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Original Article

Myelin expression factor 2 expression is associated with aggressive phenotype in triple-negative breast cancer

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Abstract: Triple-negative breast cancers (TNBCs) are characterized by an aggressive clinical course with frequent recurrence and short survival and an absence of molecular targets. Myelin expression factor 2 (MYEF2) is suggested to play a role in carcinogenesis by in silico analysis; however, the function of MYEF2 in cancer is largely unknown. The purpose of this study was to investigate MYEF2 expression in TNBC. Immunohistochemistry for MYEF2 was performed on tissue microarray sections from 132 patients with TNBC and interpreted using a semiquantitative immunoreactive score (IRS). Association of MYEF2 expression with various clinicopathologic characteristics, including patient survival, was analyzed. MYEF2 expression was localized in the nucleus of tumor cells, and positive staining was observed in 90.15% of TNBCs. Expression of MYEF2 was positively correlated with tumor size (P = 0.027), AJCC stage (P = 0.013), and Ki-67 proliferation index (P < 0.001). Survival analyses using Cox proportional regression model revealed that lymphovascular invasion, lymph node metastasis, and AJCC stage were prognostic factors for disease-free and overall survival. Patients with high MYEF2-expressing tumors showed a decreased estimated survival time (96 months) compared with patients with absent or low MYEF2 expression (101 months), however, the difference was not statistically significant (P = 0.178). Expression of MYEF2 is significantly correlated with aggressive phenotypes of TNBC, such as larger tumor size, high AJCC stage, and Ki-67 proliferation index. Further functional studies will be required to define the role of MYEF2 in cancers.

Keywords: Triple-negative breast cancer, myelin expression factor 2, immunohistochemistry, prognosis

Introduction

Triple-negative breast cancers (TNBCs), which are defined by lack of expression of estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2, constitute one of the most challenging groups of breast cancers [1, 2]. This subgroup accounts for approximately 10-15% of all breast cancers [3]. TNBCs are characterized by biologically aggressive tumor behavior with frequent recurrence in the first 1-3 years and a higher death rate than other subtypes of breast cancer [4, 5].

Myelin expression factor 2 (MYEF2) is a transcriptional repressor of the gene encoding myelin basic protein, a major component of the myelin sheath whose production is developmentally controlled during myelinogenesis [6]. MYEF2 is known to play a role in neuron and myotube differentiation, and is related to human diseases, such as dilated cardiomyopathy and cardiac hypertrophy [6, 7]. The function of MYEF2 in cancer is largely unknown. Recent bioinformatics research suggested that MYEF2 potentially plays a role in the molecular pathway of prostate cancer development, but no direct relationship between MYEF2 and the cancer has been demonstrated [8].

In the present study, we investigated MYEF2 expression by immunohistochemistry in a series of TNBCs and evaluated its association with clinicopathologic variables, including patient survival.

Materials and methods

Patients and tumor samples

A total of 132 TNBC patients who underwent curative surgery at the Hanyang University

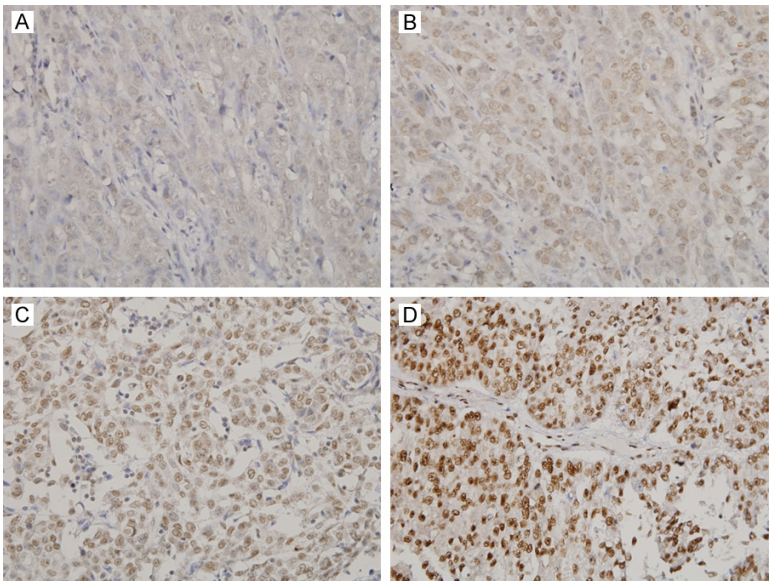

Figure 1. Representative microphotographs of myelin expression factor 2 immunostaining in triple-negative breast cancers. Negative (A), weak (B), intermediate (C) and strong (D) staining in the nuclei of tumor cells.

Hospital, Seoul, between January 2001 and December 2014 were retrospectively enrolled in this study.

