (+), p53 (+) and Ki-67 (+) patients were identified by a review of the pathological report. Median follow-up time for overall survival (OS) of 306 patients was 68.27 months (range, 3.85-75.02 months), and median follow-up time for disease-free survival (DFS) of 306 patients was 69.41 months (range, 2.14-75.02 months). Follow-ups for all patients continued until December 2013 or until mortality. The clinicopathological features of the 306 patients included are summarized in Table I.

IHC. Paraffin-embedded breast cancer TMA (Shanghai Outdo Biotech Co., Ltd.) sections (3 μm) were deparaffinized with pure xylene for 15 min and rehydrated in a descending alcohol series (from 70, 85 and 95% until pure alcohol for 5 min) at room temperature. The TMAs were subsequently submerged in citrate (pH 6.0) and autoclaved at 120˚C for 2 min, then quenched with 3% H_2O_2 for 10 min and blocked with 5% goat serum (OriGene Technologies, Inc., Beijing, China) for 10 min at room temperature. TMAs were cooled for 30 min prior to incubation with primary rabbit polyclonal antibody for Cav-1 (cat. no. 3238; dilution, 1:100; Cell Signaling Technology, Inc., Danvers, MA, USA) and a rabbit polyclonal instant antibody against EGFR (cat. no. YT1488; dilution, 1:200; Immunoway Biotechnology Company, Plano, TX, USA) overnight at 4˚C. Finally, TMAs were incubated with horseradish peroxidase-linked goat anti-rabbit secondary antibody (cat. no. PV6001; OriGene Technologies, Inc.) for 20 min at room temperature, the sections were then stained with DAB (OriGene Technologies, Inc.) to detect the proteins for 2 min at 37˚C, followed by counterstaining with hematoxylin for 5 min at 37˚C. Slides were dehydrated through an ascending series of alcohols (from 85 and 95% until pure alcohol for 5 min) and mounted. IHC and scoring were performed using a light microscope at magnifications, x100 and x400, separately for all samples by two independent investigators without any prior knowledge of the clinicopathological data. The degree of Cav-1 IHC staining was evaluated in tumor and stromal cells and scored semi-quantitatively. Cav-1 staining in tumor and stromal cells was scored semi-quantitatively as: 0, no staining; 1, either diffuse weak or focal strong staining in <30% of cells; or 2, strong staining of ≥30% cells (14). Comparison with endothelial cell staining intensity was used to assess the intensity of the immunoreactions and Cav-1 down-expression corresponded to grading scores 0 and 1. IHC staining for EGFR was scored according to the following criteria: -, 0-5%; +, 6-25%; ++, 26-50%; and +++, 51-100% of the cells stained. To optimally balance the multitude of the two sides divided on the basis of the positive staining rate, a threshold of 25% was used for EGFR. Positive values indicate that the positive staining cell rates were increased compared with the threshold value, whereas negative values indicate the rate was decreased compared with or equal to the threshold value.

Statistical analysis. Statistical calculations were performed using the SPSS statistical software package (version 19.0; IBM SPSS, Armonk, NY, USA). $P<0.05$ was considered to indicate a statistically significant difference. Correlation between expression levels was determined using the Pearson coefficient. χ^2 tests were used to analyze the association between Cav-1 and EGFR expression and clinicopathological features. Cumulative OS curves were generated according to the Kaplan-Meier estimator method, and the association between each of the variables and survival was assessed using the log-rank test in a univariate analysis. To identify independent predictors of survival, the parameters were then tested using the multivariate Cox's proportional hazards model.

Results

Expression of cytoplasmic EGFR and Cav-1 and stromal Cav-1 in breast cancer tissues. Using IHC, the expression of cytoplasmic EGFR and Cav-1 and stromal Cav-1 was detected in patient breast tumor and adjacent normal breast tissues. As presented in Fig. 1, cytoplasmic EGFR and Cav-1 were significantly overexpressed ($P<0.001$ and $P=0.007$, respectively) in tumor tissues relative to adjacent normal tissues, whereas stromal Cav-1 appeared to be downregulated ($P=0.010$). IHC analysis revealed that cytoplasmic EGFR and Cav-1 were overexpressed in 53.92% (165/306) and 44.12% (135/306), respectively, of tumor tissues, whereas stromal Cav-1 was downregulated in 38.56% (118/306) of tumor tissues.

Clinical significance of cytoplasmic EGFR and Cav-1 and stromal Cav-1 expression in breast cancer tissues. Downregulation of stromal Cav-1 in breast cancer was associated with differentiation ($P=0.050$), p53 status ($P=0.001$) and Ki-67 status ($P=0.042$), but not with any of the other clinical parameters. Overexpression of cytoplasmic EGFR was positively associated with HER-2 ($P=0.015$) and Ki-67 ($P=0.015$) expression (data not shown). Statistical correlation analysis demonstrated that EGFR expression was positively correlated with cytoplasmic Cav-1 expression ($r=0.177$; $P=0.002$), but no correlation with stromal Cav-1 expression in tumor tissues was identified; however, low expression of

Table I. Patient baseline and disease characteristics.

Feature	Patients
Total, n (%)	306 (100)
Median age, years (range)	49 (27-82)
Age, years, n (%)	
≤50	175 (57.19)
>50	131 (42.81)
Tumor size, cm, n (%)	
<2	121 (39.54)
>2 and ≤5	171 (55.88)
>5	14 (4.58)
Differentiation, n (%)	
High	20 (6.54)
Moderate	260 (84.97)
Poor	26 (8.49)
Adjuvant chemotherapy, n (%)	
Yes	249 (81.37)
No	57 (18.63)
Histological type, n (%)	
IDC	276 (90.20)
Other types	30 (9.80)
ATCC stage, n (%)	
I	63 (20.59)
II	192 (62.75)
III-IV	51 (16.66)
Lymph node status, n (%)	
No	152 (49.67)
Yes	154 (50.33)
Breast cancer subtype, n (%)	
Luminal A	145 (47.39)
Luminal B	119 (38.89)
HER-2+	18 (5.91)
Basal-like	24 (7.81)
p53 status, n (%)	
Negative	207 (67.65)
Positive	99 (32.35)
ER status, n (%)	
Negative	110 (35.95)
Positive	196 (64.05)
PR status, n (%)	
Negative	59 (19.28)
Positive	247 (80.72)
HER-2 status, n (%)	
Negative	237 (77.45)
Positive	69 (22.55)
Ki-67 status, n (%)	
Negative	48 (15.69)
Positive	258 (84.31)
Survival status, n (%)	
Deceased	56 (18.30)
Alive	250 (81.70)
Relapse status, n (%)	
Yes	40 (13.07)
No	266 (86.93)
Median disease-free survival (range)	69.41 (2.14-75.02)
Median overall survival (range)	68.27 (3.85-75.02)

IDC, invasive ductal carcinoma; ATCC, American Type Culture Collection; HER-2, human epidermal growth factor receptor-2; ER, estrogen receptor; PR, progesterone receptor.

