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Original Article

Expression and Role of Epithelial Membrane Proteins in Tumorigenesis of Hormone Receptor-Positive Breast Cancer

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Conflict of Interest
The authors declare that they have no competing interests.

Author Contributions
Conceptualization: Cha YJ; Data curation: Cha YJ, Koo JS; Formal analysis: Cha YJ; Funding acquisition: Cha YJ; Methodology: Koo JS; Resources: Koo JS; Visualization: Cha YJ; Writing - original draft: Cha YJ; Writing - review & editing: Cha YJ, Koo JS.

ABSTRACT

Purpose: Studies on the expression of epithelial membrane proteins (EMPs) in breast cancer have been rare and limited. In the present study, we aimed to evaluate the expression of EMP1, EMP2, and EMP3 in invasive ductal carcinoma (IDC) of the breast, and investigate their clinical implications.
Methods: In total, 418 IDC cases were collected, and specimens were used to construct a tissue microarray. Immunohistochemical staining of EMP1, EMP2, and EMP3 was performed and the results were analyzed in combination with the clinical data.
Results: EMP1 was expressed in > 90% of all IDC subtypes. A decreased expression of EMP2 and EMP3 was observed in triple-negative breast cancer. EMP3 expression was independently associated with human epidermal growth factor receptor 2 (HER2) positivity. HER2-negative cases exhibited a decreased EMP2 expression along with a higher histological grade and an increased proliferative index. No significant difference was found in the overall survival or disease-free survival based on the EMP expression. In HER2-negative breast cancer, EMP2 expression inversely correlated with the histological grade and proliferative index.
Conclusion: EMP2 may be involved in the early stage of tumor development in hormone-positive breast cancer.

Keywords: Breast neoplasms; EMP-2 protein, human; Immunohistochemistry; Pathology

INTRODUCTION

Breast cancer is the most common carcinoma observed in women. Early detection reveals a good prognosis in patients; however, breast cancer is still an aggressive disease that requires multidisciplinary therapeutic approaches. Sex steroid hormones, particularly estrogen and progesterone, play crucial roles in the development of breast cancer and also serve as targets by acting as selective modulators of estrogen receptor (ER) [1]. Moreover, breast cancers with an amplified human epidermal growth factor receptor 2 (HER2) can be treated with the HER2 targeting agent trastuzumab [2]. The use of preoperative neoadjuvant chemotherapy is increasing and it indicates complete pathological response of HER2-positive cancer and triple-negative breast cancer (TNBC) in relation to tumor-infiltrating lymphocytes (TIL) [3]. As aforementioned, the hormone receptor (HR), HER2, and TIL are major parameters

determining the treatment outcome and patient prognosis; however, in addition to these known factors, efforts have been made to mine a new prognostic marker and/or potential therapeutic target.

Epithelial membrane proteins (EMPs; EMP1, EMP2, and EMP3) belong to the family of peripheral myelin protein (PMP22) with highly conserved structural homology. PMP22 is highly expressed in peripheral nerves, where it is localized in the compact portion of myelin. It is crucial for normal physiological and pathological processes in the peripheral nervous system.

So far, previous studies regarding EMP1, EMP2, and EMP3 demonstrated the variable expression of EMPs in diverse solid tumors. In breast cancer, several preclinical and clinical studies have been performed; however, these studies investigated only one type of EMP with relatively few breast cancer cases without considering the biomarker-defined subtypes [4-7]. Since no study has evaluated EMP1, EMP2, and EMP3 in the large number of invasive ductal carcinoma (IDC), that is most common histologic type of breast cancer, we aimed to examine the expression of EMPs IDC using numerous cases, assess the differential expression of EMPs among the IDC subtypes, and correlate the result with clinical parameters.

METHODS

This study was approved by the Institutional Review Board of Severance Hospital, Seoul, Korea (No. 4-2018-0429). The need for informed consent was waived by the review board.

Patients

From January 2000 to December 2012, a total of 5,427 patients were diagnosed with breast cancer at Severance Hospital, and 1,957 patients received surgical resection. Except 81 patients who were treated with preoperative neoadjuvant chemotherapy, available 493 cases were subjected to tissue microarray (TMA). Among these, 75 cases with core losses were excluded, and finally a total of 418 cases from female patients were included. All patients underwent treatments according to the standard protocols. All cases were retrospectively reviewed by 2 breast pathologists (YJC and JSK) using hematoxylin and eosin (H&E)-stained slides. Histological grade was assessed using the Nottingham grading system [8]. Tumor staging was based on the 8th American Joint Committee on Cancer criteria.

Disease-free survival (DFS) was calculated from the date of the first curative surgery to the date of the first locoregional or systemic relapse or to the date of death without relapse. Overall survival (OS) was estimated from the date of the first curative surgery to the date of the last follow-up or death from any cause. Clinicopathological parameters evaluated for each case included patient age at initial diagnosis, lymph node metastasis, tumor recurrence, distant metastasis, and patient survival.

TMA

All H&E-stained slides from resected breast cancer specimens were reviewed and representative areas were marked on the slides. Tissue cores (3 mm) were extracted from the matched formalin-fixed paraffin-embedded (FFPE) tumor blocks and were placed into 6 × 5 recipient TMA blocks. Two tumor cores were collected from each case to construct the TMA.