We reviewed all hematoxylin and eosin (H&E)-stained slides, pathology reports, and other medical records to confirm the diagnosis and obtain follow-up data. The clinicopathologic parameters included American Joint Committee on Cancer (AJCC) tumor stage, primary tumor size, lymph node metastasis, histologic grade, lymphovascular invasion, perineural invasion, perinodal tumor extension in cases of lymph node metastasis, Ki-67 labeling index, disease-free survival, and overall survival. This study was approved by the Institutional Review Board of Hanyang University Hospital (IRB File No 2015-12-023-001).

Tissue microarray construction

We used a manual tissue microarrayer (QuickRay, Unitma, Seoul, Korea) to construct a tissue microarray (TMA) from archival formalin-fixed, paraffin-embedded tissue blocks. We selected well-fixed, non-necrotic areas by light microscopy of H&E-stained sections, and marked the area on the corresponding paraffin blocks. Tissue cylinders of 2-mm diameter were punched from the marked region on each donor blocks and transferred to the recipient blocks.

Immunohistochemical staining

Expression of MYEF2 was evaluated by immunohistochemical staining with 4-μm-thick sections from TMA blocks. The sections were first deparaffinized in xylene and then rehydrated through graded ethanol. For antigen retrieval, we performed autoclave heating at 100°C for 20 minutes in sodium citrate buffer (pH 6.0). Endogenous peroxidase activity was blocked with peroxidase blocking solution (S2023, Dako Cytomation, Carpinteria, CA, USA). TMA slides were incubated with primary antibodies at 4°C overnight, and then incubated with labeled polymer (DAKO REAL EnVision/HRP, K5007, DakoCytomation) for 30 min at room temperature. The primary antibody was rabbit polyclonal anti-MYEF2 antibody (ab26098, Abcam, Cambridge, UK) used at a 1:100 dilution. 3,3'-Diaminobenzidine was used as a chromogen for visualization, and Mayer's hematoxylin counterstain was applied.

Interpretation of immunohistochemical staining

MYEF2 expression was evaluated semiquantitatively by two independent pathologists who were blinded to the patients' clinicopathologic data. Nuclear MYEF2 expression was categorized in terms of staining intensity and extent. The intensity score was assigned as follows: 0 (negative), 1 (weak staining), 2 (intermediate staining), and 3 (strong staining). The proportion score was as follows: 0 (< 10%), 1 (10%-25%), 2 (26%-50%), 3 (51%-75%), and 4 (76%-100%). The product of the intensity and proportion scores was used as the final immunoreactive score (IRS). Thus, the maximum combined score was 12 and the minimum score was 0. Representative photomicrographs of MYEF2 immunostaining in TNBCs are shown in **Figure 1**.

Statistical analysis

Statistical analyses were performed using SPSS software version 19.0 (IBM Corp., Ar-

Table 1. Correlation between MYEF2 expression and clinicopathologic factors in TNBC

Clinicopathologic parameters		N	MYEF2 expression		*P* value (χ^2-test)
			Negative	Positive	
AJCC stage	Stage 1	36	23 (63.9%)	13 (36.1%)	0.027
	Stage 2&3	93	39 (41.9%)	54 (58.1%)	
Primary tumor size	≤ 2 cm	44	28 (63.6%)	16 (36.4%)	0.013
	> 2 cm	85	34 (40.0%)	51 (60.0%)	
Lymph node metastasis	Absent	71	37 (52.1%)	34 (47.9%)	0.285
	Present	58	25 (43.1%)	33 (56.9%)	
Histologic grade	Grade 1&2	34	19 (55.9%)	15 (44.1%)	0.201
	Grade 3	95	43 (45.3%)	52 (54.7%)	
Lymphovascular invasion	Absent	74	38 (51.4%)	36 (48.6%)	0.405
	Present	55	24 (43.6%)	31 (56.4%)	

monk, NY, USA). The chi-square test was used to compare the proportion of MYEF2 expression status and various clinicopathologic parameters. The difference of Ki-67 labeling index between the groups was evaluated by t-test. The Kaplan-Meier method was used to plot survival curves. Univariable and multivariable survival analyses using the Cox proportional hazards regression model were performed to identify prognostic relevance.

Results

Clinicopathologic characteristics of TNBC patients

Successful MYEF2 immunostaining was achieved for 129 cases and the remaining 3 cases were excluded from subsequent analyses. The mean patient age was 50.9 years (range, 26 to 79 years). Twenty-four patients (18.6%) suffered from local recurrence or distant metastasis, and 18 patients (14.0%) died of breast cancer. The mean overall survival from diagnosis was 52.3 months. The majority of TNBCs (73.6%) had histologic grade 3. By AJCC tumor staging, 37 cases were stage I (28.7%), 54 were stage II (41.9%), and 38 were stage III (29.4%). The primary tumor was evaluated as T1 in 34.4%, T2 in 53.9%, T3 in 6.3%, and T4 in 5.5% of patients. Lymph node metastasis was diagnosed pathologically as N0 in 55.3%, N1 in 22.0%, N2 in 8.1%, and N3 in 14.6% of patients.

