SPANDIDOS PUBLICATIONS

Clinical significance of *ApoE* expression in human gastric cancer

KATSUYA SAKASHITA[1,2], FUMIAKI TANAKA[1], XIANG ZHANG[1,2], KOSHI MIMORI[1], YUKIO KAMOHARA[1], HIROSHI INOUE[1], TETSUJI SAWADA[2], KOSEI HIRAKAWA[2] and MASAKI MORI[1]

[1]Department of Surgery, Medical Institute of Bioregulation Kyushu University, 4546 Tsurumihara, Beppu 874-0838; [2]Department of Surgical Oncology, Osaka City University Graduate School of Medicine, 1-4-3 Asahi-machi, Abeno-ku, Osaka 545-8585, Japan

Received June 5, 2008; Accepted September 3, 2008

DOI: 10.3892/or_00000146

Abstract. *ApoE* plays a key role in various biological events. The aim of this study is to clarify its clinical significance in gastric cancer. We obtained paired clinical bulk samples of tumor tissue and corresponding normal tissue from 124 gastric cancer patients. To address *ApoE* mRNA expression clearly, we selected four samples, and differentially dissected gastric cancer and normal epithelium using laser microdissection (LMD) system. *ApoE* mRNA expression was examined by real-time reverse transcription (RT)-polymerase chain reaction (PCR). ApoE protein expression was assessed by immnuno-histochemistry. The relationship between *ApoE* mRNA expression and clinicopathologic factors was statistically analyzed. RT-PCR assay for 124 bulk samples showed that *ApoE* mRNA expression was more highly expressed in gastric cancer tissue than in corresponding normal mucosa ($p<0.0001$). By RT-PCR assay of four LMD samples, *ApoE* mRNA was overexpressed in gastric cancer. Immuno-histochemistry showed that ApoE was predominantly expressed in gastric cancer. Tumors with high *ApoE* mRNA expression showed deeper tumor invasion into the muscle layer ($p<0.0001$), the serosal layer ($p<0.01$), or more positive lymph node metastasis ($p<0.05$). When assessed by Kaplan-Meier analysis, patients with high *ApoE* expression tumor had a shorter survival than those with low *ApoE* expression tumor ($p<0.05$). Moreover, multivariate analysis indicated that high *ApoE* mRNA expression was an independent indicator for muscular invasion ($p<0.01$). *ApoE* is highly expressed in gastric cancer, contributing to shorter survival. In particular, *ApoE* was closely correlated with muscular invasion, and may be a possible biomarker predicting muscular invasion of gastric cancer.

Correspondence to: Dr Fumiaki Tanaka, Department of Surgery and Molecular Oncology, Medical Institute of Bioregulation, Kyushu University, 4546 Tsurumihara, Beppu 874-0838, Japan
E-mail: fumi@beppu.kyushu-u.ac.jp

Key words: apolipoprotein E, gastric cancer, muscular invasion

Introduction

Gastric cancer is one of the most life-threatening cancers worldwide, however, clinical outcome has gradually improved over the last decades. This is mainly due to the prevalence of endoscopic technology, which allows detecting gastric cancer at early stages (1). When tumors are localized within the mucosal layer, endoscopic mucosal resection (EMR) can be successfully performed. However, because of an expansion of the criteria for endoscopic procedure, the tumors have been endoscopically resected as endoscopic submucosal dissection (ESD), even though invasion was through the submucosal layer (2). In order to determine whether gastric cancers detected at early stages are eligible for endoscopic local treatments, it is required to evaluate the presence of lymph node metastasis and the depth of tumor invasion with accuracy.

Studies on differential gene expression between gastric cancer and metastatic lymph node using microarray or serial analysis of gene expression (SAGE) technique have been published (3-5). Among them, Oue *et al* demonstrated that *Apolipoprotein E (ApoE)* is highly expressed in the metastastic lymph node (6).

ApoE is a secretory glycoprotein that mediates lipid metabolism by binding to the low-density lipoprotein (LDL) receptor (7). Its ligand-receptor binding also activates various signal transductions (8-10). Therefore, it has been suggested that *ApoE* not only regulates lipid metabolism but also plays a key role in biological events such as nerve regeneration, anti-oxidant effects, immune response and cell proliferation (11-13). Moreover, concerning cancer field, *ApoE* has been correlated with cancer development in epithelial malignancies such as ovary or prostate (14-16).

However, to the authors' knowledge, there is no available information concerning *ApoE* expression in gastric cancer. Based on previous data from other cancer studies, we hypothesized that *ApoE* would show biological activity for promotion of lymph node metastasis in gastric cancer. Since it is important in clinical applications to acquire the samples as easily as possible, we focused on *ApoE* expression in primary site, not the metastatic lymph node. The aim of this study is to clarify clinical significance of *ApoE* expression in gastric cancer.

