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.
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, PR, and HER2 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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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.
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

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

**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 (3.3)            |
| 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.

**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 (52.5)          | 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   |           |

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.

**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).
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 \( (p = 0.034) \).

| 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           |                     |                   |             |               |               |
| Negative       | 7 (3.2)             | 0 (0.0)           | 0 (0.0)     | 3 (2.4)       | 0.762         |
| Positive       | 212 (96.8)          | 38 (100.0)        | 35 (100.0)  | 123 (97.6)    | < 0.001       |
| EMP2           |                     |                   |             |               | 0.009         |
| 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           |                     |                   |             |               |               |
| 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; \( p = 0.019 \)).

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.
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, ...
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

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.
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.

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.
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

Click here to view

REFERENCES

1. Hammond ME, Hayes DF, Dowsett M, Allred DC, Hagerty KL, Badve S, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer (unabridged version). Arch Pathol Lab Med 2010;134:e48-72. PUBMED

2. Wolff AC, Hammond ME, Allison KH, Harvey BE, Mangu PB, Bartlett JM, et al. Human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline focused update. J Clin Oncol 2018;36:2105-22. PUBMED | CROSSREF

3. Denkert C, von Minckwitz G, Darb-Esfahani S, Ingold Heppner B, Klauschen F, Furlanetto J, et al. Abstract SI-09: Evaluation of tumor-infiltrating lymphocytes (TILs) as predictive and prognostic biomarker in different subtypes of breast cancer treated with neoadjuvant therapy - A metaanalysis of 3771 patients. Cancer Res 2017;77:S1-09. CROSSREF
4. Fu M, Maresh EL, Helguera GF, Kiyohara M, Qin Y, Ashki N, et al. Rationale and preclinical efficacy of a novel anti-EMP2 antibody for the treatment of invasive breast cancer. Mol Cancer Ther 2014;13:902-15.
5. Sun GG, Wang YD, Lu YF, Hu WN. EMP1, a member of a new family of antiproliferative genes in breast carcinoma. Tumour Biol 2014;35:3347-54.
6. Turashvili G, Bouchal J, Ehrmann J, Fridman E, Skarda J, Kolar Z. Novel immunohistochemical markers for the differentiation of lobular and ductal invasive breast carcinomas. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 2007;151:59-64.
7. Evtimova V, Zeilinger R, Weidle UH. Identification of genes associated with the invasive status of human mammary carcinoma cell lines by transcriptional profiling. Tumour Biol 2003;24:189-98.
8. Elston CW, Ellis IO. Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. Histopathology 2002;41:154-61.
9. Hammond ME, Hayes DF, Dowsett M, Allred DC, Hagerty KL, Badve S, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. J Clin Oncol 2010;28:2784-95.
10. Won KY, Kim GY, Kim YW, Song JY, Lim SJ. Clinicopathologic correlation of beclin-1 and bcl-2 expression in human breast cancer. Hum Pathol 2010;41:1074-12.
11. Hong XC, Fen YJ, Yan GC, Hong H, Yan CH, Bing LW, et al. Epithelial membrane protein 3 functions as an oncogene and is regulated by microRNA-765 in primary breast carcinoma. Mol Med Rep 2015;12:6445-50.
12. Sun GG, Lu YF, Fu ZZ, Cheng YJ, Hu WN. EMP1 inhibits nasopharyngeal cancer cell growth and metastasis through induction apoptosis and angiogenesis. Tumour Biol 2014;35:3185-93.
13. Ben-Porath I, Benvenisty N. Characterization of a tumor-associated gene, a member of a novel family of genes encoding membrane glycoproteins. Gene 1996;183:69-75.
14. Bredel M, Bredel C, Juric D, Harsh GR, Vogel H, Recht LD, et al. Functional network analysis reveals extended gliomagenesis pathway maps and three novel MYC-interacting genes in human gliomas. Cancer Res 2005;65:8679-89.
15. Hippo Y, Yashiho M, Ishii M, Taniguchi H, Tsutsuhi S, Hirakawa K, et al. Differential gene expression profiles of scirrhous gastric cancer cells with high metastatic potential to peritoneum or lymph nodes. Cancer Res 2001;61:889-95.
16. Ariës IM, Jerchel IS, van den Dungen RE, van den Berk LC, Boer JM, Horstmann MA, et al. EMP1, a novel poor prognostic factor in pediatric leukemia regulates prednisolone resistance, cell proliferation, migration and adhesion. Leukemia 2014;28:1828-37.
17. Wang YW, Cheng HL, Ding YR, Chou LH, Chow NH. EMP1, EMP 2, and EMP3 as novel therapeutic targets in human cancer. Biochim Biophys Acta Rev Cancer 2017;1868:199-211.
18. Wadehra M, Su H, Gordon LK, Goodglick L, Braun J. The tetraspan protein EMP2 increases surface expression of class I major histocompatibility complex proteins and susceptibility to CTL-mediated cell death. Clin Immunol 2003;107:129-36.
19. Habeeb O, Goodglick L, Soslow RA, Rao RG, Gordon LK, Schirripa O, et al. Epithelial membrane protein-2 expression is an early predictor of endometrial cancer development. Cancer 2010;116:4718-26.
20. Wadehra M, Natrajan S, Seligson DB, Williams CJ, Hummer AJ, Hedvar C, et al. Expression of epithelial membrane protein-2 is associated with endometrial adenocarcinoma of unfavorable outcome. Cancer 2006;107:90-8.
21. Ma XJ, Dahiya S, Richardson E, Erlander M, Sgroi DC. Gene expression profiling of the tumor microenvironment during breast cancer progression. Breast Cancer Res 2009;11:R7.
PUBMED | CROSSREF