Correlation between MYEF2 expression and various clinicopathologic parameters in TNBC

MYEF2 expression was absent in only 10 cases (7.6%); most cases showed immunoreactivity of various staining intensity and extent. The tumors were classified into two groups for statistical analyses; IRS = 0 to 4 (absent/low expression), IRS = 6 to 12 (high expression). We evaluated the correlation between MYEF2 expression and various clinicopathologic parameters to assess the significance of its expression in TNBC. There was no correlation between MYEF2 expression and lymph node metastasis, histologic grade, lymphovascular invasion, perineural invasion, and perinodal tumor extension. However, larger tumors (2 cm or more in size) tended to have a frequent high MYEF2 expression than smaller tumors (P = 0.013, chi-square test). The tumors with higher AJCC stage (stage II or above) correlated with high MYEF2 expression (P = 0.027, chi-square test) (**Table 1**). In addition, the high MYEF2 expression group had an increased Ki-67 labeling index (P < 0.001, t-test) (**Figure 2**).

Correlation between MYEF2 expression and TNBC patient survival

AJCC stage, lymph node metastasis, and lymphovascular invasion showed a significant effect on overall and disease-free survival in univariable analyses (**Table 2**). Patients with high MYEF2-expressing tumors showed a lower estimated survival time (85.8 months) than patients with absent or low MYEF2 expression (104.7 months) but the difference was not statistically significant (log rank test, P = 0.178) (**Figure 3**).

Discussion

Since there is no *in vitro* or *in vivo* functional study investigating the role of MYEF2 in cancer, there is no direct evidence for a role of MYEF2 in carcinogenesis or tumor progression. However, several previous studies implicate potential involvement of MYEF2 in cancer biology. Gene expression profiling analysis using six normal and four prostate cancer tissues identified MYEF2 as one of the up-regulated transcription regulators in prostate cancer com-

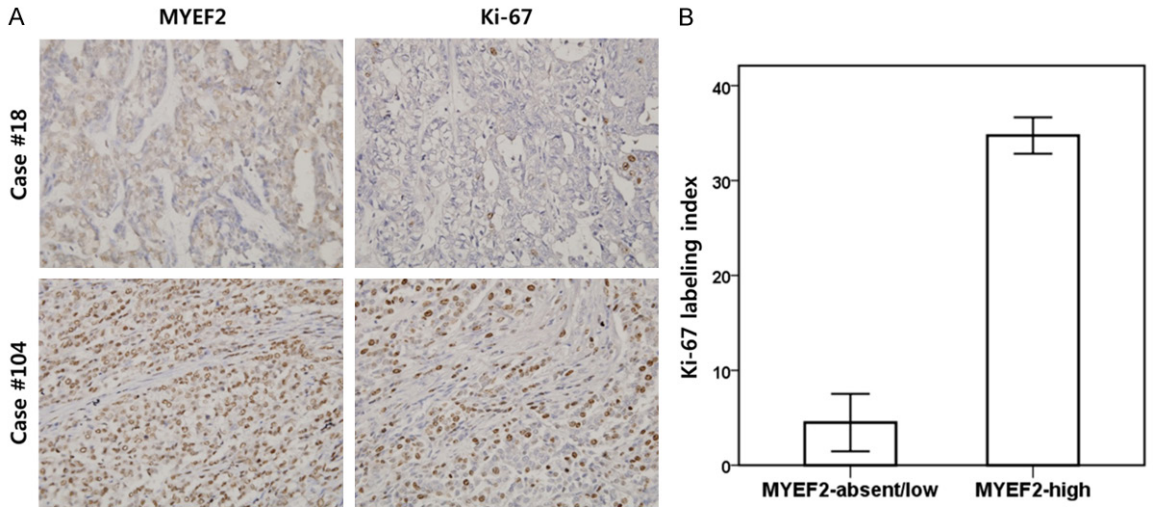

Figure 2. Correlation between MYEF2 expression and Ki-67 proliferation index. A. The tumor with negative MYEF2 shows low Ki-67-positive tumor cells, whereas MYEF2-positive tumor has high Ki-67 labeling index. B. Mean Ki-67 labeling index of TNBCs according to MYEF2 expression status. (t-test, P < 0.001).

