including ADCC ($P = 0.0005$, log-rank test) (Fig. 3A). ADCC has a better prognosis than high-grade histological subtypes of salivary gland carcinoma [21]. The correlation analysis between CNI and protein expression in ADCC (Fig. 2A, gray bars) revealed that although 85.7% of ADCCs had strong expression (+3) or moderate expression (+2) of actinin-4 protein, CNI of *ACTN4* was recognized in only one tumor. To remove the bias of the unique prognosis of ADCC, we also investigated the prognostic significance with CNI of *ACTN4* in 37 salivary gland carcinoma patients excluding ADCC. A statistically significant difference in prognosis was recognized between patients with NCN and patients with CNI ($P = 0.0112$); the overall survival of patients with CNI was worse than patients with NCN (Fig. 3C). In contrast, the actinin-4 protein expression level was not statistically correlated to overall survival in salivary gland carcinomas when including or excluding ADCC (Fig. 3B and D).

HR for death in patients with salivary gland carcinoma

We calculated the hazard ratios (HR) of some parameters, including age, gender, size, lymph node metastasis, histological grade, neural invasion, vascular invasion, actinin-4 protein expression, and CNI of *ACTN4*, for death by using univariate and multivariate Cox regression analysis.

In the patients with salivary gland carcinomas including ADCC, histological grade (HR: 4.69; 95% confidence interval [CI]: 1.50–14.61), vascular invasion (HR: 10.86; 95% CI: 3.56–33.14), and CNI of *ACTN4* (HR: 5.21; 95% CI: 1.92–14.19) remained as positive predictors by using univariate analysis, and multivariate analysis revealed that vascular invasion (HR: 7.46; 95% CI: 1.98–28.06) and CNI of *ACTN4* (HR: 3.23; 95% CI: 1.08–9.68) were independent positive predictors for death in patients with salivary gland carcinoma (Table 2).

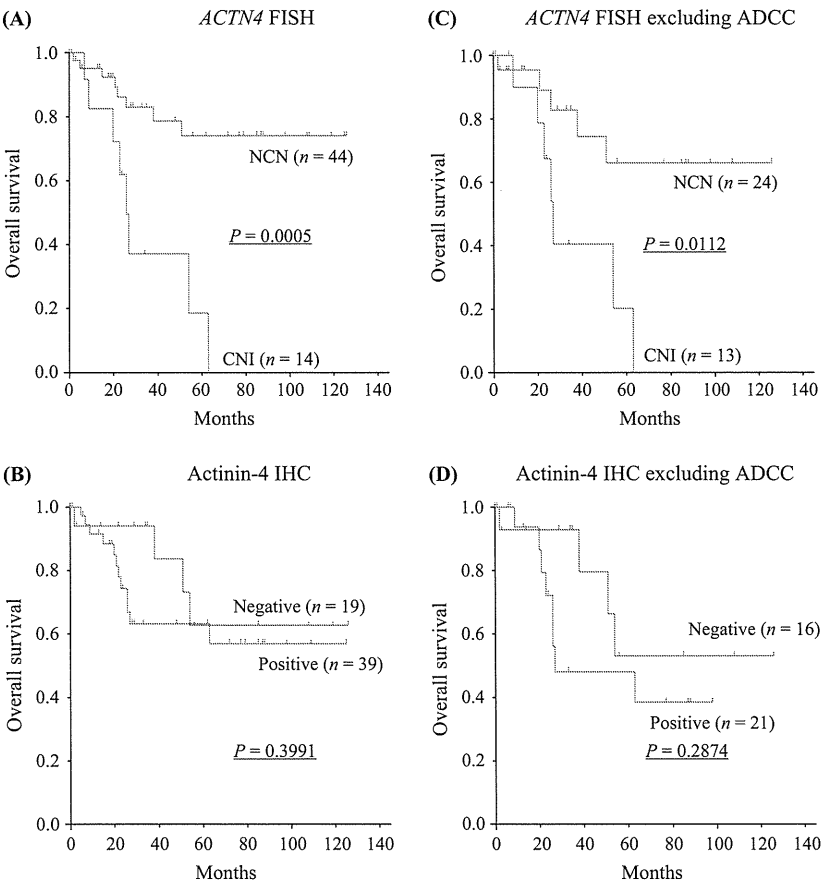

Figure 3. Overall survival curves of patients with salivary gland carcinomas, including adenoid cystic carcinoma (ADCC) (A and B) or excluding ADCC (C and D), by evaluations of fluorescence in situ hybridization (FISH) (A and C) or immunohistochemistry (IHC) (B and D). The statistical significances were recognized in evaluation of FISH between copy number increase (CNI) and normal copy number (NCN) in patients with salivary gland carcinomas including/excluding ADCC (A and C). In contrast, the statistical significance was not recognized in an evaluation of IHC in both cohorts (B and D).

Table 2. Hazard ratios for death in salivary gland cancer patients.

	Univariate analysis[1]			Multivariate analysis[1]		
Variable	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Age						
<67/≥67 years	2.69	0.93–7.78	0.067			
Gender						
Women/men	1.20	0.45–3.24	0.714			
T classification						
T1–T2/T3–T4	2.28	0.51–10.11	0.279			
Lymph node metastasis						
Absent/present	2.51	0.93–6.75	0.069			
Histological grade						
Low, intermediate/high	4.69	1.50–14.61	**0.007765**	1.32	0.31–5.45	0.701222
Neural invasion						
Absent/present	1.38	0.51–3.71	0.524			
Vascular invasion						
Absent/present	10.86	3.56–33.14	**0.000028**	7.46	1.98–28.06	**0.002958**
Actinin-4 IHC						
Negative/positive	1.64	0.53–5.10	0.394			
ACTN4 FISH						
NCN/CNI	5.21	1.92–14.19	**0.001230**	3.23	1.08–9.68	**0.035815**

HR, hazard ratio; CI, confidence interval; FISH, fluorescent in situ hybridization; IHC, immunohistochemistry; NCN, normal copy number; CNI, copy number increase.

[1]Univariate and multivariate analysis with Cox proportional hazards regression model. *P*-values of <0.05 are shown in bold.

We also calculated the HRs for death in patients with salivary gland carcinomas excluding ADCC. Univariate analysis revealed that vascular invasion (HR: 8.58; 95% CI: 2.16–34.11) and CNI of *ACTN4* (HR: 4.18; 95% CI: 1.29–13.53) were significant positive predictors for death in salivary gland carcinomas excluding ADCC. Multivariate analysis also revealed that vascular invasion and CNI of *ACTN4* were independent risk factors for both salivary gland carcinomas including and excluding ADCC (Table 3).

Discussion

The assessment of prognostic factors in salivary gland carcinoma is difficult due to its low frequency and morphological diversity [22]. Histological grading of salivary gland carcinoma is an important predictor of survival [23]. It can stratify the risk of lymph node metastases and provide information for deciding the treatment strategy, including the extent of surgery and the use of adjuvant radiotherapy [24]. In the present study, we identified a novel predictor for the prognosis of salivary gland carcinoma and found that it was significantly associated with histological grade.

Our laboratory identified the *ACTN4* gene product as an actin-bundling protein that was closely associated with cell movement and cancer invasion [3]. In a previous study, colorectal cancer cell lines in which actinin-4 was overexpressed stimulated invasive cellular protrusions and had a significantly more invasive phenotype than control cells [4]. Moreover, pancreatic and oral squamous cell carcinoma cells in which actinin-4 expression was reduced with siRNA exhibited decreased invasiveness [10, 11]. An orthotopic transplantation study of cells overexpressing actinin-4 revealed that the regional lymphatic metastasis and destructive invasion to stromal cells were significantly increased in colorectal [4] and pancreatic cancer [11].

Actinin-4 overexpression was also confirmed in solid malignant tumors that had been surgically excised, and protein expression was an unfavorable predictor of patient outcome. One cause of actinin-4 protein overexpression is *ACTN4* gene amplification. In fact, gene amplification of *ACTN4* has been detected in tumors from patients with pancreatic [11], ovarian [6, 7], and lung cancers [13], and correlations between protein expression and gene amplification have been statistically recognized in some cancers. Especially, Noro et al. reported that *ACTN4* amplification could more strictly predict poor outcome than actinin-4 protein expression in stage-I adenocarcinoma of the lung [13]. To identify the specificity of gene amplification of *ACTN4*, we previously examined the correlation of gene amplification of a gene near *ACTN4*. V-akt murine thymoma viral oncogene homolog 2 (*AKT2*) is located on 19q13, and it is near *ACTN4*. The distance between *ACTN4* and *AKT2* is 1.6 Mbp. We previously reported that coamplification of

Table 3. Hazard ratios for death in salivary gland cancer patients excluding ADCC.

	Univariate analysis[1]			Multivariate analysis[1]		
Variable	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Age						
<67/≥67 years	2.52	0.68–9.41	0.1688			
Gender						
Women/men	2.10	0.57–7.82	0.2670			
T classification						
T1–T2/T3–T4	1.68	0.36–7.81	0.5075			
Lymph node metastasis						
Absent/present	1.55	0.50–4.83	0.4483			
Histological grade						
Low, intermediate/high	2.81	0.76–10.44	0.1228			
Neural invasion						
Absent/present	1.50	0.47–4.72	0.4932			
Vascular invasion						
Absent/present	8.58	2.16–34.11	**0.0023**	9.00	2.15–37.61	**0.0026**
Actinin-4 IHC						
Negative/positive	1.91	0.57–6.43	0.2912			
ACTN4 FISH						
NCN/CNI	4.18	1.29–13.53	**0.0168**	4.35	1.28–14.87	**0.0187**

ADCC, adenoid cystic carcinoma; HR, hazard ratio; CI, confidence interval; FISH, fluorescent in situ hybridization; IHC, immunohistochemistry; NCN, normal copy number; CNI, copy number increase.

[1]Univariate and multivariate analysis with Cox proportional hazards regression model. *P*-values of <0.05 are shown in bold.