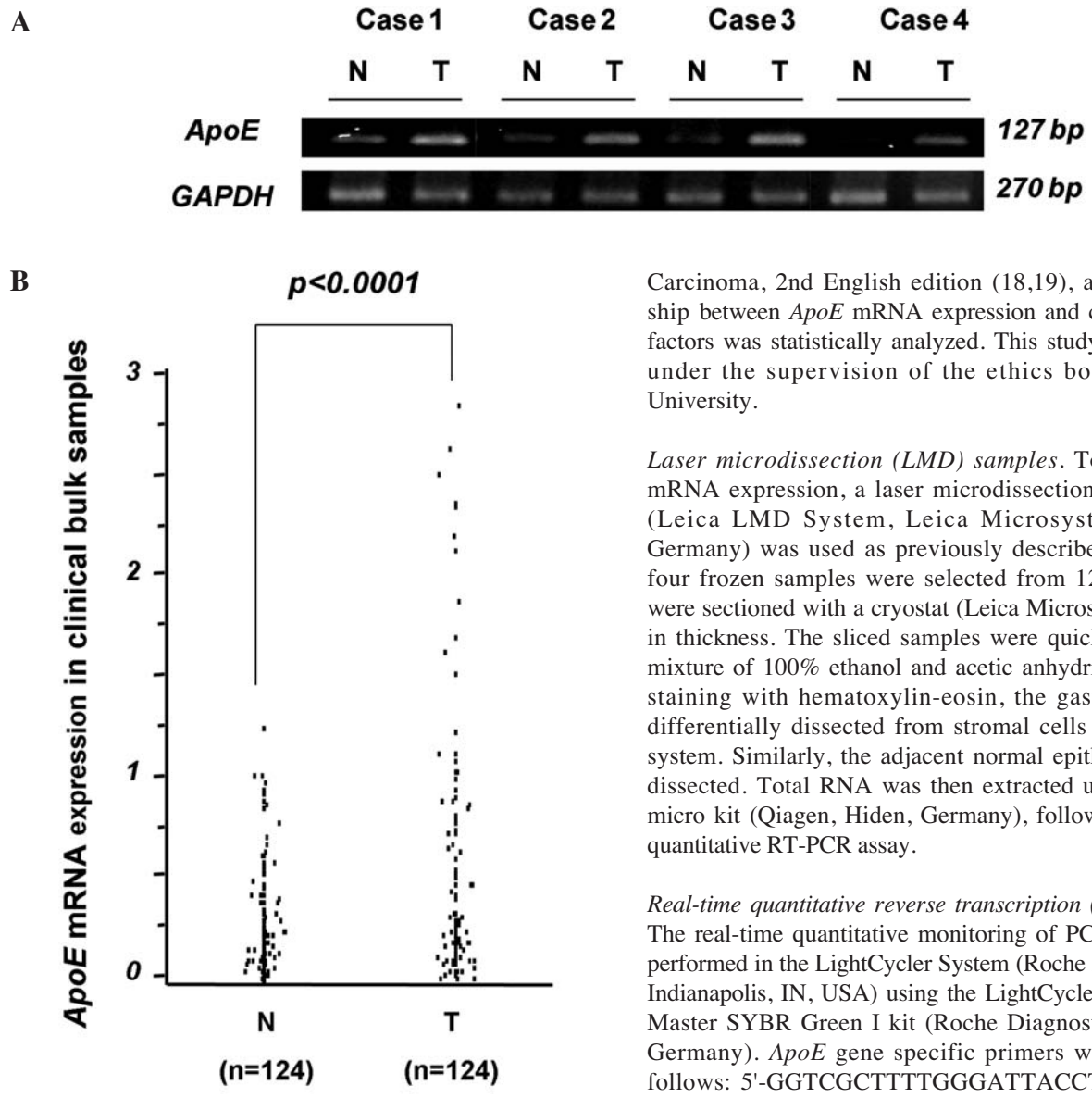

Figure 1. *ApoE* mRNA expression in gastric cancer tissue (T) and the corresponding normal tissue (N). (A) Gel images of *ApoE* expression. (B) Result of real-time RT-PCR assay for 124 clinical bulk samples. *ApoE* mRNA was expressed at significantly higher levels in tumor tissue ($p<0.0001$; Student's t-test).

Materials and methods

Clinical bulk samples. Clinical bulk samples were collected from a total of 124 patients suffering from primary gastric cancer. All patients underwent surgery without pre-operative treatments such as chemotherapy at our institute from 1989 to 2000. Immediately after the operation, bulk samples were carefully removed from tumor tissue (T) and the corresponding normal tissue (N), and frozen with RNAlater (Ambion, Austin, TX, USA) at -80˚C until RNA extraction. The cDNA was then reverse-transcribed from 8.0 μg of total RNA extracted using Isogen (Nippon Gene, Tokyo, Japan), followed by real-time quantitative RT-PCR assay (17). All of the patients were regularly observed every month, for an average follow-up period of 2.4 years. Histopathological evaluations were done with reference to the Japanese Classification of Gastric Carcinoma, 2nd English edition (18,19), and the relationship between *ApoE* mRNA expression and clinicopathologic factors was statistically analyzed. This study was conducted under the supervision of the ethics board of Kyushu University.