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Table 1. Source, clone, and dilution of antibodies

Antibody	Company	Clone	Dilution
EMP1	Abcam, Cambridge, UK	N-terminal	1:100
EMP2	Abcam, Cambridge, UK	C-terminal	1:100
EMP3	Santa Cruz Biotechnology, Santa Cruz, USA	SW-5	1:100

EMP = epithelial membrane protein.

Immunohistochemical staining and interpretation

Antibodies used in the study for immunohistochemistry (IHC) are listed in **Table 1**. Briefly, 3-μm thick tissue sections were cut from the FFPE TMA block. After deparaffinization and rehydration using xylene and alcohol graded solutions, respectively, IHC was performed on Ventana Discovery XT Automated Slide Stainer (Ventana Medical System, Tucson, USA). Cell Conditioning 1 buffer (citrate buffer, pH 6.0; Ventana Medical System) was used for antigen retrieval. Appropriate positive and negative controls were included.

Staining of all IHC markers was assessed via light microscopy. A cut-off value of 1% nuclear staining or more was considered positive for ER and progesterone receptor (PR) [9]. HER2 staining was interpreted based on the 2018 American Society of Clinical Oncology/College of American Pathologists guidelines [2]. Only strong and circumferential membranous HER2 expression (3+) was considered positive, whereas 0 and 1+ HER2 staining was regarded as negative. Cases with equivocal HER2 expression (2+) were further evaluated for HER-2 gene amplification using silver *in situ* hybridization (SISH). Positive nuclear Ki-67 staining was assessed with the positive tumor cell percentage reported as Ki-67 labeling index (LI).

To interpret the EMP1, EMP2, and EMP3 expression, IHC slides were scored by multiplying the staining intensity (1, weak; 2, moderate; 3, strong) and the staining proportion score (0%, negative; 1, < 30% positive; 2, ≥ 30% positive). Values of 0 and 1 were considered negative and those of 2 or more were considered positive [10]. Representative pictures of IHC are illustrated in **Figure 1**.

Tumor classification based on HR and HER2 status

Breast cancer subcategorized was based on biomarker status according to the IHC staining results of ER, PR, and HER2 and SISH results for HER2. The specimens were categorized as follows: ER and/or PR positive and HER2 negative (HR+HER2−), ER and/or PR positive and HER2 overexpressed and/or amplified (HR+HER2+), ER and PR negative and HER2 overexpressed and/or amplified (HER2), and ER, PR, and HER2 negative (TNBC).

Statistical analysis

Data were analyzed using SPSS software for Windows (version 18.0; SPSS Inc., Chicago, USA). A *p*-value less than 0.05 was considered as statistically significant. Student's *t*-test and Fisher's exact test were used for continuous and categorical variables, respectively. Wilcoxon signed rank test was used to evaluate the EMP expression in matched normal and cancer tissues within the same core. Kaplan-Meier survival curves and log-rank statistics were used to assess the tumor metastasis and survival time. Regression analysis was performed using binary logistic analysis.

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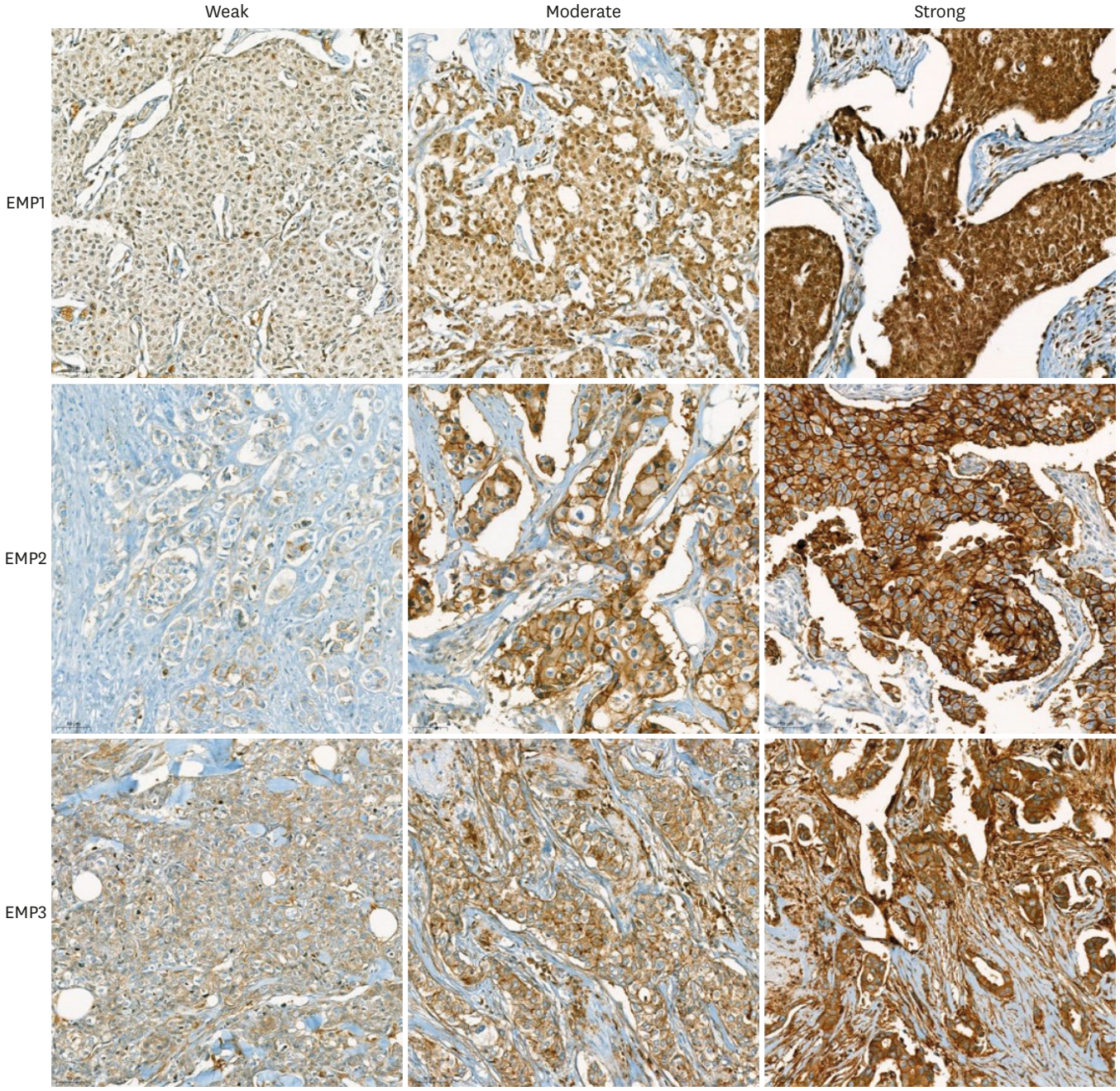