Table II. Correlation of stromal Cav-1, cytoplasmic Cav-1 and EGFR in the total group.

Variable	Stromal Cav-1	EGFR
Cytoplasmic Cav-1		
Spearman's correlation	-0.325	0.177
P-value[a]	<0.001[a]	0.002[a]
Stromal Cav-1		
Spearman's correlation		0.039
P-value[a]		0.511

[a]P<0.05; Cav-1, caveolin-1; EGFR, epidermal growth factor receptor.

Table III. Correlation of stromal Cav-1, cytoplasmic Cav-1 and EGFR in the chemotherapy group.

Variable	Stromal Cav-1	EGFR
Cytoplasmic Cav-1		
Spearman's correlation	-0.272	0.181
P-value[a]	<0.001[a]	0.005[a]
Stromal Cav-1		
Spearman's correlation		0.016
P-value[a]		0.800

[a]P<0.05; Cav-1, caveolin-1; EGFR, epidermal growth factor receptor.

stromal Cav-1 was negatively correlated with cytoplasmic Cav-1 expression in tumor tissues of total patients group (r=-0.325; P<0.001; Table II). EGFR expression was positively correlated with cytoplasmic Cav-1 expression (r=0.181; P=0.005), but no correlation with stromal Cav-1 expression in tumor tissues was identified; however, low expression of stromal Cav-1 was positively correlated with cytoplasmic Cav-1 expression in tumor tissues of the total patients group (r=-0.272; P<0.001; Table III).

Clinical significance of combined marker EGFR/stromal Cav-1 expression in breast cancer tissues. Four patient subgroups were classified according to the combined expression

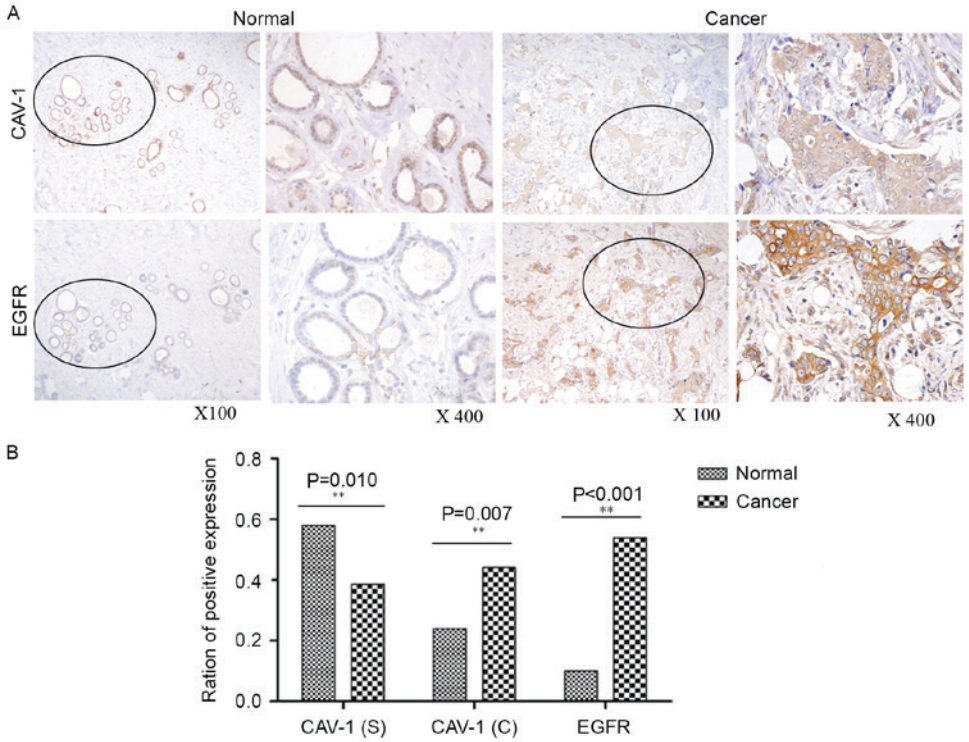

Figure 1. (A) IHC of protein expression in cancer tissues and adjacent normal tissues. High EGFR, high cytoplasmic Cav-1 expression and low stromal Cav-1 expression in tumor tissues compared with normal tissues. The magnification is indicated with the higher magnification images taken from the circled areas. (B) IHC of protein expression in tumor (n=306) and normal tissues (n=50). Compared with normal tissues, tumor tissues expressed significantly more stromal Cav-1 (P=0.01), cytoplasmic Cav-1 (P=0.007) and EGFR (P<0.001). Cav-1 (C), cytoplasmic Cav-1; Cav-1 (S), stromal Cav-1; IHC, immunohistochemistry; EGFR, epidermal growth factor receptor; Cav-1, caveolin-1.