Laser microdissection (LMD) samples. To address *ApoE* mRNA expression, a laser microdissection (LMD) system (Leica LMD System, Leica Microsystems, Wetzlar, Germany) was used as previously described (20). Briefly, four frozen samples were selected from 124 samples, and were sectioned with a cryostat (Leica Microsystems) at 8 μm in thickness. The sliced samples were quickly fixed with a mixture of 100% ethanol and acetic anhydride (18:1). After staining with hematoxylin-eosin, the gastric cancer was differentially dissected from stromal cells using the LMD system. Similarly, the adjacent normal epithelium was also dissected. Total RNA was then extracted using an RNeasy micro kit (Qiagen, Hiden, Germany), followed by real-time quantitative RT-PCR assay.

Real-time quantitative reverse transcription (RT)-PCR assay. The real-time quantitative monitoring of PCR reactions was performed in the LightCycler System (Roche Applied Science, Indianapolis, IN, USA) using the LightCycler FastStart DNA Master SYBR Green I kit (Roche Diagnostics, Mannheim, Germany). *ApoE* gene specific primers were designed as follows: 5'-GGTCGCTTTTGGGATTACCT-3' (sense) and 5'-CCTTCAACTCCTTCATGGTCTC-3' (anti-sense). To verify quality and integrity of synthesized cDNA, the *glyceraldehyde-3-phosphate dehydrogenase (GAPDH)* gene was used as an internal control: 5'-TTGGTATCGTGGAAG GACTCA-3' (sense) and 5'-TGTCATCATATTTGGCAG GTT-3' (anti-sense). Cycling conditions consisted of 36 cycles of denaturation at 95˚C for 10 sec, annealing at 66˚C (60˚C for *GAPDH*) for 10 sec and elongation at 72˚C for 8 sec. The amplicons were subjected to a temperature gradient to produce a melting curve of the products. A single peak verified the purity of each product.

The standard curve was plotted by two-fold serial dilutions of cDNAs made from Human Universal Reference total RNA (Clontech, Palo Alto, CA, USA). The amount of *ApoE* and *GAPDH* mRNA expression was calculated in proportion to that of the cDNA of Human Universal Reference total RNA. Normalization was achieved by dividing each calculated value of *ApoE* expression by the level of *GAPDH* expression.

Immunohistochemistry. ApoE protein expression was visualized with immunohistochemical staining of surgical specimens from gastric cancer patients. Sections of formalin-fixed, paraffin-embedded tissues were subjected

n-antibody reactions with mouse monoclonal against ApoE (BD Biosciences Pharmingen, San Diego, CA, USA). ApoE specific antibody was used at a dilution of 1:2000. The antigen-antibody reactions were visualized using the streptavidin-biotin-peroxidase method (LSAB Kit, Dako, Kyoto, Japan). All sections were counterstained with hematoxylin.

Statistical analysis. The study samples were classified into two groups (higher expression group: n=62, lower expression group: n=62) by the median *ApoE* mRNA expression in tumor tissue of bulk samples.

All statistical analysis was carried out using JMP 5 for Windows software (SAS Institute Inc. Cary, NC, USA) The differences between these two groups, based on clinicopathologic factors, were statistically analyzed using Student's t-test and Chi-square test. The survival curve was plotted according to the Kaplan-Meier method, and log-rank test was done to compare the survival rate. Multivariate analysis for survival was performed using the Cox proportional hazards regression model, while multivariate analysis for lymph node metastasis and muscular invasion was performed using the logistic regression model. If the probability value was <0.05, the difference was considered statistically significant.

Results

ApoE mRNA expression in bulk samples. We examined *ApoE* mRNA expression in gastric cancer. Fig. 1A shows gel images of *ApoE* mRNA expression, and Fig. 1B shows the result of real-time RT-PCR assay for 124 bulk samples. Significant difference (p<0.0001; Student's t-test) in *ApoE* mRNA expression between tumor and normal tissue was observed. In 83 of 124 patients (66.9%), the *ApoE* mRNA expression level was frequently higher in tumor tissue than in the corresponding normal tissue (the mean expression level in tumor tissue was 0.54, and 0.28 in the corresponding normal tissue).

ApoE expression in LMD samples. Fig. 2A shows *ApoE* mRNA expression in the specimens which were dissected using the LMD system. *ApoE* mRNA expression in gastric cancer was higher than expression in the adjacent normal epithelium.

Immunohistochemistry. Immunohistochemical staining revealed that ApoE protein was predominantly expressed in cancer tissue (Fig. 2B). None of the stromal cells stained positively for ApoE expression.