AKT2 and *ACTN4* did not necessarily accord for invasion of pancreatic cancer [5, 11]. The present study is the first report that CNI of *ACTN4*, including gene amplification and high polysomy of *ACTN4*, was significantly correlated to histological grade and vascular invasion in salivary gland carcinoma. CNI of *ACTN4* was recognized at a frequency greater than 20% in OC (1/1, 100%), MYC (1/1, 100%), SC (2/3, 66.7%), SDC (2/4, 50.0%), EMYC (1/3, 33.3%), ACNOS (2/7, 28.6%), CAEPA (3/11, 27.3%), and MEC (1/4, 25.0%). However, CNI of *ACTN4* was not found in patients with acinic cell carcinoma (ACCC) (0/3, 0%) and was rarely observed in patients with ADCC (1/21, 4.8%). The survival time of the patient with CNI of *ACTN4* in ADCC was 7 months from the first treatment. Despite the rare frequency of CNI of *ACTN4* in ADCC, overexpression of actinin-4 protein was recognized in 85.7% of patients with ADCC. ADCC has several cellular components constructed by ductal epithelial, myoepithelial, and basement cells, and protein expression of actinin-4 is particularly recognized in myoepithelial cells of normal salivary glands. Therefore, it was considered that the protein expression of actinin-4 was dependent on the histological phenotype; however, this was not associated with the genetic alteration that was dependent on malignant change.

-ADCC was different from other subtypes of salivary gland carcinomas, we investigated the correlation between CNI and protein expression of actinin-4 by using 37 patients with salivary gland carcinomas excluding ADCC. Significant correlations were recognized between increased copy numbers of *ACTN4* and protein expression levels of actinin-4. This data suggests that the overexpression of actinin-4 protein was stimulated by CNI of *ACTN4*. Cox regression univariate analysis revealed that histological grade, vascular invasion, and CNI of *ACTN4* were risk factors for cancer death in salivary gland carcinoma patients with or without ADCC. The HR of CNI of *ACTN4* for death was higher than the HR for the histological grade. In addition, although multivariate analysis revealed that CNI of *ACTN4* and vascular invasion were independent risk factors for tumor death, histological grade did not remain as an independent risk factor. These results suggest that CNI of *ACTN4* may have a greater impact than histological grade on patient death. Although CNI of *ACTN4* was significantly correlated with histological grade and vascular invasion, the protein expression of actinin-4 was not associated with any clinical factors in salivary gland carcinoma. In addition, protein expression could not predict an unfavorable outcome in patients with salivary gland carcinoma. Although CNI of *ACTN4* was dominantly recognized in salivary gland carcinoma with invasive phenotypes, it was recognized in only one of 21 cases with ADCC; therefore, we considered the possibility that, due to the overexpression of actinin-4 that was frequently observed in ADCC patients, protein expression is not correlated to an unfavorable prognosis in salivary gland carcinoma. In contrast,

protein expression of actinin-4 was recognized in 18 cases with ADCC. An explanation for this observation involved the discrepancy between CNI and protein expression of actinin-4 in ADCC. We analyzed the correlation between overall survival and protein expression of actinin-4 in patients excluding ADCC; although statistical significance was not recognized, it seemed that overall survival in the positive staining group had a poorer prognosis than in the negative staining group. To probe the statistical significance of protein expression of actinin-4, we considered that the power of statistical hypothesis testing was not enough. More non-ADCC salivary gland carcinoma samples are needed to prove a significant correlation between overall survival and protein expression. Moreover, although CNI can quantitatively evaluate the copy numbers of the *ACTN4* gene, our evaluation system for protein expression of actinin-4 cannot quantitatively classify the cases with protein expression of actinin-4. Therefore, we considered that CNI of *ACTN4* more strictly predicted the vascular invasion of cancer cells and poor prognosis than protein expression of actinin-4. Similar observations have also been recognized in ovarian cancer. We previously reported that the gene amplification of *ACTN4* could predict the prognosis of patients with advanced stage ovarian cancer more accurately than protein expression of actinin-4 [6].

Ettl et al. reported the occurrence of genomic aberrations of the tyrosine kinase receptors *EGFR*, human epidermal growth factor receptor 2 (*HER2*), and hepatocyte growth factor (*MET*) as well as phosphatase and tensin homolog on chromosome 10 (*PTEN*) in different subtypes of salivary gland carcinomas [25, 26], which have a strong impact on overall survival [27]. In addition, they also reported that the metastasis of cervical lymph nodes also correlated with copy number gain of *EGFR* and *HER2*, aberration of *MET*, and *PTEN* [20]. Moreover some translocation and fusion genes are found frequently in MEC t(11;19) (CRYC1-MAML2) [28] or ADCC t(6;9) (MYB-NFIB) [29] and have a prognostic impact. These genetic alterations are considered as a driver for malignant phenotype, and molecular-targeted therapy has gained attention as a new therapeutic strategy for salivary gland carcinomas. In fact, clinical trials with some inhibitors or antibodies for molecular targets, such as gefitinib (a small-molecule *EGFR* inhibitor), cetuximab (an anti-EGFR antibody), and trastuzumab (an anti-HER2 antibody), were performed for patients with salivary gland carcinomas. Although the results of phase II clinical trials of gefitinib, cetuximab, and trastuzumab have been reported [30, 31], standard molecular-targeted therapy has not yet been established for salivary gland carcinoma.

ACTN4 is located on chromosome 19q13.1 [11]. Genetic alterations of 19q13.1 and *ACTN4* have not yet been reported in salivary gland carcinoma. Although a large-scale prospective study to prove the clinical significance for *ACTN4* is necessary, we conclude that *ACTN4* is a surrogate biomarker for predicting prognosis to support histological grading in salivary gland carcinoma and that the inhibition of the biological function of *ACTN4* may impact a new therapeutic strategy for high-grade salivary gland carcinoma.

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Conflict of Interest

None declared.

References

1. Speight, P. M., and A. W. Barrett. 2002. Salivary gland tumours. Oral Dis. 8:229–240.
2. Terhaard, C. H., H. Lubsen, I. Van der Tweel, F. J. Hilgers, W. M. Eijkenboom, H. A. Marres, et al. 2004. Salivary gland carcinoma: independent prognostic factors for locoregional control, distant metastases, and overall survival: results of the Dutch head and neck oncology cooperative group. Head Neck 26:681–692; discussion 692-683.
3. Honda, K., T. Yamada, R. Endo, Y. Ino, M. Gotoh, H. Tsuda, et al. 1998. Actinin-4, a novel actin-bundling protein associated with cell motility and cancer invasion. J. Cell Biol. 140:1383–1393.
4. Honda, K., T. Yamada, Y. Hayashida, M. Idogawa, S. Sato, F. Hasegawa, et al. 2005. Actinin-4 increases cell motility and promotes lymph node metastasis of colorectal cancer. Gastroenterology 128:51–62.
5. Hayashida, Y., K. Honda, M. Idogawa, Y. Ino, M. Ono, A. Tsuchida, et al. 2005. E-cadherin regulates the association between beta-catenin and actinin-4. Cancer Res. 65:8836–8845.
6. Yamamoto, S., H. Tsuda, K. Honda, K. Onozato, M. Takano, S. Tamai, et al. 2009. Actinin-4 gene amplification in ovarian cancer: a candidate oncogene associated with poor patient prognosis and tumor chemoresistance. Mod. Pathol. 22:499–507.
7. Yamamoto, S., H. Tsuda, K. Honda, M. Takano, S. Tamai, I. Imoto, et al. 2012. ACTN4 gene amplification and actinin-4 protein overexpression drive tumour development and histological progression in a high-grade

subset of ovarian clear-cell adenocarcinomas. Histopathology 60:1073–1083.
8. Yoshii, H., K. Ito, T. Asano, A. Horiguchi, and M. Hayakawa. 2013. Increased expression of alpha-actinin-4 is associated with unfavorable pathological features and invasiveness of bladder cancer. Oncol. Rep. 30:1073–1080.
9. Koizumi, T., H. Nakatsuji, T. Fukawa, S. Avirmed, T. Fukumori, M. Takahashi, et al. 2010. The role of actinin-4 in bladder cancer invasion. Urology 75:357–364.
10. Yamada, S., S. Yanamoto, H. Yoshida, I. Yoshitomi, G. Kawasaki, A. Mizuno, et al. 2010. RNAi-mediated down-regulation of alpha-actinin-4 decreases invasion potential in oral squamous cell carcinoma. Int. J. Oral Maxillofac. Surg. 39:61–67.
11. Kikuchi, S., K. Honda, H. Tsuda, N. Hiraoka, I. Imoto, T. Kosuge, et al. 2008. Expression and gene amplification of actinin-4 in invasive ductal carcinoma of the pancreas. Clin. Cancer Res. 14:5348–5356.
12. Welsch, T., S. Keleg, F. Bergmann, S. Bauer, U. Hinz, and J. Schmidt. 2009. Actinin-4 expression in primary and metastasized pancreatic ductal adenocarcinoma. Pancreas 38:968–976.
13. Noro, R., K. Honda, K. Tsuta, G. Ishii, A. M. Maeshima, N. Miura, et al. 2013. Distinct outcome of stage I lung adenocarcinoma with ACTN4 cell motility gene amplification. Ann. Oncol. 24:2594–2600.
14. Miyanaga, A., K. Honda, K. Tsuta, M. Masuda, U. Yamaguchi, G. Fujii, et al. 2013. Diagnostic and prognostic significance of the alternatively spliced ACTN4 variant in high-grade neuroendocrine pulmonary tumours. Ann. Oncol. 24:84–90.
15. Honda, K., T. Yamada, M. Seike, Y. Hayashida, M. Idogawa, T. Kondo, et al. 2004. Alternative splice variant of actinin-4 in small cell lung cancer. Oncogene 23:5257–5262.
16. Santarius, T., J. Shipley, D. Brewer, M. R. Stratton, and C. S. Cooper. 2010. A census of amplified and overexpressed human cancer genes. Nat. Rev. Cancer 10:59–64.
17. Jouzdani, E., J. Yachouh, V. Costes, J. L. Faillie, C. Cartier, F. Poizat, et al. 2010. Prognostic value of a three-grade classification in primary epithelial parotid carcinoma: result of a histological review from a 20-year experience of total parotidectomy with neck dissection in a single institution. Eur. J. Cancer 46:323–331.
18. Ohtomo, R., T. Mori, S. Shibata, K. Tsuta, A. M. Maeshima, C. Akazawa, et al. 2013. SOX10 is a novel marker of acinus and intercalated duct differentiation in salivary gland tumors: a clue to the histogenesis for tumor diagnosis. Mod. Pathol. 26:1041–1050.
19. Cappuzzo, F., C. Ligorio, L. Toschi, E. Rossi, R. Trisolini, D. Paioli, et al. 2007. EGFR and HER2 gene copy number and response to first-line chemotherapy in patients with advanced non-small cell lung cancer (NSCLC). J. Thorac. Oncol. 2:423–429.
20. Ettl, T., M. Gosau, G. Brockhoff, S. Schwarz-Furlan, A. Agaimy, T. E. Reichert, et al. 2013. Predictors of cervical lymph node metastasis in salivary gland cancer. Head Neck. doi: 10.1002/hed.23332.
21. Seethala, R. R. 2011. Histologic grading and prognostic biomarkers in salivary gland carcinomas. Adv. Anat. Pathol. 18:29–45.
22. Cheuk, W., and J. K. Chan. 2007. Advances in salivary gland pathology. Histopathology 51:1–20.
23. Seethala, R. R., K. Cieply, E. L. Barnes, and S. Dacic. 2011. Progressive genetic alterations of adenoid cystic carcinoma with high-grade transformation. Arch. Pathol. Lab. Med. 135:123–130.
24. Seethala, R. R. 2009. An update on grading of salivary gland carcinomas. Head Neck Pathol. 3:69–77.
25. Ettl, T., S. Schwarz, N. Kleinsasser, A. Hartmann, T. E. Reichert, and O. Driemel. 2008. Overexpression of EGFR and absence of C-KIT expression correlate with poor prognosis in salivary gland carcinomas. Histopathology 53:567–577.
26. Ettl, T., C. Stiegler, K. Zeitler, A. Agaimy, J. Zenk, T. E. Reichert, et al. 2012. EGFR, HER2, survivin, and loss of pSTAT3 characterize high-grade malignancy in salivary gland cancer with impact on prognosis. Hum. Pathol. 43:921–931.
27. Ach, T., K. Zeitler, S. Schwarz-Furlan, K. Baader, A. Agaimy, C. Rohrmeier, et al. 2013. Aberrations of MET are associated with copy number gain of EGFR and loss of PTEN and predict poor outcome in patients with salivary gland cancer. Virchows Arch. 462:65–72.
28. Tonon, G., S. Modi, L. Wu, A. Kubo, A. B. Coxon, T. Komiya, et al. 2003. t(11;19)(q21;p13) translocation in mucoepidermoid carcinoma creates a novel fusion product that disrupts a Notch signaling pathway. Nat. Genet. 33:208–213.
29. West, R. B., C. Kong, N. Clarke, T. Gilks, J. S. Lipsick, H. Cao, et al. 2011. MYB expression and translocation in adenoid cystic carcinomas and other salivary gland tumors with clinicopathologic correlation. Am. J. Surg. Pathol. 35:92–99.
30. Milano, A., F. Longo, M. Basile, R. V. Iaffaioli, and F. Caponigro. 2007. Recent advances in the treatment of salivary gland cancers: emphasis on molecular targeted therapy. Oral Oncol. 43:729–734.
31. Haddad, R., A. D. Colevas, J. F. Krane, D. Cooper, B. Glisson, P. C. Amrein, et al. 2003. Herceptin in patients with advanced or metastatic salivary gland carcinomas. A phase II study. Oral Oncol. 39:724–727.

