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Expression of epithelial membrane protein (EMP) 1, EMP 2, and EMP 3 in thyroid cancer

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Summary. Purpose. Epithelial membrane protein (EMP) 1, EMP2, and EMP3 are expressed in various types of tumors and have been reported to be involved in carcinogenesis. In this study, we aimed to investigate the expression of these proteins in primary and metastatic thyroid cancer and its clinical implication.

Methods. EMP1, EMP2, and EMP3 immunohistochemistry was performed using tissue microarrays of 545 primary thyroid carcinomas [338 papillary thyroid carcinoma (PTC), 111 follicular carcinoma (FC), 69 medullary carcinoma (MC), 23 poorly differentiated carcinoma (PDC), and 4 anaplastic carcinoma (AC)] and 59 recurrent or metastatic PTCs.

Results. EMP1 showed high expression in AC, PTC, FC (P<0.001), and EMP2 was highly expressed in AC (P<0.001). EMP1 and EMP2 were not expressed in stromal cells. Expression of EMP3 in tumor cells [EMP3 (T)] was higher in PDC, PTC, and AC (P<0.001), and expression in stromal cells [EMP3 (S)] was observed only in AC and PTC (P=0.001). The expression of EMP1 (P=0.002) and EMP3 (T) (P<0.001) was higher in conventional PTC than in follicular variant PTC. PTC with *BRAF* V600E mutation showed higher expression of EMP1 (P<0.001), EMP3 (T) (P<0.001), and EMP3 (S) (P=0.012) than PTC without *BRAF* V600E mutation. In the PTC without *BRAF* V600E mutation group, expression of EMP3 (S) was associated with shorter disease free survival (P=0.004). Metastatic PTC showed higher EMP2 (3.4% vs. 0%, P=0.022) and lower EMP3 (T) (44.1% vs. 66%, P=0.001) than primary PTC.

Conclusions. Expression of EMP1, EMP2, and EMP3 is different according to the subtypes of thyroid cancer. Further studies are needed to determine their role as prognostic markers and treatment target in thyroid cancer.

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Introduction

The epithelial membrane proteins (EMP1, EMP2, and EMP3) belong to the peripheral myelin protein 22-kDa (*PMP22*) gene family, which mainly functions in the peripheral nervous system but also plays a variety of roles in various tumors (Lobsiger et al., 1996; Wang et al., 2017). Expression of EMP1 has been reported to be involved in tumorigenesis and progression through the PI3K/AKT signaling pathway in non-small cell lung cancer (Lai et al., 2012). Increased EMP2 expression in endometrial cancer cells has been shown to activate the FAK/Src pathway and increase tumor cell migration and angiogenesis (Fu et al., 2011; Gordon et al., 2013). Increases in EMP3 in the upper urinary tract urothelial carcinoma and hepatocellular carcinoma were found to promote cancer cell proliferation and migration via the ErbB2/PI3K/AKT pathway (Wang et al., 2014; Hsieh et al., 2015). These preclinical studies suggest the potential of EMPs as therapeutic targets and their physiological function and molecular interactions need to be more clarified.

Thyroid cancer is the most common endocrine malignancy, accounting for 3.1% of all cancer diagnoses worldwide, with the global incidence rate in women of 10.2 per 100,000 which is 3 times higher than in men (Bray et al., 2018). The most common subtype is papillary thyroid carcinoma (PTC) and other subtypes include follicular carcinoma (FC), medullary carcinoma (MC), poorly differentiated carcinoma (PDC), and anaplastic carcinoma (AC). These subtypes show differences in cell origin, clinical manifestation, metastatic pattern, and clinical prognosis (Fagin and Wells, 2016).

EMP1, EMP2, and EMP3 have been studied in a variety of carcinomas, but there has been little research of thyroid cancer. In the present study, we aimed to investigate the expression of EMP1, EMP2, and EMP3

in subtypes of primary thyroid cancer and recurrent/metastatic thyroid cancer and ascertain their clinical significance.

Materials and methods

Patient selection and histologic evaluation

The present retrospective study included 338 cases of primary PTC and 59 cases of recurrent or metastatic PTC who had been diagnosed and undergone surgery at Severance Hospital from January 2011 to December 2012 in primary PTC and from January 2005 to December 2012 in recurrent or metastatic PTC. Other subtypes included 207 patients diagnosed with certain subtypes after operation at Severance Hospital from January 2000 to December 2012 (111 FC, 69 MC, 23 PDC, and 4 AC). Patients who received preoperative treatment were excluded. This study was approved by the Institutional Review Board of Yonsei University Severance Hospital. Clinicopathologic data were obtained from the patients' medical records and included age at diagnosis, disease recurrence, metastasis, current status, and follow up period. All cases were made with routine hematoxylin and eosin (H&E)-stained slides and were reviewed by a thyroid pathology specialist (J.S. Koo). The tumor size, location (right or left lobe), extent (confined to the thyroid parenchyma or with microscopic extrathyroidal extension), and number of metastatic lymph nodes were also noted from review of the slides and the surgical pathology reports.

The stromal components of PTC were observed under a microscope and classified as follows; Desmoplastic type, cellular proliferation of fibroblasts and myofibroblasts constitute the tumor stroma; sclerotic type, fewer cellular components and fibrotic collagenous component constituting tumor stroma; pauci-type, almost no stromal reaction; inflammatory type, inflammatory cells consisting mainly of tumor stroma (Fig. 1).

Tissue microarray (TMA)

A representative area showing tumor and tumor stroma was selected on an H&E-stained slide, and a corresponding spot was marked on the surface of the paraffin block. Using a biopsy needle, the selected area was punched out, and a 3-mm tissue core was transferred to a 6x5 recipient block. Two tissue cores of invasive tumor were extracted to minimize extraction bias.

