SPANDIDOS PUBLICATIONS

Expression and prognostic roles of *PABPC1* in esophageal cancer: Correlation with tumor progression and postoperative survival

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Abstract. The prognosis of patients with esophageal cancer remains poor. TNM classification is not sufficient to predict their prognosis, and novel predictive markers of the prognosis of esophageal cancer patients are therefore needed. *Poly A binding protein, cytoplasmic 1 (PABPC1)* plays a role in post-transcriptional control of mRNA and may be involved in tumorigenesis. *PABPC1* expression has not been studied in esophageal cancer. Expression of *PABPC1* was quantified by real-time reverse transcription polymerase chain reaction (RT-PCR) using LightCycler in 41 primary esophageal squamous cell carcinomas (ESCCs) and their paired normal esophageal mucosa. We examined the correlation between *PABPC1* expression and the clinicopathological factors and prognosis of ESCC patients. Reduced expression of *PABPC1* was accompanied by locally invasive tumors (t-factor, p=0.0145) and more advanced tumors (pathologic stage, p=0.0264). Moreover, ESCC patients with low *PABPC1* mRNA expression had a significantly shorter postoperative survival time than those with high expression (median survival, 3.1 vs. 6.5 months, p=0.002). In esophageal cancer, reduced expression of *PABPC1* was correlated with local tumor progression and poor prognosis after surgery.

Introduction

Esophageal cancer is the sixth most common cancer in the world, of which esophageal squamous cell carcinoma (ESCC) accounts for >90% of cases. The prognosis of patients with esophageal cancer remains poor, prompting the search for new treatment strategies. Today, the overall 5-year survival rate is generally less than 50%, despite the use of multi-modality therapy. Even in early stage disease, many patients develop local tumor recurrence or distant metastasis within a short time after surgery. To develop a strategy for new treatment, it is important to understand the biological behavior of esophageal cancer. Previous studies revealed that several genes and molecules are involved in the origin and/or progression of esophageal cancer, including *TP53* (1), *deleted in esophageal cancer 1* (*DEC1*) (2), *deleted in colorectal cancer* (*DCC*) (3), *deleted in lung cancer 1* (*DLC1*) (4), *cyclin D1* (5), *transforming growth factor-beta receptor type II* (*TGFBRII*) (6), *adenomatous polyposis coli* (*APC*) (7), *survivin* (8), and *murine double minute 2* (*MDM2*) (9). However, the precise mechanisms that underlie the development and progression of ESCC are unclear.

The mRNA degradation mechanism plays an important role in controlling gene expression. Any alteration to the control of mRNA degradation will have a significant effect on cell growth control, as evidenced by the observation that overexpression of *eIF-4* transforms cells (10), and expression of a dominant-negative mutant of the double-stranded RNA-dependent kinase, which phosphorylates *eIF-2* (11), causes noncancerous cells to become tumorigenic (12).

PABPC1 mRNA is known to be involved in mRNA translation and degradation (13,14), and stabilizes the 5' cap of mRNA. In addition, *PABPC1* plays the most important role in deadenylation and protects poly (A) tail by covering the poly (A) tail. However, in yeast, it is known that depletion of the poly (A) binding protein results in the inhibition of translation initiation and poly (A) shortening (15). Poly (A) binding protein has shown to be involved in ribonuclease activity for the poly (A) tail, thus conferring *PABPC1 with* apparently contradictory functions of protection and degradation of the poly (A) tail (16). In this study, we report the correlation between *PABPC1* expression and the clinicopathological factors and prognosis of ESCC patients.

Materials and methods

Tissue samples. Samples were obtained from 41 esophageal cancer patients who had undergone surgery at the Second Department Surgery, Nagoya City University Medical School between 1996 and 2000 without pre-operative chemotherapy or radiation.

The tumors were classified according to the guidelines for the clinical and pathological studies on carcinoma of the esophagus (17). All samples for RT-PCR were immediately

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Table I. *PABPC1* mRNA expression and clinical characteristics of 41 patients with esophageal carcinoma.