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Histological Growth Pattern of and Alpha-actinin-4 Expression in Thyroid Cancer

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Abstract. *Aim: To assess the clinicopathological significance of the histological growth pattern (HGP) and α-actinin-4 (ACTN4) expression in thyroid cancer. Patients and Methods: We classified 83 thyroid cancer cases into infiltrative margin (IM) and pushing margin (PM) groups according to peripheral tumor margin contour and immunohistochemically determined ACTN4 expression. Correlations between clinical stage and clinicopathological characteristics were analyzed. Results: IM and high ACTN4 expression were observed in 39% and 49% of cancer cases, respectively. Higher clinical stage was significantly correlated with older age, higher T and N factor, preoperative recurrent laryngeal nerve paralysis (pre-RLNP), IM, and poor prognosis. Patients with stage IV disease had significantly poorer prognosis than those with stages I–III. On multivariate analysis, older age, pre-RLNP, and IM correlated with higher clinical stages. IM was significantly correlated with high ACTN4 expression. Conclusion: IM, pre-RLNP, and ACTN4 expression could be novel indicators of tumor aggression and prognostic factors of thyroid cancer.*

It is estimated that in 2008 more than 210,000 new cases of thyroid cancer were diagnosed and that approximately 35,000 patients died from thyroid cancer worldwide (1). Most thyroid cancers present with an indolent clinical course, but we sometimes encounter clinically aggressive cases that present with local recurrences or distant metastases regardless of histological type (2). These cases often require combined modality therapies and can be associated with poor outcomes.

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Key Words: Thyroid, infiltrative margin, pushing margin, alpha-actinin-4, preoperative recurrent laryngeal nerve paralysis.

Although ultrasonography, computed tomography (CT), magnetic resonance imaging, positron emission tomography/CT, fine needle aspiration cytology, or core needle biopsy are used in the diagnoses of thyroid tumors, the definitive diagnostic procedures for these tumors depend greatly on the histopathological diagnoses of the resected surgical specimens (3, 4). However, it remains difficult to evaluate the degree of malignancy to predict local recurrence or distant metastases by using histopathological analyses alone except in rare and highly malignant types (*e.g.* undifferentiated carcinomas).

The histological growth pattern (HGP) of the peripheral tumor margin is approved worldwide as one of the predictive indicators of prognoses in various malignancies (5-8). The tumor margins were mainly categorized into pushing margin (PM) and infiltrative margin (IM) groups, and a relationship between HGP and tumor aggression or lymph node metastasis in papillary thyroid cancer (PTC) was suggested (9, 10).

Alpha-actinins (ACTNs) are members of the superfamily of actin-binding proteins that cross-link actin filaments to give cells their shape (11). ACTN4 is an isoform of non-muscular ACTN, and ACTN4 overexpression has been reported to occur frequently in human epithelial cancers of various origins, such as of the breast, ovaries, pancreas, and the oral cavity (12-15). ACTN4 overexpression has been reported to be a prognostic factor in breast and ovarian cancers that correlated with lymph node metastasis in colorectal cancer (12, 13, 16). These reports suggest that a high-level ACTN4 expression is related to malignancy grade, lymph node metastasis, and patient outcome. Therefore, ACTN4 appears to be a useful molecular prognostic marker in various types of cancers (13-15, 17-19). However, no studies have investigated ACTN4 expression in thyroid cancer.

In the present study, we focused on studying the correlation between clinical stage and histopathological parameters including HGP and ACTN4 expression and evaluated their clinical significance in thyroid cancer.

Table I. *Criteria for categorizing ACTN4 expression in thyroid cancer.*

Category	Immunohistochemical findings
No expression	No immunoreaction or immunoreaction of any intensity in <10% of tumor cells
Low expression	Immunoreaction with much lower intensity than vascular endothelial cells in ≥10% of tumor cells
Moderate expression	Immunoreaction with intensity equal to or slightly lower than vascular endothelial cells in ≥10% of tumor cells
High expression	Immunoreaction with stronger intensity than vascular endothelial cells in ≥10% of tumor cells

ACTN4, α-actinin-4.

Patients and Methods

We reviewed the clinicopathological records of 83 patients who underwent initial surgical treatments for primary thyroid cancer between 1991 and 2007 at the National Defense Medical College Hospital of Japan. Information regarding the following was obtained from the patients' medical records: age; gender; Tumor, Node, and Metastasis (T, N, and M) factors; clinical stage; presence or absence of preoperative recurrent laryngeal nerve paralysis (pre-RLNP) or tracheal/prevertebral invasion; histopathological diagnosis; and prognosis. TNM classification and clinical staging of each thyroid cancer were performed according to the Union for International Cancer Control-2002 (sixth edition).

Two observers (NT and SY) reviewed all of the hematoxylin-eosin–stained slides and classified them into two HGP groups. If the extended thyroid cancer tissue displaced the surrounding tissue in a pushing manner and the cancer cells had invaded <10% of the tissue surrounding the peripheral margin of the tumor nodule, it was classified into the PM group. If the peripheral margin was poorly demarcated and the cancer cells had invaded ≥10% of the tissue surrounding the peripheral margin of the tumor nodule, it was classified into the IM group. Representative images of the PM and IM groups are shown in Figure 1.

The primary antibody used for immunohistochemistry was an anti-ACTN4 rabbit polyclonal antibody (Ab-2) raised against a synthetic peptide, as described by Honda *et al.* (16). Immunohistochemistry was performed on 4-μm thick tumor sections from representative formalin-fixed and paraffin-embedded tissue blocks. Antigen retrieval was accomplished by using a 10-mM citrate buffer (pH 6.0) and by heating of the samples in an autoclave for 10 min at a controlled final temperature of 120°C. After the endogenous peroxidase activity was blocked by incubating the slides in 3% hydrogen peroxide (v/v) in methanol for 5 min, non-specific binding was further blocked with 2% normal swine serum (v/v; Dako, Carpinteria, CA, USA) in phosphate-buffered saline for 10 min. The slides were incubated with the primary antibody overnight at 4°C and then with the secondary antibody and peroxidase with EnVision (Dako) for 1 h. Specific antigen-antibody reactions were visualized by using 3,3'-diaminobenzidine tetrahydrochloride. The nuclei were counterstained with Mayer's hematoxylin.

ACTN4 expression was classified into four categories according to immunoreaction intensity (Table I). If ≥10% of the tumor cells were stained, the tumor was judged as positive, whereas if <10% of the tumor cells were stained, the tumor was judged to have no expression. If the staining intensity of the tumor cells was stronger or much stronger than that of the vascular endothelial cells, the tumor was classified as having high expression. Conversely, if the staining intensity of the tumor cells was similar to or a little lower than that of the vascular endothelial cells, the tumor was classified as having moderate expression. Finally, if the staining intensity of the tumor cells was much lower than that of the vascular endothelial cells or only slightly evident, the tumor was classified as having low expression. Representative images of ACTN4 expression are shown in Figure 2.

Statistical analyses were performed using the JMP 10.0.0 software (SAS Institute Inc., Cary, NC, USA). To analyze the relationships between parameters, Pearson's chi square test or Fisher's exact test (two-tailed) was used. Factors with p-values <0.1 on univariate analysis were tested by using logistic regression analysis. The disease-free survival rates were calculated according to the Kaplan-Meier method, while a log-rank test was performed to examine the univariate associations among groups. Statistically significant differences were considered when $p<0.05$.