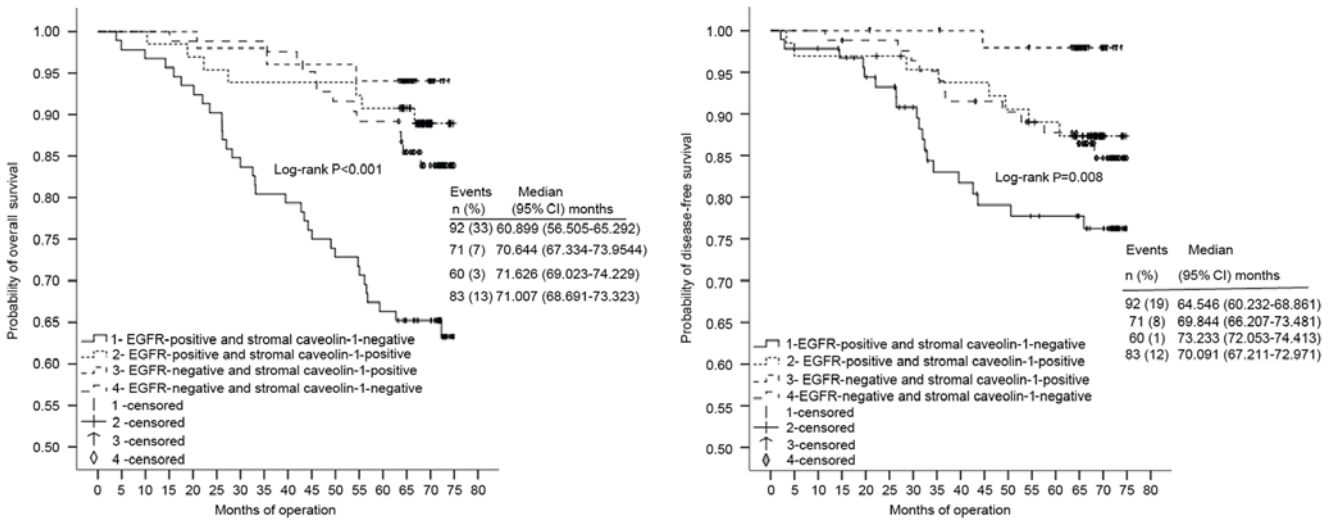

Figure 2. Line of overall survival and disease-free survival representing concurrent EGFR(+) and stromal caveolin-1(-) isolated separately from the other three lines (P<0.001 and P=0.008, respectively). EGFR, epidermal growth factor receptor; CI, confidence interval.

status of EGFR and stromal Cav-1 (Fig. 2). For OS and DFS, patient tissues with concordant high EGFR (P<0.001) and low stromal Cav-1 (P=0.008) expression (n=92) were observed isolated from those of the other three groups, classified as low EGFR/high stromal Cav-1 (n=60), high EGFR/high stromal Cav-1 (n=71) and low EGFR/low stromal Cav-1 (n=83). To simplify the data and illustrate a mechanistic framework, the four groups were consolidated into Cluster A [EGFR(+) and stromal Cav-1(-), n=92] and Cluster B [either EGFR(-) or stromal Cav-1(+), n=214]. Notably, there were significant associations with differentiation (P=0.041), p53 status (P=0.019) and PR status (P=0.036) for the Cluster A subgroup (data not shown).

OS univariate and multivariate analyses. The patients were divided into two further groups (adjuvant chemotherapy-treated patients, n=249; non-adjuvant chemotherapy-treated patients, n=57) on the basis of whether they received adjuvant

Table IV. Univariate and multivariate analyses of OS and DFS in patients with chemotherapy group (n=249).

	OS				DFS			
	Multivariate		Univariate		Multivariate		Univariate	
Variable	HR (95% CI)	P-value[a]	HR (95% CI)	P-value[a]	HR (95% CI)	P-value	HR (95% CI)	P-value
Age, years (<50 vs. ≥50)	1.586 (0.884-2.846)	0.122			1.066 (0.550-2.069)	0.85		
Differentiation (poor/moderate vs. high)	3.601 (1.516-8.555)	0.004[a]	4.463 (1.683-11.840)	0.003[a]	2.989 (1.052-8.496)	0.040		0.056
AJCC stage (III/IV vs. I/II)	12.305 (1.695-89.322)	0.013[a]	3.882 (2.237-6.739)	0.000	4.877 (1.172-20.306)	0.029	7.884 (4.029-15.426)	0.000
Histological type (IDC vs. other types)	0.503 (0.122-2.076)	0.342			0.995 (0.305-3.246)	0.994		
Breast cancer subtype		0.215				0.191		
Luminal B vs. luminal A	0.546 (0.234-1.277)	0.163			0.692(0.247-1.942)	0.485		
Basal-like/HER-2 + vs. luminal A	0.922 (0.406-2.096)	0.847			1.343 (0.498-3.617)	0.56		
ER expression (positive vs. negative)	0.449 (0.250-0.807)	0.007[a]		0.223	0.571 (0.297-1.099)	0.094		0.304
PR expression (positive vs. negative)	0.735 (0.372-1.450)	0.374			0.697 (0.328-1.482)	0.348		
Ki-67 expression (positive vs. negative)	0.656 (0.258-1.671)	0.377			1.519 (0.688-3.352)	0.301		
HER-2 expression (positive vs. negative)	2.291 (1.232-4.259)	0.009[a]		0.432	1.552 (0.729-3.301)	0.254		
p53 expression (positive vs. negative)	2.577 (1.417-4.687)	0.002[a]		0.412	2.901 (1.479-5.692)	0.002	2.738 (1.304-5.748)	0.008
Cytoplasmic Cav-1 expression (positive vs. negative)	1.197 (0.657-2.182)	0.557			0.79 (0.562-2.130)	1.094		
Stromal Cav-1 expression (positive vs. negative)	0.228 (0.096-0.539)	0.001[a]	0.296 (0.099-0.875)	0.028	0.456 (0.214-0.971)	0.042		0.433
EGFR expression (positive vs. negative)	1.893 (1.019-3.519)	0.044[a]		0.786	1.975 (0.988-3.951)	0.054		0.233
Combined markers (Cluster A vs. Cluster B)	2.885 (1.579-5.272)	0.001[a]	2.418 (1.139-5.133)	0.021	2.139 (1.107-4.134)	0.024	2.688 (1.280-5.643)	0.009

[a]P<0.05; Cluster A, EGFR+ and stromal Cav-1-; Cluster B, non-(EGFR+ and stromal Cav-1-); AJCC, American Joint Committee on Cancer; HR, hazard ratio; CI, confidence interval; OS, overall survival; DFS, disease-free survival; EGFR, epidermal growth factor receptor; Cav-1, caveolin-1.

Table V. Univariate and multivariate analyses of OS and DFS in patients with HER-2(-) expression (n=237).

