Virchows Arch (2010) 457:69–76
DOI 10.1007/s00428-010-0930-8

ORIGINAL ARTICLE

Expression of the GA733 gene family and its relationship to prognosis in pulmonary adenocarcinoma

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Received: 4 February 2010 / Revised: 23 April 2010 / Accepted: 26 April 2010 / Published online: 16 May 2010

Abstract The GA733 gene family is composed of GA733-1 (TROP2) and GA733-2 (Ep-CAM), whose expression has been examined in various carcinomas and reported to be significantly associated with prognosis. The aim of this study was to investigate the expression of GA733 gene family members and to compare their prognostic significance in pulmonary adenocarcinoma. One hundred thirty paraffin-embedded specimens of small-sized pulmonary adenocarcinoma, less than 2 cm in diameter, were categorized using the classification of small-sized pulmonary adenocarcinoma devised by Noguchi et al. (Cancer 75:2844–2852, 1995) and examined immunohistochemically using a murine monoclonal antibody against Ep-CAM and a goat polyclonal antibody against TROP2. The patient survival rate was calculated using the Kaplan–Meier method. Ep-CAM and TROP2 were similarly expressed in many small-sized pulmonary adenocarcinomas. The expression of Ep-CAM was significantly related to a favorable outcome ($p=0.0185$), whereas TROP2 tended to be expressed in cases with an unfavorable outcome ($p=0.0564$), and was significantly associated with an unfavorable outcome in nonlepidic-type adenocarcinomas ($p=0.0125$). Multivariate analysis showed that TROP2 overexpression and lymph node metastasis were independent prognostic markers. Although the two GA733 proteins share structural similarity, they appear to have opposite biological significances in small-sized adenocarcinomas. As the expression of TROP2 was detected in more poorly differentiated tumors, the protein may have oncogenic activity.

Keywords GA733 family · Adenocarcinoma · Lung · Immunohistochemistry · Prognosis

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Introduction

Lung cancer has a poor prognosis and is a major cause of death worldwide. Most lung cancer patients are initially diagnosed at an advanced stage, and few chemotherapeutic drugs elicit a satisfactory response. Therefore, there is a need to find biomarkers that are highly expressed in lung cancer from an early stage and can be used for prognostication. Lung cancer can be roughly classified into four groups: adenocarcinoma, squamous cell carcinoma, large cell carcinoma, and small cell carcinoma, among which the incidence of adenocarcinoma has been increasing recently, making it the most common histological type [1].

Previously, we established an immortalized atypical adenomatous hyperplasia cell line (PL16T) and a human

nonneoplastic bronchial epithelial cell line (PL16B) from the same patient by transfection with the gene for SV40 large T antigen [2]. We then compared the gene expression profile of PL16T with that of PL16B by suppression subtractive hybridization. Among the genes studied, GA733-1 was expressed three to 20 times higher in PL16T than in PL16B, and GA733-2 was expressed three times higher in PL16T than PL16B [2]. GA733-1 and GA733-2 belong to the GA733 gene family [3]. The GA733-1 gene is also known as TACSTD2, TROP2, M1S1, or EGP-1, whereas the GA733-2 gene is also known as TACSTD1, Ep-CAM, or KSA. Ep-CAM consists of nine exons and TROP2 is an intronless gene, formed by retroposition of Ep-CAM via an mRNA intermediate [4]. TROP2 has 49% homology with Ep-CAM, and both are type I transmembrane proteins with a single transmembrane domain [5]. Ep-CAM is a 40-kDa glycoprotein, which is reported to mediate Ca^{2+}-independent homotypic cell–cell adhesion [6]. TROP2 is a polypeptide of 35 kDa and thought to transduce an intracellular calcium signal, acting as a cell surface receptor [7]. Mutation of this gene is associated with gelatinous drop-like corneal dystrophy, an autosomal recessive disorder characterized by severe corneal amyloidosis leading to blindness [8]. Ep-CAM is expressed in many normal tissues and overexpressed in epithelial malignant tumor [9]. Ep-CAM has been highlighted as a target for immunotherapy. In addition, a correlation between Ep-CAM expression and clinical factors has been reported in squamous cell carcinoma [10], but it appears that no previous studies have analyzed the expression of Ep-CAM and TROP2 in adenocarcinoma. The aim of this study was to examine the expression of GA733 family genes in pulmonary adenocarcinomas and analyze its biological significance.

Materials and methods

Cases

We retrospectively selected 130 cases diagnosed as primary pulmonary adenocarcinoma with a maximal tumor dimension of less than 2 cm. The cause of death was related to lung cancer in all cases. The surgical resections had been performed at the National Cancer Center Hospital (Tokyo) during the period from January 1993 to December 2000. None of the patients had received chemotherapy and radiotherapy before or after surgery. Written informed consent had been obtained from all the patients (62 males and 68 females), who were aged between 38 and 82 years (median age, 60.7 years; Table 1). The patients were followed until death or up to October 12, 2007. Twenty-seven patients died during this period. In Table 1, the number of patients who died is shown in parentheses.