Immunohistochemistry

The antibodies used for immunohistochemistry (IHC) in this study were EMP1 (polyclonal, Abcam, Cambridge, UK; 1:100 dilution), EMP2 (polyclonal, Abcam; 1:50 dilution), EMP3 (clone SW-5, Abcam; 1:100 dilution), and BRAF V600E (clone VE1, Ventana, Tucson, AZ, USA; 1:50 dilution). Immunohistochemistry (IHC) was performed on 3-μm tissue microarray sections from formalin-fixed, paraffin-embedded tissue blocks using a Ventana Discovery XT automated strainer (Ventana Medical Systems, Tucson, AZ, USA). Antigen retrieval was performed using CC1 buffer (Cell Conditioning 1; citrate buffer pH 6.0, Ventana Medical System). Each TMA slide contained appropriate positive and negative controls.

Interpretation of immunohistochemical staining

Immunostaining results of EMP proteins were evaluated semi-quantitatively according to the previous methods (Choi et al., 2012). Tumor and stromal cell staining were assessed as 0, negative or weak immunostaining in <1% of the tumor/stroma; 1, focal expression in 1~10% of tumor/stroma; 2, positive in 11~50% of tumor/stroma; and 3, positive in 51~100% of tumor/stroma. Score 0 was considered negative and score 1 or more was considered positive. *BRAF* V600E was considered positive if there was unequivocal diffuse cytoplasmic staining in almost tumor cells and negative if it showed faint or weak staining or isolated nuclear staining (Koperek et al., 2012; Sun et al., 2015).

Statistical analysis

Data were analyzed using SPSS for Windows,

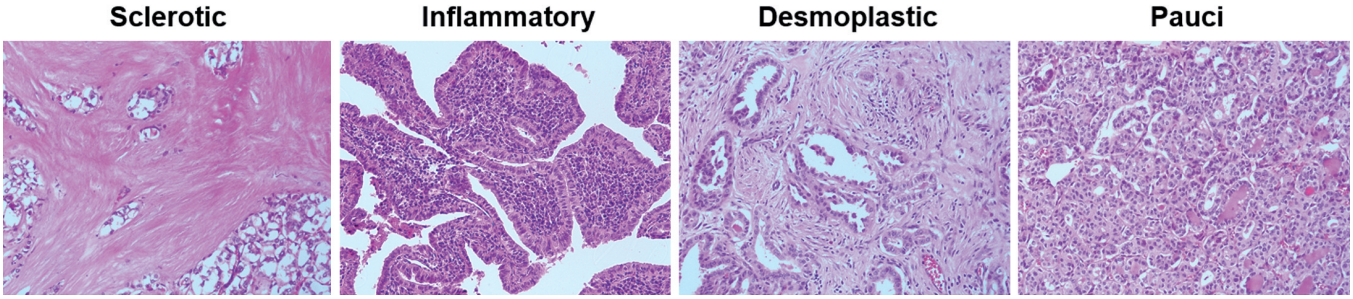

Fig. 1. Stromal components of PTC. Sclerotic type: fewer cellular components and a fibrotic collagenous component constitutes the tumor stroma. Inflammatory type: tumor stroma consists mainly of inflammatory cells. Desmoplastic type: cellular proliferation of fibroblasts and myofibroblasts constitutes the tumor stroma. Pauci-type: almost no stromal reaction. x 200.

Version 23.0 (SPSS Inc., Chicago, IL, USA). For determination of statistical significance, Student's t and Fisher's exact tests were used for continuous and categorical variables, respectively. In the case of analyzing data with multiple comparisons, a corrected p-value with the application of the Bonferroni multiple comparison procedure was used. Statistical significance was set to $P<0.05$. Kaplan-Meier survival curves and log-rank statistics were employed to evaluate time to tumor recurrence and overall survival.

Results

Basal characteristics of thyroid cancer

The basal characteristics of thyroid cancer are demonstrated in Table 1. PTC consisted of 302 conventional type (cPTC) and 36 follicular variant (FVPTC). *BRAF* V600E mutation was observed in 236 (69.8%) cases of PTC. *BRAF* V600E mutation had a higher proportion of cPTC ($P<0.001$), and the tumor border showed infiltrative features in cPTC and *BRAF* V600E mutant PTC ($P=0.009$). FC comprised of 61 minimally invasive type, 37 encapsulated angioinvasive type, and 13 widely invasive type. As invasiveness augmented, the tumor size increased ($P=0.040$) and the frequency of distant metastasis increased ($P=0.001$).

In MC, 33.3% (23/69) and 4.3% (3/69) were confirmed to have lymph node metastasis and distant metastasis, respectively. PDC showed relatively low lymph node metastasis (4.3%; 1/23), whereas 30.4% (7/23) had distant metastasis. All four ACs were over 45 years old, female, over 4 cm in size, extrathyroidal extension, and distant metastasis.

Table 1. Basic characteristics of patients with thyroid tumors.