Results

The clinicopathological profiles of 83 patients are presented in Table II. The mean age was 53.7 (range, 19-84), and the ratio of males to females was approximately 1:3. T3 (35 cases; 42%) was the most common T factor, while N1 and M1 occupied 59% (49 cases) and 5% (4 cases), respectively. Stage IVa (31 cases; 37%) accounted for more than one third of all cases, while 11 cases (13%) had findings of pre-RLNP. A total of 71 cases (86%) were histologically classified as PTC. IM and high ACTN4 expression were noted in 32 (39%) and 41 (49%) cases, respectively. Sixteen (19%) patients experienced residual or recurrent disease or died of the thyroid cancer (19%). The follow-up period was 0.5-16 years (median=5.5 years).

Out of the 83 patients, 72 underwent thyroidectomy with regional lymph node dissection, 10 underwent thyroidectomy without lymph node dissection, and one underwent open biopsy-only.

The correlation between clinicopathological findings and clinical stage in 78 thyroid cancers is shown in Table III. Unfortunately, clinical staging of the remaining 5 cases could not be identified from the medical records. Patients with stage IV disease were more frequently identified with T4 and N1 than T1-T3 ($p<0.0001$) or N0 ($p=0.0005$) staging, and more frequently had pre-RLNP ($p=0.0019$). Significantly more patients in the IM group than those in the PM group had stage IV disease ($p=0.0019$), and the prognoses of patients with stage IV than those with stages I-III disease were significantly

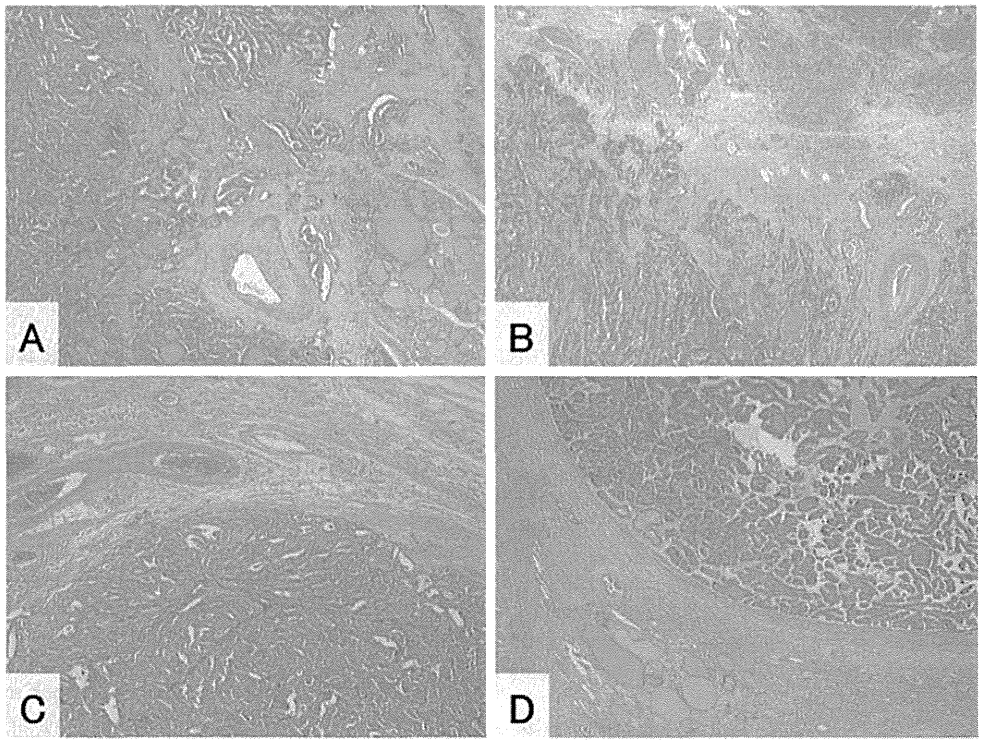

Figure 1. *Representative images of thyroid cancer cases with infiltrative margins (A and B) or pushing margins (C and D). H$E staining, (original magnification, ×40).*

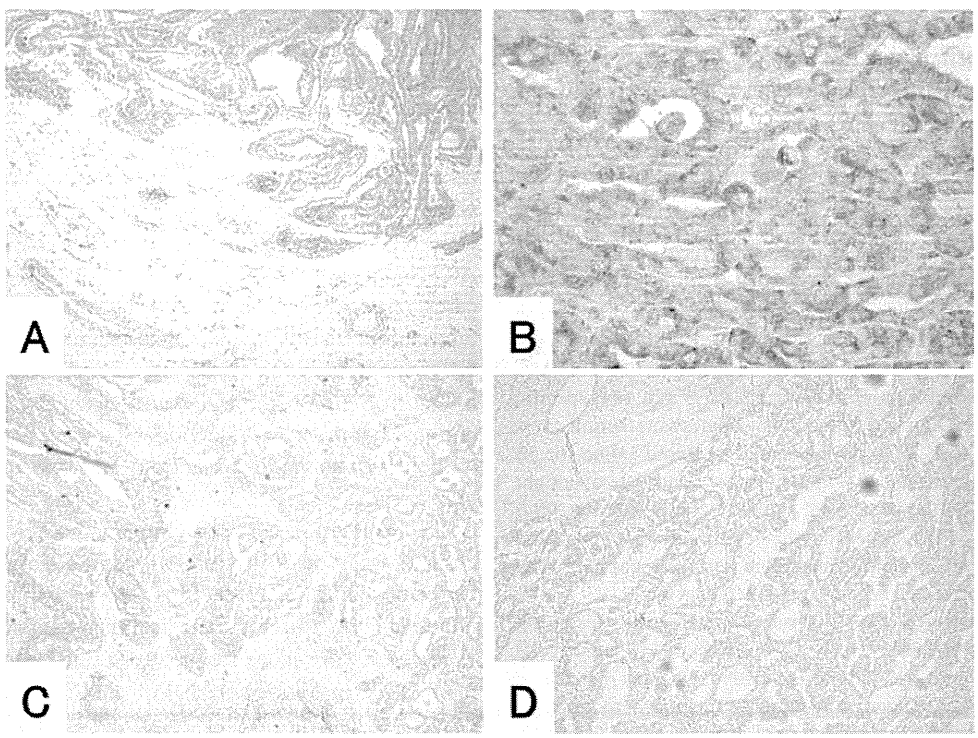

Figure 2. *Representative images of α-actinin-4 (ACTN4) immunohistochemistry of thyroid cancer. A, B: Immunostaining for high ACTN4 expression. C: Immunostaining for low ACTN4 expression. D: Immunostaining for no ACTN4 expression. Immunoperoxidase staining: ×100 magnification in A, C, and D; ×200 in B.*

Table II. *Clinicopathological data of 83 thyroid cancer cases.*

		Cases	(%)
Age (years)	<55	40	(48)
	55≤	43	(52)
Gender	Male	22	(27)
	Female	61	(73)
T	1	5	(6)
	2	13	(16)
	3	35	(42)
	4a	23	(28)
	4b	3	(4)
	Unknown	4	(5)
N	0	30	(36)
	1	49	(59)
	Unknown	4	(5)
M	0	79	(95)
	1	4	(5)
Clinical stage	I	23	(28)
	II	4	(5)
	III	16	(19)
	IVa	31	(37)
	IVb	1	(1)
	IVc	3	(4)
	Unknown	5	(6)
Pre-RLNP	–	67	(81)
	+	11	(13)
	Unknown	5	(6)
Tracheal/prevertebral invasion	–	70	(84)
	+	8	(10)
	Unknown	5	(6)
Histological type	PTC	71	(86)
	FTC	10	(12)
	ATC	1	(1)
	MTC	1	(1)
HGP	PM	51	(61)
	IM	32	(39)
ACTN4 expression	No	7	(8)
	Low	12	(14)
	Moderate	23	(28)
	High	41	(49)
Residual/recurrence/death	–	65	(78)
	+	16	(19)
	Unknown	2	(2)

Pre-RLNP, preoperative recurrent laryngeal nerve paralysis; PTC, papillary thyroid cancer; FTC, follicular thyroid cancer; ATC, anaplastic thyroid cancer; MTC, medullary thyroid cancer; HGP, histological growth pattern; PM, pushing margin; IM, infiltrative margin; ACTN4, α-actinin-4.

poorer (p=0.0102). Stage IV PTC tended to present less frequently than stage IV non-PTC, which included follicular, anaplastic, and medullary thyroid cancers (p=0.0991).

Figure 3 shows a Kaplan-Meier disease-free survival analysis demonstrating that patients with stage IV disease had poorer prognosis than those with stage I–III disease by using log-rank test (p=0.0342; HR 0.2354; 95% CI, 0.0617-0.8980).

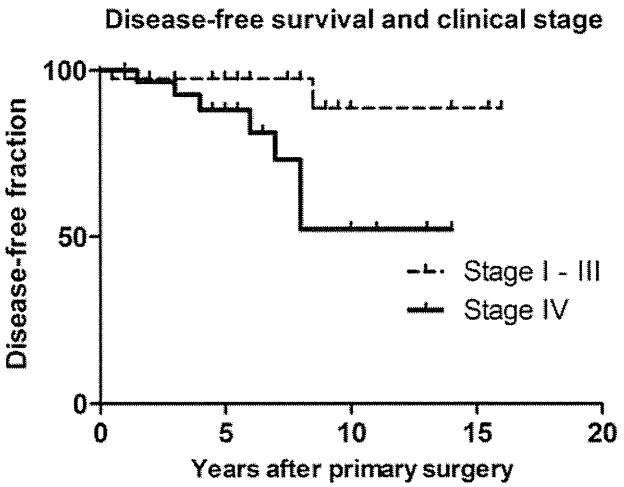

Figure 3. *Kaplan-Meier curve of disease-free survival by clinical stage indicating poorer prognosis in patients with stage IV disease than in patients with stage I-III disease.*

To identify the independent factors that influenced clinical stage in thyroid cancer, a multivariate analysis was conducted (Table III). T and N factors were excluded to avoid multivariate analysis instability and the residual/recurrence/death parameter was omitted as well since the parameter itself was the outcome. Age (p=0.0065), pre-RLNP (p=0.0141), and HGP (p=0.0132) were independent factors that correlated with the clinical stages of thyroid cancers.