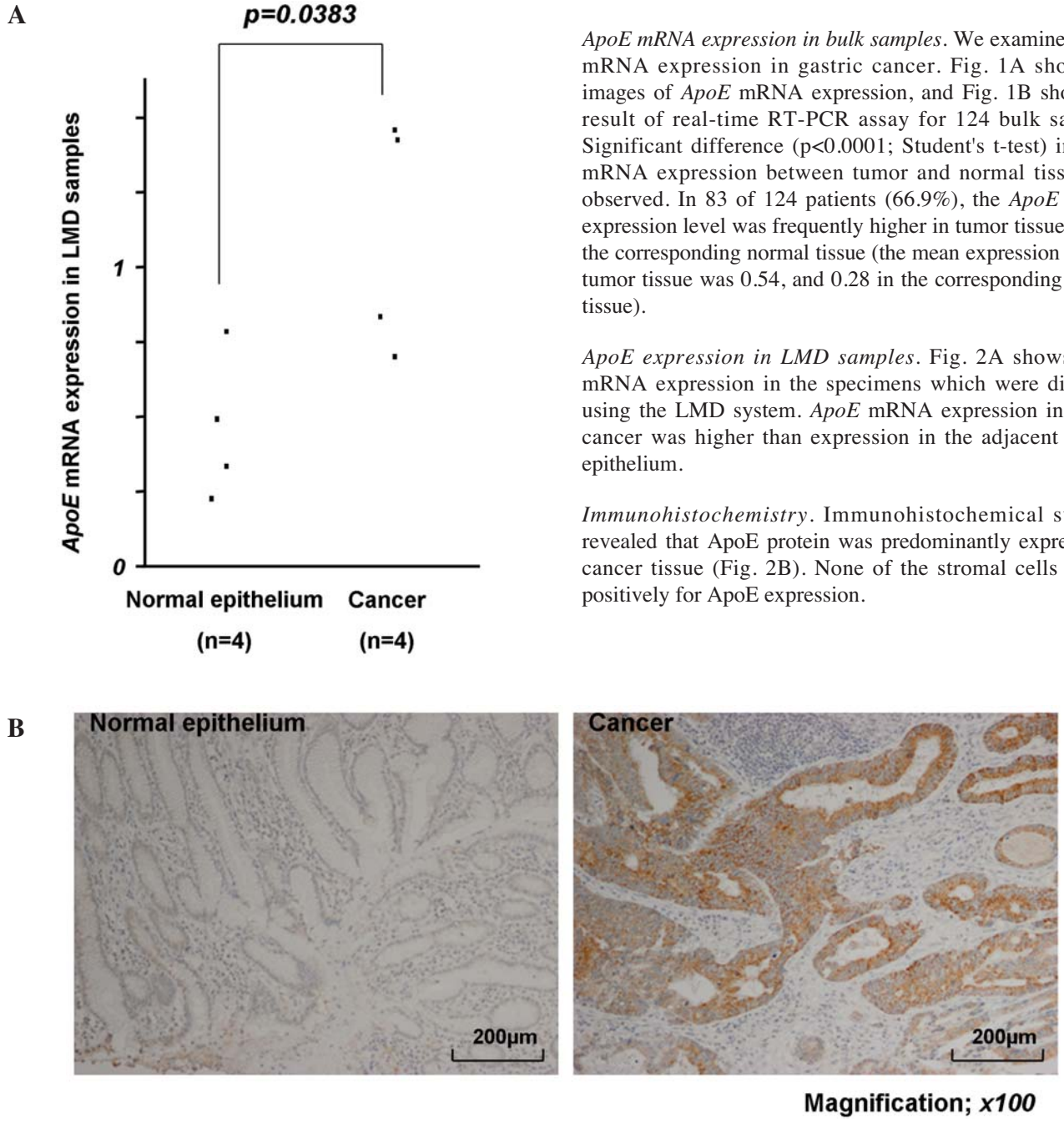

Figure 2. The responsibility of *ApoE* expression was investigated. (A) Real-time RT-PCR assay of four LMD samples. Gastric cancer and the adjacent normal epithelium were differentially dissected using an LMD system to prevent contamination with stromal cells and necrotic tissues. *ApoE* mRNA expression in gastric cancer was higher than that in adjacent normal epithelium (p=0.0383; Student's t-test). (B) A representative picture of immunohistochemical staining (original magnification, x100). ApoE protein was predominantly expressed in cancer cells, indicating that *ApoE* overexpression originated from the gastric cancer cells.