Parameters	Thyroid papillary carcinoma							Thyroid follicular carcinoma					Thyroid high grade tumor		
		Histologic subtype			BRAF V600E mutation				Minimally	Encapsulated	Widely				
	Total N=338 (%)	Follicular variant n=36 (%)	Conventional type n=302 (%)	P value	Absent n=102 (%)	Present n=236 (%)	P value	Total N=111 (%)	invasive type, n=61 (%)	angioinvasive type, n=37 (%)	invasive type, n=13 (%)	P value	MC, n=69 (%)	PDC, n=23 (%)	AC, n=4 (%)
Age (years)				0.774			0.089					0.334			
<45	152 (45.0)	17 (47.2)	135 (44.7)		53 (52.0)	99 (41.9)		50 (45.0)	31 (50.8)	15 (40.5)	4 (30.8)		21 (30.4)	4 (17.4)	0 (0.0)
≥45	186 (55.0)	19 (52.8)	167 (55.3)		49 (48.0)	137 (58.1)		61 (55.0)	30 (49.2)	22 (59.5)	9 (69.2)		48 (69.6)	19 (82.6)	4 (100.0)
Sex				0.702			0.155					0.272			
Male	67 (19.8)	8 (22.2)	59 (19.5)		25 (24.5)	42 (17.8)		28 (25.2)	12 (19.7)	11 (29.7)	5 (38.5)		22 (31.9)	10 (43.5)	0 (0.0)
Female	271 (80.2)	28 (77.8)	243 (80.5)		77 (75.5)	194 (82.2)		83 (74.8)	49 (80.3)	26 (70.3)	8 (61.5)		47 (68.1)	13 (56.5)	4 (100.0)
Tumor size (cm)				0.950			0.428					0.040			
≤2.0	266 (78.7)	28 (77.8)	238 (78.8)		76 (74.5)	190 (80.5)		34 (30.6)	23 (37.7)	11 (29.7)	0 (0.0)		52 (75.4)	8 (34.8)	0 (0.0)
>2.0, ≤4.0	65 (19.2)	7 (19.4)	58 (19.2)		23 (22.5)	42 (17.8)		48 (43.2)	27 (44.3)	13 (35.1)	8 (61.5)		14 (20.3)	9 (39.1)	0 (0.0)
>4.0	7 (2.1)	1 (2.8)	6 (2.0)		3 (2.9)	4 (1.7)		29 (26.1)	11 (18.0)	13 (35.1)	5 (38.5)		3 (4.3)	6 (26.1)	4 (100.0)
Capsular invasion												<0.001			
No								13 (11.7)	0 (0.0)	13 (35.1)	0 (0.0)				
Yes								98 (88.3)	61 (100.0)	24 (64.9)	13 (100.0)				
Vascular invasion												<0.001			
No								65 (58.6)	61 (100.0)	0 (0.0)	4 (30.8)				
Yes								46 (41.4)	0 (0.0)	37 (100.0)	9 (69.2)				
Tumor margin				0.009			0.009								
Infiltrative	285 (84.3)	25 (69.4)	260 (86.1)		78 (76.5)	207 (87.7)							44 (63.8)	17 (73.9)	4 (100.0)
Expanding	53 (15.7)	11 (30.6)	42 (13.9)		24 (23.5)	29 (12.3)							25 (36.2)	6 (26.1)	0 (0.0)
Tumor extension				0.958			0.406					<0.001			
Intrathyroidal	102 (30.2)	11 (30.6)	91 (30.1)		34 (33.3)	68 (28.8)		94 (84.7)	53 (86.9)	35 (94.6)	6 (46.2)		51 (73.9)	11 (47.8)	0 (0.0)
Extrathyroidal	236 (69.8)	25 (69.4)	211 (69.9)		68 (66.7)	168 (71.2)		17 (15.3)	8 (13.12)	2 (5.4)	7 (53.8)		18 (26.1)	12 (52.2)	4 (100.0)
Histologic subtype							<0.001								
Conventional					21 (20.6)	15 (6.4)									
Follicular					81 (79.4)	221 (93.6)									
Lymph node metastasis				0.533			0.178					0.147			
No	134 (36.9)	16 (44.4)	118 (39.1)		46 (45.1)	88 (37.7)		109 (98.2)	61 (100.0)	36 (97.3)	12 (92.3)		46 (66.7)	22 (95.7)	2 (50.0)
Yes	204 (60.4)	20 (55.6)	184 (60.9)		56 (54.9)	148 (62.7)		2 (1.8)	0 (0.0)	1 (2.7)	1 (7.7)		23 (33.3)	1 (4.3)	2 (50.0)
Distant metastasis				0.948			0.408					0.001			
No	320 (94.7)	34 (94.4)	286 (94.7)		95 (93.1)	225 (95.3)		100 (90.1)	58 (95.1)	34 (91.9)	8 (61.5)		66 (95.7)	16 (69.6)	0 (0.0)
Yes	18 (5.3)	2 (5.6)	16 (5.3)		7 (6.9)	11 (4.7)		11	3 (4.9)	3 (8.1)	5 (38.5)		3 (4.3)	7 (30.4)	4 (100.0)

MC, medullary carcinoma; PDC, poorly differentiated carcinoma; AC, anaplastic carcinoma.

Expression of EMP1, EMP2, and EMP3 in thyroid cancer

EMP1, EMP2, and EMP3 all showed different expression patterns according to histologic subtypes (Table 2). EMP1 showed high expression rate in AC (100%), PTC (86.1%), and FC (75.7%; P<0.001). EMP2 was highly expressed in AC (50%; P<0.001) and was not stained in PTC, FC, and PDC. EMP1 and EMP2 were not expressed in stromal cells. Expression rate of EMP3 in tumor cells [EMP3 (T)] was higher in PDC (95.7%), PTC (66%), and AC (50%; P<0.001) and expression in stromal cells [EMP3 (S)] was observed only in AC

Table 2. Expression of EMP1, EMP2, and EMP3 according to the histologic subtypes of thyroid cancer.

Parameters	Total N=545 (%)	PTC n=338 (%)	FC n=111 (%)	MC n=69 (%)	PDC n=23 (%)	AC n=4 (%)	P value
EMP1							<0.001
Negative	116 (21.3)	47 (13.9)	27 (24.3)	28 (40.6)	14 (60.9)	0 (0.0)	
Positive	429 (78.7)	291 (86.1)	84 (75.7)	41 (59.4)	9 (39.1)	4 (100.0)	
EMP2							<0.001
Negative	537 (98.5)	338 (100.0)	111 (100.0)	63 (91.3)	23 (100.0)	2 (50.0)	
Positive	8 (1.5)	0 (0.0)	0 (0.0)	6 (8.7)	0 (0.0)	2 (50.0)	
EMP3 (T)							<0.001
Negative	254 (46.6)	115 (34.0)	85 (76.6)	51 (73.9)	1 (4.3)	2 (50.0)	
Positive	291 (53.4)	223 (66.0)	26 (23.4)	18 (26.1)	22 (95.7)	2 (50.0)	
EMP3 (S)							0.001
Negative	519 (95.2)	313 (92.6)	111 (100.0)	69 (100.0)	23 (100.0)	3 (75.0)	
Positive	26 (4.8)	25 (7.4)	0 (0.0)	0 (0.0)	0 (0.0)	1 (25.0)	

AC, anaplastic carcinoma; FC, follicular carcinoma; MC, medullary carcinoma; PDC, poorly differentiated carcinoma; PTC, papillary thyroid carcinoma; S, stromal cells; T, tumor cells.