Patient	Age (years)	Gender	T	N	M	Stage	*PABPC1/GAPDH*	Follow-up (months)	Outcome
1	62	M	1a	0	0	0	4.972	50	Alive
2	67	M	1b	0	0	1	3.118	31	Alive
3	68	M	1a	1	0	1	7.146	26	Alive
4	46	M	1b	0	0	1	8.844	38	Alive
5	66	M	3	0	0	2	3.226	36	Dead
6	73	F	2	1	0	2	11.251	38	Alive
7	69	M	1b	2	0	2	6.710	3	Alive
8	60	M	1b	2	0	2	9.164	32	Alive
9	79	F	3	0	0	3	2.171	30	Dead
10	69	M	3	2	0	3	2.605	9	Dead
11	56	M	3	2	0	3	3.339	34	Alive
12	58	M	3	1	0	3	1.878	12	Dead
13	60	M	3	3	0	3	2.030	12	Dead
14	66	M	3	1	0	3	2.629	12	Dead
15	66	F	3	2	0	3	4.545	40	Dead
16	54	F	3	3	0	3	2.363	17	Dead
17	68	M	3	1	0	3	19.221	13	Dead
18	68	M	3	2	0	3	1.902	27	Dead
19	68	M	3	1	0	3	2.907	12	Dead
20	57	F	3	2	0	3	3.805	14	Dead
21	61	F	3	2	0	3	2.844	19	Dead
22	63	M	3	3	0	3	4.246	28	Alive
23	47	F	3	2	0	3	5.532	38	Alive
24	50	M	3	2	0	3	3.041	50	Dead
25	68	M	3	2	0	3	5.042	10	Dead
26	70	F	3	2	0	3	4.300	5	Dead
27	75	M	4	3	0	4	5.752	3	Dead
28	55	M	4	3	0	4	3.747	4	Dead
29	51	F	4	2	0	4	4.305	9	Dead
30	55	M	4	4	0	4	2.654	7	Dead
31	51	M	2	4	0	4	7.774	3	Dead
32	69	M	4	4	0	4	2.856	8	Dead
33	62	M	4	2	0	4	1.529	7	Dead
34	45	M	4	3	0	4	2.971	8	Dead
35	47	M	4	4	0	4	1.358	4	Dead
36	52	M	3	4	0	4	6.884	15	Dead
37	72	M	3	4	0	4	5.742	6	Dead
38	53	M	4	2	0	4	2.762	14	Dead
39	76	M	4	3	0	4	2.508	11	Alive
40	64	M	4	2	0	4	4.481	6	Alive
41	51	M	4	4	0	4	1.187	7	Dead

PABPC1, poly A binding protein; *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase.

frozen in liquid nitrogen and stored at -80˚C until analysis. The samples were used with written consent from the patients. The details of the 41 patients with *PABPC1* are shown Table I.

RNA extraction and RT-PCR analysis. Total RNA was extracted from the esophageal cancer tissues, and normal esophageal mucosa samples were taken as far from the tumor as possible using the Absolutely RNA™ RT-PCR Miniprep kit (Stratagene, La Jolla, CA). The concentration of total RNA was adjusted to 200 ng/ml using a spectrophotometer. Reverse transcriptional reaction was carried out at 42˚C for 90 min and 95˚C for 5 min followed by incubation at 72˚C for 15 min using 10 μg total RNA, 0.5 mg oligo (dT) primer, and Superscript II enzyme (Gibco BRL, Gaithersburg, MD). To confirm the accuracy of RNA extraction and RT-PCR, all samples were subjected to PCR amplification using the Light

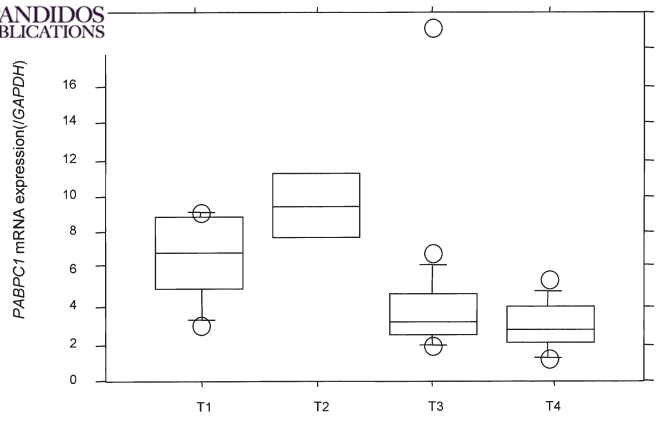

Figure 1. *PABPC1* mRNA expression in ESCCs according to the t-factor. *PABPC1* mRNA expression was significantly decreased in tumors that were more locally progressed (n=41, p=0.0145; Kruskal-Wallis test). Data represent mean ± SD (boxes) and 2 SD (bars), respectively.

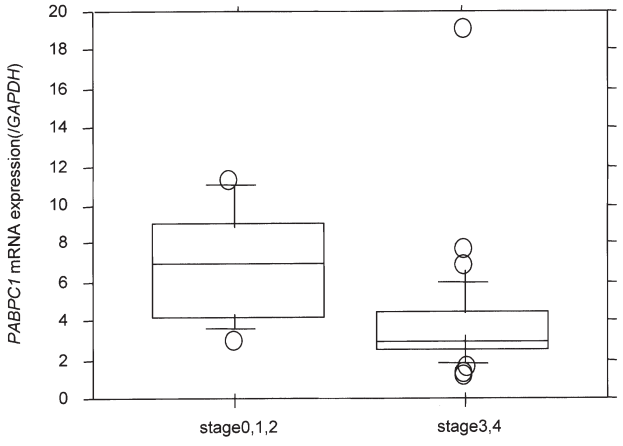

Figure 2. *PABPC1* mRNA expression in ESCCs according to stage. *PABPC1* mRNA expression was significantly lower in stages 0-2 ESCC than in stage 3/4 ESCC. (n=41, p=0.0264; Kruskal-Wallis test). Data represent mean ± SD (boxes) and 2 SD (bars), respectively.