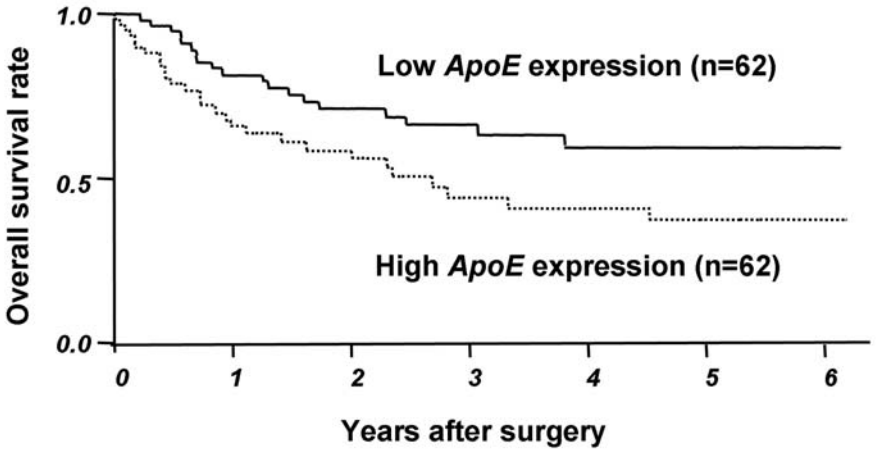

Figure 3. Kaplan-Meier survival curves were plotted based on *ApoE* mRNA expression in tumor tissue. Patients with high *ApoE* expression tumor showed significantly shorter survival than those with low *ApoE* expression tumor (p=0.0324; log-rank test).

Prognostic difference according to ApoE mRNA expression. Kaplan-Meier survival curves (Fig. 3) revealed that patients with high *ApoE* expression tumor showed significantly poorer prognosis than those with low *ApoE* expression (p=0.0324; log-rank test). The overall survival rate at five years in the high *ApoE* expression group was 58.6%, while that of low *ApoE* expression group was 36.8%.

Relationship between ApoE expression and clinicopathologic factors. The differences between the low and high expression groups based on clinicopathologic factors are summarized in Table I. The Chi-square test was used for statistical analysis. Factors such as age and gender did not significantly differ between the two groups. However, there were significant differences when deeper tumor invasion and more positive lymph node metastasis were considered.

Patients with high *ApoE* expression tumor frequently showed deeper tumor invasion than those with low *ApoE* expression tumor. Muscular invasion and serosal invasion were significantly higher in the high *ApoE* expression group than in the low *ApoE* expression group (p<0.0001 and p=0.0059, respectively). Muscular invasion was observed in 59 of 62 (95.2%) cases in the high *ApoE* expression group, and in 35 of 62 (56.5%) cases in the low *ApoE* expression group. Serosal invasion accounted for 33 of 62 (53.2%) cases in the high *ApoE* expression group, and 18 of 62 (29.0%) in the low *ApoE* expression group.

The incidence of lymph node metastasis was also significantly higher in the high *ApoE* expression group than in the low *ApoE* expression group (p=0.0156). The cases with positive lymph node metastasis were 45 of 56 (72.6%) in the high *ApoE* expression group, and 32 of 62 (51.6%) in the low *ApoE* expression group. The incidence of lymphatic permeation was also more frequently observed in the high *ApoE* expression group than in the low *ApoE* expression group (p=0.0504). No significant differences were observed in histological type, distant metastasis, venous permeation and peritoneal dissemination.

Multivariate analysis. High *ApoE* mRNA expression was not an independent indicator for overall survival, based on the results of Cox multivariate analysis (data not shown, RR:1.05, 95%CI:0.78-1.43, p=0.7565). Since *ApoE* mRNA expression was closely correlated with positive lymph node metastasis, we performed multivariate analysis for lymph node metastasis (Table II). However, high *ApoE* mRNA expression was not an independent indicator for lymph node metastasis (RR:1.50, 95%CI:0.54-4.13, p=0.4290). The most influential factors for lymph node metastasis were positive lymphatic permeation (p<0.0001) and positive serosal invasion (p=0.0088).

Since elevated *ApoE* expression was involved in muscular invasion with the most significance (Table I), a positive relationship between *ApoE* expression and lymph node metastasis would be likely observed through the relationship between *ApoE* expression and deeper tumor invasion into the gastric wall. We hypothesized that *ApoE* may play a critical role in tumor invasion into the muscle layer. Since, in this study, all tumors >5 cm in diameter showed deeper invasion than the muscle layer, tumors <5 cm (n=61) were entered into the multivariate analysis for muscular invasion (Table III). The factors affecting muscular invasion were determined by comparing high *ApoE* mRNA expression with undifferentiated type, tumor size >3 cm in diameter and a positive ulcerative area in tumor. As a result, high *ApoE* mRNA expression was revealed as an independent factor for muscular invasion (RR: 65.24, 95%CI: 6.41-2478.96, p=0.0035).

Discussion

Real-time quantitative RT-PCR assay for 124 clinical bulk samples revealed that *ApoE* mRNA expression was frequently higher in gastric cancer tissue than in the corresponding normal tissue (Fig. 1). However, it has been reported that *ApoE* is synthesized in a wide variety of peripheral cells (21,22). Furthermore, Niemi *et al* suggested that *ApoE* may be secreted from stromal cells surrounding the tumor (23). In this study, we prepared four paired specimens - gastric cancer and adjacent normal epithelium were dissected using an LMD system to avoid contamination with stromal cells and necrotic tissues - and compared *ApoE* mRNA expression in gastric cancer with that in normal epithelium (Fig. 2A). It was subsequently confirmed that overexpression of *ApoE* mRNA

The relationship between *Apo*E expression and histopathologic factors.