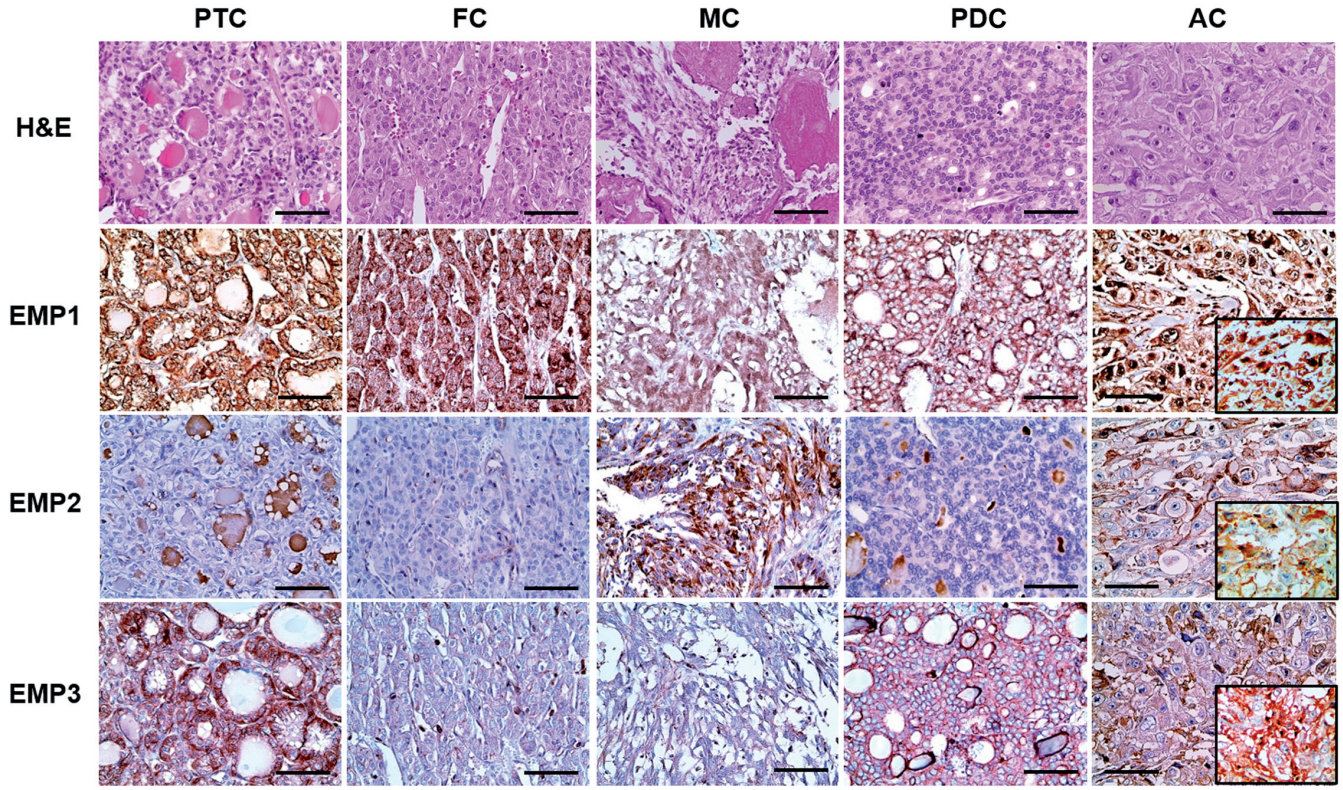

Fig. 2. Expression of EMP1, EMP2, and EMP3 in thyroid cancer. EMP1, EMP2, and EMP3 show different expression patterns according to histologic subtypes; EMP1 shows high expression in anaplastic carcinoma (AC), papillary thyroid carcinoma (PTC), and follicular carcinoma (FC). EMP2 is highly expressed in AC. Expression rate of EMP3 in tumor cells is higher in poorly differentiated carcinoma (PDC), PTC, and AC. Scale bars: 500 μm. x 200.