	OS						DFS					
	Univariate			Multivariate			Univariate			Multivariate		
Variable	HR	95% CI	P-value[a]	HR	95% CI	P-value	HR	95% CI	P-value	HR	95% CI	P-value
Age, years (<50 vs. ≥50)	0.962	(0.380-2.438)	0.962				0.782	(0.375-1.633)	0.513			
Differentiation (poor/moderate vs. well)	1.442	(0.328-6.350)	0.628				3.503	(1.059-11.593)	0.040	12.985	(3.325-50.713)	0.000
AJCC stage (III/IV vs. I/II)	3.892	(1.637-9.249)	0.002[a]	4.852	(1.919-12.271)	0.001	4.964	(1.184-20.807)	0.028	9.573	(4.463-20.534)	0.000
Adjuvant chemotherapy (yes vs. no)	1.537	(0.444-5.316)	0.497				1.333	(0.466-3.811)	0.591			
Histological type (IDC vs. other types)	0.041	(0-24.654)	0.328				0.973	(0.296-3.200)	0.964			
Breast cancer subtype			0.141						0.141			
Luminal B vs. luminal A	0.819	(0.307-2.182)	0.690				0.994	(0.288-3.434)	0.993			
Basal-like/HER-2 + vs. luminal A	1.204	(0.429-3.380)	0.724				2.049	(0.583-7.197)	0.263			
ER expression (positive vs. negative)	0.580	(0.306-1.098)	0.094				0.548	(0.268-1.118)	0.098			0.061
PR expression (positive vs. negative)	0.889	(0.409-1.935)	0.767				0.766	(0.330-1.779)	0.535			
Ki-67 expression (positive vs. negative)	0.451	(0.138-1.469)	0.186				1.328	(0.543-3.248)	0.534			
p53 expression (positive vs. negative)	2.469	(1.302-4.681)	0.006[a]			0.130	2.754	(1.344-5.646)	0.006			0.081
Cytoplasmic Cav-1 expression (positive vs. negative)	1.040	(0.552-1.961)	0.903				1.118	(0.559-2.271)	0.758			
Stromal Cav-1 expression (positive vs. negative)	0.330	(0.146-0.748)	0.008[a]			0.278	0.433	(0.186-1.005)	0.050			0.212
EGFR expression (positive vs. negative)	2.264	(1.417-4.470)	0.019[a]			0.564	2.202	(1.037-4.677)	0.040			0.192
Combined markers (Cluster A vs. Cluster B)	3.075	(1.638-5.774)	0.000[a]	3.287	(1.658-6.516)	0.001	2.611	(1.289-5.287)	0.008	5.023	(2.224-11.345)	0.000

[a]P<0.05; Cluster A, EGFR+ and stromal Cav-1-; Cluster B non-(EGFR+ and stromal Cav-1-). HR, hazard ratio; CI, confidence interval; OS, overall survival; DFS, disease-free survival; EGFR, epidermal growth factor receptor; Cav-1, caveolin-1; AJCC, American Joint Committee on Cancer; IDC, invasive ductal carcinoma; ER, estrogen receptor; PR, progesterone receptor.

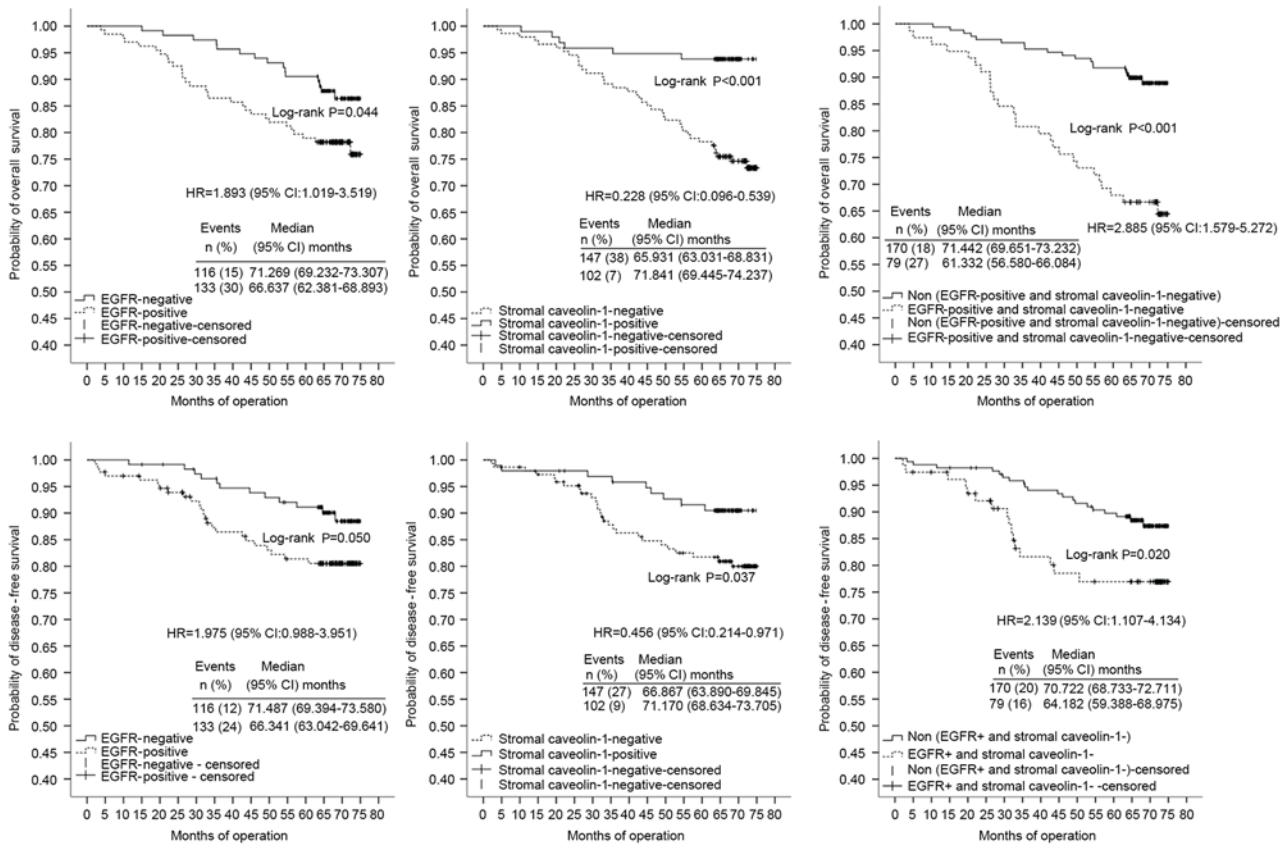

Figure 3. Kaplan-Meier estimator plots of overall survival and disease-free survival time distribution in association with EGFR (P=0.044 and P=0.050, respectively), stromal Cav-1 (P<0.001 and P=0.037, respectively) and cluster (EGFR and stromal Cav-1) (P<0.001 and P=0.020, respectively) expression in patients with chemotherapy. EGFR, epidermal growth factor receptor; Cav-1, caveolin-1; CI, confidence interval; HR, hazard ratio.