Clinicopathologic factors	High *Apo*E mRNA expression (n=62)	Low *Apo*E mRNA expression (n=62)	P-value
Age at surgery			
Mean ± SD	65.0±10.4	67.0±11.8	
<69	39 (63.9%)	34 (54.8%)	
≥70	22 (36.1%)	28 (54.8%)	
Unknown	1	0	N.S. (0.3354[a])
Sex			
Male	38 (61.3%)	44 (71.0%)	
Female	24 (38.7%)	18 (29.0%)	N.S. (0.2543)
Histological type			
Differentiated type[b]	29 (46.8%)	31 (50.8%)	
Undifferentiated type[c]	33 (53.2%)	30 (49.2%)	
Unknown	0	1	N.S. (0.6535[a])
Muscular invasion			
Absent	3 (4.8%)	27 (43.5%)	
Present	59 (95.2%)	35 (56.5%)	<0.0001
Serosal invasion			
Absent	29 (46.8%)	44 (71.0%)	
Present	33 (53.2%)	18 (29.0%)	0.0059
Lymph node metastasis			
Absent	17 (27.4%)	30 (48.4%)	
Present	45 (72.6%)	32 (51.6%)	0.0156
Lymphatic permeation			
Absent	14 (22.6%)	24 (38.7%)	
Present	48 (77.4%)	38 (61.3%)	N.S. (0.0504)
Distant metastasis			
Absent	55 (88.7%)	59 (95.2%)	
Present	7 (11.3%)	3 (4.8%)	N.S. (0.1814)
Venous permeation			
Absent	43 (69.4%)	49 (79.0%)	
Present	19 (30.6%)	13 (21.0%)	N.S. (0.2171)
Peritoneal dissemination			
Absent	50 (80.6%)	52 (83.9%)	
Present	12 (19.4%)	10 (16.1%)	N.S. (0.6381)

[a]Unknown, excluded in Chi-square test; [b]Differentiated type, including well/moderately differentiated adenocarcinoma; [c]Undifferentiated type, including signet ring cell carcinoma, mucinous adenocarcinoma and poorly differentiated adenocarcinoma. SD, standard deviation; N.S., not significant.

in bulk samples was derived from gastric cancer. An additional immunohistochemical study also showed that ApoE protein was predominantly expressed in gastric cancer (Fig. 2B). Taking into consideration Kaplan-Meier survival curves indicating shorter survival among patients with high *ApoE* expression tumor (Fig. 3), *ApoE* in gastric cancer could play a critical role in tumor progression. Consistent with our date, Chen *et al* disclosed that *ApoE* is positively correlated with the development of ovarian cancer (14). According to their study, *ApoE* produced from ovarian cancer cells may bind to the LDL receptor via autocrine or paracrine mechanism, and serve as a trigger to activate certain downstream signal transductions, resulting in tumor cell growth. Thus, we consider that ApoE may contribute to gastric cancer development in a similar manner.

The most important aspect of this study is the evidence that gastric cancers with high *ApoE* expression tended to show deeper tumor invasion or more positive lymph node metastasis

Table II. Multivariate analysis for lymph node metastasis.

Candidate indicators for lymph node metastasis	Relative risk (95%CI)	P-value
Undifferentiated type[a]	0.46 (0.15-1.33)	N.S. (0.1666)
Positive serosal invasion	5.04 (1.57-18.21)	0.0088
Positive lymphatic permeation	12.99 (4.62-41.05)	<0.0001
Positive venous permeation	3.84 (1.01-19.27)	N.S. (0.0660)
High *ApoE* mRNA expression	1.50 (0.54-4.13)	N.S. (0.4290)

[a]Undifferentiated type, including signet ring cell carcinoma, mucinous adenocarcinoma and poorly differentiated adenocarcinoma. N.S., not significant; 95%CI; 95% confidence interval.

Table III. Multivariate analysis for muscular invasion.

Candidate indicators for muscular invasion	Relative risk (95%CI)	P-value
Undifferentiated type[a]	1.43 (0.16-15.13)	N.S. (0.7483)
Tumor size (>3 cm)	3.58 (0.33-83.89)	N.S. (0.3216)
Positive excavated or ulcerative area in tumor	32.32 (3.93-755.79)	0.0053
High *Apo*E mRNA expression	65.24 (6.41-2478.96)	0.0035

This analysis was done regarding gastric cancers which were <5 cm in diameter. [a]Undifferentiated type, including signet ring cell carcinoma, mucinous adenocarcinoma and poorly differentiated adenocarcinoma. N.S., not significant. 95%CI, 95% confidence interval.