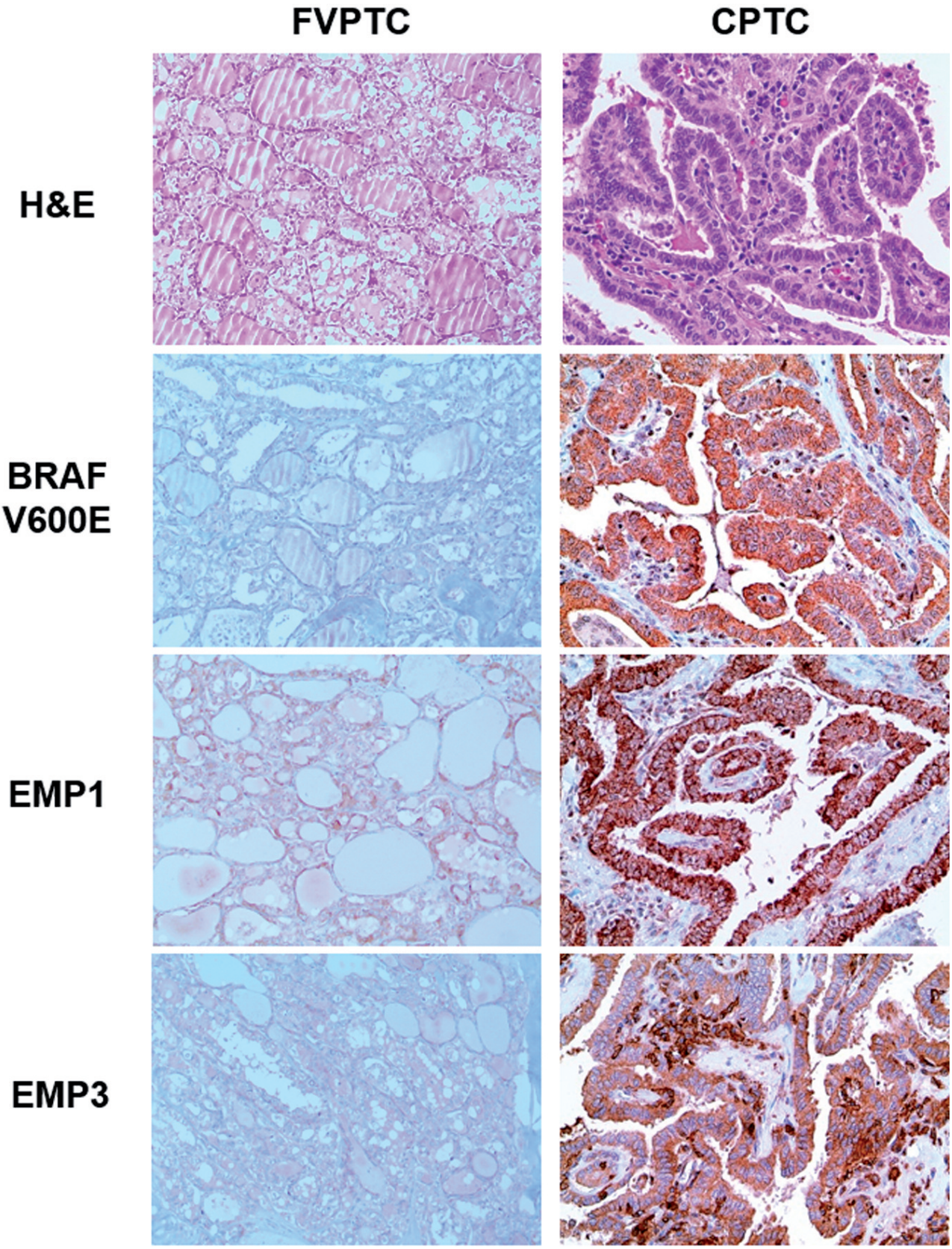

Fig. 3. Expression of EMP1 and EMP3 in papillary thyroid carcinoma. The expression of EMP1 and EMP3 in tumor cells are higher in conventional PTC (cPTC) and BRAF V600E mutated PTC than in follicular variant PTC (FVPTC) or BRAF wild type PTC. x 200.

(25%) and PTC (7.4%, P=0.001; Fig. 2).

The expression of EMP1 (P=0.002) and EMP3 (T) (P<0.001) was higher in cPTC than in FVPTC. PTC with BRAF V600E mutation showed higher expression of EMP1 (P<0.001), EMP3 (T) (P<0.001), and EMP3 (S) (P=0.012) than the PTC without *BRAF* V600E mutation (Table 3, Fig. 3). In FC, EMPs showed no significant differences according to the subtypes (Table 4).

Expression of EMP1, EMP2, and EMP3 according to stromal component types in PTC

In the PTC group without *BRAF* V600E mutation, the expression of EMP1 in tumor cells was different according to the types of stromal components (P=0.006). The rate of EMP1 positive expression was highest in the pauci-type and lowest in the sclerotic type (Fig. 4A). In BRAF V600E mutant PTC group, the expression of EMP3 (S) was relatively high in inflammatory-type (P <0.001, Fig. 4B). EMP3 was expressed in lymphocytes and plasma cells (Fig. 5).

Impact of the expression of EMP1, EMP2, and EMP3 on prognosis in thyroid cancer

Metastatic PTC showed relatively higher EMP2 (3.4% vs. 0%, P=0.022) and lower EMP3 (T) (44.1% vs. 66%, P=0.001) than primary PTC (Table 5, Fig. 6). However, in univariate analysis, EMP1, EMP2, and EMP3 expression in PTC did not significantly affect disease free and overall survival (Table 6). In subgroup analysis, EMP3 (S) was associated with shorter disease free survival (DFS) in the PTC without *BRAF* V600E mutation group (P=0.004, Fig. 7).

Table 3. Expression of EMP1, EMP2, and EMP3 according to the histologic subtype of papillary thyroid carcinoma.

Parameters	Histologic subtype		P value	BRAF V600E status		P value
	Follicular variant n=36 (%)	Conventional type n=302 (%)		Absent, n=102 (%)	Present, n=236 (%)	
EMP1			0.002			<0.001
Negative	11 (30.6)	36 (11.9)		38 (37.3)	9 (3.8)	
Positive	25 (69.4)	266 (88.1)		64 (62.7)	227 (96.2)	
EMP3 (T)			<0.001			<0.001
Negative	22 (61.4)	93 (30.8)		61 (59.8)	54 (22.9)	
Positive	14 (38.9)	209 (69.2)		41 (40.2)	182 (77.1)	
EMP3 (S)			0.073			0.012
Negative	36 (100.0)	277 (91.7)		100 (98.0)	213 (90.3)	
Positive	0 (0.0)	25 (8.3)		2 (2.0)	23 (9.7)	

S, stromal cells; T, tumor cells.