Table 2. Univariate and multivariate survival analyses for various clinicopathologic prognostic factors in TNBC

	HR	95% CI	*p*-value	HR	95% CI	*p*-value
Disease-free survival						
Histologic grade (G1/2 vs. G3)	0.692	0.303-1.583	0.383			
Primary tumor size (≤ 2 cm vs. > 2 cm)	2.275	0.848-6.104	0.103			
LN metastasis (absent vs. present)	4.470	1.770-11.290	0.002	4.167	0.972-17.866	0.055
AJCC stage (I & II vs. III & IV)	3.575	1.595-8.011	0.002	1.909	0.671-5.432	0.225
LV invasion (absent vs. present)	3.040	1.299-7.122	0.010	0.674	0.168-2.709	0.578
MYEF2 (absent/low vs. high)	3.863	0.520-28.679	0.186			
Overall survival						
Histologic grade (G1 vs. G2&G3)	0.547	0.216-1.386	0.203			
Primary tumor size (≤ 2 cm vs. > 2 cm)	2.077	0.682-6.324	0.198			
LN metastasis (absent vs. present)	7.077	2.046-24.487	0.002	9.174	1.782-47.215	0.008
AJCC Stage (I & II vs. III & IV)	4.531	1.751-11.726	0.002	2.328	.713-7.602	0.162
LV invasion (absent vs. present)	2.985	1.119-7.963	0.029	0.375	.098-1.434	0.152
MYEF2 (absent/low vs. high)	1.354	0.310-5.913	0.687			

HR, hazard ratio; CI, confidence interval; LN, lymph node; AJCC, American Joint Com mittee on Cancer; LV, lymphovascular; MYEF2, myelin expression factor 2.

pared to normal prostate tissues [9]. Another study using publically available gene expression data on large number of prostate tissues (77 normal tissues, 66 primary prostate tumors, and 24 metastatic tumors) revealed that a set of four genes (TUBB6, MYEF2, PARM1, SLC25A22) provided the best accuracy for prediction of normal tissues versus metastatic tumors and primary prostate tumors versus metastatic tumors [8].

Recently, a study using high-throughput transcriptional analysis of HT20 colon adenocarcinoma cells revealed that MYEF2 is one of the candidate genes regulated by the Wnt/β-catenin signaling pathway that is activated in many human cancers [10]. In addition, MYEF2 has been revealed as a protein associated with SOX2, which has an oncogenic role in medulloblastoma [11].

On the other hand, data from several other studies have suggested that MYEF2 functions as a tumor suppressor in cancers. A validation study of selected microRNAs in malignant melanoma revealed that microRNA-17 is one of the

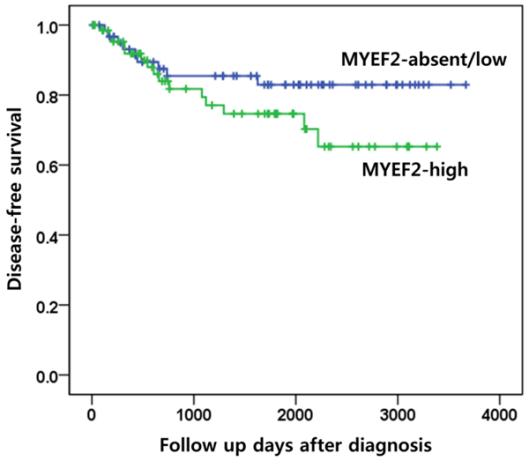

Figure 3. Cumulative disease-free survival curves according to MYEF2 expression. (Kaplan-Meier method with log-rank test, P = 0.178).

up-regulated microRNAs. This suggests that MYEF2 might show decreased expression in melanoma, since MYEF2 is a computational predictive target of microRNA-17 [12]. The modified ginseng extract (MGX) has an anti-proliferative effect on A549 non-small cell lung cancer cells. MYEF2 is one of the up-regulated genes after MGX treatment, suggesting an involvement in anti-proliferative mechanisms [13]. Finally, in serous epithelial ovarian carcinoma, expression of the RUNX1 transcription factor contributes to cell proliferation, migration, and invasion. MYEF2 is one of the genes that are up-regulated by RUNX1 knockdown in SKOV3 ovarian cancer cells [14].

In the present study, we examined the protein expression level of MYEF2 in triple-negative breast cancer using immunohistochemistry and found that MYEF2 expression was positively associated with larger tumor size, high AJCC stage and Ki-67 proliferation index. In survival analyses, patients with high MYEF2-expressing tumors had a shorter estimated survival time than patients with absent or low MYEF2 expression. These results suggest that MYEF2 might be involved in the tumor progression of TNBC. This is consistent with previous microarray data indicating that MYEF2 is upregulated and related to a well-known cancer pathway.

In conclusion, our study reveals that MYEF2 expression is associated with aggressive phenotypes of TNBCs, such as tumor size and stage and Ki-67 proliferation index. However, opposite findings implicating MYEF2 as a tumor suppressor have also been reported in the previous publications. Therefore, the functional role of MYEF2 and its potential involvement in a novel mechanism in TNBC should be investigated in future studies.

Disclosure of conflict of interest

None.

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