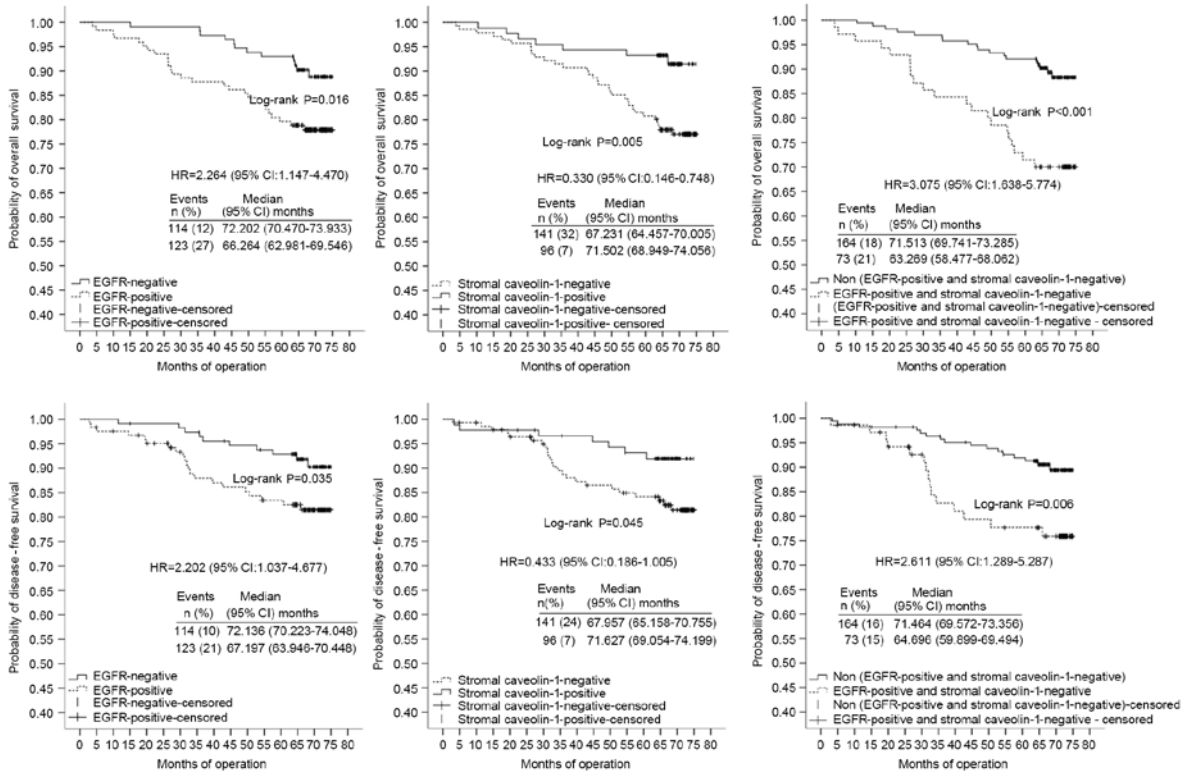

Figure 4. Kaplan-Meier estimator plots of overall survival and disease-free survival time distribution in association with EGFR (P=0.016 and P=0.035, respectively), stromal Cav-1 (P=0.005 and P=0.045, respectively) and cluster (EGFR and stromal Cav-1) (P<0.001 and P=0.006, respectively) expression in patients with human epidermal growth factor receptor 2(-) expression. EGFR, epidermal growth factor receptor; Cav-1, caveolin-1; CI, confidence interval; HR, hazard ratio.

Table VI. Univariate and multivariate analyses of overall survival in patients with HER-2(+) expression (n=69).

	Univariate			Multivariate		
Variable	HR	95% CI	P-value[a]	HR	95% CI	P-value[a]
Age, years (<50 vs. ≥50)	0.962	(0.380-2.438)	0.962			
Differentiation (poor/moderate vs. well)	1.442	(0.328-6.350)	0.628			
AJCC stage (III/IV vs. I/II)	3.892	(1.637-9.249)	0.002[a]	4.852	(1.919-12.271)	0.001[a]
Adjuvant chemotherapy (yes vs. no)	1.537	(0.444-5.316)	0.497			
Histological type (IDC vs. other types)	0.041	(0-24.654)	0.328			
Breast cancer subtype			0.141			
Basal-like /HER-2+ vs. luminal A vs. luminal B	0.823	(0.270-2.502)	0.731			
ER expression (positive vs. negative)	0.626	(0.144-2.729)	0.533			
PR expression (positive vs negative)	0.950	(0.310-2.905)	0.928			
Ki-67 expression (positive vs. negative)	1.028	(0.428-2.469)	0.951			
p53 expression (positive vs. negative)	1.192	(0.458-3.099)	0.719			0.081
Cytoplasmic Cav-1 expression (positive vs. negative)	1.023	(0.377-2.775)	0.964			
Stromal Cav-1 expression (positive vs. negative)	0.237	(0.068-0.830)	0.024[a]			0.619
EGFR expression (positive vs. negative)	1.712	(0.610-4.804)	0.307			
Combined markers (Cluster A vs. Cluster B)	4.627	(1.726-12.404)	0.002[a]	7.384	(2.522-21.714)	0.000[a]

[a]P<0.05; Cluster A, EGFR+ and stromal Cav-1-; Cluster B non-(EGFR+ and stromal Cav-1-). HR, hazard ratio; CI, confidence interval; EGFR, epidermal growth factor receptor; Cav-1, caveolin-1; ER, estrogen receptor; PR, progesterone receptor; AJCC, American Joint Committee on Cancer; IDC, invasive ductal carcinoma.