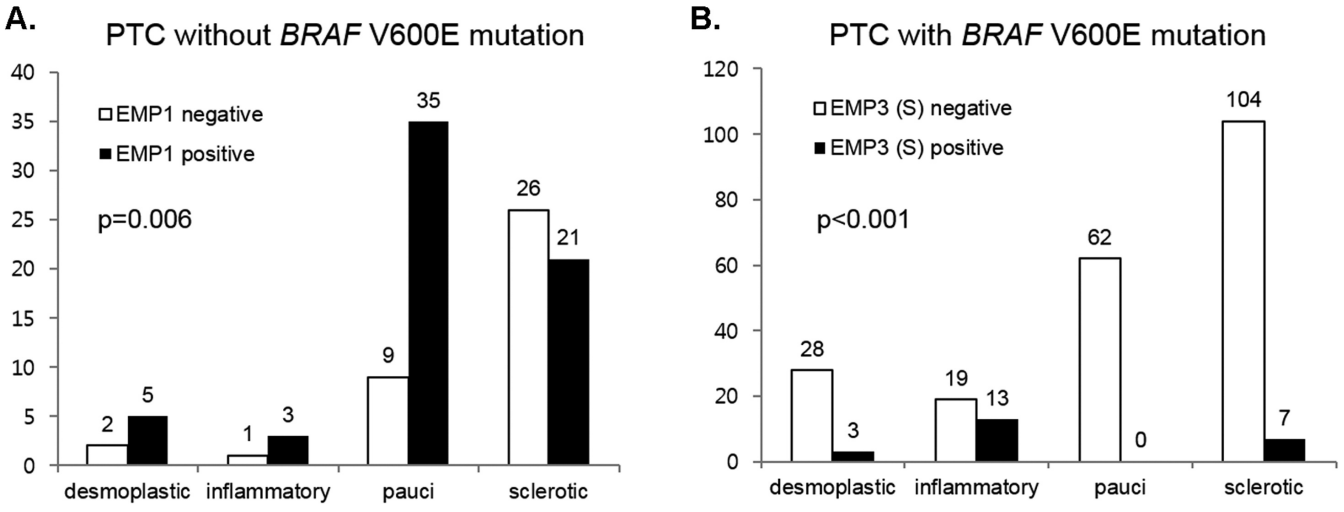

Fig. 4. Expression of EMP1, EMP2, and EMP3 in types of stromal components in PTC. **A.** In BRAF wild type PTC, the rate of positive EMP1 expression was highest in the pauci-type and lowest in the sclerotic type. **B.** In BRAF V600E mutant PTC, the expression of EMP3 in stromal cells [EMP(S)] is high in inflammatory-type.

Discussion

In this study, we examined the pattern of EMP expression in thyroid cancer and its clinicopathologic impact. There was a difference in the expression pattern of EMP according to the subtype of thyroid cancer. In PTC, expressions of EMP1 and EMP3 were relatively higher than EMP2. Conventional PTC and *BRAF* V600E mutated PTC showed higher levels of EMP1 and EMP3 than FVPTC and *BRAF* wild type PTC, respectively. FC showed relatively high expression of EMP1 and PDC showed high expression level of EMP3 in tumor cells.

The molecular pathogenesis of most thyroid carcinomas, which are differentiated thyroid carcinomas (PTC and FC), primarily involves dysregulation of the MAPK and PI3K/AKT signaling pathways (Prete et al.,

Table 4. Expression of EMP1, EMP2, and EMP3 according to the histologic subtype of follicular carcinoma.

Parameters	Total, N=111 (%)	Minimally invasive type, n=61 (%)	Encapsulated angio-invasive type, n=37 (%)	Widely invasive type, n=13 (%)	P value
EMP1					0.802
Negative	27 (24.3)	15 (24.6)	8 (21.6)	4 (30.8)	
Positive	84 (75.7)	46 (75.4)	29 (78.4)	9 (69.2)	
EMP2					N/A
Negative	111 (100.0)	61 (100.0)	37 (100.0)	13 (100.0)	
Positive	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
EMP3 (T)					0.810
Negative	85 (76.6)	48 (78.3)	27 (73.0)	10 (76.9)	
Positive	26 (23.4)	13 (21.3)	10 (27.0)	3 (23.1)	

T, tumor cells.

Table 5. Expression of EMP1, EMP2, and EMP3 in primary and recurred or metastatic papillary thyroid carcinoma (PTC).

Parameters	Total N=397 (%)	Primary PTC N=338 (%)	Recurred or metastatic PTC N=59 (%)	P-value
EMP1				0.673
Negative	54 (13.6)	47 (13.9)	7 (11.9)	
Positive	343 (86.4)	291 (86.1)	52 (88.1)	
EMP2				0.022
Negative	395 (99.5)	338 (100.0)	57 (96.6)	
Positive	2 (0.5)	0 (0.0)	2 (3.4)	
EMP3 (T)				0.001
Negative	148 (37.3)	115 (34.0)	33 (55.9)	
Positive	249 (62.7)	223 (66.0)	26 (44.1)	

T, tumor cells.

Table 6. Univariate analysis of the influence of EMP1, EMP2, and EMP3 expression in thyroid papillary cancer on disease–free and overall survival by the log–rank test.

Parameter	Number of patients /recurrence/death	Disease–free survival Mean survival (95% CI) months	Disease–free survival P-value	Overall survival Mean survival (95% CI) months	Overall survival P-value
EMP1			0.750		0.781
Negative	47/2/2	104 (98-110)		106 (103-110)	
Positive	291/16/16	106 (104-109)		107 (105-109)	
EMP3 (T)			0.657		0.127
Negative	115/7/3	105 (101-109)		109 (107-111)	
Positive	223/11/15	107 (104-110)		106 (104-109)	
EMP3 (S)			0.858		0.328
Negative	313/17/16	106 (104-109)		108 (106-110)	
Positive	25/1/2	103 (95-110)		99 (88-109)	

S, stromal cells; T, tumor cells.

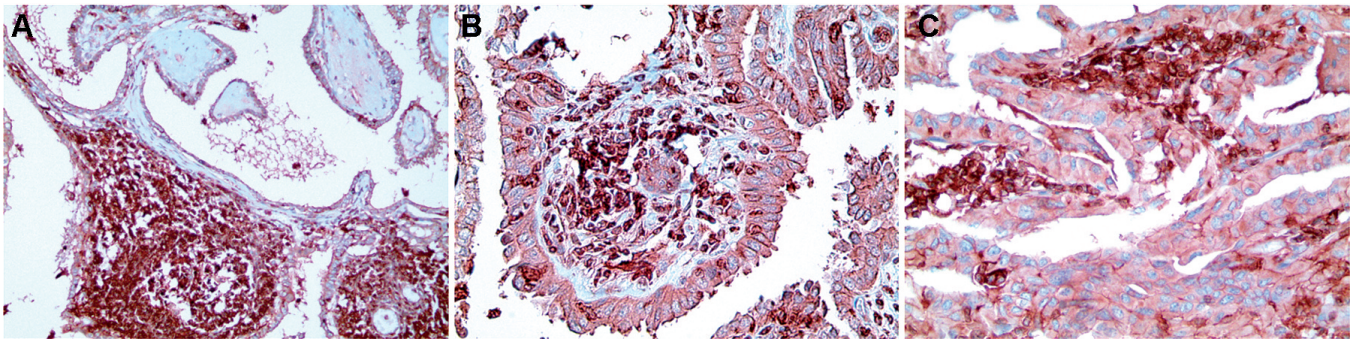

Fig. 5. Expression of EMP3 in stromal cells of PTC. Among the stromal components, lymphocytes and plasma cells showed EMP3 expression. x 200.