Tissue specimens and histological typing

All resected tumors were fixed with 10% formalin and embedded in paraffin for histologic examination. Sections were cut at a thickness of 3 μm and stained with hematoxylin and eosin and elastica van Gieson. Three pathologists (M.N. T.I. and Y.A.) examined the sections by light microscopy and classified the tumors into six histological subtypes according to the classification proposed by Noguchi et al. [11]. Briefly, they divided small-sized pulmonary adenocarcinoma into two groups: lepidic type and nonlepidic type. In addition, the lepidic type was divided into three types: type A (localized bronchioloalveolar carcinoma (LBAC)), type B (LBAC with alveolar collapse), and type C (LBAC with foci of active fibroblastic proliferation). The nonlepidic type was divided into another three types: type D (poorly differentiated adenocarcinoma), type E (acinar adenocarcinoma), and type F (true papillary adenocarcinoma). The patients with tumors classified as type A and type B showed a 100% 5-year survival rate. However, those with tumors classified as type C had a 5-year survival rate of 75%. On the other hand, the outcome of nonlepidic-type carcinomas was more unfavorable than that of lepidic-type carcinomas. Ep-CAM and TROP2 expression were determined by immunohistochemistry on paraffin-embedded tissue specimens using a mouse monoclonal antibody against Ep-CAM (ab7504, Abcam plc, Cambridge, UK, clone number Ber-EP4) and a goat polyclonal antibody against TROP2 (AF650, R&D Systems, Inc., Minneapolis, MN, USA). The antigen peptide of Ep-CAM and the protein sequence of TROP2 did not overlap. Three-micrometer-thick continuous sections were cut from paraffin-embedded tissue blocks, mounted on adhesive-coated glass slides, and then deparaffinized and rehydrated.

For staining of Ep-CAM, slides were pretreated with pronase solution (Proteinase K; Dako, Carpinteria, CA, USA) for 5 min. For staining with anti-TROP2 antibody, slides were placed in a box filled with TE buffer (pH 9.0) and autoclaved at 121°C for 10 min for antigen retrieval. After the pretreatment, the slides were removed and chilled in water.

The sections were then washed three times in wash buffer (Dako, CA, USA), followed by treatment with peroxidase-blocking solution (Dako, Glostrup, Denmark) for 5 min to quench any endogenous peroxidase. After another wash in wash buffer, the slides were incubated for 30 min at room temperature with each primary antibody (Ep-CAM; 1:10 dilution, TROP2; 1:50 dilution). After a further wash in wash buffer, the slides were incubated for 30 min at room temperature with the secondary antibody (Envision Plus dual link system peroxidase (Dako, CA, USA) for Ep-CAM and Histofine® Simple Stain MAX PO (G) (Nichirei Bioscience, Japan) for TROP2). To visualize the immunolabeled structures, the slides were reacted with

Table 1 Relationship of Ep-CAM and TROP2 overexpression and conventional clinicopathological parameters (n=130)

Characteristics	Number of patient	Ep-CAM overexpression		p value	TROP2 overexpression		p value
		No	Yes		No	Yes	
Gender							
Male	62	18	44	0.349	19	43	0.574
Female	68	25	43		24	44	
Age							
<60	60	18	42	0.49	15	45	0.07
≧60	70	25	45		28	42	
Noguchi classification							
Type A	12 (0)	4 (0)	8 (0)		5 (0)	7 (0)	
Type B	14 (0)	1 (0)	13 (0)		4 (0)	10 (0)	
Type C	63 (16)	18 (9)	45 (7)		18 (4)	45 (12)	
Type D	27 (8)	13 (4)	14 (4)		10 (0)	17 (8)	
Type E	8 (2)	3 (1)	5 (1)		4 (1)	4 (1)	
Type F	6 (1)	4 (0)	2 (1)		2 (0)	4 (1)	
Type A–C	89 (16)	23 (9)	66 (7)	0.01	27 (4)	62 (12)	0.328
Type D–F	41 (11)	20 (5)	21 (6)		16 (1)	25 (10)	
Pleural invasion							
p0	97	29	68	0.186	30	67	0.372
≧p1	33	14	19		13	20	
Vascular invasion							
v−	73	19	54	0.053	24	49	0.956
v+	57	24	33		19	38	
Nodal status							
pN0	89	24	65	0.029	29	60	0.86
≧pN1	41	19	22		14	27	
Prognosis							
Alive	103	29	74	0.02	38	65	0.071
Dead	27	14	13		5	22	

Envision Plus/HRP (DAB; Dako, CA, USA). Following incubation for 5 min, the slides were rinsed with distilled water. All sections were counterstained with hematoxylin.

Scoring of sections

Two observers (H.K. and Y.K.) scored the sections independently without any information about clinical outcome. We scored all sections in accordance with a previous report on immunohistochemistry for Ep-CAM [12]. Briefly, a total immunostaining score was calculated as the product of a proportion score and an intensity score. The proportion score represented the estimated fraction of positively stained tumor cells (0 = none; 1 = <10%; 2 = ≧10%, <50%; 3 = ≧50%, <80%; 4 = ≧80%). The intensity score represented the estimated staining intensity (0—no staining, 1—weak, 2—moderate, 3—strong). Thus, the total score ranged from 0 to 12. Ep-CAM and TROP2 overexpressions were defined as a total score exceeding 4.