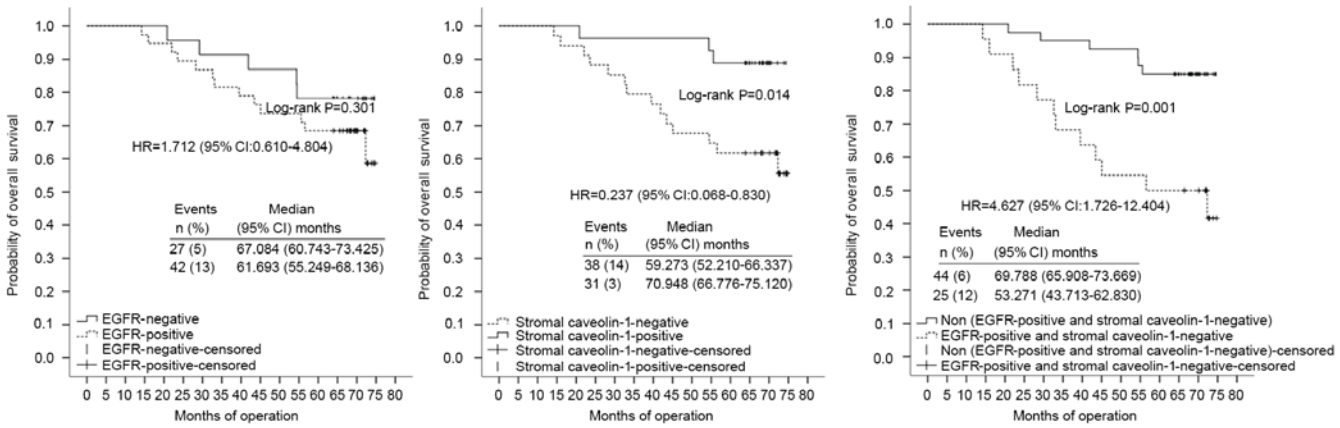

Figure 5. Kaplan-Meier estimator plots of overall survival time distribution in relation to EGFR (P=0.301), stromal Cav-1 (P=0.014) and cluster (EGFR and stromal Cav-1) (P=0.001) expression in patients with human epidermal growth factor receptor 2(-) expression. EGFR, epidermal growth factor receptor; Cav-1, caveolin-1; CI, confidence interval; HR, hazard ratio.

chemotherapy soon after surgical resection or not. In multivariate analyses using stratified Cox regression, OS rates of the adjuvant chemotherapy-treated patients were significantly lower in tissues with poor/moderate differentiation (HR, 4.463; 95% CI, 1.683-11.840), high TNM clinical stage (HR, 3.882; 95% CI, 2.237-6.739), stromal Cav-1(-) expression (HR, 0.296; 95% CI, 0.099-0.875) or those in the Cluster A subgroup (HR, 2.418; 95% CI, 1.139-5.133) (Table IV; Fig. 3). TNM clinical stage, p53 expression and combined expression of Cav-1 and EGFR markers were significant independent prognostic factors for DFS of the adjuvant chemotherapy-treated patients with HRs of 7.884 (95% CI, 4.029-15.429), 2.738 (95% CI, 1.304-5.748) and 2.688 (95% CI, 1.280-5.643), respectively (Table IV; Fig. 3). Notably, combined expression of Cav-1 and EGFR markers was the most marked independent prognostic factor in multivariate analysis, as presented in Fig. 3. For non-adjuvant chemotherapy-treated patients, EGFR, stromal Cav-1 and combined expression of Cav-1 and EGFR markers no significant correlation with OS in the multivariate analysis was identified.

Patients were divided into two groups on the basis of HER-2 and ER status. Multivariate analysis identified that differentiation, TNM clinical stage and combined markers were significant independent prognostic factors for OS of the HER-2(-) group (n=237) with HRs of 5.589 (95% CI, 1.863-16.766), 11.326 (95% CI, 1.545-83.025) and 3.287 (95% CI, 1.658-6.516), respectively (Table V;

Table VII. Univariate and multivariate analyses of overall survival in patients with estrogen receptor(+) expression (n=196).

	Univariate			Multivariate		
Variable	HR	95% CI	P-value[a]	HR	95% CI	P-value[a]
Age, years (<50 vs. ≥50)	1.929	(0.912-4.077)	0.085	2.721	(1.225-6.004)	0.014[a]
Differentiation (poor/moderate vs. well)	7.728	(2.657-22.473)	0.000[a]	13.309	(4.125-42.937)	0.000[a]
AJCC stage (III/IV vs. I/II)	9.656	(1.312-71.069)	0.026[a]	4.241	(2.225-8.087)	0.000
Adjuvant chemotherapy (yes vs. no)	0.643	(0.273-1.513)	0.312			
Histological type (IDC vs. other types)	0.298	(0.040-2.192)	0.234			
Breast cancer subtype						
Basal-like/HER-2/luminal B vs. Luminal A	0.630	(0.295-1.343)	0.231			
Basal-like/HER-2(0)	-	-	-	-	-	-
PR expression (positive vs. negative)	0.770	(0.232-2.552)	0.669			
Ki-67 expression (positive vs. negative)	0.794	(0.238-2.648)	0.708			
HER-2 expression (positive vs. negative)	1.402	(0.533-3.689)	0.494			
p53 expression (positive vs. negative)	3.000	(1.426-6.311)	0.004[a]			0.176
Cytoplasmic Cav-1 expression (positive vs. negative)	0.910	(0.429-1.934)	0.807			
Stromal Cav-1 expression (positive vs. negative)	0.349	(0.141-0.864)	0.023[a]	0.205	(0.077-0.547)	0.002[a]
EGFR expression (positive vs. negative)	1.561	(0.720-3.382)	0.259			
Combined markers (Cluster A vs. Cluster B)	2.546	(1.211-5.355)	0.014[a]			0.543

[a]P<0.05; Cluster A, EGFR+ and stromal Cav-1-; Cluster B non-(EGFR+ and stromal Cav-1-). HR, hazard ratio; CI, confidence interval; EGFR, epidermal growth factor receptor; Cav-1, caveolin-1; HER-2, human epidermal growth factor receptor; PR, progesterone receptor; AJCC, American Joint Committee on Cancer; IDC, invasive ductal carcinoma.