2020). The MAPK pathway plays a fundamental role in the regulation of cell growth, proliferation, apoptosis, and metabolic activities by regulating the expression of various genes (Xing, 2013). The PI3K/AKT pathway also plays a crucial role in the regulation of many processes, including cell growth, proliferation, apoptosis, protein synthesis, and glucose metabolism, by regulating the expression of various genes (Madhunapantula et al., 2011). Genetic changes eliciting activation of the MAPK pathway, such as *BRAF* V600E mutation, primarily lead to the development of PTC from follicular thyroid cells. Meanwhile, activation of

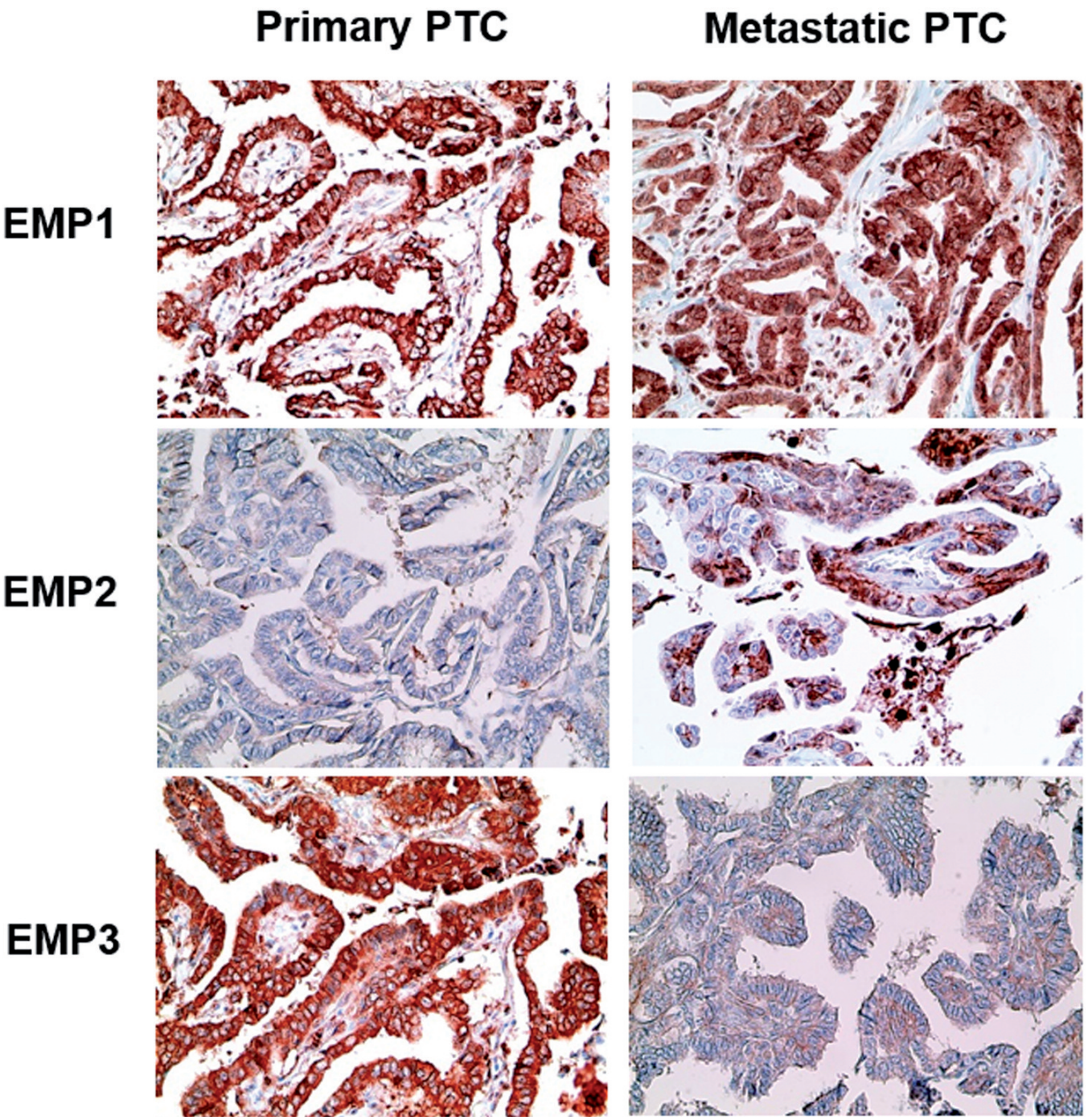

Fig. 6. Impact of the expression of EMP1, EMP2, and EMP3 in primary and recurred/metastatic thyroid papillary carcinoma. Metastatic PTC shows higher EMP2 and lower EMP3 (T) compared to primary PTC. x 200.

the PI3K/AKT pathway by genetic alterations, such as mutations in *RAS, PTEN* and *PIK3CA*, primarily drives the development of follicular thyroid adenoma and FC from follicular thyroid cells (Xing, 2013). Interestingly, research has shown that differentiated thyroid carcinoma can progress or dedifferentiate to PDC and AC via several additional mutations in p53, Wnt/β-catenin, *TERT* promoter, and *PIK3CA* (Haugen and Sherman, 2013; Prete et al., 2020).

The Cancer Genome Atlas (TCGA) Research Network has identified two major groups of PTC based on their gene expression profiles, *BRAF* V600E-like signature and *RAS*-like signature (Cancer Genome Atlas Network, 2014). *BRAF* V600E-like tumors generally have cPTC histology, high incidence of *BRAF* V600E mutation, and high levels of MAPK pathway signaling. In contrast, *RAS*-like tumors frequently exhibit follicular growth pattern and have high prevalence of *RAS* mutation and low levels of MAPK pathway signaling. In our cohort, EMP1 and EMP3 were highly expressed in PTC, especially cPTC and *BRAF* V600E mutated PTC, suggesting that they may be associated with MAPK signaling. In a recent study, over-expression of EMP1 in ovarian cancer was found to promote tumor cell proliferation and invasion via MAPK signaling (Liu et al., 2020). Furthermore, EMP3 has been reported to be associated with the MAPK pathway in gallbladder cancer (Ma et al., 2018), and further studies on the relationship between EMP1 and EMP3 and the MAPK signaling pathway in PTC are needed.