Figure 1. Representative immunohistochemistry of EMP1, EMP2, and EMP3.
EMP = epithelial membrane protein.

RESULTS

Clinicopathological data

In total, 418 cases of female IDC breast cancer were analyzed. Mean patient age was 49.5 ± 11.4 years and median follow-up was 65 months (range, 2–141 months). There were 219 HR+HER2−, 38 HR+HER2+, 35 HER2, and 126 TNBC cases. Forty-seven patients experienced recurrence and the same number of patients died. Base characteristics of patients are summarized in **Table 2**. With respect to the clinicopathological parameters, histological

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Table 2. Base clinicopathological characteristics

Characteristics	Value
Age (yr)	49.49 ± 11.41
Subtype	
HR+HER2−	219 (52.4)
HR+HER2+	38 (9.1)
HER2	35 (8.4)
TNBC	126 (30.1)
Histologic grade	
I	76 (18.2)
II	191 (45.7)
III	151 (36.1)
pT stage	
1	197 (47.1)
2	205 (49.0)
3	13 (3.1)
pN stage	
0	251 (60.0)
1	108 (25.8)
2	36 (8.6)
3	21 (5.0)
Recurrence	47 (11.2)
Death	47 (11.2)
Median follow-up period (mo)	65 (2–140.5)

Values are presented as mean ± standard deviation, number (%) or number (range).
HR+ = hormone receptor positive; HER2− = human epidermal growth factor receptor 2 negative; HER2+ = human epidermal growth factor receptor 2 positive; TNBC = triple-negative breast cancer.

grade ($p < 0.001$), recurrence ($p = 0.008$), and death ($p = 0.035$) significantly differed among the breast cancer subtypes. Most of the HR+ cases, irrespective of HER2 status, were of histological grade I or II, whereas more than half of HER2 and TNBC cases presented higher histological grades ($p < 0.001$). The HER2 subtype revealed the highest recurrence and death rates ($p = 0.035$; **Table 3**).

Expression of EMP1, EMP2, and EMP3 in IDC specimens

EMP1 demonstrated > 90% expression in all subtypes and no significant difference was found across the subtypes. EMP2 ($p < 0.001$) and EMP3 ($p = 0.009$) exhibited decreased expression in TNBC compared to that in the other subtypes. EMP2 and EMP3 presented > 80% expression in HR+ breast cancers (**Table 4**).

Table 3. Clinicopathological parameters on the basis of breast invasive ductal carcinoma subtype

Characteristics	HR+HER2− (n = 219)	HR+HER2+ (n = 38)	HER2 (n = 35)	TNBC (n = 126)	*p*-value
Histologic grade					< 0.001
I	64 (29.2)	5 (13.2)	1 (2.9)	6 (4.8)	
II	115 (25.5)	25 (65.8)	16 (45.7)	35 (27.8)	
III	40 (18.3)	8 (21.1)	18 (51.4)	85 (67.5)	
Lymph node metastasis	91 (41.7)	16 (42.1)	14 (40.0)	44 (35.2)	0.675
Recurrence	14 (6.4)	5 (13.2)	7 (20.0)	21 (16.7)	0.008
Death	17 (7.8)	4 (10.5)	8 (22.9)	18 (14.3)	0.035
Ki67 LI (%)	9.52 ± 11.44	13.08 ± 10.05	12.10 ± 2.05	24.20 ± 2.16	HR+HER2− vs. TNBC, < 0.001*
					HR+HER2+ vs. TNBC, < 0.001*
					HER2 vs. TNBC, < 0.001*

Values are presented as number (%) or mean ± standard deviation.
HR+ = hormone receptor positive; HER2− = human epidermal growth factor receptor 2 negative; HER2+ = human epidermal growth factor receptor 2 positive; TNBC = triple-negative breast cancer; LI = labelling index.
*Bonferroni *post hoc* test.