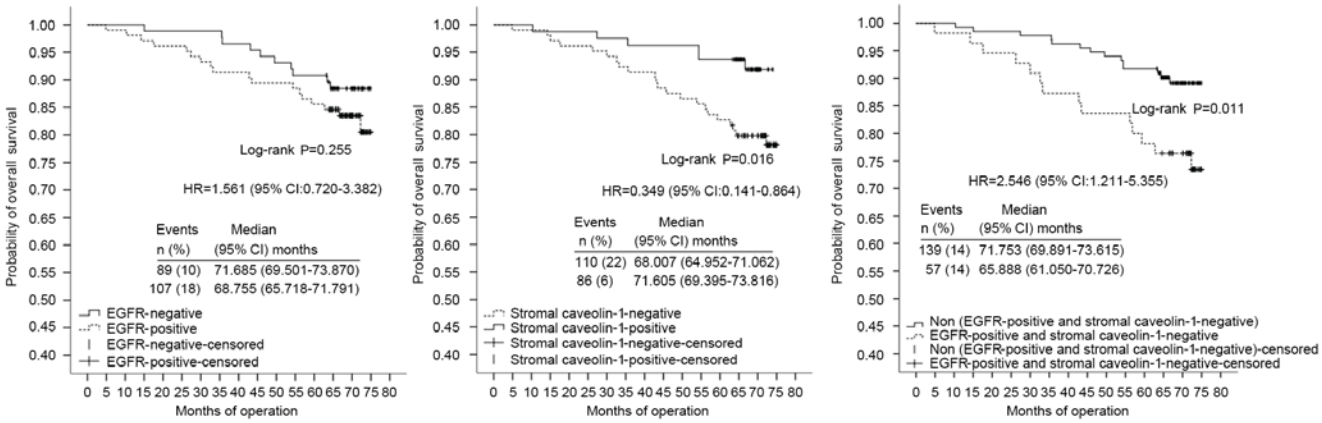

Figure 6. Kaplan-Meier estimator plots of overall survival time distribution in association with EGFR (P=0.255), stromal Cav-1 (P=0.016) and cluster (EGFR and stromal Cav-1) (P=0.011) expression in patients with human epidermal growth factor receptor-2(-) expression in patients with estrogen receptor(+) expression. EGFR, epidermal growth factor receptor; Cav-1, caveolin-1; CI, confidence interval; HR, hazard ratio.

Fig. 4). Poor/moderate differentiation (HR, 12.985; CI 95%, 3.325-50.713), high TNM clinical stage (HR, 9.573; 95% CI, 4.463-20.534) and combined markers in Cluster A (HR, 5.023; 95% CI, 2.224-11.345) were also confirmed as independent prognostic factors for low DFS of the HER-2(-) group (Table V; Fig. 4). High TNM clinical stage (HR, 4.852; 95% CI, 1.919-12.271) and combined markers in Cluster A (HR, 7.384; 95% CI, 2.522-21.714) were significant independent prognostic factors for OS in the HER-2(+) group (n=69) (Table VI; Fig. 5).

OS of the ER(+) group (n=196) was significantly decreased in patients of younger age (<50 vs. ≥50 years; HR, 2.712; 95% CI, 1.225-6.004) and in tumor tissues of poor/moderate differentiation (HR, 13.309; 95% CI, 4.125-42.937), high TNM clinical stage (HR, 4.241; 95% CI, 2.225-8.087) and stromal Cav-1(-) expression (HR, 0.205; 95% CI, 0.077-0.547) (Table VII; Fig. 6). TNM clinical stage and combined markers were significant independent prognostic factors of OS of the ER(-) group (n=110) with HRs of 3.631 (95% CI, 1.768-7.456) and 5.020 (95% CI, 2.250-11.200), respectively (Table VIII; Fig. 7). No significant correlation between EGFR, stromal Cav-1 and combined markers with DFS of ER(+) (n=188) or HER-2(+) (n=62) groups were identified in the multivariate analysis. However, the rates for DFS of the ER(-) group

Table VIII. Univariate and multivariate analyses of OS and DFS in patients with estrogen receptor(-) expression (n=110).

	OS				DFS			
	Univariate		Multivariate		Univariate		Multivariate	
Variable	HR (95% CI)	P-value	HR (95% CI)	P-value[a]	HR (95% CI)	P-value	HR (95% CI)	P-value
Age, years (<50 vs. ≥50)	1.086 (0.522-2.258)	0.825			1.073 (0.445-2.590)	0.875		
Differentiation (poor/moderate vs. well)	1.396 (0.420-4.638)	0.586			1.281 (0.297-5.526)	0.740		
AJCC stage (III/IIV vs. I/II)	3.939 (2.027-7.654)	0.000	3.631 (1.768-7.456)	0.000[a]	49.255 (11.309-214.522)	0.000	6.971 (2.855-17.023)	0.000[a]
Adjuvant chemotherapy (yes vs. no)	0.991 (0.378-2.597)	0.985			1.189 (0.348-4.057)	0.782		
Histological type (IDC vs. other types)	0.390 (0.053-2.869)	0.355			0.379 (0.046-3.144)	0.369		
Breast cancer subtype		0.3				0.300		
Luminal B vs. luminal A	1.133 (0.422-3.042)	0.805			2.390 (0.782-7.310)	0.127		
Basal-like/HER-2 + vs. luminal A	1.884 (0.804-4.415)	0.145			1.883 (0.597-5.944)	0.280		
PR expression (positive vs. negative)	1.472 (0.670-3.234)	0.336			2.040 (0.741-5.619)	0.168		
Ki-67 expression (positive vs. negative)	0.330 (0.078-1.392)	0.131			0.802 (0.233-2.759)	0.727		
HER-2 expression (positive vs. negative)	1.791 (0.861-3.725)	0.119			1.150 (0.459-2.886)	0.765		
p53 expression (positive vs. negative)	1.200 (0.562-2.563)	0.638			1.323 (0.538-3.256)	0.542		
Cytoplasmic Cav-1 expression (positive vs. negative)	1.298 (0.609-2.765)	0.5			1.388 (0.564-3.417)	0.476		
Stromal Cav-1 expression (positive vs. negative)	0.292 (0.101-0.842)	0.023		0.645	0.304 (0.089-1.037)	0.057		0.233
EGFR expression (positive vs. negative)	3.391 (1.447-7.945)	0.005		0.685	2.672 (1.025-6.965)	0.044		0.547
Combined markers (Cluster A vs. Cluster B)	4.485 (2.252-10.474)	0.000	5.020 (2.250-11.200)	0.000	3.324 (1.372-8.052)	0.008	3.475 (1.398-8.635)	0.007

[a]P<0.05; Cluster A, EGFR+ and stromal Cav-1-; Cluster B non-(EGFR+ and stromal Cav-1-); HR, hazard ratio; CI, confidence interval; OS, overall survival; DFS, disease-free survival; EGFR, epidermal growth factor receptor; Cav-1, caveolin-1; HER-2, human epidermal growth factor receptor 2; PR, progesterone receptor; AJCC, American Joint Committee on Cancer; IDC, invasive ductal carcinoma.