Statistical analysis

Differences between the expression of Ep-CAM or TROP2 and clinical variables were estimated using the χ^2 test. Survival curves in relation to Ep-CAM expression and TROP2 expression in patients overall and for lepidic type (types A, B, and C) and nonlepidic type (types D, E, and F) were drawn using the Kaplan–Meier method and compared by the log-rank test. Additionally, the influence of each variable on survival, including gender, age, pleural invasion, vascular invasion, lymph node metastasis, Noguchi classification (lepidic/nonlepidic), Ep-CAM overexpression, and TROP2 overexpression, was assessed using the Cox proportional hazards regression model in order to find independent prognostic factors. For all analyses, a p value of less than 0.05 was considered statistically significant. All statistical analyses were carried out using the SPSS statistical software package (SPSS Inc., Chicago, IL, USA).

Results

Histological typing

The 130 pulmonary adenocarcinomas were classified into six types according to the Noguchi classification: 12 tumors were classified as type A, 14 as type B, 63 as type C, 27 as type D, eight as type E, and six as type F (Table 1). The clinicopathological characteristics of all the cases are summarized in Table 1. Pleural invasion were seen in 33 cases (25.4%), vascular invasion in 57 (43.8%), and lymph node metastasis in 41 (31.5%).

Immunohistochemical detection of Ep-CAM and TROP2

Immunohistochemistry with both the Ep-CAM and TROP2 antibodies showed a positive reaction at the cell membrane (Fig. 1a, b). However, seven cases also showed cytoplasmic positivity for TROP2 (Fig. 1c). Many lepidic-type tumors (types A, B, and C) were stained strongly for Ep-CAM (66 out of 89, 74%) and TROP2 (62 out of 89, 70.0%). Ep-CAM overexpression was seen in 87 of the 130 tumors (67%), and TROP2 overexpression was also seen in 87 tumors (67%). In type C, which includes cases with both a favorable and an unfavorable outcome, Ep-CAM expression tended to be lower in the central fibrotic area (invasive area) of the tumor, in comparison with the periphery (Fig. 2b–d). Analysis of the Kaplan–Meier curves revealed that overexpression of Ep-CAM was significantly related to a favorable outcome (p=0.0185; Fig. 3a). A similar result was seen for patients with lepidic-type tumors (types A, B, and C; p=0.0014; Fig. 3b) and also in patients with type C (p=0.0029, data not shown). However, for nonlepidic-type tumors (types D, E, and F), there was no significant difference in outcome between positive cases and negative cases (Fig. 3c).

On the other hand, overexpression of TROP2 in the tumors tended to be associated with an unfavorable