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Table 4. Expression of EMP1, EMP2, and EMP3 in invasive ductal carcinoma on the basis of subtype

Characteristics	HR+HER2– (n = 219)	HR+HER2+ (n = 38)	HER2 (n = 35)	TNBC (n = 126)	*p*-value
EMP1					0.762
Negative	7 (3.2)	0 (0.0)	0 (0.0)	3 (2.4)	
Positive	212 (96.8)	38 (100.0)	35 (100.0)	123 (97.6)	
EMP2					< 0.001
Negative	43 (19.6)	4 (10.5)	8 (22.9)	60 (47.6)	
Positive	176 (80.4)	34 (89.5)	27 (77.1)	66 (52.4)	
EMP3					0.009
Negative	35 (16.0)	1 (2.6)	2 (5.7)	28 (22.2)	
Positive	184 (84.0)	37 (97.4)	33 (94.3)	98 (77.8)	

Values are presented as number (%).
HR+ = hormone receptor positive; HER2– = human epidermal growth factor receptor 2 negative; HER2+ = human epidermal growth factor receptor 2 positive; TNBC = triple-negative breast cancer; EMP = epithelial membrane protein.

The histological grade revealed no significant differences in the expression of EMP1, EMP2, and EMP3. No significant differences were found for total patients and each subgroup (**Supplementary Table 1**). Univariate logistic regression analysis (**Supplementary Table 2**) revealed that HER2 positivity was significantly associated with EMP2 (odds ratio [OR], 2.164; 95% confidence interval CI], 1.118–4.188; $p = 0.022$) and EMP3 expression (OR, 5.213; 95% CI, 1.590–17.091; $p = 0.006$). EMP3 positivity was independently associated with HER2 positivity (OR, 1.456; 95% CI, 1.272–14.462; $p = 0.019$).

Markers EMP2 and EMP3 exhibited different expression rates with respect to HER2 status (**Figure 2**). Both EMP2 and EMP3 exhibited significantly higher expression rates in association with HER2 positivity. In HER2-negative cases with higher histological grades, EMP2 expression was significantly decreased (**Table 5**). Moreover, mean Ki-67 LI was significantly higher in the EMP2-negative group among the HER2-negative cases (16.11% ± 19.59% vs. 24.79% ± 23.09%, respectively, $p = 0.001$), as listed in **Table 5**.

EMP2 expression in matched normal, ductal carcinoma *in situ* (DCIS), and invasive carcinoma in HR-positive breast cancer

EMP2 expression in matched normal, DCIS, and invasive cancer were evaluated in the HR-positive cases (**Table 6**). EMP2 expression revealed significant stepwise increase from normal to DCIS and invasive cancer ($p < 0.001$). Among the evaluable normal luminal cells in 48 cases, 17 cases presented weak EMP2 expression (expression score 1 or 2), and 6 cases with expression score 2 revealed columnar cell change. Between DCIS and invasive cancer, invasive cancer revealed slightly higher expression score compared to DCIS ($p = 0.034$).

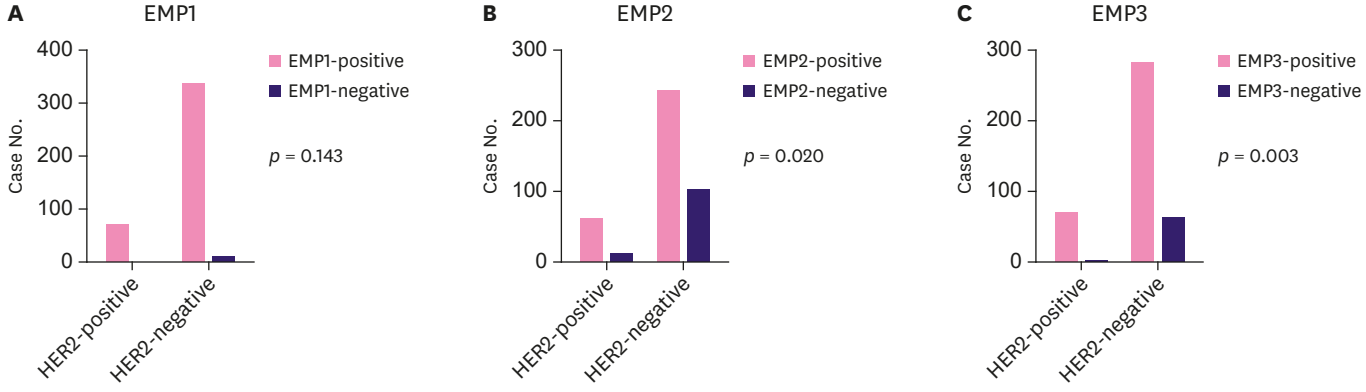

Figure 2. Expression of EMP1, EMP2, and EMP3 on the basis of HER2 status. EMP1 expression does not differ, regardless of HER2 status (A). EMP2 (B) and EMP3 expression (C) is associated with HER2 positivity.
EMP = epithelial membrane protein; HER2 = human epidermal growth factor receptor 2.