Table IV shows that significantly more ACTN4 is expressed in thyroid cancers in the IM group than in those in the PM group (p=0.0052). Furthermore, higher ACTN4 expression was observed in the peripheral margin of the tumor nodule.

Discussion

The findings of the present study indicate a significant correlation between clinical stage and age, pre-RLNP, and HGP. Additionally, HGP was significantly related with ACTN4 expression.

ACTN is a protein that weighs approximately 100 kDa and is expressed in four isoforms in mammals (ACTN1, ACTN2, ACTN3, ACTN4) (20). The overall structure of ACTN is similar to that of a dumbbell due to dimer formation at the center of the rod domain that makes actins cross-link or form bundles with the actin-binding domain located at either end (21). ACTN presumably plays an important role in binding the actin cytoskeleton to the cell membrane by combining with the integrin β chain (20). Through these mechanisms, ACTN is involved in cellular structural maintenance.

Table III. *Clinical features of clinical stage and a multivariate regression model of clinicopathological parameters that influence the clinical stage in 78 cases of thyroid cancer (5 cases of which clinical stages are unknown are excluded).*

	Clinical Stage (78)		Univariate	Multivaliate regression		
	I-III (48)	IV (35)	*p*-Value	Odds ratio	95% CI	*p*-Value
Age (years)						
<55	26	10	* 0.0050	0.219	0.0630-0.6634	* 0.0065
55≤	17	25				
Gender						
male	10	12	0.2816			
female	33	23				
T stage						
1-3	39	13	* <0.0001			
4	4	22				
N stage						
0	24	6	* 0.0005			
1	19	29				
M stage						
0	42	32	0.3205			
1	1	3				
Pre-RLNP						
–	41	25	* 0.0019	0.064	0.0023-0.6089	* 0.0141
+	1	10				
(Unknown	1	0)				
Histological type						
PTC	34	33	0.0991	0.251	0.0315-1.2313	0.0916
Non-PTC	9	2				
Tracheal/prevertebral invasion						
–	40	29	0.1314			
+	2	6				
(Unknown	1	0)				
HGP						
PM	34	15	* 0.0019	0.228	0.0618-0.7390	* 0.0132
IM	9	20				
ACTN4 expression						
No–Moderate	24	16	0.3748			
High	19	19				
Residual/recurrence/death						
–	39	23	* 0.0102			
+	2	7				

CI, Confidence interval; pre-RLNP, preoperative recurrent laryngeal nerve paralysis; PTC, papillary thyroid cancer; HGP, histological growth pattern; PM, pushing margin; IM, infiltrative margin; ACTN4, α-actinin-4. Non-PTC group in histological type includes follicular, anaplastic, and medullary thyroid cancers. *Statistically significant.

ACTN4, which was identified in 1998, is an actin-binding protein that comprises 884 amino acids and is found in non-muscular cells, as is ACTN1 (a known microfilament protein) (12). Although these isoforms share 86.7% homology at the amino acid level, their intracellular localizations differ (12). ACTN4 is widely distributed in the adjacent areas of the actin fibers, cytoplasm, or nucleus, whereas ACTN1 distribution is restricted to areas near cell adhesion molecules such as integrin or catenin at the ends of the actin fibers (20, 22, 23). Cell motility is important when cancer cells infiltrate adjacent

Table IV. *The relevance between HGP and ACTN4 expression in 83 thyroid cancer cases.*

		HGP		
		PM	IM	*p*-Value
ACTN4 expression	No-moderate	32	10	* 0.0052
	High	19	22	

HGP, Histological growth pattern; ACTN4, α-actinin-4; PM, pushing margin; IM, infiltrative margin. *Statistically significant.

tissues or migrate to distant organs via the blood and lymphatic vasculature. Under enhanced cell motility conditions, cancer cells have been reported to show essential dynamic actin cytoskeletal changes (12), which is why ACTN4 is thought to be a factor that affects invasion or metastasis.

In the case of colorectal cancer, concentrated ACTN4 expression was observed in filopodia and reported to increase cell motility significantly (16). Furthermore, ACTN4 expression was significantly correlated with regional lymph node metastases (16). Increasing numbers of reports have clarified that ACTN4 overexpression is an indicator of poor prognosis or resistance to chemotherapy in patients with breast, esophageal, pancreatic, or ovarian cancer (12, 17, 24).

To date, age, gender, T, N, and, M factors, and histology have been suggested as prognostic factors (25-28). A significant correlation between clinical stage and prognosis in thyroid cancer has been reported (29), and multivariate analysis findings in the present study findings suggest that older age, IM, and pre-RLNP are independent factors influencing poor prognosis in thyroid cancer. The significant association observed between IM and high ACTN4 expression indicates that ACTN4 is also a promising prognostic factor in thyroid cancer. Because the method used to evaluate HGP and ACTN4 expression was not technically different from current diagnostic approaches, HGP and ACTN4 expression would be useful indication criteria for the post-surgical treatment of thyroid cancer. Furthermore, it is possible to assess tumor invasiveness immunohistochemically according to the ACTN4 expression status of preoperative biopsy or cytopathology specimens. Such evaluations might contribute to decisions regarding surgical resection extent.

Herein we examined the correlation between clinicopathological findings and clinical stage in 83 cases of thyroid cancer and showed that age, IM, pre-RLNP, and high ACTN4 expression were important prognostic factors.

Conflicts of Interest

The Authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

1 Ferlay J, Shin HR, Bray F, Forman D, Mathers C and Parkin DM: Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer *127*: 2893-2917, 2010.

2 Ito Y and Miyauchi A: Prognostic factors of papillary and follicular carcinomas in Japan based on data of kuma hospital. J Thyroid Res *2012*: 973497, 2012.

3 Choi JS, Kim J, Kwak JY, Kim MJ, Chang HS and Kim EK: Preoperative staging of papillary thyroid carcinoma: comparison of ultrasound imaging and CT. AJR Am J Roentgenol *193*: 871-878, 2009.

4 Chang HY, Lin JD, Chen JF, Huang BY, Hsueh C, Jeng LB and Tsai JS: Correlation of fine needle aspiration cytology and frozen section biopsies in the diagnosis of thyroid nodules. J Clin Pathol *50*: 1005-1009, 1997.

5 Rajaganeshan R, Prasad R, Guillou PJ, Chalmers CR, Scott N, Sarkar R, Poston G and Jayne DG: The influence of invasive growth pattern and microvessel density on prognosis in colorectal cancer and colorectal liver metastases. Br J Cancer *96*: 1112-1117, 2007.

6 López JI and Angulo JC: Growth pattern in superficial urothelial bladder carcinomas. Histological review and clinical relevance. Int Urol Nephrol *41*: 847-854, 2009.

7 Tajima Y, Nakanishi Y, Ochiai A, Tachimori Y, Kato H, Watanabe H, Yamaguchi H, Yoshimura K, Kusano M and Shimoda T: Histopathologic findings predicting lymph node metastasis and prognosis of patients with superficial esophageal carcinoma: analysis of 240 surgically resected tumors. Cancer *88*: 1285-1293, 2000.

8 Berglund P, Stighall M, Jirström K, Rydén L, Fernö M, Nordenskjöld B and Landberg G: Cyclin E confers a prognostic value in premenopausal breast cancer patients with tumours exhibiting an infiltrative growth pattern. J Clin Pathol *61*: 184-191, 2008.

9 Kim KJ, Hong SW, Lee YS, Kim BW, Lee SC, Chang HS and Park CS: Tumor margin histology predicts tumor aggressiveness in papillary thyroid carcinoma: a study of 514 consecutive patients. J Korean Med Sci *26*: 346-351, 2011.

10 Jung CK, Kang YG, Bae JS, Lim DJ, Choi YJ and Lee KY: Unique patterns of tumor growth related with the risk of lymph node metastasis in papillary thyroid carcinoma. Mod Pathol *23*: 1201-1208, 2010.

11 Sjöblom B, Salmazo A, and Djinović-Carugo K: Alpha-actinin structure and regulation. Cell Mol Life Sci 65: 2688-2701, 2008.

12 Honda K, Yamada T, Endo R, Ino Y, Gotoh M, Tsuda H, Yamada Y, Chiba H and Hirohashi S: Actinin-4, a novel actin-bundling protein associated with cell motility and cancer invasion. J Cell Biol *140*: 1383-1393, 1998.

13 Yamamoto S, Tsuda H, Honda K, Kita T, Takano M, Tamai S, Inazawa J, Yamada T and Matsubara O: Actinin-4 expression in ovarian cancer: a novel prognostic indicator independent of clinical stage and histological type. Mod Pathol *20*: 1278-1285, 2007.

14 Kikuchi S, Honda K, Tsuda H, Hiraoka N, Imoto I, Kosuge T, Umaki T, Onozato K, Shitashige M, Yamaguchi U, Ono M, Tsuchida A, Aoki T, Inazawa J, Hirohashi S and Yamada T: Expression and gene amplification of actinin-4 in invasive ductal carcinoma of the pancreas. Clin Cancer Res *14*: 5348-5356, 2008.

15 Yamada S, Yanamoto S, Yoshida H, Yoshitomi I, Kawasaki G, Mizuno A and Nemoto TK: RNAi-mediated down-regulation of alpha-actinin-4 decreases invasion potential in oral squamous cell carcinoma. Int J Oral Maxillofac Surg *39*: 61-67, 2010.

16 Honda K, Yamada T, Hayashida Y, Idogawa M, Sato S, Hasegawa F, Ino Y, Ono M and Hirohashi S: Actinin-4 increases cell motility and promotes lymph node metastasis of colorectal cancer. Gastroenterology *128*: 51-62, 2005.

17 Yamamoto S, Tsuda H, Honda K, Onozato K, Takano M, Tamai S, Imoto I, Inazawa J, Yamada T and Matsubara O: Actinin-4 gene amplification in ovarian cancer: a candidate oncogene associated with poor patient prognosis and tumor chemoresistance. Mod Pathol *22*: 499-507, 2009.

18 Barbolina MV, Adley BP, Kelly DL, Fought AJ, Scholtens DM, Shea LD and Stack MS: Motility-related actinin alpha-4 is associated with advanced and metastatic ovarian carcinoma. Lab Invest *88*: 602-614, 2008.