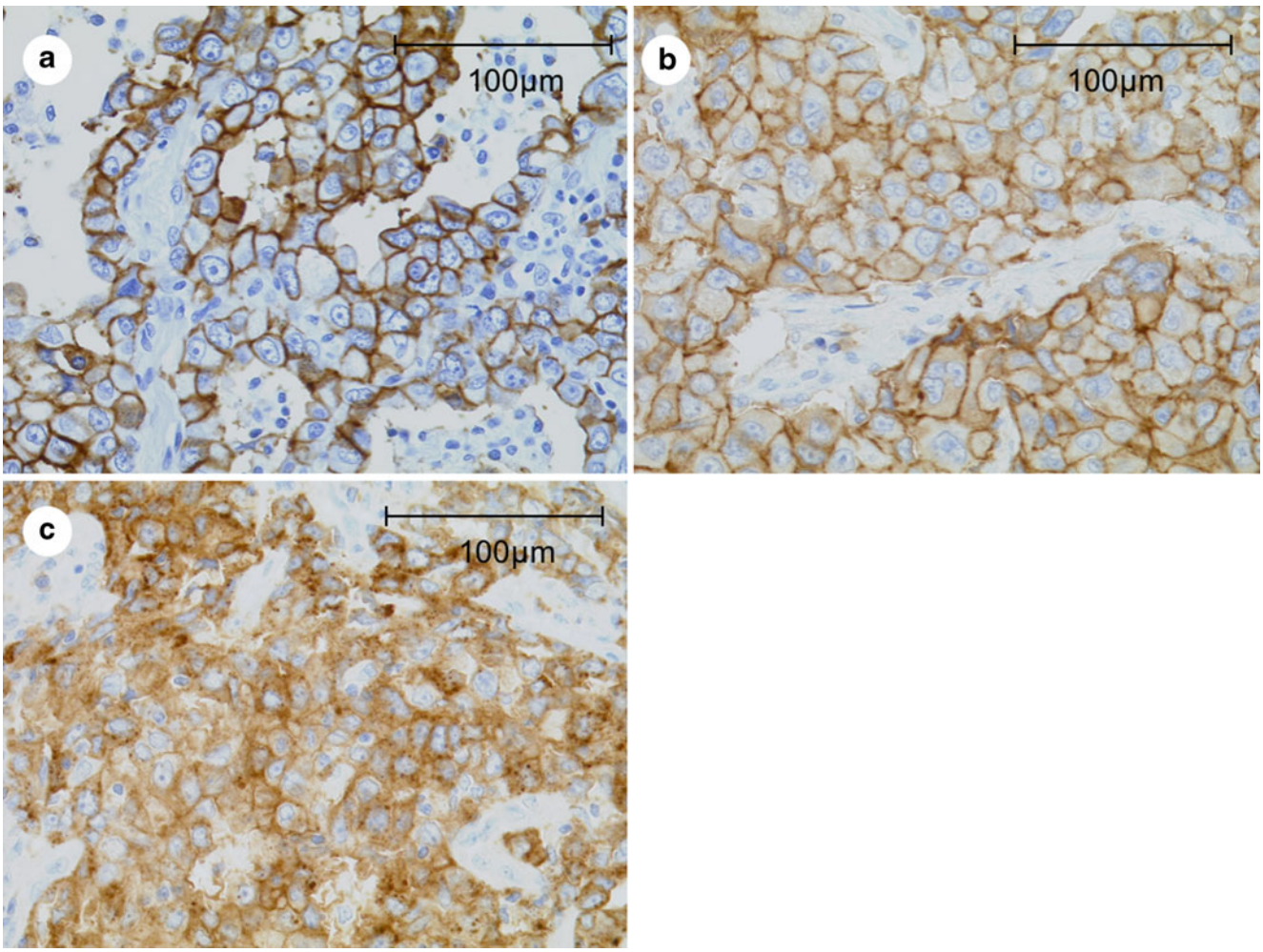

Fig. 1 Immunohistochemistry for Ep-CAM (**a**) and TROP2 (**b**, **c**). Both show a positive reaction at the cell membrane (**a**, **b**). In some cases, TROP2 also shows a positive reaction in the cytoplasm of the tumor cells (**c**). The length of the bar is 100 μm (original magnification, ×400)