22. Obermayr E, Sanchez-Cabo F, Tea MK, Singer CF, Krainer M, Fischer MB, et al. Assessment of a six gene panel for the molecular detection of circulating tumor cells in the blood of female cancer patients. BMC Cancer 2010;10:666.
PUBMED | CROSSREF

23. Md Nasir ND, Ng CC, Rajasegaran V, Wong SF, Liu W, Ng GX, et al. Genomic characterisation of breast fibroepithelial lesions in an international cohort. J Pathol 2019;249:447-60.
PUBMED | CROSSREF

24. Logullo AF, Nimir C. Columnar cell lesions of the breast: a practical review for the pathologist. Surg Exp Pathol 2019;2:2.
CROSSREF

25. Alaminos M, Dávalos V, Ropero S, Setién F, Paz MF, Herranz M, et al. EMP3, a myelin-related gene located in the critical 19q13.3 region, is epigenetically silenced and exhibits features of a candidate tumor suppressor in glioma and neuroblastoma. Cancer Res 2005;65:2565-71.
PUBMED | CROSSREF

26. Fumoto S, Hiyama K, Tanimoto K, Noguchi T, Hihara J, Hiyama E, et al. EMP3 as a tumor suppressor gene for esophageal squamous cell carcinoma. Cancer Lett 2009;274:25-32.
PUBMED | CROSSREF

27. Xue Q, Zhou Y, Wan C, Lv L, Chen B, Cao X, et al. Epithelial membrane protein 3 is frequently shown as promoter methylation and functions as a tumor suppressor gene in non-small cell lung cancer. Exp Mol Pathol 2013;95:313-8.
PUBMED | CROSSREF

28. Wang YW, Li WM, Wu WJ, Chai CY, Liu HS, Lai MD, et al. Potential significance of EMP3 in patients with upper urinary tract urothelial carcinoma: crosstalk with ErbB2-PI3K-Akt pathway. J Urol 2014;192:242-51.
PUBMED | CROSSREF

29. Mackay A, Jones C, Dexter T, Silva RL, Bulmer K, Jones A, et al. cDNA microarray analysis of genes associated with ERBB2 (HER2/neu) overexpression in human mammary luminal epithelial cells. Oncogene 2003;22:2680-8.
PUBMED | CROSSREF