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Table 5. Expression of EMPs, histological grade, and proliferative index on the basis of HER2 status

Characteristics	EMP1			EMP2			EMP3		
	Positive	Negative	*p*-value	Positive	Negative	*p*-value	Positive	Negative	*p*-value
HER2 negative (n = 345)									
Histologic grade			0.285			< 0.001			0.652
I (n = 70)	66 (94.3)	4 (5.7)		56 (80.0)	14 (20.0)		58 (82.9)	12 (17.1)	
II (n = 150)	147 (98.0)	3 (2.0)		116 (77.3)	34 (22.7)		125 (83.3)	25 (16.7)	
III (n = 125)	122 (97.6)	3 (2.4)		70 (56.0)	55 (44.0)		99 (79.2)	26 (20.8)	
Ki67 LI (%)	18.74 ± 21.16	17.70 ± 18.01	0.877	16.11 ± 19.59	24.79 ± 23.09	0.001	18.22 ± 20.71	20.90 ± 22.55	0.361
HER2 positive (n = 73)									
Histologic grade			NA			0.504			0.851
I (n = 6)	6 (100.0)	0 (0.0)		5 (83.3)	1 (16.7)		6 (100.0)	0 (0.0)	
II (n = 41)	41 (100.0)	0 (0.0)		36 (87.8)	5 (12.2)		39 (5.1)	2 (4.9)	
III (n = 26)	26 (100.0)	0 (0.0)		20 (76.9)	6 (23.1)		25 (96.2)	1 (3.8)	
Ki67 LI (%)	15.15 ± 11.23	NA	NA	15.90 ± 11.53	11.42 ± 9.05	0.209	15.45 ± 11.37	8.33 ± 2.89	0.286

Values are presented as number (%) or mean ± standard deviation.
EMP = epithelial membrane protein; HER2 = human epidermal growth factor receptor 2; LI = labelling index; NA = not available.

Table 6. EMP2 expression in matched normal, DCIS, and invasive carcinoma in hormone receptor-positive breast cancer

Characteristics	Expression score	*p*-value
Normal vs. DCIS (n = 17)	0.65 ± 0.93 vs. 4.00 ± 1.00	< 0.001
Normal vs. invasive carcinoma (n = 48)	0.40 ± 0.76 vs. 3.38 ± 1.54	< 0.001
DCIS vs. invasive carcinoma (n = 58)	3.36 ± 1.33 vs. 3.72 ± 1.47	0.034

Values are presented as mean ± standard deviation.
EMP = epithelial membrane protein; DCIS = ductal carcinoma *in situ*.

EMP1, EMP2, and EMP3 expression in IDC and prognosis

For all patients, no significant differences were observed in OS based on EMP1, EMP2, or EMP3 expression. In HR+HER2− cases, EMP1 and EMP2 positivity exhibited tendencies of better OS and DFS; however, these differences were not statistically significant (**Figures 3** and **4**). In HR+HER2− cases with positive EMP3 expression, the patients revealed significantly better DFS ($p = 0.049$; **Figure 3**). In HR+HER2+ cases, patients presented significantly separated OS and DFS in association with EMP3 expression; however, only one patient who exhibited EMP3 negativity died.

DISCUSSION

In the present study, we evaluated the expression of EMP1, EMP2, and EMP3 in IDC of the breast using a numerous HR+HER2− cases and a long follow-up period. We found different expression rates of EMP2 and EMP3 according to the HER2 status. In particular, the expression of EMP1, EMP2, and EMP3 was reduced in TNBC. We analyzed a relatively large number of TNBC cases (n = 126) compared to the previous studies [4,6,11] and found no clinical significance in expression of the EMPs; however, HER2 positivity was associated with EMP2 and EMP3 expression.

Previous studies have reported that EMP1 downregulation is associated with growth arrest [12] and cellular differentiation [13]. Although expression of EMP1 has been reported in glioma [14], gastric cancer [15], and acute lymphoid leukemia [16], it has been rarely evaluated in breast cancer [5]. Conversely, in nasopharyngeal carcinoma, an inverse correlation exists between EMP1 expression and clinical parameters such as T stage, node metastasis, and clinical stage [12]. In a previous study that used the ER-positive cell line, MCF7, EMP1 level was lower in the cancer epithelium compared to that in normal tissue and the EMP1 expression correlated with parameters associated with tumor aggressiveness (T stage, node metastasis,