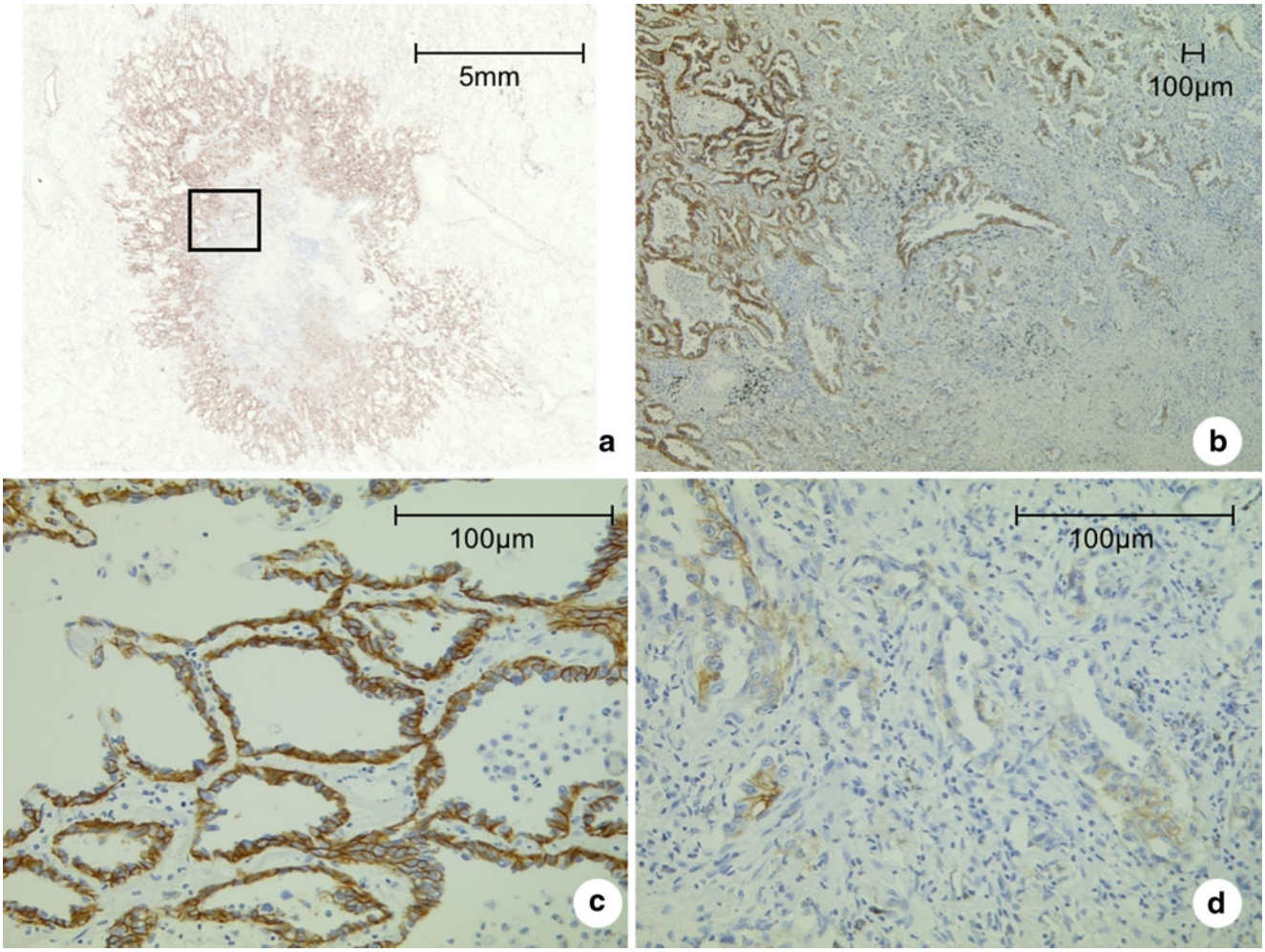

Fig. 2 Immunohistochemistry for Ep-CAM in type C adenocarcinoma. Low magnification shows a positive reaction in the marginal area (in situ lepidic growth component) of the tumor (**b**; original magnification; ×40). The expression decreased in the area showing transition from in situ lepidic growth to invasion (*boxed region*; **a**, **b**). Ep-CAM is strongly positive in the in situ lepidic growth component (**c**) but weakly positive or negative in the invasive area (**d**; original magnification; ×400)

outcome (p=0.0564; Fig. 4a). Interestingly, in nonlepidic-type tumors (types D, E, and F), TROP2 overexpression was significantly associated with an unfavorable outcome (p=0.0125; Fig. 4c). Among the seven cases positive for cytoplasmic staining, four showed a diffuse staining pattern. All of them were histologically classified as invasive lepidic-type tumor (type C), and the patients died of the disease. The other three cases showed partially positive staining for TROP2. Among them, one case was classified as invasive lepidic-type tumor (type C), and the other two cases were nonlepidic-type tumors (type D). Two of the three patients died of the disease. Therefore, in total, six of seven patients whose tumors showed cytoplasmic staining (85.7%) died of their disease.

Univariate analysis of tumor Ep-CAM and TROP2 expression in relation to clinicopathological factors revealed that Ep-CAM overexpression was correlated with the tumor growth pattern (lepidic type or nonlepidic type), lymph node metastasis, and prognosis, whereas TROP2 overexpression had no such correlation (Table 1). Multivariate analysis identified TROP2 overexpression as an independent prognostic marker (p=0.045, hazard ratio (HR) 2.884, 95% confidence interval (CI) 1.024–8.123; Table 2). Additionally, lymph node metastasis was the most independent prognostic marker (p<0.0001, HR 5.712, 95% CI 2.183–14.944).

Discussion

Many studies have addressed the relationship between Ep-CAM expression and prognosis in various cancers [10, 12–19]. For example, overexpression of Ep-CAM is reportedly correlated with an unfavorable outcome in cancers of the breast [12–15], ovary [16], gallbladder [17], and esophagus [18] and also with the presence of disseminated tumor cells