(Table I). Furthermore, *ApoE* was also closely correlated with positive pathologically lymph node permeation (Table I). These results are in agreement with our hypothesis that *ApoE* may show biological activity for promotion of lymph node metastasis in gastric cancer. However, high *ApoE* mRNA expression was not a very reliable indicator for lymph node metastasis by multivariate analysis (Table II). High *ApoE* mRNA expression showed a close correlation with muscular invasion rather than lymph node metastasis (Table III). For gastric cancers confined to mucosal or submucosal regions, endoscopic resection is a valid choice of curative treatment. Histological cell type, tumor size and the presence of ulcerative change determine whether the tumors are eligible for endoscopic resection (2). Endoscopic ultrasonography (EUS) has been clinically applied as a valuable diagnostic modality for more accurate predictions of the depth of tumor invasion. However, Akashi *et al* reported that the presence of ulcerative change in a tumor decreases correct EUS diagnosis of tumor depth (24,25). Since high *ApoE* mRNA expression was one of the factors predicting the likelihood of muscular invasion, incorporating genetic diagnosis of *ApoE* expression with EUS technology would help in selecting eligible cases for endoscopic resection.

The current study, for the first time, showed a positive correlation between biological aggressiveness of gastric cancers and high *ApoE* expression. However, we need to take note of the presence of three common isoforms, *ApoE2*, *E3* and *E4*, resulting from amino acid substitutions (26,27). Interestingly, it is known that these distinct substitutions affect the binding affinity between *ApoE* and the LDL receptor. Since ApoE achieves its roles by binding to the LDL receptor, the difference in binding affinity could cause a variety of biological behaviors for tumor growth (28-30). According to Zunarelli *et al*, the possession of *ApoE4*, which shows the greatest binding affinity to the LDL receptor, is associated with better prognosis in patients with brain tumor (31). The different *ApoE* phenotypes may have some influence on the result of this study, and future research stratified by phenotypic variation is required.

In conclusion, *ApoE* was highly expressed in gastric cancer. Patients with high *ApoE* expression tumor showed frequent deeper tumor invasion or more positive lymph node metastasis, resulting in shorter survival. In particular, *ApoE* was closely correlated with tumor invasion into the muscle layer. Although further research is required, *ApoE* may be a possible detection modality for the prediction of muscular invasion in gastric cancer.

Acknowledgements

We thank T. Shimooka, K. Ogata, M. Oda, N. Kasagi and Y. Nakagawa for their excellent technical assistance. This work was supported in part by the following grants and foundations: CREST, Japan Science and Technology Agency (JST); Japan Society for the Promotion of Science (JSPS) Grant-in-Aid for Scientific Research, grant numbers 17109013, 17591411, 17591413, 18390367, 18590333, 18659384, 18790964, 19390336 and 19591509; The Ministry of Education, Culture, Sports, Science and Technology (MEXT) Grant-in-Aid for Scientific Research on Priority Area, grant number 18015039.