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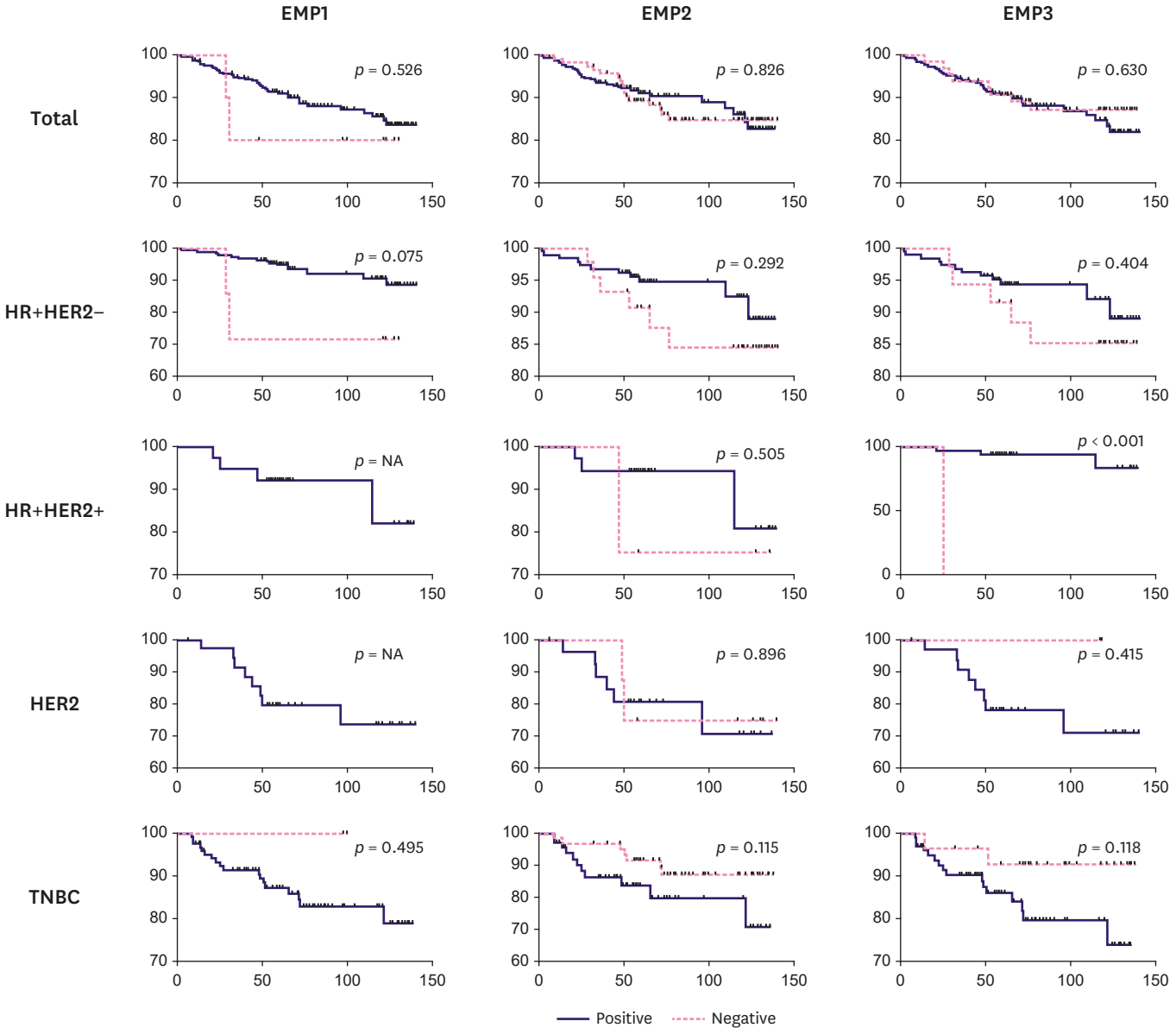

Figure 3. Overall survival on the basis of EMP1, EMP2, and EMP3 expression and breast cancer subtype. No significance is observed among the EMP1, EMP2, and EMP3 expression and breast cancer subtype. The x axis, overall survival (months); y axis, cumulative survival (%).
HR+ = hormone receptor positive; HER2− = human epidermal growth factor receptor 2 negative; HER2+ = human epidermal growth factor receptor 2 positive; TNBC = triple-negative breast cancer; EMP = epithelial membrane protein.

histological grade); however, loss of EMP1 expression correlated with poor OS [5]. The results from the study suggested the possibility that EMP1 may act as a negative regulator in ER-positive breast cancer. In the present study, most of the IDC cases expressed EMP1, particular those with HR+, which presented > 90% EMP1 expression. In contrast to the previous study, in this study, we did not find any significant difference in the EMP1 expression according to the molecular subtype, histological grade, or patient prognosis.