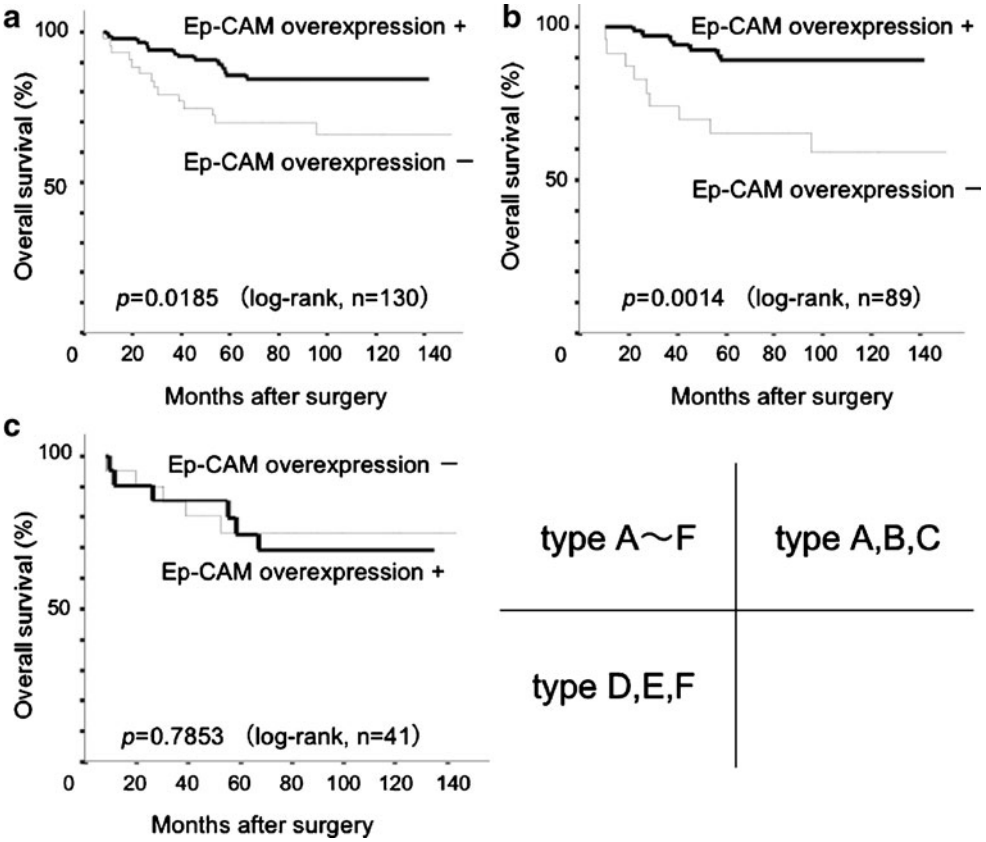

Fig. 3 Prognostic significance of Ep-CAM overexpression in 130 patients with pulmonary adenocarcinoma in relation to overall survival determined by Kaplan–Meier analysis. *Dark line*: pulmonary adenocarcinoma patients with high Ep-CAM expression. *Light line*: patients lacking, or with low Ep-CAM expression. **a** The survival curve for the total 130 patients. **b** The survival curve for patients whose tumors showed lepidic growth (types A, B, and C). **c** The survival curve for patients whose tumors showed nonlepidic growth (types D, E, and F)

in lymph nodes after complete resection of nonsmall cell lung cancer [19]. In squamous cell carcinoma of the lung, tumor expression of Ep-CAM has been reported to increase significantly with increasing involvement of regional lymph nodes and TNM stage [10]. On the other hand, it has been reported that a decrease or loss of Ep-CAM expression is correlated with an unfavorable outcome in clear cell renal carcinoma [20] and gastric cancer [21]. In the present study, Ep-CAM overexpression was correlated with a favorable outcome of pulmonary adenocarcinoma, supporting a previous study suggesting an inverse relationship between Ep-CAM overexpression and outcome of patients with squamous cell carcinoma of the lung [10].

Ep-CAM is an epithelial adhesion molecule expressed in the normal alveolar epithelium and is thought to play a role in cell–cell contact. Interestingly, Gosens et al. have shown that reduced Ep-CAM staining at the invasive margin of rectal tumor specimens was correlated significantly with tumor budding, tumor grade, and an increased risk of local recurrence [22]. They concluded that loss of membranous Ep-CAM was associated with nuclear β-catenin localization and that this contributed to reduction of cell–cell adhesions, increased migratory potential, and tumor budding. However, it is very difficult to discuss the tumor margin (budding) of lung adenocarcinoma since the direction of invasion is not easily discernible in lung cancers. Another report has indicated that adhesion induced by Ep-CAM suppresses the invasion of tumor cells in a murine model [23]. On the basis of the present findings, we hypothesize that a decrease in the expression of Ep-CAM will cause a tumor to develop an invasive nature. Suppression of Ep-CAM will lead to degradation of cell–cell attachment at the primary site and promote distant metastasis. Therefore, it is not surprising that the patients in the present series showing overexpression of Ep-CAM would have had a lower likelihood of lymph node metastasis and a more favorable outcome. The difference between our data and previous study of squamous cell carcinoma may have been due to the different histological natures of adenocarcinoma and squamous cell carcinoma. Ep-CAM was considered to be a useful prognostic marker of pulmonary adenocarcinoma and especially for identification of early invasive adenocarcinomas (type C tumors) that have an extremely favorable outcome and for which reduction surgery is warranted.

On the other hand, TROP2, like Ep-CAM, is also a member of the GA733 gene family and considered to be located on the cell membrane, but unlike Ep-CAM, its overexpression was associated with an unfavorable outcome in pulmonary adenocarcinoma. Although the number of studies is limited, overexpression of TROP2 is reportedly associated with an unfavorable outcome in colorectal