Table II. Clinicopathological data of 41 ESCC patients and *PABPC1* mRNA expression of the tumors.

Factors	No.of pts. (%)	PABPC1/GAPDH mRNA level	p-value
Samples	41	4.521±3.284	
Age			
≤65	25	4.223±2.342	
>65	16	4.986±2.736	0.4747
Gender			
Male	32	4.507±3.461	
Female	9	4.569±2.736	0.9611
Pathologic stage			
0	1	4.973	
1	3	6.370±2.941	
2	4	7.588±3.449	
3	18	4.134±3.924	(0-2 vs. 3,4)
4	15	3.768±2.021	0.0264
Tumor status			
T1	6	6.659±2.310	
T2	2	9.513±2.459	
T3	21	4.298±3.689	
T4	12	3.010± 1.369	0.0145
Lymph node status			
N0	5	4.467±2.684	
N1	6	7.462±6.756	
N2	17	4.215±1.890	
N3	8	3.310±1.226	
N4	5	4.024±3.195	0.6973
Lymphatic invasion			
Negative	5	7.122±2.437	
Positive	31	3.809±2.025	0.0022
Vein invasion			
Negative	15	5.264±2.842	
Positive	21	3.558±1.665	0.0299

Cycler-FastStart DNA Master SYBR-Green kit (Roche Molecular Biochemicals, Mannheim, Germany). Primer sequences for the *PABPC1* gene were: forward, 5-AGCAAA TGTTGGGTGAACGG-3; and reverse, 5-ACCGGTGGC ACTGTTAACTG-3. PCR protocol was initial denaturation at 95˚C for 10 min, followed by 50 cycles at 95˚C for 15 sec, annealing at 60˚C for 5 sec, and extension at 72˚C for 10 sec. The PCR product was quantified with the intensity of SYBR-Green I at 88˚C.

Statistical analysis. Data are expressed as the mean ± standard deviation. Statistical analysis was carried out using StatView software (Abacus Concepts, Berkeley, CA), and the Kruskal-Wallis and Mann-Whitney U tests were used to evaluate the significance of the difference in expression of *PABPC1* mRNA. The survival of *PABPC1* patients after surgery was examined by the Kaplan-Meier method, and the difference in the survival was analyzed using the log-rank test. A p-value <0.05 was considered to be statistically significant.

Results

We examined the *PABPC1* mRNA expression level in 41 primary esophageal cancer tissues and their paired normal esophageal tissues using LightCycler. *PABPC1* mRNA expression was detectable in the majority of ESCC samples and all samples of normal esophageal mucosa. *PABPC1* expression was standardized in reference to that of *glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*).

PABPC1 expression in the tumor varied, and there was no significant difference between *PABPC1* expression of the tumor and corresponding normal esophageal mucosa (data not shown). *PABPC1* expression in ESCC tissue was significantly correlated with tumor size and local invasiveness (t-factor) (Fig. 1). *PABPC1* expression was significantly reduced in locally advanced ESCC (t3 and t4 tumors, p=0.0145) (Fig. 1). The levels of *PABPC1* expression were significantly lower in stages 0-2 ESCC than in stage 3/4 ESCC (p=0.0264) (Fig. 2). *PABPC1* expression levels in

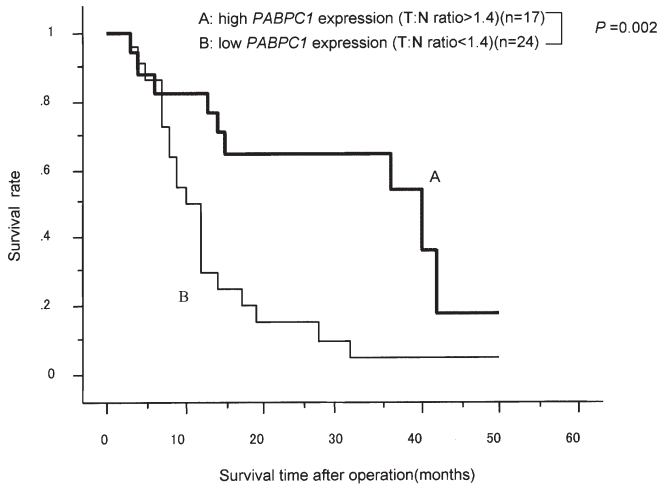

Figure 3. Survival time of ESCC patients after surgery. (A) Patients with high *PABPC1* mRNA expression (T:N ratio >1.4), and (B) patients with low *PABPC1* mRNA expression (T:N ratio <1.4). There is a significant difference between the two groups (p=0.002; log-rank test).

patients with ESCC who had lymphatic invasion were significantly lower compared with the expression levels in patients without lymphatic invasion (p=0.0022). *PABPC1* expression levels in ESCC patients with vein invasion were significantly lower compared with the expression levels in patients without vein invasion (p=0.0299).