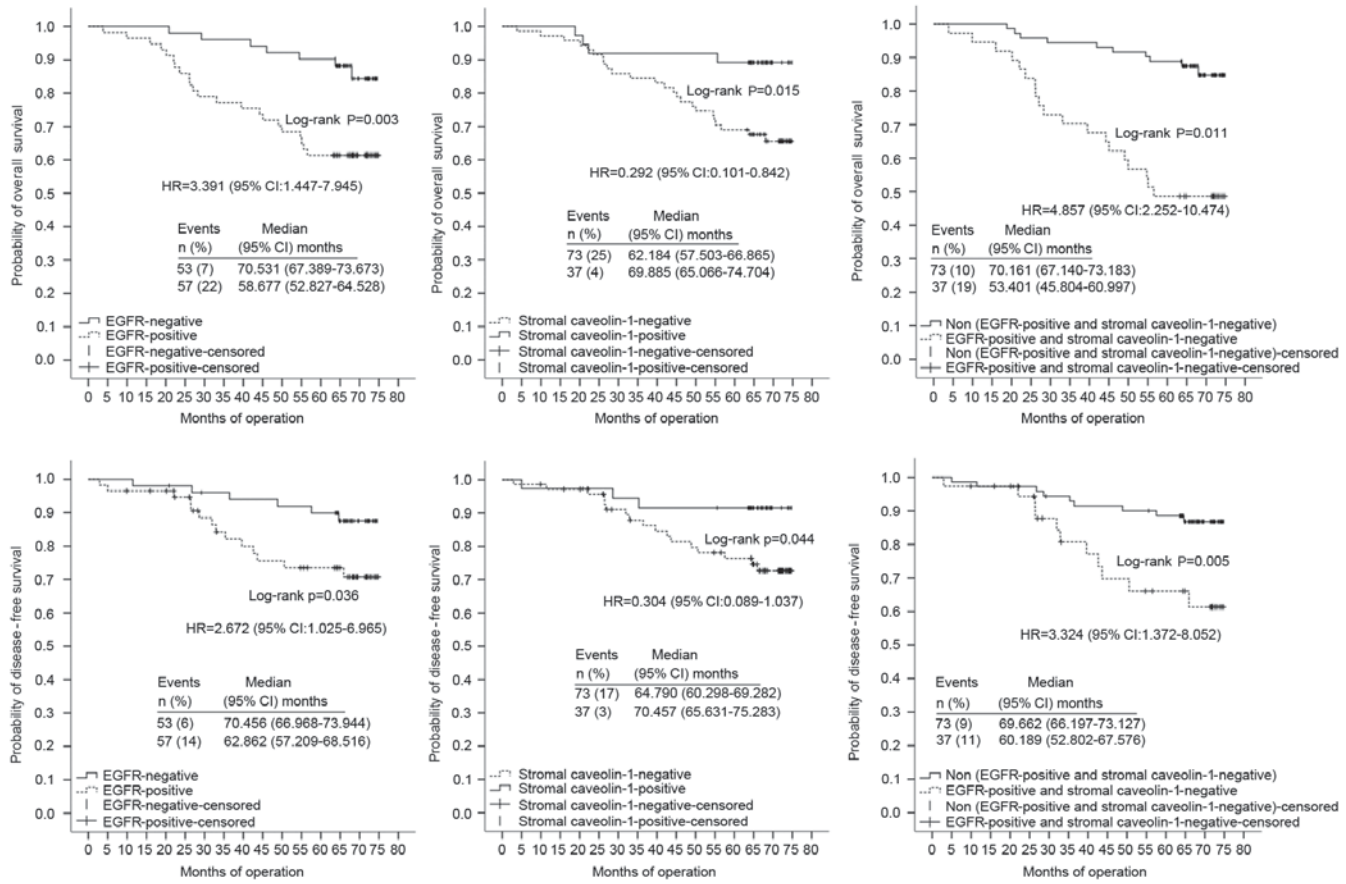

Figure 7. Kaplan-Meier estimator plots of overall survival and disease-free survival time distribution in association with EGFR (P=0.003 and P=0.036, respectively), stromal Cav-1 (P=0.015 and P=0.044, respectively) and cluster (EGFR and stromal Cav-1) (P=0.011 and P=0.005, respectively) expression in patients with estrogen receptor(-) expression. EGFR, epidermal growth factor receptor; Cav-1, caveolin-1; CI, confidence interval; HR, hazard ratio.

were significantly lower in tumor tissues of more advanced TNM clinical stage (HR, 6.971; 95% CI, 2.855-17.023) and those under combined markers in Cluster A (HR, 3.475; 95% CI, 1.398-8.635) (Table VIII; Fig. 7).

Discussion

Cav-1 is a primary scaffolding protein of the cell membrane whose abnormal stromal expression is associated with the occurrence, progression and prognosis of breast cancer (5,13), and Cav-1 negatively regulates EGFRs (17,31), further confirmed by data from the present study. In addition, it was revealed that Cav-1 is more effective as a breast cancer prognostic marker when its expression is combined with that of EGFR.

Multiple factors analysis of a series of variables revealed that, for patients in the ER(+) group, the expression of stromal Cav-1 alone is a significant prognostic marker of breast cancer. However, in the chemotherapy, HER-2(-), HER-2(+) and ER(-) groups, use of combined markers was more effective. Specifically, stromal Cav-1 expression combined with that of EGFR tends to have greater prognostic capacity than that of Cav-1 alone. Indeed, the data identified a consistent correlation among stromal Cav-1 and EGFR expression with clinical pathological features. This finding may be useful during the first step of prognostic screening as Cav-1 is readily detected in numerous breast cancer tumors.

Cav-1 is able to bind the signal transduction factor EGFR to regulate its tyrosine kinase activity and one of the notable findings is the ability of Cav-1 to regulate certain tyrosine kinase receptors (29). Inactivated EGFRs are clustered within caveolae and leave this lipid raft structure upon activation and raft internalization is regulated by Cav-1 scaffolds that indirectly regulate EGFR (28). In addition, oligomeric Cav-1 domains bind to inactive EGFR and prevent its activation (17). It was revealed that Cav-1 in the parenchyma is negatively associated with stromal Cav-1 expression and positively correlated with EGFR expression. It has been demonstrated that Cav-1 is a tumor suppressor in breast cancer (32). The combined results of these previous studies and our those of the present study suggest that Cav-1 has marked biological and clinical significance for EGFR(+) breast cancer, and the combination of stromal Cav-1 and EGFR expression in breast cancer is an improved prognostic indicator compared with either individually in tumors with a number of different receptor and pathological features.

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