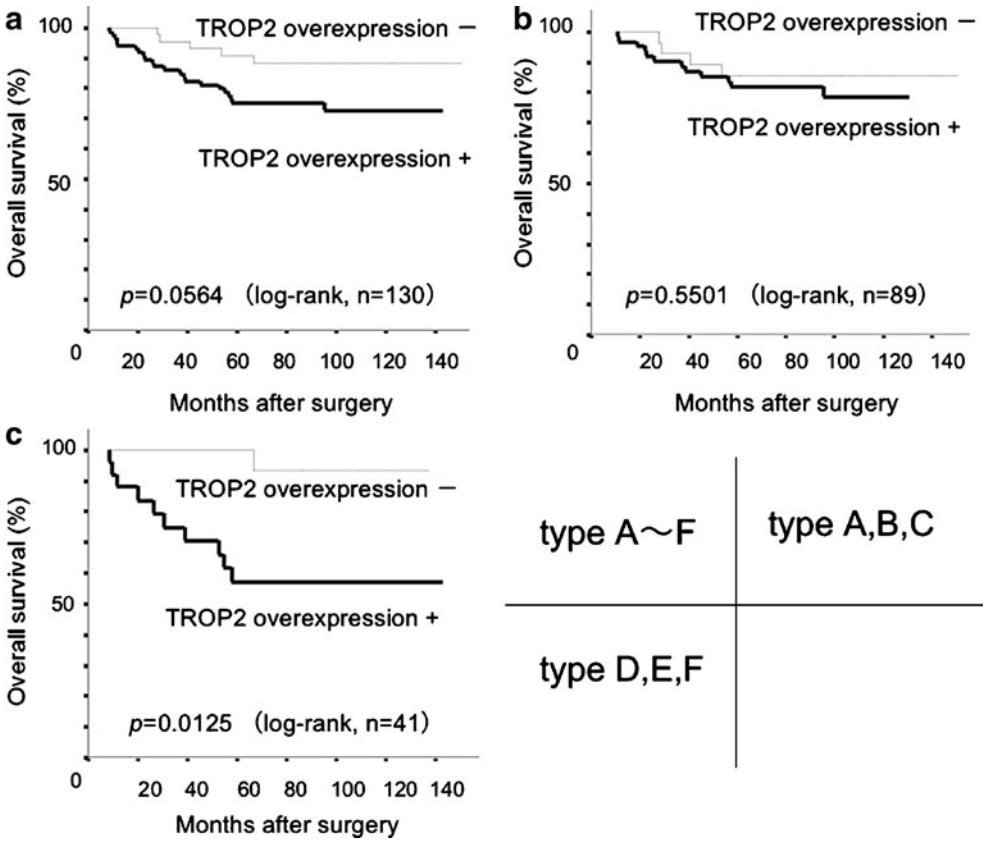

Fig. 4 Prognostic significance of TROP2 overexpression in 130 patients with pulmonary adenocarcinoma in relation to overall survival determined by Kaplan–Meier analysis. *Dark line*: pulmonary adenocarcinoma patients with high TROP2 expression. *Light line*: patients lacking, or with low TROP2 expression. **a** Survival curve for all 130 patients. **b** Survival curve for patients whose tumors showed lepidic growth (types A, B, and C). **c** Survival curve for patients whose tumors showed nonlepidic growth (types D, E, and F)

carcinoma [24], squamous cell carcinoma of the oral cavity [25], and pancreatic carcinoma [26]. Interestingly, Terrinoni et al. have indicated that *CYCLIN D1-TROP2* chimera protein in human cancer cells [27] and TROP2 may have a role in accelerating the cancer cell cycle and cell growth. As shown in Fig. 1c, expression of Ep-CAM was clearly detected at the cell membrane, whereas TROP2 was additionally expressed in the cytoplasm. Interestingly, six (85.7%) of the seven patients whose tumors showed cytoplasmic expression of TROP2 died of tumor metastasis or recurrence. Although the number of cases was limited, cytoplasmic staining of TROP2 may be associated with an unfavorable prognosis in patients with lung adenocarcinoma. The incomplete or abnormal transcription or translation of the TROP2 gene may result in cytoplasmic expression of TROP2 and correlated with its function. These findings indicate that the TROP2 gene might participate as an oncogene in pulmonary adenocarcinoma. Although Ep-CAM and TROP2 belong to the same gene family, their opposite effects in relation to the prognosis of pulmonary adenocarcinoma are intriguing. TROP2 expression was characteristically detected in nonlepidic-type advanced cancers and was correlated with tumor invasion or metastasis. Although expression of both TROP2 and Ep-CAM was shown to be associated with the outcome of lung adenocarcinoma by univariate analysis, TROP2 was the

Table 2 Multivariate analysis of survival in small adenocarcinoma

Parameter	Hazard ratio (95% CI[a])	*p* value
Gender (male vs. female)	1.488 (0.635–3.490)	0.360
Age (<60 vs. ≧60)	1.030 (0.450–2.361)	0.944
Pleural invasion (present vs. absent)	0.633 (0.257–1.556)	0.319
Vascular invasion (present vs. absent)	1.687 (0.626–4.547)	0.301
Lymph node metastasis (present vs. absent)	5.712 (2.183–14.944)	0.0001
Noguchi classification (lepidic vs. nonlepidic)	1.349 (0.549–3.312)	0.514
Ep-CAM overexpression	0.693 (0.291–1.653)	0.409
TROP2 overexpression	2.884 (1.024–8.123)	0.045

[a] Confidence interval

only independent prognostic factor indicated by multivariate analysis. It seemed that the influence of Ep-CAM expression was canceled because there was strong correlation between Ep-CAM expression and lymph node metastases. Recently, specific monoclonal antibodies against Ep-CAM (e.g., Edrecolomab) have been used for the treatment of colorectal cancer and metastatic breast cancer [28]. However, in pulmonary adenocarcinoma, it is considered that TROP2, rather than Ep-CAM, is the more powerful prognostic factor and may be a more useful therapeutic target.

Acknowledgments This work was supported in part by a Grant-in-Aid for Cancer Research (16-1) from the Ministry of Health, Labour and Welfare of Japan.

Conflict of interest statement We declare that we have no conflict of interest.