EMP2 is known to be expressed in most human tissues in patterns similar to EMP1 expression [17]. A previous study found that EMP2 is involved in cell proliferation and intercellular interaction [18]. In solid tumors, particularly endometrial cancer, EMP2 expression increases

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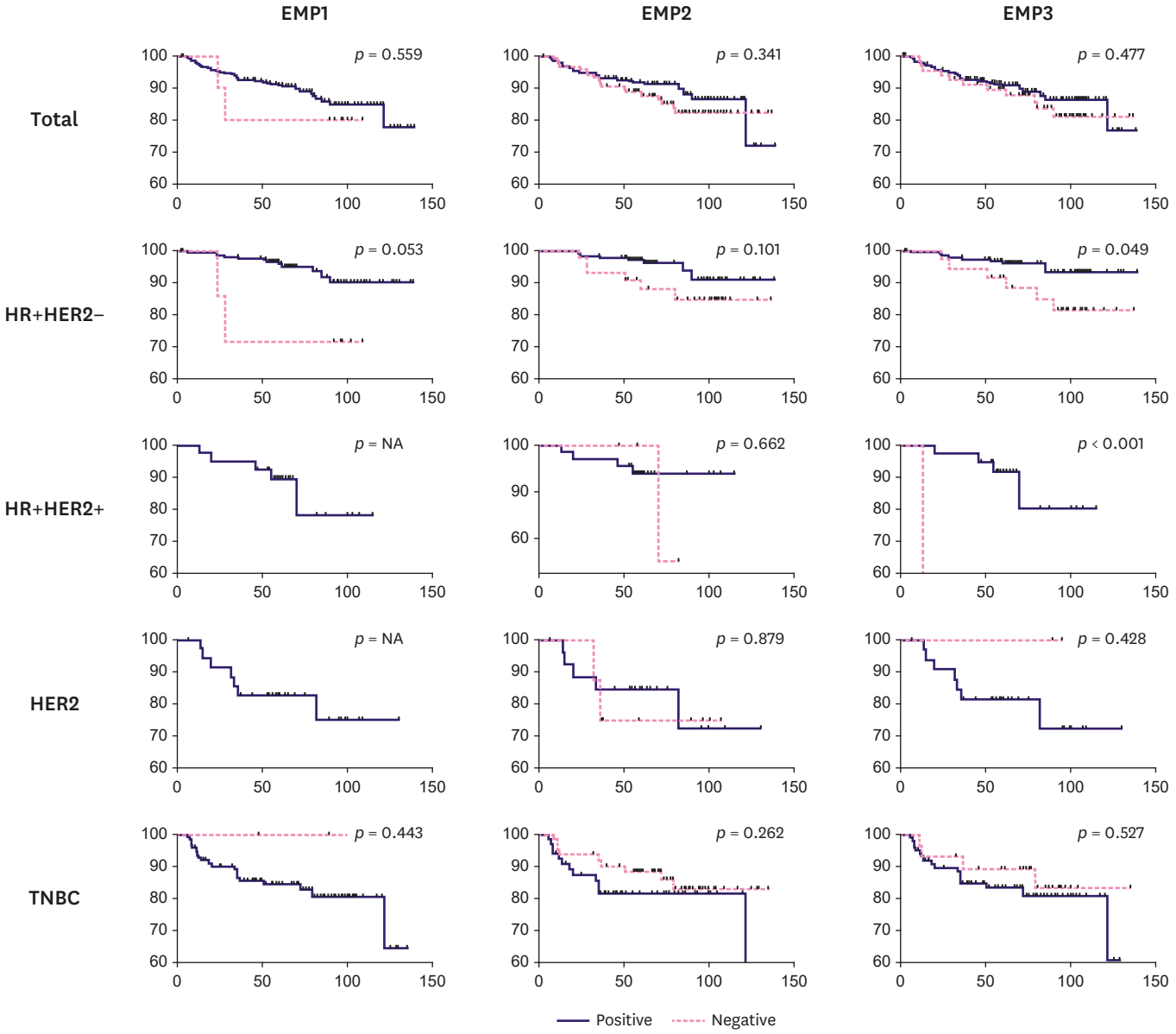

Figure 4. Disease-free survival on the basis of EMP1, EMP2, and EMP3 expression and breast cancer subtype. In the HR+HER2− group, EMP3 positive cases depict superior disease-free survival. The x axis, disease free survival (months); y axis, cumulative survival (%).
HR+ = hormone receptor positive; HER2− = human epidermal growth factor receptor 2 negative; HER2+ = human epidermal growth factor receptor 2 positive; TNBC = triple-negative breast cancer; EMP = epithelial membrane protein.

in a stepwise manner from hyperplasia to carcinoma [19] and was associated with unfavorable patient survival [20]. Fu et al. [4] evaluated 236 IDC specimens and an additional 23 TNBC specimens and detected the EMP2 expression in 63% and 70% of IDC and TNBC cases, respectively. In contrast, these investigators observed negative or minimal EMP2 expression in the normal mammary glands. The level of EMP2 correlates with focal adhesion kinase and Src activation and invasion, which are suppressed by blocking EMP2 with an anti-EMP2 immunoglobulin G1 antibody. Moreover, high EMP2 expression is associated with lymph node metastasis, and the authors suggest that EMP2 may act as a therapeutic target for breast cancer, specifically in TNBC cases; however, in the present study, we observed decreased expression of EMP2 in TNBC cases compared to other subtypes with no significant prognostic differences.