1. Alberts SR, Cervantes A and van de Velde CJ: Gastric cancer: epidemiology and treatment. Ann Oncol 14: 31-36, 2003.
2. Tanaka M, Ono H, Hasuike N and Takizawa K: Endoscopic submucosal dissection of early gastric cancer. Digestion 77: 23-28, 2008.
3. Yasui W, Oue N, Ito R, Kuraoka K and Nakayama H: Search for new biomarkers of gastric cancer through serial analysis of gene expression and its clinical implications. Cancer Sci 95: 385-392, 2004.
4. Wang L, Zhu JS, Song MQ, Chen GQ and Chen JL: Comparison of gene expression profiles between primary tumor and metastatic lesions in gastric cancer patients using laser micro-dissection and cDNA microarray. World J Gastroenterol 12: 6949-6954, 2006.
5. Terashima M, Maesawa C, Oyama K, *et al*: Gene expression profiles in human gastric cancer: expression of maspin correlates with lymph node metastasis. Br J Cancer 92: 1130-1136, 2005.
6. Oue N, Hamai Y, Mitani, *et al*: Gene expression profile of gastric carcinoma: Identification of genes and tags potentially involved in invasion, metastasis, and carcinogenesis by serial analysis of gene expression. Cancer Res 64: 2397-2405, 2004.
7. Mahley RW: Apolipoprotein E: cholesterol transport protein with an expanding role on cell biology. Science 240: 622-630, 1988.
8. Li Y, Cam J and Bu G: Low-density lipoprotein receptor family: endocytosis and signal transduction. Mol Neurobiol 23: 53-67, 2001.
9. Ho YY, Deckelbaum RJ, Chen Y, Vogel T and Talmage DA: Apolipoprotein E inhibits serum-stimulated cell proliferation and enhances serum-independent cell proliferation. J Biol Chem 276: 43455-43462, 2001.
10. Mahley RW and Rall SC Jr: Apolipoprotein E: far more a lipid transport protein. Annu Rev Genomics Hum Genet 1: 507-537, 2000.
11. Rubinsztein DC, Hanlon CS, Irving RM, *et al*: Apo E genotypes in multiple sclerosis, Parkinson's disease, schwannomas and late-onset Alzheimer's disease. Mol Cell Probes 8: 519-525, 1994.
12. Ishigami M, Swertfeger DK, Hui MS, Granholm NA and Hui DY: Apolipoprotein E inhibition of vascular smooth muscle cell proliferation but not the inhibition of migration is mediated through activation of inducible nitric oxide synthase. Arterioscler Thromb Vasc Biol 20: 1020-1026, 2000.
13. Williams H, Johnson JL, Carson KG and Jackson CL: Characteristics of intact and ruptured atherosclerotic plaques in brachiocephalic arteries of Apolipoprotein E knockout mice. Arterioscler Thromb Vasc Biol 22: 788-792, 2002.
14. Chen YC, Pohl G, Wang TL, *et al*: Apolipoprotein E is required for cell proliferation and survival in ovarian cancer. Cancer Res 65: 331-337, 2005.
15. Dupont-Wallois L, Soulié C, Sergeant N, *et al*: ApoE synthesis in human neuroblastoma cells. Neurobiol Dis 4: 356-364, 1997.
16. Venanzoni MC, Giunta S, Muraro GB, *et al*: Apolipoprotein E expression in localized prostate cancers. Int J Oncol 22: 779-786, 2003.
17. Ohmachi T, Tanaka F, Mimori K, Inoue H and Yanaga K: Clinical significance of TROP2 expression in colorectal cancer. Clin Cancer Res 12: 3057-3063, 2006.
18. Japanese Gastric Cancer Association: Japanese Classification of Gastric Cancer, 2nd English Edition. Gastric Cancer 1: 10-24, 1998.
19. Jass JR, Sobin LH and Watanabe H: The World Health Organization's histologic classification of gastrointestinal tumors. A commentary on the second edition. Cancer 66: 2162-2167, 1990.
20. Kosaka Y, Mimori K, Tanaka F, Inoue H, Watanabe M and Mori M: Clinical significance of the loss of MATS1 mRNA expression in colorectal cancer. Int J Oncol 31: 333-338, 2007.
21. Mazzone T: Apolipoprotein E secretion by macrophages: its potential physiological functions. Curr Opin Lipidol 7: 303-307, 1996.
22. Zannis VI, Cole FS, Jackson CL, Kurnit DM and Karathanasis SK: Distribution of Apolipoprotein AI, CI, CIII and E mRNA in fetal human tissues. Time-dependent induction of apolipoprotein E mRNA by cultures of human monocyte-macrophages. Biochemistry 24: 4450-4455, 1985.
23. Niemi M, Häkkinen T, Karttunen TJ, *et al*: Apolipoprotein E and colon cancer expression in normal and malignant human intestine and effect on cultured human colonic adenocarcinoma cells. Eur J Intern Med 13: 37-43, 2002.
24. Akashi K, Yanai H, Nishikawa J, Satake M, Fukagawa Y, Okamoto T and Sakaida I: Ulcerous change decreases the accuracy of endoscopic ultrasonography diagnosis for the invasive depth of early gastric cancer. Int J Gastrointest Cancer 37: 133-138, 2006.
25. Kim JH, Song KS, Youn YH, Lee YC, Cheon JH, Song SY and Chung JB: Clinicopathologic factors influence accurate endosonographic assessment for early gastric cancer. Gastrointest Endosc 66: 901-908, 2007.
26. Raffai RL, Dong LM, Farese RV Jr and Weisgraber KH: Introduction of human apolipoprotein E4 'domain interaction' into mouse apolipoprotein E. Proc Natl Acad Sci USA 98: 11587-11591, 2001.
27. Zunarelli E, Nicoll J, Migaldi M and Trentini GP: Apolipoprotein E polymorphism and breast carcinoma: correlation with cell proliferation indices and clinical outcome. Breast Cancer Res Treat 63: 193-198, 2000.
28. Kervinen K, Södervik H, Mäkelä J, Lehtola J, Niemi M, Kairaluoma MI and Kesäniemi YA: Is the development of adenoma and carcinoma in proximal colon related to Apolipoprotein E phenotype? Gastroenterology 110: 1785-1790, 1996.
29. Niemi M, Kervinen K, Kiviniemi H, *et al*: Apolipoprotein E phenotype, cholesterol and breast and prostate cancer. J Epidemiol Community Health 54: 938-939, 2000.
30. Slattery ML, Sweeney C, Murtaugh M, *et al*: Associations between apoE genotype and colon and rectal cancer. Carcinogenesis 26: 1422-1429, 2005.
31. Zunarelli E, Nicoll JAR and Trentini GP: Apolipoprotein E polymorphism and central nervous system tumors: correlation with cell proliferation indices and clinical outcome. Clin Neuropathol 19: 1-7, 2000.