19 Iida Y and Chiba H: The relationship between subcellular localization of actinin-4 and cell motility in oral squamous cell carcinoma. Oral Sci Int *1*: 30-37, 2004.

20 Otey CA, Pavalko FM and Burridge K: An interaction between alpha-actinin and the beta 1 integrin subunit in vitro. J Cell Biol *111*: 721-729, 1990.

21 Shams H, Golji J, and Mofrad MR: A molecular trajectory of α-actinin activation. Biophys J *103*: 2050-2059, 2012.

22 Otey CA, Vasquez GB, Burridge K and Erickson BW: Mapping of the alpha-actinin binding site within the beta 1 integrin cytoplasmic domain. J Biol Chem *268*: 21193-21197, 1993.

23 Knudsen KA, Soler AP, Johnson KR and Wheelock MJ: Interaction of alpha-actinin with the cadherin/catenin cell-cell adhesion complex via alpha-catenin. J Cell Biol *130*: 67-77, 1995.

24 Hatakeyama H, Kondo T, Fujii K, Nakanishi Y, Kato H, Fukuda S and Hirohashi S: Protein clusters associated with carcinogenesis, histological differentiation and nodal metastasis in esophageal cancer. Proteomics *6*: 6300-6316, 2006.

25 Akslen LA, Haldorsen T, Thoresen SO and Glattre E: Survival and causes of death in thyroid cancer: a population-based study of 2479 cases from Norway. Cancer Res *51*: 1234-1241, 1991.

26 Lundgren CI, Hall P, Ekbom A, Frisell J, Zedenius J and Dickman PW: Incidence and survival of Swedish patients with differentiated thyroid cancer. Int J Cancer *106*: 569-573, 2003.

27 Simpson WJ, Panzarella T, Carruthers JS, Gospodarowicz MK and Sutcliffe SB: Papillary and follicular thyroid cancer: impact of treatment in 1578 patients. Int J Radiat Oncol Biol Phys *14*: 1063-1075, 1988.

28 Tzavara I, Vlassopoulou B, Alevizaki C, Koukoulis G, Tzanela M, Koumoussi P, Sotsiou F and Thalassinos N: Differentiated thyroid cancer: a retrospective analysis of 832 cases from Greece. Clin Endocrinol (Oxf) *50*: 643-654, 1999.

29. Gilliland FD, Hunt WC, Morris DM and Key CR: Prognostic factors for thyroid carcinoma. A population-based study of 15,698 cases from the Surveillance, Epidemiology and End Results (SEER) program 1973-1991. Cancer *79*: 564-573, 1997.

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A retrospective analysis of factors associated with selection of end-of-life care and actual place of death for patients with cancer

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ABSTRACT

Objectives: The factors associated with end-of-life (EOL) care that patients with cancer selected and actual place of death (POD) is less elucidated. We analysed how specific EOL care, especially anticancer therapies, selected by patients with pancreatic carcinoma affected their POD in Japan.

Setting: A retrospective cohort study using clinical records of a single institute.

Participants: This study included 433 advanced or recurrent patients with pancreatic carcinoma who had completed standard chemotherapies and were receiving hospice care in the National Cancer Center Hospital between April 2008 and April 2011.

Outcome measures: We analysed statistical association factors, demographic information, geographical differences, medical environment, EOL care selection, along with actual POD using logistic regression analysis.

Results: Of the 433 patients, 147 selected palliative care units (PCUs) as the POD; 229, hospital; and 57, home with hospice care. POD selection was associated with several factors. Notably, EOL care selection, especially the use of complementary and alternative medicine (CAM), is associated with POD selection (death in PCU; OR=0.23, p=0.02).

Conclusions: This study is, to the best of our knowledge, the first to unveil that EOL care selection is associated with POD in Japan. Certain factors such as gender, medical environment and EOL care selection might influence the POD. Patients who pursue aggressive anticancer therapies, such as CAM use, were possibly deprived of a chance of early reference to a PCU.

Strengths and limitations of this study

- This study is the first to unveil that end-of-life care selection is associated with place of death in Japan.
- Patients who pursue aggressive anticancer therapies, such as complementary and alternative medicine use, were possibly deprived of a chance of early reference to the palliative care unit.
- Limitations of this study should be considered, including its retrospective nature and the involvement of a single institution. Therefore, the findings may not be entirely representative of patients receiving cancer treatment at other Japanese cancer hospitals.

INTRODUCTION

In Japan, the Cancer Control Act was established to improve the quality of life (QOL) of all patients with cancer, and disseminating palliative care was identified as one of the most important areas to be improved. To disseminate quality palliative care, palliative care units (PCUs) and palliative care team were established. Although PCU is the most common type of specialised palliative care service in Japan,[1] patients with cancer can choose their place of death (POD) as either PCUs, home with hospice or non-PCU hospitals.

Dying at a preferred place is one of the most important determinants for terminally ill patients with cancer.[2 3] In some previous reports, POD for patients with cancer was influenced by several factors such as illness, demographic variables, personal variables, social support and relationship with the physician.[4 5] Moreover, patients who optimistically estimated their prognoses are more likely to undergo aggressive treatment, but controlling for known prognostic factors, their 6-month survival is no better.[6]

Choice of cancer therapy at the EOL is becoming increasingly complex due to more options for therapy, high expectations from therapy, less toxic treatments and better supportive care. Consequences of these choices may have an enormous impact on patients and families (caregiver) and societal healthcare costs. Although less aggressive care,

especially palliative care, at the EOL is associated with better QOL near death,[7 8] patients with cancer are receiving increasingly aggressive care at the EOL.[9 10] The use of complementary and alternative medicine (CAM) as an aggressive anticancer therapy has been increasing worldwide over the past two decades, and an estimated 40–60% of adult patients with cancer use CAM, although it does not provide definite survival benefit and its users report clinically poor QOL.[11]

We hypothesised that aggressive anticancer therapy, especially CAM chosen by patients with cancer, limited their options of POD selection. Hence, we conducted this study to analyse the factors that influence POD and to show the evidence of influence of EOL care selection after standard chemotherapy on patients' POD. Moreover, we also analysed the factors that influence EOL care selection in this study.

METHODS

Selection criteria

Patients receiving hospice care at the National Cancer Center Hospital (NCCH) between April 2008 and April 2011 were selected. The inclusion criteria were as follows: confirmed as having carcinoma according to the results of histological tests, had advanced or recurrent pancreatic carcinoma, were receiving systemic palliative chemotherapy at the NCCH, failed to respond to standard chemotherapy and had discussed about EOL care with their attending physician. Prior to the start of chemotherapy, all patients included in the analysis were clearly informed that the chemotherapy being administered was not curative but aimed at prolonging their survival and palliating their symptoms. Their signed informed consent for the same was obtained. From the analysis, we excluded patients who had not been receiving standard chemotherapies or who did not choose POD. This study protocol was approved by the Institutional Review Board of the NCCH, Tokyo, Japan.

Data extraction and definition of terms

The following information was collected with regard to patients: (1) demographic (age, sex, relation with the attending physician, main family caregiver and state of disease), (2) geographical differences (distance from the cancer centre), (3) medical environment (involvement of a palliative care team, a case worker, a primary care doctor and regional healthcare cooperation during chemotherapy) and (4) EOL care selection (best supportive care (BSC), non-standard chemotherapy and CAM use).

In this study, we defined PCU as the institute has been covered by National Medical Insurance since 1990 and plays a central role in providing specialised palliative care services to patients with cancer. Since the NCCH does not have beds assigned for palliative care, patients were provided with information about PCUs near their homes or according to their wish at the start of chemotherapy or completion of standard chemotherapy. Dying at home was defined as dying at home with hospice. Other hospitals except PCUs and homes with hospice were defined as non-PCU hospitals in this study.

In this study, we defined standard therapy as gemcitabine-based or S-1-based chemotherapy. Aggressive anticancer therapy was defined as non-standard chemotherapies and CAM. Non-standard chemotherapy was defined as chemotherapy with other cytotoxic agents and included participation in a clinical trial. We used the definition of CAM adopted by the National Cancer Institute: 'CAM is the term for medical products and practices that are not part of standard medical care.' NCI categorises CAM as follows: CAM (any medical system, practice or product, ie, not thought of as standard care), complementary medicine (CAM therapy used along with standard medicine), alternative medicine (CAM therapy used in place of standard treatments) and integrative medicine (an approach that combines treatments from conventional medicine and CAM for which there is some high-quality evidence of safety and effectiveness).

Statistical analysis

We conducted statistical analyses using IBM SPSS V.18.0 (SPSS, Chicago, Illinois, USA). All patient characteristics and background factors were analysed using the logistic regression analyses. Multivariate logistic regression analyses were performed after univariate analyses to reveal strong correlation factors between POD and EOL care. p Values less than 0.05 in a two-sided test were considered significant.

RESULTS

Patient characteristics

A total of 433 patients received systemic chemotherapy for advanced and recurrent pancreatic carcinoma at the NCCH (figure 1). Of these, 147 (34%) patients chose PCU, 57 (13%) patients chose home with hospice and 229 (53%) patients chose non-PCU hospitals as their POD. In total, 357 (82%) patients chose to receive BSC and 76 (18%) patients chose aggressive anticancer treatment as EOL care. In patients with aggressive anticancer therapy, 64 (15%) patients used non-standard chemotherapies and 57 (13%) patients used CAM as EOL care (table 1).