PABPC1 expression did not have an apparent correlation with lymph node metastasis (n-factor) (Table II). According to other clinical factors, including the gender and age of ESCC patients, no apparent differences in *PABPC1* expression were recognized (Table II).

We also investigated the correlation between the *PABPC1* expression and survival time of the ESCC patients after surgery with a mean follow-up of 14.6 months. We divided the 41 ESCC patients into two groups. Group A contained patients with high expression of *PABPC1* [tumor to normal ratio (T:N ratio) >1.4, n=17], and group B had patients with low expression (T:N ratio <1.4, n=24). Patients with low *PABPC1* expression had a significantly shorter median survival time (3.117±1.667 months) after surgery than those with high *PABPC1* expression (6.502±4.014 months) (p=0.002; log-rank test) (Fig. 3).

Discussion

Poly A binding protein, cytoplasmic 1 (*PABPC1*), which is found complexed to the 3-prime poly (A) tail of eukaryotic mRNA, is required for poly (A) shortening and translation initiation (13). *PABPC1* participates in at least three major post-transcriptional events: i) mRNA biogenesis; ii) regulation of mRNA turnover; and iii) initiation of protein synthesis (18). However, exact reasons for the essential role of the *PABPC1* function remain to be elucidated.

PABPC1 is the key factor responsible for the poly (A) tail-stimulated pathway of translational initiation (18,19). Grosset *et al* reported 5 proteins that form a multiprotein complex with the major protein-coding region determinant of instability (mCRD) of the *FOS* gene: *PABPC1*, *AUF1/heterogeneous nuclear ribonucleoprotein D* (*HNRNPD*), *poly (A) binding protein interacting protein 1* (*PAIP1*), *NS1-associated protein 1* (*NSAP1*), and *upstream of N-ras* (*UNR*) (20).

In this study, we reported that decreased expression of *PABPC1* in esophageal cancer tissue is accompanied by the local progression of esophageal cancer (Figs. 1 and 2 and Table II). Patients with lower *PABPC1* expression also had a poorer prognosis (Fig. 3). Thus, the down-regulation of *PABPC1* may contribute to tumor growth in esophageal carcinoma by losing normal control of mRNA turnover. Because *PABPC1* plays critical roles in translation and mRNA stabilization/degradation, it is necessary to investigate how the deficiency of normal mRNA regulation by down-regulation of *PABPC1* mRNA might result in tumor progression and poor prognosis.

PABPC1 is located at chromosome region 8q22.2-23. Loss of heterozygosity (LOH) at 8q22 has been reported in human malignancies, such as oral cancer (21), breast cancer (22) and leukemia (23), and proposes the existence of a tumor suppressor gene in various cancers. It is speculated that the inactivation of *PABPC1* may be involved in the carcinogenesis of those organs. In esophageal cancer, we are currently investigating LOH at 8q22 (unpublished data) as a possible mechanism of PABPC1 inactivation. Although we have not examined the mutations of *PABPC1*, *PABPC1* may be a tumor suppressor. In addition to genetic changes, transcriptional silencing due to methylation in the promoter region may be responsible for the down-regulation of *PABPC1*. This possibility remains to be examined.

In esophageal cancer patients, many prognostic markers such as *cyclin D1*, *E-cadherin* and *MDM2* have been reported (5,9). We have also reported that the expression of *survivin* (8), *pituitary tumor transforming gene 1* (*PTTG1*) (24), *peroxisome proliferator-activated receptor gamma* (PPARγ) (25), *DNA fragmentation factor 45* (*DFF45*) (26), and *excision repair cross complementing 3* (*ERCC3*) (27) may be prognostic markers of ESCC. *PABPC1* has now been added as a possible prognostic indicator in ESCC patients.

Although the precise molecular mechanism of down-regulated *PABPC1* expression needs to be clarified, our data indicate that *PABPC1* may be a good molecular prognostic marker, as well as a molecular target for development of an effective drug for patients with esophageal cancer. Elucidating the function of PABPC1 may lead to a better understanding of the carcinogenic mechanism of tumor progression in patients with esophageal carcinoma.

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