References

1. Travis WD, Brambilla E, Muller-Hermelink HK et al (2004) Pathology and genetics: tumours of the lung, pleura, thymus and heart World Health Organization classification of tumours. IARC, Lyon
2. Shimada A, Kano J, Ishiyama T et al (2005) Establishment of an immortalized cell line from a precancerous lesion of lung adenocarcinoma, and genes highly expressed in the early stages of lung adenocarcinoma development. Cancer Sci 96:668–675
3. Linnenbach AJ, Wojcierowski J, Wu S et al (1989) Sequence investigation of the major gastrointestinal tumor-associated antigen gene family, GA733. Proc Natl Acad Sci USA 86:27–31
4. Linnenbach AJ, Seng BA, Wu S et al (1993) Retroposition in a family of carcinoma-associated antigen genes. Mol Cell Biol 13:1507–1515
5. Szala S, Froehlich M, Scollon M et al (1990) Molecular cloning of cDNA for the carcinoma-associated antigen GA733-2. Proc Natl Acad Sci USA 87:3542–3546
6. Litvinov SV, Bakker HAM, Gourevitch MM et al (1994) Evidence for a role of the epithelial glycoprotein 40(Ep-CAM) in epithelial cell–cell adhesion. Cell Adhes Commun 2:417–428
7. Ripani E, Sacchetti A, Corda D et al (1998) Human Trop-2 is a tumor-associated calcium signal transducer. Int J Cancer 76:671–676
8. Tsujikawa M, Kurahashi H, Tanaka T et al (1999) Identification of the gene responsible for gelatinous drop-like corneal dystrophy. Nat Genet 21:420–423
9. Balzar M, Winter MJ, de Boer CJ et al (1999) The biology of the 17-1A antigen (Ep-CAM). J Mol Med 77:699–712
10. Piyathilake CJ, Frost AR, Weiss H et al (2000) The expression of Ep-CAM (17-1A) in squamous cell cancers of the lung. Hum Pathol 31:482–487
11. Noguchi M, Morikawa A, Kawasaki M et al (1995) Small adenocarcinoma of the lung. Histologic characteristics and prognosis. Cancer 75:2844–2852
12. Gastl G, Spizzo G, Obrist P et al (2000) Ep-CAM overexpression in breast cancer as a predictor of survival. Lancet 356:1981–1982
13. Spizzo G, Obrist P, Ensinger C et al (2002) Prognostic significance of Ep-CAM and Her-2/neu overexpression in invasive breast cancer. Int J Cancer 98:883–888
14. Spizzo G, Went P, Dirnhofer S et al (2004) High Ep-CAM expression is associated with poor prognosis in node-positive breast cancer. Breast Cancer Res Treat 86:207–213
15. Schmidt M, Hasenclever D, Schaeffer M et al (2008) Prognostic effect of epithelial cell adhesion molecule overexpression in untreated node-negative breast cancer. Clin Cancer Res 14:5849–5855
16. Spizzo G, Went P, Dirnhofer S et al (2006) Overexpression of epithelial cell adhesion molecule (Ep-CAM) is an independent prognostic marker for reduced survival of patients with epithelial ovarian cancer. Gynecol Oncol 103:483–488
17. Varga M, Obrist P, Schneeberger S et al (2004) Overexpression of epithelial cell adhesion molecule antigen in gallbladder carcinoma is an independent marker for poor survival. Clin Cancer Res 10:3131–3136
18. Stoecklein NH, Siegmund A, Scheunemann P et al (2006) Ep-CAM expression in squamous cell carcinoma of the esophagus: a potential therapeutic target and prognostic marker. BMC Cancer 6:165
19. Kubuschok B, Passlick B, Izbicki JR et al (1999) Disseminated tumor cells in lymph nodes as a determinant for survival in surgically resected non-small-cell lung cancer. J Clin Oncol 17:19–24
20. Kim HL, Seligson D, Liu X et al (2004) Using protein expressions to predict survival in clear cell renal carcinoma. Clin Cancer Res 10:5464–5471
21. Songun I, Litvinov SV, van de Velde CJ et al (2005) Loss of Ep-CAM (CO17-1A) expression predicts survival in patients with gastric cancer. Br J Cancer 92:1767–1772
22. Gosens MJ, van Kempen LC, van de Velde CJ et al (2007) Loss of membranous Ep-CAM in budding colorectal carcinoma cells. Mod Pathol 20:221–232
23. Basak S, Speicher D, Eck S et al (1998) Colorectal carcinoma invasion inhibition by CO17-1A/GA733 antigen and its murine homologue. J Natl Cancer Inst 90:691–697
24. Ohmachi T, Tanaka F, Mimori K et al (2006) Clinical significance of Trop2 expression in colorectal cancer. Clin Cancer Res 12 (10):3057–3063
25. Fong D, Spizzo G, Gostner JM et al (2008) TROP2: a novel prognostic marker in squamous cell carcinoma of the oral cavity. Mod Pathol 21:186–191
26. Fong D, Moser P, Krammel C et al (2008) High expression of TROP2 correlates with poor prognosis in pancreatic cancer. Br J Cancer 99:1290–1295
27. Terrinoni A, Dell'Arciprete R, Fornaro M et al (2001) Cyclin D1 gene contains a cryptic promoter that is functional in human cancer cells. Genes Chromosomes Cancer 31:209–220
28. Schwartzberg LS (2001) Clinical experience with edrecolomab: a monoclonal antibody therapy for colorectal carcinoma. Crit Rev Oncol Hematol 40:17–24