Factors influencing POD

In multivariate logistic regression analysis using strong factors that correlated with POD in univariate analyses, patients who selected PCUs as the POD were most likely to be of female gender (OR 1.85; 95% CI 1.23 to 2.79; p=0.003) and CAM users (OR 0.23; 95% CI 0.06 to 0.81; p=0.02). Patients who selected dying at home were most likely to be supported by a case worker (OR 3.50; 95% CI 1.22 to 10.03; p=0.02) and be town dwellers (OR 2.13; 95% CI 1.18 to 3.85; p=0.01). Patients who died at non-PCU hospitals were likely to be of male gender (OR

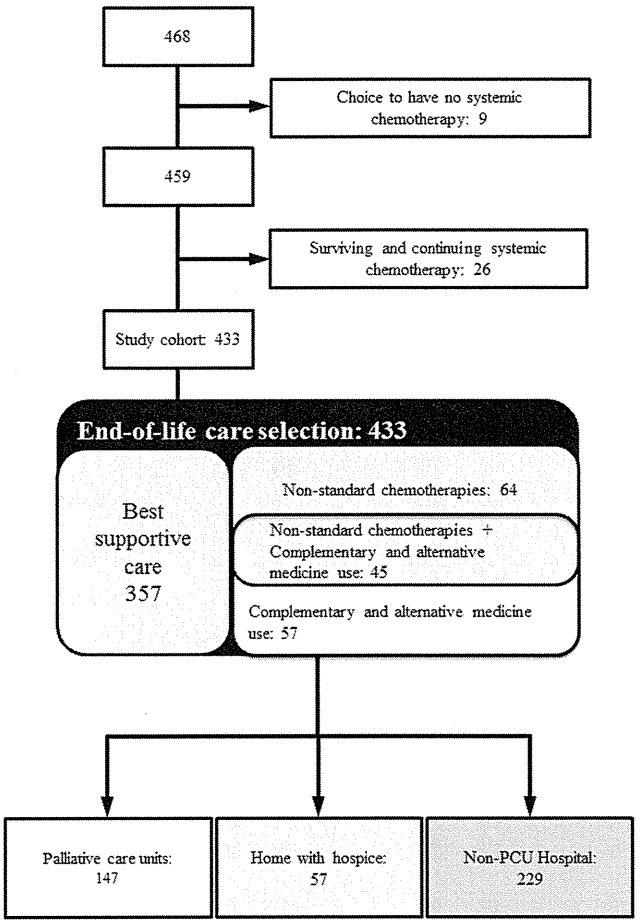

Figure 1 Patient distribution in the study. A total of 468 patients with advanced or recurrent pancreatic carcinoma were seen at the National Cancer Center Hospital. Nine patients chose best supportive care without receiving chemotherapy. Twenty-six patients are still alive and are continuing with standard chemotherapy.

1.64; 95% CI 1.10 to 2.44; p=0.02), rural dwellers (OR 1.85; 95% CI 1.24 to 2.74; p=0.002) and had involvement of a case worker (OR 2.44; 95% CI 1.43 to 4.17; p=0.001) (table 2). Although we conducted additional analyses including all variables (age, gender, caregiver, distance from the cancer centre, attending physician, state of disease, EOL selection and medical environment) into the model, the results were materially unchanged.

Factors influencing EOL care selection

Table 3 shows the results of multivariate analyses using significant factors after univariate analyses associated with EOL care selection were performed. Patients who selected BSC as EOL care were of older age (OR=1.67; 95% CI 1.02 to 2.78; p=0.04) and had recurrence after surgical resection (OR=1.82; 95% CI 1.02 to 3.23; p=0.04). Patients who selected non-standard chemotherapies were of younger age (OR=2.04; 95% CI 1.18 to 3.51; p=0.01). Patients who selected CAM use were of younger age (OR=2.57; 95% CI 1.43 to 4.63; p=0.01) and depended on the attending physician. Although we conducted additional analyses including all variables into the model, the results were materially unchanged.

DISCUSSION

The present study results indicate that EOL care selected by Japanese patients with pancreatic cancer after complete standard chemotherapy was correlated with the selection of POD. Notably, the factors of (1) demographic (gender), (2) geographical differences (distance from the cancer centre), (3) medical environment (involvement of a case worker) and (4) EOL care selection (CAM use) were strongly correlated with selection of POD. Moreover, patients' age, state of disease and dependence on the attending physician were strong factors that correlated with EOL care selection. We found that patients who selected aggressive anticancer therapy as EOL care, especially CAM use, tended to lose the opportunity to die in a PCU.

In Japan, a series of national surveys was conducted by the Ministry of Health, Labour and Welfare in 2008 to reveal the preferred place of care and POD. Home was the preferred place of care in general, with 29% of respondents reporting that they wanted to receive care at home and be admitted to a PCU if necessary, and 23% preferring to receive care at home and be admitted to a hospital if necessary. Another 11% chose home until death, while a considerable number of respondents reported that they want to be admitted to a hospice earlier and stay until death (18%) or be admitted to a hospital earlier and stay until death (10%). The distribution of POD in this study reflected the trend in the preference of Japanese patients with cancer with regard to place of care and POD.

In some previous reports, factors that influence selection of POD for patients with cancer were related to illness,[12] individual factors that account for the maintenance of patients' individuality, comparison of demographic variables and personal variables,[13] social support[4] and relationship with the physician.[5] The present study showed gender female associated with PCU as actual POD. On the other hand, a previous British report showed gender was not associated with POD.[4] In this study, EOL care selection, especially CAM use, influenced POD. Moreover, selection of best supportive care as EOL care associated with PCU as actual POD. Patients select aggressive anticancer therapies closer to death, with unintended consequences of late PCU referral.[14] Moreover, physicians can predict the survival time of their patients based on experience and clinical data.[15] On the other hand, patients pursue aggressive anticancer therapies, such as CAM use due to lack of awareness of their prognosis. Selecting a treatment mode without prediction of prognosis causes these patients to lose their chance of early reference to their preferred POD.

Table 1 Patient characteristics

		Total	PCU		Home with hospice		Non-PCU Hospital		
		Total	n	Per cent	n	Per cent	n	Per cent	
Total		**433**	**147**	**34**	**57**	**13**	**229**	**53**	**p Value***
Age									
Mean (SD)		64.8 (9.3)	65.0 (9.4)		66.5 (8.8)		64.2 (9.4)		
≥65		234	82	56	31	54	121	53	0.85
<65		199	65	44	26	46	108	47	
Gender									
Male		258	72	49	36	63	150	66	0.005
Female		175	75	51	21	37	79	34	
Close relative (caregiver)									
Spouse	+	334	110	75	44	77	180	79	0.70
	–	99	37	25	13	23	49	21	
Daughter(s) or son(s)	+	326	109	74	43	75	174	76	0.92
	–	107	38	26	14	25	55	24	
Parent(s)	+	13	5	3	1	2	7	3	0.82
	–	420	142	97	56	98	222	97	
Distance from the cancer center									
Mean (SD) (km)		32 (78.1)	32 (85.6)		16 (10.4)		36.2 (82.2)		
0–19		224	83	56	38	67	103	45	0.005
≥20		209	64	44	19	33	126	55	
Attending physician									
A		127	43	29	11	19	73	32	0.45
B		62	24	16	11	19	27	12	
C		114	35	24	16	28	63	28	
D		130	45	31	19	34	66	28	
State of disease									
Advanced		350	114	78	47	82	189	83	0.46
Recurrence		83	33	22	10	18	40	17	
End-of-life care selection									
Best supportive care	+	357	129	88	48	84	180	79	0.07
	–	76	18	12	9	16	49	21	
Non-standard chemotherapies	+	64	14	10	8	14	42	18	0.06
	–	369	133	90	49	86	187	82	
CAM	+	57	10	7	7	12	40	17	0.01
	–	376	137	93	50	88	189	83	
Medical environment									
Involvement of a palliative care team	+	44	13	9	5	9	26	11	0.69
	–	389	134	91	52	91	203	89	
Involvement of a caseworker	+	354	127	86	53	92	174	76	0.002
	–	79	20	14	4	8	55	24	
Primary care doctor	+	133	46	31	12	21	75	33	0.23
	–	300	101	69	45	79	154	67	

*Using χ^2 test for categorical variables.
CAM, complementary and alternative medicine; PCU, palliative care units.

Geographical differences in established PCUs, BSC at home and regional hospitals with palliative care teams reduce the choice of POD available to patients. According to studies conducted in Europe, patients living in rural areas have increased difficulty in accessing healthcare[12] and palliative care[16]; yet, they are more likely to die at home.[4] In the present study, the choice of dying at home with hospice increased with the closer distance from the cancer centre, which is located in the centre of Tokyo. These results support the view that geographical trends affect the choice of POD in Japan and Europe.

The present study also showed that social support and involvement of a case worker affect the selection of POD. Specifically, social support influenced death at home through arrangement of medical environment by case workers. On the other hand, involvement of a palliative care team can potentially improve the timing of referral to a PCU.[17] In this study, the palliative care team had no role in influencing the selection of POD of patients with cancer. Comprehensive cancer teams including the palliative care team, psycho-oncologist and case workers can involve patients in discussions about advance planning for care or POD.

Table 2 Factors associated with place of death: multivariate analysis

Place of death	Factors	n	OR (95% CI)	p Value*
PCU	Gender			
	Male	72	1 (Ref)	0.003
	Female	76	1.85 (1.23 to 2.79)	
	Best supportive care			
	–	18	1 (Ref)	0.13
	+	129	3.85 (0.66 to 25)	
	Non-standard chemotherapies			
	–	14	1 (Ref)	0.15
	+	133	3.00 (0.68 to 13.3)	
	CAM			
	–	137	1 (Ref)	0.02
	+	10	0.23 (0.06 to 0.81)	
Home with hospice	Distance from the cancer centre			
	0–19 km	38	1 (Ref)	0.01
	≥20 km	19	0.47 (0.26 to 0.85)	
	Involvement of a caseworker			
	–	5	1 (Ref)	0.02
	+	52	3.50 (1.22 to 10.03)	
Non-PCU Hospital	Gender			
	Male	150	1 (Ref)	0.02
	Female	79	0.61 (0.41 to 0.91)	
	Distance from the cancer centre			
	0–19 km	103	1 (Ref)	0.002
	≥20 km	126	1.85 (1.24 to 2.74)	
	Best supportive care			
	–	180	1 (Ref)	0.45
	+	49	0.53 (0.10 to 2.80)	
	Non-standard chemotherapies			
	–	42	1 (Ref)	0.37
	+	187	1.87 (0.47 to 7.35)	
	CAM			
	–	40	1 (Ref)	0.13
	+	189	2.41 (0.76 to 7.63)	
	Involvement of a caseworker			
	–	55	1 (Ref)	0.001
	+	174	2.44 (1.43 to 4.17)	

*The multivariate analysis was performed using logistic regression analysis after.
CAM, complementary and alternative; PCU, palliative care units; Ref, reference.