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HER2-negative cases revealed stepwise decreases in the EMP2 expression along with higher histological grade and increased proliferative index (Ki-67 LI). Furthermore, we analyzed the TNBC cases; however, no significant differences were found in the EMP expression and histological grades (data not shown). In the TNBC cases, tumors with histological grade I and II exhibited 63.4% EMP2 positivity. The majority of TNBC cases in the present study belonged to grade III (67.5%) and presented decreased EMP2 expression. This may explain the different results in positive rates between the previous study and the present study as the previous study did not report whether EMP2 expression differed among the histological grades. In addition to the increased EMP2 protein levels, previous studies using microarray analysis found upregulated EMP2 mRNA in breast cancer [21]. In advanced or recurrent breast cancer, EMP2 gene was detected in the blood sample of the patients [22]. Collectively, our present results in accordance with the previous findings suggest that EMP2 might play a crucial role in tumor development in hormone-dependent cancers. Although we only evaluated the expression of EMPs in IDC specimens without comparable normal tissue, the HR-negative, HER2, and TNBC subtypes exhibited decreased expression of EMP2 compared to that in the HR-positive cases. This further indirectly supports the involvement of EMP2 in the tumorigenesis of hormone-dependent cancer. Conversely, we observed decreased expression of EMP2 in the higher histologic grade HER2-negative cases, which is not in accordance with the results of previous studies. Based on our result and previous studies, EMP2 seems to be mainly involved in the early stage of cancer development in HR-positive breast cancer; however, the experimental setting and the sample number or sample types differ, and for a concrete conclusion, further analysis is required to elucidate the exact role of EMP2 in breast cancer, particularly HR-positive breast cancer and TNBC. In this study, although few cases could be assessed, in most cases, the normal luminal cells lacked EMP2 expression, whereas DCIS and invasive cancer cells of HR-positive cases expressed EMP2. Moreover, weak EMP2 expression in columnar cell change implies that EMP2 may be involved in early tumorigenesis in HR-positive cancer, as the columnar cell change has overlapped genetic alteration of ER-positive low grade lesion such as low grade DCIS and tubular carcinoma [23,24].

EMP3 has been studied in several organs and cell lines. In neuroblastoma and gliomas, hypermethylation of the EMP3 gene is suggested to play a tumor suppressor role [25]. Similarly, in an esophageal squamous cell cancer cell line, EMP3 expression is repressed [26] and in non-small cell lung cancer, EMP3 expression is inversely correlated to the TNM stage and is reduced compared to that in normal lung tissue [27]. Meanwhile, EMP3 appears to be an oncogene in urothelial carcinoma [28] and breast cancer [11]. In breast cancer, EMP3 mRNA levels are higher than those in normal tissue [7]. Furthermore, EMP3 expression is repressed by miR-765, and knockdown of EMP3 inhibits tumor invasion [11]. In the present study, EMP3-positivity was presumably related to better DFS in the HR+HER2− group, which implied a role for EMP3 as a negative regulator in HR+HER2− IDCs. In a HER2 overexpressing breast cancer cell line, MYC and EMP3 expression are upregulated, which suggests that EMP3 might interact with MYC and may function as an oncogene in HER2-positive breast cancer [29]. Additionally, we found higher expression of EMP3 in HER2-positive cases, which was in accordance with the previous results and may support EMP3 as a potential therapeutic target in treating HER2 positive breast cancer. Although EMP3 positive cases presented significantly better OS and DFS in the HR+HER2+ cases, these results may be less reliable as only one EMP3-negative patient was present in the HR+HER2+ group, who eventually died. To validate this finding, a larger cohort that includes HER2-positive cases with and without HR positivity should be studied to evaluate the effects of EMPs on patients' prognosis. Among the HER2-positive cases, the EMP3-positive cases revealed a slightly

higher proliferative index than those of the EMP3-negative cases. While this difference in the proliferative index was not statistically significant, EMP3 may affect tumor proliferation in HER2 positive breast cancer.

The present study has several limitations. First, we used TMA analysis and interpreted the IHC results, which might not fully reflect the actual gene expression status. Second, although we evaluated the specimens from numerous patients, the number of HER2 cases was relatively small, which might be a reason for the insignificant survival curves. Additionally, we only analyzed the IDC tissue without evaluating the comparable normal breast tissue, precursor lesion like carcinoma *in situ*, or other histological subtypes of invasive carcinoma. Previous studies have evaluated the expression of EMPs in carcinoma and normal tissue and have found higher expression of EMPs in carcinoma. As most of the cases (except the TNBC cases) presented high positive rates of EMP expression, the result may have context with the previous studies, although comparison between IDC subtypes revealed no statistical significance.

In conclusion, EMP1 was highly expressed, and all subtypes of IDC and EMP2 and EMP3 expression were associated with HER2 positivity. In HER2-negative breast cancer, EMP2 expression was inversely correlated with the histological grade and proliferative index. EMP2 may play a crucial role in tumor development in HR-positive breast cancer.

SUPPLEMENTARY MATERIALS

Supplementary Table 1

Expression of EMP1, EMP2, and EMP3 in invasive ductal carcinoma subtypes and correlation with histological grade

Click here to view

Supplementary Table 2

Correlation of HER2 positivity and expression of EMP1, EMP2, and EMP3 based on binary logistic regression

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