The most common somatic mutations in FC are *RAS* mutation and *PPARG* gene fusion. Mutations involving the PI3K/PTEN/AKT pathway related genes also occur (Hou et al., 2007; Wang et al., 2007). EMP1 has been reported to be involved in tumorigenesis through the PI3K/AKT pathway in non-small cell lung cancer (Lai et al., 2012) and glioblastoma (Miao et al., 2019). Considering that EMP1 has a relatively high expression rate in FC, it is possible to suggest that EMP1 participates in PI3K/AKT signaling in FC.

AC showed high expression of EMP1, EMP2, and EMP3, although the absolute number of cases (n = 4) was small, making it difficult to derive a definite meaning. Unlike PTC, FC, PDC, and AC, which originate from follicular thyroid cells, MC arises from parafollicular C cells, and *RET* alteration, a proto-oncogene, plays a crucial role in its tumorigenesis (Xing, 2013). MC did not show significant EMP expression levels relative to other thyroid cancers, which is thought to reflect the difference in major molecular alterations between them.

In this study, EMP3 was expressed not only in tumor cells of PTC and AC, but also in stromal cells. EMP expression patterns in stromal cells other than tumor cells have not been studied. Interactions between tumor cells and stromal cells play a very important role in tumor growth and progression. In the *BRAF* V600E mutant PTC group, the expression of EMP3 (S) was relatively high for the inflammatory-type. It has been shown that overexpression of EMP3 in macrophages inhibits CD8+ cytotoxic T lymphocytes (CTL) (Kusumoto et al., 2018) and that knockdown of EMP3 expression enhances the induction of CTLs, secretion of interferon-γ, and the expression of IL-2 receptor by CD8+ T cells. These findings suggest that EMP3 may regulate the immune system via inhibition of CTL induction (Ahmat Amin et al., 2019). Interestingly, we observed shorter DFS with high expression of EMP3 in stromal cells in the *BRAF* V600E non-mutated PTC group. Further studies are needed to determine the effect of EMP3 expression on stromal cells on biology of thyroid cancer.

Expression of EMP1, EMP2, and EMP3 in tumor cells did not significantly affect disease free and overall survival. In addition, EMT3 was expressed relatively low in metastatic PTC compared to primary PTC. EMP2 was expressed in a few cases (n=2) in metastatic PTC, but it is difficult to deduce the association of EMP2 with metastatic PTC. Whether EMP plays a tumor-promoting role or tumor-suppressive role and how it affects the prognosis in cancers has shown controversial results in previous studies (Ahmat Amin et al., 2019). EMP1 has been identified as an independent predictor of poor outcome by acting as a prednisolone resistance mechanism in precursor-B cell acute lymphoblastic leukemia (Aries et al., 2014), and is also demonstrated to be a biomarker of gefitinib resistance in non-small cell lung cancer (Jain et al., 2005). In contrast, it has been described as a negative regulator of tumor cell growth and metastasis in nasopharynx, stomach, breast, and

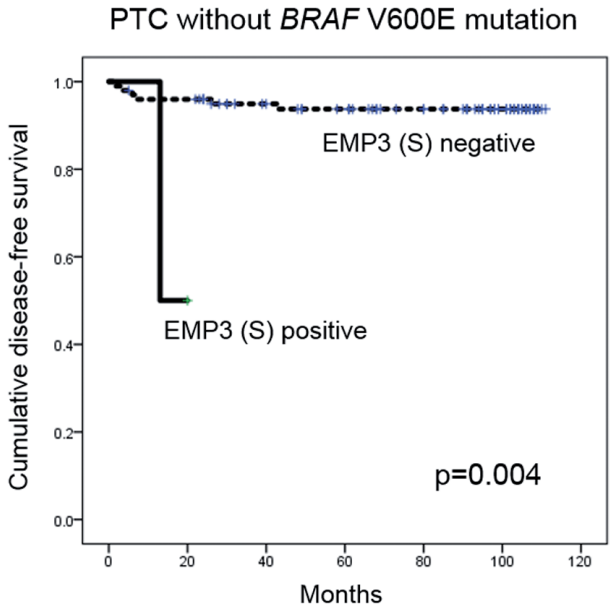

Fig. 7. Impact of the expression of EMP1, EMP2, and EMP3 on prognosis in thyroid cancer. EMP3 expression in stromal cell is associated with shorter disease free survival in the PTC without BRAF V600E mutation group.

colorectal cancers (Sun et al., 2014a-d). EMP2 acts as an oncogene in hormone-related cancers in endometrium or breast (Habeeb et al., 2010; Fu et al., 2014), while it acts as a tumor suppressor gene in hormone-non related cancers in upper urinary tract or nasopharynx (Wang et al., 2013; Ahmed et al., 2015). In addition, EMP3 is expressed in ERBB2-positive breast cancer and urothelial carcinoma (Mackay et al., 2003; Wang et al., 2014), and in hepatocellular carcinoma, cell proliferation and invasion are decreased when EMP3 is knocked down (Hsieh et al., 2015). On the other hand, it has been shown to act as a tumor suppressor gene in glioma, neuroblastoma, esophageal squamous cell carcinoma, and non-small cell lung cancer (Alaminos et al., 2005; Fumoto et al., 2009; Xue et al., 2013).

One of the clinical implications of this study is the potential role of EMP as a therapeutic target in thyroid cancer. Further research is needed in thyroid cancer, as it has been reported that inhibiting EMP2 in endometrial cancer (Shimazaki et al., 2008), ovarian cancer (Fu et al., 2010), and breast cancer (Fu et al., 2014) limits tumor progression. Above all, because EMP plays a role as a tumor progressor or suppressor depending on the type of tumor, the target strategy for EMP should also be individualized accordingly.

In conclusion, our findings demonstrated different expression of EMP1, EMP2, and EMP3 according to the subtypes of thyroid cancer. Further studies are needed to determine their role as prognostic markers and treatment target in thyroid cancer.

Authors' Contributions. EKK participated in the design of the study and performed the statistical analysis and carried out the immunoassays. JSK conceived the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

Competing interest. The authors declare no conflicts of interest.

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