The trend of use of aggressive chemotherapy increased even in older patients, and the use of PCU as simply a place to die in rather than to control symptoms became common.[9 15] In this study, 18% of patients with pancreatic cancer used aggressive anticancer treatment as EOL care. In the USA, the proportion of patients who choose cancer therapy at the EOL has increased from 13.8% to 18.5%.[18] Our study shows a similar proportion when compared with previous reports. On the other hand, in this study, the prevalence of CAM use in patients with pancreatic cancer was 13%. This rate was slightly lower than that found in previous studies.[19 20] The prevalence of CAM use was potentially affected by several factors, including primary cancer site. In terms of cancer site, the rate of CAM use was higher in patients with lung, breast and hepatobiliary cancers than in those with other cancers, including gastrointestinal cancer. Hence, the ratio of CAM use in pancreatic cancer may be lower than that in the previous report.[19] The multivariate analysis also revealed a close association between aggressive anticancer therapies and younger age. Previous studies showed that some factors, including younger age, were significant independent predictors of aggressive EOL care.[9 10 19 21]

Certain limitations of this study should be considered, including its retrospective nature and the involvement of a single institution. Therefore, the findings may not be entirely representative of patients receiving cancer treatment at other Japanese cancer hospitals; moreover, we could not determine some other factors that influenced the selection of POD by patients with cancer. Above all, the study focused on factors associated with choosing EOL care and how these factors affect POD choice, but it did not include analysis of some other factors such as

Table 3 Factors associated with end-of-life care selection: multivariate regression

End-of-life care	Factors	n	Multivariate analyses OR (95% CI)	p Value*
Best supportive care	Age			
	≥65	201	1 (Ref)	0.04
	<65	156	0.60 (0.36 to 0.98)	
	State of disease			
	Advanced	295	1 (Ref)	0.04
	Recurrence	62	1.82 (1.02 to 3.23)	
Non-standard chemotherapies	Age			
	≥65	25	1 (Ref)	0.01
	<65	39	2.04 (1.18 to 3.51)	
CAM	Age			
	≥65	20	1 (Ref)	0.002
	<65	37	2.57 (1.43 to 4.63)	
	Attending physician			
	A	24	1 (Ref)	0.03
	B	9	0.27 (0.11 to 0.62)	0.002
	C	16	0.37 (0.13 to 1.02)	>0.05
	D	8	0.38 (0.15 to 0.94)	0.04

*The multivariate analysis was performed using logistic regression analysis after.
†Univariate analyses.
CAM, complementary and alternative; Ref, reference.

income of patients, religion and timing of EOL discussions.

Physicians commonly avoid EOL care-related discussions with patients until they fail standard chemotherapy or are nearing death.[22] Physicians who have close, long-term relationships with patients often wish to avoid discussions around EOL care.[23] Physicians involved in longitudinal care, however, may be best equipped to have meaningful discussions about the patient's values and goals.[5] NCCH, all attending physicians informed their patients before starting chemotherapy that advanced pancreatic carcinoma had reduced chances of being cured and that chemotherapy was of limited use in palliation and prolongation of survival. Some patients who discussed EOL care or POD during treatment with standard chemotherapy collaterally underwent a checkup or received palliative care in community hospitals or PCUs. The selection of CAM use as EOL care by the attending physician points to the critical need to recognise the lack of discussion with patients about EOL care. Moreover, selecting EOL care after failing standard chemotherapy had a direct bearing on the selection of POD.

In conclusion, the present study provides new and important information on the factors influencing patients' choices at the EOL. To the best of our knowledge, this is the first report of an investigation on POD that focuses on EOL care selection, especially aggressive anticancer treatment including CAM, among Japanese patients with pancreatic cancer. Importantly, patients and physicians should share the same information related to survival benefits and places to receive EOL care and choose appropriate POD.

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REFERENCES

1. Morita T, Miyashita M, Shibagaki M, *et al.* Knowledge and beliefs about end-of-life care and the effects of specialized palliative care: a population-based survey in Japan. *J Pain Symptom Manage* 2006;31:306–16.
2. Steinhauser KE, Christakis NA, Clipp EC, *et al.* Factors considered important at the end of life by patients, family, physicians, and other care providers. *JAMA* 2000;284:2476–82.

3. Miyashita M, Sanjo M, Morita T, *et al.* Good death in cancer care: a nationwide quantitative study. *Ann Oncol* 2007;18:1090–7.
4. Gomes B, Higginson IJ. Factors influencing death at home in terminally ill patients with cancer: systematic review. *BMJ* 2006;332:515–21.
5. Mack JW, Cronin A, Taback N, *et al.* End-of-life care discussions among patients with advanced cancer: a cohort study. *Ann Intern Med* 2012;156:204–10.
6. Weeks JC, Cook EF, O'Day SJ, *et al.* Relationship between cancer patients' predictions of prognosis and their treatment preferences. *JAMA* 1998;279:1709–14.
7. Smith TJ, Temin S, Alesi ER, *et al.* American Society of Clinical Oncology provisional clinical ppinion: the integration of palliative care into standard oncology care. *J Clin Oncol* 2012;30:880–7.
8. Wright AA, Zhang B, Ray A, *et al.* Associations between end-of-life discussions, patient mental health, medical care near death, and caregiver bereavement adjustment. *JAMA* 2008;300:1665–73.
9. Earle CC, Landrum MB, Souza JM, *et al.* Aggressiveness of cancer care near the end of life: is it a quality-of-care issue? *J Clin Oncol* 2008;26:3860–6.
10. Ho TH, Barbera L, Saskin R, *et al.* Trends in the aggressiveness of end-of-life cancer care in the universal health care system of Ontario, Canada. *J Clin Oncol* 2011;29:1587–91.
11. Yun YH, Lee MK, Park SM, *et al.* Effect of complementary and alternative medicine on the survival and health-related quality of life among terminally ill cancer patients: a prospective cohort study. *Ann Oncol* 2013;24:489–94.
12. Hunt R, McCaul K. A population-based study of the coverage of cancer patients by hospice services. *Palliat Med* 1996;10:5–12.
13. Koffman J, Higginson IJ. Dying to be home? Preferred location of death of first-generation black Caribbean and native-born white patients in the United Kingdom. *J Palliat Med* 2004;7:628–36.
14. Matsuyama R, Reddy S, Smith TJ. Why do patients choose chemotherapy near the end of life? A review of the perspective of those facing death from cancer. *J Clin Oncol* 2006;24:3490–6.
15. Glare P, Virik K, Jones M, *et al.* A systematic review of physicians' survival predictions in terminally ill cancer patients. *BMJ* 2003;327:195–8.
16. Auchincloss AH, Van Nostrand JF, Ronsaville D. Access to health care for older persons in the United States: personal, structural, and neighborhood characteristics. *J Aging Health* 2001;13:329–54.
17. Morita T, Miyashita M, Tsuneto S, *et al.* Late referrals to palliative care units in Japan: nationwide follow-up survey and effects of palliative care team involvement after the Cancer Control Act. *J Pain Symptom Manage* 2009;38:191–6.
18. Grunfeld E, Lethbridge L, Dewar R, *et al.* Towards using administrative databases to measure population-based indicators of quality of end-of-life care: testing the methodology. *Palliat Med* 2006;20:769–77.
19. Hyodo I, Amano N, Eguchi K, *et al.* Nationwide survey on complementary and alternative medicine in cancer patients in Japan. *J Clin Oncol* 2005;23:2645–54.
20. Cassileth BR, Vickers AJ. High prevalence of complementary and alternative medicine use among cancer patients: implications for research and clinical care. *J Clin Oncol* 2005;23:2590–2.
21. Earle CC, Neville BA, Landrum MB, *et al.* Trends in the aggressiveness of cancer care near the end of life. *J Clin Oncol* 2004;22:315–21.
22. The AM, Hak T, Koëter G, *et al.* Collusion in doctor-patient communication about imminent death: an ethnographic study. *BMJ* 2000;321:1376–81.
23. Christakis NA, Lamont EB. Extent and determinants of error in doctors' prognoses in terminally ill patients: prospective cohort study. *BMJ* 2000;320:469–72.

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Twenty-six Cases of Advanced Ampullary Adenocarcinoma Treated with Systemic Chemotherapy

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Objective: Ampullary adenocarcinoma is a rare disease entity and little information regarding these tumors is available. The aim of the present study was to clarify the treatment outcome of systemic chemotherapy in patients with advanced ampullary adenocarcinoma.
Methods: This study consisted of a retrospective review of data obtained from patients diagnosed as having advanced ampullary adenocarcinoma who received non-surgical treatment at a single institution between 1997 and 2010.
Results: We identified 26 patients (15 men, 11 women; median age, 62.0 years) who received treatment for advanced ampullary adenocarcinoma. Twelve patients had Stage IV disease and 14 had recurrences. The chemotherapy regimens consisted of 5-fluorouracil-based regimens (5-fluorouracil + cisplatin, $n = 3$; tegafur-uracil + doxorubicin, $n = 5$ and tegafur, gimeracil and oteracil potassium, $n = 3$) and gemcitabine-based regimens (gemcitabine, $n = 10$ and gemcitabine + cisplatin, $n = 5$). The overall response rate was 7.7%. The median progression-free survival period was 3.2 months (2.5 months in the 5-fluorouracil group vs. 3.5 months in the gemcitabine group), and the median overall survival time was 9.1 months (8.0 months in the 5-fluorouracil group vs. 12.3 months in the gemcitabine group). The median overall survival was significantly longer in stage IV disease than in recurrent disease. The histological phenotype was determined in 10 of the 26 patients. Eight patients had intestinal-type adenocarcinomas and remaining two patients had pancreatobiliary-type adenocarcinomas.
Conclusions: The treatment outcome of patients with advanced ampullary adenocarcinoma was poor. Further development of novel treatments is necessary to improve the prognosis.

Key words: ampullary adenocarcinoma – chemotherapy – 5-fluorouracil – gemcitabine – histological phenotype

INTRODUCTION

Ampullary carcinoma is a particularly uncommon neoplasm. Between 1985 and 2005, the incidence of ampullary carcinoma in the USA was 0.7 cases per 10 000 males and 0.4 cases per 10 000 females (1), accounting for 0.5% of all gastrointestinal malignancies (2). The number of annual deaths because of ampullary carcinoma is only 100–200 in the USA and 800–900 in Japan (http://www.who.int/healthinfo/morttables/en/). This inconsistency in the number of annual deaths may be due to the different geographical regions.

Compared with other periampullary adenocarcinomas, ampullary adenocarcinomas is associated with a higher likelihood of resectability and a more favorable prognosis. Among patients who undergo radical resection, the overall 5-year survival rate ranges from 35 to 46%, which is better than that for patients with distal biliary adenocarcinomas (5-year survival